Degradation of Some Textile Dyes using Biological and Physical Treatments

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Reham Fathey
Dedication

My special gratitude to my parents, my sister Neven, my brothers Bassem and Ahmed for their forever love and support. I would like thank my dear husband, Amr, without his tremendous help and patience; I would not be of success in my life.

Reham Fathey
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Reham Fathey
Abstract

A total of twenty samples composed of ten samples of decaying eucalyptus leaves and ten soil samples were collected from El-Kanater El-Khairia district. All isolates were purified and identified to the species level. They found to be belonging to two main genera: *Aspergillus sp.* and *Penicillium sp.* The obtained fungal isolates were screened for testing their ability to decolorize Isolan dyes. The strain *Aspergillus niger* ES-5 was chosen for its highest ability to decolorize the four Isolan dyes. The biological decolorization of the textile metal azo dye was investigated under co-metabolic conditions. The decolorization capacity of the strain was influenced by the presence and/or absence of media components.

The majority of decolorization was growth related, where resulted in 90.4%, 99.6%, 95.0% and 94.6% for I.Y, I.R, I.N and I.G, respectively after 72 h, only 2.5, 1.3, 1.4 and 3.0% for I.Y, I.R, I.N and I.G, respectively were desorbed, while negligible decolorization was detected using extracellular fluid (ECF) as well as using dead pellets. The addition of the dye to fungal cultures didn’t affect the extracellular GOD production while intracellular GOD production exhibited a different profile. Pictures of the mycelia represent dye uptake over the 72 h period of decolorization. The metal detection using Energy Dispersive X-ray Spectroscopy (EDS) of the outer fungal mycelium wall and ECF were both below detection level after the decolorization process took place. Thus, decolorization process and the removal of the elements by *A. niger* ES-5 involve initial adsorption followed by entrapment of the adsorbed dye inside the fungal biomass. Gamma rays increase color intensity in I.Y, while the other three Isolan dyes showed negative decolorization efficiency till 2.5 kGy after which, slow increase in the decolorization was observed.
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AOP</td>
<td>Advanced Oxidation Processes</td>
</tr>
<tr>
<td>ATCC</td>
<td>American Type Culture Collection</td>
</tr>
<tr>
<td>BOD&lt;sub&gt;5&lt;/sub&gt;</td>
<td>Biological Oxygen Demand</td>
</tr>
<tr>
<td>BSM</td>
<td>Basal Salt Medium</td>
</tr>
<tr>
<td>C.I.</td>
<td>Color Index</td>
</tr>
<tr>
<td>COD</td>
<td>Chemical Oxygen Demand</td>
</tr>
<tr>
<td>ECF</td>
<td>Extracellular Fluids</td>
</tr>
<tr>
<td>EDS</td>
<td>Energy Dispersive X-ray Spectroscopy</td>
</tr>
<tr>
<td>EEAA</td>
<td>Egyptian Environmental Affairs Agency</td>
</tr>
<tr>
<td>ETMF</td>
<td>Egyptian Textile Manufacturers Federation</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier Transform Infrared Spectroscopy</td>
</tr>
<tr>
<td>GOD</td>
<td>Glucose Oxidase enzyme</td>
</tr>
<tr>
<td>GRAS</td>
<td>Generally Recognized As Safe</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>---------</td>
<td>-------------</td>
</tr>
<tr>
<td>I.G</td>
<td>Isolan Grey</td>
</tr>
<tr>
<td>I.N</td>
<td>Isolan Navy</td>
</tr>
<tr>
<td>I.R</td>
<td>Isolan Red</td>
</tr>
<tr>
<td>I.Y</td>
<td>Isolan Yellow</td>
</tr>
<tr>
<td>kGy</td>
<td>Kilo Gray</td>
</tr>
<tr>
<td>LiP</td>
<td>Lignin Peroxidase</td>
</tr>
<tr>
<td>MnP</td>
<td>Manganese Peroxidase</td>
</tr>
<tr>
<td>NCRRT</td>
<td>National Center for Radiation Research and Technology</td>
</tr>
<tr>
<td>PCP</td>
<td>Pentachloro Phenol</td>
</tr>
<tr>
<td>PDA</td>
<td>Potato-Dextrose Agar</td>
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</table>
Introduction
Introduction

Due to the increased demand for textile products, the textile industry and its wastewater have been increasing proportionally, making it one of the main sources of severe pollution problems worldwide. Approximately 100,000 commercial dyes and dyestuff are used in the coloring (textile, cosmetic, leather) industries and around 10-15% of all dyestuff are directly lost to wastewater. Particularly, azo dyes are the most commonly used synthetic dyes in textile, food, paper-making and cosmetic industries. However, release of residual azo dye into industrial effluents deteriorates the water quality not only because of their color which result in aesthetic problems and affects photosynthesis in aquatic plants, but also because many azo dyes from wastewater and their breakdown products are toxic and/or mutagenic to various forms of life and may cause a significant impact on human health due to their mutagenic and carcinogenic effects.

In addition to the environmental problem, the textile industry consumes large amounts of potable water. In many countries where potable water is scarce, this large water consumption has become intolerable and wastewater recycling has been recommended in order to decrease the water requirements and also recycling of dyes to be used again.

Without adequate treatment, these dyes are stable and can remain in the environment for an extended period of time. Therefore, this effluent must be treated before discharge into natural water streams.
Introduction

The most often used methods for decolorization and degradation of dyes are chemical and physical treatments whereas most of these methods have limitations such as high running cost and disposal of large amount of sludge produced during these processes.

Consequently, most investigations have focused on using the most economical and the environmental friendly approaches such as radiation technology and biological processes.

Radiation technology has been recognized as a promising process for wastewater treatment using $\gamma$-rays or accelerated electrons as a simple and efficient technique.

Industrial biological wastewater treatment systems are designed to remove pollutants from the environment using microorganisms. The microorganisms used are responsible for the degradation of the organic matter. Biological treatments have several advantages such as cheap, simple, produce smaller volumes of excess sludge and high flexibility, since it can be applied to very different types of effluents.

The textile industries is considered one of the most important industries all over the world and considered as the 5th largest source of foreign currency, but it also considered as the main sources of water pollution because the textile companies in Egypt discharge their wastewater into soak way and in few cases to stream of potable water.

Results presented by most researchers focus on white rot fungi and their degradative enzymes. Thus, the main challenge is not only to isolate a local and effective strain which has the ability to decolorize wastewater in high percentage but also to
detect the mode of action by which these fungi perform the decolorization process.

The following study is devoted to study a local brown rot fungi isolate for both basic and applied standpoint. It is categorized into two main aspects: the first is to adjust suitable conditions for fungus to achieve maximum decolorization of dye wastewater in order to detect the mechanism by which *Aspergillus niger* ES-5 decolorize the most common used dyes in Egypt. The second one is to decolorize textile effluents and try to reuse dyes which could be considered the most practical solution.

This study had the following objectives:

1- Isolation of mycobiota from selected samples.

2- Identification of the isolated mycobiota.

3- Screening of the obtained fungal isolates for decolorization of textile dyes.

4- Adjust co-metabolic condition in order to achieve maximum decolorization.

5- Determine chemical parameters before and after fungal treatment.

6- Toxicity test using *Escherichia coli*.

7- Detection mode of action by which *Aspergillus niger* ES-5 decolorize dye wastewater.

8- Physical treatment using gamma radiation of dye wastewater.

9- Decolorize effluents wastewater.

10- Re-use of entrapped dyes to dye cloth.
Literature Review
Textile dyes

Ever since clothes were woven, people have found ways to enhance their own appearances and express beauty by adorning or decorating their garments using different ornaments and paint. Painting with dyes made from natural materials has been done for thousands of years. The history of natural dyes is very interesting, mankind has used dyes for centuries and the earliest proof of use of a colorant is believed to be by Neanderthal man about 180,000 years ago (Christie, 2007).

However, the first known use of an organic colorant was much later, being nearly 4000 years ago, when the blue dye indigo was found in the wrappings of mummies in Egyptian tombs. Alexander the Great is supposed to have deceived the Persians into thinking that his army was wounded, by sprinkling his soldiers with a red dye, probably madder juice, which contains the dye alizarin. A legend recorded on coins attests that Hercules, the god of strength, discovered tyrian purple which was used by the Phoenicians in the 25th century B.C. when his dog bit a snail which stained his jaws purple (Gordon and Gregory, 1983).

Till the late nineteenth century, all the dyes were more or less natural with main sources like vegetable extracts, leaves, twigs, roots, berries, flowers of various plants, insects and mollusks, and were generally have limited range of colors and prepared on a small scale (Gupta and Suhas, 2009).
Literature Review

Although natural dyes are of a chemical and historical interest, in most dyeing operations these dyes are very expensive, need purification and do not bind well because they lack the chemical grouping necessary to react with the binding sites of a particular fabric. Therefore, they have been completely replaced by synthetic colors, which are generally purer, less expensive; moreover, the colors bind faster to the fabric (Gallop, 1987).

In 1771 Woulfe prepared picric acid by the action of nitric acid on indigo and showed that it dyed silk in bright yellow shades. In 1856 Perkin discovered mauve, the first synthetic dye to be manufactured and used for practical dyeing (Hunger, 2003).

Alizarin was the first natural dye to be produced synthetically at 1868 and at 1880 indigo had been synthesized. In 1916, an extensive technology had developed, most of it concentrated within a German cartel that held a virtual monopoly over dye production (Gallop, 1987).

Mordants are chemical substances formerly used to confer affinity for textile fibers to natural and early synthetic dyes. Alizarin, applied with an aluminum-salt mordant, was used extensively to produce bright red shades on cotton. Tannin and sodium stannate were used to form insoluble salts with basic dyes on silk. Chromium is still used but to a limited extent in the United States (U.S) and to a greater extent abroad combined with azo and basic dyes for the dyeing of wool and the printing of cotton. Only with the onset of World War II did Germany lose its position as the world’s principal supplier of dyes. Today, the U.S dye industry aided by the post World War
The acquisition of German technology has become a major exporter of dyes (Gallop, 1987).

Dye molecules are comprised of two key components: the chromophores, responsible for producing the color, and the auxochromes, which can not only supplement the chromophore but also render the molecule soluble in water and add an enhanced affinity toward the fiber (Christie, 2001).

Mishra and Tripathy (1993) classified synthetic dyes in three categories. Anionic which include direct, acid and reactive dyes. Non-ionic including disperse dyes and finally cationic which described as basic dye. The only common characteristic for these dyes is their ability to absorb light in the visible region.

On the other hand, dyes may be classified according to their chemical structure into azo dyes and anthraquinone dyes. Azo dyes characterized by being strong, good all-round properties and cost-effective, while anthraquinone dyes are weak and expensive. Dyes may also be classified on the basis of their solubility as soluble dyes which include acid, mordant, metal complex, direct, basic and reactive dyes; and insoluble dyes including azoic, sulfur, vat and disperse dyes (Dos Santos et al., 2007).

- **Azo dyes:**

Azo dyes are characterized by the presence of one or more azo bonds (-N=N-), in association with one or more aromatic structures. They are designed to convey high photolytic stability and resistance towards major oxidizing agents. They have wide variety of application in textile, food,
paper-making and cosmetic industries (Chung and Stevens, 1993 and Carliell et al., 1995).

Weber and Wolfe (1987) found that azo-and nitro-compounds are reduced in sediments and in the intestinal environment, resulting in the regeneration of the parent toxic amines, also Chang and Lin (2001) stated that the release of residual azo dyes into industrial effluents deteriorates the water quality, and may cause a significant impact on human health due to mutagenic or carcinogenic effects of some azo dyes or their metabolites. Thus, particularly in the case of azo dyes, effluent treatment becomes a serious issue because of their negative impact on water ecosystems and human health, especially that thousands of azo dyes have been developed for use on every type of fiber (Reife and Othmer, 1993 and Yesiladali et al., 2006).

- Anthraquinone dyes:

   Anthraquinone dyes constitute the second most abundant class of textile dyes, after azo dyes (Baughman and Weber, 1994). Anthraquinone dyes have a wide range of colors in almost the whole visible spectrum. Anthraquinone-based dyes are the most resistant to degradation due to their fused aromatic structures, which remain colored for long periods of time (Fontenot et al., 2003).

- Acid dyes:

   Acid dyes are used for nylon, wool, silk, modified acrylcs, paper, leather, ink-jet printing, food and cosmetics. They are generally water soluble. The principal chemical classes of these dyes are azo (including premetallized),
anthraquinone, triphenylmethane, azine, xanthene, nitro and nitroso (Hunger, 2003 and Christie, 2007).

• Metal-complex dyes:

Metal-complex dyes are primarily used on wool, they are combinations of a dyestuff and a metal (usually chrome) and they are in use since the 1940's. They were developed from the older mordant dyes and are highly light-and wash-fast, metal-based complex dyes, such as chromium-based dyes, their use could lead to the release of chromium, which is carcinogenic in nature, into water supplies (Banat et al., 1996).

• Direct dyes:

Direct dyes are used in the dyeing of cotton, rayon, paper, leather and nylon. They are water-soluble anionic dyes; generally, the dyes in this class are polyazo compounds, along with some stilbenes, phthalocyanines and oxazines (Hunger, 2003).

• Basic dyes:

Basic dyes are used for paper, polyacrylonitrile, modified nylon, modified polyesters, cation dyeable polyethylene terephthalate and in medicine too. Originally they were used for silk, wool and tannin-mordanted cotton. These water-soluble dyes yield colored cations in solution and that's why they are called as cationic dyes. The principal chemical classes are diazahemicyanine, triarylmethane, cyanine, hemicyanine, thiazone, oxazine and acridine (Hunger, 2003 and Christie, 2007).
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- **Reactive dyes:**

  Reactive dyes are water soluble, anionic dyes that require relatively simple dyeing methods; they are mainly used for dyeing cellulosic fibers, such as cotton and rayon but are also used for silk, wool, nylon and leather (Yang et al., 2005).

- **Sulfur dyes:**

  Sulfur dyes are used for cotton and rayon and have limited use with polyamide fibers, silk, leather, paper and wood. They have intermediate structures and though they form a relatively small group of dyes, the low cost and good wash fastness properties make this class important from an economic point of view (Hunger, 2003).

- **Vat dyes:**

  Vat dyes are extremely fast dyes used for cotton mainly to dye cellulosic fibers as soluble leuco salts and for rayon and wool too. These dyes are with principal chemical class containing anthraquinone (including polycyclic quinones) and indigoids (Christie, 2007).

- **Disperse dyes:**

  Disperse dyes are used mainly on polyester and to some extent on nylon, cellulose, cellulose acetate and acrylic fibers. These are substantially water-insoluble non-ionic dyes used for hydrophobic fibers from aqueous dispersion. They generally contain azo, anthraquinone, styryl, nitro and benzodifuranone groups (Hunger, 2003 and Christie, 2007).
**Effect of textile wastewater discharge on the environment:**

Prior to displaying the effect of textile wastewater on the environment, the textile manufacturing process and the kind of toxic substances generated from this process must be known.

There are main three stages involved; they are spinning, knitting or weaving and wet processing the later involves many steps like sizing, desizing, scouring, bleaching, mercerizing, dyeing, printing and finishing. Each of these operations generates huge amounts of wastewater and pollution from wet processing steps desizing is one of the largest sources of wastewater pollutants and often contributes up to 50% of the Biological Oxygen Demand (BOD) load in wastewater. The scouring process also has a high BOD and also uses the highest volumes of water in the preparatory stages. The major pollution issues in the bleaching process are chemical handling, water conservation and high pH values. Also, using pentachlorophenol (PCP) during scouring, bleaching, dyeing and printing which is removed from the fabric and discharged into the wastewater. It is toxic due to its relative stability against natural degradation processes and it is also bioaccumulative (EEAA, 2003).

But the majority of wastewater containing residual dyes is generated after dyeing and printing. Colored wastes reportedly contribute about 10-30% of the total BOD and in many cases reach 90%. Dyes also contribute about 2-5% of the Chemical Oxygen Demand (COD), while dye bath chemicals contribute about 25-35%. In addition to the high BOD and
COD values of dyes, toxicity to aquatic organisms and fish toxicity have also been reported.

Salts (e.g. sodium chloride and sodium sulphate) have been identified as a potential problem area in textile-dyeing wastewater, although the mammalian and aquatic toxicity of salts are very low. Their massive use in certain textile-dyeing process can produce wastewater with salt levels well above the regulatory limits. On the other hand, heavy metals present in textile wastewater may typically include copper, cadmium, chromium, nickel and zinc. Toxic effects of heavy metals to animal and aquatic life are dependent on the physico-chemical interaction. Dyes and pigment from printing and dyeing operation are the principal sources of colors in textile effluent. Finishing processes typically generate wastewater containing natural and synthetic polymers and a range of other potentially toxic substances (EEAA, 2003).

From the previous explanation of textile manufacturing process it is apparent that textile wastewater must be treated, although the concentration of dyes in wastewater is usually lower than the other chemicals present (less than 1 ppm for some dyes), they often receive the largest attention due to their strong color that render them highly visible even at very low concentration, thus causing serious aesthetic and pollution problems in wastewater disposal and water transparency and gas solubility in lakes, rivers and other water bodies (Kritikos et al., 2007). The removal of color from wastewater is often more important than the removal of soluble colorless organic substance, which usually contribute the major fraction of BOD (Banat et al., 1996).
There are more than 8000 chemical products associated with the dyeing process listed in the color index, while over 100,000 commercially available dyes exist with over $7 \times 10^5$ metric tons of dyestuff produced annually per year (Fu and Viraraghavan, 2001 and Park et al., 2007), approximately 100,000 commercial dyes and dyestuffs are used in the coloring (textile, cosmetic, leather) industries (Robinson et al., 2001 and Yesiladali et al., 2006). Due to inefficiencies of industrial dyeing process, 10-15% of the dyes are lost in the effluents of textile units, rendering them highly colored (Vaidya and Date, 1982 and Boer et al., 2004).

It is estimated that 280,000 tons of textile dyes are discharged every year in such industrial effluents worldwide (Maas and Chaudhari, 2005). Direct discharge of these effluents causes formation of toxic aromatic amines under anaerobic conditions in receiving media. In addition to their visual effect and their adverse impact in terms of COD, many synthetic dyes are toxic, mutagenic and carcinogenic (Jin et al., 2007), therefore, water pollution control is currently one of the major areas of scientific activity.

The frequently high volumetric rate of industrial effluent discharge in combination with increasingly stringent legislation, make the search for appropriate treatment technologies an important priority (O'Neil et al., 1999).

Effluents from the textile industries containing dyes are highly colored and are therefore visually identifiable (Kilic et al., 2007). The complex aromatic structure of the dyes is resistant to light, biological activity, ozone and other degradative environmental conditions, thus conventional
wastewater treatment remains ineffective (Kaushik and Malik, 2009). Also, anionic and non-ionic azo dyes release toxic amines due to the reactive cleavage of azo groups (Joshi et al., 2004). Presence of heavy metals like chromium, cobalt and copper in wastewater is also an environmental concern (Freeman et al., 1996). Up till now scientists have been trying to develop a single and economical method for the treatment of dyes in textile wastewater but still it remains a big challenge (Dos Santos et al., 2007 and Kaushik and Malik, 2009).

Different methods of textile wastewater treatment:

During the past two decades, several physico-chemical decolorization techniques have been reported, few however, have been accepted by textile industries (Pala and Toket, 2002 and Zhang et al., 2003).

These include physico-chemical flocculation combined with flotation, electro flotation, flocculation with Fe (II)/Ca (OH)₂, membrane-filtration, electrokinetic coagulation, electrochemical destruction, ion-exchange, irradiation, precipitation, ozonation and katox treatment method involving the use of activated carbon and air mixtures (Fu and Viraraghavan, 2001). Among all the used methods, adsorption is the most widely used methods (Abdelwahab et al., 2006 and Akkaya et al., 2007). Both adsorption and chemical coagulation/precipitation do not destroy the dyes, they rather result in phase transfer of the pollutant, and hence ultimate sludge disposal remains an unsolved problem (Yesiladali et al., 2006).
There are several physico-chemical decolorization methods used to degrade wastewater such as:

- **Chemical treatments:**

  The goal of any oxidation process is to generate and use hydroxyl free radicals (OH\(^{-}\)) as strong oxidant that react with organic contaminants and destroy compounds that can not be oxidized by conventional oxidant (Al-Kdasi et al., 2004).

  Fenton's reagent is an effective chemical means of decolorization of the textile wastewater which are resistant to biological treatment or is poisonous to live biomass but not very effective in reducing its COD unless combined with another process such as coagulation (El-Kadri et al., 2002).

  Ozone treatment is widely used in water treatment; ozone either alone or in combinations (O\(_3\)-UV or O\(_3\)-H\(_2\)O\(_2\)) is now used in the treatment of industrial effluents (Langlais et al., 1991). Since, chromophores groups which is responsible for color, can be broken down by ozone either direct or indirect formation of smaller molecules (Swaminathan et al., 2006).

  Industrial textile wastewater is usually treated by conventional methods such as photochemical oxidation (Parsa and Abbasi, 2007). UV light activates the destruction of H\(_2\)O\(_2\) into hydroxyl radicals, these radicals may attack organic molecules by abstracting a hydrogen atom from the molecule that can oxidize organic compound (RH) producing organic radical (R) (Georgiou et al., 2002; Azbar et al., 2004 and Rezaee et al., 2008).

  Sodium hypochlorite (NaOCl) attacks the amino group of the dye molecule by the Cl\(^{-}\); it initiates and accelerates azo
bond cleavage (Slokar and Le Marechal, 1997). Although sodium hypochlorite (NaOCl) added to effluent may remove residual colors effectively, its high cost and environmental problems of biological toxicity must be taken into consideration (Hsing et al., 2007).

Cucurbituril was investigated regarding its potential as a sorbent for the removal of reactive dyes from model solutions and authentic wastewater (Karcher et al., 2001).

In recent years, there has been an increasing interest in the use of electrochemical method for the treatment of wastewater; these methods have been successfully applied in the purification of textile wastewater (Parsa and Abbasi, 2007). On the other hand, the cost of electricity used is so high comparable to the price of chemicals (Robinson et al., 2001).

- Physical treatments:

  Decolorization by physical treatment is a result of two mechanisms: adsorption and ion exchange (Slokar and Le Marechal, 1997), and is influenced by many physio-chemical factors, such as, dye/sorbent interaction, sorbent surface area, particle size, temperature, pH and contact time (Kumar et al., 1998).

  Adsorption is an attractive and effective method for dye removal from wastewater, especially if the adsorbent is cheap and widely available (Eren and Acar, 2007).

  Activated carbon is the most widely used method of dye removal with great success because it's higher adsorption capacity (Amin, 2009). The adsorption on activated carbon
without pretreatment is impossible because the suspended solids rapidly clog the filter (Matsui et al., 2005).

The cellular structure of peat makes it an ideal choice as an adsorbent. It has the ability to adsorb transition metals and polar organic compounds from dye-containing effluents. Unlike activated carbon it requires no activation, and also costs much less (Poots and Mckay, 1976a).

Wood chips show a good adsorption capacity for acid dyes, although due to their hardness, it is not as good as other available sorbents and longer contact times are required (Poots and Mckay, 1976b and Nigam et al., 2000).

Many low-cost adsorbents have been investigated on fly ash for dye adsorption (Mane et al., 2007). Its adsorption capacity depends on the properties of adsorbent such as porous structure, chemical structure and surface area (Dincer et al., 2007).

Silica gel could be considered as an effective material for removing basic dyes, although side reactions such as air binding and air fouling with particulate matter, prevents it from being used commercially (Robinson et al., 2001).

Membrane filtrations were used to separate color and hazardous chemicals from textile wastewater (Arafat, 2007). It has some special features unrivalled by other methods; resistance to temperature, an adverse chemical environment, and microbial attack (Robinson et al., 2001).

Ion exchange resins were applied to decolorize the textile wastewater and to reduce its COD (Karcher et al., 2002). Ion exchange has not been widely used for the treatment of dye-
containing effluents, mainly due to the opinion that ion exchangers can not accommodate a wide range of dyes (Slokar and Le Marechal, 1997 and Robinson et al., 2001).

Electrocoagulation has been developed in recent years and employs the electrochemical reaction to produce metal ions at the anode, causing the metal ions to immediately undergo further spontaneous reactions to produce corresponding hydroxides and/or polyhydroxides at the cathode (Mollah et al., 2004).

Radiation in general is the emission of any rays or particles from a source, the energy travels through space (Uvarov and Isaacs, 1986). Radiation is classified into two main categories; ionizing radiation and non-ionizing.

Generally, use of radiolysis in environmental remediation of wastewater, contaminated soil, sediment and textile effluents is a promising treatment technology; because the effect of radiation can be intensified in aqueous solution in which the dye molecules are degraded effectively by the primary products formed from the radiolysis of water (Getoff and Lutz, 1985). The radiation dose necessary for complete decomposition of a dye depends principally on its molecular structure and reactivity towards the primary water radiolysis products (Şolpan and Gűven, 2002).

