“Evaluation Of toxicity and biodegradation of major dyes used in textile industry”

Project Report

MASTERS OF SCIENCE

(MICROBIOLOGY HONS.)

SUBMITTED BY

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Under the guidance of

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CERTIFICATE

This is to certify that the thesis entitled **"Evaluation of toxicity and biodegradation of major dyes used in textile industry"** by**Miss. Prama Rani (11611113)**, submitted to the Lovely Professional University, Punjab for the Degree of Master of Science in Microbiology is a record of bonafide research work, carried out by her in the Department of Microbiology under my supervision. I believe that the thesis fulfils part of the requirements for the award of Master of Science. The results embodied in the thesis have not been submitted for the award of any other degree.

Date: 30-11-17 Signature of Advisor

Sujata Das

Associate professor

Lovely Professional University

DECLARATION

I Prama Rani hereby declare that, this project report entitled **"Evaluation of toxicity and biodegradation of major dyes used in textile industry"**, carried out by me, under the guidance of Sujata Das, Associate Professor, Lovely Professional University, Phagwara is my own and has not been submitted to any other University or Institute or published earlier.

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TABLE OF CONTENT

LIST OF FIGURE AND TABLE

CHAPTER -1

INTRODUCTION

Implementation of fungi in the process of remediation is called mycoremediation. Fungi play vital roles in all ecosystems, regulating the flow of nutrients and energy through their mycelial networks. It can be said that they act like natural and true ecosystem engineers [1]. Among the other fungi, White-rot fungi posses a great range of different enzymes such as hydrolytic enzymes (cellulose, pectinase, xyloses) and extracellular ligninolytic enzymes (lignin peroxides, manganese peroxidase and laccase) [2]. Beside the role in wood degradation white-rot fungi can be applied for degradation of different industrial contaminants such as low molecular polycyclic aromatic carbohydrates, aromatic carbohydrates and chlorophenols [3-4], textile dyes [5-6], pesticides [7] or in recent time pharmaceuticals such as ibuprofen, clofibric acid and carbamazepine [8] or naproxen [9].

Coloured substance are mainly originated from textile sector. It is estimated that over $7*10^5$ tons of dyes produce annually and 2 percent are directly discharge into water bodies. They have mutagenic and carcinogenic effect on human being. It is essential to eliminate dye from wastewater before discharging to open environment. There are several method like physical ,chemical and biological method are used to remove the dye among all biological method by using microorganism capable of degradation or decolourizaton of dye receive increasing interest because of cost , effectiveness ,and ability to produce less sludge[10]. Crystal Violet and Malachite green are highly toxic can cause skin irritation and kidney failure [11,12,13] and also effect immune and reproductive system [14].Adsorption is a good method to remove dye from wastewater because cost ,simple operation ,high efficiency , ability to separate wide range of chemical compound by using low cost adsorbent wheat bran.

A second step should be degradation and decolourization of dye by using microorganism. There are wide range of microorganism capable to degrade these compound white rot fungus include in this group ,so we are isolating white rot fungus than screening with enzyme and also checkingtheir effect on cell line .The aim of this work were to study the adsorptive capacity of wheat bran for removal of malachite green and crystal violet.

CHAPTER – 2

Review of Literature

A dye is a coloured substance that has an affinity to the substrate to which it is being applied. Dyes may be natural or synthetic in origin. It may be soluble or insoluble in water and impart color when apply to matter such as textile ,food ,plastic ,paper etc.

