A SEMINAR PAPER

ON

BLEACHING OF DYES USING BACTERIA

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SUBMITTED TO:

Course Instructors	Major Professor
1. Dr. A. K. M. Aminul Islam Professor , BSMRAU **2. Dr. Md. Mizanur Rahman** Professor , BSMRAU **3. Dr. Md. Rafiqul Islam** Professor, BSMRAU **4. Dr. Dinesh Chandra Shaha** Assistant Professor, BSMRAU	**Dr. Md. Manjurul Haque** Professor Department of Environmental Science BSMRAU, Gazipur

SUBMITTED BY:

POLASH KISKU MARCUS

MS Student

Reg. No.: 16-11-4162

Department of Environmental Science

BANGABANDHU SHEIKH MUJIBUR RAHMAN AGRICULTURAL UNIVERSITY, SALNA, GAZIPUR- 1706

BLEACHING OF DYES USING BACTERIA

ABSTRACT

Removal of synthetic dyes is one of the main challenges before releasing the wastes discharged by textile industries. Biodegradation of dyes by bacterial consortium is one of the environmental-friendly methods used for the removal of dyes from textile effluents. In fact, biodegradation can be used due to less expensive, reliable, and produce less sludge. In study of Congo red, the result found that consortium showed maximum decolorization (98%) compare to other bacteria like *E. coli, Salmonella sp., S. aureus, Proteus sp., Psedomonas sp. and Bacillus subtilis*. But incase of methyl orange and crystal violet, *Lactobacillus casei* TISTR 1500 and *Aeromonas hydrophila* were more tolerant for bleaching respectively. P^H 7 showed maximum rate of decolouration (70%) for Congo red but incase of orange acid, 37 ºC temperature was found for *Staphylococcus hominis* RMLRT03 strain for maximum decolouration. The review study will be informed about the isolation of textile dyes degrading bacterium from a dyes contaminated environment and their decolouration rate for further analysis.

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CHAPTER 1

INTRODUCTION

Rapid industrialization has necessitated the manufacture and use of different chemicals in day to day life (Baljeet and Poonam, 2011). The textile industry is one of them which extensively use synthetic chemicals as dyes. Wastewaters from textile industries carriage a threat to the environment, as large amount of chemically different dyes are used. A significant proportion of these dyes enter the environment via wastewater (Moorthi *et al.,* 2007). Approximately 10,000 different dyes and pigments which were used industrially and over 0.7 million tons of synthetic dyes are produced yearly all over the world. (Rafi *et al.,* 1990). Pollution due to textile industry effluent has increased during recent years. Moreover, it is very difficult to treat knit industry overflows because of their high BOD, COD, heat, color, pH and the presence of the metal ions (Anjali *et al.,* 2007). The textile finishing generates a large amount of waste water containing dyes and represents one of the largest causes of water pollution (Bhatti *et al.,*2008), as 10- 15% of dyes are lost in the waste during the dyeing process. The traditional textile finishing industry consumes about 100 liters of water to process about 1 Kg of textile material. The new closed-loop technologies such as the reuse of microbial or enzymatic treatment of dyeing effluents could help reducing this enormous water pollution (Abadulla *et al.,* 2000). However, most azo dyes are toxic, carcinogenic and mutagenic (Tomas *et al.,* 2004). Azo bonds present in these compounds are resistant to breakdown, with the potential for the persistence and accumulation in the environment (Telke *et al.*, 2007).

Crystal violet is also involved in paper, food, cosmetic, and rubber industries, and for coloring of oils, lipids, varnished plastics (Chiing-Chang *et al.,* 2007; Mittal et al., 2010; Parshetti *et al.,* 2011). CV is poorly metabolized by microorganisms and has a long life in the environment (Moturi and Singaracharya 2009). However, they can be degraded by bacteria under aerobic and anaerobic conditions (Zollinger H ,1991). Recently, an excellent overview on chemical treatment technologies for wastewater recycling was brought forth, and many methods were critically examined (Gupta et al. 2012a). In fact, biological/biochemical processes (biodegradation) can be used: they are less expensive, reliable, and produce less sludge. For example, microorganisms such as *Agrobacterium radiobacter*, *Pseudomonas putida*, *Bacillus cereus*, and *Aeromonas hydrophila* could degrade CV (Chiing-Chang *et al.,* 2007; Deng *et al.,* 2008; Ren *et al.,* 2006; Parshetti *et al.,*

2011). Several physio-chemical techniques have been proposed for treatment of colored textile effluents. These include adsorption on different materials, oxidation and precipitation by Fenton's reagent, bleaching with chloride or ozone photo removal or membrane filtration (Robinson *et al.*, 2001). All these physical or chemical methods are very expensive and result in the production of large amounts of sludge, which creates the secondary level of land pollution. Therefore, economic and safe removal of the polluting dyes is still an important issue. Bioremediation through microorganisms has been identified as a cost effective and environment friendly alternative for disposal of textile effluent (Ponraj et al., 2011). In present years a number of studies have concentrated on some microorganisms capable of degrading and absorbing dyes from wastewater. A wide variety of microorganisms are reported to be capable of decolonization of dyes (Moreno et al.,2012). The present study deals with the segregation of textile dyes humiliating bacterium from a dyes contaminated environment, its ability to degrade reactive dyes. Several physicochemical methods, such as filtration, coagulation/flocculation, reverse osmosis, adsorption, oxidation, ozonation, and electrolysis have been used for the removal of dyes from wastewater effluent.