Gamma and electron beam radiation can be considered as alternate methods for the treatment of wastewater from textile industries (Şolpan and Gűven, 2002 and Şolpan et al., 2003).

In such techniques, mainly hydroxyl radicals are used as primary oxidants (Wojnárovits and Takács, 2008). The
usefulness of ionizing radiation for efficient degradation of a large variety of pollutants has been successfully demonstrated (Zhang et al., 2005).

Finally, the drawbacks of all these methods have been largely due to the high cost, low efficiency, limited versatility and disposal problems, thus most of the chemical and physical methods for treating dye wastewater are not widely applied in textile industry sites or plants (Mazmanci and Unyayar, 2005 and Jin et al., 2007).

Since many textile plants have rural locations and municipal treatment costs are expensive, both industries and scientists are becoming compelled to search for innovative novel treatments and technologies directed particularly towards the decolorization of dyes in effluents (Zee and Villaverde, 2005).

- **Biological treatments:**

  Since the removal of dyes from effluents by physico-chemical means are often very costly, though efficient accumulation of concentrated sludge creates a disposal problem. Thus, there is a need to find alternative treatments that are effective in removing dyes from large volumes of effluents, low in cost and technically attractive (Dias et al., 2003).

  Biological methods being cheap and simple to use are resorted to as the proposed solution. The ability of microorganisms to carry out dye decolorization has received much attention and is seen as a cost-effective method for removing these pollutants from the environment. Lately, fundamental work has revealed the existence of a wide variety
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of microorganisms capable of decolorizing wide range of dyes (Robinson et al., 2001).

Microbial decolorization involving suitable bacteria, algae and fungi has attracted increasing interest (McMullan et al., 2001), these microorganisms are able to biodegrade and/or bioabsorb dyes in wastewater (Fu and Viraraghavan, 2001).

Efforts to isolate bacterial cultures capable of degrading azo dyes started in the 1970's with report of a Bacillus subtilis (Horitsu et al., 1977), followed by numerous bacteria: Aeromonas hydrophila (Idaka and Ogawa, 1978), Bacillus cereus (Wuhrmann et al., 1980), Pseudomonas strains (Kulla, 1981), Proteus mirabilis (Chen et al., 1999) and Mycobacterium avium (Jones and Falkinham, 2003).

Several actinomycete strains have been reported with a capability to decolorize reactive dyes, including anthraquinone, phthalocyanine and azo groups, through adsorption of dyes to the cellular biomass without any degradation. Other Cu-based azo dyes, such as formazan-copper complex dyes, were completely decolorized through degradation by the same actinomycete strains (Zhou and Zimmermann, 1993).

Algae have been suggested to remove color from textile wastewater (Tarlan et al., 2002). The potential use of commonly available green algae belonging to species Spirogyra was investigated as viable biomaterials for biological treatment of simulated synthetic azo dye (reactive yellow 22) effluents, their ability to remove color was dependent both on the dye concentration and algal biomass (Mohan et al., 2002).
A number of simple azo dye was degraded in liquid aerated batch cultures by a strain of yeast *Candida zeylanoides* (Martins et al., 1999). Only limited studies about yeast decolorization were reported, the ability of *Kluyveromycse marxianus* IMB3 to decolorize remazol black b dye was investigated (Meehan et al., 2000).

The role of fungi in the treatment of wastewater has been extensively investigated (Azmi et al., 1998; Coulibaly et al., 2003 and Brar et al., 2006). Fungi have proved to be a suitable organism for the treatment of textile effluents and in dye removal (Banat et al., 1996). The fungal mycelia have an additive advantage over single cell organisms by solubilising the insoluble substrates by producing extracellular enzymes, due to an increased cell-to-surface ratio; fungi have a greater physical and enzymatic contact with the environment. The extracellular nature of the fungal enzymes is also advantageous in tolerating high concentrations of the toxicants (Kaushik and Malik, 2009).

Many genera of fungi have been employed for the dye decolorization either in living or dead form. Based on the mechanism involved through biodegradation, biosorption and/ or bioaccumulation. Biodegradation is an energy dependent process and involves the breakdown of dye into various by-products through the action of various enzymes. Biosorption is defined as binding of solutes to the biomass by processes which do not involve metabolic energy or transport, although such processes may occur simultaneously where live biomass is used. Therefore, it can occur in either living or dead biomass (Tobin et al., 1994). Bioaccumulation is the accumulation of pollutants by actively growing cells by metabolism (Aksu and Donmez, 2005).
Enrichment procedures were designed to obtain microbial agents suitable for decolorizing dye containing wastewater resulted in the isolation of several strains of fungi capable of decolorization. These include strains *Myrothecium verrucaria* and *Ganoderma sp.* mainly through adsorption to the fungal mycelium and effective for a wide range of dyes. It was reported that high dye concentrations resulted in low color removal (*Mou et al.*, 1991).

**Spadaro et al.** (1992) observed that aromatic rings with substituents such as hydroxyl, amino, acetamido, or nitro functions were mineralized to a greater extent than unsubstituted rings in dye decolorization by *Phanerochaete chrysosporium*. In contrast, **Paszczynski et al.** (1992) reported that the substitution pattern of five sulfonated azo dyes did not significantly influence the susceptibility of the dyes to degradation by *Phanerochaete chrysosporium*.

A strain of *Aspergillus sojae* B-10 was shown to be capable of decolorizing the azo dye amaranth, congo red and sudan III in nitrogen-poor media after 3 to 5 days of incubation (*Ryu and Weon*, 1992).

**Wong and Yu** (1999) reported that dye decolorization by *Trametes versicolor* was dependent on dye structure, anthraquinone dyes were laccase substrate while azo and indigo dyes were not the substrates for the same enzyme. **Zhang et al.** (1999) also observed that the color removal efficiency decreased with an increase in the concentration of the cotton bleaching effluent.

**Yesiladali et al.** (2006) demonstrated that *Trichophyton rubrum* LSK-27 is a promising culture for dye removal.
applications and can be a potential candidate for treatment of textile effluents under aerobic conditions leading to non toxic degradation of dye compounds.

Economical and environment friendly approaches are needed for degradation of dyes. The degradation of dyes by white rot fungi was first reported in 1983 (Glenn and Gold, 1983), since then, many white rot fungi have been studied for their decolorization ability. White rot fungi have non-specific ligninolytic enzyme system which include manganese peroxidase, lignin peroxidase and laccase and can degrade a wide range of dyes (Rani et al., 2009). These ligninolytic enzymes are extracellularly excreted by the fungi to initiate the oxidation of substrates in the extracellular environment of the fungal cells (Mester and Tien, 2000).

Several fungal cultures capable of decolorization have been isolated by Ohmomo et al. (1985) and were identified as Coriolus versicolor, Mycelia sterilia and Aspergillus fumigatus. Several other brown rot fungi capable of decolorizing a wide range of structurally different dyes were also isolated and were found to be more effective than Phanerochaete chrysosporium (Knapp et al., 1995).

Also, there are various fungi such as Aspergillus niger (Fu and Viraraghavan, 1999, 2000), Rhizopus arrhizus (Zhou and Banks, 1991, 1993), Rhizopus oryzae (Gallagher et al., 1997) which can also decolorize and/or biosorb diverse dyes. Aspergillus species are a ubiquitous group of filamentous fungi which are commonly isolated from soil, plant debris and indoor air environments, Aspergillus niger is commonly found as a saprophyte growing on dead leaves, stored grain, compost piles,
and other decaying vegetation. The spores are widespread, and are often associated with organic materials and soil (Bennett, 1979). *Aspergillus niger* includes a set of fungi which are generally considered asexual, although perfect forms which (reproduce sexually) have been found (Frisvad *et al.*, 1990).

*Aspergillus niger* grows rapidly on a variety of artificial substrates producing colonies which consist of a compact white or yellow basal felt covered by a dense layer of dark brown to black conidial heads, their mycelial hyphae are septate and hyaline (Frisvad *et al.*, 1990). Many *Aspergillus* enzymes are used in brewing and textile industries. Several researches suggest that *Aspergillus* fungi could be used to remove toxic and radioactive metals from the environment.

*Aspergillus niger* fermentation is generally regarded as safe (GRAS) by the Food and Drug Administration (FDA), also *Aspergillus niger* is used to test the efficacy of preservative treatments (Jong and Gantt, 1987). In addition, *Aspergillus niger* has been shown to be exquisitely sensitive to micronutrient deficiencies prompting the use of *Aspergillus niger* strains for soil testing (Raper and Fennell, 1965).

*Aspergillus niger* is known to produce a battery of enzymes, one of the most important enzyme is glucose oxidase. Glucose oxidase (β-D-glucose:oxygen 1-oxidoreductase) is a dimeric flavoprotein which catalyzes the oxidation of β-D-glucose with molecular oxygen (as an electron acceptor) via D-glucono delta lactone to D-gluconic acid and hydrogen peroxide (Yamaguchi *et al.*, 2007). Glucose oxidase is a glycosylated enzyme, it consists of two identical polypeptide chain subunits covalently linked by disulfide bonds (O'Malley and Weaver,
1972), each having a molecular weight of 80,000 daltons, with a total molecular weight of 160,000 daltons (Ferreira et al., 2005), it belongs to a large class of enzymes known as oxidoreductase or "redox enzymes". Many types of redox reactions require co-factors which must be regenerated or added to the reaction mixture. This is achieved in living systems via the electron-transport chains. Applications of redox enzymes in the food industry are limited to a few examples, glucose oxidase being a member of such group, containing a co-factor which is a tightly bound prosthetic group that can regenerate itself, and this is achieved by the reduction of oxygen to hydrogen peroxide (Woods and Swinton, 1991).

There are several conditions which play an important role in dye decolorization such as carbon source. Kapdan et al. (2000) stated that the most readily usable carbon source for most of the fungi is glucose. They employed starch, molasses, fructose and glucose in decolorization of everzol turquoise blue g by Coriolus versicolor and they observed that glucose showed the optimal results in which case 100% dye removal was achieved after 5 days of incubation followed by fructose 92% in 9 days. In addition, the amount of nitrogen present in the media affects dye decolorization by altering the enzyme production by fungi (Kaushik and Malik, 2009). Hatvani and Mecs (2002) tested the effect of three nitrogen sources (ammonium chloride, peptone and malt extract) on decolorization of Poly R-478 by Lentinus edodes and observed that NH₄Cl, peptone and malt extract decolorize tested dye within 18, 21 and 17 days, respectively.
It was also found that textile wastewater contains various acids, alkalis, salts or metal ions as impurities, the presence of such ions influenced the dye decolorizing capacity of the fungus (Kaushik and Malik, 2009).

Aksu et al. (2007) reported that the removal of Remazol black B by Trametes versicolor was decreased when Cr (VI) was added, where the presence of Cr (VI) in growth medium inhibited the growth and extended the growth period of fungus significantly, so both dye removal and removal rate were both delayed and decreased.

Temperature was considered an essential factor, where the studies suggested that the fungal biomass is capable of removing dyes at higher temperatures which is a requirement for fungal their applicability in treating dye wastewater (Kaushik and Malik, 2009). Zeroual et al. (2006) also reported that decolorization of bromophenol blue dye on biomass of Rhizopus stolonifer increased slightly with an increase in temperature up to 35°C, and then remained stable with further increase in temperature at 55°C. Bayramoglu and Arica (2007) reported that decolorization capacity of Trametes versicolor increased when temperature was increased from 5 to 35°C for direct blue-1 and direct red-128, this was due to the increased surface activity of each dye molecule.

In addition, pH has a great effect on fungal decolorization capacity, where, initial pH of dye solution significantly influences the chemistry of both dye molecules and fungal biomass (Fu and Viraraghavan, 2002). The pH of the solution influences the surface charge of the adsorbent which in turn influences the degree of adsorption of charged
dye groups to it (Kaushik and Malik, 2009). Kapdan et al. (2000) also reported the optimum growth pH of Coriolus versicolor at 4.5; highest decolorization efficiency (99%) was also obtained at pH 4.5 which lowered to 50% at pH 6 and 7. O'Mahony et al. (2002) reported that higher decolorization capacity of Rhizopus arrhizus for acidic dye remazol blue at pH 2 as compared to that at pH 10 was due to the increased protonation of the weak base groups at lower pH. These base groups then acquire a net positive charge and bind anionic groups of the acidic dyes. Maurya et al. (2006) also observed that sorption of methylene blue by Fomes fomentarius and Phellinus igniarius increased from 18 - 75% and 16 - 79%, respectively as the pH was increased from 3 – 11. This is due to the increase in the net electronegativity of the biosorbent due to deprotonation of different functional groups, which in terms of increases the electrostatic force between the negatively charged biosorbent and the positively charged methylene blue ions.

Importance of the textile industry in Egypt:

The textile industry is one of the oldest in the world. The oldest known textiles, which date back to about 5000 B.C. are scarps of linen cloth found in Egyptian caves. The industry was primarily a family and domestic one until the early part of the 1500’s, when the first factory system was established. During expeditions of Mohamed Ali pasha, the textile industry developed in Egypt. Today, the textile sector in Egypt consists of over 3000 companies, ranging from the very small (employing less than 8 laborers) to the very large (more than 20,000 laborers) both public and private sector companies (EEAA, 2003).
The textiles industry is the 5th largest source of foreign currency; after oil, transactions, tourism and Suez Canal. It is the second largest manufacturing sector in Egypt after food processing and represents 25% of total industrial output (excluding petroleum products). Egypt produces 25-30% of the world's cotton, although there is strong competition from United States of America (USA), China and India. Egypt also produces some of the highest quality extra fine cotton in the world, having a 35% share of the world market. There are over 2300 private sector factories which are members of the Egyptian Textile Manufacturers Federation (ETMF). There are also many small factories and workshops which are not ETMF members, as well as informal workers who are not included in any of these groups. The private sector currently dominates the market in terms of knitted fabrics and ready-made goods (EEAA, 2003).

While textile industries are very important in Egypt, the industrial wastewater they produce is considered one of the main sources of water pollution because of their toxic chemicals and organic loading (Myllylä, 1995).

About 80% of the whole country's annual industrial effluent is discharged untreated into the Nile, canals, wells, municipal sewerage system and the Mediterranean Sea. Egypt's 329 major factories continue to discharge as much as 2.5 million m$^3$ per day of untreated effluent into Egypt's water resources. The end result is that Egypt's shores and coastal fishing and tourism are being damaged, areas around industrial zones are becoming inhospitable, and water purification is becoming very costly (Sadek and Hanifa, 1994).
Most water pollution control projects implemented have been run by the public sector. Within the private sector, few industrial wastewater treatment projects have been set up, since regulations are not enforced yet. But this is likely to change over the next years the recent environmental law requires plant owners to clean up their discharges, so the industrial wastewater situation is likely to improve. The law relies not only on command and control approach with penalties, but also includes economic tools and incentives: fewer taxes on industrial wastewater equipment, subsidies etc. (Sadek and Ibid, 1992).

Cairo, with 13 million people is the largest city in the Middle East region with continuous rapid population growth and spatial expansion. Since the city is an open environmental system, Cairo's surrounding regions are burdened with heavy wastewater discharges and increasing water demand, also the city's water resources are affected by discharges from other regions. Cairo is one of the main industrial centers in Egypt: 50-64% of industrial activities are mainly located in the capital. Its public sector industries (75%) consist of chemical, textile, metal (iron and steel), food, engineering and cement production operations, and they use 162 millions m$^3$ of fresh water per year, and discharge 129 million m$^3$ per year, each day they discharge 0.75 tons of heavy metals. For example, Cairo's Shoubra El-Kheima is an industrialized district north of the city, and its industries discharge to drains (which are heavily polluted) finally flowing to the Mediterranean Sea (Myllylä, 1995). In general, water use of Egypt's chemical, iron, and steel
companies (which produce the most toxic wastes) is expected to increase.

Most of the discharge to the sewage collection systems is from domestic sources; also industries in Cairo discharge 56 million m$^3$ annually to the collection system, in many cases without pretreatment and only half of the industry had in 1992 some type of effluent treatment before discharge to the collection system. Available limited data restricts evaluation of different pollution concentrations from effluents discharge wastewater, no accurate information is available of the amount of toxic substance (Myllylä, 1995).

Therefore, government legislation is becoming more stringent in developed countries regarding the removal of dyes from industrial effluents, which is becoming an increasing problem for textile industries. Environmental protection agencies in Europe are promoting transfer prevention of pollution problems from one part of the environment to another. This means that for most textile industries, developing on site or in plant facilities to treat their own effluents before discharge is fast approaching actuality (Banat et al., 1996).

Therefore, the Egyptian Government evaluated Law 48/1982 concerning protection of the River Nile and Egypt waterways from pollution regulating the discharge of waste to the River Nile, its branches and marine environment by a permit from the Ministry of Public Works and Irrigation after fulfilling certain criteria monitored by periodic analysis (EEAA, 2003).
Materials & Methods
I. Biological treatments:

Isolation of mycobiota from selected samples:

A total of twenty samples composed of ten samples of decaying eucalyptus leaves and ten soil samples were collected from El-Kanater El-Khairia district (Dias et al., 2003). All samples were collected in clean plastic bags and transferred to the laboratory and immediately used for isolation of fungi.

Ten grams of each sample were transferred into 250 ml Erlenmeyer conical flasks containing sterilized saline (90 ml) and incubated for 15 min at 150 rpm and 35°C; serial dilutions were prepared to obtain adequate colony count. One ml of each dilution was plated on the surface of potato-dextrose agar (PDA) medium. The plates were incubated at 30°C for 3-7 days and the growing colonies were picked out for purification. The isolates were purified by re-streaking on PDA plates and were examined microscopically to check their purity (Johnson and Curle, 1972).

- Identification of the isolated mycobiota:

The purified isolates were identified to the species level according to their morphological characteristics and microscopical examination following the description given by Barnett and Hunter (1972) and Pitt and Hocking (1985).
Textile dyes used in this study:

Four textile dyes kindly supplied from (Golden Textile Factory at the 10th of Ramadan district) were used in the present study. Absorbance measurements were performed using a UV 2100 spectrophotometer (Schimadzu- Japan), at National Center for Radiation Research and Technology (NCRRT). Wave length, C.I. name and class of the used textiles dyes are listed in Table 1.

Table (1): Textile dyes used in this study.

<table>
<thead>
<tr>
<th>Dyes</th>
<th>Wave Length (nm)</th>
<th>*C.I. Name</th>
<th>Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolan Yellow</td>
<td>546.2</td>
<td>Acid yellow 232</td>
<td>Azo dye</td>
</tr>
<tr>
<td>Isolan Red</td>
<td>546</td>
<td>Acid red 414</td>
<td>Azo dye</td>
</tr>
<tr>
<td>Isolan Navy</td>
<td>464.6</td>
<td>Acid blue 335</td>
<td>Azo dye</td>
</tr>
<tr>
<td>Isolan Grey</td>
<td>464.6</td>
<td>Acid black 220</td>
<td>Azo dye</td>
</tr>
</tbody>
</table>

*C.I.= Color Index*
Screening of the obtained fungal isolates for decolorization of textile dyes:

- **Preliminary screening:**

  The ability of all isolated strains to grow in the dye containing media and/or reduce the color intensity in liquid Kirk's medium III (a medium common for induction of degrading enzymes) containing Isolan Red and Isolan Navy at 30°C and 150 rpm for 7 days was assessed (Kapdan *et al.*, 2000).

- **Decolorization in static liquid medium:**

  In order to detect the efficiency of the tested strains for decolorizing the four Isolan dyes under static condition; Kirk's medium III broth and modified basal salt medium (BSM) were prepared with 0.2 gl⁻¹ of each Isolan dye. Both were incubated at 30°C and static condition, after 7 days the color intensity was measured (Swamy and Ramsay, 1999).

- **Decolorization in shaking liquid medium:**

  In order to select the fungal isolate which has the highest decolorizing ability; Kirk's medium III was prepared with 0.2 gl⁻¹ of each Isolan dye. Tested strains were inoculated and incubated at 30°C and 150 rpm for 7 days. The color intensity was measured (Yang *et al.*, 2009). All upcoming experiments were performed in triplicates.

- **Decolorization on solid medium:**

  The efficiency of the tested strains for decolorizing the four Isolan dyes on solid medium was detected using modified
BSM, sabouraud agar for the tested strains. Two tenth gl\(^{-1}\) of each dye were added to the medium prior to sterilization. Each plate containing one of the dyes was centrally inoculated with 5 mm agar disc, removed from an actively growing fungus, all plates were incubated at 30°C for 7 days along with control plates without fungal inoculums. The uninoculated plates served as control for abiotic decolorization (Swamy and Ramsay, 1999 and Rani et al., 2009).

**Evaluation of the culture conditions for maximum decolorization:**

- **Inoculum preparation and culture condition:**

  Five mm discs of fungal mycelium of 7 days old of *Aspergillus niger* ES-5 was excised from agar plate by sterilized cork porer, each was transferred to four groups (each group containing only one type of Isolan dye) of 250 ml Erlenmeyer flasks containing 20 ml working volume of BSM.

  All flasks were incubated in a rotary shaker at 150 rpm and 30°C in complete darkness at pH 4.5 ±0.2. The decolorization percentage was calculated according to the following equation:-

\[
\frac{AD-A}{AD} \times 100
\]

Where \(A_0\) = the initial dye absorption on the day of inoculation.

\(A\) = the final dye absorption after incubation period (Rani et al., 2009).
Glucose oxidase activities for all the samples were determined and dry weight was performed at the end of each experiment.

Flasks (free of fungal inocula) were used as control under the same conditions described earlier. All experiments were performed as triplicates and the data plotted are the mean value.

- Media contents:

To obtain co-metabolic conditions for GOD production, media components were removed one by one and decolorization was monitored under the same culture conditions as represented in Table (2).

**Table (2):** Different media contents.

<table>
<thead>
<tr>
<th>Media (BSM)</th>
<th>Glucose</th>
<th>CaCO(_3)</th>
<th>*N</th>
<th>KH(_2)PO(_4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>II</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>III</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IV</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>V</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>VI</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>VII</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = the presence of the component, *N represents the total organic (peptone) and inorganic (NaNO\(_3\)) nitrogen added to the media.
Enzymatic assays:

The primary enzyme suspected for involvement in decolorization by *Aspergillus niger* is an intracellular sugar oxidase (*Watanabe et al.*, 1982). Therefore, glucose oxidase enzyme was determined in the following experiments to detect its relevance to decolorization.

**Extraction of glucose oxidase enzyme (GOD):**

- **Intracellular GOD enzyme:**

  In order to measure intracellular GOD activity; one gram of mycelia grown at the optimized conditions was washed with distilled water and pressed between two filter papers to remove excess water. One gram of washed and dried sand plus 5 ml acetate buffer were added to the wet mycelia in a cool sterilized mortar. The grinding took place in a dark area for at least 15 min. The homogenate was then centrifuged at 5000 rpm for 15 min. GOD was immediately assayed in the supernatant (*Lu et al.*, 1996).

- **Extracellular GOD enzyme:**

  In order to measure extracellular GOD activity; the culture fluid was clarified by centrifugation at 5000 rpm for 15 min. Enzyme activities were measured in the clear supernatant (*Lu et al.*, 1996).

- **Glucose oxidase assay:**

  The GOD activity was determined by titration method according to *Lu et al.* (1996) under the following conditions: 1
ml of the enzyme solution was added to 25 ml of 60 mM sodium acetate buffer pH 5.6 containing 2% β-D glucose. The mixture was shaken for 1 h at 30°C in a rotatory shaker at 200 rpm. Twenty ml of 0.1 N sodium hydroxide was added to stop the reaction. The resulting mixture was titrated to a red endpoint by 0.1 N HCl solution using phenolphthalein as an indicator.

The GOD activity (U/min/ml) could be calculated by the following formula:

$$(V_0-V) \times N \times 1000 / 60$$

In which $V_0$ = the volume of added standard HCl solution (ml) in blank.

$V$ = the volume of added standard HCl solution (ml).

$N$ = the concentration of standard HCl solution (N).

The blank assay (enzyme is not added) was performed under the same experimental conditions.

One unit of the enzyme was defined as: the amount required to oxidize 1 µmol of β-D glucose to D-gluconic acid and H₂O₂ per min at pH 5.6 and 30°C.

**Dry weight:**

Fungal growth was measured after 72 h, samples were filtered on pre-weighed filter paper Whatman No.1 and dried at 50°C overnight (Yang et al., 2009).
Materials and Methods

Glucose concentration:

The effect of glucose concentration on dye decolorization, GOD production and dry weight were investigated with various glucose concentrations: 0, 10, 20, 30, 40, 50, 60, 70 and 80 g l⁻¹.

