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Review

Role of microorganisms in biodegradation of food additive Azo dyes: A review

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Food additives Azo dyes are synthetic compounds added to foods to impart color and improve their properties. Some azo dyes have been banned as food additives due to toxic, mutagenic, and carcinogenic side effects. Long exposure to foods containing azo dye leads to chronic toxicity. Some microorganisms are capable to degrade these dyes and convert them to aromatic amines. In human body, microbiota can play a vital role in biodegradation of azo dyes by producing azo reductase. Aromatic amines are toxic, water-soluble and well absorbed via human intestine. In the current study, the role of microorganisms in biodegradation of six dyes related to azo group was discussed. These dyes are: Tartrazine E102, Sunset Yellow E110, Ponceau E124, Azorubine E122, Amaranth E123, and Allura Red E129 which are classified as the most harmful food additive dyes.

Key word: Food additive, azo dyes, microorganisms, azo reductase, aromatic amines.

INTRODUCTION

Food additives are synthetic compounds added to food for many proposes such as maintaining the product from deterioration or improving its safety, freshness, taste, texture or appearance (WHO, 2012). Color additives are extensively used in food, cosmetics, and drugs (Macioszek and Kononowicz, 2004). Colorants are widely used for giving attractive coloring properties; every year industrial factories produce about 8 million tons of food colorants. Azo dyes are one of the most famous dyes used in coloring food, cosmetics, pharmaceutical products, and in the textile industry (Lorimer et al., 2001). Azo dyes are organic compounds containing an azo group (-N=N-), but some dyes have two (diazo), three (triazo) or more (Benkhaya et al., 2020; Bell et al., 2000). These dyes are aromatic compounds that are usually stable and highly water soluble.

In the USA and European countries, some azo dyes have been banned as food additives due to toxic, mutagenic, and carcinogenic side effects (Chung, 2000).

Azo dyes can cause many diseases such as, edema of the face, tongue, neck, larynx and pharynx, contact dermatitis, respiratory disease, lacrimation, hypertension, exophthalmos, upon ingestion, blindness, rhabdomyolysis, skin irritation, chemosis, vomiting gastritis, vertigo and acute tubular necrosis supervene (Young and Yu, 1997).

Some microorganisms are capable of utilizing azo dyes and degrade them, but intermediate compounds are toxic, mutagenic, and carcinogenic (Houk et al., 1999, Helal et al., 2000; IARC, 1982). However, azo dyes do not accumulate in the human body cells; they are metabolized in the liver by azo reductase and excreted out in the urine (Hassan and Elnmer, 2017).

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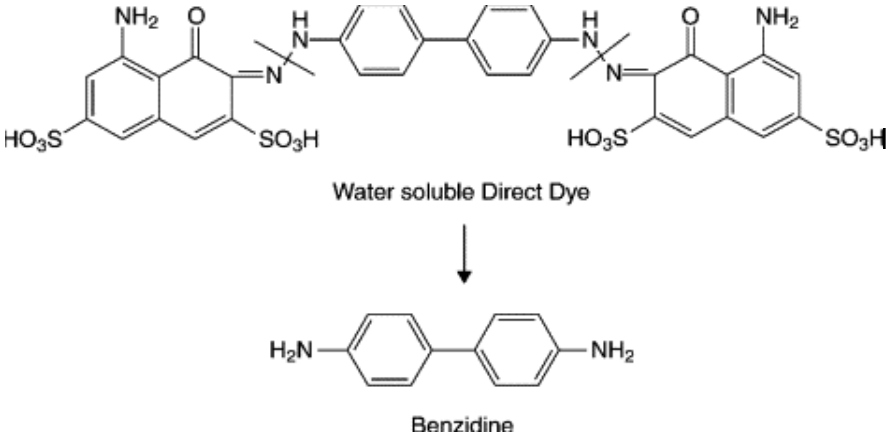

Figure 1. Reduction of azo dye to Benzidine.

The danger of these compounds is not due to the dye itself, but to intermediate products produced through biodegradation of the dyes (Varjani et al., 2020). Reduction of azo group (N=N linkage) leads to production of aromatic compounds which are more toxic and known for causing mutations and carcinogenic diseases (Puvaneswari et al., 2006; Alabdraba and Bayati, 2014).

In some cases, chronic toxicity affects cellular viability which leads the cell to absorb the dye instead of decolorizing it (Chen, 2002).

