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# Toxic Response of Nitrobacter to Orange II and Direct Blue 71

***ORANUSI, NATHANIEL A.; OGUGBUE, JASON C.**

*Department Of Microbiology, University Of Port Harcourt, P.M.B 5323, Port Harcourt*

**ABSTRACT:** Tolerance of Nitrobacter to Orange II (C.L. 15510) and Direct Blue 71 (C.I. 34140) dyes was determined by total viable count. There was decrease in percentage log survival with increase in concentration of the toxicants and increase in exposure time. Percentage survival at 20h was 84.50% and 75.50% at toxicants concentration of 100mgl$^{-1}$ and 300mgl$^{-1}$ respectively for orange II. The data obtained with direct Blue 71 for 20 were 88.96% and 83. 31% at 100mgl$^{-1}$ and 300mgl$^{-1}$ respectively. The $LC_{50}$ for Orange II at various exposure times were 48h (139.86mgl$^{-1}$) and 36h (352.79mgl$^{-1}$). The LC50 was >1,000mgl$^{-1}$ for exposure times of )-36h.The LC50 Direct Blue 71 were > 1000mgl$^{-1}$ for all exposure times. The difference in toxicity of the two dyes was attributed to difference in dye content and molecular weight. *@JASEM*

Dyes are extensively used in the textile, pharmaceutical, food, leather and cosmetic industries (Raffi *et al*, 1990). The discharge of dye wastewater into the environment causes various pollution problems (Percy *et al*, 1989; Spadaro *et al*, 1992; Ganesh *et al*, 1994; Kim and Shoda, 1999). The large number of dyes and high potential for toxicity of dyes and its component has made class of compounds the most extensively reviewed and regulated under the Toxic Substances Control Act (Hork *et al*, 1991). The toxicity of a chemical to microoganisms is normally measured in terms of growth inhibition (Norkis and Zur, 1979), oxygen consumption (Salbbert and Brawbow, 1986), ATP level (Parker and Pribyl, 1984), enzyme activity (Bilton *et al*, 1986) and colony formation on agar plate (Anderson and Ahdelgan, 1980; Cenciet *et al*, 1987; Liu *et al*; 1989).

An ideal organism for toxicity bioassay must be sensitive and convenient use. Williamson and Johnson (1981) reported that Nitobacter is an ideal organism for assessing the toxicity of toxicants in wastewaters. In Nigeria, most work carried out on toxicity of pollutants to Nitrobacter have concentrated on crude oil and by- products of crude oil refinding and chemicals used for drilling and exploration (Okpokwasili and Odokuma, 1994; Okpokwasili and Odokuma, 1996).

There is death if information on toxicity of dyes on microorganisms despite the increasing number of textile, food, cosmetic, Pharmaceutical and leather industries in Nigeria. Azo dyes are the most extensively used of these industrial dyes (Raffi *et al*; 1990).

The aim of this study is to assess the toxic response of Nitrobacter to a 20 dyes (Orange II and Direct Blue 71).

* Corresponding author

## MATERIALS AND METHOD

Soil samples from a garden soil were collected into sterile bottles and subjected to serial 10-fold dilutions in physiological saline. One milliliter of the various solutions was inoculated into Winogradsky agar plates by the spread-plate technique. The cultures were incubated at room temperature (30 ± 2° C) for 4 days. Greyish, mucoid, and flat colonies suggestive of Nitrobater were picked. These colonies were Gram stained and isolates that were Gaminagetive and pear-shaped indicative of Nitrobacter were further purified by repeated subculture onto Winogradsky agar. This procedure was adopted from Colwell and Zambruski (1972) and Okpokwasili and Odokuma (1994).

Cultures were streaked onto Winogradsky agar slants, incubated at 30 ± 2°C for 4 days, and then stored at 4°C in a refrigerator. Isolates from the slant culture were inoculated into 100ml Winogradsky broth contained in 25ml Erlenmeyer flasks. These were incubated at 30± 2°C for 3 days. These cultures were used as inoculums for the toxicity tests.

Short-term shake flask test method (Duffus, 1980) for assessing the toxicity of chemical was used. Tolerance level was assessed using Total Viable count (TVC) as an index of toxicity.

The toxicants, Orange II (C.I. 15510) and Direct Blue 71 (C.I. 34140), were purchased directly from Aldrich chemical Co; USA the dyes were used at their commercially available purity level of 90% dye content for Orange II and 50% dye content for Direct Blue 71.

A stock solution of each dye was prepared by dissolving 2.5g of each dye in 100ml Winogradsky broth contained in 250ml Erlenmeyer flask. The stock solutions were sterilizes by membrane filtration,(0.2µm pore size Acordisc). Various concentrations

(0.001, 0.10, 0.030 and 0.050g $l^{-1}$) were prepared by diluting the stock solution with sterile Winogradsky broth. Control flasks without toxicant were also set up. One milliliter of the inoculums (107 cfu $ml^{-1}$) was aseptically inoculated into 100ml of triplicate set of each dye concentration contained in 250ml Erlenmeyer flask. The cultures were incubated at 30± 2°C for 48h. At exposure time of oh 4h, 12h, 20h, 28h, 36, 44h and 48h, one milliliter was aseptically withdrawn from each flask and serially diluted (10-1 -10-3). The dilutions were inoculated onto Winogradsky agar plates and incubated at 30± 2°C for 3 days. The number of colonies, which developed, was counted. Results were expressed as colony-forming units per ml (cfu $ml^{-1}$). Controls were treated the same way as the samples.