Determination of reducing sugar:

One ml of the culture was filtered and added to 1 ml of dinitro salysalic acid (DNS) according to Miller (1959), the mixture was placed in a water bath at 100°C for 10 min after cooling, 3 ml of deionized H₂O was added and measured spectrophotometrically at 540 nm.

Optimization conditions:

The selected fungus used to degrade the four Isolan dyes. Fungal decolorization conditions were performed as described in media contents section.

- Incubation period:

The effect of time course on dye decolorization, GOD production and dry weight were investigated at various incubation periods: 0, 24, 48, 72 and 96 h.

- Initial pH:

The effect of pH on dye decolorization, GOD production and dry weight were investigated at various initial pH values: 3.5, 4.5, 5.5, 6.5, 7.5, 8.5, 9.5 and 10.5.
Materials and Methods

- **Incubation temperature:**

  The effect of temperature on dye decolorization, GOD production and dry weight were investigated at various temperatures: 25, 30, 35, 40, 45 and 50°C.

**Assessment of some chemical parameters:**

- **Determination of Chemical Oxygen Demand (COD):**

  Ten ml of the tested sample was diluted 100 times, 5 ml potassium di-chromate was added, 15 ml of sulfuric acid silver sulphate (which acts as catalyst) was added. The mixtures were placed in a semi closed COD reactor for 120 min at 148°C. After cooling ferron was added as an indicator and titrated against ferrous ammonium sulfate to a reddish end point. The same previous conditions were applied for the blank sample but the tested sample was replaced by 10 ml of distilled water (Annual Book of Standards, 1995).

  COD was calculated as follows:

  \[ V_2 = V_B - V_1 \]

  Where \( V_B \) = volume taken from ferrous ammonium sulfate in blank.

  \( V_1 \) = volume taken from ferrous ammonium sulfate of tested sample.

  \[ \text{COD (mg/l)} = V_2 \times 96 \]

- **Determination of Biological Oxygen Demand (BOD\(_5\)):**

  One hundred and fifty four ml of 100-folds diluted blank sample was added to 10 ml seeding (treated wastewater as source of microorganisms), 5 drops of allyl thiourea was added
as a nitrification inhibitor, the bottles which contained 2 NaOH pellets were sealed by rubber and a sensor was placed (Young and Baumann, 1976). Readings were taken after 5 days using WTW apparatus (United Kingdom). The tested samples were diluted (50 times) under the same previous conditions.

\[
\text{BOD}_5 \text{ (mg/l)} = \text{reading of sensor} \times \text{dilution} \times 10
\]

- **Determination of nitrate, nitrite and phosphate:**

  Ten ml of a 10-fold sample were used to determine the nitrate, nitrite and phosphate contents. The tests were performed according to the manufacturer instructions. The readings were taken after 3 min in case of nitrate and after 10 min for nitrite and phosphate using Lovibond comparator (United Kingdom).

  Value of nitrate (mg/l nitrate-nitrogen) = reading of disc \( \times \) 14/62

  Value of nitrite (mg/l nitrite-nitrogen) = reading of disc

  Value of phosphate (mg/l phosphate-phosphorus) = reading of disc \( \times \) 31/95

- **Total suspended solids (TSS):**

  Fungal supernatants containing Isolan dyes were obtained by filtration of extracellular fluid on Whatman No.1 filter paper at 0 and 72 h and were transferred into pre-weighed flasks \( (W_1) \), then all flasks were dried at 50°C overnight and weighed after cooling \( (W_2) \) (Chanin et al., 1958). The total suspended solids were calculated as follows:

  \[
  \text{Total suspended solid (mg/l)} = (W_2 - W_1) \times 50
  \]
Effect of decolorized extra cellular fluids on microbial growth for detecting toxicity:

The bioassay was based on the inhibition of the bacterial growth by the degraded components present in the decolorized media for the four Isolan dyes and was carried out on a clear supernatant after fungal decolorization in comparison to the toxicity before fungal decolorization (as control samples).

The assay involves the incubation of *E. coli* ([Ambrósio and Takaki, 2004](#)) at 35°C and 150 rpm in nutrient broth after 24 h, 2 ml of the bacterial culture was transferred onto sterile Petri dishes with nutrient agar. After 24 h, holes were punched in seeded Petri dishes which were filled with 50μl of the clear supernatant, after another 24 h the inhibition zone diameter was measured.

Detection the mode of dye decolorization by *Aspergillus niger* ES-5:

- **In vitro-decolorization:**

  The following experiment was performed to know if the decolorization mechanism of the four Isolan dyes depends only on the extracellular enzymes secreted by *Aspergillus niger* ES-5 or not. BSM IV was inoculated with *Aspergillus niger* ES-5 under the same previous conditions after 72 h, extracellular fluid (ECF) of liquid culture was filtered and used to decolorize the four Isolan dyes separately. Following the incubation for 3 days at 30°C and 150 rpm, ECF were measured spectrophotometrically each at its specific wave length ([Lorenzo et al., 2002](#)).
• **Test of dead fungal mycelium:**

In order to investigate the potential of autoclaved fungus in decolorization of the four Isolan dyes, four groups of 250 ml Erlenmeyer flasks containing 20 ml working volume of BSM IV, each group containing one type of the four Isolan dyes. All flasks were inoculated with 5 mm disc containing the actively growing mycelia *Aspergillus niger* ES-5 (pH 4.5±0.2).

Flasks were autoclaved at 121°C for 15 min, after cooling all flasks were incubated in a dark shaking incubator at 30°C and 150 rpm for 7 days and the decolorization percentage were measured (Gallagher *et al.*, 1997).

• **Adsorption:**

To investigate the efficiency of *Aspergillus niger* ES-5 to use the dye as the sole carbon source, the adsorption of dyestuff on fungal mycelium was carried out using only dyestuff, in the absence of nutrients. Incubation conditions were as previously mentioned.

The amount of adsorbed dye was calculated by subtracting the color intensity in the liquid phase at the end of the incubation period from the initial value also, the dry weight was measured (Kapdan *et al.*, 2000).

• **Ultraviolet (UV) and Fourier Transform Infrared spectroscopy (FTIR) spectral analysis:**

In order to investigate that the mode of decolorization occurred via degradation or not, ECF of liquid culture of *Aspergillus niger* ES-5 in modified BSM IV before and after
Materials and Methods

decolorization of the four Isolan dyes were compared using a Schimadzu UV-2100 spectrophotometer at 300-700 nm Aspergillus niger ES-5 after 72 h of incubation. The absorbance was plotted against the wave length (del Río et al., 2009).

FTIR was also performed. Samples were dried at 50°C over night, grinded and then mixed with potassium bromide (KBr) and finally pressed in the form of discs and analyzed by FTIR-6300 Jasco apparatus (Japan). The results were plotted as transmittance and the peaks were compared before and after decolorization to monitor the possible changes, also pure powder of dyes were mixed with KBr as previously mentioned and measured (Husseiny, 2008).

- High performance liquid chromatography (HPLC):

Samples were analyzed by a SP400 HPLC (Thermo Separation Product, USA) system equipped with a variable wave length detector adjusted to 254 nm and a reversed-phase partsil C18 column (250mm x 4.6mm). The mobile phase composed of CH3CN: H2O; (30:70) at a flow rate of 1.5 ml/min. Sulphanilic acid and aniline were used as standards and were injected for comparison to the degradation by-products.

- Desorption:

To detect if the dye molecules attach to fungal cell wall or not, dye desorption was conducted according to Miranda et al. (1996) by incubating the mycelia after decolorization in sterilized water; incubation was performed under the same decolorization conditions. The amounts of desorbed dyes were
measured spectrophotometrically and were calculated as the increase in absorbance at the specific absorbance maxima for each dye.

- **Energy dispersive X-ray spectroscopy (EDS):**

  In order to investigate if Isolan dyes were decolorized enzymatically through biotransformation in the inner side of the fungal mycelia, metal analysis was performed at NCRRT using EDS system model Oxford (United Kingdom) ISIS with SiLi detector attached to a scanning electron microscope model JEOL-5400 (Japan) for metal detection of pure dyes and dried mycelia of *Aspergillus niger* ES-5 after 72 h of decolorization. The samples were dried at 50°C for overnight, and ground to powder state, after which samples were attached to stubs using a double face adhesive tape.

- **Light microscopy:**

  This experiment was performed to investigate if Isolan dyes were only entrapped inside fungal biomass, fungal mycelia were collected at 0, 24, 48 and 72 h, the mycelia were filtered from BSM IV medium containing Isolan dyes and excess water was removed by placing the mycelia between two Whatman No.1 filter papers. The mycelia were placed in separate sterile Petri dishes, cut using a sterile scalpel and photographed using LABOMED Microscope CX RIII (Japan) connected to Benq 510 E Digital camera (China).
II. Physical treatment:

The irradiation process was performed to determine the effect of radiation on dye degradation by using Indian Gamma Chamber, 4000 A activities 9100 CU and dose rate 5 kGy/h at the National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt.

Isolan dyes were exposed to 0.5, 1.0, 1.5, 2, 2.5, 3, 6, 10, 15 and 20 kGy in separate tubes and decolorization percentage and change in pH value were determined.

• Combined physical/biological treatment:

In order to detect if combination of physical and biological treatment increase the percentage of decolorization or not, Isolan dyes were exposed to 0.5, 1, 1.5, 2 and 2.5 kGy in separate tubes and then added to the media in 250 ml Erlenmeyer flasks. All flasks were inoculated with *Aspergillus niger* ES-5 and incubated in a dark shaking incubator at 150 rpm for 72 h at 30°C, the decolorization percentage was determined as mentioned before.

**Application of *Aspergillus niger* ES-5 as decolorizing strain on mixture of the four Isolan dyes:**

To assess the ability of *Aspergillus niger* ES-5 to decolorize more than one dye, a mixture of the four Isolan dyes (0.2 gl⁻¹ of each dye) was prepared and added to BSM I and IV media as represented before in Table (2). The mixture was sterilized and inoculated with *Aspergillus niger* ES-5 in 250 ml Erlenmeyer flasks, all flasks were incubated in a dark shaking
incubator at 150 rpm (pH 4.5±0.2) for 168 h at 30°C and
decolorization percentage, GOD activity and dry weight were
determined as mentioned before (Sumathi and Manju, 2000).

Application of *Aspergillus niger* ES-5 on textile effluents:

The ability of *Aspergillus niger* ES-5 to remove color of
single dyes efficiently does not indicate the suitability of this
organism in treatment of colored textile effluents. In order to
detect the efficiency of *Aspergillus niger* ES-5 to decolorize
textile effluents, textile effluents were collected in sterile
containers from a company for textile dyeing and printing
located in the 6th of October (Cairo, Egypt). Samples were
obtained at the end of a dyeing process of cotton with yellow,
red and orange dyes, with a final olive green color, pH 10 and
were stored at 4°C for use within 24 h. The absorbance
maximum was detected using a Schimadzu UV 2100
spectrophotometer.

- **Decolorization of textile effluents under various
conditions:**

  Optimization conditions carried out to achieve
maximum decolorization, GOD production and dry
weight of textile effluents are represented in Table (3). The results were plotted after 72 h of incubation period.
Materials and Methods

Table (3): Different optimization conditions of wastewater.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Wastewater</th>
<th>BSM IV</th>
<th>pH(5.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

- **Light microscopy:**
  Photos of fungal mycelia before and after decolorization were taken as described before.

- **Measurement of some chemical parameters:**
  Some chemical parameters (COD, BOD₅, nitrate, nitrite, phosphate and total suspended solids) were detected before and after fungal decolorization as described before.

- **Energy dispersive X-ray spectroscopy (EDS):**
  Metal were detected using EDS as described before.

- **Ultraviolet (UV) and Fourier Transform Infrared spectroscopy (FTIR) spectral analysis:**
  UV was detected before, after fungal decolorization and when combined with H₂O₂ pretreatment as described before. As
well as, FTIR analysis was also performed for textile effluents before and after fungal decolorization as described before.

**Physical treatment of textile effluents:**

- **Irradiation:**

  Textile effluents were exposed to 25 kGy after adjusting its pH to 5.5 before irradiation. Decolorization percentage was determined.

- **Pre and Post treatment of textile effluents with H$_2$O$_2$:**

  According to Table (3), two groups of media were prepared and inoculated with *Aspergillus niger* ES-5. 130 μl of H$_2$O$_2$ 30% (Sigma, Germany) was added to the first group at zero time and incubated at 30°C and 150 rpm. The same amount was also added to the second group after 72 h of incubation under the same previous condition. The decolorization percentage was determined for the two groups at various times: 0, 72, 96, 168 and 240 h.

**Application: retrieve of textile dyes and reuse in dyeing of cotton and polyester:**

Two types of pre-treated cloth (cotton and polyester) were kindly provided by textile department at NCRRT. The swatches were placed in untreated dye solution as control, intracellular extraction of grinded fungal mycelia containing
Materials and Methods

four Isolan dyes and treated filtrate. Sodium carbonate (25 gl⁻¹) and sodium chloride (50 gl⁻¹) were added to the mixtures. pH was brought to 7 using NaOH (1N) in separate glass containers at 80°C for 120 min under static conditions and mixed at interval times (Hunter and Renfrew, 2008). The swatches were dried at room temperature and photographed using Benq 510 E Digital camera.

Media used in this study:

- **Potato-dextrose agar (PDA) (The Oxoid Manual, 1980):**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>gl⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potatoes</td>
<td>400</td>
</tr>
<tr>
<td>Glucose</td>
<td>20</td>
</tr>
<tr>
<td>Agar</td>
<td>15</td>
</tr>
<tr>
<td><strong>pH 5.6 ±0.2</strong></td>
<td></td>
</tr>
</tbody>
</table>

- **Sabouraud dextrose agar (The Oxoid Manual, 1980):**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>gl⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycological peptone</td>
<td>10</td>
</tr>
<tr>
<td>Dextrose</td>
<td>40</td>
</tr>
<tr>
<td>Agar</td>
<td>15</td>
</tr>
<tr>
<td><strong>pH 5.6±0.2</strong></td>
<td></td>
</tr>
</tbody>
</table>
• **Kirk’s medium III:** (Kapdan *et al.*, 2000)

\[
\begin{align*}
\text{Urea} & : 0.036 \\
\text{Potassium di-hydrogen phosphate} & : 2.0 \\
\text{Magnesium sulphate.7H}_2\text{O} & : 0.5 \\
\text{Calcium chloride} & : 0.099 \\
\text{Glucose} & : 5.0 \\
pH & : 4.5\pm0.2
\end{align*}
\]

• **Nutrient agar (The Oxoid Manual, 1980):**

\[
\begin{align*}
\text{Beef extract} & : 1.0 \\
\text{Yeast extract} & : 2.0 \\
\text{Peptone} & : 5.0 \\
\text{Sodium chloride} & : 5.0 \\
\text{Agar} & : 15 \\
pH & : 4.5\pm0.2
\end{align*}
\]

• **Basal salt medium (BSM):** (Fiedurek and Gromada, 1997)

\[
\begin{align*}
\text{Glucose} & : 80 \\
\text{Peptone} & : 3.0 \\
\text{Sodium nitrate} & : 0.5
\end{align*}
\]
### Materials and Methods

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium di-hydrogen phosphate</td>
<td>1.0</td>
</tr>
<tr>
<td>Calcium carbonate (oven sterilized)</td>
<td>35</td>
</tr>
<tr>
<td>pH 4.5±0.2</td>
<td></td>
</tr>
</tbody>
</table>

All media were autoclaved for 20 minutes at 121°C.
Results
Results

I. Biological treatments:

Isolation and identification of mycobiota from selected samples:

A total of twenty six fungal isolates were recovered. The obtained isolates were purified and identified as: *Aspergillus niger* (11 isolates), *Aspergillus flavus* (4 isolates) and *Penicillium sp.* (11 isolates) as represented in Table (4).

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

- Preliminary screening:

  The obtained fungal isolates were screened for testing their ability for textile dyes decolorization. *Phanerochaete chrysosporium* ATCC 24725 was used as standard decolorizing fungal strain to compare its ability in decolorization of Isolan Red and Isolan Navy as preliminary step to the isolated 26 strain's decolorizing ability as represented in Table (4).
Table (4): Screening of local isolates for dye decolorization in comparison with standard decolorizing fungal strain after 7 days of incubation at 30°C and 150 rpm.

<table>
<thead>
<tr>
<th>SOURCE OF ISOLATES</th>
<th>IDENTIFICATION OF ISOLATES</th>
<th>PERCENTAGE OF ISOLAN DECOLORIZATION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Isolan Red</td>
</tr>
<tr>
<td>ATCC 24725</td>
<td><em>Phanerochaete chrysosporium</em></td>
<td>82.0</td>
</tr>
<tr>
<td></td>
<td><em>Aspergillus niger-1</em></td>
<td>40.0</td>
</tr>
<tr>
<td></td>
<td><em>Aspergillus niger-2</em></td>
<td>23.0</td>
</tr>
<tr>
<td></td>
<td><em>Aspergillus flavus-3</em></td>
<td>21.0</td>
</tr>
<tr>
<td></td>
<td><em>Penicillium-4</em></td>
<td>40.4</td>
</tr>
<tr>
<td></td>
<td><em>Aspergillus niger ES-5</em></td>
<td>77.0</td>
</tr>
<tr>
<td></td>
<td><em>Penicillium-6</em></td>
<td>35.0</td>
</tr>
<tr>
<td></td>
<td><em>Aspergillus niger-7</em></td>
<td>45.0</td>
</tr>
<tr>
<td></td>
<td><em>Penicillium-8</em></td>
<td>20.0</td>
</tr>
<tr>
<td></td>
<td><em>Penicillium-9</em></td>
<td>31.0</td>
</tr>
<tr>
<td></td>
<td><em>Aspergillus niger-10</em></td>
<td>40.0</td>
</tr>
<tr>
<td></td>
<td><em>Penicillium-11</em></td>
<td>30.5</td>
</tr>
<tr>
<td></td>
<td><em>Aspergillus niger-12</em></td>
<td>67.6</td>
</tr>
<tr>
<td></td>
<td><em>Aspergillus flavus-13</em></td>
<td>12.0</td>
</tr>
<tr>
<td>Soil around eucalyptus tree</td>
<td><em>Penicillium-14</em></td>
<td>23.0</td>
</tr>
<tr>
<td></td>
<td><em>Penicillium-15</em></td>
<td>25.4</td>
</tr>
<tr>
<td></td>
<td><em>Penicillium-16</em></td>
<td>19.0</td>
</tr>
<tr>
<td></td>
<td><em>Penicillium-17</em></td>
<td>23.0</td>
</tr>
<tr>
<td></td>
<td><em>Aspergillus niger-18</em></td>
<td>31.0</td>
</tr>
<tr>
<td></td>
<td><em>Aspergillus niger-19</em></td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td><em>Aspergillus niger-20</em></td>
<td>35.0</td>
</tr>
<tr>
<td></td>
<td><em>Aspergillus niger-21</em></td>
<td>23.0</td>
</tr>
<tr>
<td></td>
<td><em>Penicillium-22</em></td>
<td>15.0</td>
</tr>
<tr>
<td></td>
<td><em>Aspergillus niger-23</em></td>
<td>30.0</td>
</tr>
<tr>
<td></td>
<td><em>Penicillium-24</em></td>
<td>25.0</td>
</tr>
<tr>
<td></td>
<td><em>Aspergillus flavus 2</em></td>
<td>25.0</td>
</tr>
<tr>
<td></td>
<td><em>Aspergillus flavus 3</em></td>
<td>31.0</td>
</tr>
</tbody>
</table>

- Selection of the best isolate for maximum decolorization:

The results indicated that only four 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 thus, they were selected for tests on the four Isolan dyes.
From Table (5) and Fig (1) it could be noticed that the four different isolates revealed different decolorization percentage of the four Isolan dyes but *Aspergillus niger* ES-5 was the only fungus capable of decolorization of the four Isolan dyes at a rate above 50%, thus *Aspergillus niger* ES-5 was employed for further investigation in the up coming experiments.

**Table (5):** Selection of the best isolate for maximum decolorization of the four Isolan dyes after 7 days of incubation at 30 °C and 150 rpm.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Percentage of Isolan dye decolorization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I.Y</td>
</tr>
<tr>
<td><em>Phanerochaete chrysosporium</em> ATCC 24725</td>
<td>75.0</td>
</tr>
<tr>
<td><em>Aspergillus flavus</em> 2</td>
<td>45.0</td>
</tr>
<tr>
<td><em>Aspergillus flavus</em> 3</td>
<td>50.0</td>
</tr>
<tr>
<td><em>Aspergillus niger</em> ES-5</td>
<td>91.0</td>
</tr>
<tr>
<td><em>Aspergillus niger</em> -12</td>
<td>91.0</td>
</tr>
</tbody>
</table>


**Fig. (1):** Relative comparison between the most decolorizing fungal isolates and *Ph. chrysosporium* using the four Isolan dyes.
• **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 (6) and Fig. (2).

**Table (6):** 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.

<table>
<thead>
<tr>
<th>Dyes</th>
<th>Percentage of decolorization for <em>Ph. chrysosporium</em> ATCC 24725</th>
<th>Percentage of decolorization for <em>A. niger</em> ES-5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Static condition</td>
<td>Shaking condition</td>
</tr>
<tr>
<td>I.Y</td>
<td>30.5</td>
<td>75.0</td>
</tr>
<tr>
<td>I.R</td>
<td>60.1</td>
<td>82.0</td>
</tr>
<tr>
<td>I.N</td>
<td>40.6</td>
<td>65.0</td>
</tr>
<tr>
<td>I.G</td>
<td>30.8</td>
<td>38.5</td>
</tr>
</tbody>
</table>
Fig. (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:**

  The decolorization ability of *A. niger* ES-5 and *P. chrysosporium* ATCC 24725 were investigated on solid agar plates, it is evident that both *Aspergillus niger* ES-5 and *Phanerochaete chrysosporium* ATCC 24725 were unable to decolorize all the four Isolan dyes on solid-agar plates as presented in Table (7).

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

<table>
<thead>
<tr>
<th>Dyes</th>
<th><em>Phanerochaete chrysosporium</em> ATCC 24725</th>
<th><em>Aspergillus niger</em> ES-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.Y</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>I.R</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>I.N</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>I.G</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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:**

  Since, different dyes have different molecular structures; therefore a microorganism capable of decolorizing one dye may have variant capacities for other dyes, each having a different enzymatic system to attack the dye in question.

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

  The structural genes coding for the production of many enzymes are normally inactive in the absence of the enzyme substrate. When the substrate is added, the structural gene is turned on and the enzyme is produced, this is called induction. Sugars added to the media are inducers to sugar oxidases. Induced enzymes are produced only when the substrate they attack is present in the medium.

  The effect of glucose on the percentage of decolorization, glucose oxidase activity and fungal growth were studied, two types of basal salt media were prepared BSM I and BSM II as shown in Table (2), the results in Table (8) and Fig. (3) showed that BSM II containing no glucose gave 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%, I.N gave 80 and 29.3% and I.G gave 87 and 30% in BSM I and BSM II, respectively at the end of 72 h.
Results

Table (8): Effect of glucose on dye decolorization by *Aspergillus niger* ES-5 at 30°C and 150 rpm throughout 168 h.

<table>
<thead>
<tr>
<th>Dyes</th>
<th>Percentage of decolorization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I.Y</td>
</tr>
<tr>
<td>Time (h)</td>
<td>BSM I</td>
</tr>
<tr>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>24</td>
<td>28.1</td>
</tr>
<tr>
<td>48</td>
<td>15.6</td>
</tr>
<tr>
<td>72</td>
<td>84.4</td>
</tr>
<tr>
<td>168</td>
<td>84.4</td>
</tr>
</tbody>
</table>

Fig. (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.
Similarly, glucose oxidase activity and fungal growth were also affected negatively by the removal of glucose as represented in Tables (9,10) and Figs. (4,5).

**Table (9):** Effect of glucose on the production extracellular GOD by *Aspergillus niger* ES-5 in BSM at 30°C and 150 rpm.

<table>
<thead>
<tr>
<th>Dyes</th>
<th>Extracellular GOD in BSM I (U/ml)</th>
<th>Extracellular GOD in BSM II (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.Y</td>
<td>9.2</td>
<td>0</td>
</tr>
<tr>
<td>I.R</td>
<td>8.1</td>
<td>0</td>
</tr>
<tr>
<td>I.N</td>
<td>9.5</td>
<td>0</td>
</tr>
<tr>
<td>I.G</td>
<td>10.8</td>
<td>0</td>
</tr>
</tbody>
</table>

**Fig. (4):** Effect of glucose on the production of extracellular GOD by *Aspergillus niger* ES-5 in BSM at 30°C and 150 rpm.
Table (10): Effect of glucose on dry weight by *Aspergillus niger* ES-5 in BSM at 30°C and 150 rpm.

