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Biological Treatments : Isolation of Mycobiota from Selected Samples

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ABSTRACT

Introduction: The contemporary world's fast industrialization and urbanisation have both led to an increase in the quantity of garbage that is discarded into the environment, which in turn contributes to the problem of pollution.

Aim of the study: the main aim of the study is to Biological Treatments: Isolation of Mycobiota From Selected Samples

Material and method: All of the samples were gathered in sterile plastic bags, transported to the laboratory, and put to use straight away in the process of isolating fungus.

Conclusion: There are many different industries that employ dyes, including the leather tanning business, the textile industry, the paper industry, food technology, agricultural research, light-harvesting arrays, photoelectrochemical cells, hair colouring, and cosmetics.

Keywords: Mycobiota, Biological Treatments

I. INTRODUCTION

1.1 OVERVIEW

The contemporary world's fast industrialization and urbanisation have both led to an increase in the quantity of garbage that is discarded into the environment, which in turn contributes to the problem of pollution. Water is necessary for the continued existence of life on earth and for its own survival. It is one of the principal sources of environmental toxicity, and it also has an effect on the soil microflora and the aquatic ecosystem. Waste water and sewage are both discharged from industrial facilities, and these wastes are then carried into bodies of water. The production of enormous volumes and high concentrations of aqueous waste effluents is the aspect of the textile dyeing business that does the greatest damage to the environment as a result of its operations. The introduction of dye effluents that include refractory residue into water bodies such as rivers and lakes. The textile industry, the cosmetics industry, paper mills, pulp industries, dyeing and dye intermediates industries, and bleaching industries are responsible for the production of more than 80,000

tonnes of dyes and pigments annually. These industries also produce residual dyes from a variety of other sources. Particularly in the textile sectors, more than 70 percent of India's total trash was created by those businesses. After China, India is the country that exports the second most dyestuffs and intermediates in the world. The use of dyestuffs is highest in the textile sector, accounting for about 80% of total consumption. Industrialization is essential to the economy of a country because it acts as a catalyst for growth at the national level. However, there are additional issues that arise as a consequence of the disposal of waste products from industrial processes into the natural environment. The persistence (poor biodegradability) and toxicity of many of these compounds creates an issue for the environment. The effluent that is not treated is one of the principal sources of metal dyes, phenol, and aromatic amines that are taken by humans. Phenol and many aromatic amines are known to cause cancer and mutations in people. Internal organs such as the kidney, liver, and gastrointestinal system are also impacted by dye exposure.

1.2 MICROBIAL DEGRADATION OF DYES

Numerous sectors, including the textile, leather, paint, food, cosmetic, and paper industries, rely heavily on dyes as an essential supply of colour. According to the chemical structure of the chromophore, there are roughly twenty-five different kinds of dye groups that may be used. There are over a thousand different dyes that fall under the category of textile dyes, which are used to colour a wide range of garments. Dye precursors are referred to as dye intermediates. With the assistance of a number of different chemical processes, one may produce them from the raw ingredients, which include naphthalene and benzene. The contamination of water bodies is caused by the discharge of effluents from municipalities and other industrial sources into bodies of water. Pollution has a negative impact on the environment, and this has the potential to pose direct or indirect health concerns to all kinds of life that exist on earth. There are many different categories that may be applied to dyes based on their composition and use. Because dyes have a high capacity for solubilization in water, it is difficult to remove them using the procedures that have traditionally been used. Dye used in textiles often comprises colours, which not only harms creative expression but also prevents light from diffusing through water, which in turn lowers the concentration of dissolved oxygen and slows the pace at which aquatic organisms may photosynthesise. The removal of dyes and other pollutants from industrial wastewater may be accomplished using a variety of processes, including physico-chemical, biological, chemical, and physical approaches. The technique of biological therapy is uncomplicated, economical, and kind to the natural world, which are only a few of its many merits. There is also a vast variety of microorganisms accessible, all of which are simple to keep alive and need nothing in the way of preparation. In addition to the aforementioned strategies for dye degradation, the periphyton biofilm or periphytic biofilm system may also be used for the breakdown of dyes. Dye manufacture is the most significant contributor to environmental pollution caused by the industry as a whole owing to the leakage of toxic dyes into bodies of water throughout the manufacturing process. The capacity to mineralize and/or decolorize a wide variety of dyes is possessed by a wide variety of microorganisms, including algae, yeast, bacteria, and fungus. Both pure and mixed microbial cultures may be used in the process of treating effluent from dye manufacturing. It has been observed that a mostly mixed culture of microorganisms may accomplish effective dye degradation as a result of synergistic metabolic processes.

