

Bacillus Subtilis Bacteria as a Biosorbent for Dye Removal from Industrial Water Effluents

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ABSTRACT

World population growth and increasing needs to various industries have led to the accumulation of a wide variety of contaminants in the environment and natural resources. The use of synthetic dyes is increasing in many areas. More than 10,000 chemically different dyes are being manufactured. Synthetic dyes have been widely used in many industries such as textile, tannery, food, pharmaceutical, pulp and paper, paint, plastics, electroplating, and cosmetics industries. The effluent discharge from these industries has destructive effects on the environment and human health; reducing sunlight penetration and gas solubility in aqueous ecosystems and mutagenic and carcinogenic effects in living organisms. Interest in the pollution potential of textile dyes has been primarily prompted by concern over their possible toxicity and carcinogenicity. Even though physico-chemical methods are effective in dye removal, problems such as the overall cost, regeneration, secondary pollutants, limited versatility, interactions with other wastewater constituents, and residual sludge generation limit their usage. As an alternative, biological treatments are a relatively inexpensive way to remove dyes from wastewater. The use of biological methods such as bioaccumulation and biosorption through bioflocculation technique is suitable for the removal of such dyes from wastewaters. In this study, bacterial strain of *Bacillus subtilis* has been used for the removal of methyl violet dye from an Egyptian textile effluent. More than 98 % removal is achieved through application of bioflocculation technique. Complete characterization for the textile effluent before and after treatment with bacterial strain has been done including TSS, TDS and turbidity. At the same time, physicochemical characterization of *Bacillus subtilis* bacterial strain was studied.

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1. Introduction

Increasing population numbers, a changing climate, intensive agricultural practices, economic growth and urbanization will undoubtedly continue to make the issue of water scarcity a global priority for years to come. With average economic growth, the 2030 Water Resource Group reports that the worldwide water supply-to-demand gap is likely to reach approximately 40% by 2030 unless significant efficiency gains can be made. The Intergovernmental Panel on Climate Change (IPCC) predicts that by the year 2050, around 60% of the world's population could experience severe water shortages, with 33% thought to be already under stress. In Europe, competing demands for limited and sometimes over-exploited water resources concern more than a few Member States: water scarcity and droughts already affect one third of the EU territory across different latitudes. Tackling the water gap challenge while achieving good status of all water bodies will require a transformation of the sector based on the combination of new technological, organizational and management approaches to supply, conservation, reuse and recycling in agricultural, industrial, urban and domestic contexts [1].

The progressive accumulation of more and more organic compounds in natural waters is mostly a result of the

development of chemical technologies towards organic synthesis and processing. The population explosion and expansion of urban areas have had an increased adverse impact on water resources, particularly in regions in which natural resources are still limited. Currently, water use or reuse is a major concern which needs a solution. Population growth leads to a significant increase in default volumes of wastewater, which makes it an urgent imperative to develop effective and low-cost technologies for wastewater treatment [2].

In the textile industry, organic dyes are commonly used in a wide range of industrial applications. Therefore, it is very important to reduce the dye concentration of wastewater before discharging it into the environment. Discharging large amounts of dyes into water resources, organics, bleaches, and salts, can affect the physical and chemical properties of fresh water. Dyes in wastewater that can obstruct light penetration and are highly visible, are stable to light irradiation and heat and also toxic to microorganisms. The removal of dyes is a very complex process due to their structure and synthetic origins [4]. Dyes that interfere directly or indirectly in the growth of aquatic organisms are considered hazardous in terms of the environment. Nowadays a growing awareness has emerged on the impact of these contaminants on

ground water, rivers, and lakes [3–5]. The application of microorganisms for dye wastewater removal offers considerable advantages which are the relatively low cost of the process, its environmental friendliness, the production of less secondary sludge and completely mineralized end products which are not toxic [6].

2. Materials and Methods

2.1. Physicochemical Characterization of Textile Effluent

Effluent samples were collected in pre-sterilized polypropylene bottles from textile industries periodically and conventional parameters such as pH, TSS, TDS, Temp., COD and BOD were characterized as per the procedure recommended by standard method for the examination of water and wastewater [7].

