Bacterial Degradation and Detoxification of Recalcitrant Colouring Pollutants from Textile Wastewater for Environmental Safety

THESIS

SUBMITTED TO
BABASAHEB BHIMRAO AMBEDKAR (A CENTRAL)
UNIVERSITY, LUCKNOW

BABASAHEB
BHIMRAO
AMBEDKAR
UNIVERSITY
• LUCKNOW •
प्रज्ञा शील करुणा
ESTABLISHED 1996

FOR THE DEGREE OF

Doctor of Philosophy

IN

Environmental Microbiology

Submitted By

Roop Kishor

(Enrolment No. 379/15)

Under the supervision of

Dr. Ram Naresh Bharagava

(Assistant Professor)

DEPARTMENT OF ENVIRONMENTAL MICROBIOLOGY
SCHOOL FOR ENVIRONMENTAL SCIENCES
BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
(A Central University, NAAC Accreditation ‘A’ Grade)
VIDYA VIHAR, RAEBARELI ROAD, LUCKNOW-226025
UTTAR PRADESH, INDIA

APRIL, 2022

Dedicated to

my Beloved Parents

BABASAHEB
BHIMRAO
AMBEDKAR
UNIVERSITY
LUCKNOW
प्रज्ञा शील करुणा
ESTABLISHED 1996

Babasaheb Bhimrao Ambedkar University
(A Central University)
Vidya Vihar, Rae Bareli Road, Lucknow - 226 025.
बाबासाहेब भीमराव अम्बेडकर विश्वविद्यालय
विद्या विहार, रायबरेली रोड, लखनऊ - 226 025

DR. RAM NARESH BHARAGAVA
Assistant Professor
Department of Environment Microbiology
E-mail: bharagavarnbbau11@gmail.com

Letter No.: ....
Date: ....

CERTIFICATE

This is to certify that the thesis entitled **"Bacterial Degradation and Detoxification of Recalcitrant Colouring Pollutants from Textile Wastewater for Environmental Safety"** submitted by **Mr. Roop Kishor** is an original research work and has not been previously submitted in part or full for the award of any other degree or diploma to this or any other university.

The thesis submitted to Babasaheb Bhimrao Ambedkar University, Lucknow satisfies all the requirements as stipulated in the *Doctor of Philosophy (Ph.D.) Regulations - 1999 as amended in 2008/2010/2013* and it is fit for submission and evaluation for the award of the degree of Doctor of Philosophy of the University.

Date: 20/04/2022

(Dr. Ram Naresh Bharagava)
Supervisor

Dr. R. N. Bharagava
PhD
Assistant Professor
Deptt. of Environmental Microbiology
Babasaheb Bhimrao Ambedkar University
(A Central University)
Rae Bareli Road, Lucknow-25

20/4

Head of Department

विभागाध्यक्ष
पर्यावरणीय सूक्ष्मजैविकीय विज्ञान विभाग
बाबासाहेब भीमराव अम्बेडकर विश्वविद्यालय
(केंद्रीय विश्वविद्यालय), लखनऊ, २२६०२५

STUDENT DECLARATION

I, **Roop Kishor** hereby declare that the work, which is being presented in the thesis entitled **"Bacterial Degradation and Detoxification of Recalcitrant Colouring Pollutants from Textile Wastewater for Environmental Safety"** in the partial fulfilment of requirements for the award of degree of **Doctor of Philosophy (Ph.D)** and submitted in the **Department of Environmental Microbiology, Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow 226025, Uttar Pradesh, India** is an authentic record of my own work carried out during the period from February 2018 to February 2022 under the supervision of **Dr. Ram Naresh Bharagava,** Assistant Professor, **Department of Environmental Microbiology, Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow 226025, Uttar Pradesh, India.**

The thesis is essentially free from all kinds of plagiarism and the work has not been previously submitted in part or full for the award of any other degree or diploma to this or any other university.

Roop Kishor
20/04/2022

Roop Kishor
Ph.D Scholar (Enrol No. 379/15)
Department of Environmental Microbiology
Babasaheb Bhimrao Ambedkar University
(A Central University), Vidya Vihar, Raebareli Road
Lucknow 226 025, Uttar Pradesh, India
Mobile No. 9410043601
E-mail: roopkishor1095@gmail.com

ACKNOWLEDGEMENTS

Undertaking this Ph.D. has been a truly life-changing experience for me and it would not have been possible to do without the continuous support and guidance that I received from many peoples.

A special mention goes to my dynamic and enthusiastic supervisor, **Dr. Ram Naresh Bharagava**, Assistant Professor for his tremendous academic support and several wonderful opportunities that he provided during the course of my study. Many thanks go out to his dedicated help, advice, discussion, inspiration, encouragement and continuous support during my study. I am also thankful to **Dr. R. N. Bharagava**, for his constant faith in my lab work and providing fantastic lab training and nurturing my enthusiasm for environmental microbiology and support during a comfortable stay when so generously hosted me in Lucknow. I have very fond memories of my time there.