II. LITERATURE REVIEW

Zafar, Sadia & Bukhari (2022) The term "dyes" refers to synthetic aromatic compounds with a variety of functional groups. These colourful substances are often released in effluents and are very harmful to

aquatic life. Basically, the dye business began by obtaining its materials from natural plant and insect sources before abruptly switching to artificial synthesis. The decrease in photosynthetic activity brought on by dyes alters the natural balance of our environment. 900,000 tonnes of dyes of all types are typically produced in China and utilised in a variety of sectors, including food, textile, food, paper, and leather. Untreated wastewater seriously harms aquatic life by eutrophicating bodies of water, changing the colour of the water, and depleting oxygen levels. The main source of environmental degradation nowadays is dye wastewater. Recent years have seen the development of a robust research programme to investigate the decolorization and biodegradation of dyes in both aerobic and anaerobic environments. The chemistry, toxicity, and microbial biodegradation/decolorization are discussed in this paper. To provide the framework for how to handle dye pollution, several current research are also covered, along with new methodology and strategies. Overall, microorganisms are an important basis for green chemistry to remove hazardous dyes from industrial wastewater due to their great biodegradation capacity and efficiency.

Gopal, Adithya & Franco, Valan M.F. (2022) Due to the toxic dyes that the chemical and textile industries are discharging into the land and rivers, environmental contamination has increased. The removal of harmful dyes from water bodies and the land at the same time has compelled the scientific community to search for appropriate methods for waste water treatment. In recent years, the removal of colours from wastewater and effluent has been made possible via photochemical processes. A short overview of inorganic chemistry-based removal techniques for hazardous dyes is provided.

Daphedar, Azharuddin & Kakkalameli (2022) Different kinds of colourful pigments have historically been extracted naturally from biological sources such as shells, flowers, insects, and so on. These natural coloured compounds (dyes) are now being replaced by synthetic dyes. On the other hand, these synthetic dyes or coloured compounds continue to exist in the environment owing to their ongoing use for diverse purposes. For instance, industrial wastewater includes a variety of pollutants, such as dyes. Many of them (artificial colours) have been discovered to be hazardous to living things. The removal of dye(s) using microorganisms has received increased attention lately. These techniques were reasonably priced for getting rid of such toxins from the environment. In order to decolorize synthetic colours from industrial effluent, numerous researchers isolated microorganisms from environmental samples. Additionally, the genetically modified bacteria discovered a greater degradative/decolorize ability to target substances in the natural surroundings. There are very few evaluations on particular dye treatment methods, including chemical, bacterial, and/or fungal methods. Here, we provide enlightening literature findings on the removal of various colours by microorganisms, including bacteria (including anaerobic and aerobic), fungus, GEM, microbial enzymes, and greenly generated nanoparticles. This current literature review will assist environmental managements in identifying such contaminants in nature and in the process of decolorizing dyes.

Kuok Ho, Daniel Tang & Tang (2022) Comparing biological removal of dyes to physicochemical techniques of treating dye effluents, biological removal of dyes has been supported for its ease of use, cost-effectiveness, and minimal operating needs. This article compares the effectiveness of biological colour removal utilising bacteria, algae, and fungus, including yeasts, in addition to outlining new developments in the area. The majority of the academic publications analysed in this report were released between 2010 and 2021. Numerous colours might be broken down by bacteria, it was discovered. The efficiency with which one colour was broken down by various microorganisms varied. Similar to this, different bacterial species may destroy various colours. Although fungi are thought to degrade

colours more quickly than bacteria, this research reveals that bacteria are just as effective at doing so. It's also important to note that certain yeast species have been found to be particularly effective at decolorizing dyes. Pure bacteria cultures were often found to have lower dye-decolorizing effectiveness than mixed bacteria or bacteria-fungus cultures. Algae may need more contact time and are less effective in decolorizing pigments than bacteria and fungus. The effectiveness of dye biodegradation may be improved by recent innovations including genetic engineering and the immobilisation of microorganisms and enzymes. However, there are obstacles that must be removed before biological removal of dyes may be employed in a practical way. The complexity of optimization, the inability to completely decolorize dyes, the potential for the formation of toxic byproducts during dye decolorization, safety concerns regarding immobilisation materials, the cost and technical viability of biological removal of dyes are some of the major limitations. This review is significant because it highlights the major limitations of the present biological dye removal method, which might open the door for new developments in this field of study.

