ISOLATION OF DYE DEGRADING MICROORGANISMS FROM TEXTILE EFFLUENTS

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ABSTRACT

AIM: To isolate probable Novel dye-degrading microorganisms from textile effluents.

METHODOLOGY: For the collected textile effluents, the enrichment of microorganisms was carried out using a dilute nutrient medium simulating the natural environment in hopes of isolating 'yet uncultured' dye-degrading microorganisms. The inocula were then spread on dilute media containing dye.

RESULTS: After incubation, almost all the plates showed large patches of decolorization, indicating the capacity of microorganisms to degrade azo dye.

INTERPRETATION: There is a possibility that the patches of decolorization seen could be due to yet uncultured dye-degrading microorganisms. In the future, this study can be taken further to identify and characterize the microorganisms isolated. It is worth finding the decolorization potential of these microbes at varying concentrations of the dyes. A study can also be carried out wherein different inocula can be mixed and the collective decolorizing capacity of a consortium could be analyzed.

INTRODUCTION

In the early 19th century, dyes used for coloring fabric materials were primarily obtained from natural sources. These natural sources were either of vegetable (i.e., weld, madder, indigo) or animal origin (i.e., cochineal, shellfish) or chemical types such as flavonoids (yellow), anthraquinones (red) and indigoids (blue and violet) [1]. Dyes play a vital role in various branches of the dyeing and textile industries. There are two groups of dyes available: Natural dyes and Synthetic dyes. Among the above classification of dyes, Synthetic Dyes i.e., Azo Dyes are the most frequently used dyes in dyeing industries. These dyes can be easily identified by their azo groups [2]. Textile industries consume synthetic dyes on a large scale, thus contributing to the economic growth of the country. The downside of these industries is that during the manufacturing process, only 5% of the raw materials used are contained within the garments while the remaining 95% of the materials which majorly consist of dyes are released into nearby water bodies.

This thick, ink-like water is frequently let off through pipes which cannot be traced back to their sources, meaning factories can commit this offense anonymously.

Among the different classes of dyes used for manufacturing colored garments, Azo dyes are considered to be recalcitrant, non-biodegradable, and persistent [3],[4]. Hence, the degradation of these dye-based effluents is one of the most challenging jobs due to environmental concerns [5]. These dyes are lethal to living organisms as they are toxic and carcinogenic. Disposal of these azo dyes from textile processing industries into water bodies causes various environmental hazards like reduction in dissolved oxygen and reduction in water transparency [6] along with adverse effects on germination and growth of the plant species. Dyes contain various aromatic compounds, metal salts, chlorides, etc. These components affect photosynthesis in aquatic plants by preventing light penetration and are also toxic to aquatic fauna and flora. Hence, removal of these dyes from textile effluents is necessary [7], [8].

Generally, physical or chemical methods are used to get rid of azo dye residues in wastewater effluents. However physical methods are time-consuming, labor intensive, and maybe even expensive, while chemical methods despite being faster and easier to use are harmful to the aquatic flora and fauna. As compared to these methods, nowadays biological methods for degrading azo dyes have proved to be beneficial as they are cost-effective and environment friendly.

Extensive studies have been carried out where dye degrading capacities of different microorganisms such as bacteria [9], fungi [10], actinomycetes[11], algae[12], etc. have been reported. Among previous studies, it was found that the laccase from the fungus Pleurotus ostreatus showed 95.72% decolorization of Remazol Brilliant violet 5R dye [13]. It was also found that the use of mixed bacterial cultures showed much better decolorization as compared to pure cultures[14]. To aim for a unique and different approach from current studies, we tried employing strategies like the use of a dilute nutrient medium and natural environmental components for enrichment and isolation of probable novel yet uncultured microorganisms.

The Ulhas and Waldhuni are among 53 of the most polluted rivers in Maharashtra and 351 of the most polluted ones across the country according to the Central Pollution Control Board. A major source of this pollution are the textile effluents let off into these rivers by textile industries. Ulhas supplies drinking water to over three million people in Maharashtra's Badlapur-Thane belt [15]. Thus, it is essential to find a solution to the pollution of the Ulhas and Waldhuni rivers. The following study focuses on studying the dye-degrading capacity of microorganisms present in soil.

MATERIALS AND METHODS:

SELECTION OF DYES:

The two dyes used in this study were Indigo Carmine AR and a local red-colored dye. Indigo Carmine was specifically selected for this study as it is the most widely used dye in textile industries, particularly in the manufacturing of blue jeans. The Indigo carmine used was of analytical grade having molecular formula $C_{16}H_8N_2Na_2O_8S_2$ and chemical structure [Fig.(1)] [16]. The red dye was a local dye used regularly in small-scale textile dyeing processes.

This dye was previously decolorized in a complete medium (nutrient broth) by the inocula collected and to check if the same was possible in a dilute medium we used this dye in the present study.