However, implementation of physical/chemical methods have the inherent drawbacks of being economically unfeasible (as they require more energy and chemicals), being unable to completely remove the recalcitrant azo dyes and/or their metabolites, generating a significant amount of sludge that may cause secondary pollution problems (Forgacs et *al.*, 2004). However, the microbial decolorization also known as biobleaching and degradation of dyes has gained the considerable interest of researchers because it is inexpensive, eco-friendly, and produces less amount of sludge (Oturkar *et al.*, 2013). Among the microorganisms, bacteria are more effective to bleach a wide range of azo dyes (Saratale *et al.*, 2011). However, bleaching of wastewater containing dyes using bacteria is quite a new concept for Bangladesh. So, it is important to understand the types of bacteria and different factors involving discoloration of dyes for its better utilization, application and further study.

Objectives

- To have an overall idea regarding dyes and their ecological significance
- To overview the different types of bacteria and their action during decolorization
- To review the effects of different physical parameters during bleaching of dyes

CHAPTER 2

MATERIALS AND METHODS

This seminar paper is exclusively a review paper. It has been prepared by reviewing the various articles published in different Books, Proceedings, Abstracts, Review papers, Journals, Online Resources, MS thesis and PhD Dissertations etc. available in the library of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh. Valuable suggestions from honorable major professor and other resource personnel were taken into account to enrich the paper. After collecting necessary information, it was compiled and arranged chronologically for the fulfillment of the objectives.

CHAPTER 3

REVIEW OF FINDINGS

3.1 Dye

A color additive is a substance capable of conveying its color to a given substrate, such as paint, paper or cotton, in which it is present. Dyes and pigments are the most significant colorants used to add a color or to change the color of something. A dye must be soluble in water which called application standard, in the coloration process. It also shows some substantively for the dyed material and from the aqueous solution it absorbed. Pigments are the colorants composed of particles that are insoluble in the application standard (Broadbent 2001). Pigments and dyes are used all over the world as colorants in the fabric industry, pharmaceutical, food, cosmetics, plastics, paint, ink, photographic and paper industries. Statistics shows that over 10,000 different dyes and pigments are used industrially and over 70,0000 tons of synthetic dyes are each year produced in the world (Chequer *et al.*, 2013).

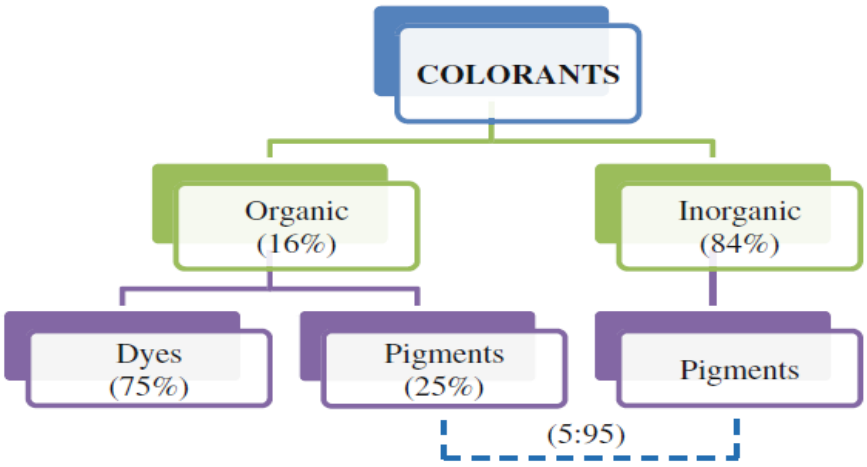

Figure1. Share of colorants from organic and inorganic classes (Zollinger,2003)

3.2 Dyes and Pigments

Both pigments and dyes are used to provide color to all sorts of substances and it is important to humans from the dawn of history. The similarity and dissimilarity among them is that dyes are soluble in the substrate and thus disperse at a molecular level, while pigments are insoluble and are dispersed as particles. Conventional pigments provide small color than dyes, but they are less light stable and not permanent (Rothon 2012). From the ancient time, man has been fascinated to

color the objects of daily use employing inorganic salts or natural pigments of vegetable, animal, and mineral origins. These dyes are well known and the chemical complexes used for coloring fabrics, leather, plastic, paper, food items, cosmetics, etc. Dyes are of two different types, i.e. synthetic and natural. Petroleum compounds synthetic dye, whereas plant, animal, and mineral matters produces natural dyes are (Singh and Bharati 2014). It is understood that colorants are normally include both pigments and dyestuff. Pigments known as inorganic salts and oxides, such as iron and chromium oxides, which are usually dispersed in crystal or powder form in an application medium Dyes (also called dyestuff) are conventionally understood to refer to organic molecules dissolved, as molecular chromophores, in the application medium. Examples are azo dyes, coumarin dyes, and perylene dyes (Zhang 2010).

Dyes are colored substances describe everywhere that have affinity to the substrates to which they are being applied (Pereira and Alves 2012). Dyes are soluble and go through an application process which, at least temporarily, by absorption, solution, and mechanical retention destroys any crystal structure, or covalent chemical bonds (Nwokonkwo 2013). pigments are different colored, black, white or fluorescent particulate and physically and chemically unaffected by, the vehicle or substrate in which they are incorporated, organic or inorganic solids which usually are insoluble defined by Color Pigment Manufacturers Association. They alter appearance by selective absorption and/or by scattering of light (Merchak 2012).