2.2. Isolation and Growing of Bacteria

Bacterial strain was isolated from surface of Egyptian phosphate ore through vigorous agitation of phosphate sample with 0.4% sodium chloride, NaCl, solution for 30 min on a rotary shaker at 30°C, and allowed to settle. The supernatant obtained was serially diluted with sterile water and spread on the surface of nutrient agar plates which were incubated at 30 °C. About eight bacterial isolates were isolated, purified by streaking on nutrient agar plates, then transferred to nutrient agar slopes stored at 4°C and subcultured monthly. The efficiency of these isolates was screened using a laser particle size analyzer [8–10]. Based on the later test, the most promising bacterial isolate has been selected to conduct this study.

2.3. Morphological and Gram Staining Identification

Microscopic examination and gram staining of the selected bacterial isolate were carried out.

2.4-Bio-Chemical Identification

The selected bacterial isolate was identified using the BIOLOG GEN III Micro-plate microbial identification system. A pure culture was grown on biologi recommended agar media and incubated at 30° C. Inoculum were prepared where the cell density was in the range of 90-98%T. precisely 100 µl of the cell suspension was transferred by multichannel pipette into the wells of biologi micro-plate. The plates were incubated for 36 hours at 30° C into the Omni-Log incubator/reader. The biologi micro-plate tests the ability of an organism to utilize or oxidize a pre-selected panel of 95 different carbon sources. The dye tetrazolium violet is used to indicate utilization of substrates. A panel of 95 different substrates gives a very distinctive and repeatable pattern of purple wells for each organism in which the manufacturers literature terms a "Metabolic Fingerprint". Finally; micro plate was read using Biologi's Microbial Identification Systems software through biology reader [10, 11].

2.5. Microorganism Growth and Preparation for Biosorption

The nutrient broth was prepared using the prescribed growth medium containing beef extract 1.0g, yeast extract 0.1g, peptone 5.0g, sodium chloride 5.0g and distilled water 1.0 litre. The bacterial culture was sterilized in an autoclave maintained at 15 lbs for 15 minutes and maintained as per the guidelines of MTCC.

2.6. Screening Efficient Dye Decolorizing Bacterial Isolates

The ability of decolorization of each isolate was tested in the liquid medium. Media inoculated with the respective inocula were incubated at 35°C for 24 h. After 24 h, the respective cells were harvested by medium centrifugation at 10000 rpm for 10 minutes. Then decolorization was

determined with the help of spectrophotometer at 597nm. Un-inoculated blanks were run to determine abiotic decolorization.

3. Results and Discussion

3.1. Physicochemical Characterization of Textile Effluent

Table 1 represents the results of characterization of the textile effluents containing methyl violet dye. The dye-containing effluents as seen are characterized by high alkalinity, biological oxidation demand, chemical oxidation demand, and total dissolved solids with dye concentrations generally below 1 g L⁻¹. This inadequate disposal of untreated dye-containing effluents in water bodies causes serious direct and indirect impacts on the environment and human health. Direct impacts are as, color change, poor sunlight penetration, water pollution, and suppression in the reoxygenation capacity. The indirect impacts are as killing of aquatic life, microtoxicity and damage to the immune system of human beings. However, dye-containing effluents are very difficult to treat, since this type of dyes are recalcitrant molecules with a complex aromatic structure, resistant to aerobic digestion, and stable to oxidation agents [12–14].

Table 1. Physicochemical Characterization of Textile Effluent.

#	Parameter	Effluent
1	Color	Dark black
2	Temperature	38
3	pH	8
4	Total Dissolved Solids (TDS) mg/l	750
5	Total Suspended Solids (TSS) mg/l	500
6	Chemical Oxygen Demand (COD) mg/l	710
7	Biological Oxygen Demand (BOD) mg/l	220

3.2- Identification of Bacterial Isolates

Biologi identification indicated that bacterial isolate is *Bacillus subtilis*. Microscopic examination revealed that cells are Gram-positive, catalase-positive bacterium. *Bacillus Subtilis* is rod-shaped, and can form a tough, protective endospore, Fig. 1. Colonies of *Bacillus Subtilis* are yellowish, flat, opaque, and dry, with lobate or crenate edges, Fig.2.