I am thankful to all the faculty members of the Department of Environmental Microbiology, **Prof. Ram Chandra (**Head), **Prof. Rajesh Kumar**, **Dr. Jay Shankar Singh, Dr. Vinay Singh Baghel**, **Dr. Pankaj Kumar Arora, Dr. Digvijay Verma, Dr. Ravi Gupta** and **Dr. Harish Chandra,** Assistant Professor, for the academic help, discussion, encouragement and support throughout this study.

I gratefully acknowledge the funding received towards my Ph.D from the **University Grants Commission (UGC)** and **Department of Science and Technology (DST), Government of India (GOI)** New Delhi, India as financial support; without which this study could not have been undertaken.

My thanks also go out to the academic and personnel support that I received from **Dr. Gaurav Saxena,** Assistant Professor**,** Shoolini University, Solan (Himachal Pradesh)**, Dr. Surabhi Zainith,** Assistant Professor, BFIT Technical Campus, Dehradun, (Uttarakhand) and **Dr. Jay Prakash**, Research Associate, Babasaheb Bhimrao Ambedkar University, Lucknow. I am also thankful to my best friend **Mr. Ajeet Singh,** Senior Research Fellow (SRF), Department of Physics, Babasaheb Bhimrao Ambedkar University, Lucknow, India, for helping me in various ways during my study. The good time spent with them can never be forgotten and will be cherished throughout life.

I am also thankful to my juniors, **Miss. Anuradha Devi** and **Miss. Cristina Saran,** Doctoral Fellow, Department of Environmental Microbiology, Babasaheb Bhimrao Ambedkar University, Lucknow.

I am also thankful to my seniors **Mr. Adarsh Kumar, Mr. Sheel Ratana** and **Miss. Shweta Ambust,** Doctoral Fellow, Department of Environmental Microbiology, Babasaheb Bhimrao Ambedkar University, Lucknow, India for their great help during the study.

I am also thankful to **Mrs. Shweta Bharti, Mrs. Prerna, Mr. Kshitij Singh, Mr. Vinay Kumar, Mr. Ajay Prakash, Miss. Apoorva Dixit** and **Mr. Abhishek Shekhawat,** Doctoral Fellow, Department of Environmental Microbiology, Babasaheb Bhimrao Ambedkar University, Lucknow, India for their great help during the study.

I am also very grateful to all the dedicated non-teaching and technical staff members, especially **Mr. Sarju Singh, Mr. Rahul Srivastava, Mrs. Bhumika Ranjan, Miss Nancy Jaiswal** and **Mrs. Sunita Pal,** Technical Assistant at the office of my department, who were always so helpful and provided me with their assistance throughout this study

Finally, I would like to convey my deepest love and heartfelt thanks to my parents and my sister and brother for their constant support, unfailing patience, contagious love, forgiveness, selflessness, endless support and always inspiring me in all efforts that brought me to this stage. At the end, I can say that this experience had been a rich and rewarding one from which I have learnt that:

Roop Kishor
20/04/2022

Roop Kishor

Babasaheb Bhimrao Ambedkar University
(A Central University), Lucknow-226025,
Uttar Pradesh, India

Date:

CONTENTS

List of Tables

List of Figures

List of abbreviations

AB	:	Azure B
ABTS	:	2,2-Azino-bis-3-Ethylbenzothiazoline-6-Sulfonic acid
ADHD	:	Attention deficit hyperactivity disorder
ADMI	:	American Dye Manufacturing Institute
AOPs	:	Advanced oxidation processes
BLAST	:	Basic local alignment search tool
BOD	:	Biological oxygen demand
BT	:	Biological treatment
BT-TWW	:	Bacterial treated textile wastewater
COD	:	Chemical oxygen demand
CMC	:	Carboxymethyl cellulose
cm	:	Centimeter
Conc.	:	Concentration
CPCB	:	Central pollution control bard
CR	:	Congo red
CV	:	Crystal violet
CS	:	Carbon disulphide
CWs	:	Constructed wetlands (CWs)
CTMs	:	Combined treatment methods
dATP	:	Deoxyadenosine triphosphate
dCTP	:	Deoxycytidine triphosphate
DGGE	:	Denaturation gradient gel electrophoresis
dGTP	:	Deoxyguanosine triphosphate
DCM	:	Dichloromethane
DO	:	Dissolve oxygen
DBT	:	Dibutyltin
DW	:	Distilled water
EC	:	Electro coagulation
EC	:	Electrical conductivity
EDCs	:	Endocrine disrupting chemicals
EO	:	Electro-chemical oxidation
EPA	:	Environmental Protection Agency
FAS	:	Ferrous Ammonium Sulphate
FT-IR	:	Fourier Transform-Infrared Spectroscopy
GC	:	Gas Chromatography
GC-MS	:	Gas Chromatography-Mass Spectroscopy
GI	:	Germination index;
GP	:	Germination percentage
HCB	:	Hexachlorobenzene
HMs	:	Heavy metals
HP-LC	:	High Performance-Liquid Chromatography

