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Biodegradation of various dyes; Eosin Y, Methyl Green, Crystal Violet, Basic Fuchsin and Brilliant Blue R

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Capstone Approval Page

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Biodegradation of various dyes; Eosin Y, Methyl Green, Crystal Violet, Basic Fuchsin and Brilliant Blue R

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ABSTRACT:

A simple and practical biological process for the decolorization and degradation of dyes is described. In this study, an aerobic Gram positive bacterium was used for the decolorization and degradation of different types of dyes, like triphenyl methane, fluorine and acid dyes. The dyes used in this study were Eosin Y (fluorine), Methyl Green (triphenyl methane), Brilliant Blue R (acid dye), Crystal violet (triphenyl methane), and Basic Fuchsin (triphenyl methane). The bacterium was able to use these dyes as a sole source of carbon for its growth. Absorption spectral studies provided the evidence for decolorization. The color loss is not reversible upon exposure to air, indicating that the dyes are indeed degraded by the bacterium. Since the bacterium is capable of decolorizing different types of dyes, it could be commercially exploited for its application in treatment of textile effluents.

Introduction

The use of synthetic dyes has greatly increased since 1856, when the first synthetic dye, mauevin, was discovered. Now over 100,000 dyes are being generated worldwide with an annual production of over 7 x 10^5 metric tons. Dyes are extensively used in textile, paper and pharmaceutical industries. They are also used for coloring plastics, gasoline, varnish, fats, oil and waxes. Food and cosmetic industries also use different kinds of dyes. Usage of dyes also extends into medicinal fields and biological staining. Most of the dyes are highly recalcitrant compounds, difficult to degrade biologically.

Classification of dyes

Dyes may be classified in several ways, according to chemical constitution, application class and end use. The different types of the dyes are:

Acid dyes are water-soluble anionic dyes that are applied to fibers such as silk, wool, nylon and modified acrylic fibers using neutral to acid dyebaths.

Basic dyes are water-soluble cationic dyes that are mainly applied to acrylic fibres, but find some use for wool and silk. Basic dyes are also used in the coloration of paper.

Direct or substantive dyeing is normally carried out in a neutral or slightly alkaline dyebath, at or near boiling point, with the addition of either sodium chloride (NaCl) or sodium sulfate (Na_2SO_4). Direct dyes are used on cotton, paper, leather, wool, silk and nylon. They are also used as pH indicators and as biological stains.

Mordant dyes require a mordant, which improves the fastness of the dye against water, light and perspiration.. Most natural dyes are mordant dyes. The most important mordant dyes are the

synthetic mordant dyes, or chrome dyes, used for wool; these comprise some 30% of dyes used for wool, and are especially useful for black and navy shades. The mordant, potassium dichromate, is applied as an after-treatment.

Vat dyes are essentially insoluble in water and incapable of dyeing fibers directly. However, reduction in alkaline liquor produces the water soluble alkali metal salt of the dye, which, in this leuco form, has an affinity for the textile fibre. Subsequent oxidation reforms the original insoluble dye.

Reactive dyes utilize a chromophore containing a substituent that is capable of directly reacting with the fibre substrate. The covalent bonds that attach reactive dye to natural fibers make it among the most permanent of dyes. "Cold" reactive dyes, such as Procion MX, Cibacron F, and Drimarene K, are very easy to use because the dye can be applied at room temperature. Reactive dyes are by far the best choice for dyeing cotton and other cellulose fibers at home or in the art studio.

Disperse dyes were originally developed for the dyeing of cellulose acetate, and are substantially water insoluble. They can also be used to dye nylon, triacetate, polyester and acrylic fibres.

Azo dyeing is a technique in which an insoluble azoic dye is produced directly onto or within the fibre. This is achieved by treating a fibre with both diazoic and coupling components. With suitable adjustment of dyebath conditions the two components react to produce the required insoluble azo dye. This technique of dyeing is unique, in that the final colour is controlled by the choice of the diazoic and coupling components.

Dyes can also be divided into different gropus based on the nature of their chromophore and they are:

- Category:Acridine dyes, derivates of acridine
- Category:Anthraquinone dyes, derivates of anthraquinone
- Arylmethane dyes
    - Category:Diaryl methane dyes, based on diphenyl methane
    - Category:Triarylmethane dyes, derivates of triphenyl methane
- Category:Azo dyes, based on -N=N- azo structure
- Category:Cyanine dyes, derivates of phthalocyanine
- Category:Diazonium dyes, based on diazonium salts
- Category:Nitro dyes, based on a $-NO_2$ nitro functional group
- Category:Nitroso dyes, based on a -N=O nitroso functional group
- Category:Phthalocyanine dyes, derivates of phthalocyanine
- Category:Quinone-imine dyes, derivates of quinone
    - Category:Azin dyes
        - Category:Eurhodin dyes
        - Category:Safranin dyes, derivates of safranin
    - Indamins
    - Indophenols
    - Category:Oxazin dyes, derivates of oxazin
    - Category:Oxazone dyes, derivates of oxazone
    - Category:Thiazin dyes, derivates of thiazin
- Category:Thiazole dyes, derivates of thiazole
- Xanthene dyes, derived from xanthene
    - Fluorene dyes, derivates of fluorene
        - Category:Pyronin dyes
        - Category:Rhodamine dyes, derivates of rhodamine
    - Category:Fluorone dyes, based on fluorone

Dyes and pollution

Dyes are released into the environment either from their manufacturer or from the industries in which they are extensively used..

