ROLE OF AZOREDUCTASE IN AZO-DYE TREATMENT

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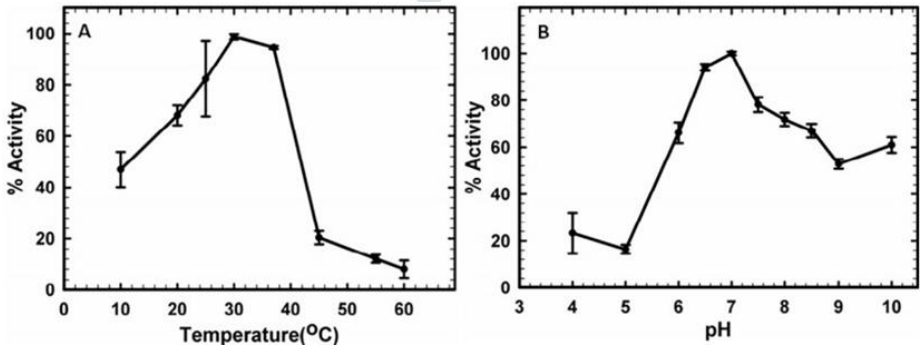
Abstract: Azo dyes one of the major dyes that are used in textile industries they have a unique bond called azo bond (N=N) which is also a functional group, found mostly in synthetic dye together with some aromatic ring structures. As we know the treatment of textile is done by this dyes along with articles of lather, and some other foods. Degrading the azo linkage of azo dyes at the first stage is a crucial process in the decolorization of this dyes. The microbial enzyme use of azoreductase which are accepted in a biological way is environmentally helpful and demonstrated to degrade azo dyes having a variable pH, great temperature stability and wide substrate specificity. In this review a type of report and information on the degradation of different type of azo dyes is made. Capabilities of a strain from azoreoductase on the degradation of an azo dye was further proven and investigated. In this review, plate assay method showing the degradation and decolorization of methyl red and Congo red and strain isolation, isolating the enzymes which will be most efficient for degradation for the dyes Acid Yellow 17 (AY17) and Disperse Blue 64 (DB64).

Index Terms: Azo dye, azoreductase, dye treatment.

I. INTRODUCTION

Azo dyes is an organic compound which bears the functional group which consist of an N=N bond and on either side of the N bone consist of aryls, Azo dyes consist 70% of the demand in the global textile industrial demand. Since it has a genetic complexity of causing mutation, the every year disposal of azo dyes causes a problem to the wellbeing of the environment and the ecosystem. This azo dyes are used widely in various industry such as the textile industry, food industry printing industry and many more [1, 2]. Several physical methods and chemical methods like the chemical method of adsorption and various treatment in pair of ion and ion extraction have been used through various aerobic and anaerobic approaches which are very costly and also making a huge amount of sludge. Due to this the study of decolorization of azo dyes now focuses on other methods like biological approach. Some microorganism have been proved to have helped in the decolorizing process of azo dyes this microorganism are from bacteria and fungi and through the process of biodegradation this methods are seen to be working and efficient [3-4]. Some removal of the dye was being done by the process of bio sorption on the cell membrane and the removal of the dye was possible with the help of different functional group present during the process. From this we can see that the degradation through biological method and microorganism have a potential advantage in the treatment of water. With the uses of Azoreductase the canalization of the dependent NAD(P)H with the help of amines degrade the azo compound and after this the process than continued to cleave the azo linkage in the azo dye (-N=N-), which than can be called the degradation of an azo dye[5-6]. This review presents an insight and evaluating the properties of some of the enzymes from azoreductase found in other microorganism. It will also shed a light on its classification method and the degradation process.

The known enzymes are stable at a pH of 5-9 along with alkalophilic azoreductases, thermal stability is at interval temperature that varies at 25–85 °C. The optimum study of pH was done in a pH scale of 4-10 at a temperature of 30°C and 70°C. Activity of this enzymes at different temperatures were made. A researcher found that the dependent NADH which has a no favin and can be activated at a temperature of 70°C. Azoreductase stability at basic pH scale and better temperatures might build them a lot of proficient for an industry because their useful function for pharmacy. As extremely equilibrium and are efficient azoreductases are hopeful in helping the treatment of further azo dyes. [7-8].



°C

Figure 1: Temperature and hydrogen ion concentration optimum: (A) shows the optimum operating temperature i.e. 30°C-37°C (B) Shows the optimum ion concentration at 4 pH to 10 pH is 7.2 [8]

II. METHODS OF DEGRADATION AND DECOLORAIZATION

2.1 Degradation using plate asay

Here we will be talking about the decolorization for two type of azo dye i.e. Methyl red and Orange G, the method of decolorization and which enzymes we are using to decolorize this Azo dyes, we will also see the comparison of their decolorization by plate assay method for different temperature and pH.