IARC	**:**	International Agency for Research for Cancer
IR	**:**	Infrared
LiP	**:**	Lignin peroxidase
LLE	**:**	Liquid-liquid extraction
MB	**:**	Methylene blue
MBR	**:**	Membrane bioreactor
MFCs	**:**	Microbial fuel cells
MG	**:**	Malachite green
MnP	**:**	Manganese peroxidase
MO	**:**	Methyl orange
MTs	**:**	Membrane technologies
MF	**:**	Microfiltration
MR-VP	**:**	Methyl red Voges proskauer’s
MSM	**:**	Mineral salt medium
NAM	**:**	Nutrient agar medium
NF	**:**	Nanofiltration
NCBI	**:**	National Council for Biotechnological Information
NIST	**:**	National Institute of Standards and Technology
ND	**:**	Not detected
NPE	**:**	Nonyl ethoxyphenol
NPEs	**:**	Nonylphenol ethoxylates
NP	**:**	Nonylphenol
ORR	**:**	Oxygen reduction reaction
PCR	**:**	Polymerase chain reaction
PCP	**:**	Pentachlorophenol
PAHs	**:**	Polyaromatic hydrocarbons
PCBs	**:**	Polychlorinated biphenyls
PFCs	**:**	Perfluorinated chemicals
PFOS	**:**	Perfluorooctane sulphonate
POPs	**:**	Persistent organic pollutants
PVA	**:**	Polyvinyl alcohol
RAPD	**:**	Random Amplified Polymorphic DNA
RBCs	**:**	Red blood cells
RCPs	**:**	Recalcitrant coloring pollutants
RT	**:**	Retention time
RO	**:**	Reverse osmosis
RL	**:**	Root length
rpm	**:**	Revolution per minute
rRNA	**:**	Ribosomal Ribonucleic
SCCPs	**:**	Short-chain chlorinated paraffins
SS	**:**	Suspended solids

SD	:	Standard deviation
SL	:	Shoot length
TBP	:	Tributylphosphate
TBT	:	Tributyltin
TPhT	:	Triphenyltin
TCEs	:	Trichloroethane
TIs	:	Textile industries
TIWW	:	Textile industry wastewater
TWW	:	Textile wastewater
TOC	:	Total organic carbon
TDS	:	Total dissolved solids
TS	:	Total solid
TSS	:	Total suspended solids
TP	:	Tap water
UF	:	Ultrafiltration
UK	:	United Kingdom
UNEP	:	United Nation Environment Programme
USA	:	United States of America
USEPA	:	United States Environmental Protection Agency
UT-TWW	:	Untreated textile wastewater
UV	:	Ultra violet
VOCs	:	Volatile organic compounds
WBCs	:	White blood cells
WWTPs	:	Wastewater Treatment Plants
OD	:	Optical density
PAGE	:	Polyacrylamide Gel Electrophoresis
SDS	:	Sodium dodecyl sulfate
TEMED	:	N, N, N’ N’-tetramethylethylenediamine

List of Symboles

~ : Approximately equal
< : Less than
> : Greater than
= : Equal
± : Plus - minus
As : Arsenic
°C : Degree Celsius
% : Percentage
µL : Microliter
Co : Copper
Cd : Cadmium
Cr : Chromium
Fe : Iron
Fig : Figure
g/L : Gram per liter
H : Hour
K : Potassium
M : Molar
m^3 : Cubic meter
Mg : Magnesium
mg/L : Milligram per liter
IN : India
µS/cm : micro-siemens percentimeter
mS/cm^{-1} : Millisiemens per centimeter
min : Minute
mL : Milliliter
Mm : Millimolar
mM : Micromolar
Mn : Manganese
nm : Nanometer
P : Phosphorous
Pb : Lead
pH : Power of hydrogen
US$: United States dollar (USD)
v/v : Volume by volume
w/v : Wight by volume
Zn : Zinc
µL : Microliter
µm : Micromolar
l : Wavelength
Kbp : Kilo base pair
kDa : Kilo dalton
m/z : Mass-to-charge ratio

