

# The investigation of anti-bacterial activity of Reactive Red-120

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## ABSTRACT

Reactive dyes are resistant to natural biodegradation, due to the aromatic rings in their structure. Among dyes used in textile industry, Reactive Red-120 (RR-120) is one of the frequently used dyes in textile industries and is a potential threat to the aquatic environment due to its poor biodegradability. The aim of this research is to analyze the antibacterial activity of Reactive Red-120 on *E. coli* and *Bacillus subtilis* with MIC, MBC and disc diffusion tests. In this study dilution test was used to finding the minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of the Reactive red-120. For disc diffusion assay test substance were tested at

125 µg, 250 µg, and 500 µg concentrations. MIC values of Reactive red-120 against *Bacillus subtilis* is 100 In this study dilution test was used to finding the minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of the Reactive red-120. For disc diffusion assay test substance were tested at 125, 250, and 500 µg concentrations. MIC values of Reactive red-120 against *Bacillus subtilis* is 100 µg/ml, and MBC values was 1200 µg/ml, MIC values of Reactive red-120 against *E. coli* is 50 µg/ml, and MBC values is 800 µg/ml. The result of this study showed that Reactive red-120 has not antimicrobial activity against *Bacillus subtilis* and *E. coli*, because diameter of inhibition zone at all concentration was zero mm. So, it can be concluded that Reactive red-120 might not pose a potential risk for nature. However, it must be evaluated with different new studies.

**Key Words:** Reactive red-120; *E. coli*; *Bacillus subtilis*; Anti-bacterial activity.

Dye is a material that has an affinity to the substrate to which it is being applied. The dye is usually used in solution, and may need a mordant to remedy the fastness of the dye on the fiber (1). The generality of natural dyes is reproduced from plants, fungi and lichens. Plant-based dyes such as woad, indigo and saffron were important trade in the economies of Asia and Europe (2). Archaeological proofs showed that, particularly in India and Phoenicia, dyeing has been widely achieved for over 5,000 years. The detection of synthetic dyes late in the 19th century ended the large-scale market for natural dyes. The first human accomplished organic aniline dye, mauveine, was found serendipitously by William Henry Perkin in 1856, the result of a failed attempt at the total synthesis of quinine. Other aniline dyes followed, such as fuchsine, safranin, and induline. Many thousands of synthetic dyes have since been prepared (3,4). Dyes are classified according to their used and chemical schema, and are composed of a group of atoms known as chromophores, responsible for the dye color. These chromophore-containing centers are based on various functional groups, such as azo, anthraquinonoid, methine, nitro, aryl methane, carbonyl and others. Also, electrons withdrawing or donating substituents so as to generate or intensify the color of the chromophores are denominated as auxochromes (5-7).

Azo dyes are organic compounds including the functional group R-N=N-R', in which R and R' are generally aryl. They are a commercially important family of azo compounds containing the linkage C-N=N-C. Azo dyes are pervasively used to treat textiles, leather articles, and some foods (8,9). Some classes of azo dyes include disperse Dyes, metal-complex dyes, reactive dyes and substantive dyes. Also called direct dyes, substantive dyes are used for cellulose-based textiles, which include cotton. The dyes bind to the textile by non-electrostatic forces. In another sorting, azo dyes can be classified according to the number of azo groups (10). Many azo dyes are nontoxic, although some azo dyes such as dinitroaniline orange, ortho-nitroaniline orange, or pigment orange 1, 2, and 5 showed that mutagenic and carcinogenic effects on several test systems (11,12). In a reactive dye, a chromophore consists of substituent that reacts with the substrate. Reactive dyes majority use in dyeing of cellulose same cotton or flax, but also wool is dyeing with reactive dyes. Reactive dyeing is the most significant method for the coloration of cellulosic fibres. Reactive dyes can also be applied on wool and nylon; in the latter case they are applied under weakly acidic conditions (13). Reactive Red remains a popular platform for reactive dyes. Generally, reactive dyes are resistant to natural biodegradation, due to the aromatic rings in their structure (14). Among dyes used in textile industry, Reactive Red-120 (RR-120) is one of the frequently used dyes in textile industries and is a potential threat to the aquatic environment due to its poor biodegradability (15). The

aim of this research is to analyze the antibacterial activity of Reactive Red-120 on *E. coli* as a gram negative bacterial strain and *Bacillus subtilis* as a gram positive bacterial strain with MIC, MBC and disc diffusion tests.

## MATERIALS AND METHODS

### Bacterial strains and culture conditions

The bacterial strains used as test organism were *Escherichia coli* as a gram negative bacterial strains and *Bacillus subtilis* as gram positive bacterial strains obtained from Department of Biology of Pamukkale University. All bacteria were cultured aerobically at 37°C in LB agar and LB broth medium. Before experimental use, cultures from agar medium were sub cultivated in liquid media, incubated for 12 h (37°C).

In this study, LB Broth, LB agar, Mueller Hinton broth and Mueller Hinton agar were purchased from Merck and the test substance, Reactive Red-120 was purchased from Sigma-Aldrich and its properties and molecular structure is shown in Figures 1 and 2.