<table>
<thead>
<tr>
<th>Dyes</th>
<th>Dry weight in BSM I (g/100ml)</th>
<th>Dry weight in BSM II (g/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.Y</td>
<td>4.05</td>
<td>1.97</td>
</tr>
<tr>
<td>I.R</td>
<td>4.10</td>
<td>2.24</td>
</tr>
<tr>
<td>I.N</td>
<td>3.40</td>
<td>1.85</td>
</tr>
<tr>
<td>I.G</td>
<td>3.10</td>
<td>2.49</td>
</tr>
</tbody>
</table>

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

From the obtained results, glucose could be considered as the main media components which has a great effect on Isolan dyes decolorization percentage, fungal growth and GOD production where, glucose could be considered as GOD substrate.
Effect of different glucose concentrations on decolorization percentage, GOD production and dry weight:

From Tables (11, 12, 13 and 14) and Figs. (6,7) it is clear that 30 g/l of glucose could result in maximum decolorization as well as, highest GOD values when compared to the other glucose concentrations. On the other hand, as represented in Tables (11, 12, 13, and 14) and Fig. (8) the results clearly indicated the positive influence of primary growth substrate on growth rates and biomass concentration of the fungus.

**Table (11):** Effect of different glucose concentrations on dye decolorization of Isolan Yellow, extracellular GOD activity and dry weight by *Aspergillus niger* ES-5 incubated in BSM IV at 30 °C and 150 rpm.

<table>
<thead>
<tr>
<th>Glucose Conc. (g/L)</th>
<th>Percentage of decolorization after 72 h</th>
<th>Extracellular GOD activity (U/ml)</th>
<th>Dry weight (g/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero</td>
<td>23.00</td>
<td>0.0</td>
<td>0.0603</td>
</tr>
<tr>
<td>10</td>
<td>78.10</td>
<td>1.7</td>
<td>0.4640</td>
</tr>
<tr>
<td>20</td>
<td>62.50</td>
<td>2.0</td>
<td>0.6630</td>
</tr>
<tr>
<td>30</td>
<td>90.00</td>
<td>3.5</td>
<td>0.8250</td>
</tr>
<tr>
<td>40</td>
<td>56.25</td>
<td>2.2</td>
<td>1.4220</td>
</tr>
<tr>
<td>50</td>
<td>55.90</td>
<td>2.5</td>
<td>1.4020</td>
</tr>
<tr>
<td>60</td>
<td>53.00</td>
<td>2.7</td>
<td>1.8720</td>
</tr>
<tr>
<td>70</td>
<td>79.40</td>
<td>2.9</td>
<td>1.9020</td>
</tr>
<tr>
<td>80</td>
<td>85.70</td>
<td>3.0</td>
<td>1.5090</td>
</tr>
</tbody>
</table>
### Table (12): Effect of different glucose concentrations on dye decolorization of Isolan Red, extracellular GOD activity and dry weight by *Aspergillus niger* ES-5 incubated in BSM IV at 30 °C and 150 rpm.

<table>
<thead>
<tr>
<th>Glucose Conc. (g/L)</th>
<th>Percentage of decolorization after 72 h</th>
<th>Extracellular GOD activity (U/ml)</th>
<th>Dry weight (g/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero</td>
<td>9.5</td>
<td>0.0</td>
<td>0.091</td>
</tr>
<tr>
<td>10</td>
<td>63.4</td>
<td>1.6</td>
<td>0.558</td>
</tr>
<tr>
<td>20</td>
<td>69.9</td>
<td>1.9</td>
<td>0.890</td>
</tr>
<tr>
<td>30</td>
<td>99.0</td>
<td>4.5</td>
<td>1.155</td>
</tr>
<tr>
<td>40</td>
<td>82.8</td>
<td>2.1</td>
<td>1.385</td>
</tr>
<tr>
<td>50</td>
<td>47.0</td>
<td>2.5</td>
<td>1.458</td>
</tr>
<tr>
<td>60</td>
<td>79.5</td>
<td>2.6</td>
<td>1.997</td>
</tr>
<tr>
<td>70</td>
<td>85.9</td>
<td>3.0</td>
<td>2.097</td>
</tr>
<tr>
<td>80</td>
<td>85.9</td>
<td>3.6</td>
<td>2.387</td>
</tr>
</tbody>
</table>

### Table (13): Effect of different glucose concentrations on dye decolorization of Isolan Navy, extracellular GOD activity and dry weight by *Aspergillus niger* ES-5 incubated in BSM IV at 30 °C and 150 rpm.

<table>
<thead>
<tr>
<th>Glucose Conc. (g/L)</th>
<th>Percentage of decolorization after 72 h</th>
<th>Extracellular GOD activity (U/ml)</th>
<th>Dry weight (g/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero</td>
<td>29.1</td>
<td>0.00</td>
<td>0.059</td>
</tr>
<tr>
<td>10</td>
<td>78.9</td>
<td>1.42</td>
<td>0.340</td>
</tr>
<tr>
<td>20</td>
<td>80.0</td>
<td>1.50</td>
<td>0.680</td>
</tr>
<tr>
<td>30</td>
<td>98.0</td>
<td>4.00</td>
<td>0.890</td>
</tr>
<tr>
<td>40</td>
<td>75.2</td>
<td>1.60</td>
<td>1.020</td>
</tr>
<tr>
<td>50</td>
<td>75.8</td>
<td>1.62</td>
<td>1.140</td>
</tr>
<tr>
<td>60</td>
<td>80.4</td>
<td>2.00</td>
<td>1.530</td>
</tr>
<tr>
<td>70</td>
<td>72.8</td>
<td>2.69</td>
<td>1.930</td>
</tr>
<tr>
<td>80</td>
<td>87.1</td>
<td>3.10</td>
<td>2.270</td>
</tr>
</tbody>
</table>
Table (14): Effect of different glucose concentrations on dye decolorization of Isolan Grey, extracellular GOD activity and dry weight by *Aspergillus niger* ES-5 incubated in BSM IV at 30 °C and 150 rpm.

<table>
<thead>
<tr>
<th>Glucose Conc. (g/L)</th>
<th>Percentage of decolorization after 72 h</th>
<th>Extracellular GOD activity (U/ml)</th>
<th>Dry weight (g/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero</td>
<td>39.5</td>
<td>0.0</td>
<td>0.106</td>
</tr>
<tr>
<td>10</td>
<td>78.0</td>
<td>1.50</td>
<td>0.516</td>
</tr>
<tr>
<td>20</td>
<td>77.6</td>
<td>1.56</td>
<td>0.770</td>
</tr>
<tr>
<td>30</td>
<td>93.0</td>
<td>4.20</td>
<td>1.069</td>
</tr>
<tr>
<td>40</td>
<td>78.6</td>
<td>2.25</td>
<td>1.156</td>
</tr>
<tr>
<td>50</td>
<td>69.9</td>
<td>2.30</td>
<td>1.811</td>
</tr>
<tr>
<td>60</td>
<td>75.6</td>
<td>2.43</td>
<td>1.329</td>
</tr>
<tr>
<td>70</td>
<td>55.5</td>
<td>3.45</td>
<td>1.878</td>
</tr>
<tr>
<td>80</td>
<td>88.1</td>
<td>4.00</td>
<td>2.532</td>
</tr>
</tbody>
</table>

Fig. (6): Effect of different glucose concentrations on the four Isolan dyes decolorization by *Aspergillus niger* ES-5.
Fig. (7): Effect of different glucose concentrations on the production of extracellular GOD activity by *Aspergillus niger* ES-5.

Finally, from economic point of view 30g/l of glucose would be used in the upcoming experiments.
• **Carbon source consumption:**

Fungi consume and grow on readily available carbon sources at the initial stages of growth, after which they produce secondary metabolites and extracellular enzymes for biodegradation of dyestuff at low concentrations of carbon or nitrogen sources.

Thus, the time required for *Aspergillus niger* ES-5 to consume the glucose present in basal salt media to produce extracellular enzymes must be detected, the following experiment was conducted to estimate the consumed sugar throughout the incubation time needed for decolorization. The results in Table (15) and Fig. (9) indicated that the majority of sugar was consumed after 72 h of incubation, while a small fraction was exhausted after 96 h.

**Table (15):** Estimate of residual reducing sugar concentration by *Aspergillus niger* ES-5 incubated in BSM IV at 30°C and 150 rpm throughout 96 h.

<table>
<thead>
<tr>
<th>Dyes</th>
<th>Reducing sugar at various time (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 h</td>
</tr>
<tr>
<td>No dyes</td>
<td>0.148</td>
</tr>
<tr>
<td>I.Y</td>
<td>0.133</td>
</tr>
<tr>
<td>I.R</td>
<td>0.189</td>
</tr>
<tr>
<td>I.N</td>
<td>0.172</td>
</tr>
<tr>
<td>I.G</td>
<td>0.178</td>
</tr>
</tbody>
</table>
Results

Fig. (9): Residual reducing sugar concentration by *Aspergillus niger* ES-5 incubated in BSM IV at 30°C and 150 rpm as affected by different dyes throughout 96 h.

- **Effect of glucose only on decolorization percentage, GOD and dry weight:**

  If the absence of glucose is considered limiting step in decolorization, would its presence alone in the media help in decolorization? A media with glucose only (BSM V) was prepared in order to determine the ability of *Aspergillus niger* ES-5 to decolorize the four Isolan dyes with glucose only and determine GOD and dry weight in comparison with BSM I as shown in Table (2).

  The results in Table (16) and Fig. (10) revealed that the percentage of decolorization of: I.Y gave 84.4, 22.2, I.R gave 89.5, 18.1, I.N gave 80, 23.3 and I.G gave 87, 21.3% in BSM I and BSM V, respectively at the end of 72 h, also GOD and dry weight gave lower values in BSM V when compared with BSM I as shown in Tables (17, 18) and Figs. (11, 12).
Results

Table (16): Effect of media containing glucose only on dye decolorization by *Aspergillus niger* ES-5 at 30°C and 150 rpm throughout 168 h.

<table>
<thead>
<tr>
<th>Dyes</th>
<th>Percentage of decolorization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I.Y</td>
</tr>
<tr>
<td>Time (h)</td>
<td>BSM I</td>
</tr>
<tr>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>24</td>
<td>28.1</td>
</tr>
<tr>
<td>48</td>
<td>15.6</td>
</tr>
<tr>
<td>72</td>
<td>84.4</td>
</tr>
<tr>
<td>168</td>
<td>84.4</td>
</tr>
</tbody>
</table>

Fig. (10): Isolan dyes decolorization by *Aspergillus niger* ES-5 in media containing glucose only (a) Isolan Yellow, (b) Isolan Red, (c) Isolan Navy and (d) Isolan Grey.
Results

Table (17): Effect of media containing glucose only on the production of extracellular GOD by *Aspergillus niger* ES-5 at 30°C and 150 rpm.

<table>
<thead>
<tr>
<th>Dyes</th>
<th>Extracellular GOD in BSM I (U/ml)</th>
<th>Extracellular GOD in BSM V (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.Y</td>
<td>9.2</td>
<td>0.5</td>
</tr>
<tr>
<td>I.R</td>
<td>8.1</td>
<td>1.7</td>
</tr>
<tr>
<td>I.N</td>
<td>9.5</td>
<td>1.2</td>
</tr>
<tr>
<td>I.G</td>
<td>10.8</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Fig. (11): Effect of media containing glucose only on the production of extracellular GOD by *Aspergillus niger* ES-5.
Table (18): Effect of media containing glucose only on dry weight by *Aspergillus niger* ES-5 at 30°C and 150 rpm.

<table>
<thead>
<tr>
<th>Dyes</th>
<th>Dry weight in BSM I (g/100ml)</th>
<th>Dry weight BSM V (g/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.Y</td>
<td>4.05</td>
<td>2.00</td>
</tr>
<tr>
<td>I.R</td>
<td>4.10</td>
<td>2.50</td>
</tr>
<tr>
<td>I.N</td>
<td>3.40</td>
<td>1.45</td>
</tr>
<tr>
<td>I.G</td>
<td>3.10</td>
<td>1.60</td>
</tr>
</tbody>
</table>

Fig. (12): Effect of media containing glucose only on dry weight by *Aspergillus niger* ES-5.

Results showed that, although glucose is considered as an essential component in the decolorization process, GOD production and fungal growth, but it must be supported with other media components to work in synergism in order to achieve the maximum activity for the fungi.
Results

- **Calcium carbonate:**

  The effect of CaCO$_3$ on the percentage of decolorization, glucose oxidase activity and fungal growth must be detected thus, two types of basal salt media were prepared BSM I and BSM IV as shown in Table (2), from the results could noticed that the presence of CaCO$_3$ resulted in lower effect in Isolan dye decolorization than media lacking CaCO$_3$. Where, I.Y gave 84.4 and 90.4%, I.R gave 89.5 and 99.6%, I.N gave 80 and 95% and I.G gave 87 and 94.6% in BSM I and BSM IV, respectively at the end of 72 h as presented in Table (19) and Fig (13).

**Table (19):** Effect of CaCO$_3$ on dye decolorization by *Aspergillus niger* ES-5 in BSM at 30°C and 150 rpm throughout 168 h.

<table>
<thead>
<tr>
<th>Dyes</th>
<th>Percentage of decolorization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I.Y</td>
</tr>
<tr>
<td><strong>Time (hours)</strong></td>
<td><strong>BSM I</strong></td>
</tr>
<tr>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>24</td>
<td>28.1</td>
</tr>
<tr>
<td>48</td>
<td>15.6</td>
</tr>
<tr>
<td>72</td>
<td>84.4</td>
</tr>
<tr>
<td>168</td>
<td>84.4</td>
</tr>
</tbody>
</table>
Results

Fig. (13): Effect of CaCO₃ on Isolan dyes decolorization by *Aspergillus niger* ES-5 (a) Isolan Yellow, (b) Isolan Red, (c) Isolan Navy and (d) Isolan Grey.

On the other hand, in case of glucose oxidase activity and fungal growth contradictory results to decolorization were achieved as represented in Tables (20, 21) and Figs. (14, 15). GOD production and dry weight in BSM I was detected to be higher than those obtained by BSM IV.
Results

Table (20): Effect of CaCO₃ on the production of extracellular GOD by *Aspergillus niger* ES-5 in BSM at 30°C and 150 rpm.

<table>
<thead>
<tr>
<th>Dyes</th>
<th>Extracellular GOD in BSM I (U/ml)</th>
<th>Extracellular GOD in BSM IV (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.Y</td>
<td>9.2</td>
<td>3.2</td>
</tr>
<tr>
<td>I.R</td>
<td>8.1</td>
<td>4.3</td>
</tr>
<tr>
<td>I.N</td>
<td>9.5</td>
<td>4.4</td>
</tr>
<tr>
<td>I.G</td>
<td>10.8</td>
<td>4.5</td>
</tr>
</tbody>
</table>

Fig. (14): Effect of CaCO₃ on the production of extracellular GOD by *Aspergillus niger* ES-5 in BSM at 30°C and 150 rpm.
Table (21): Effect of CaCO₃ on dry weight by *Aspergillus niger* ES-5 in BSM at 30°C and 150 rpm.

<table>
<thead>
<tr>
<th>Dyes</th>
<th>Dry weight in BSM I (g/100ml)</th>
<th>Dry weight in BSM IV (g/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.Y</td>
<td>4.05</td>
<td>1.0</td>
</tr>
<tr>
<td>I.R</td>
<td>4.10</td>
<td>1.3</td>
</tr>
<tr>
<td>I.N</td>
<td>3.40</td>
<td>1.1</td>
</tr>
<tr>
<td>I.G</td>
<td>3.10</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Fig. (15): Effect of CaCO₃ on dry weight by *Aspergillus niger* ES-5 in BSM at 30°C and 150 rpm.

The results indicated that, dye decolorization by *A. niger* ES-5 is independent to CaCO₃ addition in Isolan dye decolorization, while the addition CaCO₃ in the growth medium substantially induced glucose oxidase activity as well as fungal growth. CaCO₃ prevents pH decrease at or below pH 4 where GOD production was entirely excluded therefore, CaCO₃ could be considered as an automatic pH controller for GOD production and as a mechanical support for fungal growth.
• **Nitrogen source:**

The potential ability of *Aspergillus niger* ES-5 to consume nitrate in BSM IV was measured before and after decolorization as shown in Table (22).

Table (22): Percentage of the consumption of nitrate by *Aspergillus niger* ES-5 in the presence of the four Isolan dyes.

<table>
<thead>
<tr>
<th>Dyes</th>
<th>Percentage of consumption of nitrate by <em>Aspergillus niger</em> ES-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.Y</td>
<td>54</td>
</tr>
<tr>
<td>I.R</td>
<td>54</td>
</tr>
<tr>
<td>I.N</td>
<td>46</td>
</tr>
<tr>
<td>I.G</td>
<td>80</td>
</tr>
</tbody>
</table>

On the other hand, the effect of peptone and sodium nitrate on the percentage of decolorization, glucose oxidase activity and fungal growth were detected, basal salt media was modified (BSM VI) and the results obtained was compared to those obtained when using BSM I as presented in Table (2). Results in Table (23) and Fig. (16) represented that BSM VI resulted in lower efficiency in Isolan dye decolorization than BSM I, where, I.Y gave 84.4 and 27.8%, I.R gave 89.5 and 23.1%, I.N gave 80 and 47.8% and I.G gave 87 and 86.2% in BSM I and BSM VI, respectively at the end of 72 h thus, the results indicated that the presence of nitrogen sources have a positive effect on the efficiency of *Aspergillus niger* ES-5 to decolorize Isolan dyes, therefore, it could be considered as an essential factor in the decolorization process.
Table (23): Effect of N-source on dye decolorization by *Aspergillus niger* ES-5 in BSM at 30°C and 150 rpm throughout 168 h.

<table>
<thead>
<tr>
<th>Dyes</th>
<th>Percentage of decolorization</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I.Y</td>
<td>I.R</td>
<td>I.N</td>
<td>I.G</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time (h)</td>
<td>BSM I</td>
<td>BSM VI</td>
<td>BSM I</td>
<td>BSM VI</td>
<td>BSM I</td>
<td>BSM VI</td>
<td>BSM I</td>
<td>BSM VI</td>
</tr>
<tr>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>24</td>
<td>28.1</td>
<td>0.0</td>
<td>41.0</td>
<td>0.0</td>
<td>70.6</td>
<td>0.0</td>
<td>76.0</td>
<td>0.0</td>
</tr>
<tr>
<td>48</td>
<td>15.6</td>
<td>16.7</td>
<td>88.0</td>
<td>21.5</td>
<td>77.5</td>
<td>48.9</td>
<td>81.0</td>
<td>86.2</td>
</tr>
<tr>
<td>72</td>
<td>84.4</td>
<td>27.8</td>
<td>89.5</td>
<td>23.1</td>
<td>80.0</td>
<td>47.8</td>
<td>87.0</td>
<td>86.2</td>
</tr>
<tr>
<td>168</td>
<td>84.4</td>
<td>5.6</td>
<td>85.5</td>
<td>20.0</td>
<td>77.0</td>
<td>47.8</td>
<td>79.8</td>
<td>83.0</td>
</tr>
</tbody>
</table>

Figure (16): Effect of N-source on Isolan dyes decolorization by *Aspergillus niger* ES-5 (a) Isolan Yellow, (b) Isolan Red, (c) Isolan Navy and (d) Isolan Grey.
Glucose oxidase activity was also affected by nitrogen source as represented in Table (24) and Fig. (17), GOD in BSM I gave higher value than BSM VI where, peptone and sodium nitrate were used for glucose oxidase production by different microorganisms.

**Table (24):** Effect of N-source on the production of extracellular GOD by *Aspergillus niger* ES-5 in BSM at 30°C and 150 rpm.

<table>
<thead>
<tr>
<th>Dyes</th>
<th>Extracellular GOD in BSM I (U/ml)</th>
<th>Extracellular GOD in BSM VI (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.Y</td>
<td>9.2</td>
<td>0.50</td>
</tr>
<tr>
<td>I.R</td>
<td>8.1</td>
<td>0.80</td>
</tr>
<tr>
<td>I.N</td>
<td>9.5</td>
<td>0.42</td>
</tr>
<tr>
<td>I.G</td>
<td>10.8</td>
<td>0.30</td>
</tr>
</tbody>
</table>

**Fig. (17):** Effect of N-source on the production of extracellular GOD by *Aspergillus niger* ES-5 in BSM at 30°C and 150 rpm.
On the other hand, the fungal growth was not affected by the absence of nitrogen sources where fungal dry weight in BSM I is very close to that obtained in BSM VI as represented in Table (25) and Fig. (18), and this indicated that nitrogen source is not considered as a rate limiting step for mycelial growth.

**Table (25):** Effect of N-source on dry weight by *Aspergillus niger* ES-5 in BSM at 30°C and 150 rpm.

<table>
<thead>
<tr>
<th>Dyes</th>
<th>Dry weight in BSM I (g/100ml)</th>
<th>Dry weight BSM VI (g/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.Y</td>
<td>4.05</td>
<td>4.50</td>
</tr>
<tr>
<td>I.R</td>
<td>4.10</td>
<td>4.45</td>
</tr>
<tr>
<td>I.N</td>
<td>3.40</td>
<td>3.50</td>
</tr>
<tr>
<td>I.G</td>
<td>3.10</td>
<td>2.95</td>
</tr>
</tbody>
</table>

**Fig. (18):** Effect of N-source on dry weight by *Aspergillus niger* ES-5 in BSM at 30°C and 150 rpm.
This experiment proved that GOD contributes in the decolorization process where the absence of nitrogen resulted in nearly 55% drop for some dyes (I.Y, I.R), 30% for I.N and no effect for I.G at 72 h.

- **Potassium dihydrogen phosphate:**

  Generally, the majority of the studies were concerned only with the concentration and the general effect of potassium dihydrogen phosphate on color removal by fungi, but this experiment studied the combined effect of N-source with phosphate group and C-source with phosphate group.

  Results in Table (26) showed that *Aspergillus niger* ES-5 consumes all phosphate contents in BSM IV.

**Table (26):** Percentage of the consumption of phosphate by *Aspergillus niger* ES-5 in the presence of the four Isolan dyes.

<table>
<thead>
<tr>
<th>Dyes</th>
<th>Percentage of consumption of phosphate by <em>A. niger</em> ES-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.Y</td>
<td>100</td>
</tr>
<tr>
<td>I.R</td>
<td>100</td>
</tr>
<tr>
<td>I.N</td>
<td>100</td>
</tr>
<tr>
<td>I.G</td>
<td>100</td>
</tr>
</tbody>
</table>

  The results in Table (27) and Fig. (19) showed that the effect of KH\(_2\)PO\(_4\) with N-source gave higher values than KH\(_2\)PO\(_4\) with C-source in term of decolorization, while contrary results were obtained in case of GOD as well as dry weight Tables (28, 29) and Figs. (20, 21).
Table (27): Effect of BSM III and BSM VII on dye decolorization by *Aspergillus niger* ES-5 at 30°C and 150 rpm throughout 168 h.

<table>
<thead>
<tr>
<th>Dyes</th>
<th>Percentage of decolorization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I.Y</td>
</tr>
<tr>
<td>Time (h)</td>
<td>BSM III</td>
</tr>
<tr>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>24</td>
<td>0.0</td>
</tr>
<tr>
<td>48</td>
<td>8.7</td>
</tr>
<tr>
<td>72</td>
<td>21.7</td>
</tr>
<tr>
<td>168</td>
<td>15.0</td>
</tr>
</tbody>
</table>

Fig. (19): Effect of BSM III and BSM VII on Isolan dyes decolorization by *Aspergillus niger* ES-5 (a) Isolan Yellow, (b) Isolan Red, (c) Isolan Navy and (d) Isolan Grey.
Table (28): Effect of BSM III and BSM VII on the production of extracellular GOD activity by *Aspergillus niger* ES-5 at 30°C and 150 rpm.

<table>
<thead>
<tr>
<th>Dyes</th>
<th>Extracellular GOD in BSM III (U/ml)</th>
<th>Extracellular GOD in BSM VII (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.Y</td>
<td>0</td>
<td>0.3</td>
</tr>
<tr>
<td>I.R</td>
<td>0</td>
<td>0.2</td>
</tr>
<tr>
<td>I.N</td>
<td>0</td>
<td>0.1</td>
</tr>
<tr>
<td>I.G</td>
<td>0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Fig. (20): Effect of BSM III and BSM VII on the production of extracellular GOD by *Aspergillus niger* ES-5 at 30°C and 150 rpm.
Table (29): Effect of BSM III and BSM VII on dry weight by *Aspergillus niger* ES-5 at 30°C and 150 rpm.