Table: 1 The relationship between color and wavelength of absorbed light

Wavelength absorbed (nm)	Color absorbed	Color observed
400–435	Violet	Yellow-Green
435–480	Blue	Yellow
480–490	Green-Blue	Orange
490–500	Blue-Green	Red
500–560	Green	Purple
560–580	Yellow-Green	Violet
580–595	Yellow	Blue
595–605	Orange	Green-Blue
605–700	Red	Blue-Green

Source:(Burkinshaw, 2016).

3.3 Ecological significant of dyes

Due to the fact that the dyes are synthesized to be chemically and photolytically stable, they are very much visible (detected in concentration < 1 mg/l) and found in natural environments. Consequently, the release of potentially hazardous dyes in the environment can be an ecotoxic risk and can be affect human by the food chain (Van der Zee and Villaverde, 2005). Dye concentrations below 1 mg/l cannot affect Algae growth and fish. Basic and acid dyes are the most toxic dyes for algae and fishes are. In the living thing tests only a few dyes showed LD50 values below 250 mg/kg body weight, whereas a majority showed LD50 values between 250 and 2000 mg/kg body weight (Van der Zee and Villaverde, 2005). Sensitization to dyes has been seen in textile industry since 1930, when 20% of the workers dyeing cotton with red azoic dyes, developed occupational eczema (Giusti et al., 2004). The majority of sensitizing dyes, present in clothes, practically all are same group of disperse dyes. The explanation is probably that the attachment of molecules from disperse dyes is weak, as they are more easily available for skin contact (Weisburger, 2002). The azo dyes propensity to bioaccumulate has been extensively investigated in fish (Erickson and McKim 1990). However other factors may be important for the uptake as diffusion resistance, molecular size, respiratory volume and gill perfusion (Jung et al., 1992). The eradication rates for hydrophobic elements are low. Uptake and clearance between fish and water is a first-order exchange process for hydrophobic chemicals it is often been shown. Water-soluble dyes like acid, reactive and basic dyes generally are not bioaccumulated (Van der Zee, 2002). The majority of dyes, if highly disinfected are not mutagenic. However, many of the commercial available dyes may, due to impurities, show metabolic activation and mutagenic activity in vitro (Adedayo et al.,2004). For increasing the solubility of the dyes used in the textile industry, they usually contain one or more sulphonated groups. Due to this fact, sulphonic containing dyes generally have a low genotoxic potential (Jung et al., 1992). The labile dye linkage may easily undergo enzymatic breakdown in mammalian organisms, including man. After cleavage of the azo-linkage, the component aromatic amines are absorbed in the intestine and excreted in the urine (Bressler et al., 2000). Many studies have been conducted showing the toxic potential of aromatic amines from azo dyes (Weisburger, 2002).

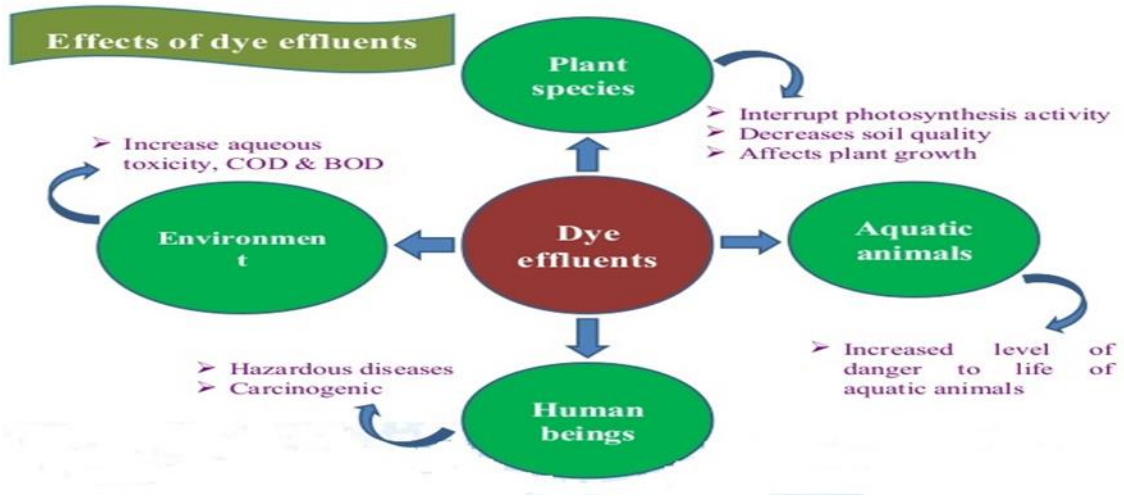

Figure 2: Effects of textile dye effluent in the environment (Weisburger, 2002)

