

PAPER 27 – COMPARATIVE STUDY OF BIOLOGICAL DEGRADATION OF CRYSTAL VIOLET BY MICROBIAL MIXED CULTURES FROM COW DUNG AND SOIL

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ABSTRACT

The removal of dyes such as crystal violet (CV) from effluents is a major concern worldwide due to their toxic and carcinogenic nature. Recently, biodegradation through microorganisms has emerged as a potential method for eliminating these pollutants. This study was carried out to investigate the extent and the kinetic parameters of biological degradation of crystal violet by mixed-microbial cultures from cow dung and soil. A solution containing 100 mg/l of CV was prepared and added to a reactor inoculated with cow dung and another with soil. The reactors were stirred continuously for 300 mins with concentration of CV measured every 15 mins. For 1 g/l initial inoculum size, 92% and 75 % of CV degradation was achieved with cow dung and soil respectively. Adsorption test showed both cow dung and soil have negligible adsorption capacities. Kinetic parameters like maximum growth rate (m_{max}) and half-saturation constant (K_s) were estimated to be 2.65 day⁻¹ and 0.45 g/l for cow dung. However, 0.82 day⁻¹ and 1.33 g/l as m_{max} and K_s for soil were estimated respectively. These results showed mixed microbial cultures from cow dung and soil can effectively degrade CV. It also suggests that cow dung is a more efficient inoculum than soil in degrading CV.

KEYWORDS: Crystal Violet, biodegradation, kinetic parameters, microorganisms, cow dung, soil

5. INTRODUCTION

The presence of dyes in effluents of various industries like textile, cosmetics, pharmaceuticals, rubber and plastic poses a serious threat to the environment when they are discharged without sufficient level of treatment (Hussain et al., 2020; Singha et al., 2021). Dye-coloured effluent directly discharged into rivers and lakes does not only introduce aesthetic problems but, also obstruct penetration of light, as a result hindering aquatic plant photosynthesis, reducing dissolved oxygen concentration and creating toxic conditions to aquatic flora and fauna (Dutta & Bhattacharjee, 2022). The environmental issues surrounding the presence of colour in effluent is a continuing problem for dyestuff manufactures, dyers, finishers and water companies, because increasingly stringent colour consent standards are being enforced by regulatory bodies to reduce the quality of colour in effluent and water courses (Kannan & Sundaram, 2001; Al-Tohamy et al., 2022). Crystal violet (CV) is one of the most used dyes in textile and pharmaceutical industries. It is very notorious due to its structural stability and resistance to heat and light. Crystal violet is toxic, mutagenic and carcinogenic (Mudhoo et al., 2020; Patil et al., 2022). This detrimental effect means eliminating crystal violet from effluents cannot be over emphasized.

There are various physicochemical techniques for the treatment of effluents containing dyes such as adsorption, precipitation, reverse osmosis, chemical oxidation, photodegradation, advanced oxidation, coagulation, membrane filtration, electrochemical processes, and ultrasonic irradiation (Abdi et al., 2020; Samsami et al., 2020). However, most of these methods of decolorizing and treating dye-containing effluents are not widely used due to high investment cost, formation of sludge, formation of hazardous by-products and intensive energy requirements (Abdi et al., 2020; Al-Tohamy et al., 2022). Physicochemical methods of dye removal are effective only if the effluent volume is small and still generates toxic sludge. This has therefore resulted in considerable interest in the use of biological techniques for the treatment of these effluents. Biodegradation and bioremediation, especially through bacteria, is becoming an emerging and important sector in effluent treatment. Recently, microbial degradation of textile effluent has been reported as eco-friendly and more economical than physicochemical methods (Shah, 2013).