Abstract

Recalcitrant coloring pollutants (RCPs) are well reported to causes serious threats to water and soil ecosystem. These are highly toxic to plant growth and human/animal health. However, an eco-friendly, cost-effective and efficient treatment method is required to degrade/detoxify the RCPs from textile wastewater (TWW). In present study, a new bacterial consortium RKS-TEX267 was developed using three pollutants degrading bacteria, *Bacillus albus* (RKS2), *Bacillus megaterium* (RKS6) and *Bacillus paramycoides* (RKS7) and used in the degradation and detoxification of RCPs from real TWW. The consortium (RKS-TEX267) showed 99.28% decolorization of RCPs with significantly reduction in COD (88.77%), BOD (93.99%), TOC (72.53%), phenol (85.90%) and heavy metals like Cr (72.35%), Cd (79.09%), As (65.88%) and Pb (83.87%) within 24h at optimized conditions. The optimum conditions were recorded to be temperature 30±2 °C, pH 7, inoculum size 10%, salt concentration (NaCl) 1%, static condition, glucose and yeast extract. During the RCPs treatment, lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase enzyme activities were recorded indicating that these enzymes are involved in RCPs degradation by the isolated bacterial strains. The various RCPs present in TWW and their metabolites produced during the treatment process were identified by FT-IR and GC-MS analysis. In addition, the toxicity studies showed that metabolites of RCPs allowed 100-90% seed germination with significantly higher improvement in root length, shoot length and biomass production of *Vigna radiata* and *Vigna mungo* indicating that the bacterial consortium RKS-TEX267 was effective in degradation and detoxification of RCPs from real TWW. Thus, this newly developed bacterial consortium RKS-TEX267 can be used effectively in degradation and detoxification of industrial wastewater pollutants.

Keywords: Recalcitrant coloring pollutants; Bacterial degradation; Enzymatic analysis; FT-IR and GC-MS analysis; Metabolites characterization, Toxicity

Chapter 1

Introduction

1. Introduction

Textile industries (TIs) are one of the oldest and largest industrial sectors worldwide (Sharma and Malaviya, 2021). These play a major role by contributing to the global economy in many developing countries like China, India, Pakistan, Brazil, Bangladesh and Malaysia (Paździor et al., 2019; Kishor et al., 2021a). In India, there are ~68202 textile looms, regularly operating in various states such as Gujrat, West Bengal, Madhya Pradesh, Punjab, Maharashtra, Haryana, Rajasthan, Odisha, Uttar Pradesh and Tamil Nadu (Khandare and Govindwar, 2015; Kadam et al., 2018; Afrin et al., 2021). Worldwide, TIs contribute ~5% of the total textile trade, ~7% of total world export, ~$2,000 billion of market value and around 120 million employments for urban and rural peoples (Satish, 2018; Vieira et al., 2021). It also shares a total 13% of exports and 5th largest source of foreign currency (Kaur et al., 2018; Kishor et al., 2021b). But unfortunately, these are also the major contributor of environmental pollution and health hazards (Chandanshive et al., 2020; Khan et al., 2021). TIs utilize a large volume of groundwater and a wide range of synthetic chemicals at various stages like sizing, bleaching, dyeing, printing, washing and finishing during the production of valuable textile products (Cao et al., 2019; Kishor et al., 2021a).

TIs discharge a large volume of highly polluted wastewater into the environment (Afrin et al., 2021). In major textile countries, the textile wastewater (TWW) is discharged into the rivers, which finally opens into the sea. For example, in India, Kanpur city is a major hub of textile industries (TIs), which discharge a large volume of wastewater into drains and canals, which opens into the Ganga River and finally, emptying into the Way of Bengal (Samuchiwal et al., 2021; Kishor et al., 2021a). The wastewater discharged from TIs is very complex and contains various toxic recalcitrant coloring pollutants (RCPs/dyes), dissolved solids, organic pollutants and various metal ions (Khandare and Govindware, 2015; Kaur et al., 2018; Afrin et al., 2021). TWW also contains a high load of salts, alkalis, binders, dispersants, volatile organic compounds (VOCs), surfactants, chlorobenzenes, dioxin, phthalates, phenols, pentachlorophenol, detergents and heavy metals (Kaur et al., 2018; Chandanshive et al., 2018). These persist in environment for long time and cause severe threats to the environment and living organisms (Khan et al., 2021; Samuchiwal et al., 2021).

Different natural fibers such as jute, cotton, silk, wool and a variety of synthetic fibers like polyamide, polyester, viscose, nylon and acrylic are converted into textile products by using various chemicals such as sizing, brightening, anticreasing, sequestering, stabilizers, softening and finishing agents (Kaur et al., 2018; Kishor et al., 2021a; Samuchiwal et al., 2021). Thousands of synthetic dyes/RCPs are used in TIs during the dyeing process. Globally, ~7×10^7 tons of different dyes are produced, more than 10,000 tons of dyes are used in TIs and ~280,000 tons of dyes are released into the environment annually (Paździor et al., 2019; Vieira et al., 2021). Dyes are synthetic, recalcitrant and heterocyclic in nature, which are widely used in dyeing, textile, paint, leather, pulp and paper, medicine and cosmetic industries as a coloring agents (Kaur et al., 2018; Kishor et al., 2021c).

