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Bio-Degradation of Crystal Violet Dye Using Bacillus Pumilus and Micrococcus Lylae

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ABSTRACT

World population growth and increasing needs to various industries have led to the accumulation of contaminants in the environment and natural resources. Synthetic dyes have been widely used in many industries. The contamination of receiving water bodies by types of dyes constitutes a major environmental concern as they are extremely toxic, recalcitrant, and exhibit a tendency to bioaccumulation. As an alternative for different applied technologies, such as precipitation, ion exchange, adsorption, electrochemical processes, and membrane processes, biological treatments are a relatively inexpensive way to remove dyes from wastewater. These methods have the advantage over such as low operating cost, minimization of the concentration of pollutant and high efficiency in detoxifying very dilute effluents. In this paper, two types of bacteria were tested in the removal of crystal violet dye from textile effluent. Complete physico-chemical characterizations of the effluent have been measured. Bio-Log identification indicated that the two bacterial isolates are *Bacillus Pumilus* and *Micrococcus Lylae*. Removal efficiency was 89.47 % and 88.4% respectively. Complete characterizations of such type of bacteria have been tested.

1. Introduction

In recent years, detoxification of heavy metal ions, dyes and removal of different types of impurities from water resources and industrial waste water has gained much importance worldwide. This is due to the increased concern about communities and the environment in addition to the tighter National/International regulations on water pollution. The treatment of industrial wastewater using processes such as precipitation, ion exchange, electrochemical reduction, evaporation, reverse osmosis, and biological method need high capital cost and recurring expenses such as chemicals, which are not suitable for small scale industries. Therefore, there is an increasing need to develop adsorbent materials that are not only economically effective at removing different types of pollutants, but that can also be easily synthesized from natural and waste materials.

Various pollution remediation techniques concentrate on removal of only one type of pollutant, which may either be positively charged or negatively charged. Currently, combinations of cation and/or anion exchangers with inorganic cohesion precipitants are generally used for simultaneous removal of both species of pollutants. However, these materials are expensive; and they release huge amounts of counter anions such as Cl^- and $SO4^{2-}$ ions into the treated system. This may require further treatment. There is an increasing need, therefore, to develop adsorbent materials that are not only economically effective at removing both types of pollutants, but that can also be easily synthesized from natural and waste materials [1-3].

Effluents contain large amounts of dye chemicals in the textile industry that cause water pollution.

Therefore, it is very important to reduce the dye concentration of wastewater before discharging it into the environment. Dyes in wastewater that obstruct light penetration are stable and toxic. [4–9]. Although various wastewater treatment methods including physical, chemical, and physicochemical have been studied, in recent years many studies have focused on biological methods with some microorganisms such as fungi, bacteria and algae [10-11]. The aim of this work is to adapt the biotechnology process for degradation of crystal violet dye from Egyptian textile effluents.

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 [12].

2.2. Isolation and Growing of Bacteria

Bacterial strains were isolated from the surface of Egyptian kaolin ore through vigorous agitation of kaolin 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 30ºC. Eighteen bacterial isolates were isolated, purified by streaking on nutrient agar plates, then transferred to nutrient agar slopes stored at 4C° and subcultured monthly. The efficiency of these isolates was screened using a laser particle size analyzer [13-15].

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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 biolog 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 biolog micro-plate. The plates were incubated for 36 hours at 30°C into the Omni-Log incubator/reader. The biolog 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 Biolog's Microbial Identification Systems software through biology reader [15,16].

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^{o}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 Disscusion

3.1. Physicochemical Characterization of Textile Effluent

Table 1 represents the results of characterization of the textile effluents containing crystal violet dye. The dye-containing effluents are of high alkalinity, biological oxidation demand, chemical oxidation demand, and total dissolved solids with dye concentrations generally below 1 g L^{-1}. This causes serious direct and indirect impacts on the environment and human health. Direct impacts are as, color change, poor sunlight penetration and suppression in the reoxygenation capacity. The indirect impacts are as killing of aquatic life and damage to the immune system of human beings. [17-21].

Table 1. Physicochemical Characterization of Textile Effluent.

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

3.2. Identification of Bacterial Isolates

Biolog identification indicated that bacterial isolates are *Bacillus Pumilus* and *Micrococcus Lylae*. Microscopic examination of the two bacterial isolates revealed that cells are spore-forming, gram positive rods for *Bacillus Pumilus* while non-spore-forming cocci for *Micrococcus Lylae*, Figs. 1 and 2. It occurs singly and forms pairs, short chains, and small groups. Colonies of *Bacillus Pumilus* are yellowish, flat, opaque, and dry, with lobate or crenate edges, Fig.3, while those of *Micrococcus Lylae* are glistening, raised, with entire margins, Fig. 4.

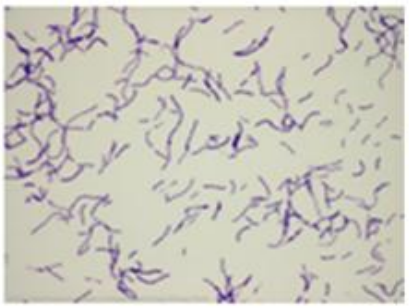

Fig.1. Gram Stain of *B. Pumilus* (1000X).