3.4 Dye removal techniques

Currently, the methods of textile wastewater treatment involve physical andchemical processes as film filtration, coagulation or flocculation, precipitation, flotation, adsorption, ion exchange, ion pair removal, ultrasonic mineralization, electrolysis, chemical reduction and advanced chemical oxidation (Gogate and Pandit 2004a). The forward-thinking oxidation processes include chlorination, bleaching, ozonation, Fenton oxidation, photocatalytic oxidation and wet-air oxidation (Gogate and Pandit 2004b). There is also the possibility that due to excessive chemical use a secondary pollution problem will be arise. Biological and mixed treatment systems that can effectively remove dyes from large volumes of wastewater at very short economy are a preferable alternative (Robinson et al., 2001a). Biological techniques include biodegradation in aerobic, anaerobic, anoxic or combined anaerobic and aerobic treatment processes with bacteria (Shrivastava et al., 2005). For this reason, several factors determine the technical and economic viability of each single dye removal technique as dye type, wastewater composition, dose and costs of required chemicals, operation costs (energy and material), environmental fate and handling costs of generated waste products. Usually, the use of one individual process may not be sufficient to obtain complete decolorization because each technique has its limitations. Combination of different techniques dye removal strategies consist (Van der Zee and Villaverde, 2005). The various dye removal techniques and their pro and cons are summarized in figure 3 and Table 2 Respectively.

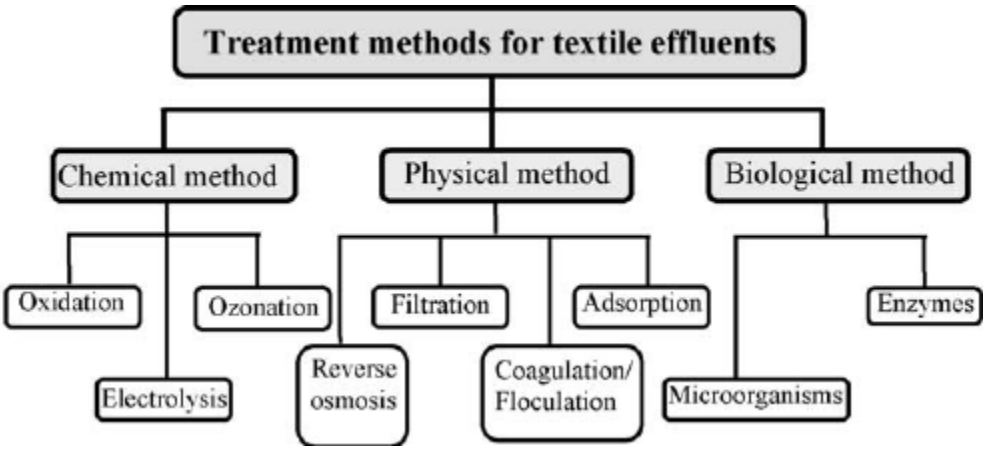

Figure 3: Color removal techniques used in textile industries (Pearce *et al.,* 2003).

Table 2. Current treatment technologies for color removal involving physical and/or chemical processes

Methods	**Pros**	**Cons**
Oxidation	Rapid process	Requires higher energy, by products
Adsorption	Good removal of dyes	Requires disposal
Membrane technologies	Removes all types of dyes	Concentered sludge formation
Coagulation	Comparatively economic	Huge sludge formation
Flocculation	Economically feasible	Huge sludge formation

(Sources: Pearce *et al.,* 2003).

3.5.1 Congo Red

Congo red is one kind of sodium salt of benzidinediazo-bis-1-naphtylamine-4-sulfonic acid (chemical name: C32H22N6Na2O6S2). It is a minor diazo dye. Congo red is soluble in water, producing a red colloidal solution; In organic solvents its solubility is better. It has a strong, though apparently non-covalent affinity to cellulose fibres. However, the use of Congo red in the cellulose industries (cotton textile, wood pulp & paper) has long been reckless, mainly because of its harmfulness.

3.5.2 Isolation and identification of bacterial strains

Six different bacterial strains were isolated from different soil samples collected from the dyeing shop areas. The pure bacterial isolates IS1, IS2, IS3, IS4, IS5 and IS6 are identified as *E. coli, Salmonella sp., S. aureus, Proteus sp., Psedomonas sp. and Bacillus subtilis* by respectively biochemical characteristics. These bacterial strains were cultured independently and as a consortium in 250 ml conical flask with 100 ml of nutrient broth. At 18 hrs. bacterial strains showed maximum growth.

At pH range from 3-11 at 300μg/ml of CR these six monocultures and consortium were inoculated in a nutrient. At pH 7, maximum decolourization was observed in consortium followed by 65% and 62% in IS6 and IS5 respectively. At pH 5, consortium showed only 50%, IS6 showed 37% decolourization, and maximum decolourization was observed at neutral pH.

3.5.3 Effect of dye concentration on decolourization

The decolourizing activity of the bacterial consortium was studied by using CR dye at different initial concentrations varying from 100 to 500 μg/ml. The maximum decolourization was detected at 300 μg/ml.

Table 3: Congo red dye decolourization (conc. 100μg/ml)

Sr. no.	Incubation time (hrs)	Decolourization percentage (%)						
		IS1	IS2	IS3	IS4	IS5	IS6	Consortium
1	24	56	58	52	65	61	64	67
2	48	71	74	67	78	72	69	78
3	72	81	83	73	95	87	79	97
4	96	88	88	77	97	90	89	97.5

(Source: Perumal *et al*., 2012)

In bacterial consortium found 98 % decolourization at 100 μg/ml concentration of Congo Red dye at pH 7 and temperature 37°C. IS5 monoculture showed 90 % decolourization at 100μg/ml. At 500 μg/ml percent (%) dye decolourization was dropped to 30%.