For example, 1,4-diamino benzene is intermediate compound and a major component of azo dye, it releases when azo dyes degrade (Figure 1). Benzidine causes human and animal tumors and contact allergen. Human intestinal microform, skin microflora, and environmental microorganisms can degrade azo dyes .Reduction of azo compounds can also occur by human liver azoreductase, or by non-biological factors (Chang, 2016).

Non-biological factors such as temperature, pH, oxygen level and structure and concentration of dye help in biodegradation process (Ajaz et al., 2019; Varjani et al., 2020).

A lot of studies discussed the capacity of these microbes to degrade food additive azo dyes; the aim of this study is to focus on:

(i) Chemical structure, properties and safety of different azo dyes that are used as a food additive such as Tartrazine E102, Sunset Yellow E110, Ponceau E124, Azorubine E122, Amaranth E123, and Allura Red E129 classified as the most dangerous additive dyes base on their side effects on human being.
(ii) Role of microorganisms in biodegradation of additive food azo dyes.

TARTRAZINE (E102)

Tartrazine (E102) is one of the most famous artificial colors; it is also referred to as FD&C yellow #5. The molecular weight is 534.3, and the formula is $C_{16}H_9N_4Na_3O_9S_2$. Figure 2 shows chemical structure of Tartrazine.

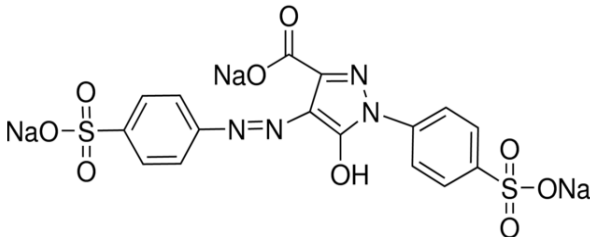

Figure 2. Chemical structure of Tartrazine.

The acceptable daily intake (ADI) is defined as the amount of a chemical that can be taken daily for an entire lifetime without any adverse reaction. The ADI of Tartrazine is up to 7.5 mg/kg bodyweight (FAO,WHO, 1964). It is used as a food additive to improve properties and give the color for food products.

Many reports recorded that Tartrazine can cause chronic urticarial and asthma (Lockey, 1959). Researches have also mentioned that Tartrazine dyes can cause some problems in children such as allergies, hyperactivity, learning impairment, irritability and aggressiveness as results of eating them from some foods like sweets and ice cream (Romieu, 2005).

A lot of studies discussed the ability of microorganisms to degrade Tartrazine. Some studies focus on human intestinal microflora in degradation of TZ. Human intestinal microflora is a group of microbes found in the small intestine (Sherwood et al., 2013).

Human gut microbes can produce azoreductase enzymes that degrade azo dyes, Azoreductases breakdown azo bonds (R-N = N-R') and produce colorless aromatic amines (Guillou et al., 2016). Aromatic amines dissolve in water and are absorbed easily via human intestine (Bomhard and Herbold, 2005).

Perez-Diaz and McFeeters (2009) investigated the ability of *Lactobacillus casei* and *Lactobacillus paracasei* in a modification of the azo dye, Tartrazine. They found 14 other lactic acid bacteria (LAB) that are capable of removing the food coloring Tartrazine.

Oranusi and Njoku (2005) discussed the ability of *Streptococcus faecalis* and *Escherichia coli* isolated from human intestinal microflora in biotransformation of food dyes (Tartrazine and Quinoline yellow). Isolated bacteria were maintained in media containing these dyes. Decolorization in aerobic conditions was higher than anaerobic conditions. Microorganisms can produce cytoplasmic flavin reductases and redox equivalents by metabolism of soluble starch and transfer electrons to the chromophoric group of the dyes.

Fungi also can degrade food additive dyes and remove their toxicity. Das and Das (2017) studied the effect of twelve types of fungi (*Irpex lacteus, Aspergillus species, Penicillium geastrivorus, Datronia sp., Myrothecium roridum, Polyporus arcularius, Fomitopsis feei, Pleurotus ostreatus, Trametes versicolor, Trametes hirsuta, fomes fomentarius* and *Ganoderma lucidum)* on decolorization and detoxification of different types of toxic azo dyes. Results showed the capacity of these fungi in biodegradation of different food additives. Toxicological assays by using daphnids showed a significant reduction of toxicity after dye decolorization of 12 types of fungi.