Dyes such as azo, vat, direct, reactive, sulphide, acidic and basic dyes are extensively used in TIs (Khan et al., 2021). Around 70% of dyes are not fixed to fibers and get discharged into the river, pond, streams and lakes along with the wastewater (Kaur et al., 2018; Chandanshive et al., 2020; Vieira et al., 2021). TWW is a very complex matrix and contains a variety of toxic metal ions like arsenic (As), lead (Pb), chromium (Cr), antimony (Sb), cadmium (Cd) and mercury (Hg) (Khandare and Govindwar, 2015; Kishor et al., 2021b). Metal ions are used in the production of color pigment of textile dyes (Ceretta et al., 2020; Kishor et al., 2021a). These are transported to a long distance along with the wastewaters, persist in environment (water/soil) for a long time (Bilinsika et al., 2019). RCPs are highly toxic to environment and living organisms due to ecotoxicological effects and bioaccumulation in terrestrial and aquatic life (Khandare and Govindwar, 2015; Khan and Malik, 2017; Khan et al., 2021).

In aquatic system, RCPs cause detrimental effects on water quality by the coloration of water, reduced sunlight penetration, decrease in photosynthetic activity of aquatic plants, dissolved oxygen content and increase in BOD and COD levels leading to anoxic conditions that adversely affect fauna/flora and microbial diversity (Cao et al., 2019; Khan et al., 2021). In soil, these reduce soil fertility, crop yield and biodiversity by causing soil salinity and soil pollution (Chandanshive et al., 2017; Noman et al., 2020). RCPs cause carcinogenic, mutagenic, genotoxic, cytotoxic and allergenic effects to all forms of life (Kaur et al., 2018; Samuchiwal et al., 2021). RCPs accumulate in living tissues through the food chain, pose severe effects on human and animal health such as diarrhea, liver, neuromuscular, hemorrhage, dermatitis, central nervous system disorder and kidney malfunctioning (Khan et al., 2021; Kishor et al., 2021a).

RCPs also cause hypersensitivity, allergies, dermatitis and intestinal cancer in living beings (Khan and Malik, 2017). According to the National Occupational Exposure Survey of the USA, around 69563 workers have been potentially exposed to methylene blue (MB) dye at their working place from 1981 to 1983 (Kishor et al., 2021c; Khan et al., 2021). In Pakistan, China and India, TWW is commonly utilized for the irrigation of agricultural lands, which leads to higher risks of bioaccumulation in crops and finally reached into the successive level through the biomagnification process (Noman et al., 2020; Kishor et al., 2021a). An overview of different stages of wastewater generation in TIs, its toxicity in human health and environment and various treatment approaches are summarized in **Fig. 1.1.** Thus, there is a need to adequately treat RCPs/dyes from TWW before their final discharge into the water bodies for the protection of environment and public health.

Different physico-chemical methods like adsorption, coagulation, flocculation and oxidation have been reported for the treatment of industrial wastewaters (Kaur et al., 2018; Ceretta et al., 2020). But, these methods are only capable to convert pollutants from one form to another form and generate a large amount of toxic sludge as a secondary pollutant causing serious threats to environment and public health (Paździor et al., 2019; Samuchiwal et al., 2021). Membrane treatment technologies like nanofiltration (NF), reverse osmosis (RO), microfiltration (MF) and ultrafiltration (UF) have been reported for the treatment of TWW (Khan et al., 2021; Kishor et al., 2021a). But, these methods require high pressure, expensive materials and produced undesired products (sludge) (Hubadillah et al., 2020).

Advanced oxidation processes (AOPs) including, Fenton, photo-Fenton, photo-catalytic, sono-catalytic, electro-coagulation and electro-oxidation methods have been employed in treatment of RCPs (Paździor et al., 2019; Noman et al., 2020; Vieira et al., 2021). These approaches are faster and more effective, but may not be suitable due to their high cost, incomplete mineralization and generation of various carcinogenic and mutagenic intermediates (Zhuang et al., 2020; Kishor et al., 2021a). Further, to improve the treatment efficiency, various combined treatment methods (CTMs) ((biological + physico-chemical), (biological + AOPs treatment) and physico-chemical + AOPs) have been also used in treatment of RCPs (Paździor et al., 2019; Kishor et al., 2021a; Vieira et al., 2021). But, CTMs require major changes to the existing wastewater treatment plants available with RCPs. Therefore, an eco-friendly and economically feasible wastewater treatment method with high treatment efficiency will be more preferable and acceptable to RCPs.

The biological treatment (BT) is an eco-friendly, cost-effective, less sludge generation and efficient method to remediate RCPs (Ceretta et al., 2020; Vieira et al.,