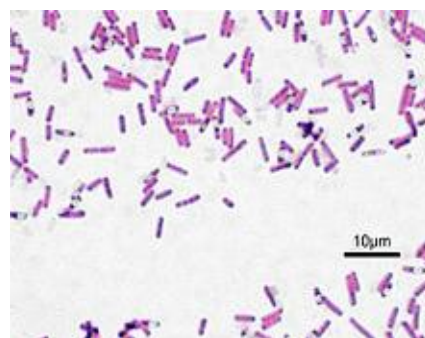


Fig 1. Gram stain of B. Subtilis.

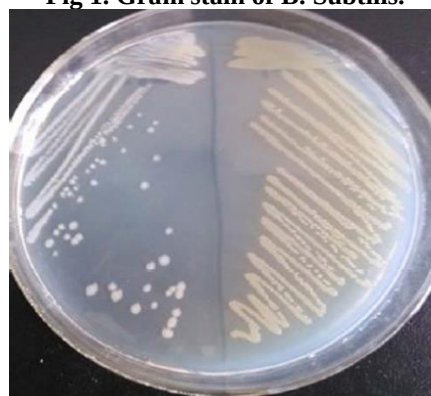


Fig 2. Colonies of B. Subtilis.

3.3- Aerobic Degradation of Crystal Violet Dye

The main idea of all biological methods of wastewater treatment is to provide contact with bacteria (cells), which feed on the organic materials in the wastewater, and thereby reduce its biological oxygen demand (BOD). In other words, the purpose of biological treatment is BOD reduction. The natural process of microbiological metabolism in aquatic environment is capitalized on in the biological treatment of wastewater. Under proper environmental conditions, the soluble organic substances of the wastewater are completely destroyed by biological oxidation. A part of it is oxidized while the rest is converted into biological mass in the biological reactors. The decolorization ability of *B. Subtilis* in an aqueous effluent containing the representative textile finishing dye (methyl violet) was investigated. It has been observed that the decolorization efficiency for dye reached almost 98 % in less time than 24 h, which points out the suitability of the selected microorganism. Optimum conditions were determined to be pH 7.0 and 30°C. Solution pH generally influences both the biomass surface dye binding sites and the dye chemistry in the medium [15-16].

A survey of literature suggested that increasing the dye concentration decreasing the decolorization efficiency of bacteria. This may be due to the harmful or toxic effect of dye onto bacterial cells and inadequate biomass concentration. The initial concentration of dyes is an important parameter and a main limiting factor, since a given mass of adsorbent can only adsorb a fixed mass of dye. The increase in the initial dye concentration at a constant flow rate increases the slope of breakthrough curve and decrease the throughput (output) until breakthrough. This may be caused by high initial concentration saturating the adsorbent more quickly, thereby decreasing the breakthrough time. In general, an increasing initial dye concentration increased the critical bed depth of adsorption column and increases the adsorption capacity [17]. The effect of water/dye ratio on the decolorization efficiency indicated that maximum removal was obtained at lower concentration of dye accompanying with maximum removal of COD. *Bacillus Subtilis* succeeded in 98% color removal and 95 % COD removal, Table 2.

Table 2. Effect of Dye Concentration on the Removal Efficiency.

Water/Dye Ratio	% removal	% COD removal
2:1	98	95
1:1	80	55
1:2	65	44

4. Conclusions

The use of biological methods is suitable for the removal of pollutants from wastewaters. These methods are of low operating cost. *Bacillus Subtilis* bacterial isolate was tested in the removal of methyl violet dye from textile effluent. The results indicated that maximum removal was obtained at lower concentration of dye 98 % accompanying with maximum removal of COD 95 %.

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