<table>
<thead>
<tr>
<th>Dyes</th>
<th>Dry weight in BSM III (g/100ml)</th>
<th>Dry weight in BSM VII (g/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.Y</td>
<td>0.022</td>
<td>2.70</td>
</tr>
<tr>
<td>I.R</td>
<td>0.024</td>
<td>2.70</td>
</tr>
<tr>
<td>I.N</td>
<td>0.037</td>
<td>2.00</td>
</tr>
<tr>
<td>I.G</td>
<td>0.023</td>
<td>1.95</td>
</tr>
</tbody>
</table>

Fig. (21): Effect of BSM III and BSM VII on dry weight by *Aspergillus niger* ES-5 at 30°C and 150 rpm.

From all the previous experiments, the following conclusions were drawn: Glucose concentration could be decreased from 80 to 30 g/l without affecting decolorization percentage, glucose is an essential component because it is considered as the inducer for GOD production which is clearly
relevant to the decolorization of the four Isolan dyes. Calcium carbonate did not influence the decolorization process thus, it could be removed from BSM components without affecting the decolorization process, nitrogen-sources were needed to increase decolorization percentage and GOD production, and also potassium dihydrogen phosphate must be added for its importance in GOD production, thus BSM IV was given the highest decolorization percentage in comparison with the other types of BSM as shown in Image (1).

![Image (1): Effect of BSM IV on Isolan dyes decolorization by Aspergillus niger ES-5 (a1) Isolan Yellow before fungal treatment, (a2) Isolan Yellow after fungal treatment, (b1) Isolan Red before fungal treatment, (b2) Isolan Red after fungal treatment, (c1) Isolan Navy before fungal treatment, (c2) Isolan Navy after fungal treatment and (d1) Isolan Gray before fungal treatment, (d2) Isolan Gray after fungal treatment.](image-url)
Results

Optimization conditions:

- **Incubation period:**

  There is a lag period for fungal adaptation always needed in order to achieve maximum decolorization, glucose oxidase production and dry weight.

  The data obtained and represented in Tables (30, 31, 32, 33 and 34) and Fig. (22), illustrate the effect of different incubation periods on the percentage of decolorization, glucose oxidase production and dry weight under the previous media components.

  The results proved that dye decolorization increased with the increase in time, and reached a steady state at 72 h. Also, the extracellular GOD showed a gradual increase in the time course, reaching a maximum level of 3.10, 4.375, 4.0 and 3.59 U/ml for I.Y, I.R, I.N and I.G, respectively at 72 h of incubation, after which the increase was considered negligible. The assay for intracellular GOD shows a difference in the pattern of production, the addition of dye causes an increase by time, contrary to intracellular GOD in control cultures (no dye) which reaches its peak at 48 h then declines. While, the extracellular GOD in both cultures follow the same pattern exactly reaching its maximum at 96 h while, in the absence of Isolan dyes *Aspergillus niger* ES-5 produced higher concentrations of GOD reaching 6.00 U/ml than in the presence of Isolan dyes.

  The mycelial dry weight in cultures containing the dyes was negatively affected compared to that of control cultures. Thus, this is an indication that Isolan dyes did not stimulate fungal growth and that fungi could not utilize Isolan dyes as a
carbon source. The fungal biomass exhibited increase with time indicating a direct relationship between incubation time and the growth of the organism and its production.

**Table (30):** Effect of time course on GOD activity, the production of intracellular GOD and dry weight by *Aspergillus niger* ES-5 incubated in BSM IV (without the dye) at 30°C and 150 rpm.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Extracellular GOD (U/ml)</th>
<th>Intracellular GOD (U/ml/d.wt)</th>
<th>Dry weight (g/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (h)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
<td>1.22</td>
</tr>
<tr>
<td>24</td>
<td>0.25</td>
<td>2.63</td>
<td>1.62</td>
</tr>
<tr>
<td>48</td>
<td>1.00</td>
<td>4.25</td>
<td>1.98</td>
</tr>
<tr>
<td>72</td>
<td>4.33</td>
<td>1.67</td>
<td>3.31</td>
</tr>
<tr>
<td>96</td>
<td>6.00</td>
<td>0.50</td>
<td>3.10</td>
</tr>
</tbody>
</table>

**Table (31):** Effect of time course on dye decolorization of Isolan Yellow, GOD activity and dry weight by *Aspergillus niger* ES-5 incubated in BSM IV at 30°C and 150 rpm.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Percentage of decolorization</th>
<th>Extracellular GOD (U/ml)</th>
<th>Intracellular GOD (U/ml/d.wt)</th>
<th>Dry weight (g/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (h)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.155</td>
<td>0.00</td>
<td>0.00</td>
<td>0.19</td>
</tr>
<tr>
<td>24</td>
<td>16.100</td>
<td>0.33</td>
<td>0.50</td>
<td>0.26</td>
</tr>
<tr>
<td>48</td>
<td>74.200</td>
<td>0.83</td>
<td>2.10</td>
<td>0.70</td>
</tr>
<tr>
<td>72</td>
<td>93.500</td>
<td>3.10</td>
<td>2.90</td>
<td>0.75</td>
</tr>
<tr>
<td>96</td>
<td>93.500</td>
<td>3.20</td>
<td>2.95</td>
<td>0.90</td>
</tr>
</tbody>
</table>
Results

**Table (32):** Effect of time course on dye decolorization of Isolan Red, GOD activity and dry weight by *Aspergillus niger* ES-5 incubated in BSM IV at 30°C and 150 rpm.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Time (h)</th>
<th>Percentage of decolorization</th>
<th>Extracellular GOD (U/ml)</th>
<th>Intracellular GOD (U/ml/d.wt)</th>
<th>Dry weight (g/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>2.3</td>
<td>0.000</td>
<td>0.000</td>
<td>0.377</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>75.9</td>
<td>0.375</td>
<td>0.625</td>
<td>0.710</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>97.6</td>
<td>1.075</td>
<td>1.875</td>
<td>1.320</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>99.1</td>
<td>4.375</td>
<td>3.986</td>
<td>1.120</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>99.1</td>
<td>4.421</td>
<td>3.900</td>
<td>1.230</td>
</tr>
</tbody>
</table>

**Table (33):** Effect of time course on dye decolorization of Isolan Navy, GOD activity and dry weight by *Aspergillus niger* ES-5 incubated in BSM IV at 30°C and 150 rpm.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Time (h)</th>
<th>Percentage of decolorization</th>
<th>Extracellular GOD (U/ml)</th>
<th>Intracellular GOD (U/ml/d.wt)</th>
<th>Dry weight (g/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1.31</td>
<td>0.00</td>
<td>0.00</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>16.64</td>
<td>0.30</td>
<td>0.83</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>92.90</td>
<td>2.00</td>
<td>1.75</td>
<td>1.31</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>95.80</td>
<td>4.00</td>
<td>3.00</td>
<td>1.30</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>95.80</td>
<td>4.08</td>
<td>3.06</td>
<td>1.50</td>
</tr>
</tbody>
</table>
Table (34): Effect of time course on dye decolorization of Isolan Grey, GOD activity and dry weight by *Aspergillus niger* ES-5 incubated in BSM IV at 30°C and 150 rpm.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Percentage of decolorization</th>
<th>Extracellular GOD (U/ml)</th>
<th>Intracellular GOD (U/ml/d.wt)</th>
<th>Dry weight (g/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (h)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.81</td>
<td>0.000</td>
<td>0.00</td>
<td>0.17</td>
</tr>
<tr>
<td>24</td>
<td>50.9</td>
<td>0.167</td>
<td>0.54</td>
<td>0.70</td>
</tr>
<tr>
<td>48</td>
<td>84.6</td>
<td>0.830</td>
<td>1.42</td>
<td>1.23</td>
</tr>
<tr>
<td>72</td>
<td>92.4</td>
<td>3.590</td>
<td>2.23</td>
<td>1.30</td>
</tr>
<tr>
<td>96</td>
<td>92.6</td>
<td>3.700</td>
<td>2.50</td>
<td>1.94</td>
</tr>
</tbody>
</table>

Fig. (22): Effect of time course on (a) extracellular GOD, (b) intracellular GOD, (c) percentage of decolorization and (d) dry weight of Isolan dyes by *Aspergillus niger* ES-5 incubated in BSM IV at 30°C and 150 rpm.
The results obtained show that 72 h was the optimum incubation period for the upcoming experiments.

- **Initial pH:**

  The pH of an aqueous medium is a very important factor, it is affected by two criteria: firstly, since dyes are complex aromatic organic compounds having different functional groups and unsaturated bonds, they have different potential at different pH, resulting in the pH dependent net charge on the dye molecules. Secondarily, the surface of fungi consist of many functional groups which are pH dependent.

  The results in Tables (35, 36, 37 and 38) and Figs. (23, 24, 25 and 26) showed that in case of I.Y the highest percentage of decolorization was at pH 3.5, maximum value of extracellular and intracellular GOD was achieved at pH 4.5 while the greatest value of fungal growth was at pH 7.5. In case of I.R the highest percentages of decolorization, the maximum value of extracellular and intracellular GOD were at pH 7.5, while the greatest value of fungal growth was at pH 6.5, in case of I.N the highest percentages of decolorization, the maximum value of extracellular GOD and the greatest value of fungal growth was at pH 5.5 while, the maximum value of intracellular GOD was achieved at pH 4.5. In case of I.G the highest percentage of decolorization was at pH 6.5, the maximum value of extracellular and intracellular GOD was achieved at pH 7.5, while the greatest value of fungal growth was at pH 6.5.
Table (35): Effect of different initial pH values on the percentage of decolorization by *Aspergillus niger* ES-5 incubated in BSM IV at 30°C and 150 rpm.

<table>
<thead>
<tr>
<th>pH</th>
<th>percentage of decolorization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I.Y</td>
</tr>
<tr>
<td>3.5</td>
<td>92.70</td>
</tr>
<tr>
<td>4.5</td>
<td>91.00</td>
</tr>
<tr>
<td>5.5</td>
<td>88.00</td>
</tr>
<tr>
<td>6.5</td>
<td>68.96</td>
</tr>
<tr>
<td>7.5</td>
<td>84.20</td>
</tr>
<tr>
<td>8.5</td>
<td>82.50</td>
</tr>
<tr>
<td>9.5</td>
<td>73.80</td>
</tr>
<tr>
<td>10.5</td>
<td>51.70</td>
</tr>
</tbody>
</table>

Fig. (23): Effect of different initial pH values on the percentage of decolorization by *Aspergillus niger* ES-5 incubated in BSM IV at 30°C and 150 rpm.
Table (36): Effect of different initial pH values on the production of extracellular GOD activity by *Aspergillus niger* ES-5 incubated in BSM IV at 30°C and 150 rpm.

<table>
<thead>
<tr>
<th>pH</th>
<th>I.Y</th>
<th>I.R</th>
<th>I.N</th>
<th>I.G</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5</td>
<td>0.2</td>
<td>2.70</td>
<td>0.41</td>
<td>1.08</td>
</tr>
<tr>
<td>4.5</td>
<td>3.0</td>
<td>4.10</td>
<td>4.20</td>
<td>4.60</td>
</tr>
<tr>
<td>5.5</td>
<td>2.9</td>
<td>4.40</td>
<td>4.30</td>
<td>4.50</td>
</tr>
<tr>
<td>6.5</td>
<td>0.5</td>
<td>5.10</td>
<td>0.58</td>
<td>1.33</td>
</tr>
<tr>
<td>7.5</td>
<td>0.4</td>
<td>5.58</td>
<td>0.58</td>
<td>4.67</td>
</tr>
<tr>
<td>8.5</td>
<td>0.4</td>
<td>0.50</td>
<td>0.42</td>
<td>0.75</td>
</tr>
<tr>
<td>9.5</td>
<td>0.2</td>
<td>0.58</td>
<td>0.30</td>
<td>0.75</td>
</tr>
<tr>
<td>10.5</td>
<td>0.1</td>
<td>0.92</td>
<td>0.50</td>
<td>1.10</td>
</tr>
</tbody>
</table>

Fig. (24): Effect of different initial pH values on the production extracellular GOD activity by *Aspergillus niger* ES-5 incubated in BSM IV at 30°C and 150 rpm.
Table (37): Effect of different initial pH values on the production of intracellular GOD activity by *Aspergillus niger* ES-5 incubated in BSM IV at 30°C and 150 rpm.

<table>
<thead>
<tr>
<th>pH</th>
<th>I.Y</th>
<th>I.R</th>
<th>I.N</th>
<th>I.G</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5</td>
<td>1.00</td>
<td>2.90</td>
<td>1.25</td>
<td>0.83</td>
</tr>
<tr>
<td>4.5</td>
<td>1.90</td>
<td>3.80</td>
<td>2.80</td>
<td>2.10</td>
</tr>
<tr>
<td>5.5</td>
<td>1.10</td>
<td>3.70</td>
<td>1.58</td>
<td>2.00</td>
</tr>
<tr>
<td>6.5</td>
<td>1.20</td>
<td>4.60</td>
<td>1.67</td>
<td>1.33</td>
</tr>
<tr>
<td>7.5</td>
<td>1.25</td>
<td>4.75</td>
<td>1.60</td>
<td>2.58</td>
</tr>
<tr>
<td>8.5</td>
<td>1.42</td>
<td>1.50</td>
<td>1.17</td>
<td>0.33</td>
</tr>
<tr>
<td>9.5</td>
<td>1.25</td>
<td>1.50</td>
<td>0.50</td>
<td>0.17</td>
</tr>
<tr>
<td>10.5</td>
<td>1.75</td>
<td>0.67</td>
<td>1.33</td>
<td>0.67</td>
</tr>
</tbody>
</table>

Fig. (25): Effect of different initial pH values on the production of intracellular GOD activity by *Aspergillus niger* ES-5 incubated in BSM IV at 30°C and 150 rpm.
Table (38): Effect of different initial pH values on dry weight by *Aspergillus niger* ES-5 incubated in BSM IV at 30°C and 150 rpm.

<table>
<thead>
<tr>
<th>pH</th>
<th>I.Y</th>
<th>I.R</th>
<th>I.N</th>
<th>I.G</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5</td>
<td>0.70</td>
<td>0.91</td>
<td>0.72</td>
<td>0.47</td>
</tr>
<tr>
<td>4.5</td>
<td>0.64</td>
<td>0.96</td>
<td>0.70</td>
<td>0.80</td>
</tr>
<tr>
<td>5.5</td>
<td>0.83</td>
<td>0.80</td>
<td>1.08</td>
<td>0.90</td>
</tr>
<tr>
<td>6.5</td>
<td>0.70</td>
<td>0.97</td>
<td>0.56</td>
<td>0.93</td>
</tr>
<tr>
<td>7.5</td>
<td>0.93</td>
<td>0.79</td>
<td>0.49</td>
<td>0.54</td>
</tr>
<tr>
<td>8.5</td>
<td>0.64</td>
<td>0.91</td>
<td>0.86</td>
<td>0.61</td>
</tr>
<tr>
<td>9.5</td>
<td>0.54</td>
<td>0.50</td>
<td>0.82</td>
<td>0.45</td>
</tr>
<tr>
<td>10.5</td>
<td>0.64</td>
<td>0.52</td>
<td>0.79</td>
<td>0.72</td>
</tr>
</tbody>
</table>

Fig. (26): Effect of different initial pH values on dry weight by *Aspergillus niger* ES-5 incubated in BSM IV at 30°C and 150 rpm.
It is evident that dye structure has a great effect on the adjustment of pH where each dye has different optimal pH to obtain maximum decolorization process, glucose oxidase activity and fungal growth.

- **Incubation temperature:**

  Microbial growth and product formation are the result of a complex series of chemical reactions, and like all chemical reactions, they are influenced by temperature.

  The results obtained are drown stated in Tables (39, 40, 41 and 42) and Fig. (27). 30°C was the optimum temperature for maximum decolorization percentage, highest GOD activity and dry weight. It was also noted that all these parameters decreased with further increase of temperature up to 45°C in all four Isolan dyes.

**Table (39):** Effect of different incubation temperatures on dye decolorization of Isolan Yellow, GOD activity and dry weight by *Aspergillus niger* ES-5 incubated in BSM IV at 150 rpm.

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>Percentage of decolorization</th>
<th>Extracellular GOD (U/ml)</th>
<th>Intracellular GOD (U/ml/d.wt)</th>
<th>Dry weight (g/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>92.3</td>
<td>0.42</td>
<td>2.00</td>
<td>0.524</td>
</tr>
<tr>
<td>30</td>
<td>93.5</td>
<td>3.10</td>
<td>1.80</td>
<td>0.800</td>
</tr>
<tr>
<td>35</td>
<td>86.7</td>
<td>1.92</td>
<td>1.75</td>
<td>0.800</td>
</tr>
<tr>
<td>40</td>
<td>83.3</td>
<td>1.83</td>
<td>1.75</td>
<td>0.370</td>
</tr>
<tr>
<td>45</td>
<td>3.85</td>
<td>0.67</td>
<td>1.00</td>
<td>0.101</td>
</tr>
<tr>
<td>50</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.022</td>
</tr>
</tbody>
</table>
Table (40): Effect of different incubation temperatures on dye decolorization of Isolan Red, GOD activity and dry weight by *Aspergillus niger* ES-5 incubated in BSM IV at 150 rpm.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Temperature °C</th>
<th>Percentage of decolorization</th>
<th>Extracellular GOD (U/ml)</th>
<th>Intracellular GOD (U/ml/d.wt)</th>
<th>Dry weight (g/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25</td>
<td>82.8</td>
<td>1.50</td>
<td>1.83</td>
<td>0.288</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>99.1</td>
<td>4.38</td>
<td>3.99</td>
<td>1.120</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>97.5</td>
<td>2.00</td>
<td>2.08</td>
<td>0.782</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>97.8</td>
<td>1.58</td>
<td>1.75</td>
<td>0.570</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>3.86</td>
<td>0.50</td>
<td>0.90</td>
<td>0.181</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.116</td>
</tr>
</tbody>
</table>

Table (41): Effect of different incubation temperatures on dye decolorization of Isolan Navy, GOD activity and dry weight by *Aspergillus niger* ES-5 incubated in BSM IV at 150 rpm.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Temperature °C</th>
<th>Percentage of decolorization</th>
<th>Extracellular GOD (U/ml)</th>
<th>Intracellular GOD (U/ml/d.wt)</th>
<th>Dry weight (g/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25</td>
<td>95.2</td>
<td>1.50</td>
<td>1.5</td>
<td>0.648</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>98.7</td>
<td>4.00</td>
<td>3.00</td>
<td>1.300</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>97.7</td>
<td>1.92</td>
<td>2.17</td>
<td>0.461</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>95.8</td>
<td>1.90</td>
<td>2.08</td>
<td>0.300</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>10.9</td>
<td>0.83</td>
<td>1.10</td>
<td>0.084</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.011</td>
</tr>
</tbody>
</table>
Table (42): Effect of different incubation temperatures on dye decolorization of Isolan Grey, GOD activity and dry weight by *Aspergillus niger* ES-5 incubated in BSM IV at 150 rpm.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Percentage of decolorization</th>
<th>Extracellular GOD (U/ml)</th>
<th>Intracellular GOD (U/ml/d.wt)</th>
<th>Dry weight (g/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature °C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>77.1</td>
<td>1.80</td>
<td>1.75</td>
<td>0.512</td>
</tr>
<tr>
<td>30</td>
<td>95.3</td>
<td>3.59</td>
<td>2.25</td>
<td>1.300</td>
</tr>
<tr>
<td>35</td>
<td>92.4</td>
<td>2.75</td>
<td>2.00</td>
<td>1.000</td>
</tr>
<tr>
<td>40</td>
<td>89.0</td>
<td>2.25</td>
<td>1.95</td>
<td>0.800</td>
</tr>
<tr>
<td>45</td>
<td>5.3</td>
<td>0.67</td>
<td>1.20</td>
<td>0.179</td>
</tr>
<tr>
<td>50</td>
<td>0.0</td>
<td>0.00</td>
<td>0.00</td>
<td>0.024</td>
</tr>
</tbody>
</table>

Fig. (27): Effect of different incubation temperatures on (a) extracellular GOD, (b) intracellular GOD, (c) percentage of decolorization and (d) dry weight of Isolan dye, by *Aspergillus niger* ES-5 incubated in BSM IV at 150 rpm.
Assessment of some chemical parameters:

Results represented in Table (43) showed that the levels of COD, BOD and total suspended solids of the four Isolan dyes before and after treatment exhibit variation and that COD and BOD removal depend on the strength of the dye.

**Table (43):** Effect of *Aspergillus niger* ES-5 on COD, BOD and total suspended solids (TSS) reduction.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Dyes</th>
<th>Percentage of COD reduction</th>
<th>Percentage of BOD reduction</th>
<th>Percentage of TSS reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I.Y</td>
<td>35.3</td>
<td>16.8</td>
<td>62.9</td>
</tr>
<tr>
<td></td>
<td>I.R</td>
<td>34.4</td>
<td>26.7</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>I.N</td>
<td>50</td>
<td>42.8</td>
<td>68.3</td>
</tr>
<tr>
<td></td>
<td>I.G</td>
<td>37.9</td>
<td>19.6</td>
<td>41.4</td>
</tr>
</tbody>
</table>

Effect of decolorized extra cellular fluids on microbial growth for detecting toxicity:

Toxicity tests confirm the safety of treated wastewater resulted from biological treatment by *Aspergillus niger* ES-5.

Results in Table (44) showed that there is no inhibition zones are formed indicating the lack of toxicity of the solution resulted from decolorization by *A. niger* ES-5 which indicates the lack of aromatic amine formation.
Table (44): Toxicity test of treated fungal dyes solution by *E. coli* expressed as inhibition zone.

<table>
<thead>
<tr>
<th>Dyes</th>
<th>Inhibition zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.Y</td>
<td>-</td>
</tr>
<tr>
<td>I.R</td>
<td>-</td>
</tr>
<tr>
<td>I.N</td>
<td>-</td>
</tr>
<tr>
<td>I.G</td>
<td>-</td>
</tr>
</tbody>
</table>

(-): Lack of inhibition zone (no toxicity).

Detection the mode of dye decolorization by *Aspergillus niger* ES-5:

There are different mechanisms by which fungi could be decolorized different types of dyes and could summarized into biodegradation, biosorption and bioaccumulation.

The mode of decolorization of the four Isolan dyes by the fungal strain *Aspergillus niger* ES-5 were as following:

- **In vitro-decolorization, Extracellular fluids (ECF):**

Results in Table (45) showed that the decolorization percentages ranged from 0 to 7, which is considered very low compared to those obtained using live fungal plugs. In this table, it is evident that a negligible decolorization is recorded in the four Isolan dyes when incubated with extracellular fluid. i.e., extracellular glucose oxidase is not the main reason for the oxidation and decolorization of Isolan dyes.
Table (45): Effect of extracellular fluids (ECF) on dyes decolorization by *Aspergillus niger* ES-5 at 30°C and 150 rpm.

<table>
<thead>
<tr>
<th>Dyes</th>
<th>Percentage of decolorization</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.Y</td>
<td>7.000</td>
</tr>
<tr>
<td>I.R</td>
<td>0.000</td>
</tr>
<tr>
<td>I.N</td>
<td>1.103</td>
</tr>
<tr>
<td>I.G</td>
<td>3.240</td>
</tr>
</tbody>
</table>

It could be observed that high percentage of dye decolorization obtained *in vivo* (whole cell) process (results shown in Table 19) are not the same as those obtained *in vitro* (crude enzyme) processes suggesting that other factors are required for the decolorization process.

- **Fungal mycelia:**

  *Aspergillus niger* is known for its ability to sorb dyes and metal via the macromolecules in the mycelial wall.

- **Test of dead fungal mycelium:**

  Dead cells do not require nutrient supply, pH adjustment or temperature; furthermore, dead cells could be stored, or used for extended periods. Results in Table (46) showed that dead fungal biomass gave negligible decolorization in all four Isolan dyes indicating that the autoclaved fungi did not adsorb the dye. These observations confirm that the decolorization process by fungal biomass takes place through microbial metabolism and is linked to microbial growth.
Table (46): Effect of dead fungal inoculums on dye decolorization incubated in BSM IV at 30°C and 150 rpm after 7 days.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>percentage of decolorization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I.Y</td>
</tr>
<tr>
<td><em>Aspergillus niger</em> ES-5</td>
<td>4.2</td>
</tr>
</tbody>
</table>

b- Living biomass:

b-I- Adsorption (Decolorization in the absence of supplements):

*Aspergillus sp.* successfully used dyes as their sole carbon and nitrogen sources.

Table (47) shows that there was an increase in color intensity which may be due to polymerization of dyes and that no fungal growth was observed in the biosorption flasks. These results, reflect that decolorization of dyes by *Aspergillus niger* ES-5 requires the addition of nutrients to the media in order to grow properly and to break the chromophore centers of the dyes. Hence, an enzyme is suspected to be involved in the decolorization process. *Aspergillus niger* ES-5 is not only incapable of utilizing the Isolan dyes, but also the lack of enzyme causes polymerization of the dyes under study.
Table (47): Dye biosorption of different dyes on *Aspergillus niger* ES-5 mycelia.