### Antibacterial activity assay

McFarland Standards are used to standardize the proximate number of bacteria in a broth suspension by comparing the turbidity of the test suspension with that of the McFarland Standard. A McFarland Standard is a chemical solution of barium chloride and sulfuric acid; the reaction between these two chemicals results in the production of a fine precipitate, barium sulfate (16,17).

*E. coli* and *Bacillus subtilis* colonies were selected from solid medium using a sterile inoculation loop and inoculated into 10 ml of Mueller-Hinton broth in a sterile tube and mixed well. Turbidity levels of bacterial suspension were equated in accordance with the turbidity of 0.5 McFarland turbidity standards, which were prepared commercially. The accuracy of bacterial suspension was approved using a spectrophotometer analysis. Sterile Mueller-Hinton broth was used and measured at a wavelength of 625 nm as a blank solution for comparison (18).

Tube dilution test as described by Caburian and Osi with some modifications was used to finding the minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of the Reactive red-120 against the *E. coli* and *Bacillus subtilis* (19). Thirteen sterile glass tubes (12 ml) were numbered 1 to 13. Different concentrations of Reactive red-120 (final concentrations of 1.56 µg/ml to 6400 µg/ml) were added to different tubes with 4 ml Mueller Hinton broth. So, a microbial suspension

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of 0.5 McFarland of bacterial strains were cultured in Mueller Hinton broth containing different Reactive red-120 concentrations tubes. The tubes were then incubated at 37°C for 12 hours. The tube with the lowest concentration (highest dilution) of the reactive red at which no visible growth or turbidity was observed was reported as the minimum inhibitory concentration of the oil on the test bacteria. The tubes were shaken to homogenize the contents and 150 µL of the contents of each tube was sub cultured by spreading Mueller-Hinton agar plastic petri dishes. The plates were incubated for 12 hours and then observed for any growth of colonies. Minimum bactericidal concentration was determined as the lowest concentration of Reactive red-120 at which no colony observed following the sub culturing onto Mueller Hinton agar mediums (20).

For disc diffusion assay Reactive red-120 were dissolved in water until a concentration of 25 mg/ml was reached. Bacterial inoculum was set according to the McFarland 0.5 standard, and then 150 µl of bacterial suspension was taken using a sterile micropipette from each bacterial inoculum to be overlaid on the Mueller Hinton agar. In this research reactive red-120 was tested at 125, 250, and 500 µg concentrations. Distilled water was used as negative control and each test set was repeated 3 times. Plates were incubated at 37°C and the inhibition zones were measured after 24 hours (21).

## RESULTS

The MIC and MBC of Reactive red-120 on *Bacillus subtilis* and *E. coli* were determined by dilution (1.56 µg/ml to 6400 µg/ml) method. The result of this study showed that MIC values of Reactive red-120 against *Bacillus subtilis* is 100 µg/ml, and MBC values was 1200 µg/ml, MIC values of Reactive red-120 against *E. coli* is 50 µg/ml, and MBC values is 800 µg/ml.

In this study we observed that the *E. coli* (as a gram negative bacterial strain) was more sensitive to Reactive red-120 than *Bacillus subtilis* (as Gram-positive bacterial strains).

In our research, Reactive red-120 concentrations (125, 250, and 500 µg) were selected for disc diffusion assay. The disc diffusion values of Reactive red-120 against *Bacillus subtilis* and *E. coli* is shown in Figures 3 and 4. The result of this study showed that Reactive red-120 has not antimicrobial activity against *Bacillus subtilis* and *E. coli*, because diameter of inhibition zone at all concentration of Reactive red-120 against two bacterial strains was zero mm.

## DISCUSSION

Azo dyes, a type of textile colorant, are integral to the textile industry and make up 70% of commercial dyes. Research has shown that some Azo dyes pose very serious health risks to humans if they are used in particular textiles and if they get into certain water supplies. The textile industry is a heavy polluter of waste gas, solids, water and noise. Wastewater is the most environmentally damaging, and the effluent from textile plants is classified as the most polluting of all the industrial sectors (22).

The results of this study showed that Reactive red-120 has not antibacterial effect on *Bacillus subtilis* and *E. coli*. According to our knowledge, it was the first study that addresses the antibacterial effects of Reactive red-120 against *Bacillus subtilis* and *E. coli*. We are using different dye in our life. Some of this dye is for food coloring and some of these are for textile industries. We are using textile and food dye in our life but some of dyes have toxic effects, for this reason we need to study about these substances. Chromosome aberration

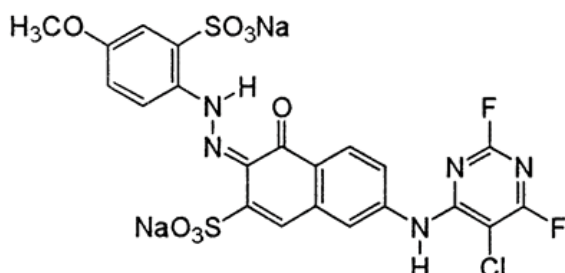


Figure 1) The structure of reactive red-120

CAS Number: 61969-31-1

PubChem CID: 19700

Chemical formula: C<sub>21</sub>H<sub>12</sub>ClF<sub>2</sub>N<sub>5</sub>Na<sub>2</sub>O<sub>8</sub>S<sub>2</sub>

Molar mass: 645.91 g/mol

Solubility in water: soluble

Synonyms: Sandoz Drimarene Scarlet K2G Granulated.

