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Role of Azoreductases in Bacterial Decolorization of Azo Dyes

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Abstract

Azo dyes are largest class of synthetic dyes used in textile industries. The disposal of wastewater from textile industry may have serious environmental concern. Bacterial decolorization of azo dyes from textile wastewater is an environment friendly and cost effective treatment system. Various species of bacteria produces azoreductase enzyme which have shown great potential to decolorize the textile azo dyes via reductive cleavage of azo bond under anaerobic or aerobic conditions.

Keywords: Bacteria; Azo dyes; Azoreductase; Decolorization; Wastewater

Introduction

Synthetic dyes are used in textile, cosmetics, food, paper, and printing industries. Among the different groups of synthetic dyes, azo dyes are extensively used in textile industry. The textile industry produced large amount of wastewater contaminated with the dyestuffs due to the high quantities of water used in the dyeing processes [1]. Improper textile wastewater disposal in aquatic ecosystems are aesthetically unacceptable causing severe environmental problems. These effluents can alter the physical, chemical and biological nature of the receiving water body. In addition, azo dyes and their metabolic intermediates are toxic to humans as well as other animals [2]. Therefore, removal of dyes from textile wastewaters is necessary prior to their disposal into water bodies. Several conventional physico-chemical techniques have been developed for color removal from dyes containing effluents, but these methods have many disadvantages and limitations due to their high cost, low efficiency and inapplicability to a wide variety of dyes. Therefore, it is necessary to develop an efficient, cost-effective and viable alternative method for the decolorization of dyes in textile wastewater. The bioremediation perspective can overcome these limitations because it is cost competitive, produces less amount of sludge and eco-friendly alternative to the conventional physico-chemical treatment. Several microorganisms including, bacteria, fungi, algae and yeast have been reported for decolorization of dyes [3]. However, bacterial systems are much more useful for the treatment of textile wastewater due to their ubiquitous nature and adaptation to survive in extreme environmental conditions. Bacterial decolorization of azo dyes is generally based on the azoreductases (oxidoreductase enzyme) which is produced by different trophic groups of bacteria. These azoreductases are generally categorized as flavin dependent or flavin independent azoreductases on the basis of their function. These enzymes catalyzed the reductive cleavage of azo bond which leads to the decolorization of azo dyes into their corresponding aromatic amines [4].

Bacterial decolorization of textile azo dyes

Diverse groups of bacteria are frequently applied for decolorization and complete mineralization of dyes because they are easy to cultivate, grow rapidly and express azoreductases which are generally responsible for decolorization of dyes [5]. The process of decolorization by bacterial system may be anaerobic or aerobic. The initial step in the bacterial decolorization of azo dyes is the reductive cleavage of azo bond by an enzymatic biotransformation reaction which leads to the formation of colorless aromatic amines. The resulting toxic aromatic amines are further degraded to simpler non-toxic forms under aerobic or anaerobic conditions [6].

Mechanism of azoreductase in bacterial decolorization of azo dyes

Azo dyes are electron-deficient xenobiotic compounds because of their azo linkage (-N=N-) [7]. Under the appropriate conditions, these dyes can be degraded by azoreductases in the presence of reducing equivalents, e.g., NADH and NADPH as an electron donars [8]. In general, reduction of -N=N- bond (azo linkage) with help of an azoreductase enzyme is the initial step in bacterial decolorization of azo dyes (Figure 1). This involves transfer of four electrons from NADH to the azo linkage of dye via FMN in two sequential steps. In each step, two electrons are transferred to the azo dye (terminal electron acceptor) resulting in dye decolorization and the formation of colorless aromatic amines [9].

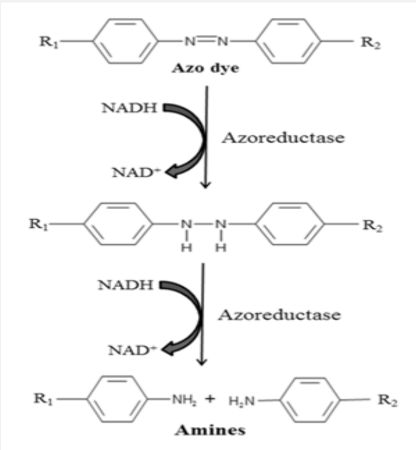

Figure 1: Decolorization of azo dye by azoreductase.

Bacterial decolorization of textile azo dyes under anaerobic condition

Decolorization of azo dyes under anaerobic conditions is a simple and non-specific process. Bacterial decolorization under anaerobic conditions involves the reduction of the highly electrophilic azo bond in the dye molecule, by a variety of soluble cytoplasmic azoreductases with low-substrate specificity [10]. The presence of azoreductases in anaerobic bacteria was first reported in the genera *Clostridium* and *Eubacterium* that decolorized sulfonated azo dyes [11]. Azoreductases from these strains were oxygen-sensitive and were produced constitutively and released extracellularly. Dye decolorization under anaerobic conditions requires complex organic carbon/energy source. It might be attributed to non-specific extracellular reactions occurring between reduced compounds generated by the anaerobic biomass [12]. Many of the bacterial cultures were able to grow aerobically but decolorization was achieved only under anaerobic conditions. Many bacterial species such as *Bacillus*, *Citrobacter*, *Pseudomonas*, *Clostridium*, *Staphylococcus*, and *Micrococcus* were found to be effective in the anaerobic decolorization of a number of azo dyes [2,13,14].

Bacterial decolorization of textile azo dyes under aerobic condition

Azo dyes are not readily metabolized and persistent under aerobic conditions because the presence of oxygen usually inhibits azo bond reduction activity [15]. However, some bacterial strains possess the ability to reduce the azo linkage by reductive mechanisms under aerobic condition [16]. These bacteria are generally specific towards their substrate and need long-term adaptation with the substrate. The adaptation involves long term aerobic growth of bacteria in continuous culture condition in presence of the simple azo compound. The adapted bacteria synthesize an oxygen-insensitive or aerobic azoreductase specific for azo compound which can reductively cleave the azo group under aerobic condition [17]. These intracellular azoreductases showed high specificity to the dye structures and required NADPH and NADH as cofactors for activity. There are only very few bacteria that are able to grow on azo compounds as the sole carbon source. These bacteria reductively cleave –N=N– bonds and utilize the resulting amines as the source of carbon and energy for their growth. Species of *Bacillus, Sphingomonas, Pseudomonas* and *Listeria* were reported for decolorization of azo dyes under aerobic condition [7,18,19].

Conclusion

Azo dyes constitute the largest class of synthetic dyes used in textile industry which is largest generator of wastewater contaminated with the azo dye. The discharge of these colored effluents into the environment affects the all forms of life. It is, therefore, necessary to select the best option for removal of azo dyes from textile wastewater. In recent years, decolorization of azo dyes by bacteria is gaining more attention. An enzyme azoreductase which is produced by many species of bacteria play a major role in decolorization of azo dyes.

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