Application of *Cotylidia pannosa* in dye decolourization of textile effluents

Submitted in partial fulfillment of 4 year degree programme B.Tech.

IN

BIOTECHNOLOGY

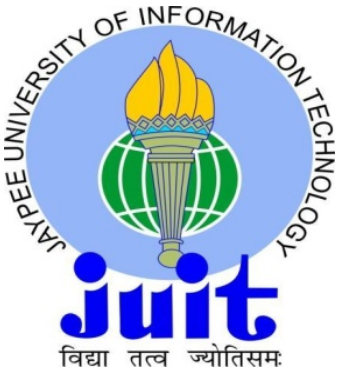
By

Kencho Wangdi (141803)

UNDER THE SUPERVISION OF

DR. GUNJAN GOEL

ASSOCIATE PROFESSOR



DEPARTMENT OF BIOTECHNOLOGY AND BIOINFORMATICS

JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY

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CERTIFICATE

This is to certify that **Mr. Kencho Wangdi** have carried out the undergraduate project work on **"Application of *Cotylidia pannosa* in dye decolourization of textile effluents"** under my supervision from July 2017 to May 2018. The work presented in this project report is original and has not been submitted anywhere else for any other degree.

(Signature of Supervisor)

Dr. Gunjan Goel

Date (…/05/2018)

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First and foremost, I would like to thank the one above all, the **God**, for answering my prayers and for giving me the strength, thank you so much Dear Lord.

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Lastly but not the least; I would like to thank my beautiful family for their love, blessing, and encouragement, even though we have been miles apart. Special thanks are due for **my parents** for all their sacrifices and hard work that has made me what I am today.

Date: …/05/2018

Kencho Wangdi (141803)

DECLARATION

I hereby declare that the dissertation entitled "**Application of *Cotylidia pannosa* in dye decolourization of textile effluents**" submitted towards fulfillment for the award of degree of Bachelor of Technology in Biotechnology at **Jaypee University of Information Technology** is based on the results of studies carried out under the guidance and supervision of **Dr. Gunjan Goel**. This dissertation or no part of this has been submitted elsewhere for the award of any degree or diploma.

Kencho Wangdi
(141803)

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CHAPTER-1

INTRODUCTION

INTRODUCTION

Dyes make the world look colorful that cheers everyone. But on the other side it's a serious pollutant to the environment. Tons of dyes are produced each year. About one million ton of azo dyes are produced each year which is commonly used in textiles, pharmaceuticals and chemical industries [Hao et al., 2000]. In India every type of dye and pigment is produced and about 80000 tons of dyes are produced in India. India is also the second highest exporter of dye stuffs. Textile industry is one of the significant industries that admission an extensive volume of water and colorant amid wet handling and creates impressive measures of colorants as waste items alongside different chemicals. The principle factors in charge of arrival of color gushing into water bodes is ill-advised color take-up of color amid wet preparing and their level of obsession with the substrate and it likewise relies upon a few factors, for example, profundity of the shade, application technique, material to alcohol proportion, and pH and so forth. Roughly 2% of the colors created specifically released in water, and around 10% is lost in the coloring forms. It is evaluated that roughly 20% of the colors and colorants enter the water bodies during wet handling. The nearness of these mixes in the water bodies makes genuine ecological issues and health issues because of poisonous and cancer-causing nature to amphibian life and to human life. The arrival of colorants and colors in water bodies has made a worldwide worry because of their monstrous lethality towards sea-going life and humanity [Geo et al., 2010]. Nearness of shading obstructs light entrance, decreases photosynthetic movement and furthermore chelates metallic particles that outcomes in miniaturized scale lethality to fish and other sea-going life forms. The nearness of harmful and perilous substances made by these colors prompts oxidation and decrease in water which additionally builds the requirement for expulsion colors from wastewater. Therefore, material effluents and shades are significant concern should have been dealt with before releasing into nature to keep these potential risks.

Colors in light of their protection from biodegradation under aerobic conditions experience reductive part of their holding under anaerobic conditions which prompts arrival of sweet-smelling amines. The significant issue with anaerobic decolorization is they experience microbiologically a nonspecific procedure, in this way both coloring technologists and shading physicists confront distinctive difficulties like accomplishing most extreme depletion and

obsession levels to adjust the coloring formulas and color outline. The textile industries and other industries confront difficulties to satisfy the prerequisite of government enactment and administrative offices to guarantee consistence with natural issues. The sorts of intricacy and prerequisites differ all inclusive and consistently. Because of such certainties, researchers and specialists are concentrating on various procedures and strategies on expulsion of colors from material modern wastewaters by utilizing distinctive treatment systems like physical, physio-chemical, chemical, and natural techniques. The physical strategies and in addition the chemical techniques have indicated effective and urging patterns however both the techniques suffer downsides and financial components. Organic treatment techniques i.e. biological treatment are considered as a monetarily and eco cordial suitable choice. The organic method for expelling material colors from the material wastewater has been fundamentally explored and it has indicated better and viable route in decolorizing material effluents than accessible physical and physio-chemical strategies [Boyter 2007], [Teli 2008], [Singh and Arora 2011].

By keeping the entire above discussed things in mind the present work focuses on the biological treatment of dyes using *Cotylidia pannosa*. So the present work entitled: *"***Application of *Cotylidia pannosa* in dye decolourization of textile effluents"** was done with following objectives:

- Growth and maintenance of *C. pannosa*
- Formulation of synthetic dye containing textile effluent
- Application of *C. pannosa* on synthetic dye containing textile effluent

CHAPTER -2

REVIEW OF LITERATURE

2.1 Dyes

"The best shading in the entire world is the one that looks great on you." From the old circumstances, individuals have been utilizing diverse colors and colorant for coloring materials, their skins and environment. Colors are even utilized for painting.

2.2 History

All colors were extracted from nature till mid of the nineteenth century. In 1856, the English scientific expert W.H. Perkin was first man to combine the engineered color, Mauveine. This innovation was the pickup venture in revelation of different engineered colors. In this way, in the start of twentieth century, the manufactured colors started to supplant regular colors [Welham 2000].

2.3 Dye Order

Colors can be ordered in a wide range of ways. They can be grouped by their structures, their method for holding, as per their responses, physical powers. They can be characterized in view of wellspring of materials, nature of their individual chromophores, and as per their techniques for application.

2.4 Classification in View of Wellspring of Materials

- Natural dyes
- Synthetic dyes

2.4.1 Natural dyes

These colors are gotten from plant parts (like roots, leaves, bark, berries, and wood), spineless creatures, or minerals. Some natural sources incorporate organisms and lichens.