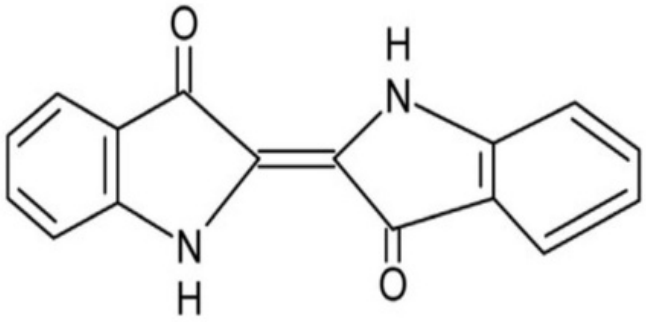

Fig. (1): Chemical structure of Indigo Carmine

PROCUREMENT OF DYES:

Indigo Carmine AR (CAS-No.:860-22-0) was procured from LOBA CHEMIE PVT.LTD INDIA, while the red-colored dye was procured from a local dyer. The stock solution of Indigo Carmine AR was prepared by dissolving 0.1g of powdered dye in 10ml distilled water and the red local dye solution was used as it is as stock solution. The stock solutions of both dyes were sterilized by autoclaving before use.

PROCUREMENT OF INOCULUM:

Soil samples to be used as inoculums were collected in clean and dry glass bottles. One of the soil samples was collected from soil present in a canal heavily polluted with untreated textile effluents, at Vicko Naka, Dombivli East, near the MIDC industrial belt (19.02129°, 73.111076°). (USED AS INOCULUM 1). Two other soil samples were also collected from similarly contaminated soils from the Dombivli and Airoli Creek areas. These soil samples were mixed and used as INOCULUM 2.

PROCUREMENT OF NATURAL ENVIRONMENTAL COMPONENT:

In the case of INOCULUM 1 water sample was collected from the same canal from where the inoculum was acquired. This water sample (EV 1) was added to the enrichment medium as a component of the natural environment after sterilization by autoclaving. In the case of INOCULUM 2, due to the inability to obtain a water sample the inoculum itself was used as a natural environmental component (EV 2) after proper sterilization by autoclaving.

MEDIA COMPOSITION:

To increase the probability of isolating yet uncultured bacteria, a dilute nutrient medium simulating the natural environment was used. Nutrient broth basal medium (HiMedia) containing (g/lit): Yeast extract 3.0, peptone 5.0, NaCl 8.0 in 1000ml distilled water having pH 6.5+2 was used in 1:100 diluted form after sterilization by autoclaving.

ENRICHMENT PROCESS:

An enrichment medium (1:100 diluted) was prepared by dissolving 0.026g of nutrient broth powder in 200 ml distilled water. This 200ml of broth was distributed in 6 test tubes to prepare 3 systems. Each system consisted of 1 TEST and 1 CONTROL. [Fig. (2)]

SYSTEM 1: dilute nutrient broth+ EV 1+ stock solution of indigo carmine AR.

SYSTEM 2: dilute nutrient broth+ EV 2+ stock solution of red dye.

SYSTEM 3: dilute nutrient both+ EV 2+ stock solution of indigo carmine AR.

[265µl of dye stock solution was added to obtain a 1:100 dilution of dye in each test tube]

After this, inoculums were added to all 3 TEST test tubes. In TEST 1 we added INOCULUM 1; while in TEST 2 &3 INOCULUM 2 was added.

Immediately after inoculation of the medium, Optical Density was determined using a colorimeter at a wavelength of 620nm for SYSTEM 1, 3, and at 530nm for SYSTEM 2. All 6 tubes were then incubated at room temperature (30+2°C) in sunlight. Optical density was regularly determined at an interval of 3 days. Determination of optical density (O.D) helped in calculating the decolorization taking place in each system by using the formula:

$$\% \text{ DECOLORIZATION} = \frac{\text{O.D initial} - \text{O.D final.}}{\text{O.D initial}} \times 100$$

Fig. (2): systems set up for enrichment

RESULTS:

After 48 hours of incubation, one of the nutrient agar plates containing 1:2 dye concentration showed small patches of decolorization. The results of dye degradation are shown in Fig. 3(a, b). After 72 hours the patches of decolorization increased in size. After a total incubation of 5 days all the plates having both 1:2 and neat concentration of dye showed large patches of decolorization which extended all over the plates. This study thus indicates the capacity of microorganisms grown in dilute media to degrade azo dyes to an extent. Limitations of the study include recreation of the exact enrichment conditions on a large scale. Along with this development of pure cultures of the obtained isolates was difficult due to the extremely small size of the colonies.