<table>
<thead>
<tr>
<th>Dyes</th>
<th>Color intensity (increase of color after 72h)</th>
<th>Dry weight (g/100ml) after 72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.Y</td>
<td>34.37%</td>
<td>0.0370</td>
</tr>
<tr>
<td>I.R</td>
<td>16.00%</td>
<td>0.0193</td>
</tr>
<tr>
<td>I.N</td>
<td>8.63%</td>
<td>0.0210</td>
</tr>
<tr>
<td>I.G</td>
<td>8.20%</td>
<td>0.0065</td>
</tr>
</tbody>
</table>

b-II- Decolorization in the presence of supplements:

From all the previous experiments it was observed that the use of live biomass resulted in the decolorization of approximately 90.4, 99.6, 95 and 94.6% in I.Y, I.R, I.N and I.G, respectively (as represented in Table (19)) indicating that many decolorization processes probably are performed through growth of related enzymes. Thus, the decolorization by living cells may involve a more complex enzyme system such as extracellular or intracellular oxidases.

- Ultraviolet visible (UV-Vis) analysis:

The UV-Vis spectra results, (Fig. 28) showed that there was a decrease in the visible range (400-800 nm) which is characteristic for the dye color and formation of the peak at 270 nm, suggesting that decolorization occurred through breakdown of the azo bond responsible for the dye color.
Fig. (28): UV-Visible spectrum of the four Isolan dyes cultures before (0 h) and after biological treatment (72 h), (a) UV-Visible spectrum of Isolan Yellow, (b) UV-Visible spectrum of Isolan Red, (c) UV-Visible spectrum of Isolan Navy and (d) UV-Visible spectrum of Isolan Gray.

- **Fourier Transform Infrared spectroscopy (FTIR) spectral analysis:**

  FTIR spectra can indicate certain specific functional groups present in a dye molecule. However, it must be combined with other techniques of instrumental analysis to identify the actual chemical composition of the compound.

  It is important to realize if after the cleavage of azo bond, there was another degradation in the Isolan dyes. FTIR analysis was carried out on dried samples. To detect the presence or
absence of the most important chemical groups in the four Isolan dyes before and after biological treatment. The data are represented in Figs. (29, 30, 31 and 32).

Aromatic rings which appear at (800-400 cm\(^{-1}\)), azo bond (1500-1400 cm\(^{-1}\)) and aniline (1650-1560 cm\(^{-1}\)) (Yuen et al., 2005).

![FTIR of Isolan Yellow](image)

**Fig. (29):** FTIR of Isolan Yellow (a) before and (b) after fungal treatment.

In case of I.Y, it could be observed that the aromatic rings decrease in intensity, indicating the decrease in concentration, but could still be detected; this indicates that they were not fully degraded. Azo bond and aniline were also decrease in intensity, indicating the decrease in concentration.
**Fig. (30):** FTIR of Isolan Red (a) before and (b) after fungal treatment.

The aromatic rings in I.R were shifted and increased in intensity while the azo bond and the aniline decreased in intensity.

**Fig. (31):** FTIR of Isolan Navy (a) before and (b) after fungal treatment.
The aromatic rings of I.N are represented by two peaks before biological treatment one of them was disappeared (609 cm\(^{-1}\)) while the other one was shifted which indicated changes in dye structure. The azo bond peaks before biological treatment disappeared after treatment which indicated breakage of it, the aniline intensity was increased which indicated further degradation of azo bond.

![FTIR of Isolan Grey (a) before and (b) after fungal treatment.](image)

**Fig. (32):** FTIR of Isolan Grey (a) before and (b) after fungal treatment.


- **High performance liquid chromatography (HPLC):**

  HPLC has been used for analysis of various dyes and metabolites from various degradation procedures.

  The suggested mechanism at which *Aspergillus niger* ES-5 degraded azo dyes was confirmed by HPLC. The suspected Isolan dyes structure is diphenol azo -6-sulphonic acid.
Therefore, the suspected by products after decolorization could be aniline and sulphanilic acid.

The results in Table (48) indicated that *Aspergillus niger* ES-5 break the azo bond -N=N- to aniline -NH₂ after 48 h. In case of I.Y and I.R, another degradation of aniline to aniline derivatives after 72 h was noticed as shown in FTIR analysis. While, in case of I.N and I.G, aniline appeared after 48 h and increased at 72 h which indicates that the fungus under study is breaking more azo bonds by time.

On the other hand, sulphanilic acid appeared after 48 h in I.Y, while it appeared after 72 h in I.R, I.N and I.G.

**Table (48):** HPLC analysis for I.Y, I.R, I.N and I.G after 48 and 72 h.

<table>
<thead>
<tr>
<th>Substance</th>
<th>I.Y¹</th>
<th>I.Y²</th>
<th>I.R¹</th>
<th>I.R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aniline</td>
<td>18.77494 X10⁵</td>
<td>15.56100 X10⁵</td>
<td>16.99520 X10⁵</td>
<td>12.79277 X10⁵</td>
</tr>
<tr>
<td>Sulphanilic</td>
<td>26.5896 X10⁴</td>
<td>22.7281 X10⁴</td>
<td>-</td>
<td>22.1354 X10⁴</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Substance</th>
<th>I.N¹</th>
<th>I.N²</th>
<th>I.G¹</th>
<th>I.G²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aniline</td>
<td>15.07784 X10⁵</td>
<td>16.79829 X10⁵</td>
<td>12.34473 X10⁵</td>
<td>15.46722 X10³</td>
</tr>
<tr>
<td>Sulphanilic</td>
<td>-</td>
<td>11.76464 X10⁵</td>
<td>-</td>
<td>30.7607 X10⁴</td>
</tr>
</tbody>
</table>

c- Desorption:

Some of the dyes are externally adsorbed by *Aspergillus niger* ES-5. Therefore, desorption could allow to detect the extent of external adsorption on the fungal wall.

The results obtained in Table (49) showed the minimal amounts of dyes released to the medium. This excludes biosorption of dyes to the mycelial wall as the major mechanism of dye removal by this fungus. But does not exclude it as one of the potential mechanisms because of the nature of the macromolecular structure of the fungal wall which could bind to the dye molecules through physico-chemical binding. Yet it is not the main mode for Isolan dyes decolorization regardless of the desorbing solution.

**Table (49):** Determine the amount of desorbed dyes by *Aspergillus niger* ES-5 at 30°C and 150 rpm.

<table>
<thead>
<tr>
<th>Dyes</th>
<th>Increase in absorbance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.Y</td>
<td>2.5</td>
</tr>
<tr>
<td>I.R</td>
<td>1.3</td>
</tr>
<tr>
<td>I.N</td>
<td>1.4</td>
</tr>
<tr>
<td>I.G</td>
<td>3.0</td>
</tr>
</tbody>
</table>
d- Intracellular GOD and Bioaccumulation:

As shown in the results obtained previously in co-metabolic condition section, a large part of GOD enzyme is located on the inner side of the mycelia (periplasmic).

The results plotted represent the data for both pure powder and dried mycelia with supernatant after decolorization element content before and after decoloization, from Table (50) one could obseve that all element content were below the detection limit i.e., the dyes could be entrapped inside the fungal biomass and not biotransformed.

Table (50): Concentration (%) of sulfur, chromium, copper and zinc for each dye before (Initial) and after 72 h (Final) incubation with A. niger ES-5 using EDS.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>6.8</td>
<td>ND</td>
<td>17.5</td>
<td>ND</td>
<td>6.63</td>
<td>ND</td>
<td>4.37</td>
<td>ND</td>
</tr>
<tr>
<td>Cr</td>
<td>9.49</td>
<td>ND</td>
<td>8.92</td>
<td>ND</td>
<td>4.97</td>
<td>ND</td>
<td>8.12</td>
<td>ND</td>
</tr>
<tr>
<td>Cu</td>
<td>1.07</td>
<td>ND</td>
<td>1.11</td>
<td>ND</td>
<td>0.58</td>
<td>ND</td>
<td>1.51</td>
<td>ND</td>
</tr>
<tr>
<td>Zn</td>
<td>-</td>
<td>-</td>
<td>0.96</td>
<td>ND</td>
<td>-</td>
<td>-</td>
<td>0.82</td>
<td>ND</td>
</tr>
</tbody>
</table>

(-): Absence of Zn in I.Y and I.N, ND: Not detected after decolorization.

The results are backed up by the visual observation of the fungal cells which did not maintain their natural color, thus, microscope examination are carried out on the fungal mycelia to investigate the fungal biomass intracellularly as shown in images (2, 3, 4, 5 and 6).
Results


I.Y after 24 h  I.Y after 48 h  I.Y after 72 h


I.R after 24 h  I.R after 48 h  I.R after 72 h

### Results

<table>
<thead>
<tr>
<th>I.N after 24 h</th>
<th>I.N after 48 h</th>
<th>I.N after 72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image5.jpg" alt="Image" /></td>
<td><img src="image5.jpg" alt="Image" /></td>
<td><img src="image5.jpg" alt="Image" /></td>
</tr>
</tbody>
</table>

**Image (5):** Showing I.N uptake by *Aspergillus niger* ES-5 mycelia after 24, 48 and 72 h of incubation.

<table>
<thead>
<tr>
<th>I.G after 24 h</th>
<th>I.G after 48 h</th>
<th>I.G after 72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image6.jpg" alt="Image" /></td>
<td><img src="image6.jpg" alt="Image" /></td>
<td><img src="image6.jpg" alt="Image" /></td>
</tr>
</tbody>
</table>

**Image (6):** Showing I.G uptake by *Aspergillus niger* ES-5 mycelia after 24, 48 and 72 h of incubation.

Results indicate that *Aspergillus niger* ES-5 could uptake the dyes inside the fungal biomass (bioaccumulation) without biotransformation and that the dominant mechanism for decolorization would be the bioaccumulation of the dye molecules.
II. Physical treatment:

The conventional aerobic biological process, e.g. activated sludge process, cannot readily treat textile wastewater, because most commercial dyes are toxic to the microorganisms, and they result in sludge bulk while, gamma radiation has been considered as a promising process for the treatment of textile wastewater.

Results in Table (51) showed that gamma rays increase color intensity in I.Y which, could be resulted from dimmerization of the parent compound causing polymerization. On the other hand, the other three Isolan dyes showed negative decolorization efficiency till 2.5 kGy after which, a slow increase in the decolorization was observed.

**Table (51):** Effect of gamma radiation on dyes decolorization.

<table>
<thead>
<tr>
<th>Dyes</th>
<th>Percentage of increase in color intensity</th>
<th>Percentage of decolorization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I.Y</td>
<td>I.R</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3.0</td>
<td>52</td>
<td>21.5</td>
</tr>
<tr>
<td>6.0</td>
<td>54</td>
<td>37.3</td>
</tr>
<tr>
<td>10.0</td>
<td>54</td>
<td>47.8</td>
</tr>
<tr>
<td>15.0</td>
<td>55</td>
<td>53.1</td>
</tr>
<tr>
<td>20.0</td>
<td>56</td>
<td>59.4</td>
</tr>
</tbody>
</table>
The pH values of irradiated and non-irradiated dye solution were measured.

Results in Table (52) showed that the pH of the solution decreased gradually with the irradiation dose.

**Table (52):** Effect of gamma radiation on the dyes pH for tested Isolan dyes.

<table>
<thead>
<tr>
<th>Radiation dose</th>
<th>I.Y</th>
<th>I.R</th>
<th>I.N</th>
<th>I.G</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7.9</td>
<td>7.97</td>
<td>7.94</td>
<td>7.98</td>
</tr>
<tr>
<td>0.5</td>
<td>7.8</td>
<td>7.9</td>
<td>7.9</td>
<td>7.8</td>
</tr>
<tr>
<td>1.0</td>
<td>7.8</td>
<td>7.87</td>
<td>7.89</td>
<td>7.8</td>
</tr>
<tr>
<td>1.5</td>
<td>7.7</td>
<td>7.85</td>
<td>7.8</td>
<td>7.7</td>
</tr>
<tr>
<td>2.0</td>
<td>7.6</td>
<td>7.80</td>
<td>7.75</td>
<td>7.6</td>
</tr>
<tr>
<td>2.5</td>
<td>7.5</td>
<td>7.79</td>
<td>7.75</td>
<td>7.6</td>
</tr>
<tr>
<td>3.0</td>
<td>7.3</td>
<td>7.72</td>
<td>7.7</td>
<td>7.54</td>
</tr>
<tr>
<td>6.0</td>
<td>7.4</td>
<td>7.64</td>
<td>7.7</td>
<td>7.5</td>
</tr>
<tr>
<td>10.0</td>
<td>7.0</td>
<td>7.2</td>
<td>7.48</td>
<td>7.21</td>
</tr>
<tr>
<td>15.0</td>
<td>7.1</td>
<td>7.13</td>
<td>7.45</td>
<td>7.03</td>
</tr>
<tr>
<td>20.0</td>
<td>7.1</td>
<td>7.11</td>
<td>7.03</td>
<td>6.91</td>
</tr>
</tbody>
</table>

- **Combined physical/ biological treatment:**

  A promising alternative to a complete oxidation of biorefractory wastewater is the application of radiation as a pretreatment for biological oxidation to convert initially non
biodegradable compounds to more easily biodegradable intermediates, the results in Table (53) showed that combined treatment gave approximately the similar value of decolorization by biological treatment only.

**Table (53):** Effect of combined treatment on dyes decolorization by *Aspergillus niger* ES-5.

<table>
<thead>
<tr>
<th>Dyes</th>
<th>Control</th>
<th>0.5</th>
<th>1</th>
<th>1.5</th>
<th>2</th>
<th>2.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.Y</td>
<td>93.8%</td>
<td>93.7%</td>
<td>93.5%</td>
<td>93.8%</td>
<td>93.3%</td>
<td>93.8%</td>
</tr>
<tr>
<td>I.R</td>
<td>99.8%</td>
<td>99.5%</td>
<td>99.3%</td>
<td>99.3%</td>
<td>99.3%</td>
<td>99.5%</td>
</tr>
<tr>
<td>I.N</td>
<td>98.0%</td>
<td>97.5%</td>
<td>97.1%</td>
<td>97.0%</td>
<td>98.0%</td>
<td>98.0%</td>
</tr>
<tr>
<td>I.G</td>
<td>95.5%</td>
<td>95.4%</td>
<td>95.0%</td>
<td>95.2%</td>
<td>94.0%</td>
<td>95.1%</td>
</tr>
</tbody>
</table>

Control: Not radiated

**Application of *Aspergillus niger* ES-5 as decolorizing strain on mixture of the four Isolan dyes:**

Usually, fungi are able to decolorize one dye, but in textile factory dyes wastewater usually contain a mixture of several dyes result from printing stage. *Aspergillus niger* ES-5 was previously tested for its ability to decolorize different azo dyes, the strain proved efficient in decolorizing different textile dyes under glucose oxidase conditions of a total dye concentration of 200 mg/l. These results were encouraging for their use in decolorization of mixture of the four Isolan dyes.

From the co-metabolic section, it could be observed that, BSM I and IV gave maximum decolorization therefore, examine the efficiency of *Aspergillus niger* ES-5 to decolorize
dyes mixture in both medium. From Table (54), it could be noticed that the extent of decolorization achieved by BSM I and BSM IV scored 86.3 and 84%, respectively at the end of 72 h. As well as, it could be observed that the mixture did not have a negative effect on glucose oxidase activity produced by *Aspergillus niger* ES-5 while, it reaches 7 and 2 U/ml in BSM I and BSM IV, respectively as well as fungal growth.

**Table (54):** Effect of mixture of the four Isolan dyes on percentage of decolorization, extracellular GOD and fungal growth in two different BSM medium by *Aspergillus niger* ES5.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Percentage of decolorization</th>
<th>Extracellular GOD(U/ml)</th>
<th>Dry weight (g/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BSM I</td>
<td>BSM IV</td>
<td>BSM I</td>
</tr>
<tr>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>72</td>
<td>86.3</td>
<td>84.0</td>
<td>7.0</td>
</tr>
<tr>
<td>168</td>
<td>86.0</td>
<td>84.0</td>
<td>2.5</td>
</tr>
</tbody>
</table>

**Application of Aspergillus niger ES-5 on textile effluents:**

- **Decolorization of textile effluents under various conditions:**
  
  It is known that culture conditions affect fungal physiology and the expression and activity of the ligninolytic enzymes which is why the conditions for decolorization should
be optimized to produce best results. Therefore, the results of experiments carried out to evaluate the ability of *Aspergillus niger* ES-5 to decolorize cotton textile wastewater under four different conditions illustrated in Table (3).

The results in Table (55) and Fig. (33) showed that, under the chosen four culture conditions, the addition of the media components and pH adjustment were both limiting steps for the maximum decolorization to take place. Using one parameter alone did not contribute much to the decolorization process, probably due to bioaccumulation which was evident microscopically by observing the greenish tint inside fungal mycelia as shown in Image (7).

**Table (55):** Effect of different conditions on decolorization, extracellular GOD activity and dry weight by *Aspergillus niger* ES-5 of textile dye effluent.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Percentage of decolorization</th>
<th>Extracellular GOD activity (U/ml)</th>
<th>Dry weight (g/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>75</td>
<td>0.5</td>
<td>2.14</td>
</tr>
<tr>
<td>2</td>
<td>7.5</td>
<td>Zero</td>
<td>0.15</td>
</tr>
<tr>
<td>3</td>
<td>2.1</td>
<td>Zero</td>
<td>0.87</td>
</tr>
<tr>
<td>4</td>
<td>2.2</td>
<td>Zero</td>
<td>0.93</td>
</tr>
</tbody>
</table>
Results

Fig. (33): Decolorization of textile effluent under different conditions after 72 h incubation.

<table>
<thead>
<tr>
<th>Wastewater after 24 h</th>
<th>Wastewater after 48 h</th>
<th>Wastewater after 72 h</th>
</tr>
</thead>
</table>

Image (7): Showing wastewater uptake by *A. niger* ES-5 mycelia after 24, 48 and 72 h of incubation.

The maximum decolorization achieved in textile effluents by *Aspergillus niger* ES-5 was lower than those obtained using Isolan dyes separately. The weak decolorization of these effluents could be explained by the influence of salts, inhibitory molecules (sulphur compounds, surfactants, heavy
metals, bleaching chemicals), carbon and nutrients within these solutions.

The initial pH of textile effluent was 10, at such pH an inhibition occurred to decolorization process, thus pH should be adjusted to 5.5. Also nutrient deficiency affected decolorization process, thus, nutrients must be added to initiate decolorization efficiency of *A. niger* ES-5. Therefore, condition (1) as represented in Table (3) was selected. The production of GOD (Fig. 34) and mycelial growth (Fig. 35) were only detected under optimal conditions for GOD production.

![Graph showing GOD production](image)

**Fig. (34):** GOD production of *A. niger* ES-5 after 72 h incubation with textile effluent under different conditions.
Results

Fig. (35): Dry weight of *A. niger* ES-5 after 72 h incubation with textile effluent under different conditions.

- **Measurement of some chemical parameters and elements assay:**

  Result in Table (56) showed that although the presence of dyes is considered the main reason for increasing the COD and BOD in textile effluents, yet the removal of 75% from the textile wastewater effluent by the strain under study did not induce the expected decrease of COD and BOD levels.

  Metals were detected using EDS before and after decolorization process and some of the metals present in the treated effluent were removed. The Na and Cl probably present from the extensive use of salt during the dyeing process were increased, these salts represent one of the major problems of textile effluents due to the use of NaCl and Na$_2$SO$_4$ and other salts used in this process also, it could be observed that COD
Table (56): Some tested parameters and elements measured after decolorization by *Aspergillus niger* ES-5 in textile effluents.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Percentage of reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD</td>
<td>8.6</td>
</tr>
<tr>
<td>BOD</td>
<td>13.4</td>
</tr>
<tr>
<td>Na</td>
<td>13.8</td>
</tr>
<tr>
<td>Al</td>
<td>60.7</td>
</tr>
<tr>
<td>Cl</td>
<td>0.6</td>
</tr>
<tr>
<td>Ca</td>
<td>1.5</td>
</tr>
<tr>
<td>Co</td>
<td>100</td>
</tr>
<tr>
<td>Nitrate</td>
<td>ND</td>
</tr>
<tr>
<td>Nitrite</td>
<td>ND</td>
</tr>
<tr>
<td>Phosphate</td>
<td>ND</td>
</tr>
<tr>
<td>TSS</td>
<td>46.3</td>
</tr>
</tbody>
</table>

(ND) results not detected due to color of textile effluent which prevented use of kits.

- **Fourier Transform Infrared spectroscopy analysis (FTIR) of textile wastewater:**

  FTIR analysis carried out for textile wastewater (Fig. 36) indicated that in the area of aromatic rings (800-400 cm⁻¹), two peaks were disappeared and the other were shifted and decreased in intensity. As well in azo bond region (1500-1400 cm⁻¹), two peaks disappeared which indicate wastewater color
removal. Also aniline in region (1650-1560 cm$^{-1}$), one peak was shifted and decreased in intensity and the other one was disappeared which indicated that further degradation were occurred.

![FTIR of textile wastewater](image)

**Fig. (36):** FTIR of textile wastewater (a) before and (b) after fungal treatment.

**Physical treatment of textile effluents:**

- **Irradiation:**

  As shown in physical treatment section of the four Isolan dyes that 20 kGy did not give accepted results as expected, higher radiation dose (25 kGy) was applied.

  Table (57) shows low efficiency of gamma rays in textile wastewater decolorizing ability.
Table (57): Effect of gamma radiation on textile wastewater decolorization.

<table>
<thead>
<tr>
<th>Radiation dose (kGy)</th>
<th>Percentage of decolorization</th>
</tr>
</thead>
<tbody>
<tr>
<td>25.0</td>
<td>41.6</td>
</tr>
</tbody>
</table>

- **Pre and Post treatment of textile effluents with H₂O₂:**

  In Table (58) and Fig. (37), the use of hydrogen peroxide prior to the inoculation of *Aspergillus niger* ES-5 showed a lag time for decolorization which reached 4 days, compared to the control cultures which showed 82.7% removal after the same duration. Nevertheless, both eventually nearly exhibited the same percentage of removal after 10 days of incubation and the post treatment stopped further decolorization.

  Also, the visual observation of the cultures indicated that the presence of hydrogen peroxide in the culture is not a contributor for enhancement of decolorization because of its negative effect on *Aspergillus niger* ES-5 growth as observed from the minimal increase in fungal dry weight for each culture compared to that of the control.
Table (58): Effect of hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) inoculated at zero time of incubation (pre-treatment) and after 72 h of incubation (post- treatment) on decolorization of textile dyes effluent by \textit{Aspergillus niger} ES-5 under condition 1.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Percentage of decolorization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
</tr>
<tr>
<td>pretreated</td>
<td>0</td>
</tr>
<tr>
<td>Post treated</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig. (37): The use of pre and post oxidation treatment of the dye cultures combined with biological treatment using \textit{Aspergillus niger} ES-5 and compared to control dye cultures.

The results obtained after UV-Vis spectrophotometric analyses and which are represented in Fig. (38), showed that there was a decrease in the visible range which is characteristic
for the dye color. Inspecting the fungal growth after 168 h of incubation and measuring the decolorization (93.2%), it was obvious that the cells did not maintain their natural color, but obtained the color of the wastewater, this indicates the possibility of adsorption as a mechanism involved in the decolorization process.

**Fig. (38):** UV-Visible spectrum of dye cultures before (0 h) and after biological treatment (96 h) and in combination with hydrogen peroxide posttreatment (96 h H₂O₂).

**Application: retrieve of textile dyes and reuse in dyeing of cotton and polyester:**

The following work was conducted to retrieve the dyes and use them for dyeing of cotton and polyester swatches and to compare them with those dyed using the actual dyes before bioremediation.

The results in both Images. (8,9) represent the swatches after the dyeing process for cotton (a) and polyester (b), the color of the swatches are nearly the same for cotton and polyester
dyed with original dye powder and those dyed with extracted dyes after intracellular accumulation in *A. niger* ES-5 mycelia.

**Image (8):** Cotton swatches dyed with (a1) initial dye solution, (a2) intracellular extraction of grinded fungal mycelia and (a3) ECF after treatment.