2.4.2 Synthetic dyes (Engineered colors)

90% of hues accessible in advertise are engineered colors. Manufactured colors were utilized wherever from industry level to home level. This is on account of they are less expensive,

quicker to create and simple to apply to texture [Zollinger 1987]. The Color Index segregates 15 diverse application classes:

2.4.3 Diverse kinds of color utilized and their application in material ventures.

Table 2.1 Sort of dyes and their application

Sort of dye	Application of each dyes
Acidic dye	Nylon, silk, and fleece
coordinate dye	Viscose, common fiber for example cotton
Vat dye	Viscose, cotton, fleece, and silk)
Disperse/ scatter fiber	Nylon, polyester, acrylic, tri-acetate acid derivation, di-acetate acid derivation
Basic dye	Jute, acrylic
Receptive dye	Cotton, fleece, silk, gooey, nylon
Sulfur dye	Cotton, viscose
Pigment	Cotton, artificial fiber
Stringent dye	Cotton, fleece, silk
Mineral	Cotton, fleece, silk
Azoic dye	Cotton, viscose
Aniline black	Cotton
Rapid/dye	Cotton
Onium dye	Cotton, jute
Rapidson dye	Cotton

2.5 Production and Release Measurements of Colors

As per an investigation on colors done by Ollgaard the interest for colors and natural shade is expanding at high rate. It was $10.6 billion of every 2008. India delivers around 80,000 tones of colors and shades every year and stood second biggest exporter of color and natural colors among creating nations after China. Material industry is one of the biggest utilization of color, at almost 80% [Ollgaard et al., 1998]. The fundamental course through which colors wind up accessible in the earth is by wastewater. To discover the relative level of colors in material gushing is by considering the level of obsession of various color classes.

Table2.2 Evaluated level of obsession and overall offer of colors.

Dye class	Types of Fiber	Fixation (%)	Loss in squander water (%)	Overall deal in tons
Corrosive	Polyamide	80-90	10-20	100-200
Basic	Acrylic	90-100	0-10	45
Direct	Cellulose	75-95	5-25	65
Scatter	Polyester	95-100	0-5	160
Metal complex	Wool	90-95	5-10	Not known
Reactive	Cellulose	50-85	15-50	115
Vat	Cellulose	90-95	5-10	101

2.6 General procedures in dying industry

Dying process and procedure changes from everyday and even hour to hour due to the batch wise idea of the coloring procedure and is in this way hard to portray. The structure is controlled by

the procedure included, fiber composes, and chemicals utilized. The most articulated varieties incorporate the shade of the wastewater and the sort of color contained in it. The solid shade of material squanders is the hardest segment to treat. The gushing commonly contains an extensive number of segments to treat. The gushing regularly contains an expansive number of mixes as exhibited by one report that, on examination of wastewater streams from four processing plants, emphatically recognized 314 mixes, decided the halfway structure of 94, and identified an extra 107 obscure mixes. The important contaminations in material effluents are aromatics, halogenated hydrocarbons and metals.

2.7 General procedures associated with dying textile/material are separated into two procedures.

2.7.1 Dry process

The dry procedure comprises of 1.Opening, blending, and, mixing 2.Carding, 3.Combing, 4.Spinning, 5.Weaving. By and large, in this process, negligible water is utilized.

2.7.2 Wet process

The wet procedure comprises of 1.Singeing, 2.Desizing, 3.Kiering, 4.Bleaching, 5.Mercerizing, and 6.Coloring. These procedures are subjected to a progression of tasks, which require considerable amounts of water at each stage.

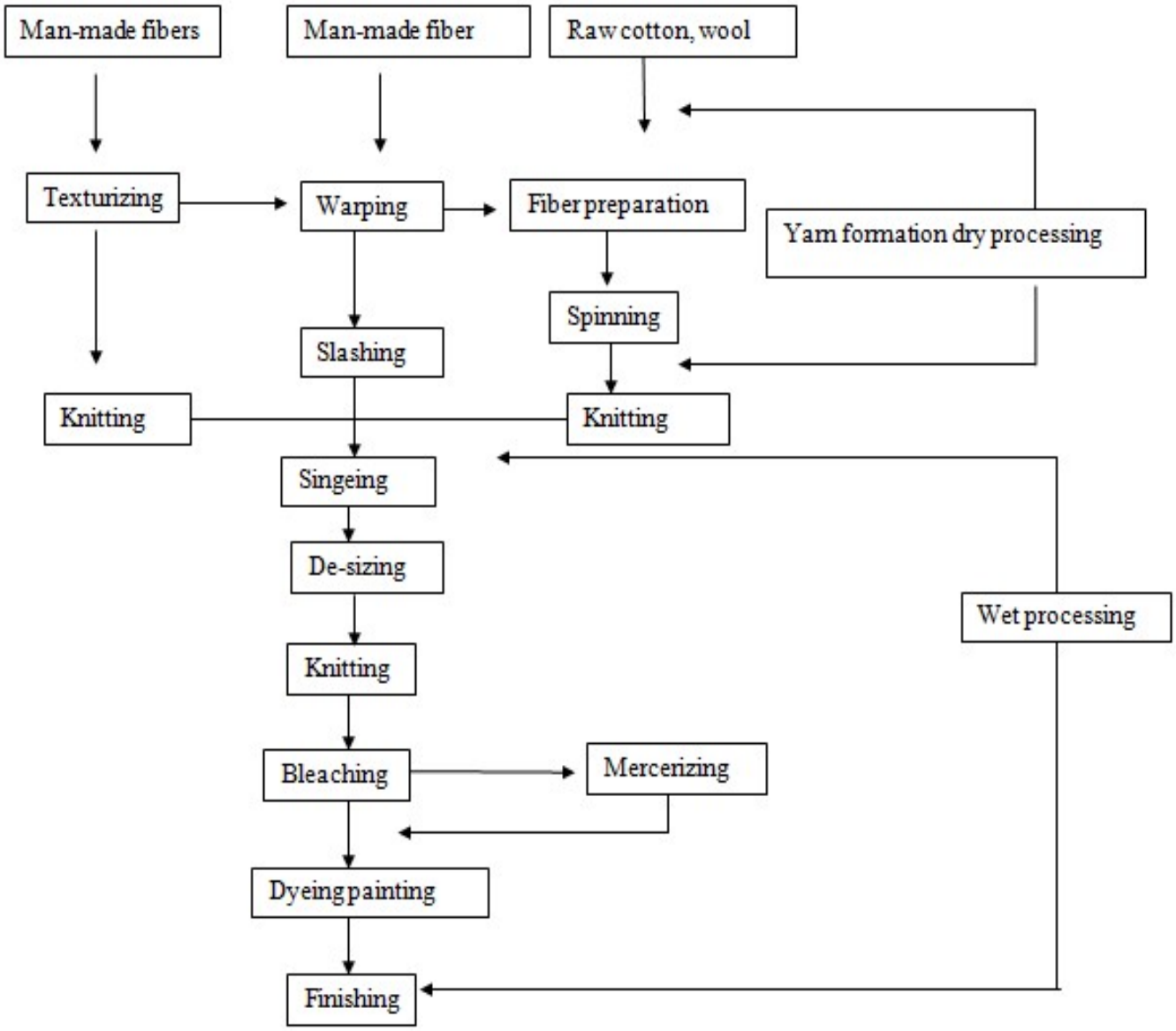


Fig2.1 Flowchart of general wet processing in dying industries

2.8 Toxicity of material effluents

Numerous colors can be effortlessly obvious at low (1 mg l-1) fixations in water. The color fixation wastewater after material handling range in 10-200 mg l-1. In this manner, the arrival of material effluents in untamed waters presents tasteful issue. The release of the wastewater in a water bodies may cause the eco-toxic peril. Because of tremendous utilization of colors, it causes

critical size of contamination. The International Agency for Research on Cancer (IARC) has recognized different sorts of colors like Benzedrine being related with the tumor in people [Anonym 1982]. Benzedrine is cancer-causing in nature and influences assortment of mammalian species, including people [Robins 1980]. Diverse kinds of colors have been tried for mutagenicity utilizing ame's bioassay and a few of them are observed to be cancer-causing and mutagenic to both amphibian creatures and people [Venturini and Tamaro 1979] [Mathur et al., 2005]. A phytotoxicity examine has uncovered the poisonous idea of material emanating and color containing wastewater [Parshetti et al., 2006].