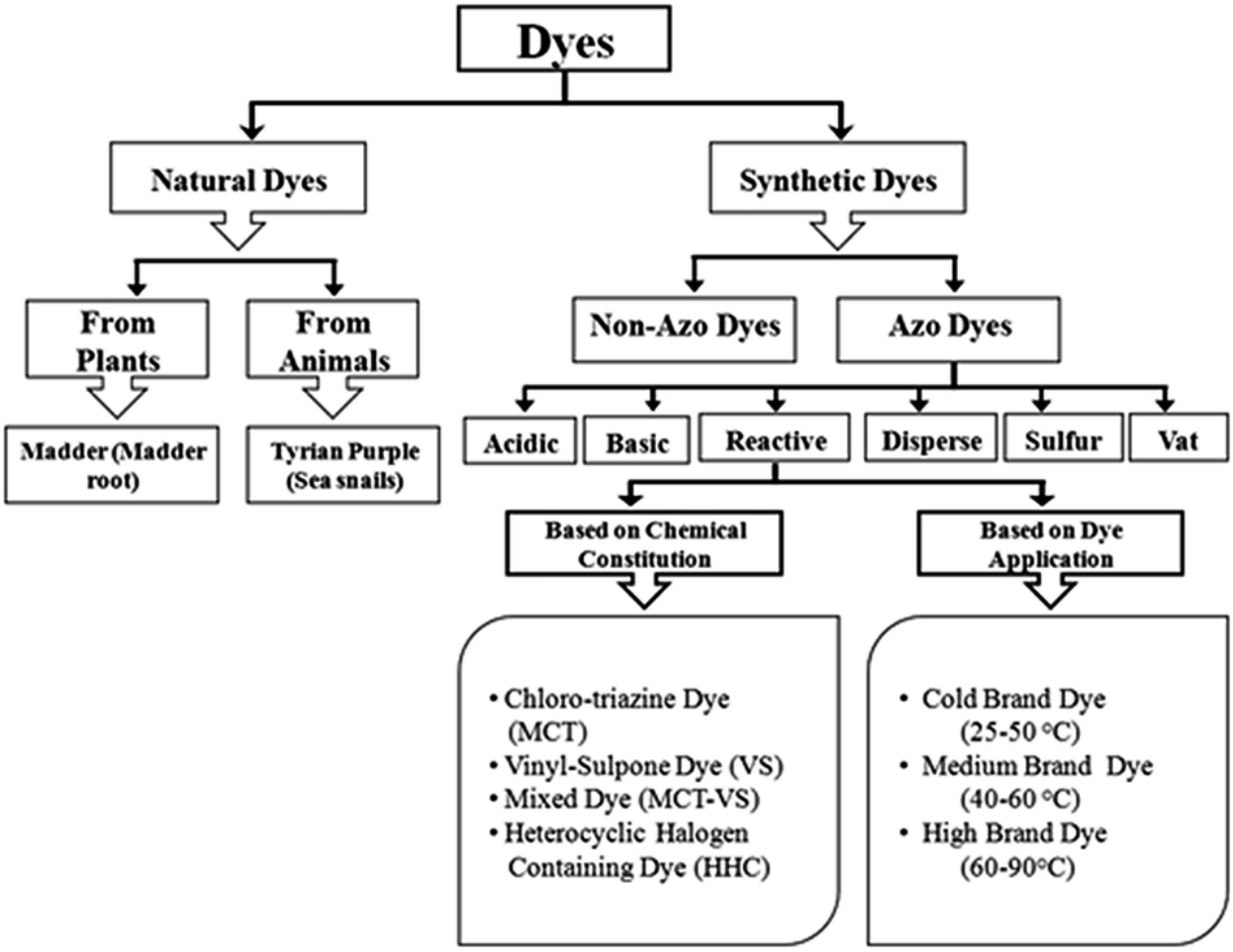

FIG. NO .1 (ROYAL SOCIETY OF BIOCHEMISTRY)

Dispersive dyes

They are water insoluble nonionic dye ,Primarily use for polyester and acetate fiber. These dyes are highly carcinogenic and mutagenic to human cell and also cause permanent injury to cornea, skin irritation, effect reproductive and immune system. Some common example of dispersive dyes are-

Crystal violet –

is also know as gentian violet used for dyeing of cotton and silk and manufacture of paints and inks. It is also use as biological stain and active ingredient in gram stain [15]. CV is highly carcinogenic to mammalian cell , cause skin irritation, digestive tract, lead to kidney failure[15] .