**Image (9):** Polyester swatches dyed with (b1) initial dye solution, (b2) intracellular extraction of grinded fungal mycelia and (b3) ECF after treatment.
Discussion
The wastewater released from the textile industries containing dyes are highly colored and are therefore visually identifiable (Kilic et al., 2007). The complex aromatic structure of the dyes is resistant to light, ozone and other degradative environmental conditions. Therefore, conventional wastewater treatment remains ineffective. Also, anionic and non-ionic azo dyes result in toxic amines due to the reactive cleavage of azo groups (Joshi et al., 2004). Up till now, scientists have been trying to develop a single and economical method for the treatment of dyes in the textile wastewater, but still it remains a big challenge (Dos Santos et al., 2007). There are various methods for the treatment of textile wastewater for the removal of dyes, they broadly fall into three categories: physical, chemical and biological. These methods have been extensively reviewed (Hao et al., 2000; Robinson et al., 2001; Forgacs et al., 2004 and Joshi et al., 2004). The major disadvantage of physico-chemical methods has been largely due to the high cost, sludge generation, low efficiency, limited versatility, interference by other wastewater constituents and the handling of the waste generated (Kaushik and Malik, 2009).

Thus, the majority of research is directed towards biological treatment which provides reliable results, less sludge and more eco-friendly treatment (Ramalingam et al., 2010).

Although the majority of decolorization studies employ white rot fungi as the sole and efficient group of fungi capable of color removal yet, brown rot fungi was also reported to have the ability to decolorize diverse dyes (Fu and Viraraghavan, 2000 and Ramalingam et al., 2010). There are a wide variety
of microorganisms which are capable of efficient decolorization, these microorganisms inhabit different environments; decaying eucalyptus leaves (Dias et al., 2003), soil and water samples (Abd-El-Rahim et al., 2003), sludge from aerobic textile wastewater bioreactors (Kumar et al., 2006) or mild rice straw (Jin et al., 2007).

In this study, twenty six isolates were screened for maximum decolorization of Isolan Red and Isolan Navy as a preliminary step, four of which represented the highest isolates with decolorizing ability. The used Isolan dyes in this study belong to the metal reactive azo dye group and are classified as anionic acid dyes. They were selected for this study because of their wide and common use in the cotton textile industry in Egypt and throughout the world. The biodegradability of azo dyes vary depending on two factors; the first one is the presence of very specific changes in their molecular structures, these changes in dye structures could significantly affect the decolorization rate (Park et al., 2007), the second is the different mechanisms of degradation and decolorization of the dyes which followed by different fungal groups (Wilkolazka et al., 2002).

In the present study, *Aspergillus niger* ES-5 was the only fungus capable of decolorizing the four Isolan dyes at a rate above 50%, thus was employed for further investigations. Several researches suggested that *Aspergillus* could be used to remove toxic and radioactive metals from the environment (Baker, 2006).

Abd-El-Rahim et al. (2003) and Ibrahim et al. (2008) were in agreement with the obtained results where *Aspergillus niger* was chosen to decolorize different dyes.
The growth of the fungus, enzyme production and subsequent dye decolorization was affected by culture conditions such as static and shaking condition, solid agar technique, media components, incubation period, pH, temperature, presence of heavy metals etc.. (Kaushik and Malik, 2009).

In the present study, a higher color removal was observed in shaking cultures because of better oxygen transfer, where dissolved oxygen is considered to be an important factor which affects the decolorization process, and nutrient distribution as compared to the stationary cultures. Also, the visual observation of the statically incubated cultures revealed formation of filamentous "mats" at the surface of the growth medium by day four for all statically grown cultures accompanied with visible sorption of the dyes onto the fungal mat. The formation of mat at the surface in static cultures restricts O\textsubscript{2} transfer to the cells beneath the surface and in the medium (Swamy and Ramsay, 1999; Wilkolazka et al., 2002; Yesilada et al., 2002; Machado et al., 2006; Rigas and Dritsa, 2006 and Parshetti et al., 2007).

The screening for decolorization isolates could be performed through direct culture or solid agar, which could be used as an initial evaluation to determine the decolorization ability of the tested strains (Swamy and Ramsay, 1999 and Mohorčič et al., 2006). In the present study, Aspergillus niger ES-5 was tested for its decolorization ability in agar plates as compared to the standard isolate Phanerochaete chrysosporium ATCC 24725. Both strains could not produce oxidizing and radical generating enzymes which play a role in dye
decolorization under solid agar plate condition. Also, the lack of halo zone formation on solid agar plates could be attributed to the dye chemical structure as Machado et al. (2006) stated. On the other hand, there was a heavy fungal growth in all dye plates which indicated the irrelevance of growth to decolorization; Wilkolazka et al. (2002) reported that some dyes inhibit fungal growth causing a reduction in the growth rate, dye decolorization progresses more slowly than radial growth and the decolorization halo for some dyes did not occupy the entire diameter of the plate even when the incubation time was prolonged to 21 days.

Many Aspergillus niger enzymes are used in food, brewing and textile industries, also a variety of these enzymes are important in the breakdown of plant lignocellulose and other biotechnological applications (Baker, 2006). A number of oxidases (glucose oxidase, glyoxal oxidase, veratryl oxidase, etc) are responsible for hydrogen peroxide production. It has been shown that hydrogen peroxide plays a key role in radical generation processes which attack aromatic compounds (Eichlerova et al., 2006).

The brown rot fungus Aspergillus niger is known to produce glucose oxidase enzyme (GOD), a dimeric protein which catalyzes the oxidation of β-D-glucose into D-glucono-1, 5-lactone producing gluconic acid and hydrogen peroxide (Fiedurek and Gromada, 2000 and Betancol et al., 2006). Glucose oxidase production by Aspergillus niger has been reported to be increasing with aeration and shaking (Petruccioli et al., 1995 and Kona et al., 2001). The decolorization process may be due to the presence of such enzyme, therefore, the decolorization process was studied under glucose oxidase inducing conditions.
There are a limited number of recent studies which have been directed to the effects of media components on the GOD production (Mirón et al., 2008). Glucose oxidase is induced by several conditions such as carbon, nitrogen, phosphorus source along with calcium carbonate (Kona et al., 2001). The most readily usable carbon source for most of the fungi is glucose (Kaushik and Malik, 2009).

The addition of glucose in the medium was critical to initiate both decolorization process and fungal growth and it could be considered as a principal inducer of glucose oxidase production. The presence of glucose in the media was found to be the main media component which has a great effect in Isolan dye decolorization percentage by Aspergillus niger ES-5. Caridis et al. (1991), Sumathi and Manju (2000), Assadi and Jahangiri (2001), Rojek et al. (2004) and Ramzan and Mehmood (2009) are in agreement with the obtained results where, decolorization process was attained through the addition of glucose and Aspergillus niger ES-5 failed to perform high decolorization percentage in the absence of glucose.

The addition of glucose initially induces a high level of decolorization (Miranda et al., 1996), however increasing glucose concentrations, do not necessarily enhance the decolorization process. About 30g/l glucose concentration resulted in higher decolorization percentage and GOD activity in Aspergillus niger ES-5, this indicates that decolorization is affected by the carbon source present to a certain extent, after which, a plateau is reached where excess glucose makes no positive contribution to the decolorization process. On the other hand, Assadi and Jahangiri, (2001) stated that very low glucose concentration adversely affects the decolorization process.
There is a relation between carbon source consumption and decolorization. Metabolism suppression may occur in the presence of dyes; however, in the presence of glucose, metabolism was not been affected. The disappearance of glucose in the first phase of culture, could be attributed to the need of *Aspergillus niger* ES-5 to use glucose for growth through the action of GOD which results in gluconic acid production, once the glucose has been exhausted in the primary stage, the microorganism could use gluconic acid that have accumulated during the first stage as a source of carbon in the second phase (Mirón *et al*., 2002). At 24 h, *Aspergillus niger* ES-5 exhibited a rapid consumption of glucose in the absence of Isolan dyes while in the presence of dyes, more time is needed. This could be attributed to the lag in growth due to the chemical nature of the dyes which may retard fungal growth resulting in the consumption of carbon source (Jin *et al*., 2007). Abd-El-Rahim *et al*. (2008) reported that *Aspergillus niger* nearly consumed all the sugar after 3 days of incubation and the unconsumed sugar was exhausted after 89 h of incubation.

Although glucose is considered an essential component in the decolorization process, GOD production and fungal growth, it must be supported by other media components to work in synergism in order to achieve the maximum activity for the fungi. It could also be concluded that the decolorization profile is highly dependent on the glucose present in the media. Glucose is considered a potent inducer for GOD induction (Witteveen *et al*., 1992), this suggests the involvement of metabolic enzymes in the decolorization process and confirms the involvement of GOD, if not as a directly involved enzyme in the decolorization process, at least as a co-metabolic enzyme.
Other media components seem to contribute in the decolorization process as well.

The addition of calcium carbonate to the growth medium substantially induced GOD activity. Calcium carbonate was added to prevent pH drop and had been also shown to be extremely important due to gluconic acid formation during fermentation. Moreover, it has been suggested that it might give some kind of mechanical support to fungal mycelium for proper growth (Sabir et al., 2007).

Although fungal decolorization is assumed to be linked to GOD inducers, yet the presence of calcium carbonate did not contribute in the decolorization process of Isolan dyes by Aspergillus niger ES-5. There are a number of explanations for the increase of decolorization in media lacking calcium carbonate. Isolan dyes are anionic in nature, and fungal biomass carries negative charges on their surface (Volesky, 1990). In the absence of calcium carbonate, the pH of media becomes acidic (4.5) thus, increasing the number of protons which could neutralize net negative charges of the culture and change part of the negatively charged surface to positively charged. This increases the electrostatic attraction between fungal biomass and dye molecules therefore, increasing the decolorization. But when calcium carbonate is added, the pH increases thus, increase OH⁻ and as a result the repulsion increase between fungal biomass and dye molecules. This results in decreasing the decolorization. Moreover, there are visual observations that some of the dyes were adsorbed to calcium carbonate particles, indicating partial involvement for some of the decolorization via adsorption.
An increase in pH could cause an increase in net electronegativity of the biosorbent due to deprotonation of different functional groups. This increases the electrostatic force between the negatively charged biosorbent and the positively charged dye ions (Maurya et al., 2006).

The role of calcium carbonate in BSM for Aspergillus niger ES-5 is one of controversy, it is assumed that it is used for both GOD induction, fungal growth and maintenance of pH throughout the duration of the experiment (Fiedurek and Gromada, 2000). The absence of calcium carbonate from the medium caused a shift in the metabolic pathway of Aspergillus niger ES-5. It was reported by Liu et al. (2001) that calcium carbonate is accompanied by a metabolic shift from the glycolytic pathway to direct oxidation of glucose by GOD. While the addition of calcium carbonate induces the production of GOD, the percentage of decolorization in its absence is also high therefore, this confirms that GOD is important in the decolorization process but not the main reason for decolorization.

Organic and inorganic nitrogen sources which are added to growth media, affect dye decolorization by altering the enzyme production by fungi. The obtained results could indicate that the presence of nitrogen sources has a positive effect on the efficiency of Aspergillus niger ES-5 to decolorize the four Isolan dyes, therefore, it could be considered as an essential factor in the decolorization process (Rojek et al., 2004 and Kaushik and Malik, 2009).

Peptone and sodium nitrate were the common organic and inorganic nitrogen sources used for glucose oxidase production by different microorganisms (Hatzinikolaou and Macris, 1995).
When different nitrogen sources were tested for their effect on *Aspergillus niger* growth and total glucose oxidase production, the results indicated that peptone was the suitable nitrogen source. Also, low concentration of peptone had a positive effect on the decolorization process and glucose oxidase production (Hatzinikolaou and Macris, 1995).

Previous studies indicated that sodium nitrate is the optimal inorganic nitrogen source, not only for enzyme biosynthesis, but also for facilitating the diffusion rate of the enzyme into the medium (Gorniak and Kaczkowski, 1973). This is probably the reason for the increase in extracellular GOD by *A. niger* ES-5 in BSM I which contains more nitrogen source than BSM VI. Sodium nitrate is an important factor for GOD production but, its addition in high concentrations led to the decrease in decolorization percentage. Oranusi and Ogugbue (2005) explained why the decolorization decreased with increasing concentration of sodium nitrate. As the concentration of azo bonds increase, the cleavage of the azo bond becomes slower. The aromatic amines resulting from the cleavage of the azo bond could be further metabolized to provide the nitrogen requirements for the organism. This will result in detoxification of these toxic, mutagenic and carcinogenic amines. The organism preferentially metabolizes the nitrate present, until the medium becomes nitrogen-limited.

Microorganisms need inorganic nutrients for their growth and metabolism as much as they need organic supplements. Phosphate is a key nutrient of regulatory importance in microbial metabolite production (Hardy *et al.*, 1998). A combination of nitrogen-source with phosphorous source was
greater than the effect of combination of carbon source with phosphorous source in the decolorization process of Isolan dyes by *Aspergillus niger* ES-5 on the other hand, this particular combination did not enhance neither GOD nor the fungal dry weight.

A correlation between potassium dihydrogen phosphate and total GOD was detected by Gromada and Fiedurek (1996) whereas Mirón et al. (2008) optimized the production of GOD by *Aspergillus niger* through combing nitrate and phosphorous.

The incubation period is an important factor for the decolorization process, there is a wide variation in the optimal incubation period at which effective decolorization took place, and this depends on the tested strains (Yang et al., 2009).

The major decolorization occurs during the exponential growth phase of the fungus (Sumathi and Phatak, 1999). As the culture time increase, the decolorization percentage increases to reach a steady state. Extracellular, intracellular GOD and dry weight are linked to culture incubation period.

The optimal incubation period coincided with the maximum decolorization time for the four Isolan dyes and also with the production of glucose oxidase by *Aspergillus niger* ES-5 indicating a strong connection between decolorization and GOD production which also has been recorded for other *Aspergillus niger* strains (Tahoun, 1993; Fiedurek, 1998 and Ibrahim et al., 2008).
The change in initial pH affects the ionization of essential active sites of amino acid residues, which are involved in substrate binding and catalysis. The ionization of these residues may cause distortion of active site cleft and hence indirectly affecting the enzyme activity (Zia et al., 2007).

The pH of the medium should be adjusted for effective decolorization of textile wastewater therefore, influencing their interaction. The initial pH of the dye solution strongly influences the chemistry of both dye molecules and fungal biomass in an aqueous solution. It is seen that for the majority of the fungi the optimum pH for dye decolorization lies in the acidic range (Shahvali et al., 2000; Fu and Viraraghavan, 2002 and Kaushik and Malik, 2009).

*Aspergillus niger* ES-5 could decolorize Isolan dyes at a wide pH range depending on the type of the dye used. Moreover, as pH increases, the percentage of decolorization decreases and this could be attributed to the concentration of OH− which increases at elevated pH values, consequently increasing the repulsion between colored anions of dye molecules and negative charges of the fungal biomass, resulting in a decrease in the decolorization capacity, GOD activity and fungal growth (Miranda et al., 1996; Hatzinikolaou et al., 1996; Liu et al., 1999 and 2001; Assadi and Jahangiri, 2001; O'Mahony et al., 2002; Maurya et al., 2006; Vijaykumar et al., 2006; Iqbal and Saeed, 2007; Parshetti et al., 2007; Ibrahim et al., 2008 and Husseiny, 2008).

One of the most important parameters for dye decolorization is the incubation temperature; the optimum incubation temperature for fungi differs from one strain to
another. The mesophilic range is traditionally used in fungi, since it is generally thought that maintaining high temperature would be uneconomical, while degradation within the psychrophilic range is too slow (Fu and Viraraghavan, 2001 and Keharia et al., 2004).

The optimum temperature for *Aspergillus niger* ES-5 growth coincided with the maximum decolorization percentage, GOD activity profile and dry weight in all four Isolan dyes. Higher temperatures caused a decrease in all those parameters, probably due to the production of large amount of metabolic heat thereby inhibiting microbial growth and enzyme formation (Assadi and Jahangiri, 2001; Toh et al., 2003; Vijaykumar et al., 2006; Iqbal and Saeed, 2007; Sabir et al., 2007 and Ibrahim et al., 2008). The optimum temperature for the four Isolan dyes was 30°C.

The presence of dyes contributes to high levels of suspended solids (SS), high chemical oxygen demand (COD) and biological oxygen demand (BOD) (Dos Santos et al., 2007). The removal of color from water bodies is considered relatively more important than the removal of soluble colorless organic substances, which contribute to the major fraction of biochemical oxygen demand (Rajamohan and Karthikeyan, 2006).

The levels of total suspended solids, COD and BOD values showed variation before and after treatments of the four Isolan dyes and this variation may be due to the changes in the composition of the dyes at the end of the color removing process (Al-Kdasi et al., 2004). Although *Aspergillus niger* ES-5 showed high efficiency in Isolan dyes decolorization, a low
efficiency of COD and BOD reduction was shown. This indicates that the removal of dyes is not necessarily correlated with the decrease in BOD and COD. On the other hand, other biological systems which are efficient in treating high BOD and COD effluents fail to remove color (Dubrow et al., 1996).

Toxicity study revealed that dye biodegradation by the isolated culture resulted in detoxification of the dye as shown from the absence of significant total cell death (Kumar et al., 2006). High concentration of dyes shows a toxic effect that adversely affects fungal performance (Yesilada et al., 2003). A. niger ES-5 showed a lack of toxicity of the decolorized solution suggesting the lack of aromatic amines. This could be verified by the high fungal and bacterial growth of both the decolorizing fungus and the bacteria tested for toxicity (Cha et al., 2001).

For a long time fungi have been the center of attention in different biodegradation and decolorization processes focusing specifically on white rot fungi regarding the involvement of enzymatic degradation pathways, inducers and inhibitors controlling the decolorization process, while research involving brown rot fungi is still limited to primitive affirmation of the presence or absence of decolorization (Kaushik and Malik, 2009). Studies on Aspergillus niger in specific has been more focused on industrial enzymes rather than its use in dye decolorization. In this study, Aspergillus niger ES-5 proved to be suitable for the decolorization of metal azo dyes, presenting the highest percentages of decolorization with the aid of glucose as an inducer for GOD production.
There are a number of modes of fungal decolorization which could be grouped into biodegradation, biosorption and bioaccumulation (Kaushik and Malik, 2009). Common modes of decolorization depend on biodegradation through enzymatic actions; the ligninolytic enzymes are common in decolorization by white rot fungi (Banat et al., 1996), internal biotransformation was reported in Trametes versicolor (Blanquez et al., 2004), external adsorption on the mycelial walls are common for Aspergillus and Trichoderma sp. (Abed-El-Rahim et al., 2003) and finally, adsorption which occur by dead fungi (fungal biomass) through ion exchange (Miao, 2004).

In vitro decolorization failed to take place for the four Isolan dyes by the extracellular fluid of Aspergillus niger ES-5, this could be attributed to the complex structure of dyes which might interfere with the correct docking of a dye molecule in the enzyme active site (Nagai et al., 2002). On the other hand, the presence of electron-withdrawing substituents present in the dye could enhance the redox potential of the dye molecule, thus making them less susceptible to enzymatic oxidation (Xu, 1996 and Dias et al., 2003). Thus, it could be concluded that the dye structure obviously controls the enzyme decolorizing activity in vitro (Dias et al., 2003). It could be observed that high percentage of dye decolorization obtained in vivo (whole cell) could not be obtained in the in vitro (extracellular form) suggesting that other factors could be required for the decolorization process (Romero et al., 2006 and Vanhulle et al., 2007).
The mechanism of decolorization by autoclaved cells is limited to physico-chemical interactions (adsorption, external deposition and ion-exchange), their biosorptive capacities may be greater than, equal to, or less than living cells. A few studies were dedicated to the removal of dyes using dead fungal biomass (Fu and Viraraghavan, 2002 and Maurya et al., 2006).

Although autoclaving could cause a disruption of fungal cell wall resulting in an increase in porosity and exposure of latent sites, consequently increasing the dye adsorption (Gallagher et al., 1997), yet autoclaved biomass of Aspergillus niger ES-5 showed negligible decolorization in all four Isolan dyes suggesting that autoclaved fungi fail to adsorb the dye. These observations confirm that the decolorization process by Aspergillus niger ES-5 biomass took place through microbial metabolism and is linked to microbial growth.

There is a limit to the extent of color removal by biosorption (Knapp et al., 1995), in this case, biodegradation and/or bioaccumulation is considered to be the main modes for decolorization (Kapdan et al., 2000). Aspergillus niger ES-5 showed a limited capacity for biosorption and also failed to utilize the four Isolan dyes as the sole carbon source, although some fungi are capable of utilizing dyes as their sole carbon source, they do not seem to be able to carry out decolorization (Marchant et al., 1994; Nigam et al., 1995 a,b and Jin et al., 2007). The addition of nutrients to the culture media was required to perform decolorization, therefore, it is suggested that Aspergillus niger ES-5 requires energy and viability for color removal of the four Isolan dyes. The addition of media
supplements is known to aid in enhancing the decolorization process and is therefore considered metabolic related (Jin et al., 2007).

Obtaining a full UV-Visible spectrum of the decolorized samples could reveal the mode of decolorization. A decrease in the visible region (400-800 nm) indicates a decrease in dye color, while the formation of peak at 270 nm indicates that color removal occur through break down of azo bond which is responsible for dye color. The presence of a peak at 270 nm and the absence of the peak at visible region are probably due to color removal and aromatic amines formation from cleavage of azo bond (Pinheiro et al., 2004 and Zille et al., 2005).

The FT-IR analysis is usually carried out to detect the presence of functional groups. The Isolan dye decolorization by Aspergillus niger ES-5 seems to vary among the four Isolan dyes depending on their chemical structure. The decrease in the intensity of aromatic ring, azo bond and aniline could indicate that azo bonds are attacked to produce aniline and aniline derivatives, suggesting an additional degradation step.

HPLC analysis was used to detect the degradation by-products. Both aniline and sulphanilic acid were expected to be the by products present after the cleavage of the azo bond of the four Isolan dyes (according to the basic structure of the dyes). An increase in the degradation process might take place by time and is indicated by the increase in aniline and sulphanilic acid to there derivatives (Kumar et al., 2006).

Thus, degradation mechanism can be summed up as follows:
Along the study performed on the four Isolan dyes, it is suspected that either biotransformation or bioaccumulation is involved in the decolorization process by *Aspergillus niger* ES-5. The relationship between the decolorization and GOD production has been established and the synergistic profile of growth, temperature and decolorization has been confirmed. It is known that the bulk of GOD is present in the cell wall periplasmic fraction (*Witteveen et al.*, 1992). While the breaking of the metal complex bond in the inner side of fungi, the release of the components to the external media is an indication for the biotransformation of the metal dyes (*Blanquez et al.*, 2004). Therefore, it is suggested that the Isolan dyes disappeared inside the mycelia. Detecting the metal content of the four Isolan dyes by Energy Dispersive X-ray Spectroscopy (EDS) revealed the absence of metals on the surface of *Aspergillus niger* ES-5 after the decolorization process, this indicates that the dyes were entrapped inside the fungal biomass, such suggestion was confirmed by the
Investigation of fungal biomass intracellularly by light microscope. The visual observation of the dye transfer through the mycelial wall by time indicates the movement of the dye molecules and their intracellular compartmentalization (Ambrósio and Takaki, 2004 and Kumar et al., 2006). The photos revealed that the dyes were indeed entrapped inside the fungal biomass without any change in their color intensity. This is a proof of bioaccumulation of dyes and not biotransformation. Intracellular storage is a phenomenon which occurs normally in growing cells (Glombitza and Iske, 1987). Moreover, the decolorization process and the removal of elements by Aspergillus niger ES-5 involve initial adsorption followed by entrapment of the adsorbed dye inside the fungal biomass.

Radiation technology has been recognized as a promising process for wastewater treatment (Wang et al., 2006), the use of γ-rays or accelerated electrons is a simple and efficient technique to destroy chemicals present in wastewater (Földváry and Wojnárovits, 2007).

There are different modes in the decolorization of dyes by gamma rays alone as a physical treatment. The most common modes are irradiation of dilute aqueous solution. Radiation results in the formation of radical species, in which both hydroxyl radicals (OH) and hydrated electrons (e\textsubscript{aq}) form together with smaller amount of H\textsuperscript{+} atom (Spinks and Woods, 1990). Hydroxyl radical (OH), is a powerful oxidant which is added to unsaturated bond, in contrast, (e\textsuperscript{aq}) is a strong reducing agent whose reactivity depends on the availability of a suitable vacant orbital. Reactivity of organic molecules toward
Discussion

(e$^{-}_{aq}$) is enhanced by electron-withdrawing substituents which are adjacent to double bonds or attached to aromatic rings (Buxton et al., 1988). The hydrated electrons and the H$^-$ atom are suggested to react with the azo group and destroy the conjugation through the –N=N– double bond causing decolorization with nearly 100% efficiency (Wojnárovits et al., 2005a, b and 2007).