Khan, Rana Rashad Mahmood & Qamar (2022) Technology growth has caused a huge impact in the environment. Pollutants are being released into the environment directly by industries. For mankind, water contamination is a major problem. The development of techniques for handling wastewater produced by biological and industrial wastes. By introducing colours into water supplies, the textile industry puts the health of all living things at risk. The main component of wastewater from the textile sector is azo dyes. The photodegradation of Congo red, the most well-known Azo dye, is the main topic of this review paper. Both biological (using microorganisms) and chemical (using nanoparticles) techniques of Congo red degradation are being researched. The majority of the bacterial and fungal species used in the biological technique. Bacillus sp., Pseudomonas sp., and Staphylococcus lentus sp. all effectively break down Congo red dye. Fungal cell walls include functional compounds like phosphates and hydroxyls that facilitate effective dye breakdown. Because nanoparticles do not produce polycyclic chemicals during the breakdown of dyes, they are preferred for this purpose. Due to their wide band gaps, several bimetallic catalysts, including ZnO and TiO2, have shown strong photocatalytic capabilities. It is preferable to employ nanoparticles that are simple to separate following photodegradation. Doping enhances the degrading ability of nanocatalysts because Gd3+ doped cobalt ferrite nanoparticles have greater removed capacities than undoped cobalt ferrite nanoparticles.

III. METHODOLOGY

3.1 Biological Treatments: Isolation Of Mycobiota From Selected Samples

The El-Kanater El-Khairia area provided the location for the collection of a total of twenty samples, ten of which consisted of decaying eucalyptus leaves and ten of which were soil samples. All of the samples were gathered in sterile plastic bags, transported to the laboratory, and put to use straight away in the process of isolating fungus. After transferring ten grammes of each sample into 250 ml Erlenmeyer conical flasks containing sterilised saline (90 ml), the flasks were placed in an incubator for fifteen minutes at a speed of 150 rpm and a temperature of 35 degrees Celsius. Serial dilutions were prepared in order to obtain an adequate colony count. On the surface of a medium consisting of potato-dextrose agar (PDA), one millilitre of each dilution was plated. Following an incubation period of three to seven days at a temperature of thirty degrees Celsius, the developing colonies on the plates were selected for further purification. The isolates were re-streaked on PDA plates in order to purify them, and then they were inspected under a microscope to ensure that they were pure.

4. RESULTS

4.1 Isolation and identification of mycobiota from selected samples:

We were able to recover a total of twenty-six different fungus isolates. After undergoing purification and identification, the acquired isolates were found to be of the following species: Aspergillus niger (11 isolates), Aspergillus flavus (4 isolates), and Penicillium sp. (11 isolates), as shown in Table 4.1.

4.2 Screening of the obtained fungal isolates for decolorization of textile dyes:

• Preliminary screening:

The fungal isolates that were collected were put through a screening process to determine whether or not they had the capacity to decolorize textile colours. Phanerochaete chrysosporium ATCC 24725 was employed as the standard decolorizing fungal strain to assess its ability in decolorization of Isolan Red and Isolan Navy as a preparatory step to the isolated 26 strain's decolorizing capacity. The results of this comparison are summarised in Table 4.1.

Table 4.1 Following seven days of incubation at 30°C and 150 rpm, local isolates were tested for dye decolorization in contrast to a reference decolorizing fungal strain.

SOURCE OF ISOLATES	IDENTIFICATIONOF ISOLATES	PERCENTAGE OF ISOLAN DECOLORIZATION	
		Isolan Red	Isolan Navy
ATCC 24725	*Phanerochaete chrysosporium*	82.0	65.0
Soil around eucalyptus tree	*Aspergillus niger*-1	40.0	4.0
	Aspergillus niger-2	23.0	15.0
	Aspergillus flavus-3	21.0	16.0
	Penicillium-4	40.4	0.0
	Aspergillus niger ES-5	77.0	50.0
	Penicillium-6	35.0	5.5
	Aspergillus niger-7	45.0	11.0
	Penicillium-8	20.0	15.0
	Penicillium-9	31.0	6.25
	Aspergillus niger-10	40.0	9.5