AZORUBINE E122

Azorubine or carmoisine is a synthetic azo dye; it is red food color and very soluble in water. The ADI is 4 mg/kg/day. The molecular weight is 502.431 and the formula is $C_{20}H_{12}N_2Na_2O_7S_2$. Figure 3 shows the chemical structure of AZ.

Azorubine shows possible effects on human health such as allergic reactions, rashes, skin swelling and hyperactivity, while some researches have shown no evidence of carcinogenic or mutagenic.

Kiayi et al. (2019) investigated the ability of *Saccharomyces cerevisiae* ATCC 9763 in degradation of Azorubine. Azorubine (Carmoisine) was removed from the aqueous medium after incubation with *Saccharomyces* for seven hours under anaerobic conditions. Results of spectrophotometry and chromatography confirmed biodegradation products of carmoisine into aromatic amines.

In the study of Au and Dzulkafli (2012), it was found that *P. ostreatus* has the capability in decolorizing of four different food dyes: Carmoisine Red, Tartrazine Yellow, Brilliant Blue, and Fast Green. UV-Spectrophotometer data confirmed complete decolorization of 10 ppm food dyes by *P*. *ostreatus*. *P. ostreatus* recorded percentages of degradation of the dyes with Carmoisine (81.34%), Tartrazine (45.36%), Fast Green (28.99%) and Brilliant Blue (19.82%) during aerobic conditions.

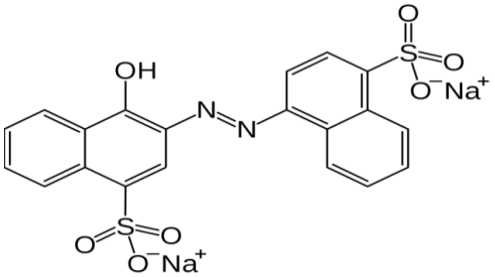

Figure 3. Chemical structure of Azorubine.

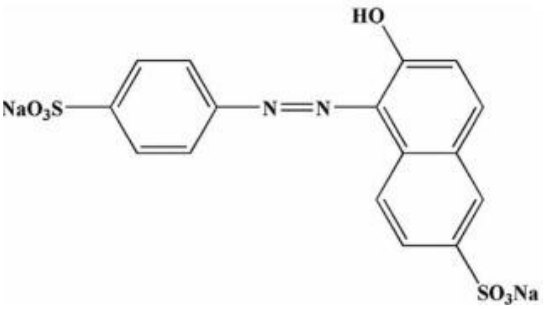

Figure 4. Chemical structure of sunset yellow.

SUNSET YELLOW E110

Sunset Yellow is a synthetic azo dye used for coloring foods, it is also known as FD&C Yellow 6; its formula ($C_{16}H_{10}N_2Na_2O_7S_2$,), Molecular weight is 452.38 g/mol, chemical structure is shown in Figure 4. The ADI is 4 mg/kg bw/day.

Sunset Yellow may cause anxiety migraines, eczema, immunosuppression and asthma (Sarıkaya et al., 2012).

Muntholib et al. (2015) studied the ability of *Actinobacillus* sp. in degradation of sunset yellow dye and p-cresol. Bacterial inoculum in the growth phase and stationary phase were tested to degrade 70 ppm of sunset yellow in M9 medium. Changes in the dye concentration were measured by using a spectrophotometer at a wavelength of 482 nm. Results show *Actinobacillus* sp. inoculum in stationary phase is more effective in degrading sunset yellow (11.88%) compared to the percentage of growth phase inoculum (2.02%).

In the study of Elbanna et al. (2017), 120 lactic acid bacterial strains and 10 bacterial intestinal isolates were examined to study their effect on degradation of sunset yellow (E110) and carmoisine (E122). High-performance liquid chromatography (HPLC) data of sunset yellow (E110) and carmoisine (E122) under anaerobic conditions showed degradation products by human intestinal bacteria that they are chemically classified as toxic aromatic amines.