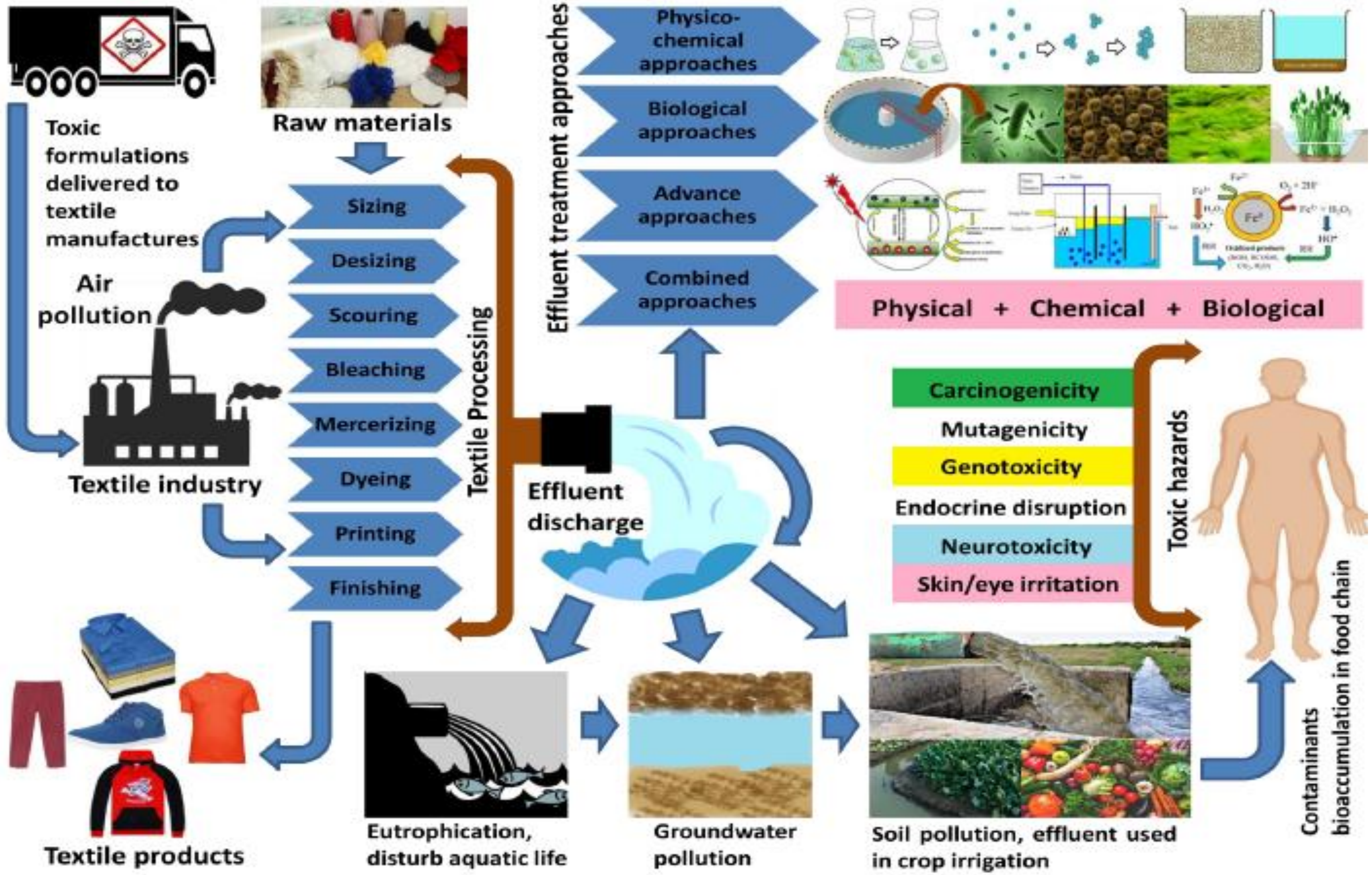


Fig. 1.1: An overview of different stages of wastewater generation in TIs, its toxicity in human health and environment and various treatment approaches.

2021). BT uses the metabolic and enzymatic versatility of bacteria, archaea, fungi, algae, actinomycetes, yeast and plants to degrade RCPs (Cao et al., 2019; Kishor et al., 2021b). Among these agents, bacteria and fungi are widely reported in treatment of RCPs (Cao et al., 2019a). Fungi showed limited decolorization rate, take a long time and cannot use pollutants for their growth (Buntic et al., 2017; Vieira et al., 2021). But, bacteria have a high capability to remediate RCPs (Paździor et al., 2019). Also, bacteria are easy to culture, fast-growing on different substrates and also convert pollutants into less toxic and inorganic compounds. For example, several bacterial strains such as *Pseudomonas, Sphingomonas, Aeromonas*, *Serratia, Pigmentiphaga, Bacillus* and *Shewanella* have been reported in treatment of RCPs from TWW (Cao et al., 2019; Kishor et al., 2021b; Afrin et al., 2021; Vieira et al., 2021).

In past, most of the studies were focused on the removal of specific pollutants like methyl orange (MO) (Ayed et al., 2010), azure B (AB) (Haq et al., 2018), crystal violet (CV) (Bharagava et al., 2018) and methylene blue (MB) dye (Kishor et al., 2021c), from TWW by bacteria. But, TWW is a very complex matrix and contains a mixture of RCPs, organic compounds and metal ions (Kaur et al., 2018; Zhuang et al., 2020). Therefore, a single/pure culture of any biological agents cannot efficiently degrade and decolorize RCPs in real TWW because single/pure culture cannot adapt to complex substances and variable environments (Kurade et al., 2012; Paździor et al., 2019; Vieira et al., 2021). However, the consortium treatment is a more suitable and effective tool to efficiently degrade TWW pollutants as compared to pure/single strains (Ayed et al., 2010; Kurade et al., 2012). The consortium treatment showed high efficiency in degradation due to the synergistic role of bacterial strains and their catabolic enzymes that can effectively degrade/decolorize a mixture of RCPs from TWW (Kurade et al., 2012; Vieira et al., 2021; Kishor et al., 2021a).