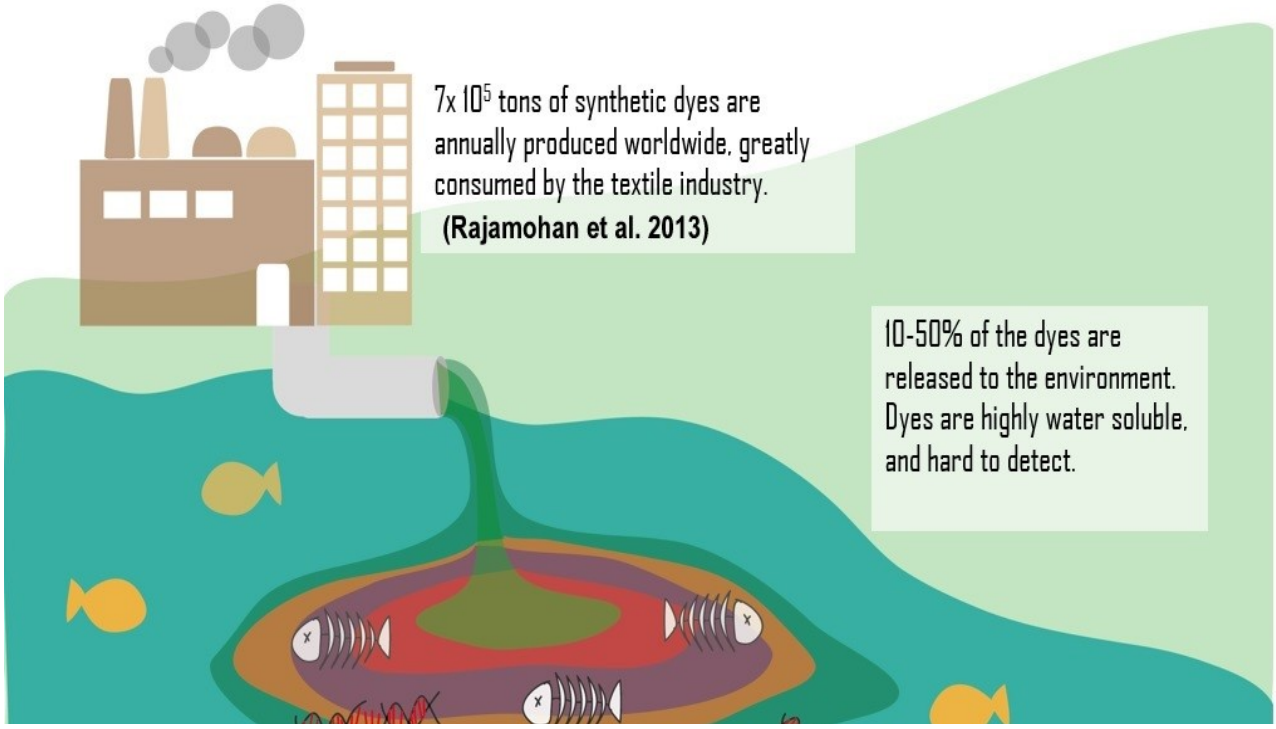


Fig 2.2 Effects of dye towards environment

2.9 Dyes used during the experiment and their application

2.9.1. Congo red

Congo red, which has a complex sub-atomic structure with different diazo fragrant gatherings, is broadly utilized as a part of material industry as an anionic color. Congo red color is one of imperative azo colors. It is shaded substances have complex compound structures and high atomic weights. The synthetic structure is the sodium salt of benzidinediazo-bis-1-naphtylamine-4-sulfonic corrosive. The color's synthetic structure and its fundamental attributes are appeared in Fig. It is very dissolvable in water and relentless in the earth, once released into a regular habitat. Along these lines, the examination on Congo red is fascinating not just to be conceivable poisons of mechanical effluents yet additionally in light of the fact that it is a decent model of complex contaminations [Tapalad et al., 2008].

Application of congo red in other fields

In histology and microscopy

Congo red is utilized for recoloring in amyloidosis, and for amyloid in the plants, and for the external film of gram-negative microorganisms. Apple-green birefringence of congo red recolored arrangements under spellbound light is demonstrative of the nearness of amyloid fibrils. Also, Congo red is utilized for the diagnostics of the *Shigella flexneri* serotype 2a, where the color ties the bacterium's one of lipopolysaccharide structure. Besides, congo red may likewise be utilized to prompt articulation of the sort III emission arrangement of *Shigella flexneri*, realizing the discharge of IpaB and IpaC, which shape translocation pores inside host cell film, enabling effector proteins to go through and change the host cell's organic chemistry. The color can likewise be utilized as a part of motion cytometry tests for the location of Acanthamoeba, Naegleria, and other amoebal growths [Wolfgang Rieper et al., 2005]. Congo red can be utilized as a pH marker [Roderich Raue et al., 2005].

2.9.2. Crystal violet

In textile industry, a noteworthy class of business colors is crystal violet (CV), which is utilized for various purposes, for example, for dermatological specialist, veterinary medication, organic recoloring, added substance in poultry bolster to lessen spread of growth, form and intestinal parasites and for material passing on and paper printing. In any case, because of poor treatment methods for the sanitization of crystal violet containing wastewater from businesses, it is much of the time distinguished in surface water. This color is cationic in nature and is more lethal than anionic color as it can undoubtedly connect with adversely charged film surfaces and can go into cells and amass in cytoplasm **[**Rehman et al., 2000**].**

Applications of crystal violet beside textile industry

Nonmedical

Crystal violet can be utilized to color paper and segment of naval force blue and dark inks for printing, ball-point pens, and inkjet printers. It is likewise used to colorize various items, for example, manures, liquid catalysts, cleansers, and calfskin. The color is likewise utilized as a histological stain, especially in gram recoloring for grouping microbes. When leading DNA gel electrophoresis, crystal violet can be utilized as a nontoxic DNA recolor as another option to fluorescent, intercalating colors, for example, ethidium bromide. Utilized as a part of this way, it might be either joined into the agarose gel or connected after the electrophoresis procedure is done. Utilized at a 0.001% focus and permitted to recolor a gel after electrophoresis for 30 minutes, it can identify as meager as 16ng of DNA. Through utilization of a methyl orange counter stain and a more perplexing recoloring strategy, affectability can be enhanced further to 8 ng of DNA. When crystal violet is utilized as a contrasting option to fluorescent stains, it isn't important to utilize bright enlightenment; this has made crystal violet prominent as a methods for keeping away from UV-actuated DNA obliteration when performing DNA cloning in vitro.