Synthesis of dyes

Dyes are synthesized in a reactor, filtered, dried, and blended with other additives to produce the final product. The principal air pollutants from dye manufacturing are volatile organic compounds (VOCs), nitrogen oxides (NOx), hydrogen chloride (HCI), and sulfur oxides (SOx). Liquid effluents, resulting from equipment cleaning after batch operation, can contain toxic organic residues. Wastewater generation rates are of the order of 1-700 liters per kg (1/kg) of product. Biochemical oxygen demand (BOD) and chemical oxygen demand (COD) levels of reactive and azo dyes can be of the order of 25 kg/kg of product and 80 kg/ kg of product, respectively. Major solid wastes of concern include filtration sludges, process and effluent treatment sludges, and container residues (Source World Bank, 2002; http://www.cleantechindia.com/eicimage/210602_24/Dye-GUIDELINE.html).

Industrial use of dyes

Worldwide, some 280,000 tons of textile dyes are discharged per annum (Willetts and Ashbolt, 2000). It is estimated that at least 10-15% of dyes are lost during the dyeing processess into the effluent (Nigam *et al.*, 1996a & b). About 90% of reactive textile dyes entering activated sludge sewage treatment plants will pass through unchanged and will be discharged to rivers (Abadulla *et al.*, 2000). The largest waste stream from most textile mills involved in washing, bleaching, and dyeing is wastewater. Textile mill wastewater is often contaminated with process chemicals (dye, salt, bleach, detergent, etc.), oil, and energy from hot water

discharges. As a result, wastewater discharge permit limits, such as BOD (biological oxygen demand), COD (chemical oxygen demand), aquatic toxicity, and metals content, are often difficult to meet. Water usage in a typical mill can easily top 40,000 gallons per day costing more than $30,000 annually in water and sewer fees (www.sate.ga.us, 2002).

Toxicity of dyes and Bioremediation

The human health impact due to the wide usage of dyes, especially the azo ones and the carcinogenicity of their degradation products, has caused concern for a number of years. The environmental and subsequent health effects of dyes released in the textile industry wastewater are becoming subject to scientific scrutiny, which has resulted in the banning of many synthetic colorants and also the import of commercial consumer goods like textiles, leather and other items dyed with azo dyes, based on carcinogen amines (Hildenbrand *et al.,* 1999).

Though a number of physical and chemical remediation processes are available like treatment with ozone (Chen, 2000), photo-oxidation (Tanaka *et al,.* 2000), UV/Fe system (Deng *et al.,* 2000), adsorption by carbon (Al-Degs *et al.,* 2000), treatment with $MgCl_2$ (Tan *et al.,* 2000), with Zero valent Iron (Nam and Tratnyek, 2000), due to their high cost and maintenance, they are not widely applicable in the rural and small-scale textile plants (Davis and Burns, 1990). Biodegradation or biological treatment of the dye effluents offers a simple and cost economic treatment process. Many organisms have been isolated and reported for the degradation of several classes of textile dyes. The treatment technologies include anaerobic treatment with either a bacterium (Knapp and Newby, 1999; Nigam and Merchant, 1995) or fungi (Fu & Viraraghavan, 2001) or sequential microbial treatment with an anaerobic-aerobic system

(Nuttapun *et al.*, 2000) or by an aerobic treatment system with different co-metabolites (Sarnaik and Kanekar, 1999; Sani and Banerjee, 1999)

AIM

The aim of this project was to study the aerobic decolorization and degradation of various types of dyes. The different dyes used in this study were Eosin Y (fluorine), Methyl Green (triphenyl methane), Brilliant Blue R (acid dye), Crystal violet (triphenyl methane), and Basic Fuchsin (triphenyl methane).

The selected microorganism for the decolorization of dyes was a Gram positive bacterium which was capable of growing on azo and triphenylmethane dyes, like malachite green. The methods used in the project included determination of growth rate of the organism, rate of decomposition of the dye, and extraction and analysis of products of degradation by spectrophotometry and mass spectrometry.

Properties and toxicity of the dyes used in this study

Methyl Green (Fig. 1a)

Methyl Green is also known as Light Green and Basic Blue 20. The dye is a dark red to brown crystalline powder and belongs to the triarylmethane class. The empirical formula of the dye is $C_{26}H_{33}N_3Cl_2$ and the molecular weight is 458.5g/mol. The dye is very soluble in water and readily soluble in ethanol. The maximum absorptions of the dye occur at 630-634 nm and 420nm. Methyl green differs from crystal violet in that it contains seven methyl groups compared to crystal violets six. The seventh group is easily lost and the dye reverts to crystal

violet. The dye is used in biological stains (Methyl Green, http://stainsfile.info/StainsFile/dyes/42585.htm).

The toxicity of methyl green has not been fully studied. The contact of the dye on the skin or in the eyes may cause irritation. The ingestion of the dye can cause irritation of the gastrointestinal tract. The inhalation of the dye may cause irritation of the respiratory tract and the mucous membrane (Material Safety Data Sheet Methyl green MSDS, http://www.sciencelab.com/xMSDS-Methyl_green-9927668).

Basic Fuchsin (Fig. 1b)

Basic fuchsin is also known as Magenta and Fuchsin. The dye is a mixture of the three chemically related dyes pararosanilin, rosanilin, and magenta II. The dye is a green crystalline powder and belongs to the triarylmethane class. The empirical formula and molecular weight are unknown because the mixture of the three dyes varies. The dye is soluble in water and ethanol. The maximum absorption of the dye occurs between 540-555nm. Basic fuchsin is used in biological stains. The dye, specifically pararosanilin, is used to produce Schiff's reagent. Schiff's reagent reacts with aldehydes to form a red color (Basic fuchsin, http://stainsfile.info/StainsFile/dyes/basfuch.htm).

