Screening and isolation of crystal violet dye decolorizing microorganisms from soil

DISSERTATION SUBMITTED TO THE

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Submitted by

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DECLARATION

I Sunita Kumari Haiburu, B.Sc. Biotechnology hereby declare that the work presented in the dissertation entitled **"screening and isolation of crystal violet dye decolorizing microorganisms from soil"** in partial fulfillment of the requirement for the award of the degree of B.Sc. Biotechnology, Department of Biotechnology, School of Health & Allied Science, Arka Jain University, Jharkhand is an authentic record of my own work done during the period of six months from January 2022 to May 2022, under the supervision of Dr. Jyoti Khurana, Department of Biotechnology, Arka Jain University.

I also declare that I have not submitted the matter embodies in this dissertation for the award of any other degree or diploma.

Place: Jamshedpur (Sunita Kumari Haiburu)

Date: AJU/190305

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ARKA JAIN University
Jharkhand (Jamshedpur)

CERTIFICATE

The dissertation entitled **"screening and isolation of crystal violet dye decolorizing microorganisms from soil"** submitted by Sunita Kumari Haiburu for the degree of B. Sc. Biotechnology submitted to Department of Biotechnology, School of Health & Allied Science, Arka Jain University, Jharkhand is the result of bonafied research done under my supervision. The dissertation conforms to standard envisaged by the regulations of university. In my judgment the work is original and the dissertation presented is adequate and merit consideration for the award of the degree for which it is submitted.

Dr. Jyoti Khurana

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(Sunita Kumari Haiburu)

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INTRODUCTION

INTRODUCTION

Crystal violet or gentian violet (also known as methyl violet 10B)is a triarymethane dye used as histological stain and in Gram's method of classifying bacteria .crystal violet has antibacterial,antifungal,and anthelmintic properties and was formerly important as a topical antiseptic. The medical use of the dye has been largely superseded by more modern drugs,although it is still listed by the World Health Organization .crystal violet when dissolved in water has a blue-violet colour with an absorbance maximum at 590nm.

The textile dyeing industry came into existence for above 4000years.fabrics dyed with indigo and madder have been found in the tombs of prednastic Egypt.There and a few matter extracted from insects and tropical woods formed the only source of dyes awaiting the middle of the middle of the last century.Dyes were obtained from natural source and only important members like king and priests could possess colored fabric.Natural colorinr agent are mainly of inorganic origin such as clays,earth, minerals,metal salt .

Crystal violet ,a typical cationic dye,used as a biological stain,dematological agent,temporary hair colorant,dyeing cotton ,wools and in diverse other profitable textile processes .crystal violet ,also known as hexamethyl paraosaniline chloride,is a basic dye with molecular formula .The IUPAC name of crystal violet is Tris(4(dimethylamino)phenyl) methyalium chloride of blue – violet colour in appearance with 205C as melting point and 40C as freezing point . How ever , the structure and colour of crystyal violet largely depend on the pH and temperature of medium, which formulates it a valuable acid- base indicator plus and excellent dye . The predominant from a crystal violet is the monovalent cation which form the major structural form and is in solid state as well as in aqueous solution across broad range of pH value ranging from 1 to 13 .

Crystal violet is delocalized by the mechanism of resonance of three nitrogen atom. The decolorization of charge across the double bond in The benzene ring stabilizes the carbonation and is responsible for vibrant purple colour of crystal violet dye in stongly basic solution , the monovalent cation slowly combine with hydro oxide ion and forms an un based colourless product (CVOH) .The dye can also be manufactured by the condensation of formaldehyde and dimethyaline to give a leuco dye , reduced from crystal violet.When dissolve in water, crystal violet give blue violet colour with maximum absorbance of 590nm and an extinction coefficient of 87000m-1 cm-1.Dye colour largely defend on the acidity of media as at a pH of 1.0, the dye give green with absorption maximum 420 nm and 620nm,while in a strongly acidic solution (pH of -1) the dye is yellow with absorption maximum 420nm .

Industries such as textile, leather, paint, acrylic, cosmetics , plastic,pharmaceutical,manufacturing etc, used dye as colouring agent which consumes substance volume of water during the processing of product .Crystal violet is not only used in colouring plastic ,gasoline , varnished , fats , oil and wax but also used for dyeing nylone , polyacrylonitrile –modified nylone and wool.

The aim of the present work was to study the bacteria potential for decolorization of crystal violet dye.The enzymes involved in the decolorization process were identified and the effect of various parameters on dye decolorization production were investigated.

REVIEW
OF
LITERATURE

REVIEW OF LITERATURE

DYES: A dye is **a colored substance that chemically bonds to the substrate to which it is being applied**. This distinguishes dyes from pigments which do not chemically bind to the material they color. Dye is generally applied in an aqueous solution, and may require a mordant to improve the fastness of the dye on the fiber.

The majority of natural dyes are derived from non-animal sources: roots, berries, bark, leaves, wood, fungi and lichen. In the 21st century, most dyes are synthetic, i.e., are man-made from petrochemicals The process was pioneered by J. Pullar and Sons in Scotland. Other than pigmentation, they have a range of applications including organic dye lasers,optical media and camera sensors.