In biomedical research

In biomedical research, crystal violet can be utilized to recolor the cores of follower cells. In this application, crystal violet functions as an intercalating color and permits the evaluation of DNA which is corresponding to the quantity of cells.

In crime scene investigation

In crime scene investigation, crystal violet was utilized to create fingerprints. Crystal violet is likewise utilized as a tissue recolor in the planning of light microscopy sections. In lab, arrangements containing crystal violet and formalin are frequently used at the same time fix and stain cells developed in tissue culture to save them and make them effectively noticeable, since most cells are dreary. It is additionally infrequently utilized as a modest method to put recognizable proof markings on lab mice; since numerous strains of lab mice are pale skinned person, the purple shading remains on their hide for half a month.

Therapeutic

Tincture of Crystal violet used to treat rakish cheilitis on the two corners of the mouth. Crystal violet has antibacterial, antifungal, antihelminthic, antitrypanosomal, antiangiogenic, and antitumor properties. It is utilized therapeutically for these properties, specifically for dentistry, and is otherwise called "pyoctanin" (or "pyoctanine"). It is ordinarily utilized for:

- Marking the skin for surgery readiness and hypersensitivity testing;
- Treating Candida albicans and related parasitic contaminations, for example, thrush, yeast diseases, different sorts of tinea (ringworm, competitor's foot, athlete tingle);
- Treating impetigo; it was utilized basically before the coming of anti-microbials, yet helpful to people who might be sensitive to penicillin.

Veterinary

In view of its antimicrobial action, crystal violet could be utilized for treatment of skin and eye contaminations in domesticated animals, and itch in angle. In any case, it isn't acknowledged for use in aquaculture in most created nations [Yang et al., 2001].

2.9.3. Coomassie Brilliant Blue

Coomassie Brilliant Blue is generally utilized as color in textile and wool industry. The effluents from these ventures have leftover measures of this color. The effluents are to be dealt with for expelling this color before arranging the effluents into the water bodies. As the color is non-degradable in nature; it gets collected in the water bodies and swings to be a potential contamination as it is poisonous in nature. It is accounted for in writing that the utilization of waters containing this color causes extreme eye issues, dangerous to oceanic life, and makes aggravation the mucous layers and upper respiratory tract of the living creatures. Further, it is probably going to make issues with respect inward breath, skin,and ingestion [Ravindhranath et al., 2006]. The reports accessible in expelling this potential poisonous color from squander waters are rare.

Other uses of coomassie brilliant blue

Medicinal uses

In 2009, Brilliant Blue G was utilized as a part of logical examinations to treat spinal wounds in lab rats. It acts by decreasing the body's common swelling reaction, which can make neurons in the zone kick the bucket of metabolic pressure. Testing on the rats demonstrated powerful. Two gatherings of harmed rats were tried, with one gathering given the color as a treatment for the spinal wounds and the other gathering was most certainly not. The aftereffects of the test demonstrated that in contrast with the rats that had not gotten the color, the rats that were treated with the color could move around better as compared to the rats without the color treatment. Testing is still in advance to decide if this treatment can be utilized adequately in people. The current tests have directed the color inside 15 minutes of damage, yet to be successful in a genuine setting, where it might set aside time for a patient to achieve the crisis room, the treatment should be viable not withstanding when managed up to two hours after damage. The main revealed reaction was that the rats briefly turned blue.

Under the exchange name Brilliant Peel, Brilliant Blue G is utilized as a stain to help specialists in retinal surgery.

Application in forensics

Through an investigation done at the University of Albany, it was demonstrated that the capacity of the coomassie color to target amino acids with sweet-smelling gatherings (phenylalanine, tyrosine, tryptophan) and essential side chains (lysine, arginine and histidine), permits Bradford test to be utilized for unique finger impression examination. Bradford test was effectively used to distinguish the natural sex of the unique mark. Female examples were appeared to have a higher absorbance contrasted with men tests when tried at comparative wavelengths. This gives a less complex strategy to unique mark investigation by diminishing the quantity of amino acids should have been broke down from 23 to 6, and having almost no measure arrangement, in contrast with the ninhydrin concoction examine which requires test planning, for example, warming and protein course [Steinberg 2009].

2.9.4. Xylenol orange

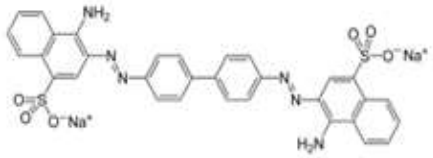
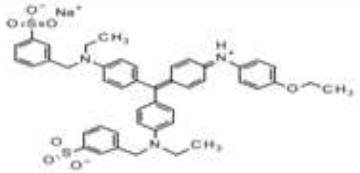
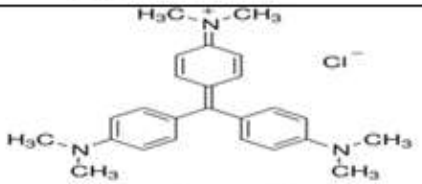
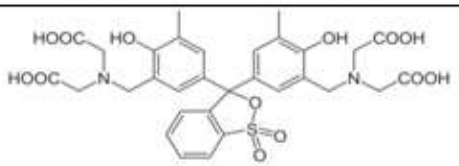
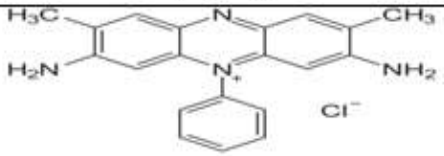
Xylenol orange is one of the commonly utilized color in material, paper, and, nourishment industry to include appeal and flavor. These colors are normal water contaminations and it might be every now and again found in follow amounts in modern waste water. It's essence in water, even at low fixations, is exceptionally obvious and bothersome. At the point when these shaded effluents enter waterways or any surface water framework they annoy natural movement [Chil et al., 2010].

2.9.5. Safranin

Material coloring technique is a central wellspring of sullying of water in charge of the persistent contamination of nature. Safranin is considered as an exceedingly lethal substance however they are used in nourishment industry, material industry, paper industry, elastic industry, and so on. Nearness of high convergence of safranin in amphibian framework tremendously affects the strength of human, creatures and plants [Kajal et al., 2014]. Pollution of safranin in water can cause hypersensitive dermatitis, skin disturbance, growth, and transformation in altruistic being. Endeavors have been as of now started to take out safranin from water. Numerous strategies have

been accounted for in writing for end of colors from squander water, for example, photograph reactant debasement, compound corruption, micellar improved filtration, cation trade film, electrochemical debasement, adsorption/precipitation process, coordinated synthetic natural corruption, and incorporated iron [Kajal et al., 2014].

Table2.3 The chemical structures and their molecular formula of different dye used for the study.