Basic fuchsin is hazardous in case of skin contact, ingestion, and inhalation. Contact may cause skin irritation. It may be absorbed through the skin in harmful amounts. Effects from skin absorption may be similar to that of inhalation and ingestion. Contact may cause skin sensitization, an allergic reaction, which becomes evident upon re-exposure to this material. Contact on the eye may cause eye irritation and corneal damage. Inhalation may cause respiratory tract irritation. Ingestion causes gastrointestinal tract irritation with colicky pain,

nausea, vomiting, diarrhea, and dryness of the throat. Inhalation may affect respiration and cause cyanosis. Exposure from skin absorption, inhalation or ingestion may cause methemoglobinemia and cyanosis. Symptoms of methemoglobinemia may include: grayish/bluish coloring of the skin, which may also appear with out signs of cardiac or pulmonary insufficiency, navy blue to black mucous membranes, dyspnea, shortness of breath, central nervous system effects - headache, dizziness, lethargy, ataxia, vertigo, muscle contraction or spasticity, weakness, faintness, disorientation, confusion, tinnitus, drowsiness, convulsions, tremor, seizures, paresthesias, muscle pain, coma-, cardiovascular system effects - heart blocks, and arrhythmias, tachycardia, vascular dystonia, cardiovascular collapse-, sluggish pupillary reaction, weakness of vision, photophobia. It may also affect the urinary system (oliguria, renal insufficiency, kidney damage, hemoglobinuria, painful micturition, hematuria, methemoglobinuria), liver, metabolism (weight loss), blood (anemia, chocolate colored blood), spleen, thyroid, and pituary gland. The chronic exposure to the dye is expected to have a carcinogenic and mutagenic effect. The dye is mutagenic to mammalian somatic cells, bacteria, and yeast. Chronic exposure may cause damage to the blood, liver, spleen and thyroid (Material Safety Data Sheet Basic fuchsin MSDS, http://www.sciencelab.com/xMSDS-Basic_fuchsin-9923017).

Crystal Violet (Fig. 1c)

Crystal violet is also known as Gentian violet, Methyl violet 10B, and Basic violet 3. The dye is a dark green powder and belongs to the triarylmethane class. The empirical formula of the dye is $C_{25}H_{30}N_3Cl$ and the molecular weight is 408g/mol. The dye is 1.68% soluble in water and 13.87% soluble in ethanol. The maximum absorption of the dye occurs between 588-593nm.

Crystal violet is used as a biological stain. The dye is the deepest blue of all the Methyl violets, making it the most suitable for the Gram's stain, a procedure for primary classification of bacteria (Crystal Violet, http://stainsfile.info/StainsFile/dyes/42555.htm).

Crystal violet is hazardous in case of ingestion or inhalation and is slightly hazardous in case of skin contact. The acute health effects from exposure are as follows. Skin contact may cause mild skin irritation. It can stain the area of contacted skin. Contact in the eyes causes moderate to severe irritation with immediate severe pain. Eye contact causes blepharospasm, purple staining of the cornea and conjunctiva causing permanent corneal/eye damage. Inhalation may cause upper respiratory tract and mucous membrane irritation. Ingestion causes gastrointestinal tract irritation with nausea, vomiting, hypermotility, diarrhea, and abdominal pain. Digestion may affect respiration (acute pulmonary edema) and behavior (ataxia). Severe systemic poisonings have not been repeated in humans, but animal studies have shown blood pressure rise and death from respiratory paralysis during IV administration. The chronic health effects from expose are as follows. Prolonged or repeated ingestion may cause peritonitis and may affect metabolism (weight loss). The dye is mutagenic for bacteria and yeast and may affect genetic material in humans. Exposure may cause adverse reproductive effects, birth defects (teratogenic), and may cause cancer based on animal test data (Material Safety Data Sheet Crystal Violet MSDS, http://www.sciencelab.com/xMSDS-Crystal_Violet-9927176).

Eosin Y (Fig. 1d)

The dye is also commonly known as Acid red 87. Eosin is a red fluorescent dye in the form of a triclinic crystal. The dye is a bromide derivative of fluorescin, tetrabromofluorescein. The empirical formula of Eosin Y is $C_{20}H_6O_5Br_4Na_2$. The molecular weight of Eosin Y is

691.88g/mol. The dye is a reddish brown crystalline powder. The solubility of the dye in water is 40 %, in glycerol 80%, in methanol 25% and 3% in ethanol. The maximum absorption of eosin Y with UV-Vis is 524.8nm (Eosin Y, http://omlc.ogi.edu/spectra/PhotochemCAD/html/EosinY-JZL.html).

Eosin Y is used in dyeing textiles, ink manufacturing, coloring cosmetics, coloring gasoline, and as a toner. The sodium or potassium salt of eosin is used in biological stains to color red blood cells bright red and to impart a red color to cytoplasmic materials and connective tissues (Eosin Y, http://www.chemicalland21.com/specialtychem/finechem/EOSIN%20Y.htm).

Eosin Y is a toxic dye. The dye is toxic to mucous membranes and is an irritant to the eyes, the skin, and the respiratory system. The repeated or prolonged exposure can produce target organ damage.

Brilliant Blue R-250 (Fig. 1e)

It is also known as Acid Blue 83. It's an acidic dye. The empirical formula of the dye is $C_{45}H_{45}N_3O_7S_2Na$ and the molecular weight is 826g/mol. It's a deep blue fine crystal with a faint reddish blue color. It dissolves in water completely. The dye is used extensively for staining protein in SDS-PAGE.