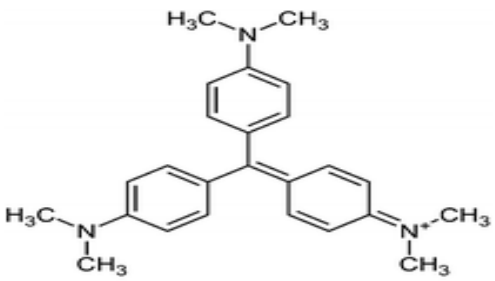

FIG .NO. 2(ROYAL SCOIETY OF BIOCHEMISTRY)

Malachite Green –

Malachite green is an N-metylated diaminotriphenyl methane dye ue for coloring and antiful agent in fish farming industry [14] .Malachite green is highly toxic to human effect immune and reproductive system [14][16] also banned in several country and not approve by US Food and drug administration [14].

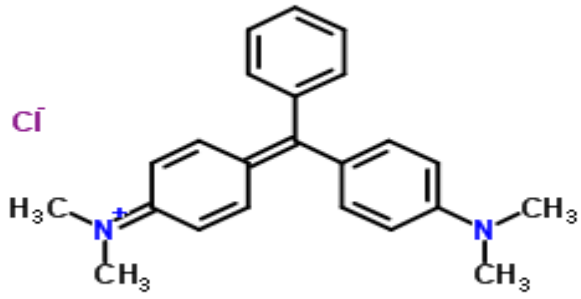

FIG. NO. 3 Malachite Green

(ROYAL SCOIETY OF BIOCHEMISTRY)

Adsorption

Nowdays removal of dye by adsorption method become popular. The substance that adsorb on surface is adsorbate and substance which is adsorb is know as adsorbent. Adsorption capacity is equally depend on physical and chemical characteristic like temperature , pH , contact time , adsorbent dosage etc[17] .

Different Types of Adsorption Isotherm

There is a connection between adsorbate and adsorbent . Adsorption isotherm is a physical process studied through graph. Amount of adsorption between adsorbate and adsorbent function at constant temperature[17] .There are different models for adsorption study are-

1) **Linear Isotherm**
2) **Freundlich Isotherm**

3)Languir Isotherm

Linear Isotherm

The concentration of adsorbate is less in film and bulk water with some adsorbent. Because surface area of activated carbon is high so concentration of adsorbate also increase as adsorption proceeds but less valid for activated cabonadsoption system.

Freundlich Isotherm

Empirically derived model and at every step has its own energy of adsorption surface is heterogenous. Kf and n are Freundlich constant and n tell us rate of adsorption process[17] .

Languir Isotherm

In this isotherm surface of adsorbent there should be a finite capacity and every surface should have same energy of adsorption and surface is homogenous. Adsorbent adheres to adsorbate in single layer.Languir isotherm is represented in linear form and qo is monolayer adsorption capacity (mg/g) and K_L Languir constant [17].

Nowdays resercher are working with different types of adsorent are-

1 Commercial Adsorbent

1.1 Silica gel

Silica gel mainly originate from by decreasing pH of alkali silicate. Non toxic and many researcher are investigated adsorption of textile dye with silica and adsorption rate was good but main prolem is their cost because silica is expensive [40].

2 Low Cost Adsorbant

2.1 Wheat bran

Wheat is a one of the major crop grow throught the world .Also produce some by product like wheat bran, wheat husk adsorption rate of wheat bran is very high and cost is not a problem. Adsorption of Malachite green by wheat bran investigatsd by resercher[27].

3 Industrial Municipal Waste

3.1 Fly Ash

It is a solid waste of thermal plant depend on coal burning. The cost is very low main problem is that their adsorption capacity is low [41].

4 Natural Material

4.1 Natural clay

Clay mainly use for adsorption are Kaolinite, bentonite, sepioliteetc are used for dye removal . Their adsorption rate is high on basic dye as compare to acidic dye because ion present on dye are character for clay[42].