Table 4: Congo red dye decolourization (conc. 300 µg/ml)

Sr. no.	Incubation time (hrs)	Decolourization percentage (%)						
		IS1	IS2	IS3	IS4	IS5	IS6	Consortium
1	24	48	42	49	52	55	51	65
2	48	56	49	58	60	59.5	57	70
3	72	61	58	63	65	62	62	72
4	96	63	60	63.5	68	64	63	73

(Source: Perumal *et al*., 2012)

Bacterial consortium found 98 % decolourization at 100 µg/ml concentration of CR dye at pH 7 and temperature 37°C. *Proteus* sp showed 90 % decolourization at 100µg/ml. This value was almost similar to that reported for CR dye decolourization among various concentrations (50 to 250 ppm). *Proteus* sp showed 90 % decolourization at 50 ppm (Perumal *et al*., 2012).

Table 5: Congo red dye decolourization (conc. 500 µg/ml)

Sr. no.	Incubation time (hrs)	Decolourization percentage (%)						
		IS1	IS2	IS3	IS4	IS5	IS6	Consortium
1	24	21	20	22	23	24	22.5	34
2	48	23	21	24	25	26	25	36
3	72	25	23	25	27	28	26	38
4	96	26	23.5	25.6	29	29	26.9	39

(Source: Perumal *et al*., 2012)

The effect of pH also perceived. At pH 7, 70% CR decolourization was showed in consortium followed by 65% and 62% in *Proteus and Pseudomonas* respectively. At pH 5, consortium observed 50%, *Pseudomonas* gave 37% decolourization, and maximum decolourization was observed in neutral pH. This value was almost same to that reported for dyes decolourization, showed 70 % decolourization at pH 7 (Aileen *et al*.,2009).

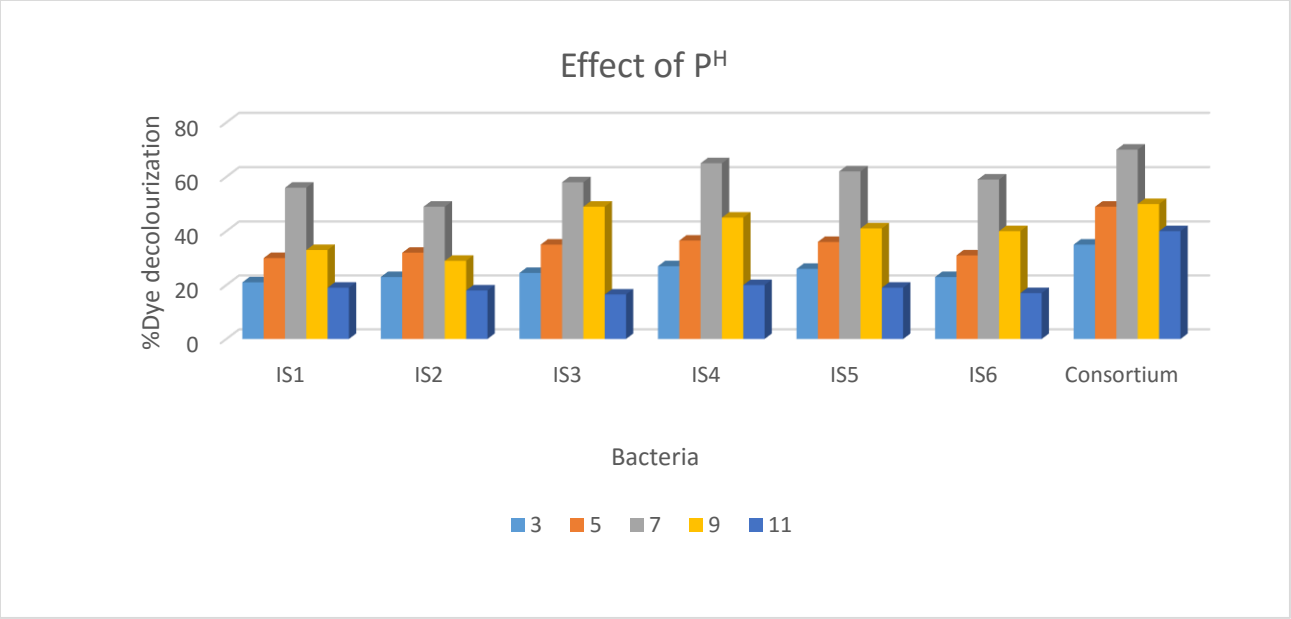

Figure 4: Effect of P^H on dye decolourization (300µg/ml) for 48 hours. (Source: Aileen *et al*., 2009)

3.6.1 Methyl Orange

Methyl orange is a pH indicator generally used in titration because of its strong and separate colour variance at different pH values. MO shows pink colour in acidic medium and it shows yellow colour in basic medium. Because it changes colour at the pH of a mid-strength acid, that is why it is usually used in titration. Unlike a worldwide indicator, methyl orange does not have a full spectrum of colour change, but has a sharp end point.