Information on the human health effects from exposure to this substance is limited. Upon inhalation it causes irritation to the respiratory tract. Symptoms may include coughing, shortness of breath. Upon ingestion it may cause irritation to the gastrointestinal tract and the symptoms may include nausea, vomiting and diarrhea.

Materials and Method

Chemicals and media

Eosin Y (dye content 94%) and basic fuchsin (99%) was obtained from Fisher Scientific Co., methyl green and crystal violet (96%) from Matheson Coleman and Bell, N.J., and Brilliant Blue-R-250 (95%) from Sigma Chemical Co. The minimal medium used was as described by Spencer and Guest (1973).

Growth studies and decolorization assay

The bacterium was grown at 37°C shaking at 250 rpm for all growth studies. To study the minimum inhibitory concentration, the bacterium was inoculated in different concentrations of the dye, 0.1%, 0.5%, 1%, 1.5%, 2%, 2.5% and 3%. Growth was measured spectrophotometrically after 48h.

To study the growth of the bacterium the culture was grown aerobically in minimal glucose medium. A 5% (v/v) of overnight grown culture was inoculated in 50 ml of minimal dye medium contained in a 250 ml conical flask. The dye concentration used was 0.5%. Samples were removed at regular time intervals and were store in the freezer until further processed. Growth was measured by determining the total protein content. The samples were centrifuged for 5 min at 14,000 rpm. The cell pellets were then boiled in 0.5 ml of 1 M NaOH for 20 min and the protein content was estimated by the Lowry method (Nachiyar and Rajkumar, 2003)

For Eosin Y, the growth was measured using a Klett meter. To study the growth of the bacterium, the culture was grown aerobically in minimal glucose medium. A 5% (v/v) of overnight grown culture was inoculated in 50 ml of minimal dye medium contained in a 500 ml sidearm flask. The two dye concentrations used were 0.5 and 1%. Growth was monitored in the Klett meter. The dye was tested as a sole source of carbon by omitting the carbon source

(glucose, 20 mM) from the dye amended minimal medium. Uninoculated medium was used as the control.

Decolorization of the dye could be seen visually and was also measured spectrophotometrically using a Shimadzu UV-2401PC double beam spectrophotometer (Tokyo, Japan). The culture supernatant was removed at regular time intervals during the biotransformation and the absorption spectra were recorded. Both the culture supernatants and the controls were diluted to 10 or 1000 times to record the absorption spectra.

Extraction of the dye for Mass spectral analysis

To identify the intermediates formed during biotransformation and to analyze the amount of dye utilized by the bacterium, the dye was extracted from the acidified culture using ethyl acetate. One ml of the culture was collected at regular time intervals (6 h) during the growth of the bacterium and stored at 4°C until extraction. The collected samples were centrifuged at 10,000 rpm for 5 minutes to pellet the cells. To the 1 ml of the acidified supernatant, an equal volume of ethylacetate was added and vortexed for 30 s. It was then centrifuged and the organic phase containing the dye was removed. This extraction was repeated three times. The ethylacetate extract was neutralized with 0.1 N NaOH to pH 7.0 and the UV spectrum of the dye was recorded. The extracts will be further analyzed by Liquid chromatography-Mass spectrometry (LC-MS).

Results and Discussion

1. Decolorization of Eosin Y

Eosin Y was used by the bacteria as a sole source of carbon, which is evident from fig. 2. In the presence of 20 mM glucose a diauxic growth pattern was observed (Fig. 2). Initially, up to

8 h, the bacterium grew without a lag phase. This shows the utilization of glucose as the carbon source. After 8 h there was a long lag period, up to the 50th h. The diauxic shift occurred as the glucose was being depleted. The organism then grew using the dye as the carbon source. A log phase from 50 to 66 h was observed; after which an extended stationary phase was observed.

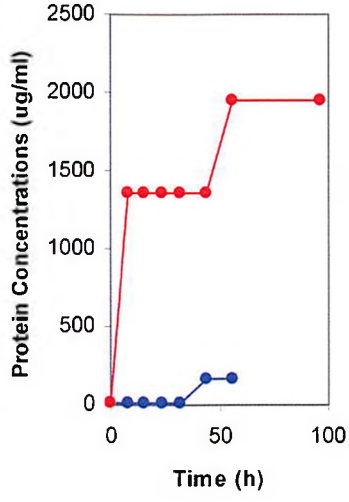


Figure 2. Growth curves of the bacterium in minimal Eosin Y media in the presence and absence of glucose. Blue: without glucose; Red: with Glucose

When growth was observed via the Klett meter reading, the bacterium was able to grow using 0.5% or 1% concentration of the dye (Fig.2D). There was a lag period for 8 h in case of 1% and a 4 h lag with 0.5% concentration of the dye. The bacterium stopped growing at 68 h in 0.5% dye; whereas in case of 1%, there was a lag phase from 56 h to 73 h. The growth of the bacterium in the dye media with glucose as a carbon source showed a diauxic growth curve (Fig. 2C). Initially, up to 8 h, the bacterium grew without a lag phase. This shows the utilization of glucose as the carbon source. After 8 h there was a long lag period, up to the 20th h. The diauxic shift occurred as the glucose was being depleted. The organism then grew using the dye as the

carbon source. A log phase from 20 to 36 h was observed with both concentrations of the dye. After which an extended stationary phase was observed.