Dyes	Molecular formula	Chemical structure
Congo red	$C_{32}H_{22}N_6Na_2O_6S_2$	
Coomassie brilliant blue	$C_{47}H_{50}N_3NaO_7S_2$	
Crystal violet	$C_{25}N_3H_{30}Cl$	
Xylenol orange	$C_{31}H_{32}N_2O_{13}S$	
Safranin	$C_{20}H_{19}N_4$+Cl-	

2.10 Dye expulsion systems

Material effluents and wastewater can be cleaned utilizing distinctive corruption procedures. A few procedures like physical, physiochemical, and natural methods can be utilized to debase shading from the color containing wastewater.

2.10.1 Physical strategies

In physical strategies, methods like electro dialysis, sedimentation, illumination, screening, nano-filtration, switch osmosis, are utilized to expel colors shape squander water and effluents. These procedures are essentially utilized amid the procedure of elucidation.

2.10.2 Synthetic Treatment

Diverse techniques utilized as a part of synthetic treatment are ozonation, neutralization, and chlorination. Ozonation is able for deciding of hues that are responsive, helps in lessening of substance oxygen request, and release of toxic contaminants from the mechanical color effluents. The fundamental drawback of ozonation is length of time.

Chlorination: It is most normally utilized strategy. In this procedure solid oxidizing concoction; chlorine (Cl2) is utilized to eliminate microscopic organisms and furthermore treats wastewater.

Neutralization: In this procedure acid or base is added to recover lack of bias of the water.

Table2.4: Advantages and detriments of some chemical technique

Methods	Advantages	Disadvantages
1.Ozonization	Volume is unaltered, remained in vapor state.	Life span is short(20 sec)
2.Photochemical	Production of sediment is off	Metals, acids, halides created as side-effects
3.Electrochemical	Disruption of non-aimless mixes	Cost of energy supply is high
4.Electrocoagulation	Decolourization can be achieved	No disappointment of framing metallic (OH) mists in squander water.

2.10.3 Physio-chemical techniques

Film filtration, ozonation, coagulation/flocculation, precipitation, buoyancy, adsorption, particle trade, particle match extraction, mineralization, electrolysis, propelled oxidation (chlorination, fading, fenton oxidation, photograph synergist oxidation, and compound diminishment are some physiochemical strategies utilized for color decolorization.

2.10.4 Natural techniques

The Biological method for color evacuating depends on the microbial biotransformation of colors. The diverse kinds of microorganisms utilized for the color decolorization are actinomycetes, green growth and microscopic organisms. Numerous specialists and researcher have led diverse approaches to corrupt colors utilizing parasites, green growth, actinomycetes and unadulterated or blended societies of microbes or their catalysts. Lignolytic and non-lignolytic growths were normally utilized by the numerous specialists for the decolorization of color wastewater. White rot fungi (lignolytic) are thought to be the best in color corruption. The normally utilized lignolytic organisms are *Phanerochaete chrysosporium*, *Trichophyton rubrum* LSK-27, *Ganoderma* sp. [Yesiladal et al., 2006], and [Nilsson et al., 2006]. A non-lignolytic organisms, yeast for example, *S.cerevisiae*, is commonly used non-lignolytic organisms for the decolorization of material effluents and wastewater [Meehan et al., 2000] and [Donmez 2002]. Amid the lignolytic procedure of color decolorization compounds, for example, laccase, peroxidase like, lignin peroxidase, manganese peroxidase are associated with biodegradation of colors [Pajot et al., 2007]. The normal variables influencing color decolonization are; static/dynamic, pH, and concentration. The ideal pH for the color decolorization run in the range of 7 and 8. [Patil et al., 2008], [Kalyani et al., 2008].

2.11 Mechanism of dye decolorization

The parasitic and bacterial degradation of the material effluents under anaerobic and oxygen expending conditions occurs by breaking and separating of chromophoric gathering. This breakage is a direct result of different frameworks, for instance, mixes, sub-nuclear weight, and substance diminish by biogenic reductase. The occasion of these reactions can be both intracellular and extracellular.

2.11.1 Direct enzymatic shading decolorization

The infectious decolorization of hues were connected with the relationship of various reductive mixes, for instance, azoreductase, MG reductase, and oxidative chemicals like lignin peroxidase, manganese, laccase, and polyphenol oxidase [Parshetti et al., 2006], [Kalme et al., 2007], [Kalyani et al., 2009].

2.11.2 Mediated natural shading decolorization

High nuclear weight sulfonated azo hues can't experience the cell film [Levine 1991]. It was prescribed that the decreasing of these hues could occur through the instrument that isn't liable to the vehicle in to the cell film [Dos Santos et al., 2007]. 1-amino 2-napthol, one of the constituent amines of an azo shading, AO7, extended its decolorization rate, maybe by intervening the trading of diminishing reciprocals [Mendez-Paz et al., 2005]. The development of designed electron bearers, for instance, anthraquinone-2,6-disulphonate could moreover unimaginably overhaul the decolorization of various azo hues [Van der Zee et al., 2001]. Uncovered the primary instance of an anaerobic cleavage of azo hues by redox center individuals surrounded in the midst of the high-affect corruption of a xenobiotic compound. Even the extension of culture filtrates of these cells could enhance anaerobic decolorization by cell suspensions created without NS. The redox intermediates delivered in the midst of high-affect debasement of fragrant blends could enhance decolorization [Keck et al., 1997]. The development of culture supernatants containing metabolites decolorizing *E. coli* NO3 strain updated its azo shading decolorization rate [Chang et al., 2004].

2.11.3 Dye decolorization using regular and inorganic blends

Color decolorization can happen from totally substance responses with inorganic mixes, for example, sulfide and ferrous atom that are shaped as convincing delayed consequences of metabolic responses under anaerobic conditions. It has been exhibited that H_2S age by SRB achieved the extracellular decolorization of the azo dyes [Diniz et al., 2002]. Sulfate affected shading diminishing associated with biogenic sulfide plan under methanogenic conditions. Without sulfur blends, shading decolorization expeditiously occurred inside seeing granular slop, demonstrating the criticalness of enzymatic instruments. An examination of decolorization vitality in the bunch reactors and in the exploration office scale anaerobic slop bed reactors indicated relative criticalness of blend shading diminishing frameworks in high rate anaerobic bioreactors [Van der Zee et al., 2003]. Distinctive inducers and stabilizers of oxidoreductive proteins, for instance, $CaCO_3$, indole, otolidine, veratrole and vanillin overhauled dye decolorization [Dawkar et al., 2008].

2.12 Common strategies to decolorize the dye

2.12.1 Bisorption: In bisorption they decolorize dyes by adsorption, proclamation, molecule exchange, mineralization, and use as carbon source.

2.12.2 Enzymatic degradation: In enzymatic debasement they take after two unmistakable part.

- Non-particular delignification in which they degrade cellulose, lignin, and hemicelluloses portions where as in
- Selective delignification they degrade lignin and hemicelluloses prior to cellulose.