5 Biosorbent

5.1 Biomass

For biosorption process different types of microorganism are used such as algae , yeast , bacteria and fungi they can decolourize dye at high rate .Many resercher investigated decolourization of dye with different organisms[29][30][31].

Some other example of low cost adsorbent such as saw dust [18] rice hush ash[19] bagasse fly ash [20] water orange peel and peanut hull [21] bottom ash zeolite [22] coconut sheel [23] groundnut shell [24] rice straw [25] wheat bran[26] etc .

Low cost adsorbent should be economically feasibile , locally avaliable , good adsorption capacity etc .The cost of these adsorbent lesser when compare to commercial activated carbon so generally cost effective and right selection of adsorbent which give high adsorption rate are key factor play important role in selection of suitable adsorbent.

By using fixed batch coloum study 3 resercher together investigate biosorption of Artocarpus heterophyllus (jackfruit) leaf powder for removal of crystal violet from aqueous solution and study was carried out at different parameter like ph , contact time , temperature , initial dye concentration.For isotherm study use Langmuir isotherm model maximum biosorption 43.39 mg/g at ph 7.0 , 293 k temperature , 120 min contact time , also follow pseudo second order kinetic model and fixed batch coloum time increase with increase bed height and decrease with flow rate and conclude that jackfruit leaf powder is promising biorbent for removal of crystal violet[15].

Another Adsorption study on removal of methylene blue from aqueous solution by using wheat bran at temperature 25 -45 ˚c ,initial concentration of methylene blue 100-500 mg per Litre. Adsorption study was describe by Langmuir model , Freundlich and Redlich –Peterson model. Apply pseudo first and second order to test experimental data and second order provide better adsorption than first [27].

In 2006 by using batch technique remove malachite green and adsorbed on wheat bran and check effect on different parameter like ph , temperature , contact time etc and maximum adsorption is observe at ph 7-9 , 90 percent dye is removed .For adsorption study use Freundlich isotherm. They also degrade malachite green with White rot fungus strain Fomes Sclerodermus and Phanerochaete chrysosporium.

Malachite green is completely degraded by F . sclerodermus at ph 5 with high lignase production . Adsorption of wheat bran ,growth , enzyme production , dye degradation are influence by ph[26].

Biological Method of Decolourization

As compare to physical and chemical method Biological method for degradation of textile dyes has minimum impact on envirnment and cost effectiveness[28]. The most widely studied microorganism are Bacteria[29] , Fungi[30] , Algae[31] for decolourization of textile dye.

Fungi

Many well known strain of fungi are to degrade textile dyes by producing 3 enzyme . The capability of fungi for degradation due to presence of highly oxidative and non –specific nature of lignolytic enzyme , lignin peroxidase because they inhibit the presence of carbon and nitrogen[32].

White Rot Fungus

White rot fungus belong to Basidiomycetes abel to degradation of lignin in nature. White rot fungus produce enzyme such as lignin peroxidase , manganese peroxidase and laccase during lignin degradation [33].

Lignin peroxidase

Isolate from Phanerochaete chrysosporium in 1983and relatively nonspecific to its substrate and oxidize in the presence of hydrogen peroxide. Molecular weight varies from 38-36 kDa. Degrade by oxidation process and interact with lignin polymer by veratryl alcohol. It is a secondary metabolite of white rot fungus and act a cofactor of enzymes[34].

Manganese peroxidase

Belong to a second peroxide group in basidiomycetes. Molecular weight 3-6.5 kDa. They also require hydrogen peroxidase for oxidation of Mn^{2+} ion to highly reactive Mn^{3+}. The redox potential of manganese peroxidase is lower than lignin peroxidase[35].

Laccases

first describe by Yoshida 1883 and eco-friendly enzyme . Belong to blue multicopper oxidase enzyme . The active site of laccases comprise four copper atom either directly or indirectly. Laccase are glycoprotein with molecular weight 15 -30 kDa . Fungal laccase have low carbohydrate content 10-20% mainly belong to Ascomycetes , Deuteromycetes , and Basidiomycetes etc.