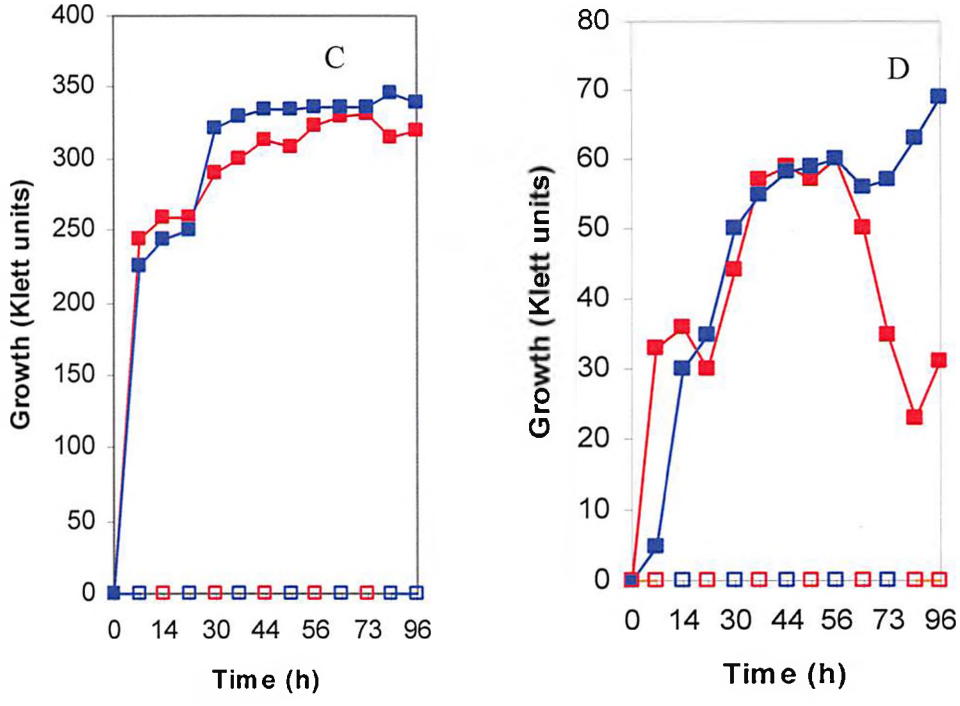


Fig. 2C & D. The corrected growth curves of the bacterium in minimal Eosin Y medium as measured by a Klett meter. C, minimal Eosin Y medium with glucose as carbon source; D, minimal Eosin Y medium; Solid blue square, 1% dye concentration; solid red square, 0.5%, open blue and red squares, controls

The decolorization of Eosin Y was observed spectrophotometrically (Fig. 3). There was a decrease in the absorption spectra as the time increased, clearly indicating that the dye was being decolorized by the bacterium. The decolorization of Eosin Y previously has been reported by Nerud *et al.* (2004), using *Pleurotus ostreatus*. In their study the decolorization was observed

only in the presence of an external carbon source. Whereas in our study, we have clearly demonstrated that the bacterium is capable of using Eosin Y as a sole source of carbon.

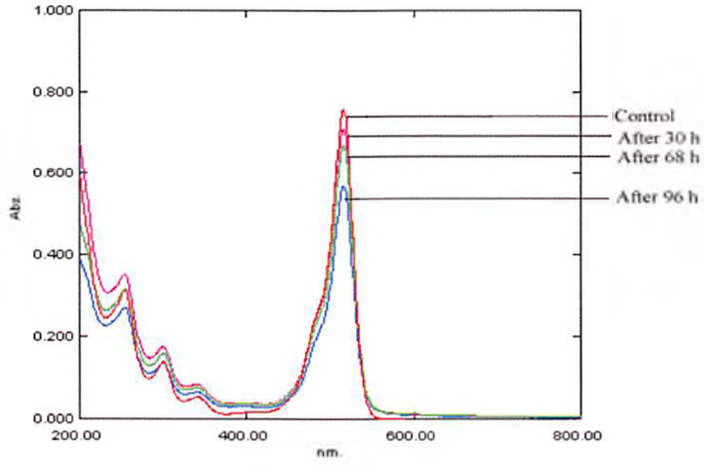


Figure 3. UV-Vis spectra of Eosin Y (0.5%, diluted 1000 times) at different time intervals during the growth of the bacterium in the dye medium.

2. Decolorization of Methyl Green

In case of methyl green, the decolorization study was performed only in the absence of glucose. The bacterium was able to utilize the dye as a sole source of carbon and grew as evident from fig. 4. There was a lag phase up to 8 h after which the bacterium grew slowly using the dye as the carbon source.

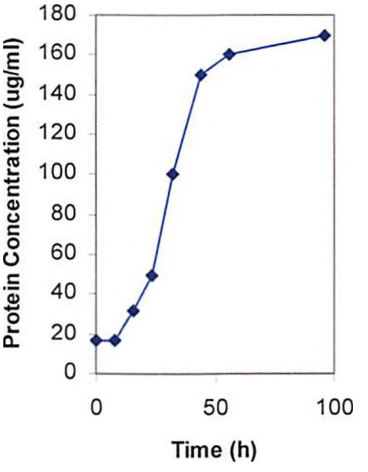


Figure 4. Growth curve of the bacterium in minimal Methyl Green medium.

The absorption spectra of methyl green after 8 h of growth showed an increase in absorbance at 630 nm and a decrease at 420nm. As the time increased, the absorbance at 630 nm decreased whereas the absorbance at 420 nm increased (Fig. 5). This alteration in absorbance may be due to the degradation of the dye, along with the accumulation of intermediates formed during the process of degradation.