To date, there are very few studies reported on the degradation of RCPs from TWW by the microbial consortium (Kurade et al., 2012; Khan et al., 2021). In this respect, in present work, we aimed to develop a new, cost-effective and potential bacterial consortium using identified potential pollutants degrading bacterial strains isolated from TWW and sludge samples for the effective treatment of RCPs from real TWW. Various environmental and nutritional parameters were optimized to maximum the treatment efficiency of developed bacterial consortium. Before and after bacterial treatment, various physicochemical parameters of TWW were analyzed. Further, lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase enzyme produced during the RCPs degradation were also quantified and characterized by SDS-PAGE analysis. In addition, the parent compounds (RCPs) present in real TWW and their metabolites were characterized by Fourier Transform-Infrared Spectroscopy (FT-IR) and Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The toxicity of RCPs and their metabolites were also assessed to evaluate the environmental safety. The present study has been compiled into the following chapters:

The first chapter has introduced basic information on the topic of the thesis and mainly focused on the TIs and their role in the national economy and employment generation. This chapter introduced wastewater generation and its pollutants and characteristics, pollution and toxic impacts on environment and living beings. This chapter also introduced the status of wastewater treatment and highlights the need for the adequate treatment of RCPs from real TWW.

Chapter two of this study has described the objectives of this study, which were targeted to complete this research work successfully in a more organized and manageable way.

Chapter three of this study has described the review of literature that provides details on TIs, production stages, wastewater generation and its characteristics and pollutants (RCPs), pollution and toxicity profile on environment and living organisms. This chapter described the various chemicals used in textile production process and introduced various treatment methods for the degradation of RCPs from TWW. This chapter also deals various analytical tools used in identification and characterization of RCPs and their metabolites.

Chapter four has described the physico-chemical analysis of TWW collected from Handloom Bhandar located at Unnao, Uttara Pradesh, India.

Chapter five has focused on the isolation, screening, characterization and identification of bacterial strains capable for the degradation of RCPs from textile wastewater.

Chapter six has described the development and optimization of a potential bacterial consortium for the effective degradation of RCPs from textile wastewater.

Chapter seven described the detection and characterization of RCPs and their metabolites from untreated and treated TWW by FT-IR and GC-MS analysis.

Chapter eight described the detection and characterization of enzyme responsible for the degradation and detoxification of RCPs.

Chapter nine described the toxicity of TWW before and after bacterial treatment by using terrestrial /aquatic test models for environmental safety.

Chapter ten has summarized the findings of the thesis. This section has mentioned the brief findings of each objective.

Chapter eleven described the concerned references cited in whole thesis. The reference section has been written in a standard format and all the important references related to the topic have been included.

Chapter 2

Objective

Objectives

The objectives of this study are as follows:

1. Physico-chemical analysis of textile wastewater (TWW) collected from textile industry.

1.1. Collection of textile wastewater from textile industry

1.2. Physico-chemical analysis of collected textile wastewater sample

1.3. Heavy metals analysis of collected textile wastewater

2. Isolation, screening and characterization of bacteria capable for the degradation of recalcitrant colouring pollutants (RCPs) in textile wastewater.

2.1. Isolation and purification of bacterial strains from TWW and sludge samples

2.2. Screening of potential bacterial strains capable for the degradation of RCPs

2.3. Characterization and identification of potential bacterial isolates

2.3.1. Morphological characterization

2.3.2. Biochemical characterization

2.3.3. Molecular identification

3. Development and optimization of a potential bacterial consortium for the effective degradation of recalcitrant colouring pollutants (RCPs) in textile wastewater.

3.1. Development and optimization of a bacterial consortium

3.1.1. Biointeraction study of isolated bacterial strains

3.1.2. Development of a potential bacterial

3.1.3. Optimization of the newly developed bacterial consortium at various environmental and nutritional parameters

3.2. Degradation of RCPs from TWW by the newly developed bacterial consortium RKS-TEX267 at optimized conditions

4. Detection and characterization of recalcitrant colouring pollutants (RCPs) and metabolites in untreated and bacteria treated TWW using FT-IR and GC-MS analysis.

4.1. Sample preparation and characterization of RCPs and metabolites in untreated and treated TWW by FT-IR analysis

4.2. Sample preparation and characterization of RCPs and metabolites in untreated and treated TWW by GC-MS analysis

5. Detection and characterization of enzyme responsible for the degradation and detoxification of recalcitrant colouring pollutants (RCPs).