3.6.2 Decolorization of Methyl orange by growing cells

At different concentrations of the dye (100, 200, 400, and 800 mg/l) the effect of the initial concentrations of methyl orange on the decolorization was studied. The bacterial strain vigorously decolorized in the first 12 h. After that, the decolorization was inhibited at 800 mg/l of methyl orange with a final concentration of 650 mg/l until the last cultivation. The bacterial biomass increased and reached a stationary phase within 36 h with a yield of 1244 mg/l, although the pH value of the culture medium was below 4 at hour 12. On the other hand, complete decolorization was obtained in the growing cultures with 100 and 200 mg/l of methyl orange within 18 and 30 h, respectively (Fig.5). The study provides evidence that a growing culture of *Lactobacillus casei* TISTR 1500 shows highre efficiency in dye utilization than the incubation of *Oenococcus oeni*

ML34, which requires a longer incubation (48 h) to reach 93% decolorization (ElAhwany 2008).

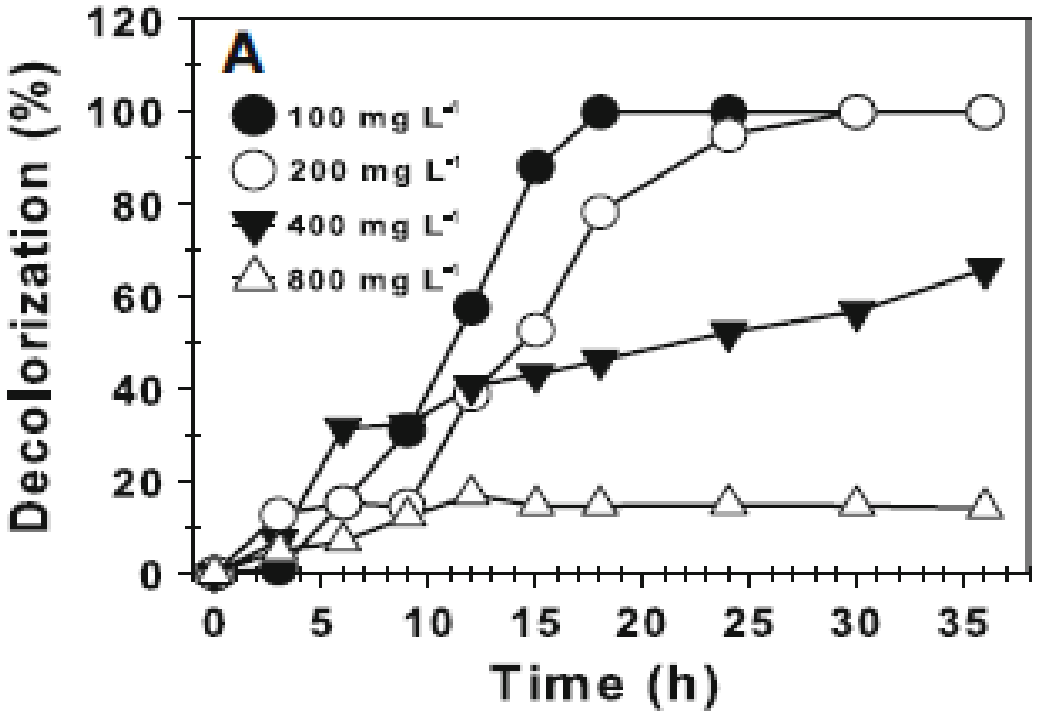

Figure 5: Decolorization (%) of methyl orange during the cultivation of Lactobacillus casei TISTR 1500 incubated at 35 ºC under static conditions. (Source: El Ahwany 2008)

As shown in Table 6, the max. individual decolorization rate of methyl orange shows 14.2 mg/gCell/h when the bacterial strain TISTR1500 was cultivated in medium containing 400 mg/l of the dye. The rate fallen to 8.7 mg/gCell/h when 800 mg/l of methyl orange was added to the medium.

Table 6: Specific decolorization rate of methyl orange by *Lactobacillus casei* TISTR 1500

Methyl Orange concentration (mg/L)	Specific decolorization rate (mg/gCell/h)
100	0.8 ± 0.04
200	2.8 ± 0.2
400	14.2 ± 0.23
800	8.7 ± 0.14

(Source: El Ahwany 2008)

3.6.3 Decolorization of methyl orange by a low cell density of washed cell suspensions

The strain of TISTR 1500 at different initial concentrations varied between 25 and 200 mg/l under static conditions removed of methyl orange as shown in Fig. 6. The reaction mixture needed a extended period of incubation. In the reaction mixture with methyl orange up to 100 mg/l, decolorizations were happened in the first 12 h of incubation. Complete decolorization was attained in the reaction mixtures with 25, 50, and 75 mg/l of methyl orange within 48 h, and the lowest dye decolorization was obtained in the mixture with the dye at 200 mg/l (22% dye removal). Same data and conclusions were observed previously, suggesting that dye utilization is contrariwise proportional to the dye concentration due to the inhibitory effects of high dye junk concentrations (Ozdemir *et al.,* 2008).