	Penicillium-11	30.5	15.0
	Aspergillus niger-12	67.6	40.7
	Aspergillus flavus-13	12.0	10.5
Leaves of eucalyptus	*Penicillium*-14	23.0	9.0
	Penicillium-15	25.4	8.0
	Penicillium-16	19.0	15.0
	Penicillium-17	23.0	14.0
	Aspergillus niger-18	31.0	0.0
	Aspergillus niger-19	8.0	0.0
	Aspergillus niger-20	35.0	10.0
	Aspergillus niger-21	23.0	6.25
	Penicillium-22	15.0	7.5
	Aspergillus niger-23	30.0	13.0
	Penicillium-24	25.0	11.0
	Aspergillus flavus 2	25.0	20.0
	Aspergillus flavus 3	31.0	19.6

Selection of the best isolate for maximum decolorization:

According to the findings, only four of the isolates (Aspergillus flavus 2, Aspergillus flavus 3, Aspergillus niger ES-5, and Aspergillus niger-12) represented the highest decolorization ability of Isolan Red and Isolan Navy. Because of this, only these four isolates were chosen for further testing on the other four Isolan dyes. Due to the fact that Aspergillus niger ES-5 was the only fungus that was able to decolorize the four Isolan dyes at a rate that was greater than 50%, it was chosen to be the subject of additional research in the upcoming experiments. This information can be gleaned from Table 4.2 and Fig. 4.1. The four different isolates each revealed a different decolorization percentage of the four Isolan dyes.

Table 4.2 After 7 days incubation at 30 °C and 150 rpm, the isolate with the highest capacity for decolorizing all four Isolan dyes was chosen

Isolates	Percentage of Isolan dye decolorization			
	I.Y	I.R	I.N	I.G
Phanerochaete chrysosporium ATCC 24725	75.0	82.0	65.0	38.5
Aspergillus flavus 2	45.0	25.0	20.0	27.5
Aspergillus flavus 3	50.0	31.0	19.6	13.5
Aspergillus niger ES-5	91.0	77.0	50.0	50.0
Aspergillus niger-12	91.0	67.6	40.7	57.5