Fig. (3-a): Plate on day 0

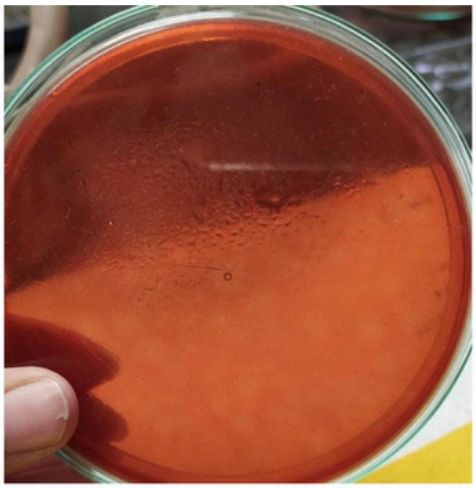

Fig. (3-b): Plate after 48 hours

DISCUSSION:

In recent years, the ever-increasing environmental pollution has become a major global dilemma. Currently, numerous studies are being carried out to deal with this environmental pollution. More than physical and chemical methods, biological methods of dealing with various pollutants are gaining popularity. Over the period of time, it was discovered that microorganisms have the capacity to degrade various pollutants. The approach of biological degradation of azo dyes using microorganisms has been recently gaining recognition due to its advantages. A large number of studies have been carried out to study the dye-degrading potential of various microorganisms. Not just microorganisms from soil or water but microorganisms from various other systems have also been studied for their dye-degrading potential. A large number of studies dealing with azo dye residues have been reported to date: [17], [18], [19], and [20].

By referring to the results of many such studies we too decided to check the potential of microorganisms in the degradation of two different dyes. In the present study, we first enriched the microorganisms in a 1:100 dilute medium containing a small portion of the natural environment.

In spite of the fact that microbial diversity is extremely vast, 99% of microorganisms in nature are still unidentified. These unidentified bacteria most probably have a wide spectrum of capacities that are still unexplored. In hopes of isolating such "yet-uncultured; novel bacteria" and exploring their potential to degrade azo dyes, a dilute media strategy was used in this study.

Another major advantage of using a dilute medium is that at low nutrient concentrations, k-strategists which have low nutritional requirements flourish abundantly as compared to the fast-growing opportunistic r-strategists. In this way, we were able to selectively enrich and isolate only the yet uncultured k-strategists with dye degrading capacity, while limiting the growth of fast-growing r-strategists that would otherwise conveniently outgrow and mask the slow-growing k-strategists. This is helpful as oligophiles or uncultured species fit best into the role of k-selected species due to their low growth rate, slow growth in the enrichment medium and high affinity to the substrate [21],[22]. We also added a portion of the natural environment in the enrichment medium as several studies have provided evidence that not all bacteria from a given environment can grow on laboratory media. An explanation for this is the failure to recreate essential aspects (nutrients, pH, osmotic conditions, temperature, or many more) of their environment in the laboratory. A solution to this is using the environment itself as an aid in growing the microorganisms, which can be done by stimulating the natural environmental conditions in the laboratory [23].

After incubation of the enrichment medium for about 13 days, all 3 systems showed varying degrees of decolorization. However significant decolorization was only seen in SYSTEM 2 and thus only SYSTEM 2 was taken forward for isolation.

ISOLATION OF DYE-DEGRADING MICROORGANISMS:

Enrichment medium from SYSTEM 2 was spread on Sterile Nutrient Agar plates containing different concentrations i.e. 1:2 and stock concentration (NEAT) of the local red dye. Nutrient agar plates were prepared in 1:100 diluted form.

All the plates were incubated at room temperature (30+2°C) and were observed periodically.

It has been seen that with increasing dye concentration, time taken for decolorization increases[24]. This is similar to what we observed in the present study wherein decolorization was first observed on plates with 1:2 concentration of dye and then on plates with neat dye concentration.

In numerous studies, greater decolorization was observed under shaking enrichment conditions as compared to static conditions. This could be because, in a static incubation environment, oxygen transfer is restricted to the surface of the broth because of which cell cultures which tend to settle at the bottom of the tubes experience rapid depletion of oxygen[25].In the present study shaking incubation conditions for enrichment were not practiced. Incorporation of these conditions in the assay could improve the extent of decolorization observed.

CONCLUSION

In this study, we enriched and isolated microorganisms in a dilute medium by taking the aid of their natural environment and observed their decolorization ability in agar media plates containing dye. Exploring more sensitive assays can be a valuable approach to identifying microorganisms with the best decolorizing potential. By varying factors like temperature, pH, inoculum size, concentration of dye, incubation time, etc optimum conditions for maximum decolorization can be achieved. Using this study as a reference, organisms from different environmental samples can be isolated, characterized and studied for their dye-degrading potential. The decolorization of dyes by microorganisms in a dilute medium indicates the presence of probable novel microorganisms. Isolation of these microorganisms and extraction of their dye-degrading enzymes could help in generating novel bioremediation strategies to reduce water pollution by azo dyes.

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Manasi Naik, Ishita Mopkar, and Ramkishan Kumbhar are students belonging to the Department of Microbiology at VPM's B.N.Bandodkar of College of Science (Autonomous), Thane. This paper represents a culmination of extensive research, analysis, and critical thinking on the subject matter by the three of them.