There is a hypothesis which suggests that the main reaction of ‘OH addition to any of the double bonds present in the aromatic ring. Because ‘OH is an electrophilic reagent, it attacks mainly the electron rich–part of the molecules, thus, (‘OH) is not effective in dye color removal. Moreover, there is a small (~10%) contribution from the H$^-$ atom reaction, although, e$^{-}_{aq}$ is very effective in decolorization but is less active in further degradation of the products formed which could be similar to the parent compounds (Sharma et al., 2003 and Wojnárovits et al., 2005 a, b). gamma rays increase color intensity in case of I.Y and this could result from azobenzene compounds, where, hydrazyl radicals are produced from the rapid protonation of the corresponding anions. ‘CH$_2$OH radical did not react with azobenzene while (CH$_3$)$_2$‘CHOH could reduce azobenzene at the azo bond after which, these reduced azobenzene substrates react with the parent compound to give a dimer molecule. Therefore, leading to dye polymerization, this in return causes an increase in the color intensity (Wojnárovits and Takács, 2008). While the other three Isolan dyes used were affected by low doses of irradiation, sometimes high radiation doses might be required to degrade dye molecules, this could be attributed to the broad variation in pH (Ma et al., 2007). The reaction takes
place with $e^-_{aq}$, H' and ‘OH while in such pH $e^-_{aq}$ is converted to ‘OH in the reaction: $e^-_{aq} + H_2O \rightarrow OH$.

Dye molecules are not degraded efficiently by the primary products formed from the radiolysis of water. The reactivity of reducing species, $e^-_{aq}$, H' and ‘OH could be strongly affected by the molecular structure of the used dyes (Wojnárovits and Takács, 2008).

Also, the pH of the solution was gradually decreased with the increase of radiation dose suggesting the formation of acidic substances, and this change in pH values depended on the structure of dye molecules. This in agreement with Solpan and Güven, (2002); Zhang et al., (2005); Kim et al., (2007) and Wojnárovits and Takács, (2008).

Combination of radiation and conventional methods, such as biological treatment is considered to be the most promising technique in the future (Jo et al., 2006). Gamma radiation treatment could break down the molecular structure of the substance and converts the non-biodegradable substance to biodegradable compounds (Ma et al., 2007), but in the present study, the combined treatment of both physical and biological treatment for the four Isolan dyes gave approximately the similar value of decolorization as given by single biological treatment.

The knowledge of general removal technique of single species of dyes by microorganisms is important, but relatively little is known about the combined effect of more than one dye on the fungal performance and the simultaneous removal of dyes from a mixture of dye solution (Aksu, 2005). Aspergillus niger ES-5 was studied in both decolorization of single dyes
and mixture of the four tested dyes. The present study revealed high decolorization for the mixture of the four Isolan dyes and this result represents a non-specific biological process which may be vital for the treatment of textile mill discharge effluent containing the mixture of dyes rather than a single dye (Sumathi and Manju, 2000 and Machado et al., 2006).

While, textile wastewater doesn't only contain dyes, but includes a large variety chemical additives which render the process an environmental challenge not only in terms of the huge quantities of colored liquid waste produced but also because of their chemical composition (Al-Kdasi et al., 2004 and Selcuk and Meric, 2006). Therefore, the ability of microorganisms to remove color of single dyes efficiently does not necessarily indicate the suitability of this organism in treatment of colored textile effluents (Swamy and Ramsay, 1999), the wide range of pH, the high salt concentrations, the high temperature of effluent and the presence of chemical structures of additives, surfactants, fixatives and heavy metals complicate the bioremediation process (Ledakowicz and Gonera, 1999; Mester and Tien, 2000 and Dos Santos et al., 2007).

Aspergillus niger ES-5 showed high decolorization capacity towards individual textile dyes and textile wastewater effluent, although not in the same efficiency. This decrease in decolorization could be a result of the presence of other recalcitrant wastewater compounds which could be responsible for the lower decolorization achieved in textile effluents when compared to single synthetic dyes (Assadi and Jahangiri, 2001 and Coulibaly et al., 2003).
Discussion

Since, pH of the media affect the decolorization performance by \textit{A. niger} ES-5 for single dye, thus, there is a need to adjust initial pH for textile effluent. This is common in other fungi (Prasad and Joyce, 1991; Mittar et al., 1992 and Mehna et al., 1995). Since textile effluent is nutrient deficient (Oke et al., 2006), the addition of nutrients to textile wastewater could activate microbial cells, hence increasing the decolorization process.

The photos of the fungal biomass intracellularly before and after decolorization of textile effluent revealed the transfer of the effluent color through the mycelial wall by time, this indicates that adsorption of textile effluent on fungal mycelia was followed by bioaccumulation.

The major pollutants present in textile wastewater are the high suspended solids, chemical oxygen demand, biological oxygen demand, heat, color and other soluble substances (Dae-Hee et al., 1999). COD, BOD and metal removal from textile effluent depended on the structure of the wastewater and the fungus involved (Coulibaly et al., 2003 and Al-Kdasi et al., 2004). The decolorization efficiency of \textit{A. niger} ES-5 is not necessarily correlated with the decrease in COD and BOD as indicated by other work (Dubrow et al., 1996).

Advanced oxidation processes (AOPs) such as photocatalytic oxidation, Fenton, UV, gamma radiation and \textit{H}_2\textit{O}_2 have been applied individually or combined with other treatments in order to generate hydroxyl free radicals, (OH') as strong oxidant could attack textile wastewater and dyes molecules non-selectively with a high reaction rate (Mohey El-Dein et al., 2006).
The effect of radiation on textile wastewater could be attributed to the attack of the ring structure of the dyes which are characterized by strong aromaticity and high chemical stability. Due to this high chemical stability under natural conditions their decomposition are rendered very slow; therefore, require a strong attack (Wojnárovits and Takács, 2008).

*Aspergillus niger* produces simultaneously GOD and catalase, both produced and act upon hydrogen peroxide present in the media (Fiedurek and Gromada, 2000). Hydrogen peroxide is the end product for metabolizing glucose by GOD, along with gluconic acid (Watanabe *et al.*, 1982), from this standpoint, the presence of excess hydrogen peroxide, through pre or post addition in the media, might cause a feedback inhibition for GOD synthesis, thus resulting in decreased decolorization.

Also, by analogy, the reactivity of the azo linkage is very low compared to other groups. The aromatic rings are more reactive towards ozone than azo linkage (Matsui, 1996). This suggestion may be another explanation for the delayed and limited decolorization occurring during the pre-oxidation step, after which, hydrogen peroxide disintegrates in the medium, the decolorization by *Aspergillus niger* ES-5 takes place at a later stage. Therefore, the use of hydrogen peroxide as a pre or post treatment is not effective.

According to Asad *et al.* (2007), decolorization is proven to take place via biodegradation if the bacterial mats maintain their color and changes in the UV-Vis range do not decrease in proportion (Chen *et al.*, 2003 and Ambrósio and Takaki,
Therefore, it is believed that decolorization of cotton textile effluent by *Aspergillus niger* ES-5 takes place via biodegradation and adsorption, this means that *Aspergillus niger* ES-5 follows the same trend of decolorization of individual dyes as well as decolorizing cotton textile effluent.

The outcome of the dye bioremediation process is commonly limited to the removal of dyes from the effluent. The information published merely focuses on the water quality, the decrease in color or the microbial ability to perform the decolorization process. Aside from the fact that dyes could be recovered by desorption only if the mode of removal is adsorption, the bioaccumulation or bioremediation in general are never regarded as modes for dyes retrieval since desorption is based on physico-chemical bonding ([Kaushik and Malik, 2009](#)), while the previously mentioned methods are biological.

In the present study, *Aspergillus niger* ES-5 was efficiently capable of biologically accumulating different Isolan dyes in their periplasmic fraction along with the intracellular GOD, this result led us to consider the retrieval of the entrapped captive dyes, this is considered an economical application which bridges the gap between solving the problem of dye release in effluents and cost effective re-use of dyes. The retrieved dyes were capable of binding to both cotton and polyester cloth, the colors of the cloth swatches were lighter than the initial dye. This indicates that the entrapped dyes could be re-used to dye pastel shades of the same colors, hence decreasing the costs and minimizing dyes usage.
Summary
Summary

Dyes are used in textile industry, leather tanning industry, paper production, food technology, agricultural research, light-harvesting arrays, photoelectrochemical cells, hair coloring and cosmetics. Due to the large amounts used, the most significant industrial use is in textile dyeing. Azo dyes are the most widely used among synthetic dyes, representing almost 70% of the textile dyestuff produced. They are easy to synthesize, have low cost, stable, can be used to color several materials (textile, leather, plastic, food) and allow a great variety of colors and shades. They have in their molecule one or more azo groups.

The most problematic feature in the dyeing industries is the dye release to the environment in the form of wastewater. The uncontrolled release of these compounds in the environment causes severe problems. Since they are designed to be chemically and photolytically stable, they are highly persistent in natural environments. Dyes, through decreasing light absorption, may significantly affect photosynthetic activity of aquatic life and may be toxic due to the presence of aromatics or heavy metals.

In this study; a survey was carried out on twenty six fungal strains were isolated from decaying eucalyptus leaves and soil around eucalyptus tree and identified into two genera: *Aspergillus* and *Penicillium*.

The isolates were preliminary screened for their maximum decolorization of I.R and I.N. The best four
decolorizing ability isolates were further tested on the four Isolan dyes in order to obtain the local isolate with maximum decolorization ability and were compared with \textit{Phanerochaete chrysosporium} ATCC 24725 as the standard strain. \textit{Aspergillus niger} ES-5 was the only fungus capable of decolorization of the four Isolan dyes above 50\% thus was chosen for further investigation.

Glucose oxidase is considered one of the major enzymes produced by \textit{Aspergillus niger} thus GOD was suggested to be involved mainly or partially in the dye decolorization process and this suggestion was further confirmed experiments where results showed that \textit{Aspergillus niger} ES-5 was unable to decolorize the four Isolan dyes in both agar plates and in static condition.

The following study had two objectives: the first is to detect the most suitable conditions for \textit{Aspergillus niger} ES-5 to reach maximum decolorizing ability of Isolan dyes in order to detect the mode of decolorization. The second is to decolorize textile effluents and attempt of the dyes again.

Several experiments were carried out in order to study the effect of media contents on decolorization, glucose can be considered as the main media components, and is considered a potent inducer for GOD induction, the concentration required was modified to 30 g/l instead of 80 g/l, calcium carbonate did not influence the decolorization process, thus it was deleted from the media contents. Nitrogen source (peptone and sodium nitrate) and potassium dihydrogen phosphate must be added for their importance in GOD production.
Aspergillus niger ES-5 could decolorize the four Isolan dyes at pH range 3.5-7.5 depending on the type of the used dye, also 72 h incubation period is the optimum time needed for maximum decolorization, the results were 93.5, 99.1, 95.8 and 92.4% for I.Y, I.R, I.N and I.G, respectively and 30°C is the optimum temperature.

Biological oxygen demand, chemical oxygen demand and total suspended solids were detected before and after decolorization and concluded that there are no correlation between decolorization and decrease in BOD and COD.

The reductive azo linkage by bacteria resulted in the formation of aromatic amines which can be highly toxic more than initial dye thus toxicity test were carried out to determine the toxicity of treated wastewater resulted from biological treatment by E. coli, the results proved the safety of treated wastewater.

The dose from 0 to 2.5 kGy had no effect on all Isolan dyes but above 2.5 to 20 kGy had an effect on I.R, I.N and I.G except I.Y did not appear any response for all gamma radiation doses, on the other hand the combined physical and biological treatment gave approximately the similar value of decolorization as given by biological treatment only.

Studies on Aspergillus niger in specific has been more focused on industrial enzymes rather than its use in dye decolorization, thus in order to know the mechanism by which Aspergillus niger ES-5 is capable of decolorizing textile dyes several experiment were carried out.
Generally, common modes of decolorization depend on biodegradation through enzymatic actions and/or sorption through dead biomass (adsorption) or living biomass (biosorption) or/and bioaccumulation or/and biotransformation. Each decolorization mode was tested to finally know what *Aspergillus niger* ES-5 mode of decolorization.

Enzymatic action was tested by using extracellular fluids (ECF) in vitro and the results showed negligible decolorization therefore extracellular glucose oxidase is not the main reason for the decolorization of the Isolan dyes. Adsorption was tested and also showed negligible decolorization, therefore, this confirm that the decolorization process by fungal biomass takes place through microbial metabolism and is linked to microbial growth, as previously reported that living biomass resulted in decolorization of approximately 90.4, 99.6, 95 and 94.6% in I.Y, I.R, I.N and I.G, respectively, indicating that decolorization probably was performed through growth related enzymes.

The element content of the four Isolan dyes on the mycelial wall and ECF was tested by Energy Dispersive X-ray Spectroscopy (EDS) was tested and the results detect that the element were below limit in dried mycelia, this indicated that dyes entrapped inside fungal biomass and this suggestion was confirmed by photos revealed that the dyes were bioaccumulated not biotransformed.

FTIR, UV and HPLC analysis were carried out and concluded that *Aspergillus niger* ES-5 was able to breakdown azo bond and convert it to aniline and sulphanilic acid and that another confirmation that *Aspergillus niger* ES-5 was able to
decolorize the Isolan dyes but, failed to reach complete degradation.

From all the previous experiments, the following conclusion could be drawn, the decolorization of the Isolan dyes by *Aspergillus niger* ES-5 appears to be a complex process comprising different removal mechanisms which include bioaccumulation as the major decolorization method and biodegradation along with physico-chemical biosorption by whole fungal biomass as the subsidiary mode of removal.

*Aspergillus niger* ES-5 was previously tested for its ability to decolorize different azo dyes, the strain proved efficient in decolorization ability these results are encouraging for their use in decolorization of both mixture of the four Isolan dyes and textile wastewater, media contents was adjusted to reach maximum decolorizing ability where in case of mixture of the four Isolan dyes BSM IV gave 84%, where BSM IV and pH5.5 was suitable to decolorize textile wastewater and gave 75%.

COD, BOD and elements assay in textile wastewater were detected after fungal treatment.

The ability of both gamma radiation and hydrogen peroxide were tested to show their ability to decolorize textile wastewater.

FTIR analysis were carried out for textile wastewater after fungal treatment and showed that breakdown of azo bond which responsible for wastewater color.
Nevertheless, the fact that *Aspergillus niger* ES-5 was capable of biologically accumulating different Isolan dyes in their periplasmic fraction, this conclusion led to the trials for retrieval of the periplasmic captive dyes and uses them for dyeing of cotton and polyester swatches. The results was the highlight of application which bridges the gap between solving the problem of dye release in the effluents and cost effective use of dyes in cases of needing pastel shades in the dyeing process.
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Arabic Summary
الملخص العربي

تستخدم الأصباغ في العديد من الصناعات مثل صناعة المنسوجات ودبياغ الجلود وإنتاج الورق وتقنيات الأغذية والبحوث الزراعية والخلايا الضوكروميائية وتطوين الشعر ومستحضرات التجميل في حين تعتبر صناعة المنسوجات من أكثر الصناعات استخداماً للصبغات حيث تعتبر صبغة الأرو هي الأكثر شيوعاً من بين الأصباغ الصناعية والتي تمثل تقريباً 70% من الصبغات المستخدمة في صناعة المنسوجات، حيث إنها تمتاز بأنها سيئة الصنع وقليلة الكثافة وثابتة وتمتلك مجموعة كبيرة من الألوان والفلال واللوك يمكن استخدامها لتوليد مواد مختلفة مثل المنسوجات والجلود والبلاستيك والمواد الغذائية.

المشكلة الأكثر شيوعاً في صناعة المنسوجات هي الخروج العشوائي لهذه المركبات للبيئة في شكل مياه صرف صحي مما يسبب مشاكل خطيرة حيث أنها مصممة لتكون مستقرة كيميائياً ولا تحلل بالضوء فهي عالية الثبات في البيئة الطبيعية بالإضافة إلى أن هذه الأصباغ تقلل من امتصاص الضوء فذلك تؤثر تأثيراً كبيراً على النشاط الضوئي للحياة المائية ويمكن أن تكون سامة بسبب وجود المواد العطرية والمعادن الثقيلة.

في هذه الدراسة تم الحصول على ستة وعشرون سلالة فطرية تم عزلهم من أوراق شجرة الكافور والترية حول شجرة الكافور ووجد أن كل الفطريات المعزولة تنتمي لجنس هما الاسمبلس وبيبسيلوم.

تم الاختيار الأولي لجميع العزلات لتحديد قدرتها على إزالة اللون من صبغتي الأيسولان الصفراء والزرقاء، ثم تم اختيار أفضل أربع سلالات وتحديد قدرتها على إزالة اللون من الأصباغ الأربعة من أجل الحصول على عزلة محلية Phanerochaete chrysosporium لها قدرة عالية على إزالة اللون ومقارنتها بسلالة قياسية ودلت النتائج إلى أن الاسمبلس نيجير-5 هي الفطر ES-5.
ال الوحيد قادر على إزالة اللون من الأربع صبغات بنسبة 50% لذلك تم اختيارهما لإجراء بعض التجارب البيوكيميائية والفيزيائية.

يُعتبر الجلوكوز أوكسيديز واحد من الإنزيمات الرئيسية التي يفرزها فطر الأسبروجلес نيجر لذلك اقترح إمكاني أن تشارك بشكل رئيسي أو جزئي في عملية إزالة اللون، و هذا الاقتراب قد تم دراسته بالعديد من التجارب، وأشارت النتائج إلى أن الأسبروجلес نيجر ES-5 غير قادر على إزالة اللون من الصبغات الأربعة عند استخدام طريقة الحفظ في إبطاء الإجار.

لهذه الدراسة هدفين: الأول وهو الكشف عن الظروف الأكثر ملاءمة للاسبروجلес نيجر LE5 ليصل إلى أقصى قدرة له إزالة اللون من أجل الوصول إلى ميكانية إزالة اللون، والثاني هو إزالة اللون من مياه صرف النسيج الناتجة من المصانع ومحاولة استخدام الأصبغ مرة أخرى في عملية الصباغة.

أجريت عدة تجارب من أجل دراسة تأثير مكونات البيئة على إزالة اللون، واعتبر الجلوكوز من المكونات الرئيسية للبيئة وكذلك يعتبر محفز قوي لإفراز الجلوكوز أوكسيديز ولكن تم إضافته بتراكيز 30 جرام في اللفت بدلاً من 80 جرام في اللورد، وكذلك تم حذف كربونات الكالسيوم من مكونات البيئة حيث إنها لم تؤثر على عملية إزالة اللون في حين أن النتائج أثبتت أنه يجب إضافته كل من مصدر البيترين (البيتون، نترات الصوديوم) والأمونيوم داي هيدروجين فوسفات وذلك لأهميتها في إنتاج الجلوكوز أوكسيديز.

و أثبتت التجربة أن الأسبروجلес نيجر ES-5 له القدرة على إزالة اللون LE5 عندما يكون الأساس لизмيدروفين يتراوح من 0.5 إلى 7.5، اعتناعاً على نوع الصبغة المستخدمة، وأيضاً تم تحديد 72 ساعة كفترة الحساسة اللازمة لإزالة اللون وكذلك 30 درجة مئوية هي درجة الحرارة المثلى لإتمام هذه العملية.
تم الكشف عن BOD و COD، و مجموع المواد الصلبة في البيئة المحتوية على كل صبغة على حدة قبل وبعد إزالة اللون، و وجد أنه لم يكن هناك علاقة بين قدرة النتر على إزالة اللون و قدرته على خفض COD و BOD.

أثبتت العديد من الأبحاث أن اختزال رابطة الأوز بواسطة البكتيريا قد تؤدي إلى تكوين الأمينات العطرية التي يمكن أن تكون أكثر سمية من الصبغة الأولية، و بالتالي تم اختبار سمية المياه المعالجة بنطوط الأسبريلسن نيجر 5-ES بواسطة بكتريا E.coli، وأظهرت النتائج سلامة المياه المعالجة بيولوجيًا.

تم اختبار مدى تأثير أشعة جاما على إزالة اللون، و قد ثبت أن الجرعة من صفر إلى 2.0 كيلوجرامات ليس لها تأثير على الأربعي صبغات، ولكن الجرعة من 2.0 إلى 20 كيلوجرامات لها تأثير على كل الصبغات، مما عدا صبغة الأبسولات الصفراء التي لم تظهر أي استجابة لكل الجرعات المستخدمة من أشعة جاما و من ناحية أخرى وجد أن الدمج بين المعالجة الفيزيائية و البيولوجية لها نفس قيم إزالة اللون التي تمت بالمعالجة البيولوجية فقط.

تركزت العديد من الدراسات والأبحاث التي تم إجرائها على الأسبريلسن نيجر على الإنزيمات الصناعية التي تفرزها هذه السلاسلية أكثر من استخدامها لإزالة اللون، و الميكانيكية المستخدمة و ذلك و من أجل معرفة هذه الآلية تم إجراء العديد من التجارب، و بشكل عام، الآلية الشائعة لإزالة اللون تعتمد على التكسير البيولوجي من خلال النشاط الإنزيمي و/أو الاستجابة من خلال الكتلة الغيرية لميتيه أو الحياة و/أو الالترام البيولوجي و/أو التشوه البيولوجي و تم اختبار كل آليه لإزالة اللون لمعرفة أي آلية يستخدمها الأسبريلسن نيجر 5-ES لإزالة لون الصبغات.

و تم اختبار النشاط الزنبوري بواسطة استخدام السوائل الخارجية من الخلية الغذرية لإزالة لون الصبغات الأربعة، و كانت النتائج سلبية لذلك تم استنتاج أن إنزيم الجلوكوز أوكسيديز ليس هو السبب الرئيسي لإزالة اللون، و كذلك تم اختبار
الاستئصال بواسطة الكئة الفطرية المثبتة لإزالة لون الصباغات الأحمره، و كانت النتائج أيضا لا تذكر مما يؤكد أن عملية إزالة اللون تتم من خلال الكئة الفطرية الحية من خلال عملية التمثيل الغذائي الميكروبي المرتبط بنمو الميكروب.

ولقد تم اختيار محتوى العناصر للأصباغ الأحمره الموجودة في كل من الجدار الخفي والسائل الخارج من الخليفة الفطرية بواسطة الأشعة المستوية للطاقة الطبيعية المشتتة، و أوضح أنه لم يمكن قياس وجود العناصر، مما يشير إلى أن الصباغات محتوية داخل الكئة الفطرية. وهذا الاستنتاج تم تأكيده بواسطة صور من الميكروسكوب الضوئي التي أوضح أن الصباغات قد تم احتجازها داخل الكئة الفطرية مما يؤكد حدوث تراكم بيولوجي و ليس تحول بيولوجي.

و كذلك تم استخدام الأشعة تحت الحمراء، والأشعة فوق البنفسجية و جهاز الفصل الكروموجرافى المائل و ذلك لتأكيد أن الاستيرجس نيجر - ES-5 كان القدرة على كسر رابطة الأزو المسؤولة عن اللون و تحويلها إلى أتيلين و حمض السلفانليك.

يمكن استنتاج من التجارب السابقة أن عملية إزالة لون الصباغات الأحمره بواسطة الاستيرجس نيجر ES-5 يبدو أنها عملية مفيدة تضم أنواع مختلفة تشمل التراكم البيولوجي كأداة رئيسي لإزالة اللون. و كذلك التكسير البيولوجي مع الاستئصال الكيميائي و الفيزيائي بواسطة الكئة الفطرية الحية كأداة مساعدة لإزالة اللون.

إثبت الاستيرجس نيجر ES-5 كفاءة في إزالة اللون من الصباغات الأحمره كل على حدة، و هذه النتائج كانت مشجعة لاستخدامها لإزالة اللون لكل من خليط من الصباغات الأحمره. و كذلك مياه صرف النسيج حيث أن مكونات البيئة تم ضبطها للوصول إلى الحد الأقصى لإزالة اللون.

تم قياس BOD و COD و محتوى العناصر لمياه صرف النسيج بعد المعالجة البيولوجية باستخدام الاستيرجس نيجر ES-5، و كذلك تم اختيار مدى...
تأثير أشعة جاما و فوق أكسيد الهيدروجين على إزالة اللون و كذلك تم فحص مياه صرف النسيج بواسطة الأشعة تحت الحمراء بعد المعالجة الفطرية و أظهرت النتائج كسر رابطة الأزو المسئولة عن اللون.

دلت النتائج إلى أن الاستيرنج نيجر ES-5 قد حجز الصبغة و هذه النتيجة أدت إلى محاولة استرجاع هذه الصبغة المهجورة و إعادة استخدامها لصبغة القطن و البوليستر، و بذلك يكون قد تم حل مشكلتي مياه صرف النسيج وأيضا تقليل تكلفة استخدام صبغات في الحالات التي تحتاج إلى ظلال في عملية الصبغة.
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و أوجه الشكر لكل من ساهم في إخراج هذه الرسالة
تحلول بعض الأصعاب المستخدمة في محاصيل النسيج

باستخدام المعالجة البيولوجية والفيزيائية

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