Bioremoval of Crystal Violet Dye from Egyptian Textile Effluent

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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 contamination of receiving water bodies by heavy metals constitutes a major environmental concern as these contaminants are extremely toxic, recalcitrant, and exhibit a tendency to bioaccumulate . Although heavy metals can be removed from industrial wastewater by a range of physicochemical treatment technologies such as precipitation, ion exchange, adsorption, electrochemical processes, and membrane processes; however, regulatory standards are not always sufficient.*

As an alternative, biological treatments are a relatively inexpensive way to remove dyes from wastewater. Bacteria has been tested for its ability to accumulate heavy metals and dyes. The use of biological methods such as bioaccumulation and biosorption is suitable for the removal of pollutants from wastewaters. 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 physic-chemical characterization of the effluent as pH, Temp., color, TDS, TSS, COD and BOD 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 characterization of such type of bacteria, isolated from Egyptian kaolin ore surface, including gram stain, growth curve, Biolog microbial identification, Scanning Electron Microscope, SEM as well as organic compounds production have been studied.

Keywords: *Biotechnology, bioprocessing, bacterial isolates, FTIR, TDS*

1- INTRODUCTION

As an essential resource for life, sustainable growth and healthy ecosystems, water has been high on the world research agenda since the early years of the Community's research, technological development (RTD) Framework Programmes (FPs). 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].

A reevaluation of the issue of environmental pollution made at the end of the last century has shown that wastes such as medicines, disinfectants, contrast media, laundry detergents, surfactants, pesticides, dyes, paints, preservatives, food additives, and personal care products which have been released by chemical and pharmaceutical industries, are a severe threat to the environment and human health on a global scale [2]. 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 [3].

Especially in the textile industry, effluents contain large amounts of dye chemicals which may cause severe water pollution. Also, 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 [5–8]. The utilization of wastewater for irrigation is an effective way to dispose of wastewater [9]. Although various wastewater treatment methods including physical, chemical, and physicochemical have been studied, in recent years a wide range of studies have focused on biological methods with some microorganisms such as fungi, bacteria and algae [10]. 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 [11].

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 strain was isolated from surface of 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 30 ºC. 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]. 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. Inoculuma 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 oC 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 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}. These 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 [17-21].

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

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.

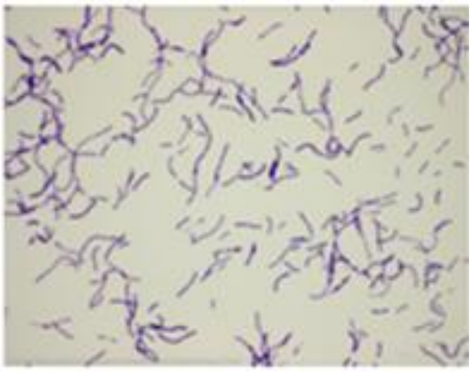

Fig.1. Gram stain of *B. Pumilus* (1000X)

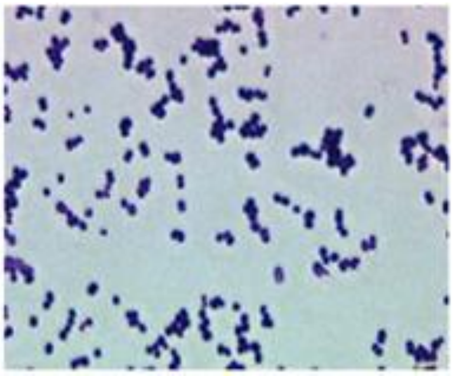

Fig.2. Gram stain of *M. lylae* (1000X)

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.

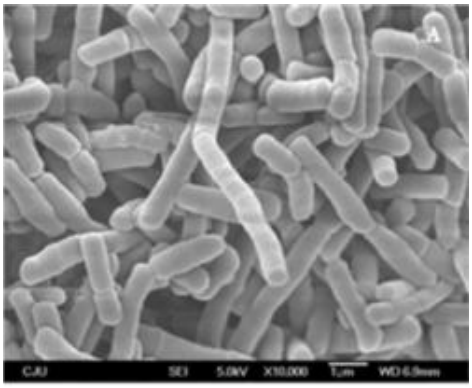

Fig.5. A typical SEM image of B. Pumilus

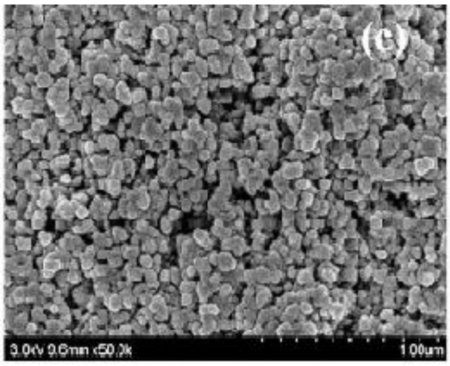

Fig.6. A typical SEM image of *M. lylae*

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-glucosamine	+	-
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). 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 biological treatment system usually consists of biological reactors and a settling tank to remove the produced biomass or sludge [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 35oC. 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

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 [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	% removal	% COD removal
M. lylae	1:2	89.47	85
	1:1	70.50	60
	0.5:1	60.15	43.5
B. Pumilus	1:2	86.88	84.2
	1:1	66.26	55
	0.5:1	48.50	23.46

4- CONCLUSIONS

The use of biological methods 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,

REFERENCES

[1] "Membrane Technologies for Water Applications", Project Information, European Commission, Environmental Technology Pollution Prevention, 2010.