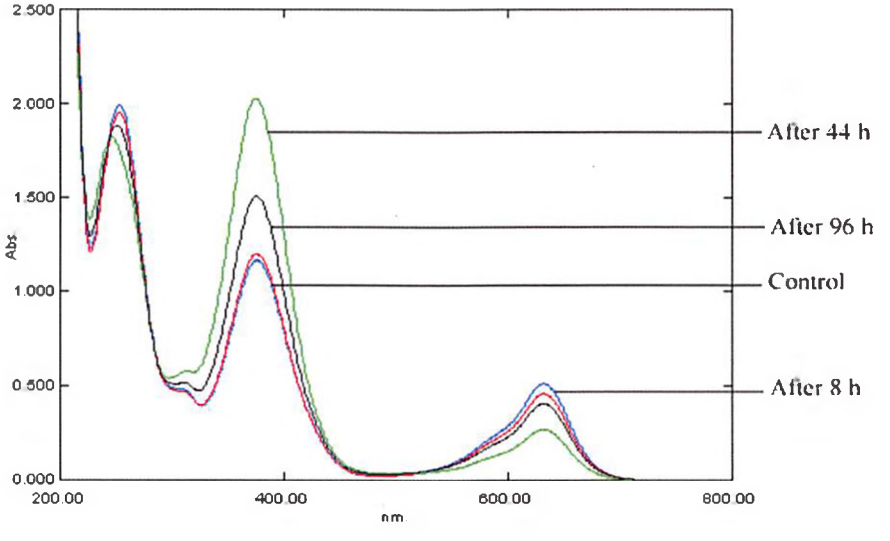


Figure 5. UV-Vis spectra of Methyl Green (0.5%, diluted 10 times) at different time intervals during the growth of the bacterium in the dye media.

The decolorization of methyl green was previously studied by using the lignin peroxidase purified from *Phanerochaete chrysosporium* (Ollikka *et al.*, 1993) and by using manganese peroxidase from *Lentinula* (*Lentinus*) *edodes* (Boer *et al.*, 2004). In our study, methyl green was used by the bacterium as a sole carbon source for its growth.

3. Decolorization of Brilliant Blue R

The decolorization of Brilliant Blue R was studied in the dye medium in the absence of glucose. The growth studies indicated that there was an initial lag phase up to 8 h; afterwards the bacterium grew slowly using the dye as the carbon source (Fig. 6).

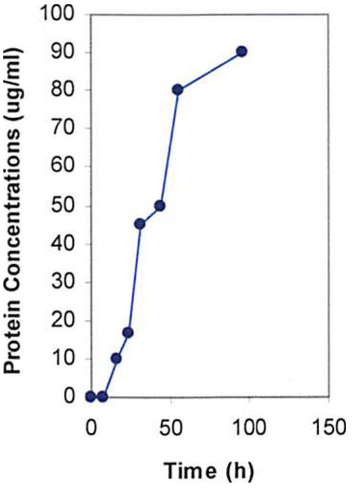


Figure 6. Growth curve of the bacterium in minimal Brilliant Blue R medium.

The bacterium was able to decolorize Brilliant Blue R as evident by the decrease in the absorption spectra over time (Fig. 7). To the best of our knowledge there have been no reports on the decolorization or degradation of Brilliant Blue R.

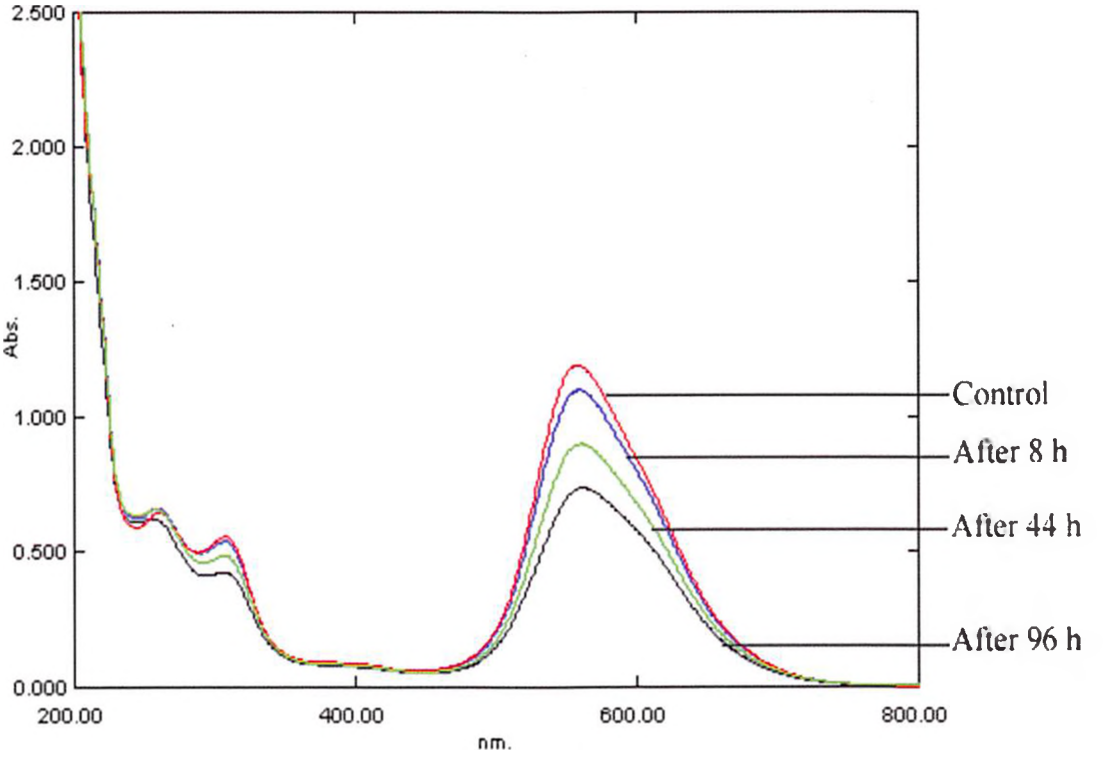


Figure 7. UV-Vis spectra of Brilliant Blue R (0.5%, diluted 10 times) at different time intervals during the growth of the bacterium in the dye media.