The color of a dye is dependent upon the ability of the substance to absorb light within the visible region of the electromagnetic spectrum (380-750 nm). An earlier theory known as Witt theory stated that a colored dye had two components, a chromophore which imparts color by absorbing light in the visible region (some examples are nitro, azo, quinoid groups) and an auxochrome which serves to deepen the color. This theory has been superseded by modern electronic structure theory which states that the color in dyes is due to excitation of valence π-electrons by visible light.

HISTORY OF DYES

The uses of colorants by mankind for painting and dyeing of their surroundings, their skins and their cloths dates back to the dawn of civilization. Until the middle of the 19th century, all colorants applied were from natural origin. For example, inorganic pigments such as soot, manganese oxide, hematite and ochre have been utilized within living memory; organic natural colorants have also a timeless history of application, especially as textile dye. These dyes are all aromatic compounds, originating from plants but also from insects, fungi and lichens.

Synthetic dye manufacturing started in 1856, when the English chemist W.H. Perkin in an attempt to synthesize quinine, obtained instead a bluish substance with excellent dyeing properties that latter known as aniline purple, Tyrian purple or mauveine. Perkin patented his invention and set up a production line. This concept of research and development was strongly stimulated by Kekule's discovery of the molecular structure of benzene in 1865. In the beginning of the 20th century, synthetic dyestuffs had almost completely supplanted natural dyes.

Textile dyeing dates back to the Neolithic period. Throughout history, people have dyed their textiles using common, locally available materials. Scarce dyestuffs that produced brilliant and

permanent colors such as the natural invertebrate dyes Tyrian purple and crimson kermes were highly prized luxury items in the ancient and medieval world. Plant-based dyes such as indigo, saffron, and madder were important trade goods in the economies of Asia and Europe. Across Asia and Africa, patterned fabrics were produced using resist dyeing techniques to control the absorption of color in piece-dyed cloth. Dyes from the New World such as cochineal and logwood were brought to Europe by the Spanish treasure fleets, and the dyestuffs of Europe were carried by colonists to America.

Dyed flax fibers have been found in the Republic of Georgine in a prehistoric cave dated to 36,000 BP. Archaeological evidence shows that, particularly in India and Phoenicia , dyeing has been widely carried out for over 5,000 years. Early dyes were obtained from animal , vegetable or mineral sources, with no to very little processing. By far the greatest source of dyes has been from the plant kingdom, notably roots, berries, bark, leaves and wood, only few of which are used on a commercial scale.

The first synthetic dye, mauve, was discovered serendipitously by William Henry Perkin in 1856. The discovery of mauveine started a surge in synthetic dyes and in organic chemistry in general. Other aniline dyes followed, such as fuchsine, safraning, and indulines. Many thousands of synthetic dyes have since been prepared.

The discovery of mauve also led to developments within immunology and chemotherapy. In 1863 the forerunner to Bayer AG was formed in what became Wuppertal, Germany. In 1891 Paul Ehrlich discovered that certain cells or organisms took up certain dyes selectively. He then reasoned that a sufficiently large dose could be injected to kill pathogenic microorganisms, if the dye did not affect other cells. Ehrlich went on to use a compound to target syphilis, the first time a chemical was used in order to selectively kill bacteria in the body, he also used methylene blue to target the plasmodium responsible for malaria

Dyes consist of two essential components

CHROMOPHORE

A chromophores is the part of a molecular responsible for its colors.The color that is seen by our eyes is the one absorbed by the reflecting object within a certain wavelength spectrum of visible light.The chromophores are atomic configuration that contain delocalized electrons.usually they are represented as nitrogen, carbon,oxygen and sulphur that have alternate single and double bonds.By incorporating the delocalized electron in these configuration into the delocalized electron in the aryl rings of aromatic compound the energy contained in the electron cloud can be modified .If the energy incorporated into the electron cloud is changed, then the wavelenghth of radiation it absorbs will also change. If this change in the wavelength to be absorbs is sufficient to cause any absorption at all within the visible range,then the compound will be coloured and the configuration of chromophoric are : -C=C-, -C=N-,-C=O-,-NO2,-N=N-.

AUXOCHROME

An auxochrome is a group of atoms attached to a chromophore which modifies the ability of that chromophore to absorb light.They themselves fail to produce the colour;but when present along with the chromophores in an organic compound intensifies the colour of the the chromogen.For the example (-OH),(-NH2),(-CHO)and (-SCH3).A chromogen without auxochrome can never act as dye.Auxochromes are group that attach to non-ionizing compound yet retain their ability to ionize the addition of both auxochrome and a chromophore result in a much alteration of the absorption maxima of the compound.

CLASSIFICATION OF DYES

Dyes may be classified according to either by the chemical structure or by the usage or application method.Dyes are classified by the chemical structure,which has many advantage .It readily identifieds dyes as belonging to a group that has characteristics and there is manageable number of chemical groups.The usage classification is advantageous to consider the classification of dyes by use and method of application before considering chemical structure.

BASIC DYES:

Basic dyes are water soluble dyes .Which yield colored cation, mainly used for silk ,wool and tanning modanted cotton but they have no affinity towards cellulosic fabrics.Basic dyes are used along with a mordant for fibres such as cotton,linen, acetate,nylon and polyester.Basic dyes show moderate light and wash fastness. For dye preparation, the dyestuff is mixed with equal amount of acetic acid followed by warm water under constant stirring .The predominant chemical classes of cationic dyes are diazahemicyanine,triarylmethane,oxazine and thiazine.

