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Feasibility of using irradiation to degrade a toxic dye compound

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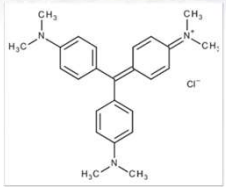

Fig. 1. Crystal violet, $C_{25}H_{30}ClN_3$

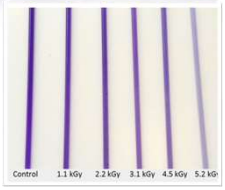

Fig. 2. CV 300 mg/l, control and after irradiation.

INTRODUCTION

Crystal Violet (CV) is a triphenylmethane dye widely used for dyeing cotton, silk and paper (Fig. 1). It has carcinogenic and mutagenic effects other than antibacterial, antifungal, and anthelmintic properties. The toxicity of this compound represents a great risk to the ecosystem and need to be treated before being discharged as wastewater into the environment. However, the complex aromatic molecular structures of CV make them more stable and more problematic to degrade.

The removal of this synthetic dye is of great concern because of the difficulty in treating such effluent by conventional methods. In this study gamma radiation was investigated as a method for degrading CV from water. Absorbance, concentration, toxicity in eukaryotic cells and bacteria's were evaluated.

MATERIAL AND METHODS

CV solution (300 mg/l) in glass bottles were irradiated in a Cobalt-60 gamma-radiation facility. Absorbance (1/10 dilution) and concentration were measured in a UV-vis spectrometer. Antimicrobial susceptibility in *Bacillus cereus*, *Bacillus subtilis*, *Enterococcus faecalis*, *Escherichia coli*, *Listeria monocytogenes* and *Staphylococcus aureus* were analyzed using the CLSI, (2012), guidelines for the microdilution method and minimum inhibitory concentration (MIC) was defined. Cytotoxicity was tested in Vero Cells after 24 h of treatment with MEM and different concentration of irradiated CV solution. Cell viability was determined with crystal violet staining assays and absorbance analysis at 570 nm in a microplate reader. Cytotoxic concentration 50% (CC_{50}) was calculated comparing treated cells with cell control.

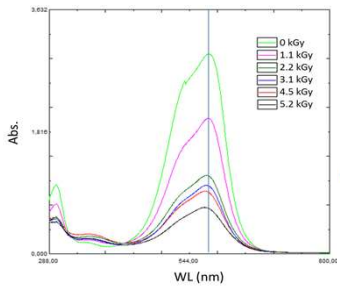

Fig. 3. Irradiation of CV: variation of absorbance

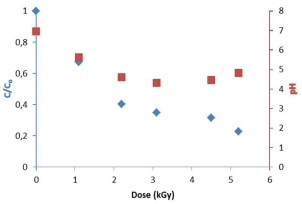

Fig. 4. CV variation of concentration and pH.

RESULTS AND DISCUSSIONS

The absorbed doses of CV solution were 1.1, 2.2, 3.1, 4.5 and 5.2 kGy. The dose rate was between 11,5-16,2 kGy/h. Visually, a partial discoloration of the solution was observed (Fig. 2). The absorption spectra showed a height reduction on the major peaks at 300 nm and 580 nm, as the dose increase, indicating that the concentration of CV is reduced (Fig. 3). This results agreed with previous reports from Li et al., 2016, who analyzed the degradation pathways of the CV and indicated that this is due the formation of leukocarvinol CV. An small peak appears in the irradiated solution at 359 nm, indicating the possible formation of intermediate metabolite 4,4`-bis(dimethylamino)benzophenone or 4-(dimethylamino) benzophenone (Ju et al., 2011).

In the Fig. 4 it is showed how the CV concentration decreased as function of the radiation dose. At 1.1 kGy, 66% of the initial concentration was conserved, while at 5.2 kGy was the 20%. This graph also shows the variation in pH from 6.9 to 4.8.

Antimicrobial activity showed a reduction in a dose-based effect in all the microorganisms tested. *Staphylococcus aureus* MIC increase from 1.5 mg/l to 37.5 mg/l when the solution was irradiated with 5.2 kGy. Similar results were obtained with *L. monocytogenes* (40-80 mg/l), *B. cereus* (6.2-100 mg/l), *B. subtilis* (50-120 mg/l), *E. faecalis* (20-140 mg/l) and *E. coli* (50- no inhibition at 150 mg/l) (Fig. 5).

The cell viability results from CV control solution demonstrated the severe inhibitory effects by this compound; the CC_{50} was 8.8 mg/l. In contrast, the solution treated with the incremental irradiation doses reduced the toxicity. The results for CV-1.1 kGy was CC_{50} 85.6 mg/l and 109 mg/l for CV-5.2 kGy (Fig. 6). Cell morphology is shown in Fig.7. Vero cells treated with CV-20 mg/l of non-irradiated solution had non-viable cells (Fig.7.B) and the cells were shrinked and detached from the flask. Cells exposed to 80 mg/l- 5.2 kGy had 80% viability, just few cells were detached and rounded (Fig.7.C).

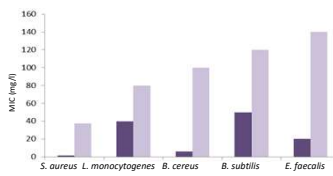

Fig. 5. Antimicrobial activity.

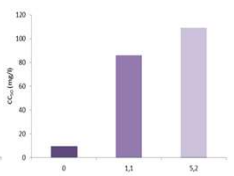

Fig. 6. Citotoxicity in Vero Cells.

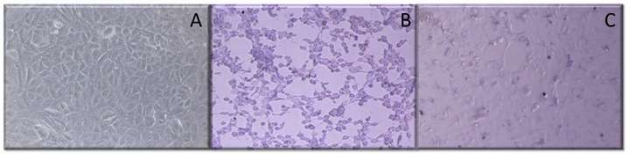

Fig. 7. Cell morphology of control Vero cells (A), Vero cells CV 20 mg/l, 0 kGy (B), cells with CV 80 mg/l + 5.2 kGy (C).

CONCLUSIONS

This study provided both, the analytical results were it is showed the CV degradation produced by irradiation, and also the reduction on the toxicity of the CV-solution, by indirect analyses, like citotoxicity test and antimicrobial activity. These preliminary studies represents a potential alternative treatment for dye wastewater.

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