4. Decolorization of Crystal Violet

The bacterium was able to grow in crystal violet media both in the presence and absence of glucose (Fig. 8). A diauxic shift was observed when the bacterium grew in the media containing both the dye and glucose. The bacterium was able to grow up to 60 h in case of the media lacking glucose, utilizing the dye as a sole source of carbon.

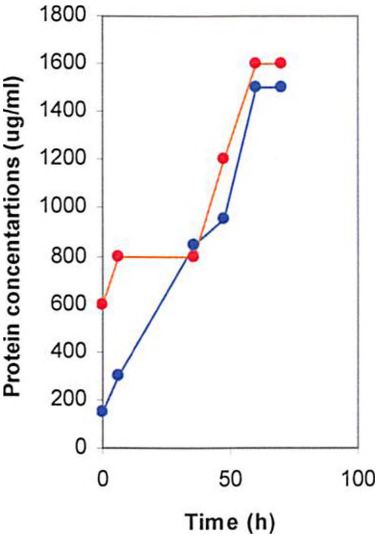


Figure 8. Growth curves of the bacterium in minimal Crystal violet media in the presence and absence of glucose. Blue: without glucose; Red: with Glucose

The UV-Vis spectra of crystal violet showed a significant decrease in the absorption spectra versus time (Fig. 9). Most studies on the decolorization of crystal violet have been reported using fungi, *Phanerochaete chrysosporium* (Bumpus and Brock, 1988), and by oxidative red yeast (Kwasniewska, 1985). Bacterial degradation of crystal violet was reported by Yatome *et al.* by *Bacillus subtilis* (1991) and *Nocardia corallina* (1993), and by waterborne pathogenic mycobacteria by Jones and Falkinham (2003). In all the previous studies, either degradation or decolorization was observed only as a co-metabolic process, whereas in our study we report the decolorization and degradation of crystal violet as the sole carbon source.

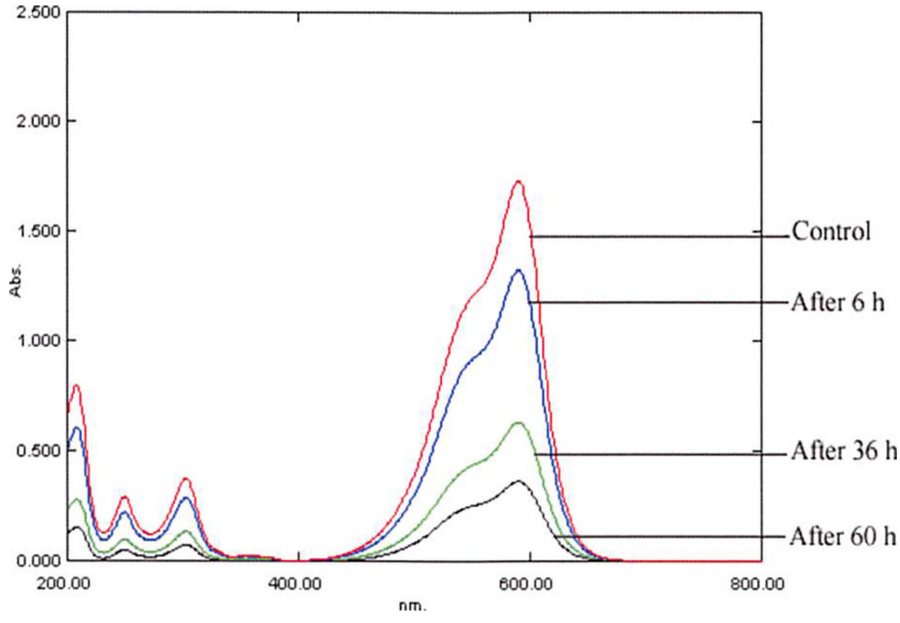


Figure 9. UV-Vis spectra of Crystal violet (0.5%, diluted 1000 times) at different time intervals during the growth of the bacterium in the dye media.

5. Decolorization of Basic Fuchsin

Basic fuchsin is a mixture of three chemically related dyes, magenta II, pararosanilin and rosanilin. The bacterium was able to grow using basic fuchsin as a carbon source both in the presence and absence of glucose (Fig. 10). This clearly indicates that the bacterium may also be able to use the three dyes individually as carbon sources.

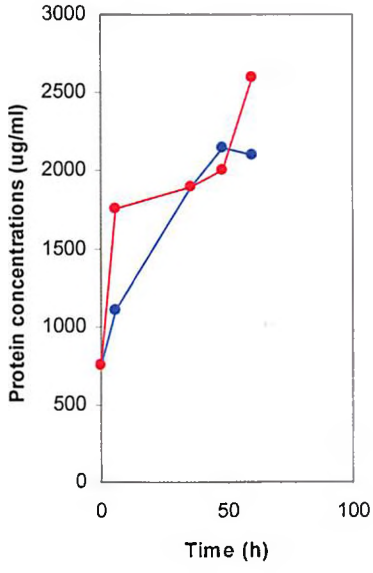


Figure 10. Growth curves of the bacterium in minimal Basic fuchsin media in the presence and absence of glucose. Blue: without glucose; Red: with Glucose

In case of decolorization of Basic fuchsin, the absorption spectra increased upon the increase in time. This kind of spectra may be attributed to the presence of three different dyes and may be due to the accumulation of intermediates from the degradation of one or more dyes. Further studies on individual dyes are necessary to confirm the exact pattern of decolorization of basic fuchsin by the bacterium.