For substrate oxidation hydrogen peroxidase is not require .Laccase oxidize phenol , aniline , N- hydroxybenzotriazole (HBT) and 2,2'-azino-bis(3-ethylbenzothiozoline-6-sulphonic acid) (ABTS) degrade dye andother substane. The lignolytic ezyme are produced during secondry metabolism of limited nitrogen[34].

Different Fungal Strain used for decolorization of textile dye-

Serial Number	**ORGANISM**	**DYE**
1	Phanerochaete chrysosporium	Crstal violet [36]
2	Ganoderma lucidan	Malachite green, Crystal violet [37]
3	Polpous elegans	Malachite green, Crystal

		violet [38]
4	Aspergillus niger	Malachite green, Crystal violet [39]
5	Pleurotus ostreatus	Malachite green, Crystal violet [37]

In 2006 first they adsorb malachite green on wheat bran by adsorptio study than degrade dye .Also discus about white rot fungus there are so many microorganism abel to degrade dye. But white rot fungus have ligninase system and have three enzyme : lignin peroxidase , Manganese peroxidase and laccase . They taken Fomes Sclerodemeus strain of white rot fungus basidiomycete.They study degradation of dye observe on wheat bran by F.Scleerodrermus[26].

CHAPTER – 3

METHEDOLOGY

ISOLATION

Collection of sample –

White rot fungus we are using for isolation purpose is isolate from dead decaying wood, soil and mushroom area near Jalandhar and Ludhiana, India. The sample was collected in sterile plastic bag sealed and brought to the lab aseptically for further use.

Isolation of sample-

Collected sample is washed with distilled water and cut aseptically in laminar air flow and inoculated on petri plate containing processed wheat bran and wheat bran agar media. Wheat bran agar media for 100 ml distilled water containing 4% agar agar and 1.5% processed wheat bran and o.1% antibiotic (streptomycin). Incubate at 30˚c temperature for 5 days. Distinct fungal colonies were isolate and repeatedly subculture until pure culture was obtained.

ADSORPTION

Collection of Adsorbent –

Wheat bran sample is taken from local market and brought to lab for further use.

Processing of Adsorbent –

Sample we brought from market is washed with distilled water to remove adhere particle .The cleaned material is kept under sunlight for dry. The dry mass was kept in hot air oven for sterilization , After sterilization blend sample to make fine core particle and used in adsorption experiment as such without any further modification.

Collection of Adsorbate

Dye sample Crystal violet , Malachite green is obtained from vardhman textiles.CV =C25 H30 CIN3, Molecular weight -407.99 and IUPAC Name –4-Tris(Dimethyl amino)phenyl)methlium chloride. MG = C30 H25 CIN2, Molecular weight – 364.911 and IUPAC name – 4[4-(Dimethylamino)phenyl](phenyl)methylene}N,N-dimethyl-2,5-cyclohexadien-1-iminiumchloride.

Preparation of Stock Solution

For 100 ml of distilled water we take 1gm of dye sample.

Experiment Procedure-

From stock solution taking 100g/l dye

↓

Wheat bran 200mg for 20ul(19.8ml D.W+0.2 ml of dye)

↓

Incubation 3hrs /6hrs in shaking incubator at 120 rpm and 30˚C temperature

↓

Dispense out 2ml of each solution into eppendrof tube (3 eppendrof for each)

↓

Centrifuge for 15 min at 5000rpm

↓

Check optical density of supernatant

Adsorption studies

We done dsorption study by using Batch adsorption method at different parameters like pH, adsorbent dosage , contact time , temperatute etc. For each experiment 20ml of crystal violet solution of known concentration taken in 100ml of Erlenmeyer flask and agitated with wheat bran at temperature 30 degree celcis. For pH , adsorbent dosage and temperature incubation period is 3 hours but for contact time incubation upto 8 hours. After incubation sample were centrifuged and supernatant was analyzed by using Light spectrophotometer at maximum wavelength 590 nm. Blank can be set by distilled water and adsorbent[15].