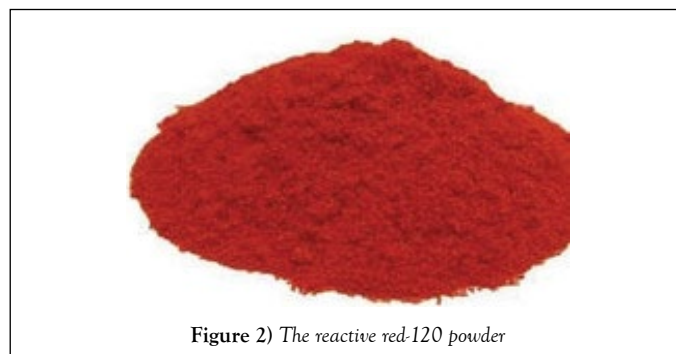


Figure 2) The reactive red-120 powder

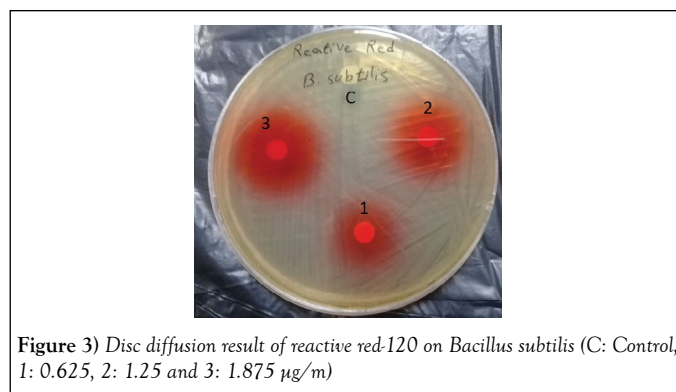


Figure 3) Disc diffusion result of reactive red-120 on *Bacillus subtilis* (C: Control, 1: 0.625, 2: 1.25 and 3: 1.875 µg/ml)

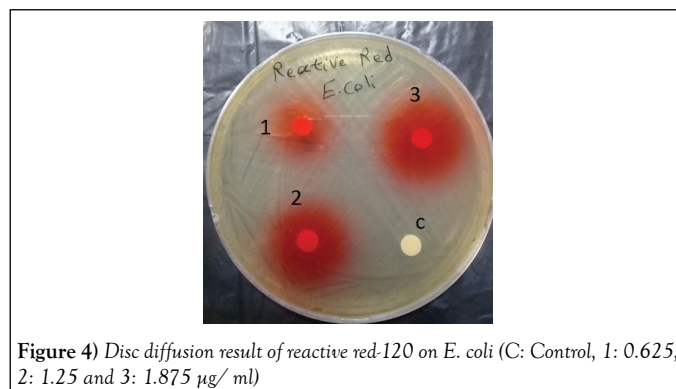


Figure 4) Disc diffusion result of reactive red-120 on *E. coli* (C: Control, 1: 0.625, 2: 1.25 and 3: 1.875 µg/ml)

(CA) assay is one of methods to measure cytotoxicity and genotoxicity of a test substance (23). For example, we studied cytotoxic, genotoxic and protective effects of a food coloring (4-MEI) with CA assay and another method, the results of these studies showed that 4-MEI have cytotoxic, anticancer and antioxidant effect but don't have genotoxic and antigenotoxic effect on different test system (24-27). There are many examples for textile dye that has toxic or antibacterial property against different bacterial strains. Leme and et al. reported that reactive green 19 textile dye has genotoxic effect on human dermal equivalent cells (28). In another research the genotoxic effect of Disperse Red 1 was studied in mouse germ cells. The results obtain from this study was showed the harmful activity of this dye on reproductive health (29). Similarly, antimicrobial effect of *Quercus infectoria* was studied on *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa*. The results obtained from this study showed that *Quercus infectoria* have antimicrobial activity on different bacterial strains (30). In contrast to Reactive red-120, some of dyes have antimicrobial activity on gram positive and gram negative bacterial strains. MAJHI et al. observed that the dye obtained from *Morinda citrifolia* have antimicrobial activity on *Klebsiella pneumoniae*, *Escherichia coli*, *Candida albicans* and *Aspergillus sp.* (31). Gul and Bakht reported that turmeric dye inhibited *Escherichia coli* and *Staphylococcus aureus* growth by disk diffusion analysis (32).

## CONCLUSION

There was no report regarding to the antimicrobial effect of Reactive red-120 in the available literatures. As our result, Reactive red-120 doesn't have antibacterial effect on *Bacillus subtilis* and *E. coli*. So, it can be concluded that Reactive red-120 might not pose a potential risk for nature. However, it must be evaluated with different new studies.

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