In delignification process the white rot fungi produces display of proteins, for instance, laccases, peroxidases like lignin peroxidase and manganese peroxidase. These mixes licenses white rot fungi to capably hydrolyze woody materials and hold clear sugars required for their improvement and framework.

2.13 Effects of dye effluents

Colors can prompt number of natural and wellbeing dangers which are as per the following;

I. Many colors and their breakdown items are cancer-causing, mutagenic and lethal to life.
II. Damage to the sea-going condition.
III. Triple essential growths including kidney, urinary bladders, and liver of color specialists have been accounted for.
IV. The exceedingly harmful and mutagenic color diminishes light entrance and photosynthetic movement causing oxygen insufficiency and constraining downstream gainful uses.
V. Inhibits movement and development of microorganism, especially in high focus.
VI. Azo colors have lethal impacts, particularly cancer-causing and mutagenic. They entering the body by ingestion and are utilized by intestinal microorganisms causing DNA harm. [Rajamohan et al., 2013]

CHAPTER-3

MATERIALS AND METHODS

3.1 Materials and methods

3.1.1 Chemicals used

Chemicals used during the projects are: acetic acid(CH_3CHOOH), urea($(NH_2)_2$ CO), potassium phosphate(KH_2PO_4), sodium bicarbonate($NaHCO_3$), Magnesium sulfate heptahydrate($MgSO_4.7H_2O$), calcium chloride($CaCl_2$), ferric chloride hexahydrate($FeCl_3.6H_2O$), sodium hydroxide(NaOH), hydrochloric acid(HCl) and glucose($C_6H_{12}O_6$).

3.1.2 Media used

A. PDA- potato dextrose agar
    - Potato infusion→200gm
    - Dextrose→20gm
    - Agar →20gm
    - Distilled water→1 liter
B. PDB-potato dextrose broth
    - Potato infusion
    - Dextrose
    - Distilled water

3.1.3 Apparatus used:

All the apparatus used for the experiments were autoclaved and sterilized. The different types of apparatus used are conical flasks, beaker, measuring cylinder, petri plates, cotton plugs, tray, and pipette and tip box.

3.1.4 Preparation of the inoculums

The culture of *Cotyledia pannosa* was revived on PDA plates and was incubated at 30˚C for 3-5 days.

Prepared stimulated textile effluents (STE) in lab (Table 3.1) and were autoclaved. STE were divided into 10 conical flasks and in each two flask different dyes were added which makes 5 pair of flask with 5 different dyes. Different dyes used were shown in table 2.3.

Inoculated 5 flask containing different dyes with 4-5 plugs of the active culture and they were marked as test and rest 5 flasks were taken as references. Incubated at 30˚C and 100 rpm. Results are noted after every 24 hours for 15 days

3.2 Composition of simulated textile effluent (STE)

Table3.1 Chemicals used to make STE

Chemicals	Amount in mg/1000ml
CH_3CHOOH (99.9%)	0.150ml
$(NH_2)_2CO$	100.0mg
KH_2PO_4	67.0mg
$NaHCO_3$	840mg
$MgSO_4.7H_2O$	38.0mg
$CaCl_2$	21.0mg
$FeCl_3.6H_2O$	7.0mg
$C_6H_{12}O_6$	6000mg

Simulated effluent was made by adding per liter of water (distilled) and pH of the effluent was adjusted to 8 using 0.1M HCl and NaOH. Followings are the chemical composition added to make the effluent.

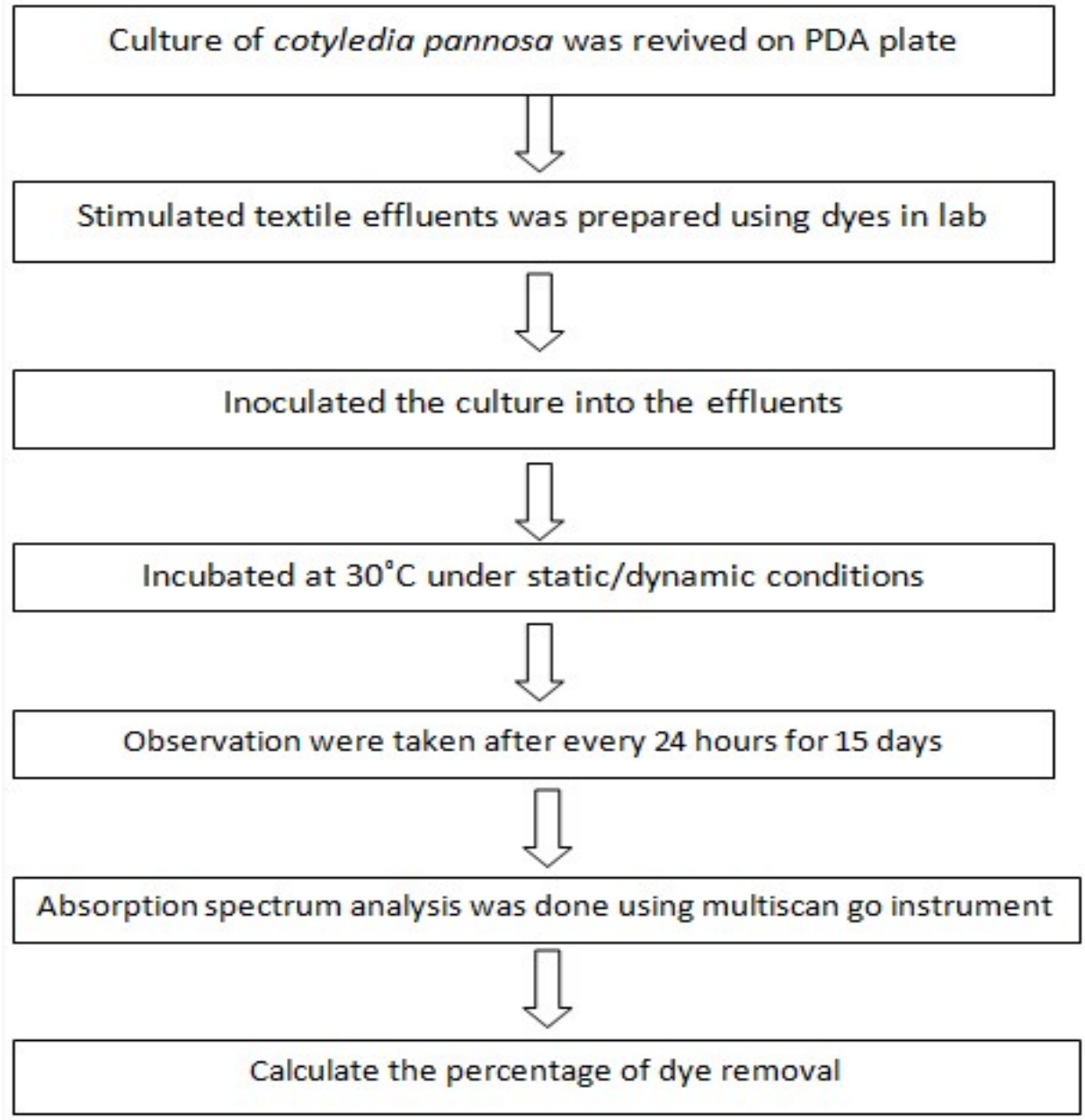


Fig 3.1 Flowchart showing the general procedure

3.3 Assimilation range investigation

The assimilation range investigation was finished utilizing Multiscan Go (Thermofischer) instrument. The range extend utilized was 380-780 nm.