DIRECT DYES:

Direct dyes are used in the dyeing of cotton and rayon,paper,leather and to some extent to nylon. When a dye colours the fabric directly without the help of any fixing agent,the dyes is said to be a direct dye. Direct dyes are water soluble. They are easy to produce,simple to apply and cheap in cost of production and application. Direct dyes are anionic in nature and have greater affinity for cellulosic fibres.They are used to dye cellulose fibres without a mordant in bright shades.A leveling agent such as sodium carbonate is added for even dyeing. At the end of dyeing,exhaustion agent such as salt is added which helps the dyes can be applied to wide variety of textile materials such as apparel,linings and automotive fabrics. Most direct dyes have good fastness to light but poor wash fastness.

REACTIVE DYES:

Dyes that react with the fibres and form covalent bonds are reactive dyes.They become an intergral part of the fibre. They are water soluble and are used to dye cellulose ,protein and polyamide fibres.They produce full range of bright shade across the spectrum.They exhibit excellent wash fastness and good light fastness properties.Dyeing of fabric with reactive dyes involves 3 steps,namely exhaustion of dye (NaCl or Glauber's salt),fixation of dye (sodium carbonate or sodium hydroxide)and washing off.

ACID DYES :

Water soluble dyes that required acid (sulphuric,acetic,formic acid etc.,)in dye bath to dye silk or wool are called as acid dyes.These acid dyes produce negative ions(anions or acidic groups) which react with positive ions of protein fibres and get attached to the fibre through electrovalent bonds.Acid dyes are similar to direct dyes however they cannot be applied to cellulose fibre due to slight variations structure.

MORDANT OR CHROME DYES:

Natural dyes and some synthetic dyes do not have affinity towards fibres. With the help of some chemicals,they can be used to dye fibres. These chemicals are called mordants or mordant dyes. Mordant dyes have affinity for both fibre and dye and form a linkage between the dye molecule and the fibre.

DISPERSE DYES:

Disperse dyes are insoluble in water. Their solubility is increased by increasing temperature and by adding dispersing agents. They are suitable for dyeing hydrophobic fibres like nylon, polyester,acrylic and other synthic fibres. Disperse dyes are non ionic or neutral in nature. They have an excellent fastness to washing and sunlight exposure.

VAT DYES:

Vat dyes are used for cotton ,mainly to cellulosic fibres as soluble and wool. These are water soluble with predominant chemical class containing anthraquinone and indigoids.

SULPHUR DYES:

These dyes are used extensively for cotton and rayon with limited usage for polyamide fibres,silk,leather,paper and wool. Low cost and good wash fastness properties make this class an importance and economic one.

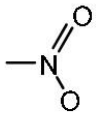
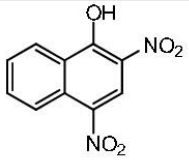
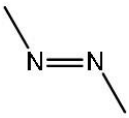
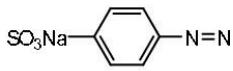
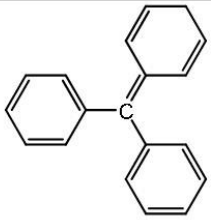
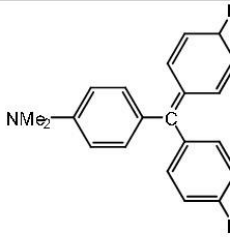
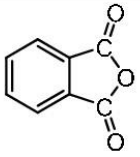
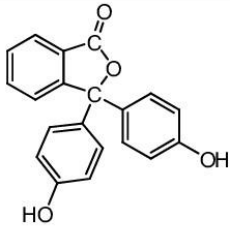
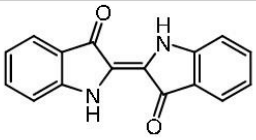
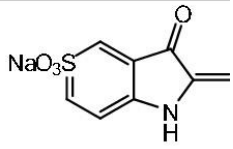
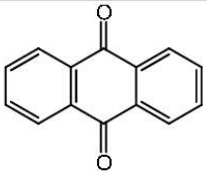
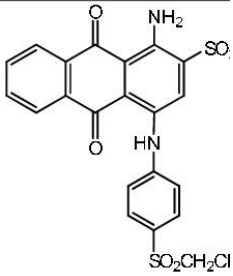
AZOIC DYES or NAPTHOL DYES:

The largest classes of dyes used in industry are azo dyes .There dyes are versatile class of dyes which have been used extensively than any other class. Azo dyes are water soluble synthetic organic compounds possessing the characteristic –N=N-,which links the chromophore and auxochrome to from colored molecular of great versity.