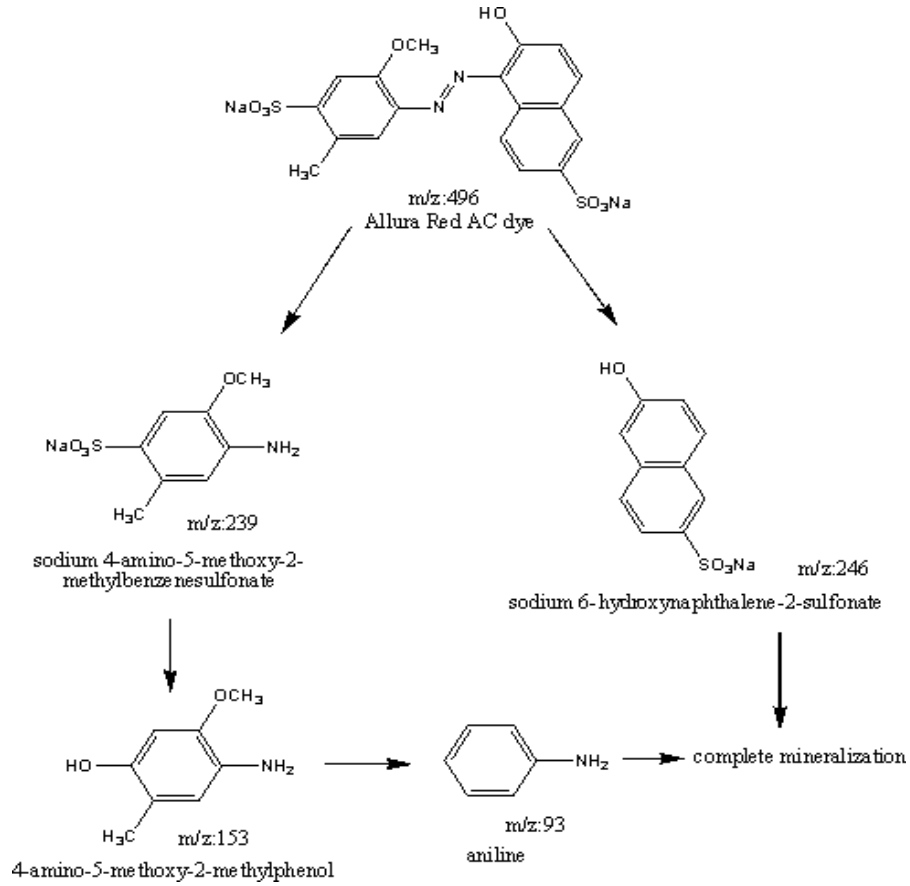

Figure 5. Proposed mechanism of ARAC by Ochrobactrum anthropi HAR08 (Kale and Thorat, 2014).

ALLURA RED AC (E129)

Allura red (E129) is synthetic diazo colorant and one of the most widely used dyes in food, drug, paper, cosmetic and textile industries. It is also known as FD&C Red 40. The formula is $C_{18}H_{14}N_2Na_2O_8S_2$ and molecular weight is 496.42 g/mol. The ADI is 0 to 7 mg/kg body weight (bw)/day.

Kale and Thorat (2014) studied the ability of *Ochrobactrum anthropi* (HAR08) in decolorizing of azo dye Allura Red AC. Percentage of biodegradation of (ARAC) recorded 95% in nutrient medium within 24 h. Degradation of Allura red was confirmed by FTIR spectroscopy and GC-MS techniques. It found that dye completely mineralized. Pathway of biodegradation of ARAC dye has been proposed Figure 5.

AMARANTH E123

Amaranth has been classified as an ionic dye. It can be applied to food, leather, paper, wood, synthetic and natural fibers (Anjaneya et al., 2013; Shahmoradi et al., 2011). It is known also as FD&C Red No. 2. Its formula is $C_{20}H_{11}N_2Na_3O_{10}S_3$ and molecular weight 604.473 g/mol. Chemical structure is shown in Figure 6. The ADI is 0.15 mg/kg / day body weight (bw)/day. It can cause acute and chronic toxicity. Amaranth dye can cause allergic, respiratory diseases, and human and animal tumors (Mittal et al., 2005). This dye could be mutagenic agent, genotoxic and carcinogenic (Jabeen et al., 2013; Jadhav et al., 2013). Considering its potential for hazardous toxicity, amaranth dye has been banned in many countries (Jadhav et al., 2013; Karkmaz et al., 2004).

Basu and Kumar (2015) studied the effect of food colorant on hemoglobin protein. They found significance conformational interaction changes between the dye and the protein by using 3D fluorescence techniques and FTIR. The interaction of amaranth with hemoglobin may enable realizing the toxic effects of azo dyes.