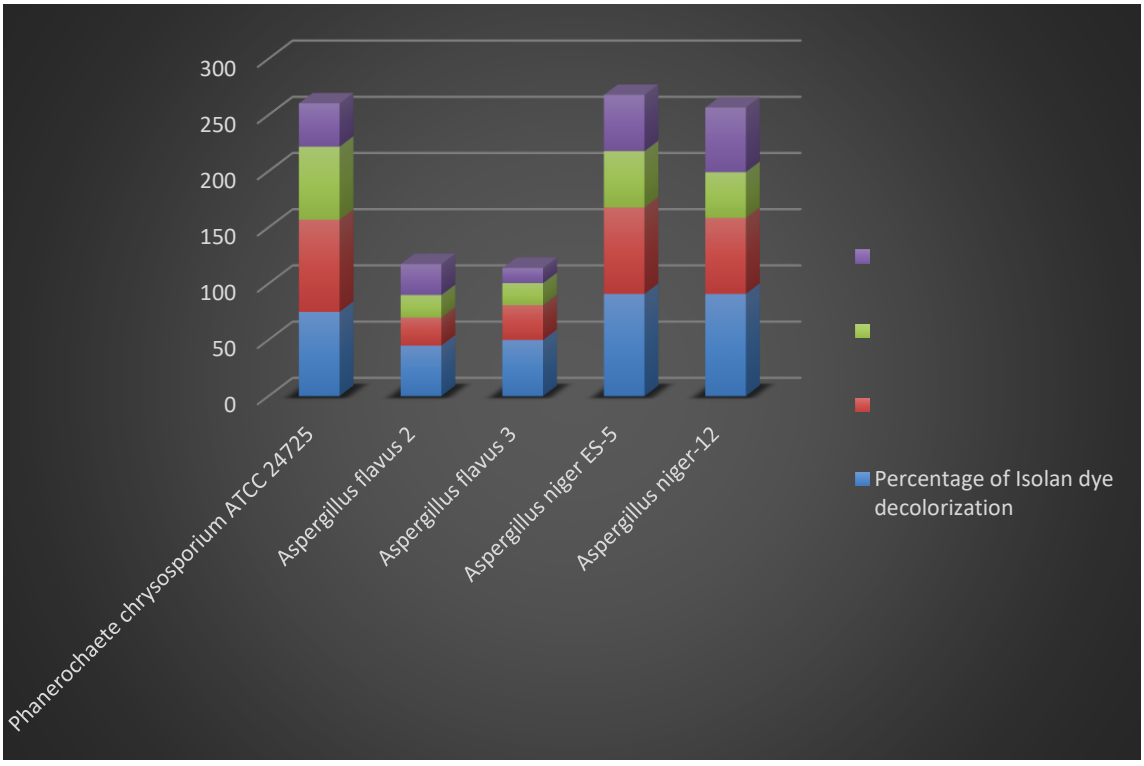


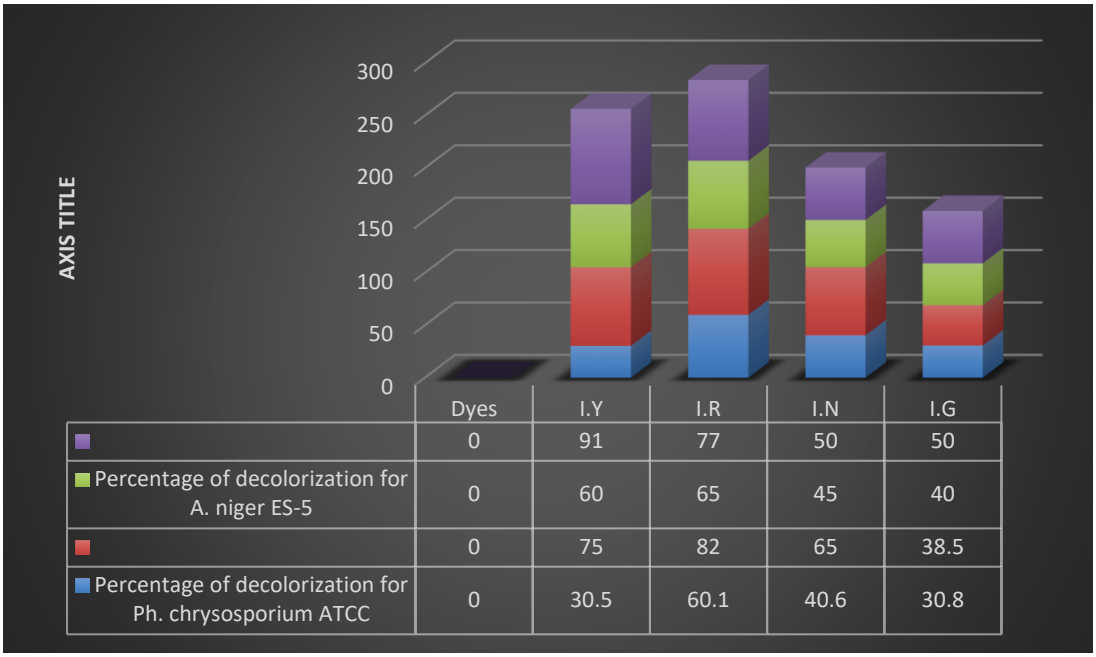
Fig. 4.1 The four Isolan dyes were used to do a comparative study of the most decolorizing fungus isolates and Ph. chrysosporium

Effect of culturing method on the decolorization of the Isolan dyes:

Efficiency of Aspergillus niger ES-5 to decolorize the four Isolan dyes under both static and shaking conditions (at 150 rpm) in comparison with Phanerochaete chrysosporium ATCC 24725, results represented that under static conditions, Aspergillus niger ES-5 is capable of decolorizing the four Isolan dyes in higher percentages than Phanerochaete chrysosporium ATCC 24725, but the results obtained by both fungi is low in comparison with the results obtained in case of shaking condition where, static culture produce aerial mycelia which prevent oxygen transfer as presented in Table 4.3 and Fig. (2).

Table 4.3 Effect of static and shaking (150 rpm) condition on the four Isolan dyes decolorization by Phanerochaete chrysosporium ATCC 24725 and Aspergillus niger ES-5 at 30°C, after 7 days of incubation.

Dyes	Percentage of decolorization for *Ph. chrysosporium* ATCC 24725		Percentage of decolorization for *A.niger* ES-5	
	Static condition	Shaking condition	Static condition	Shaking condition
I.Y	30.5	75.0	60.0	91.0
I.R	60.1	82.0	65.0	77.0
I.N	40.6	65.0	45.0	50.0
I.G	30.8	38.5	40.0	50.0



Dyes	I.Y	I.R	I.N	I.G
	0	91	77	50	50
Percentage of decolorization for A. niger ES-5	0	60	65	45	40
	0	75	82	65	38.5
Percentage of decolorization for Ph. chrysosporium ATCC	0	30.5	60.1	40.6	30.8

Fig. 4.2 Effect of static and shaking conditions on the decolorization of the four Isolan dyes by Phanerochaete chrysosporium ATCC 24725 and Aspergillus niger ES-5.