CHAPTER-4

RESULT AND DISCIUSSION

Batch Adsorption Studies-

Effect of pH on adsorption

pH is important parametrer because it effect adsorbent surface charge, chemistry of dye solution , etc . Crystal violet is a basic dye have lowerpeak value 0.8 and up to 7.0 pH adsorption capacity increase and highly adsorb at pH 6, 7and 9.Same result was reported from literature absorption of crystal violet by Artocarpus heterophyllus[15]. At low pH there is a repulsion between positively charged dye and negative charge adsorbent due to protonation so capacity to remove dye is decreased as pH increases deprotonation occur and strong electrostatic force occur between dye and adsorbent removal efficiency of dye is increased.

Effect of temperature

Adsorption of Crystal violet at different temperature from 30 degree celsius to 90 degree celsius shown that maximum adsorption is occur at low temperature like 30, 50 and 40 as the temperature increase adsorption rate is decreases because may be binding capacity of dye and adsorbent may decrease.

Effect of Contact time

Adsorption of Crystal violet at different contact time is decreases with increase in contact time and in first 60 minute adsorption rate is high but as time increases rate adsorption of dye is slow down and maximum adsorption rate is upto 120 minute after this there is gradually decrease same result found in adsorption of Cystal violet by NaoH –modified rice hush and by Actocarpus heterophyllus[15].

Effect of Adsorbent dosage

The amount of adsorbent taken from 0. 5 gram to 3 gram and initial concentration of dye is 100g/L effect of adsorption rate is gradually decreases as adsorbent dosage increases and maximum adsorption rate upto 1.5 gram and high adsorption rate was observed in 0. 5 gram adsorbant. The increase in adsorption at low adsorbent due to availability of more adsorption site and another reason aggregation because leas to decrease the total surface area of adsorbent.similar result was observed in removal of rective dyes using wheat bran.

Crystal violet pH	Incubator	Shaking incubator
2	0.535	0.216
3	0.218	0.121
4	0.163	0.149
5	0.263	0.171
6	0,323	0.219
7	0.320	0.234
8	0,310	0.101
9	0,676	0.377
10	o.180	0.086

Crystal violet adsrbant dosage	incubator	Shaking incubatyor

0.5g	1.145	0.389
1g	0.581	0.337
1.5g	0.428	0.421
2g	0.377	0.423
2.5g	0.336	0.411
3g	0.325	0.393

Crystal violet (contact time)	Incubator	Rotational
30 min	1.532	1.709
1 hr	1.389	0,348
1.5hr	0.989	0,200
2hr	0.874	0.229
2.5hr	0.852	0.231
3hr	0.681	0.285
3.5hr	0.670	0.280
4hr	0.503	0.238
4.5hr	0.558	0.197

5 hr	0.463	0.207
5.5hr	0.376	0,151
6hr	0,371	0,147
6.5hr	0,357	0,130
7hr	0.354	0.189
7hr.5	0.360	0.127
8h r	0.390	0.111

Crystal violet (temperature)	Optical density
30	0.645
40	0.392
50	0.432
60	0.368
70	0.320
80	0,342
90	0.307

Isolation of white Rot Fungus

The isolates belong to group of basidiomycetes and collected from local area shown a fungal growth on both processed wheat bran and wheat bran media agar