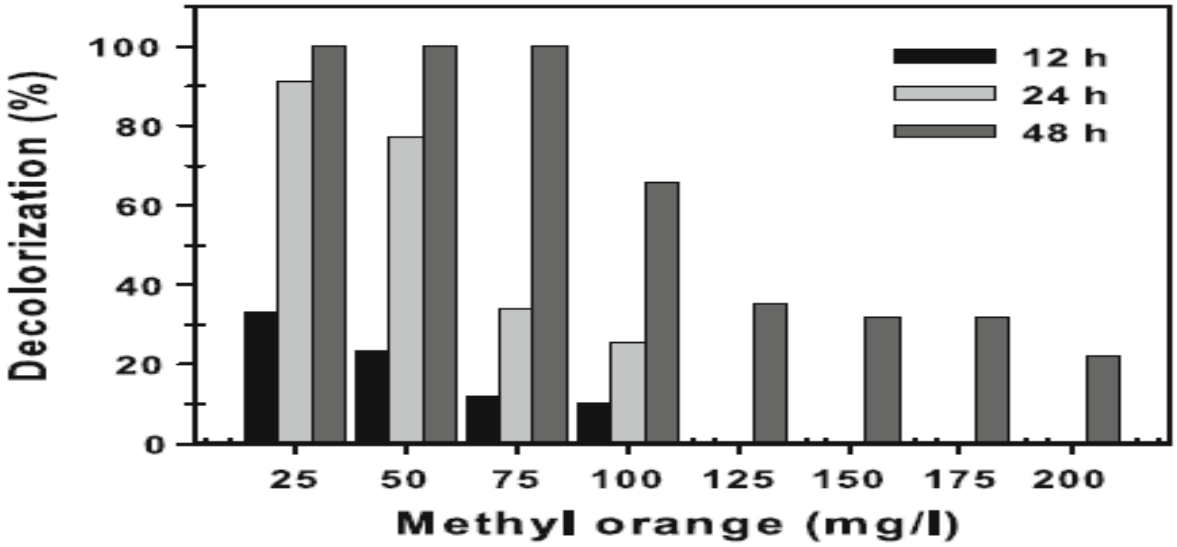

Figure 6: Effect of initial dye concentrations on decolorization by a low cell density of washed cell suspensions. (Source: Ozdemir *et al.,* 2008)

3.7.1 Decolorization of Acid Orange by *Staphylococcus hominis* RMLRT03 strain

During the growth stage of cell oxygen has a significant effect on the physiological characteristics of the cells (Bragger et al.,2004). The presence of oxygen can either favor or inhibit the microbial degradation of dyes. The *Staphylococcus hominis* RMLRT03 strain gives 85.52% decolorization of Acid Orange in static condition but in shaking condition it gives only 32.47% decolorization figure7.

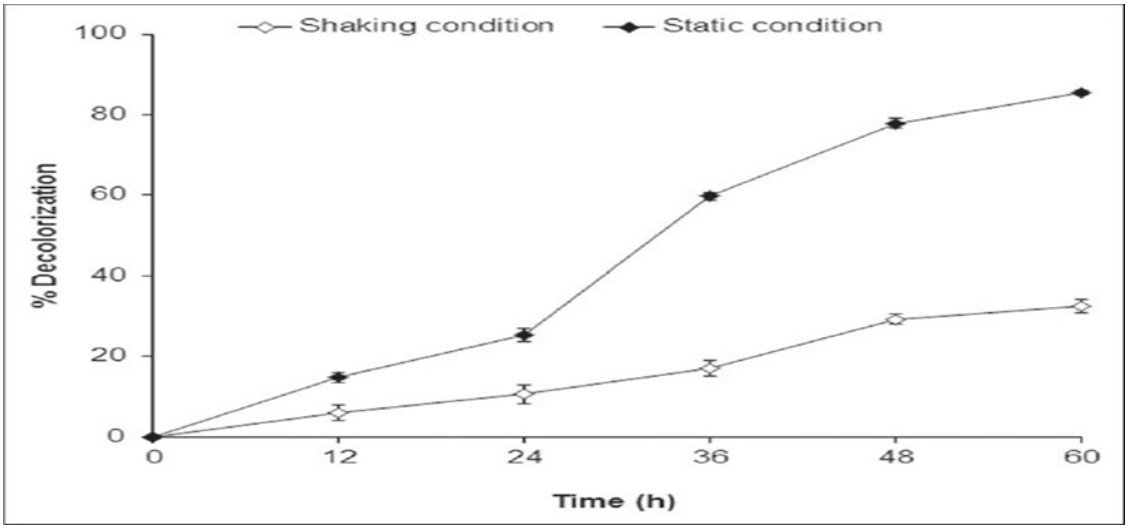

Figure-7: Effect of shaking and static on decolorization of Acid Orange (Bragger *et al.*, 2004).

3.7.2 Effect of temperature on decolorization of Acid Orange

The pH and temperature are important factor for the optimum physiological enactment of microbial cultures and decolorization of dyes. the cell growth and various biochemical and enzymatic mechanisms are affected by these factors. The temperature required to produce the maximum rate of color removal tends to correspond with the optimum cell culture growth temperature of 30–37^0 C. The deterioration in color removal activity at higher temperatures can be attributed to the loss of cell viability or to the denaturation of the dye reductase enzyme (Oturkar *et al.*, 2013).