[2] M. Grassi, G. Kaykioglu, V. Belgiorno, and G. Lofrano, Removal of emerging contaminants from water and wastewater by adsorption process. In: Emerging Compounds Removal from Wastewater: Natural and Solar Based Treatments, G. Lofrano, ed., Springer Briefs in Green Chemistry for Sustainability, 2012.

[3] A. E. Segneanu, C. Orbeci, C. Lazau, P. Sfirloaga, P. Vlazan, C. Bandas, and I. Grozescu, 2013. Waste water treatment methods. In: Water Treatment. W. Elshorbagy and R. K. Chowdhury, eds., InTech Publishing, 53–80.

[4] S. N. B. S. Jaafar, 2006. Dye removal using clay. Adsorption study, Faculty of Chemical Engineering and Natural Resources. University College of Engineering & Technology Malaysia degree of Bachelor of Chemical Engineering.

[5] C. Fernandez, M. S. Larrechi, M. P. Callao, 2010. An analytical overview of processes for removing organic dyes from wastewater effluents. Trends inAnalytical Chemistry 29(10), 1202–1211.

[6] U. G. Akpan, B. H. Hameed, 2009. Parameters affecting the photocatalytic degradation of dyes using TiO2-based photocatalysts: A review. Journal of Hazardous Materials 170, 520–529.

[7] F. P. van der Zee, S. Villaverde, 2005. Combined anaerobic–aerobic treatment of azo dyes – A short review of bioreactor studies. Water Research 39, 1425–1440.

[8] K. Kummerer, 2009. The presence of pharmaceuticals in the environment due to human use: Present

knowledge and future challenges. J Environ Management 90, 2354–2366.

[10] K. Gomes, Wastewater Management. Global Media, 2009.

[11] A. Srinivasan, and T. Viraraghavan, 2001. Decolorization of dye wastewaters by biosorbents: A review. Journal of Environmental Management 91, 1915–1929.

[12] E. Forgacs, T. Cserhati, G. Oros, 2004. Removal of synthetic dyes from wastewaters: A review. Environment International 30, 953–971.

[13] S. Saleh, (2013), Role of Becteria in beneficiation of iron ore. M.Sc.Thesis, Helwan University, HU.

[14] Abdel-Khalek, N. A., Selim, K. A., Abdallah, S. S., Yassin, K. E., 2013, "Bioflotation of low Grade Egyptian Iron Ore using Brevundimonasdiminuta Bacteria: Phosphorus removal", Elixir Bio Technology, 63, 18666-18760.

[15] N. A. Abdel-Khalek, K. A. Selim K. E. Yassin, ,Samah S. Abdallah''Bio-flotation of Egyptian Phosphate using Desulfovibrio desulfuricans Bacteria"., Journal of Mining World Express Volume 4 Issue 1, 2015

[16] N.A.Abdel- Khalek, K.A. Selim, S.E. Mohammed, H.H.E. Hendawy, R.M. Elbaz, " Interaction Between Kaolinite and Staphylococcus Gallinarum Bacteria", Journal of Mining World Express 3, 46-52, 2014.

[17] Vilinska, A.; Rao K.H. and Forssberg, K. S. E. (2007). Selective coagulation in chalcopyrite/pyrite mineral system using Acidothiobacillus group bacteria. Advanced Maternal Research. vol. 20-21, pp. 366-370.

[18] M.A.M. Salleh, D.K. Mahmoud, W.A.W.A. Karim, A. Idris, Desalination, Vol. 280, p. 1, 2011.

[19] V.K. Gupta, Suhas, Journal of Environmental Management, Vol. 40, p. 2313, 2009.

[20] G. Mezohegyi, F.P. Van der Zee, J. Font, A. Fortuny, A. Fabregat, Journal of Environmental Management, Vol. 102, p. 148, 2012.

[21] A. Srinivasan, T. Viraraghavan, Journal of Environmental Management, Vol. 91, p. 1915, 2010.

[22] A.K. Verma, R.R. Dash, P. Bhunia, Journal of Environmental Management, Vol. 93, p. 154, 2012.

[23] M. N. Hedaoo, A. G. Bhole, N. W. Ingole, and Y.-T. Hung, Biological wastewater treatment. In: Handbook of Environment and Waste Management: Air and Water Pollution Control. Y.-T. Hung, L. K. Vang, N. K. Shammas, eds., World Scientific, 2012.

[24] Y. Fu, T. Viraraghavan, 2000. Removal of a dye from an aqueous solution by fungus Aspergillus niger. Water Quality Research Journal of Canada 35(1), 95–111.

[25] Y. Fu, and T. Viraraghavan, 2001. Removal of Acid Blue 29 from an aqueous solution by Aspergillus niger. American Association of Textile Chemists andColorists Review 1(1), 36–40.

[26] M. Arica, and G. Bayramoglu, 2007. Biosorption of Reactive Red 120 dye from aqueous solution by native and modified fungus biomass preparations of Lentinus sajor-caju. Journal of Hazardous Materials 149, 499–507.

[27] T. O'Mahony, E. Guibal, J. M. Tobin, 2002. Reactive dye biosorption by Rhizopus arrhizus biomass. Enzyme Microb. Technol. 31, 456–463.

[28] "Dye Removal Using Some Surface Modified Silicate Minerals" K.A. Selim, M. A. Youssef, F. H. Abd El-Rahiem and M. S. Hassan, Elixir Applied Chemistry 63 (2013) 18659-18665

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