Table 1.Classification of dyes on the basis of application

	Class	Application	Examples
1	Acid dyes	Nylon, wool, silk, modified acrylics, paper, leather, food, inkjet printing and cosmetics.	Acid red 88, Acid red 18
2	Cationic (Basic) Dyes	Poly acrylonitrile, paper, modified polyesters, modified nylons, cation dye able polyethylene terephthalate, wool, silk, tannin mordanted cotton and medicine.	Crystal Violet, Methylene Blue, Safranin, Basic fuschin
3	Disperse Dyes	Nylon, polyester, cellulose, acrylic fibers and cellulose acetate.	Disperse Red 1, Disperse Orange 37
4	Direct Dyes	Rayon and cotton, leather, paper and nylon.	Congo Red, Brilliant Blue, copper blue 2R
5	Reactive Dyes	Wool, nylon, cotton and other cellulosic.	Reactive Black 5, Reactive Orange 16
6	Solvent Dyes	Gasoline, plastics, oils, lubricants and waxes.	Solvent Red 1, Solvent Red 49, Solvent Red 24, Solvent Red 111
7	Sulfur Dyes	Cotton and rayon, paper, leather, silk and wood.	Sulfur Brilliant Green, Sulfur black 1
8	Vat Dyes	Cotton, rayon and wool.	Vat red 10, vat violet 13 and vat orange 1.

Table 2 .Classification based on chemical composition and chromophore group

Class	Chromophore	Example
Nitro dyes		 Acid Yellow 24
Nitroso dyes		 Fast Green O
Azo dyes		 Methyl Orange
Trimethylmethane dyes		 Basic Violet
Phthalein dyes		 Phenolphthalein
Indigo dyes		 Acid Blue 71
Anthraquinone dyes		 Reactive Blue 19

Adsorption of Crystal Violet into an Agricultural

Agricultural waste can be exploited for the adsorption of dyes, due to their low cost, availability, cost-effectiveness, and efficiency. This is prepared from agricultural waste was investigated in this study as a novel adsorbent for the elimination of dye molecules. Removal of methylene blue (MB) as a cationic model dye by GS from aqueous solution was studied under different experimental conditions. The adsorbent was first subjected to morphologic characterization by scanning electron microscope. The influence of variables including pH, concentration of the dye and amount of adsorbent, particle size, contact time and temperature on the dye removal has been investigated. Three kinetic models were used to describe the sorption process. Three isotherm models were applied to evaluate the sorption equilibrium, and its thermodynamic parameters were calculated. More than 85% removal efficiency was obtained within 200 min at adsorbent dose of 0.04 g per 10 ml for initial dye concentration of 100 mg ml− 1. The maximum sorption capacity was found to be 256.41 mg g− 1 at pH 7 and at 303.16 K. The sorption kinetic data were found to be in accordance with pseudo-second order kinetics. Calculations of various thermodynamic parameters indicate the endothermic and spontaneous nature of the sorption process. Desorption experiments were conducted for regenerating garlic straw which exhibited higher desorption capacity after sorption MB using HCl at pH 2. This study showed that GS as a low-cost adsorbent had a great potential for the removal of MB as an alternative eco-friendly process. prepared from agricultural waste was investigated in this study as a novel adsorbent for the elimination of dye molecules. Removal of methylene blue (MB) as a cationic model dye by GS from aqueous solution was studied under different experimental conditions. The adsorbent was first subjected to morphologic characterization by scanning electron microscope. The influence of variables including pH, concentration of the dye and amount of adsorbent, particle size, contact time and temperature on the dye removal has been investigated. Three kinetic models were used to describe the sorption process. Three isotherm models were applied to evaluate the sorption equilibrium, and its thermodynamic parameters were calculated. More than 85% removal efficiency was obtained within 200 min at adsorbent dose of 0.04 g per 10 ml for initial dye concentration of 100 mg ml− 1. The maximum sorption capacity was found to be 256.41 mg g− 1 at pH 7 and at 303.16 K. The sorption kinetic data were found to be in accordance with pseudo-second order kinetics. Calculations of various thermodynamic parameters indicate the endothermic and spontaneous nature of the sorption process. Desorption experiments were conducted for regenerating garlic straw which exhibited higher desorption capacity after sorption MB using HCl at pH 2. This study showed that GS as a low-cost adsorbent had a great potential for the removal of MB as an alternative eco-friendly process.

CRYSTAL VIOLET

Power of crystal violet

Crystal violet is an organic chloride salt that belongs to the triphenylmethane type alkaline dyes. This monochloride salt whose scientific name is hexamethyl chloride rose aniline has a wide bio and industrial applications including developing fingerprints, dying ink, as an antifungal, antibacterial & anthelmintic agent, and as a histological stain. Other names used to describe crystal violet include Basic Violet 3, Gentian Violet, Hexamethyipararosaline chloride, and Methyl Violet 10B.

Its powder form appears as a dark green compound with metallic luster but appears purple when dissolved. When in an aqueous or alcohol solution, crystal violet has a (purple) blue-violet color, with the color largely depending on the acidity of the solution. The different colors of the dye are because of the different charged states of the dye molecule.

Charles Lauth was the first scientist to synthesize methyl violet in 1866. Crystal violet is one of the components of methyl violet. Crystal violet was first independently synthesized by Alfred Kern while working in Basel. For the easier synthesis of this product, he entered into a collaboration with a German chemist Heinrich Caro. Kern also discovered that diethylaniline could be used to synthesize violet dye, which is commonly known as basic violet.