Biodegradation utilizes microorganisms such as bacteria, fungi and protozoa to breakdown the organic wastes or contaminants into smaller less harmful substances. Such biological processes are cheaper, produce less sludge, cost effective processes and have the potential to remove or decolorize the dye completely (Kalyani et al., 2009; Hussain et al., 2020). Microorganisms from different sources such as animal waste, food waste, sewage, sludge, cow dung, soil, etc. have the potential to achieve a higher degree of dye-degradation and can process a complete

mineralization of dyes under optimal conditions (Asad et al., 2007). Although the ability of isolated bacteria to metabolize CV dye has been investigated by a number of researchers (Chen et al., 2007; Mondal et al., 2010; Parshetti et al., 2011), biodegradation of crystal violet and other dyes can be less effective because of the inherent characteristics of dyes like anti-septic and anti-fungal nature, complex chemical structure, toxicity, high weight, and strong molecular bonds (Mani & Bharagava, 2016). To date, there is little information, if any, on the extent of crystal violet degradation using mixed microbial cultures (unacclimated) and the biokinetic parameters.

Thus, this present study work was carried out to investigate the comparative effectiveness of crystal violet degradation using mixed microbial cultures from cow dung and soil. The study also aimed to estimate and compare the main kinetic parameters of biodegradation for both cow dung and soil.

6. METHODOLOGY

2.1 Materials and apparatus

The materials and main apparatus used include: analytical grade crystal violet (Sigma Aldrich, Germany), analytical grade ammonium chloride (99.5% Sigma Aldrich, Germany), magnetic stirrer (TOP-SH2, China), UV-vis spectrophotometer (Zuzi 4201/20, France) and weighing balance (Ohaus SP202, Scoutt Pro, USA). Distilled water was produced using water distiller (SZ-96 Mon Scientific, Nigeria). Other apparatus used were stopwatch and glassware.

2.2 Collection and preparation of inoculum

Cow dung and soil are the inoculum used in this study. They were collected from a farm used by the Center for Dryland Agriculture at Bayero University Kano. The cow dung and soil were collected and separately sieved to minimize stones and dirt and also to obtain uniform particle size of < 250 μ m. The samples were then stored in airtight plastic containers prior to usage.

2.3 Preparation of dye solution and calibration

A 1000 mg/l stock solution of dye was prepared by dissolving crystal violet powder in distilled water. NH_4Cl was also added in trace amount (100 mg) to the solution. Afterwards, six (6) samples of 0, 2, 4, 6, 8 and 10 ppm of CV were prepared by diluting the stock solution with appropriate volumes of distilled water. The absorbance of each of the six samples was measured using a UV spectrophotometer at a 565 nm wavelength.

2.4 Biodegradation experiment

2.4.1 Adsorption studies

Two parallel batch reactors with 500 ml working volume were set up and operated at room temperature (25°C) to allow the treatment of the 100 mg/l CV dye solution with cow dung and soil simultaneously. Mixing was carried out using a magnetic stirrer. Adsorption capacity was calculated for cow dung and soil by mixing 100 mg/l of CV solution in 500 ml reactors with 0.5 g of cow dung in the first reactor and 0.5 g of soil in the second reactor. The reactors were allowed to mix continuously for 30 minutes and the concentration of CV in the reactor was measured afterwards. The adsorption capacity (q_{ad}) and adsorption coefficient (K_{d}) for both cow dung and soil were calculated using equations 1 and 2 below (Habib et al., 2004):

$$q_{\text{ads}} = (C_0 - C_{\text{eq}}) \frac{V}{m} \dots\dots\dots(1)$$

$$K_{\text{d}} = q_{\text{ads}} / C_{\text{eq}} \dots\dots\dots(2)$$

where C_0 and C_{eq} are the initial and equilibrium CV concentration (ppm), V is the volume of the reactor (0.5 l), m is the mass of inoculum (g) and K_{d} is the adsorption coefficient (l/g).

2.4.2 Biodegradation studies

The biodegradation studies began by mixing 100 mg/l of CV in 500 ml reactors with 0.5 g of inoculum in separate reactors. The reactors ran continuously for 5 hrs. In each reactor, 5 ml of well-mixed solution was withdrawn every 15 minutes to determine the concentration of CV. The same experiment was repeated with a higher inoculum size of 1.5 g for both cow dung and soil.