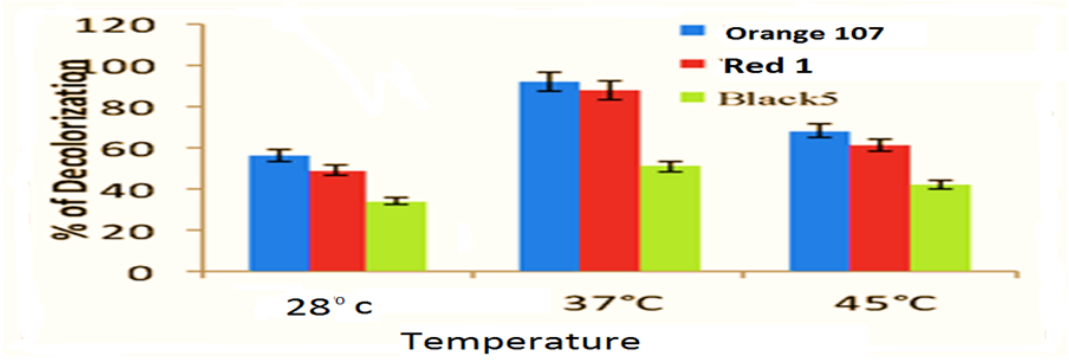

Figure 8: Effect of temperature on decolorization of Acid Orange by *Staphylococcus hominis* RMLRT03 strain (Oturkar *et al.*, 2013)

3.8.1 Crystal violet

Crystal violet (also known as methyl violet 10B or hexamethyl pararosaniline chloride) is a triarylmethane dye and used as a histological stain and in Gram's method of classifying bacteria. Crystal violet has antibacterial, antifungal, and anthelmintic assets and it is formerly important as a topical antiseptic. The medical use of the dye has been mostly superseded by more present drugs, though it is still listed by the World Health Organization (Wikipedia).

3.8.2 Biodegradation of crystal violet by *Aeromonas hydrophila*

The effect of the state of bacterial cells on the bleaching of CV by *A. hydrophila* is shown in Fig. 9. At static conditions at pH 7.5 and 37 ºC, *A. hydrophila* (0.8 $\times 10^9$ - CFU m/L) in stationary growth phase bleached 42 % of CV (50 mg/L) for 12-h incubation. The un-inoculated sample showed no evidence of bleaching, indicating that the nutrient broth and the incubator have no effect on the decolorization. These biodegradation results are equal to those obtained by Chimezie and Sawidis, who reported that *A. hydrophila* removes 30 % of CV (50 mg /L) at 30 ºC for 24 h of static incubation. A. radiobacter degraded 80 % of a less concentrated CV solution (10 mg /L) within 8 h while *P. putida* decolorized 80 % of CV (25 mg/ L) after 7 days (Parshetti et al. 2011).

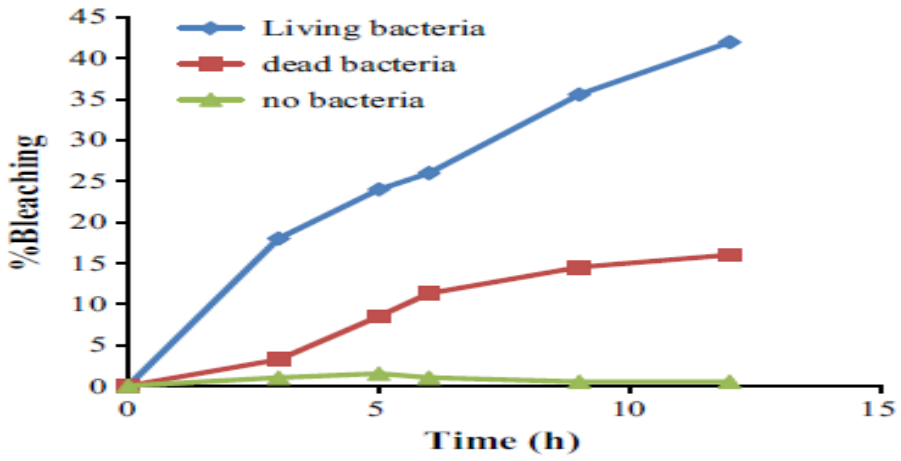

Figure 9: Effect of the state of bacterial cells on bleaching, T = 37 ºC, pH 7.5 (*A. hydrophila*). (Source: Saleh *et al.*, 2014)

In this study, the bacterial cells were small colored; therefore, a part of ruin could result from adsorption. To confirm this hypothesis, only 16 % bleaching rate was obtained with heat-killed

bacteria (Fig. 9), and this part was due to the adsorption. This bleaching rate corresponds to an increase in the pore surface area of the bacterial cells during autoclaving and then to the formation of new adsorption sites on the bacteria surface (Ogugbue Chimezie *et al.*, 2012). Hence, the bleaching rate resulting from adsorption caused by living bacteria in static condition should be very small (Saleh et al. 2014).

CHAPTER 4

CONCLUSIONS

- Dyes are extensively used in the textile, rubber product, color, printing, pharmaceutical and paper manufacturing. These are highly detectable and persistent in natural environment. Consequently, the release of potentially hazardous dyes in the environment it can be an eco-toxic and harmful for human which entered through the food chain. This is one of the major cause of environmental pollution.

- For the Congo red study, consortium showed maximum decolorization (98%) compare to other bacteria like IS1, IS2, IS3, IS4, IS5 and IS6. But incase of methyl orange and crystal violet, *Lactobacillus casei* TISTR 1500 and *Aeromonas hydrophila* were more tolerant for bleaching respectively.

- 70% Congo Red decolouration was observed at PH 7 and 37 ºC temperature was best for *Staphylococcus hominis* RMLRT03 strain to decolouration of orange acid.

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