Jakob Stilling, a German ophthalmologist, discovered the antiseptic properties of crystal violet in 1890. He collaborated with E. Merck & Co. to market the dye, which was probably a mixture of aniline dyes, as an antiseptic. Later in 1902, Conradi and Drigalski discovered that the effect of the dye was strong on most gram-positive bacteria but had little effect on gram-negative bacteria such as Bacillus Typhi (Salmonella typhi) and Bacillus Coli (Escherichia coli). John Churchman published a more detailed study of the dye on the different strains of bacteria in 1912.

APPLICATION OF CRYSTAL VIOLET

Crystal violet is used as a textile and paper dye,and is a component of navy blue and black inks for printing,ball-point pen,and inkjet printers.It is sometimes used to colourize diverse products such as fertilizer,antifreeze,detergent and leather.When crystal violet is used as an alternative to fluorescent stains,it is not necessary to use ultraviolet illumination;this has made crystal violet popular as a means of avoiding UV-induced DNA destruction when performing DNA.

Crystal violet was used to develop Fingerprints.Crystal violet is also used as a tissue stain in the preparation of light microscopy section.In laboratory,solutions containing crystal violet and formalin are often used to simultaneously fix and stain cells grown in tissue culture to preserve them and make them easily visible.

Medical use

Gentian violet has antibacterial,antifungal and antitumor properties.It is used medically for these properties,in particular for dentistry, and is also known as"pyoctanin".It is commonly used for:

*Marking the skin for surgery preparation and allergy testing.

*Treating candida albicans and related fungal infections,such as thrush,yeast infection, various types of tinea(ringworm,athlete's foot,jock itch)

*Treating impetigo;it was used primarily before the advent of antibiotics,but still useful to persons who may be allergic to penicillin.

Veterinary

Because of its antimicrobial activity ,it is used to treat ich in fish.However,it usually is illegal to consumption.

Biological reserch

Crystal violet can be used as an alternative to coomassie brilliant blue(CBB)in staining of proteins separated by SDS-PAGE,reportedly showing a 5x improved sensitivity vs CBB.

Synthesis

Crystal violet is one of the components of methyl violet,a dye first synthesized by Charles Lauth in 1861.Form 1866,methyl violet was manufactured by the Saint-Denis-based firm of Poirrier de Chappat and marketed under the name "Violet de Paris".Crystal violet itself was first synthesized in 1883 by Alfred Kern (1850-1893) working in basel at the firm of Bindschedler and Busch .To optimize the difficult synthesis which used the highly toxic phosgene,Kern entered into a collaboration with the German chemist.

TOXIC EFFECTS OF CRYSTAL VIOLET

Crystal violet is reported to mammalian cells as well as to aquatic flora and fauna.It can cause moderate eye imitation ,conjunctiva or permanent injury to comea and causes skin imitation or digestive tract imitation,if absorbed through skin in harmful amount. But in extreme cases,may lead to respiratory and kidney failure. The various kind of toxic recalcitrant organic compound, the crystal violet containing wastewater released from different has high BOD and COD values which decrease the significant amount of dissolved oxygen. There is no such universally useful method available to tread the dye wastes because of the complex chemical structures of the dyes. Due to their toxic,mutagenic and carcinogenic properties as well as their contribution to the decolorization of natural waters,the release of dyes and their metabolites into the environment is a source of concern.

HARMFUL EFFECT OF CRYSTAL VIOLET

Crystal violet ,has been extensively used in human and veterinary medicine as a biological stain,as a textile dye in textile processing industries and also used to provide a deep violet color to paints and printing ink. Crystal Violet is also used as a mutagenic and bacteriostatic agent in medical solutions and antimicrobial agent to prevent the fungal growth in poultry feed. Inspite of its many uses,Crystal Violet has been reported as a recalcitrant dye molecule that persists in environment for a long period of time and pose toxic effects. It acts as a mitotic poison,potent carcinogen and a potent clastogene promoting tumor growth in some species of fish. Thus,crystal violet is regarded as a biohazard substance. Although,there are several physic-chemical method such as a adsorption,coagulation and ion-pair extraction reported for the removal of crystal violet from industrial wastewaters and also produce large quantity of sludge containin secondary pollutants. However,biological methods are regarded as cost-effective and eco-friendly for the treatment of industrial wastewaters,but these methods also have certain limitation. Therefore,there is an urgent need to develop such eco-friendly and cost-effectively remove the dye from industrial wastewaters for the safety of environment,as well as human and animal health.

Health hazards of crystal violet

It may be fatal or cause blindness if swallowed. Harmful if inhaled. May be harmful if absorbed through the skin. May cause eye and skin irritation. May cause respiratory tract irritation. May cause central nervous respiratory tract irritation. May cause central nervous system depression .May cause liver and kidney.

Potential Health Effects

Eye: Causes moderate eye irritation. Vapors may cause eye irritation. It may cause painful sensitization to light. This product contains a cationic dye. similar dyes have caused permanent injury to the cornea and conjunctiva in documented exposure cases with human or rabbit eyes.

Skin: May cause skin irritation. May be absorbed through the skin in harmful amounts.

Ingestion: May cause irritation of the digestive tract . May cause respiratory failure. May cause vascular collapse and damage. May cause kidney failure.

Inhalation: May cause respiratory tract irritation. May cause visual impairment and possible permanent blindness. May cause effects similar to those described for ingestion.