Decolorization on solid medium:

Both Aspergillus niger ES-5 and Phanerochaete chrysosporium ATCC 24725 were unable to decolorize all four Isolan dyes on solid-agar plates, as shown in Table 4.4. This is obvious from the results of the investigation into the decolorization capabilities of both of these fungi on solid agar plates.

Table 4.4 Solid-plate dye decolorization by Phanerochaete chrysosporium ATCC 24725 and Aspergillus niger ES-5 at 30°C for 7 days.

Dyes	*Phanerochaete chrysosporium* CC24725	*Aspergillus niger* S-5
.Y		
.R		
.N		
.G		

Where (-): No inhibition zones; (+): Partial decolorization and (++): Complete decolorization.

Evaluation of the culture conditions for maximum decolorization:

Effect of media contents on dye decolorization, GOD and dry weight:

As a result of the fact that many dyes have distinctive molecular structures, a microorganism that is effective at decolorizing one dye may not be as effective at decolorizing other dyes. This is due to the fact that each dye requires a unique enzyme combination in order to be broken down.

Effect of glucose on the decolorization percentage, GOD and dry weight of the tested fungi:

In the absence of the enzyme substrate, the structural genes that provide the coding for the manufacturing of many enzymes are generally inactive. Induction refers to the process by which the enzyme is created after the addition of the substrate, which causes the structural gene to become active and triggers the production of the enzyme. Inducers for sugar oxidases are sugars that have been introduced to the medium. Only when the substrate that the enzymes attack is present in the media will the enzymes be induced to create themselves. The effect of glucose on the percentage of decolorization, glucose oxidase activity, and fungal growth was investigated. Two types of basal salt media, BSM I and BSM II, were prepared as shown in Table (3.2). The results in Table 4.5 and Fig. 4.3 demonstrated that BSM II containing no glucose gave an insignificant value in Isolan dye decolorization when compared to BSM I. I.Y gave 84.4 and 20%, I.R gave 89.5 and 7%,

Table 4.5 The effect of glucose on the decolorization of dye by Aspergillus niger ES-5 over 168 hours at 30 degrees Celsius and 150 revolutions per minute.

Dyes	Percentage of decolorization							
	I.Y		I.R		I.N		I.G	
Time (h)	BSMI	BSM II	BSMI	BSM II	BSMI	BSM II	BSMI	BSM II
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24	28.1	15.0	41.0	13.6	70.6	16.2	76.0	50.0
48	15.6	6.6	88.0	6.8	77.5	11.1	81.0	30.0
72	84.4	20.0	89.5	7.0	80.0	29.3	87.0	30.0
168	84.4	20.0	85.5	7.0	77.0	10.5	79.8	28.0