Both test and controls were dissected. All analyzed results were given in observation and result section

3.4 Analytical

All decolorization tests were completed at different circumstances. STE included with various colors was utilized as a control to decide abiotic shading misfortune amid the test. Fittings of 4-5 circle of precultured *C.pannosa* was added to 100 mL of STE included with focuses 25mg of congo red, crystal violet, R-coomassive brilliant blue, xylenol orange and 2ml (20mg) of safranine. The biodecolorization by the contagious culture was watched for 15 days. To screen the decolorization procedure, the examples were pulled back occasionally following 24 hours and were estimated utilizing spectrophotometer at the range of 370-780nm and was compared uninoculated control.

3.4.1 Equation used to compute percentage of color evacuation

$$\text{Dye decolorization}(\%) = X\text{-}Y/X$$

Where X= initial absorption and Y= final absorption of dye

CHAPTER-4

RESULTS AND DISCUSSIONS

OBSERVATIONS

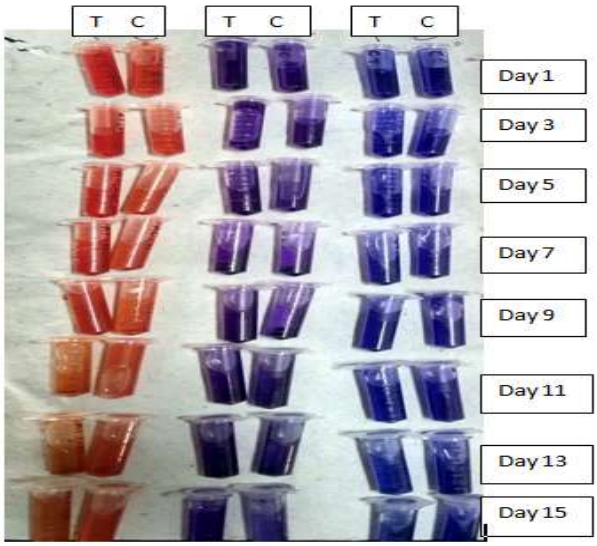


Fig 4.1 *Cotylidia pannosa* growth. **Fig4.2 Dye decolorization of congo red, R-coomassie brilliant blue, and crystal violet.**

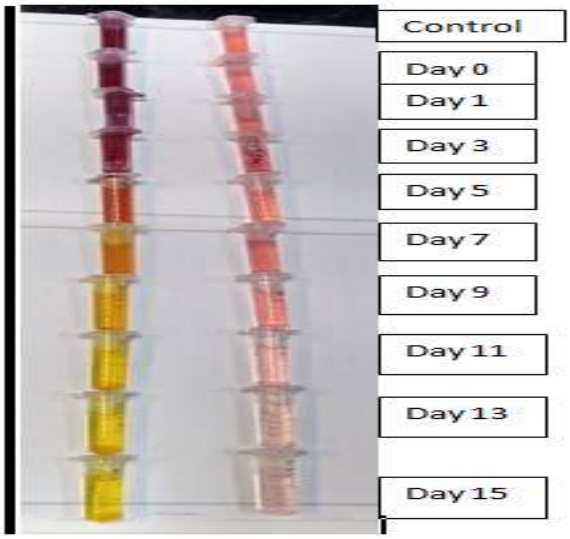
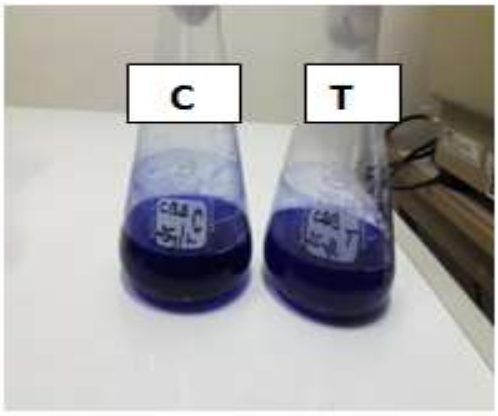


Fig4.3 Dye decolorization of Xylenol orange and safranin. **Fig4.4. Cogo red decolourization.**



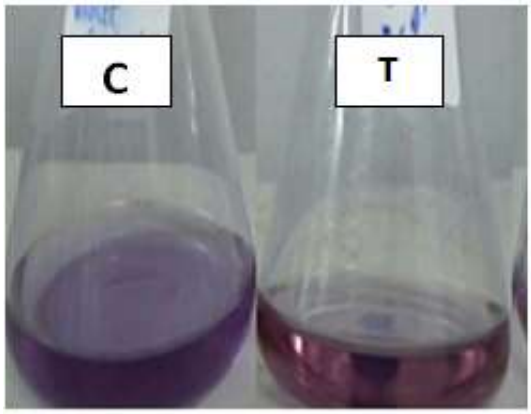
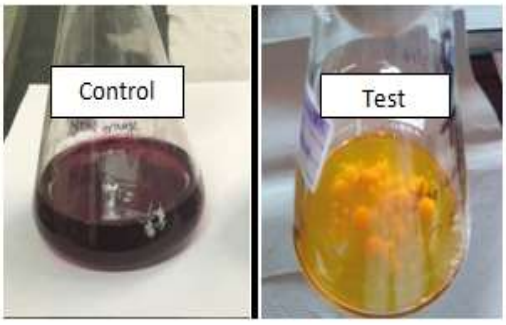


Fig4.5 R-Coomassie brilliant blue decolourization. **Fig4.6 Crystal violet decolourization.**



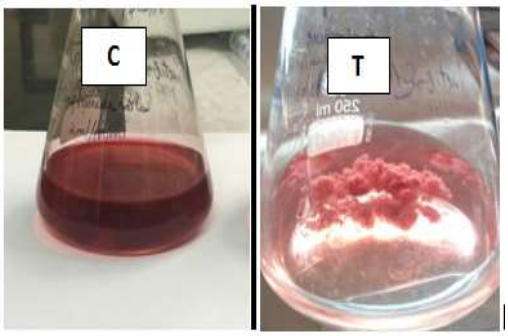


Fig 4.7 Xylenol orange decolorization. **Fig4.8 Safranine decolorization.**

The results of dye decolourzation experiments have shown the significant role of *Cotylidia pannosa* in dye decolourization. The absorption spectra (380-780nm) for congo red(Fig4.4), crystal violet(Fig4.5), R-coomassive brilliant blue(Fig4.6), xylenol orange(Fig4.7), and safranine(Fig4.8) containing effluent has shown descending patron in 15 days. Maximum absorption for congo red was observed at 500nm, crystal violet between 570-600nm, R-coomasie brilliant blue at 560nm, xylenol orange between 560-600nm, and safranine at 520nm.