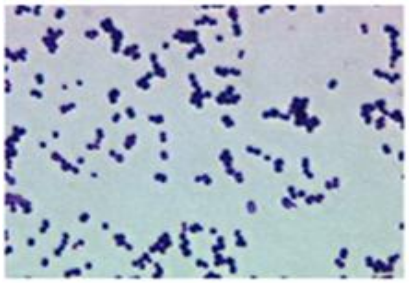

Fig.2. Gram Stain of *M. Lylae* (1000X).

Fig.3. Growth of *B. Pumilus*.

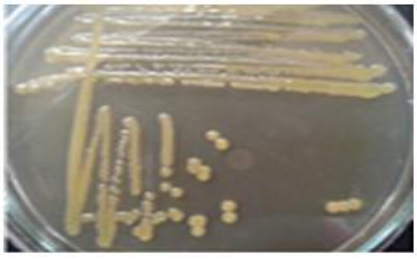

Fig.4. Growth of *M. Lylae*.

SEM was used to reveal the morphology of both bacterial isolates SEM microimages confirmed that *B. Pumilus* cells are in rod form while *M. Lylae* cells are in cocci form, Figs. 5 and 6.

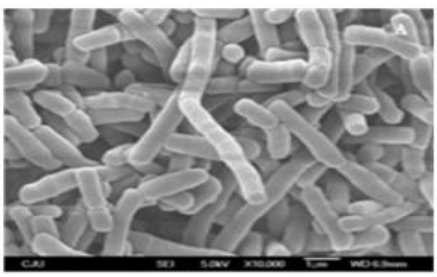

Fig.5. A typical SEM image of *B. Pumilus*.

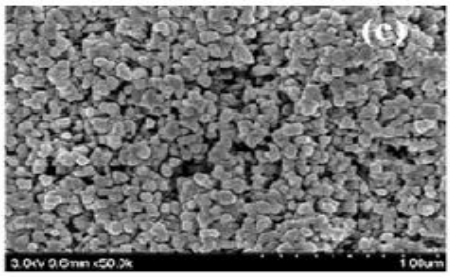

Fig.6. A typical SEM image of *M. Lylae*

Table 2 represents a comparison between acid productions from organic compounds by isolated *B. Pumilus* (column A) and those of reported by *M. Lylae*, (column B).

Table 2. Organic Compounds Produced by both *Bacillus Pumilus* and *Micrococcus Lylae*.

Organic Compound	*B. Pumilus*	*M. lylae*
Beta-galactosidase	+	-
Citrate utilization	+	-
Hydrolysis of esculin	+	+
Hydrolysis of gelatin	+	-
Hydrolysis of casein	+	-
N-acetil-D-ghscosamine	+	-
l-arabinose	+	-
Amygdaline	+	-
Arbutin	+	-
D-cellobiose	+	-
D-fructose	+	-
Galactose	+	-
Glucose	+	-
Glycerol	+	-
Beta-gentibiose	+	-
D-mannose	+	-
D-raffinose	+	-
Ribose	+	-
Sucrose	+	-
Salicin	+	-
Trehalose	+	-
D-xylose	+	-
Catalase	-	+
Oxidase	-	+
Citrate	-	+

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). The natural process of microbiological metabolism in aquatic environment is capitalized on in the biological treatment of wastewater. The soluble organic substances of the wastewater are completely destroyed by biological oxidation [22]. The decolorization ability of the two bacterial isolates *B. Pumilus and M. Lylae* in an aqueous effluent containing the representative textile finishing dye (crystal violet) was investigated. It has been observed that the decolorization efficiency for dye reached almost 86.88 % and 89.47 % respectively in less time than 18 h, which points out the suitability of the selected microorganism, Table 3. Optimum conditions were determined to be pH 7.0 and 35^{o}C. On the other hand, the ionic forms of the dye in solution and the surface electrical charge of the biomass depend on solution pH. Therefore, solution pH generally influences both the biomass surface dye binding sites and the dye chemistry in the medium [23-26].

Table 3. Effect of Bacteria Type on Crystal Violet Dye Decolorization.

Bacteria used	Absorbance Before	Absorbance After	% removal
M. Lylae	3	0.316	89.47
B. Pumilus	3	0.3935	86.88

The initial concentration of dyes is an important parameter and a main limiting factor. The increase in the initial dye concentration at a constant flow rate increases the slope of breakthrough curve and decrease the throughput (output). The increasing of the dye concentration will cause a decrease in the decolorization efficiency of bacteria. This may be due to the harmful or toxic effect of dye onto bacterial cells and inadequate biomass concentration [27]. 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. *M. Lylae* succeeded in 89.47% color removal and 85 % COD removal while *B. Pumilus* succeeded in 86.47% color removal and 84.2 % COD removal, Table 4.

Table 4. Effect of Dye Concentration on the Removal Efficiency.

Bacteria used	Water/Dye Ratio	% Color Removal	% COD Removal
M. Lylae	2:1	89.47	85
	1:1	70.50	60
	0.5:1	60.15	43.5
B. Pumilus	2:1	86.88	84.2
	1:1	66.26	55
	0.5:1	48.50	23.46

4. Conclusions

The use of biological method is suitable for the removal of pollutants from wastewaters. These methods are of low operating cost. Two types of bacteria, *Bacillus Pumilus* and *Micrococcus Lylae*, were tested in the removal of crystal violet dye from textile effluent. The results indicated that maximum removal was obtained at lower concentration of dye accompanying with maximum removal of COD. *M. Lylae* succeeded in 89.47% color removal and 85 % COD removal while *B. Pumilus* succeeded in 86.47% color removal and 84.2 % COD removal,

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