Amaranth dye is degraded by human intestinal microflora and its toxic or carcinogenic effects may derive from the microorganisms' degradation products (Ahmad and Kumar, 2011). Chan et al. (2012) investigated pathways metabolism of Amaranth dye by microaerophilic–aerobic consortium bacteria as

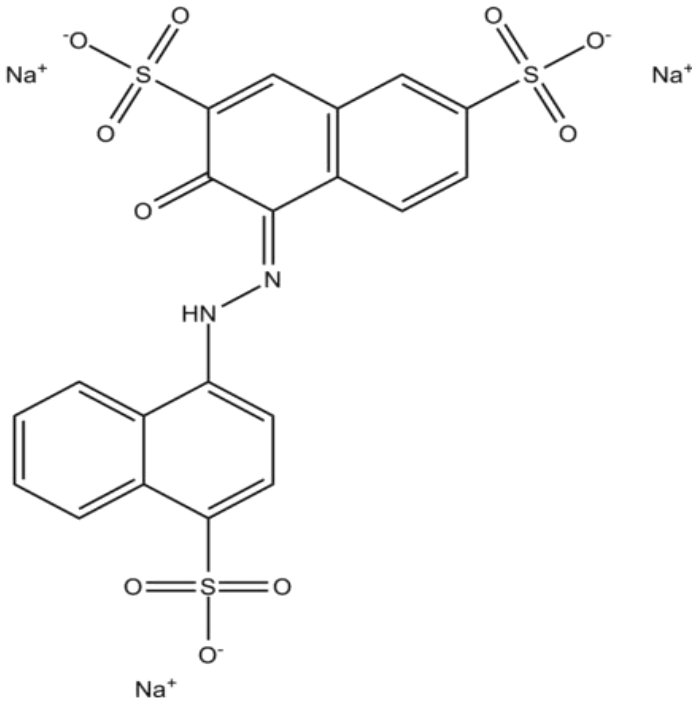

Figure 6. Chemical structure of Amaranth.

intermediates. During biodegradation of Amaranth dye by *C. freundii* and *E. cloacae* in aerobic conditions, azo linkage breakdown and produce intermediates that mineralized through metabolic pathways including benzoyl-CoA, protocatechuate, salicylate, gentisate, catechol and cinnamic acid. The steps of biodegradation of Amaranth by isolate NAR-2 are: Azo reduction, deamination, desulfonation and aromatic ring cleavage.

PONCEAU E124

Ponceau is widely used in coloring of some food such as soups, wine, cider sauces, preserves, etc. Its formula is $C_{20}H_{11}N_2Na_3O_{10}S_3$, molecular weight is 604.47 g/mol. The chemical structure is shown in Figure 7. The ADI is 0.7 mg/kg /day. It affects human health and causes urticarial, rhinitis and asthma.

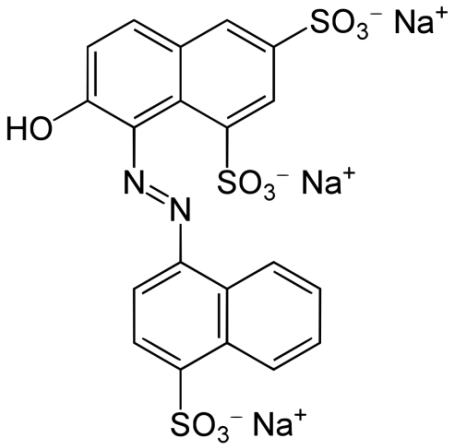

Figure 7. Chemical structure of Ponceau.

Masarbo et al. (2019) studied the decolorization of Ponceau 4R by 3 bacterial stains *Bacillus* sp. AK1, *Lysinibacillus* sp. AK2 and *Kerstersia* sp. VKY1 individually and in consortia. Spectrophotometry and chromatography analysis confirmed the products of biodegradation of Ponceau 4R and the formation of 4-aminonaphthalene-1-sulphonic acid and 5-amino-6-hydroxynaphthalene-2, 4-disulphonic acid as the products of azo bond breakage.

Cheng et al. (2016) studied the ability of sixty-three strains of white-rot fungi to degrade four types of textile azo dyes; Direct Blue 71 (C.I. 34140), Orange G (C.I. 16230), Ponceau 2R (C.I. 16450), and Biebrich Scarlet (C.I. 26905). Isolate *Coriolopsis* sp was only able to degrade four dyes with optimization of parameters such as temperatures, pH, nitrogen and carbon sources.

Omar (2008) studied the ability of green algae, diatoms, and cyanobacteria strains in degradation of Tartrazine and Ponceau. The results show that reduction of color depends on number of azo group in the dyes and the type of strain of algae. Biodegradation of azo dyes is related to activity of Azo reductase which is responsible

for reduction of azo linkage and produce aromatic amines.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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