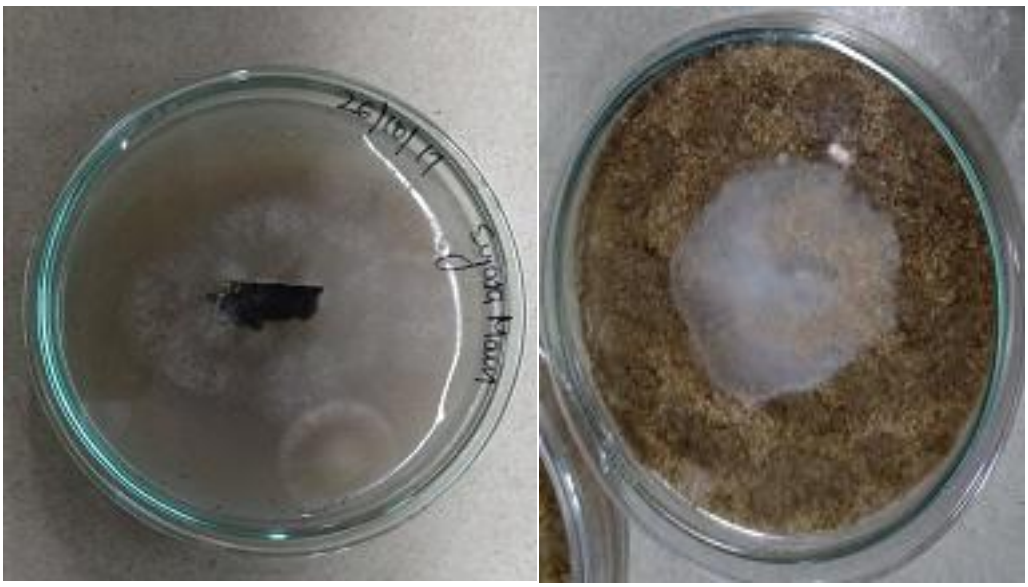

FIG. NO. 4 Growth of white rot fungus on wheat bran agar and processed wheat wheat bran.

Conclusion

In Batch adsorption method we conclude that adsorption capacity of low cost adsorbent is more as compared to other adsorbants. The cellulose content of adsorbant is very efficient in adorption of dyes.

CHAPTER -5

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TOPIC APPROVAL PERFORMA

School of Bio Engineering and Bio Sciences

Program : P263-H::M.Sc. (Hons.) (Microbiology)

COURSE CODE : BTY796 **REGULAR/BACKLOG :** Regular **GROUP NUMBER :** BSRGD0148

Supervisor Name : Sujata Das **UID :** 12521 **Designation :** Assistant Professor

Qualification : _____ **Research Experience :** _____

SR.NO.	NAME OF STUDENT	REGISTRATION NO	BATCH	SECTION	CONTACT NUMBER
1	Prama Rani	11611113	2016	B1610	8427346897

SPECIALIZATION AREA : Microbiology **Supervisor Signature:** _____

PROPOSED TOPIC : Evaluation of toxicity and biodegradation of major types of dyes used in textile industries

Qualitative Assessment of Proposed Topic by PAC		
Sr.No.	Parameter	Rating (out of 10)
1	Project Novelty: Potential of the project to create new knowledge	7.00
2	Project Feasibility: Project can be timely carried out in-house with low-cost and available resources in the University by the students.	8.00
3	Project Academic Inputs: Project topic is relevant and makes extensive use of academic inputs in UG program and serves as a culminating effort for core study area of the degree program.	7.67
4	Project Supervision: Project supervisor's is technically competent to guide students, resolve any issues, and impart necessary skills.	8.00
5	Social Applicability: Project work intends to solve a practical problem.	7.67
6	Future Scope: Project has potential to become basis of future research work, publication or patent.	7.67

PAC Committee Members		
PAC Member 1 Name: Dr. Ashish Vyas	UID: 12386	Recommended (Y/N): Yes
PAC Member 2 Name: Himanshu Singh	UID: 11691	Recommended (Y/N): Yes
PAC Member 3 Name: Dr. Joydeep Dutta	UID: 14336	Recommended (Y/N): Yes
PAC Member 4 Name: Dr. Umesh Goutam	UID: 14691	Recommended (Y/N): NA
DAA Nominee Name: Mamta Sharma	UID: 18431	Recommended (Y/N): NA

Final Topic Approved by PAC: **Study on biotransformation of dispersive dyes used in textile industries**

Overall Remarks: Approved (with major changes)

PAC CHAIRPERSON Name: 11840::Dr. Neeta Raj Sharma **Approval Date:** 28 Mar 2017