2.5 Calculation of kinetic parameters

The half saturation constant (K_s) and maximum growth rate (m_{max}) on crystal violet for the microorganisms in cow dung and soil in the reactor were calculated using the Monod model:

$$r_x = \frac{\mu_{MAX} S}{K_s + S} X \dots\dots\dots(3)$$

$$-r_s = \frac{\mu_{MAX} S}{K_s + S} \frac{X}{Y} \dots\dots\dots(4)$$

where r_x indicates the cell growth rate (mg biomass/l/h), r_s is the substrate utilisation rate (mg CV/l/h), X the cell concentration (mg /l), S the CV concentration, m_{max} the maximum growth rate (h^{-1}), K_s the half-saturation constant (mg CV/l) and Y the biomass growth yield on CV (mg biomass/mg CV). The m_{max} and K_s were calibrated on the basis of the time profiles of CV Concentration during the biodegradation experiment. The values of m_{max} and K_s calculated using non-linear regression of the Monod equations. Y was assumed to be equal to 0.47 mg biomass/mg substrate, on the basis of the biomass yield reported in many textile effluent studies (Insel et al., 2002; Henze et al., 2008).

3 RESULTS AND DISCUSSION

3.1 Adsorption studies

The aim of the adsorption experiments was to quantify the contributions of CV removal due to adsorption during operation of the bioreactors. A contact time of 30 minutes was chosen because adsorption is generally considered to be a fast process (Zhao et al., 2008). The results of adsorption studies for cow dung and soil are shown in Table 1. The amount of CV adsorbed on cow dung is lower than 2 mg CV/g and the adsorption coefficient was less than 0.02 l/g. The CV adsorbed on soil was much lower than that of cow dung as 0.47 mg CV/g soil. The adsorption coefficient of soil was also about seven times lower than that of cow dung. This is a clear indication that CV adsorption on the inoculum used in this study is very low and therefore can be considered negligible. Hence, it can be said that any reduction in CV concentration in the biodegradation experiments is solely due to microbial action.

Table 1: Adsorption of crystal violet on the cow dung and soil used in this study

Inoculum	Inoculum Concentration (g/l)	CV adsorbed (mg CV/g inoculum)	K_d (l/g)
Cow Dung	1	1.465	0.014
Soil	1	0.473	0.0019

The adsorption coefficient (K_d) between CV and the inoculum obtained in this study is in agreement with the values reported by Fent et al. (2003) in a different study, which observed K_d values (units converted for consistency) between 0.0055 and 0.074 l/g. The results in Table 1 also shows that cow dung has better adsorption potential as compared to soil.

3.2 Biodegradation experiment

Figures 1 and 2 show the concentration profiles of CV in the reactors with 1 g/l and 3 g/l initial inoculum size respectively.

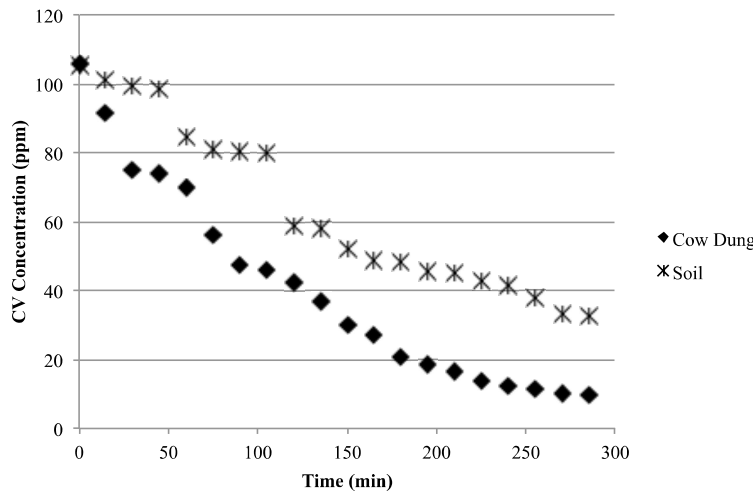


Figure 1: CV concentration profile in the reactors inoculated with 1 g/l Cow dung and 1 g/l Soil