Chronic: Prolonged or repeated skin contact may cause dermatitis. Chronic inhalation and ingestion may cause effects similar to those of acute inhalation and ingestion.

BIOLOGICAL DECOLOURISATION OF CRYSTAT VIOLET

Biological decolourisation of triphenylmethane dyes are widely in the literature.The degradation of triphenylmethane dye by pseudomonas pseudomallei intact cells without identifying the degradation products.

Decolorisation by bacteria:

Bacteria have ability to decolorize synthetic commercial dyes used for textile dyeing. Degrading bacteria from textile effluents and to evaluate the capability to decolorize commercially used textiles dyes. Industrial effluents carrying dyes are prominent environmental threats to recevinig water bodies. Related with decolorization triphenylmethane under laboratory setting .decolorizing bacterial isolates on supplemented with different conc.of tpm dyes.for secondary screening decolorization experiment were achieved under submerged fermentative condition using two thermotolerant bacterial isolates viz. bacillus subtilis B2d and B.licheniformis B3e strains .Plackett-Burman design for 11factors to screen the most influential parameter affecting decolorisation of CV by either bacterial strain .Under optimal fermentation condition,
The bacterial isolate B2d showed significantly higher decolosization than of crystal violet .

Decolorisation of algae

Crystal violet using blue green algae and green algae in order to assess the decolorisation Of these algae after incubation for 2 and 24 hr in three dyes concentrations at 10, 50 and 100 ppm. Chlorella has achieved the highest percentage of crystal violet decolorization after 2 hr at 10 ppm, but after 24 hr Scenedesmus gave the highest percentage of decolorization at 10 ppm. The highest proportion of malachite green decolorization after two hours using Chlorella at 50 ppm, but after 24 hr the higher decolorization percentage of malachite green obtained by M. aeruginosa at 100 ppm follow ed by Scenedesmus sp. at the same concentration. The green algae showed a high capacity for crystal violet decolorization than blue-green algae. Measuring the activity of laccase, manganese peroxidase and tyrosinase enzymes indicated that. gave the highest laccase activity in all dyes concentrations, while the other two enzymes haven't any activity. Chlorophyll-a and phaeophytine-a values obtained showed significant differences between most treatments; the most negatively affected species was which showed decreasing in chl-a content in the two dyes concentrations. In conclusion; the decolorization process of dyes by algae had been done by different mechanisms; one of them was enzymatic degradation. have a high ability to decolorize the two dyes so they might be used in wastewater treatment of fish farms contains these carcinogenic dyes as antifungal agents.

Decolorization of yeasts

Few studies have been carried out on dye decolorization by yeasts .compared to bacteria and filamentous fungi,yeasts have many advantages they not only grow rapidly like bacteria,but like bacteria,but like filamentous fungi they also have the ability to resist unfavorable environments.Microbial decolorizaion and degradation of direct violet 51by Candida albicans isolated from industrial effluents was reported.

The effective degradation of hazardous contaminants remains an intractable challenge in wastewater processing,especially for the high concentration of salty azo dye wastewater.However ,some unique yeast symbionts identified from the termite gut system present an impressive function to deconstruct some aromatic compounds,which imply that they may be valued to work on the dye degradation for various textile effluents.In this investigation,a newly isolated and unique yeast strain,Sterigmatomyces halo.

Deolorization of fung

Fungi were isolated and examined to decolorize crystal violet dye,frequently used as textile dye and in microorganism staining,using basal salt medium under static condition at 30C.Themore effective fungus gave less dry weight and lower pH indicating that the process was directed toward the decolorization process giving acidic products rather than microbial growth.The effective fungus was identified as Aspergillus niger and was used in the rest of experiment.Increasing the incubation period more than 10 days did not improve the decolorization process, while the best pH was 5.5.The decolorization process was effective (up to 84.6%) with the examined range of dye concentration (10-40 ppm).Sucrose content,as a carbon source,more than 1% did not improve the decolorization process (80.9%).Using ammonium sulfate as a nitrogen source, instead of sodium nitrate in the original basal medium,lowered the decolorization process,while using corn steep liquor enhanced the biodegradation up to 96.1%.Although the dye violet color vanishes in acid medium because of decreasing the possibility of extending the benzene chromophore as a resultof binding the nitrogen lone pair in the ammonium ion,the UV-Vis spectra analysis of the bioassay products proved that the decolorization is due to the biodegration of the dye rather than the resonance factor in acidic medium or biosorption by fungus mycelia.

White rot fungi: the use of Phanerochaete chrysosporium and Tinctoporia sp to decolourise the lignin containing pulp and paper wastewater was reported as early as in 1980 (Eaton et al., 1980; Fukuzumi 1980). Since then P.chrysosporium has been examined for decolorization of pulp mill wastewaters and various dyes by many researchers (Bilgic et al., 1997; Cammarota and Sant Anna, 1992; Lankinen et al., 1991; Tatarko and Bumpus 1998; Young & Yu 1997; Ollika et al., 1993; Pasti-Grigsby et al., 1992, Spadaro et al., 1992; Glenn and Gold 1983).

In addition to P.Chrysosporium other white rot fungi, also capable of decolourising dyes include Trametes Versicolor (Wong and Yu, 1999, Young and Yu, 1997), Coriolus versicolor (Knapp and Newby 1999, Knapp et al., 1995) and Funalia trogii (Yesilada et al., 1995).

