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Bioremediation of crystal violet using air bubble bioreactor packed with *Pseudomonas aeruginosa*

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Abstract

Seven water and sediment samples were collected and tested for decolorizing crystal violet. *Pseudomonas aeruginosa* was the most effective isolate for dye decolorization. The LC_{50} of the crystal violet (115 mg/l) was measured using *Artemia salina* as a biomarker. The effect of different heavy metals on crystal violet decolorization was investigated. Cd^{2+} and Fe^{3+} ions showed marginal enhancement of the decolorization process, the rate was 1.35 mg/l/h compared to (1.25 mg/l/h) for the control. Phenol and *m*-cresol showed no effect on crystal violet decolorization, meanwhile *p*-cresol and *p*-nitrophenol reduced the decolorization rate to 1.07 and 0.01 mg/l/h, respectively. *P. aeruginosa* cells were immobilized by entrapment in agar-alginate beads. The beads were cultivated and reused in Erlenmeyer flask and in an air bubble column bioreactor and they enhanced the crystal violet decolorization rate to 3.33 and 7.5 mg/l/h, respectively.

Keywords: Crystal violet; Decolorization; Biotoxicity; Bioreactor; *Pseudomonas*

1. Introduction

Crystal violet is a triphenylmethane dye used as a biological stain or as a dermatological agent. For many years it was used as oral medication for treatment of pinworms and other tropical diseases because of its great effect in controlling fungal growth under varying conditions (Zollinger, 1987). Investigations on crystal violet in vitro concluded that this dye was a mitotic poisoning agent. In addition in vivo studies proved that, crystal violet should be regarded as a biohazard substance (Au et al., 1978).

Unfortunately, wastewater treatment facilities are often unable to remove commercial dyestuffs including crystal violet dye and this effluent contaminates aqueous habitats (Micheals and Lewis, 1985). The textile and dyestuff industrial wastes are generally treated by physicochemical methods (including; adsorption, chemical precipitation and flocculation, oxidation by chlorine, H_2O_2 and ozone electrolysis, electrochemical treatment and ion pair extraction) which are significantly different in color removal, volume capability, operating speeds and capital costs. It was found that 15% of triphenylmethane dyes, which are currently manufactured are lost to waste water as a result of inefficiency in the chemical and physical treatment processes (Zollinger, 1987).

In 1981, Yatome et al. (1981) reported that four triphenylmethane dyes (basic fuchsin, methyl violet, crystal violet and Victoria blue) were biodegraded using *Pseudomonas pseudomollei* 13NA. The half-decolorization

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time was 54 h for both methyl violet and crystal violet when 4.08 mg/l dye were applied. In addition, Yatome et al. (1991) noticed that the decolorization of several triphenylmethane dyes could be obtained using *Bacillus subtilis*, *Escherichia coli* and some species of *Pseudomonas* (*P. cepacia, P. cruciviae* and *P. stutzeri*). They reported *B. subtilis* remarkably decolorized 2.85 mg/l of crystal violet after 24 h.

The present study, aims to isolate a local bacterial strain able to decolorize the crystal violet with an efficient rate and in presence of other environmental stresses like phenols and heavy metals. Also this selected biological system will be applied as free and immobilized cells in batch cultures and in an air bubble column system in order to examine the effect of the culturing conditions on the crystal violet decolorization process.

2. Materials and methods

2.1. Sampling

Seven water and sediment samples were collected from different locations: Kafer El-Dawar Dyestuffs and Chemicals Company, El-Beida Dyers Company, El-Shokeer (Red Sea), the Suez Gulf (Red Sea), East harbor (Mediterranean Sea), El Shatby (Mediterranean Sea), and Abu Qir gulf near El Amya pumping station (Mediterranean Sea). These samples were tested for crystal violet decolorization activity. The most effective sample was collected from Abu-Qir gulf near El-Amya pumping station, receiving many industrial waste effluents especially those from Kafer El-Dawar Dyestuffs and Chemicals company, and El-Beida Dyers company, Alexandria. The water samples were collected in 0.5 l sterile blue cap bottles, while the sediment samples were collected in sterile Petri dishes (9 cm in diameter), All samples were kept in an ice box and the isolation process was carried out within 24 h.

2.2. Cultivation

The culture medium used for optimal growth and maximum decolorization of crystal violet was composed of (g/l): yeast extract 5; $(NH_4)_2SO_4$ 0.5; KH_2PO_4 2.7 and Na_2HPO_4 4.5. For a solid medium 20 g/l agar was added, crystal violet dye was used in a concentration of 2.5–15 mg/l. The medium was adjusted at pH 7, 100 ml medium in 250 ml Erlenmeyer flasks was inoculated with 5 ml bacterial suspension (10^7 cell/ml with optical density = 1.0 at 550 nm) and incubated at 37 °C until complete decolorization was achieved (Sani et al., 1999).

2.3. Isolation and identification

One ml of each collected water sample was spread under aseptic conditions on Petri dishes (12 cm in diameter) containing a solid culture medium (supplemented with 2.5 mg/l crystal violet). Colonies were selected according to their ability to decolorize crystal violet (forming clear zones). A successive streaking of these isolates onto crystal violet plates allowed the isolation of pure cultures that could decolorize the dye. The most potent bacterial isolate for crystal violet decolorization was identified according to Bergey's Manual, section-4 "Gram negative aerobic rods and cocci"(Sneath et al., 1986) and Steel (1993). Where staining, microscopic examination and several biochemical tests using API 20E identification kits were carried out.

2.4. Estimation of cell growth

The dry weight of the suspended culture was determined according to a calibration curve made between the dry weight of the bacterial cell mass and the optical density of the broth (turbidity) measured spectrophotometrically at 550 nm. Hereto a 3 ml sample was taken from the culture medium aseptically. For the immobilized cell cultures the bacterial dry weight was detected at the end of each experiment, where, the beads were collected washed with sterile distilled water and dried to constant weight at 70 °C. The dry weight was calculated as a difference with the dry weight of non-inoculated beads.

2.5. Decolorization of different crystal violet concentrations

Different crystal violet concentrations (2.5, 5, 10, 15, 20, 25, 30, 40, and 50 mg/l) were added separately to 250 ml Erlenmeyer flasks containing100 ml culture medium. The cultures were inoculated with 5 ml of 10^7 cell/ml bacterial suspension (OD = 1.0) and incubated under shaking condition at 37 °C.The samples were extracted using *n*-butanol (1:2) and the remaining of the crystal violet dye was estimated spectrophotometrically at 592 nm.

2.6. The toxicity bioassay

For determining the toxicity and the LC_{50} value of the crystal violet dye the Probit method was used according to Reish and Oshida (1987). Various crystal violet concentrations (5, 10, 15, 30, 50 and 70 mg/l) were prepared in 10 ml of filtered and sterilized seawater samples, obtained from the seawater tap of the aquarium of the National Institute of Oceanography and Fisheries, Alexandria. The biotoxicity of the crystal