2.1.1 Azo dyes

Methyl Red

Methyl red $C_{15}H_{14}N_3NaO_2$ which are also called Acid Red 2, is a dye used for indication which then turns red in acidic solutions [9, 10].

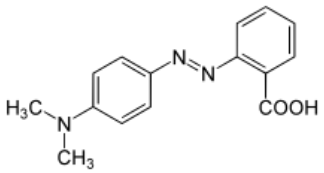


Figure 2. Structure of Methyl Red [10]

Congo red

Congo red is a type of dye that is used for indication is element salt of benzidinediazo-bis-1-naphthylamine-4-sulfonic. It's also considered as an Azo dye cause of its diazo bond. [11]

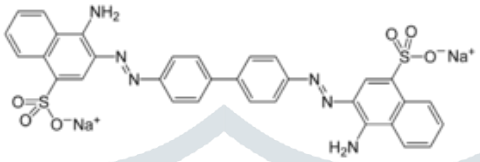


Figure 3. Structure of Congo red [12]

2.1.2 Plate assay method

Montira Leelakriangsak and Sukallaya Borisut had research and worked on a title "The process and method of the degradation of azo dyes using the enzyme azoreductase AzoR1" B. a subtilis strain was used in this study and were known to be a type of a main enzymes strain i.e JH642 and azoR1 and also found an overexpression strain ORB7106: azoR1.ORB7106 was made by a method of transforming of azoR1-lacZ which is a fusion of two types of strain where the DNA helps with its very on strain to disrupts. The type of method used was that of plate assay method, this method was easy and also was a fast screening method. ORB7106 was growing well when presence could be fled from the chemical group dyes and altogether tested concentrations (10, 50, a hundred and two hundred mg l-1). The ability of the detoxification of azoreductases was found by the formation of a shaped of halo surrounding every colony by plate assay at intervals of forty eight hours. The microorganism was ready to decolor aerobically MR and CR were efficiently decolorized over a good vary of hydrogen ion concentration (5-9) and temperatures (25-40°C).

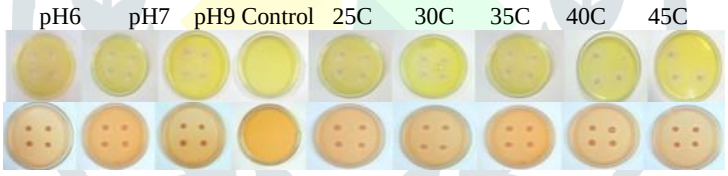


Figure 4: Effect of pH (between 5, 7, 9) and the temperatures (between 25°, 30°, 37°, 40° and 45°) of the amount of decolorization done to the given dyes. First fig is that of Methyl Red and second fig is that of Congo red [13]

2.2 Strain isolation

According to the research of Hesham et al, isolation of a bacterial species to decolorize a dye was carried out through his process [14]. An amount of water approximately about 10 mL were suspended in a medium (MBS) consisting of 90 mL. MBS was then mixed with or added upon with a BB64 OR AY17 (100 mg) a carbon source was than required so glucose (0.1%) was added to act as the source. An orbital shaker was used for the incubation of flasks at a speed of 150 rpm on a temperature scale of 30 C. A new flask which contains 90 ml of the original medium which was then added with the mentioned dye in the above sentences in this 10% of enriched cultures were added which were taken from the incubated flask after 7 days of incubation. The step that have been mentioned were future repeated a total of 5 times so that we could obtain a decoloring enzyme which is well adapted. For the final stage an individual selection of colonies were required for the purification and evaluation of the ability of decolorizing a dye, so in this cultures were moved to an agar medium which was mixed or added on with dye (100 mg) at a temperature of 30 C with a pH of 7.0, and finally the selection process was able to begin.

2.2.1 Measuring azodye degradation by the UV-vis Spectrophotometer

An overnight culture were inoculated in MBS medium added with glucose (0.1%) Along with the tested dye (100 mg) on a temperature of 30 C. The decolorization percentage was calculated from the given equation below [15].

$$\% \text{ of decolorization} = \frac{A0 - At}{A0} *$$

At = absorbance of dye at time t

A0 = initial absorbance of dyes

2.2.2 Dragadation of azodyes by Pseudomonas strain

Different Pseudomonas strains undergone isolation process selected from various dye and they were classified according to their capability to degradate an azodye. From this two strain shows a hyper capability to the degradation of an azo dyes those two strain are ASU3 AND ASU6, having higher extent of degradation (>90%), using the equation of Uv-vis spectrophotometer we can calculate the percentage of degradation so for this two azodyes were taken i.e AY17 and DB64 at 398 nm and 600 nm respectively. Using the strain ASU3 and ASU6 to degradate the two given dyes the result are shown as 61.2% and 96.8% of degradation for DB64 within a time of 48 h, whereas for the dye AY17 we got the degradation percentage to be at 38.6% and 91.2% respectively.