(1996) observed that the percentage of adsorbed colour was in the range 10-25% in the study with Aspergillus niger.

Boussaid (1995) used Sagenomella striatispora to remove colour from pulp-mill effluent and reported that only 12% colour adsorption was observed in a total of 74% colour removal. For dead cells, the mechanism is biosorption, which involves physico-chemical interactions such as adsorption, deposition and ion exchange. Zhou and Banks (1991) studied the humic acid adsorption by dead Rhizopus arrhizus and they concluded that adsorption was a biphasic process (which followed the Freundlich isotherm model); the first was fast and independent of metabolic energy while the second was slow and dependent on metabolic energy. They observed that no chemical reaction occurred between cell wall and humic acid, just a physical adsorption according to the results of infrared spectra. Banks and Parinson (1992) reported that active sites for humic acid adsorption on fungal biomass in R.arrhizus were on the fungal cell wall and were most probably the chitin/chitosan components. Zhou and Banks (1993) reported

that chitin/chitosan was the major active component of R. arrhizus for humic acid adsorption. Gallagher et al. (1997) used Rhizopus oryzae biomass to adsorb reactive Brilliant red in solution and observed that both Freundlich and Langmuir isotherm models fitted biosorption well, which indicated adsorption by combined mechanisms onto a heterogeneous surface (Fu and Viraraghvan, 2001).

MATERIALS AND METHODS

Material and Methods

Collection of Samples:

Soil sample were collected from forest soil in Jamshedpur,was carried out by serial dilution and plating appropriate of sample on nutrient agar plates.screening was done to select microorganisms than decolourize triraymethane dye so solid media containing different concentration of these dyes. The soil sampal were air-dried and stores in room temperature for use in this experiment.All chemical and biological media used in this experiment were procured from Hi-media laboratories Pvt.Ltd.Mumbai respectively.

Isolation and screening of microorganism decolourizing of crystal violet

Nutrient medium

The survival and growth of microorganism depend on available nutrients and a favorable growth environment.In the laboratory,the nutrient preparation that are used for culturing microorganisms are called media.Conical flask of 500ml capacity were taken;4g luria broth and add 200ml of distilled water added in the conical flask 3.6g of agar-agar is added in the flask,pH will be checked and maintained at 7.0.Cotton plug should be placed and covered with the conical flask with aluminium foil.The conical flask is placed in autoclave (121°C for 15 min at 15psi).After autoclaving,cool the flask of sterile agar in a 48° to 50°C water bath. Line up the desired number of sterile petri plates on the bench of the laminar air flow.Remove the aluminum foil cap from the flask and briefly flame the flask's neck. Liquefy nutrient agar ,cool to 50°C and pour 20-25ml medium into the petri plates.

Dye stuff used:

The crystat violet used in this study was procured from the Hi-Media laboratories Pvt.Ltd. Stock solution was prepared by dissolving 0.1g of crystal violet and 5ml of distilled water .The dye solution was sterilized by membrane filtration,since crystal violet may be unstable to moist heat sterilization .All chemical used were
Of the highest purity available and of an analytical grade. The dye solution is transferred to the nutrient medium.

Serial dilution:

The vast numbers of bacteria are found in soils,a small sample of soil are serially diluted in water,prior to being plate on agar within a Petri plate.100ml distilled water is measured and

taken in a beaker .1g of soil is taken in the beaker and shake the beaker and mix uniformly and label the soil solution.Label the test tube"10^-1","10^-2","10^-3",and"10^-4" Than add 9ml of distilled water to each of the tubes, using one of the pipettes.Transfer 1 ml of the solution in bottle to the tube labeled "10^-1",using a new pipettes.Transfer 1ml of the solution in the "10^-2"test –tube to be "10^-3"tube with a new pipette.Repeat this method to transfer solution from"10^-4"tube to the "10^-5"tube and seal it with cotton plugs.

Spread plate methods:

The speart -plate technique is an easy direct way for isolation of microorganism .In this technique,a small volume of dilute soil mixture of containing 100 to 200 cells or less is transferred to the center of an agar plate and is spread evenly over the surface the surface with a sterile ,L-shaped glass rod .spread the soil sample evenly over the agar surface with the sterilized spreader,making sure the entrie surface of the plate has been covered .The glass rod is normally sterilized by dipping in alcohole and flamed to burn off the alcohol .After incubation ,some of the dispersed cell develop into isolated colonies .A colony is a large the number of bacterial cells on soild medium,which is visible to the naked eye as a discrete entity .In this procedure ,one assumes that a colony is derived from one cell and therefore represents a clone of a pure culture.

Streaking

Streaking is a technique used to isolate a pure stain from a single species of microorganism ,often bacteria Samples can then be taken from the resulting colonies with the help of inoculating loop and a microbiological culture can be grown on a new plate so that the organism can be identified, studied or tested.

Decolorisaton Assay:

A loopful of bacterial culture was inoculated containing 100ml of nutrient broth and incubated at37°C for 24h.Then 1ml of 24h old culture was inoculated in100 ml of nutrient medium contanining 50ppm of crystal violet and re-incubated at37°C till complete decolourization occurs .suitable control without any inoculums was also run along with flask 1.0 ml of sample control withdrawn every 12 h and centrifuged at 10,000 rpm for 15min .The extent of decolorisation by microbial cultures and solid waste were determined by spectrophotometer at maximum absorbance of crystal violet .The percentage of dye decolrisation was calculated using the modified method .