SPECTRUM ANALYSIS

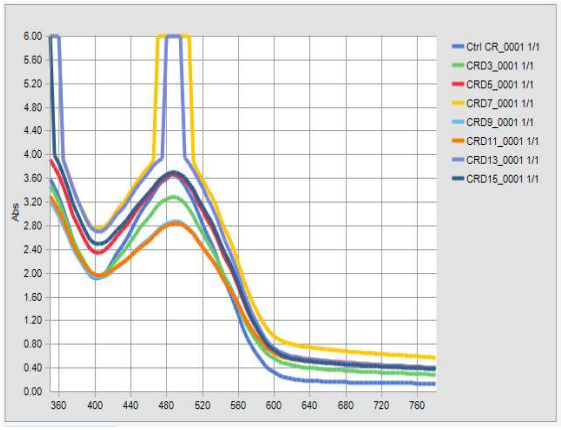


Fig 4.9 Congo red (Max. abs.500nm)

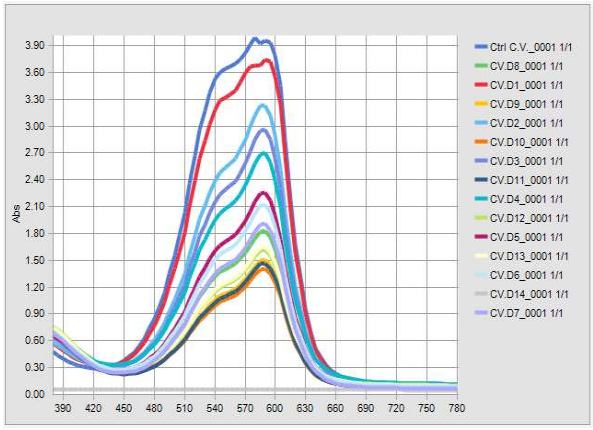


Fig 4.10 Crystal violet (Max. abs.570-600)

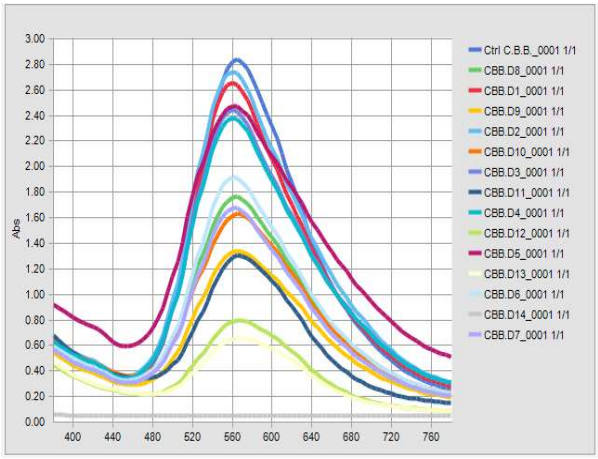


Fig 4.11.R-coomassie brilliant blue (Max. abs 560)

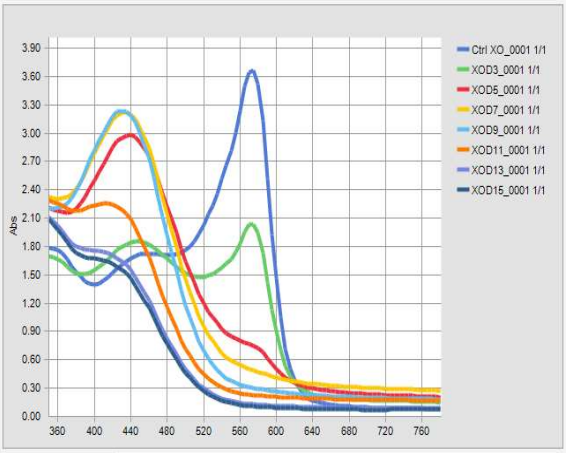


Fig 4.12. Xylenol orange (Max.abs 560-600)

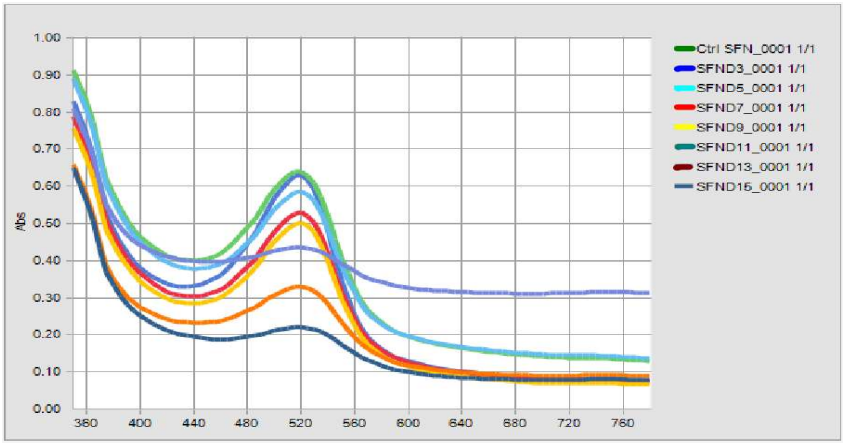


Fig4.13 Safranin decolorization (Max.abs 520)

CALCULATIONS

Table4.1 Optical density of different dye used

Days	Congo red	Crystal violet	R-coomissie brilliant blue	Xylenol orange	Saffranine
0	-	4	2.85	1.9	0.65
1	-	3.75	2.68	1.58	0.64
3	-	3.2	2.5	0.40	0.59
5	-	2.25	2.45	0.38	0.57
7	-	1.89	1.67	0.34	0.54
9	-	1.80	1.32	0.25	0.50
11	-	1.60	1.3	0.22	0.45
13	-	1.5	0.8	0.21	0.34
15	-	1.20	0.67	0.19	0.20

Table4.2 Calculation of dye decolorization efficiency(%)

Days	Congo red %	Crystal violet %	R-coomissie brilliant blue %	Xylenol orange %	Safranin %
Day 0	-	0	0	0	0
Day1	-	6.25	5.96	16.84	1.53
Day3	-	20	12.28	78.94	9.23

Day5	-	43.75	14.03	80	12.3
Day7	-	52.75	41.40	82.10	16.92
Day9	-	55	53.68	86.84	23.07
Day11	-	60	54.38	88.42	30.7
Day13	-	62.5	71.92	88.94	47.69
Day15	-	**70**	**76.49**	**90**	**69.23**

CHAPTER 5:

CONCLUSION

CONCLUSION

Recent literature designates that albeit an immensely colossal number of lab-scale studies have been conducted on decolourization of textile dye solutions by biological methods however there is a need to develop a method that can be applied on industrial scale effluents. So in the present study we tried to investigate the potential of *C. pannosa* for efficient treatment of dye containing textile effluents. In our study found that *C. pannosa* can significantly remove dyes like xylenol orange, safaranine, crystal violet and R-coomasive brilliant blue upto 90%, 69.23%, 70% and 76.49% respectively. However furthers more studies are required to make an efficient biodegradation process for treatment of dye containing effluents using *C. pannosa*.

CHAPTER-6

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