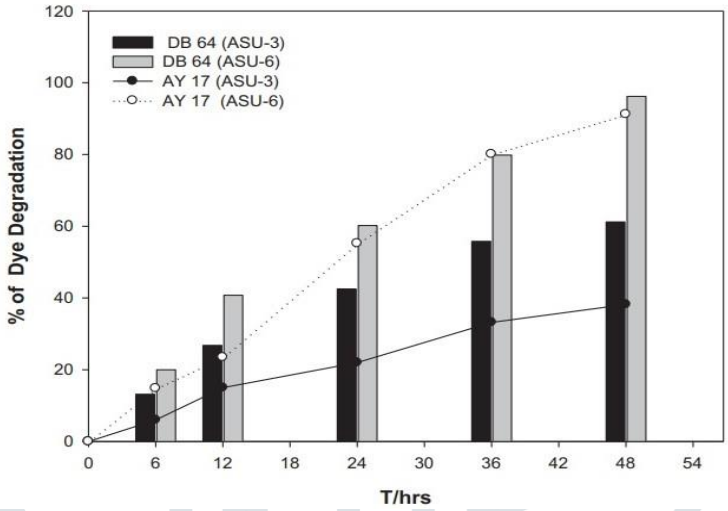


Figure 5. Percentage of degradation by ASU3 and ASU6 on the dyes DB64 and AY17 under a temperature of 30C and pH 7.0 [16].

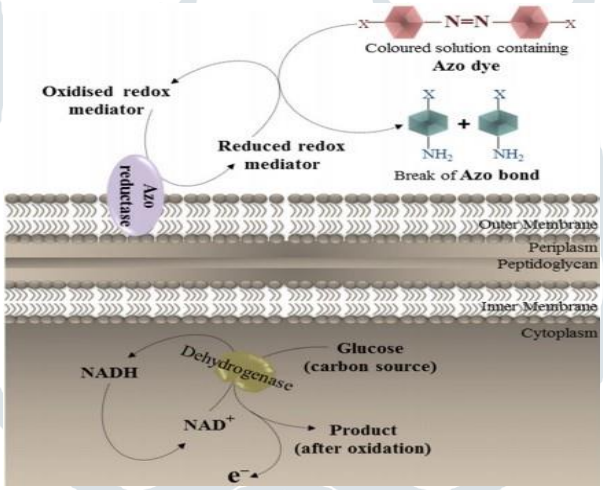


Figure 6: The process and working principle of the reduction and degradation process of azo dyes by using azoreductases [22]

III. DEGRADATION AND DETOXIFICATION PROCESS BY AZOREDUCTASE

The process of an organism to be able to cut back the radical dyes and the nitro group of some compounds was shown since a few years. As we all know Azoreductases are one of the most used enzyme that are used in the process of degrading the N to N double bond that are seen in the azo dyes, which than efficiently cut back group in nitro-aromatics. A low relative molecular mass is needed to decrease the link between NADH and FADH as they act as electron doner of a reaction [17]. On the premise of molecule use, this catalyst is of 3 types: victimisation NADH solely, victimisation NADPH solely, or both.

The cleavage of radical bond (−N=N−) was done by the enzyme which than sends four electrons as a reducing equivalent. On every stage, another pair of electron gets transfer which acts as an associate to the other electron acceptor all this are transfer to the azo dye which causes decolorization which than creates a colorless liquid. Resulting in an intermediate in toxic aromatic alkane that later degraded by aerobic method. In this condition [18], a redox mediator is used by the enzyme to associate electron shuttle (Fig. 2). The mediators metabolic results of sure substrate employed by organisms, like anthraquinonesulfonates. A Non-sulfonated are known to be degraded by a liquid of protoplasm azoreductase [19].Anaerobic condition is a lot better than the later one, as the enzyme are oxygen-sensitive. Therefore, in aerobic condition, the enzyme comes up to gas and the mediator shown a decline either than that on the dye. Often, a regular or a few enzyme can also get transformed into a dye degrading enzyme under a few strange atmosphere, as an example, at times, flavin enzyme are acting and becoming as azoreductase which than use FADH as a reaction intercessor. [20, 21].

4. Conclusion:

The review article kindly discusses the uses and roles of the enzyme called Azoreoducases their methodes and understanding of how it works on the degradation, decolorization and the detoxification of the dyes called azo dyes for the

treatment of the pollution that is caused by the dyes. The Decolorizing process by this enzymes was tested and is contributed by plate assay method as well as isolation methodwas confirmed and were efficiently decolorized.

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