Figure 1 shows that CV degradation was faster with cow dung as compared to soil when the initial inoculation size was 1 g/l. It further shows that about 92 % of CV was degraded within 300 minutes. However, only about 75% degradation of CV was achieved with soil. It was also observed that the CV concentration dropped by about 30 % and 20 % within the first 60 minutes for cow dung and soil respectively. On the other hand, as observed in Figure 2, increasing the inoculation size from 1 g/l to 3 g/l significantly improved the degradation rate of CV in the reactor with soil, achieving almost 93% degradation within 200 minutes. It can also be observed that increasing the inoculum size of both cow dung and soil virtually doubled the rate of CV degradation where 65% and 42% of the CV removal was achieved within 60 minutes respectively. It is important to observe that most studies reported in the literature were carried out using isolated bacteria (pure culture). Roy et al. (2018) reported 81.25 % degradation of CV after 72 hours using isolated bacteria. Ayed et al. (2009) also achieved up to 92% decolorization of CV within 3 hours using isolated bacteria. In another study, Parshetti et al. (2011) achieved 100 % decolorization of CV within 5 hours using isolated radiobacter. Indeed, this study has shown that mixed microbial cultures (not isolated) can achieve high CV degradation similar to the ones achieved with pure cultures.

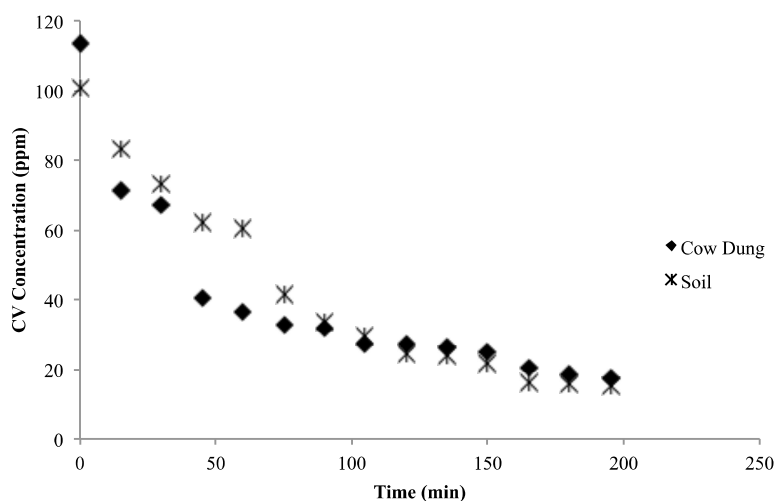


Figure 2: CV concentration profile over in the reactors inoculated with 3 g/l Cow dung and 3 g/l Soil.

3.3 Kinetic parameters of growth

The data generated from the batch operation of the reactors and the time profiles collected were used to estimate the maximum growth rate of the microorganisms growing on CV. Figures 3 and 4 show the estimated m_{max} and K_s values for cow dung and soil respectively. The m_{max} for cow dung was estimated to be 2.65 day^{-1} , which is about three times higher, compared to soil with 0.82 day^{-1} . In terms of K_s , soil was estimated to have higher value than cow dung (1.33 vs 0.45 g/l respectively). This is expected because higher degradation rate corresponds to higher m_{max} and lower K_s (Henze et al., 2008). Typical values of m_{max} reported in literature ranges from $0.22 - 13.2 \text{ day}^{-1}$ and K_s vary from $0.005 - 2.93 \text{ g/l}$ (Sollfrank & Gujer, 1991; Pala & Tokat, 2002; Al-Malack, 2006; Rajagopalu & Kanmani, 2008). In general, the values of m_{max} and K_s obtained in this study are within the range of the reported values for these parameters in the literature.