Percentage log survival was calculated using the method of Williamson and Johnson (1981).

$$Percentage\ \log\ survival = \frac{\log C}{\log c} x100$$

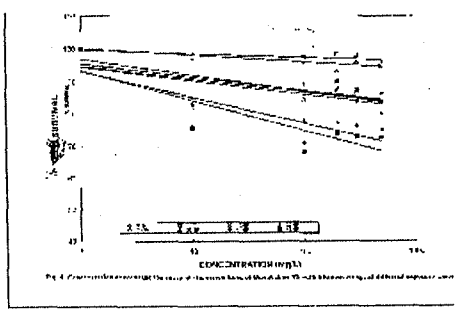
Where

C =count in each toxicant concentration
$^{C}$=count in the control (zero toxicant concentration)

The LC50 was calculated from the graph percentages log survival against concentration.

## RESULTS AND DISCUSSION

Figs1-4 shows the results obtained on the tolerance of the test organisms to the dyes. Generally, percentage log survival decreased with increasing dye concentration and exposure time as shown in figs. 1 and 2 for Orange II and figs. 3 and 4 for Direct Blue 71. Brief increase in percentage log survival of test of organisms exposed to Orange II at 0-4h exposure time was obtained with all concentrations except 10mgl-1 (figm1). The brief crease in percentage log survival was also obvious when Direct Blue 71 was exposed to test organisms at 0-4h exposure time with concentrations of 100mgl -1, 200mgl-1 and 300mgl -1 (fig 3). The LC50 for Orange II at various exposure times (fig. 2) were as follows: 4h (139.86mgl-1); 44h (181.80mgl-1) and 352.79mgl-1 at 36h. The LC50 was> 1000mgl-1 for exposure times of 0--28h. The LC50 for Direct Blue 71 were all > 1000mgl-1 (fig.4). The colony counts in the controls (no dye added) increased with time. The toxic effect may be attributed to inhibition of nitrite utilization and/or cell death. Pelzar *et al* (1984) speculated that dye exert inhibitory effects by interfering with cellular oxidation process. The brief increase in percentage log survival (0-4h) of test organism may be as result of recovery of the microbial cells from initial shock effects before the onset of toxic effects by the dyes.



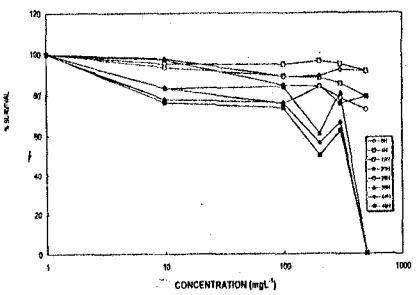
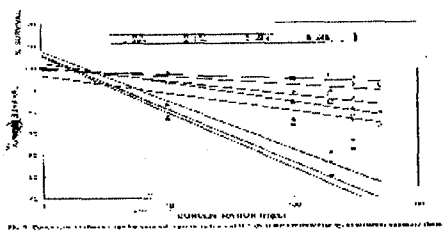
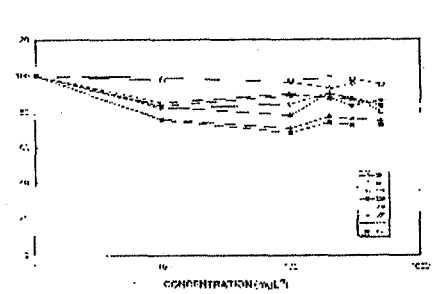



Fig. 1: Effects of Orange II on the percentage log survival of Nitrobacter sp.





Yongjiie -He and Bishop (1994) reported that Acid Orange 7 inhibited all stages of nitrification process and decrease substrates utilization. Orange II is a monoazo dye with 90% dye content and molecular weight of 350.33 while, Direct Blue 71 is a triazo dye with 50% dye content and molecular weight of 1029.88. the higher toxicity of Orange II may be attributed to its high dye content and lower molecular weight as the molecules may be transported into cell faster than Direct Blue 71 with higher molecular weight.

Moreover, decrease in percentage log survival of the organisms was observed with increase in concentration of dyes. Bagby and Sherrad (1981) showed a conceptual model of the effect of heavy metals on biological reactions in which concentration of metal ions is the controlling factor for inhibiting biological responses.

Results obtained from this experiment have shown that Nitrobacter has a low tolerance to the azo dyes and the level of tolerance decrease with time. Hence, the exposure of these Nitrifying bacteria, which play a vital role in Nitrogen cycle and thus soil and water fertility, to these dyes could adversely affect their activities in the biogeochemical cycle of nitrogen.

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