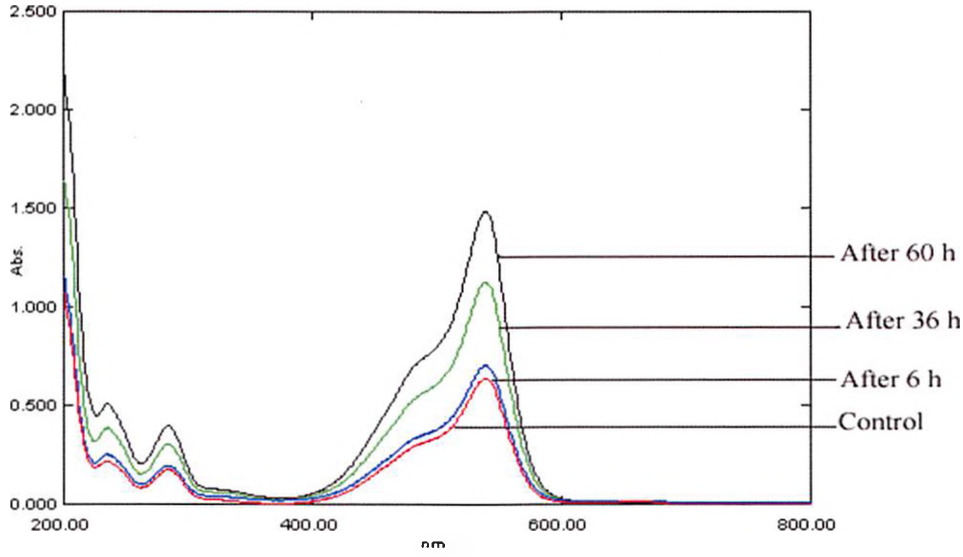


Figure 11. UV-Vis spectra of Basic Fuchsin (0.5%, diluted 10 times) at different time intervals during the growth of the bacterium in the dye media.

Figure 1a. Structure of Methyl Green

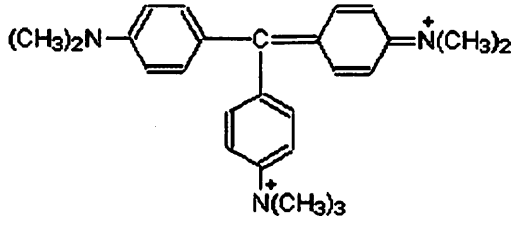
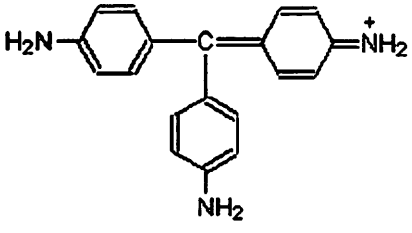
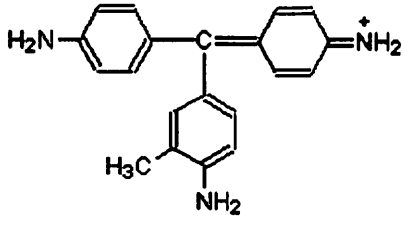


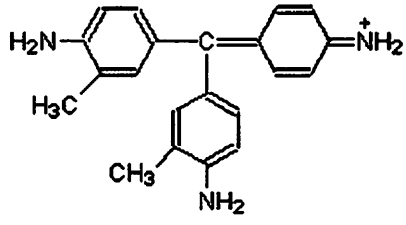
Figure 1b. Structure of Basic Fuchsin



Pararosanilin



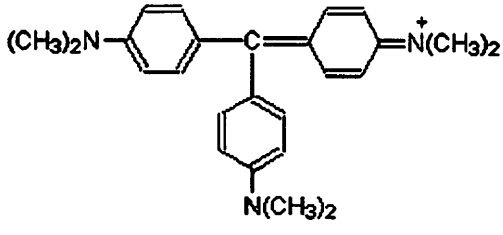
Rosanilin



Magenta II

Figure 1c. Structure of Crystal Violet;

Figure 1d. Structure of Eosin Y



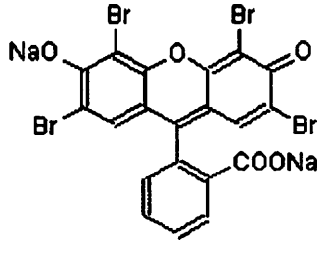
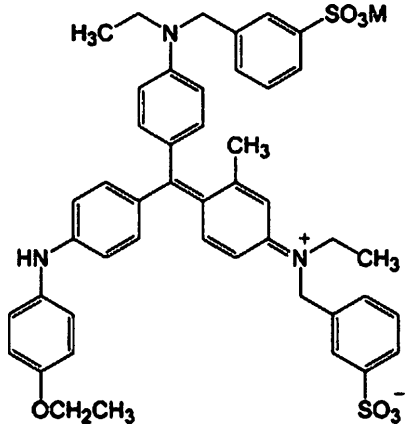


Figure 1e. Structure of Brilliant Blue R -250



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