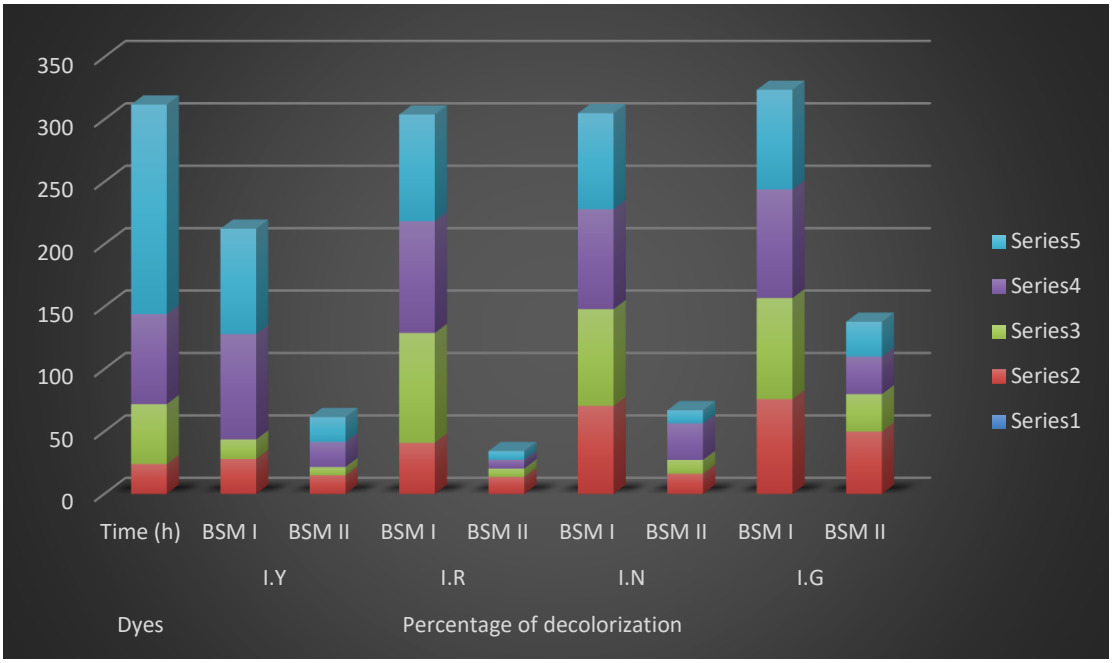


Fig. 4.3 Effect of glucose on Isolan dyes decolorization by Aspergillus niger ES-5 (a) Isolan Yellow, (b) Isolan Red, (c) Isolan Navy and (d) Isolan Grey.

The elimination of glucose also had a detrimental impact on glucose oxidase activity and the formation of fungal colonies, as shown in Tables 4.6, 4.7, and Figures 4.6 and 4.7, respectively (4.4, 4.5).

Table 4.6 Effect of glucose on the production extracellular GOD by Aspergillus niger ES-5 in BSM at 30°C and 150 rpm.

Dyes	Extracellular GODin BSM I (U/ml)	Extracellular GOD inBSM II (U/ml)
I.Y	9.2	0
I.R	8.1	0
I.N	9.5	0
I.G	10.8	0

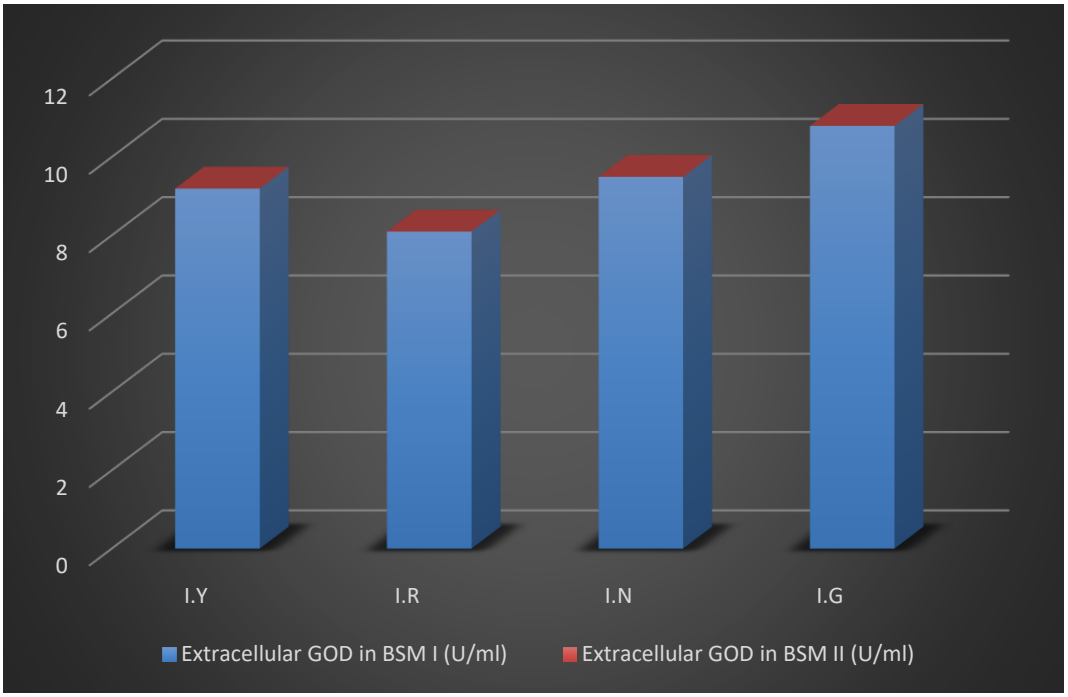


Fig. 4.4 Effect of glucose on the production of extracellular GOD by Aspergillus niger ES-5 in BSM at 30°C and 150 rpm.