The percentage decolorisation was determined as follows:

$$\%\text{Decolorization} = \frac{(\text{Initial OD} - \text{Final OD}) \times 100}{\text{Initial OD}}$$

Where,

Initial O.D.refers the initial dye concentration.

Final O.D.refers the residule dye concentration.

Decolorisation experiment were perfomed in triplicates.

Bacterial strain and culture conditions:

Bacterial strain that showed maximum decolorisation percentage of crystal violet was aerobically cultured in the nutrient medium having a label of (10-4).The pH was adjusted to 7.0.for frequent use the culture was maintained by transfer to a fresh medium at 24h intervals. When required for the long periods ,it was maintained by subculturing once every seven days on slants, prepared by solidifying the media with agar.

Factors affecting Decolorisation of crystal violet :

1.Decolorisation at different concentration of crystal violet and incubation periods:

The decolorisation of crystal violet was tested at different concentration(25,50,100,150,200,300 mg/L).The 0.1 g of crystal violet dye was added to 5 ml distilled water.The flask containing dye was inoculated at 37°C on shaking incubator .The decolorization percentage had been determined after 3,7and 14 days in incubation.

Effect of pH:

The decolorisation studies of CV were carried out at pH values 2,3,4,5,6,7and 8 by adjusting pH of the medium using buffer for acidic and alkaline.The dye was added to flask containing 5ml of distilled water and the flask containing dye was inoculated.the decolorisation was determined after 3days of incubation at 37°C on shaking incubator.

Effect of temperature:

The decolorisation of crystal violet was tested at different temperatures 25,30,33,37,40,50,60°C by inoculating which represent of each bacterium.The decolurisation was determined after 3 days.

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

The,release of dyes through soil waste water into the ecosystem form various industries,the textile,cosmetic ,paper ,leather,pharmaceutical and food industries is not only a source of asesthetic pollution but also can cause human health disordes and adversely affects the aquatic life.In view of increasing concern from perspective of environmental safety and health,physico – chemical and biological techniques are to be costantly explored for decolourisation and degradation of dyes.

Dyes used

Dye is used for studing crystal violet and many other dyes .they are widely used in textile,pharamaceutical products ,biological stains,leather,paintsand food industries.The dye was weighed and stocksolution of 0.1mg/ml was sterlised using disposable syringe.

Forest soil around Gamharia was screened for crystal violet dye for decolorisation .Soil sample was us dilution and all dilution when plated on media supplemented with 25ppm of crystal violet,it was observed that10-4 dilution was giving distant colonies, when kept for 20hr of incubation,total 8 colonies were giving halozone and these were streaked on media plates.Among all isolates,the best 4 strain were choosen to study the decolorisation and the other factors affecting CVdecolorisation.

serial dilution for soil sample was used for spreading in prepared media plates.

Media plates were prepared in laminar hood were checked for comination for keeping them in incubation for16 h.And then one plate was used as control as no dilution was spreaded.

Spread plates were kept for incubation at 37℃ for 16 to 24 h and bacterial colonies were observed.

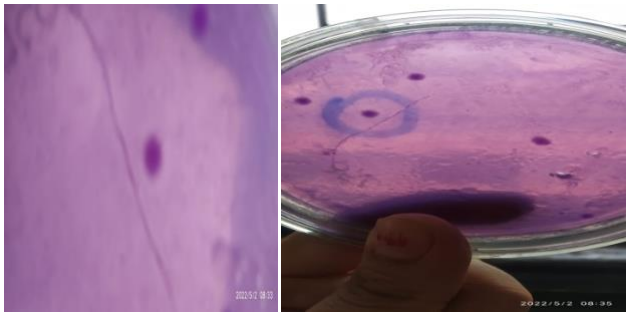


Fig 1petriplates showing bacterial colonies in different dilution

Colonies which were giving holozone were selected and further tested on media plate containing 25ppm to 40 ppm crystal violet.

The decolourizing activity of the best 4 isolate was studied using crystal violet at different initial concentrations varying from 25 to 40 mg/l. All 4 bacterial strains used in the present study having the potential of decolorizing crystal violet but with variation in their capacities .Decolorization of crystal violet after different incubation periods also have been determined.colony no.4 was giving maximum decolourisation after 48 h.

The result revealed that decolorization was also strain dependent i.e, Decolorization percentage depends on bacterial strain .The most potent strains for decolorization of crystal violet dye was used for further production on minimal media and high concentration of dye .

CONCLUSION

In this study,the isolated bacillus spp. CV-S1 has demonstrated potentiality for its Crystal violet dye degaradation. Now a days, increasing growth of industries is creating high risk to the environment as the toxic and hazardous chemical coming from the effluents. Therefore ,microbial approaches are well suited to address this issue.In this regard ,our study may enlighten to degrade highly toxic crystal violet dye .we are taking consideration to augment the capacity of those crystal violet isolates in dye degradation by optimizing various parameters as well as improve their dye degradation by slowly acclimatize them in increasing dye concentration.

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