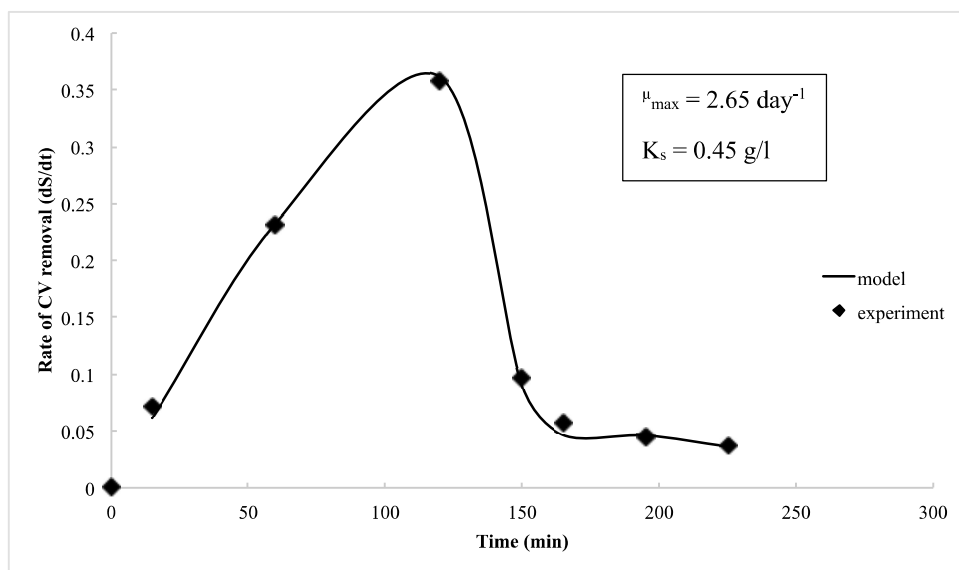


Figure 3: Rate of CV degradation over time. Comparison of experimental and model data for batch experiment with cow dung. The estimated m_{max} and K_s are reported on the graph

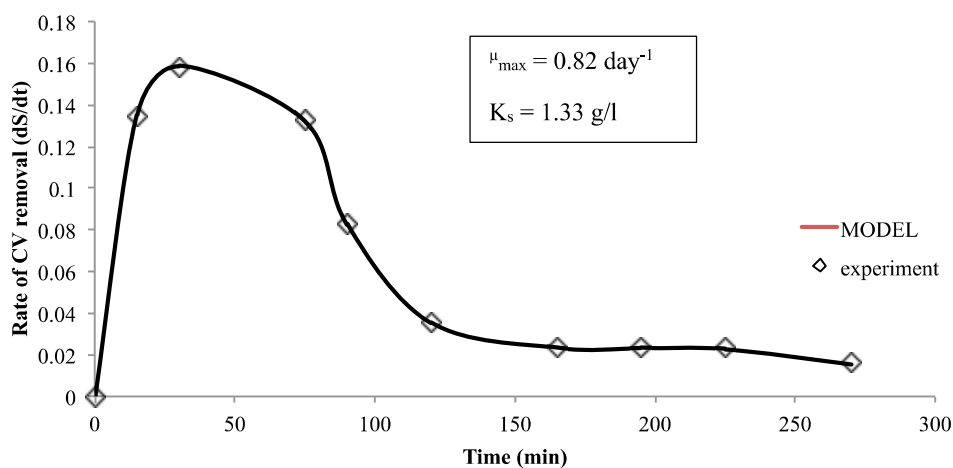


Figure 4: Rate of CV degradation over time. Comparison of experimental and model data for batch experiment with soil. The estimated m_{max} and K_s are reported on the graph

4 CONCLUSION

This study has shown that crystal violet can be degraded by mixed microbial cultures inoculated from cow dung and unacclimated soil in a batch reactor. The observed extent of CV degradation with cow dung was 90 – 92 % for cow dung and 75 – 93 % with soil. CV adsorption on cow dung and soil used as inoculum was measured and was found that adsorption was a negligible for CV removal in this case. This study shows that increasing the initial inoculum size increases the rate of degradation for both cow dung and soil. Kinetic parameters like maximum growth rate and half saturation constant were estimated to be 2.65 day^{-1} and 0.45 g/l for cow dung. The values for soil were found to be 0.82 day^{-1} and 1.33 g/l , and these are in agreement with the literature. The results obtained suggest that cow dung is a more efficient inoculum than soil in degrading CV.

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