Table 4.7 Effect of glucose on dry weight by Aspergillus niger ES-5 in BSM at 30°C and 150 rpm.

Dyes	Dry weight in BSMI (g/100ml)	Dry weight in BSMII (g/100ml)
I.Y	4.05	1.97
I.R	4.10	2.24
I.N	3.40	1.85
I.G	3.10	2.49

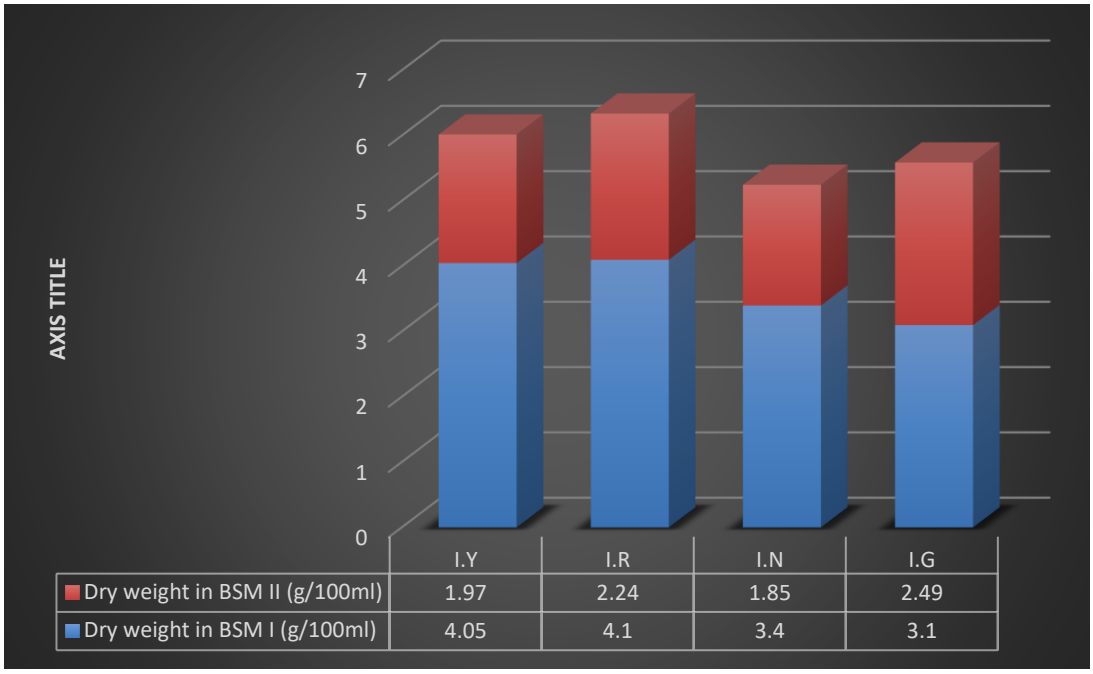


Fig. 4.5 Effect of glucose on dry weight by Aspergillus niger ES-5 in BSM at 30°C and 150 rpm.

As can be seen in Tables 4.6 and 4.7 and Figures 4.6 and 4.7, respectively, the removal of glucose also had a negative effect on the activity of glucose oxidase and the growth of fungal colonies (4.4, 4.5).

There are many different industries that employ dyes, including the leather tanning business, the textile industry, the paper industry, food technology, agricultural research, light-harvesting arrays, photoelectrochemical cells, hair colouring, and cosmetics. The dyeing of textiles is by far the most important use in industry due to the vast volumes that are utilised. The azo dye family has the largest market share among synthetic dyes, accounting for close to 70 percent of all textile dyestuff manufactured. They are simple to manufacture, have a cheap cost, are stable, may be used to colour a number of materials (including textiles, leather, plastic, and food), and enable for a wide range of colours and tones to be achieved. They contain one or more azo groups in their molecular structure. The discharge of dye into the surrounding environment in the form of wastewater is the aspect of the dying industry that causes the greatest environmental damage. The unchecked emission of these substances into the environment is a major contributor to the severity of the situation. They have been developed to be chemically and photolytically stable, which contributes to their high level of persistence in their natural settings. Because they reduce the amount of light that is absorbed, dyes may have a major impact on the photosynthetic activity of aquatic life. Furthermore, dyes may be hazardous because they contain aromatic compounds or heavy metals.

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