5.1. Preparation of cell-free extract

5.2. Enzyme assays

5.3. SDS-PAGE analysis

6. Toxicity evaluation of textile wastewater before and after bacterial treatment by using terrestrial /aquatic test models for environmental safety.

6.1. Toxicity of untreated and bacterial treated TWW on the seed germination and seedling growth of Vigna radiata and Vigna mungo

Chapter 3

Review of Literature

3. Introduction

Textile industries (TIs) play a major role by contributing to the global economy in many developing countries (Paździor et al., 2019). Worldwide, TIs contribute ~5% of the total textile trade, ~5% of total export, ~$2,000 billion of market value and around 120 million employments for urban and rural peoples (Satish, 2018; Kishor et al., 2021a). TIs discharge a large volume of highly polluted wastewater into the environment. TWW is characterized by the dark color, high pH, BOD, COD, TDS and TOC (Afrin et al., 2021; Kishor et al., 2021b). TWW has various RCPs (dyes), organic compounds and metal ions (Khan and Malik, 2017). More than thousands of RCPs (dyes) are employed in TIs. ~70% of dyes are not fixed to fibers and discharged along with the wastewater into the environment (Paździor et al., 2019; Khan et al., 2021). RCPs are highly toxic to environment and living organisms. In water ecosystem, RCPs reduced sunlight penetration power, photosynthetic activity and dissolved oxygen content and thus, increased COD and BOD levels and gives dark color, leading to eutrophication and perturbations (Kaur et al., 2018; Kishor et al., 2021a). In soil ecosystem, these causes negative impact on soil property and biodiversity by bioaccumulation of metal ions and RCPs (Noman et al., 2020; Chandanshive et al., 2020). RCPs are also toxic to animals and plants (Kishor et al., 2021b).

Thus, it is imperative to adequately treat TWW before its final disposal into the environment (Kaur et al., 2018; Cao et al., 2019). However, the increasingly stringent environmental regulations are also forcing the TIs to improve the treatment processes applied at wastewater treatment plants and also explore alternative methods for the better treatment of TWW. Therefore, this literature review provides detailed knowledge on different textile processing steps, wastewater generation, its nature and chemical composition, environmental impacts and health hazards along with the various existing and AOPs for the better treatment of TWW. It also deals with various analytical techniques used to detect and characterize TWW pollutants and their metabolites produced during the wastewater treatment processes, key issues and challenges.

3.1. Textile Industry: An overview

Textile industries (TIs) are one of the largest industry in worldwide. It plays a major role by contributing global economy in many developing countries like China, India, Pakistan, Brazil, Bangladesh and Malaysia (Paździor et al., 2019; Khan et al., 2021). In India, TIs are one of the oldest industries in country and the world's second-largest producer of textile products/fabrics. There are ~68202 textile looms regularly operating in various states such as Gujrat, West Bengal, Madhya Pradesh, Punjab, Maharashtra, Haryana, Rajasthan, Odisha, Uttar Pradesh and Tamil Nadu (Cao et al., 2019; Afrin et al., 2021).

It also shares total 13% of exports and 5th largest source of foreign currency (Tara et al., 2019). It contributes ~4% of the country's National Gross Domestic Product (GDP), 27% of the country's export income and ~14% of the overall Index of Industrial Production-IIP (Samuchiwal et al., 2021; Kishor et al., 2021a). These industries also act as a second largest employer after agriculture providing ~ 45 million employments directly and 60 million indirectly to rural and urban peoples (Satish 2018). In India, the textile market is estimated to be ~ US$108 billion and expected to reach ~US$209 billion by the end of 2021 (Khan et al., 2021; Srivastava and Bandhu, 2022). TIs are contributing to a target of $100 billion economy by 2025 with annual growth of 20% (Srivastava and Bandhu, 2022) Moreover, TIs also share nearly 24% of the world’s spindle capacity, 8% of global rotor capacity and have the highest loom capacity with 61% of the world's market share economy.

3.2. Different stages, processes and chemicals used in textile industries

Textile manufacturing is a complex process, which involves different stages and chemicals for the production of a variety of products. These stages require a large volume of water and a number of chemicals. However, the different stages of textile manufacturing are as below.

Sizing: It is the first step in production of textile from man-made or natural fibers like polyester, silk, jute, cotton and wool by adding special carboxymethyl cellulose (CMC), polyvinyl alcohol (PVA), polyacetate and polycyclic acids. These substances provide high potency to fibers.

Desizing: It is the 2nd step, which uses enzymes and many auxiliary chemicals to remove unwanted chemicals and sizing materials and enhance the absorbency of fibers. Currently, bacterial enzymes and mineral acids are more prevalent than traditional methods in desizing applications.

Scouring: It is a cleaning process used to remove impurities from fibers. In this process, alkali agents such as glycerol, ethers, sodium hydroxide, detergent and soap are used for the removal and washing of impurities like fats, waxes, oils and surfactants as well as non-cellulosic materials.

Bleaching: It is a chemical process used for the removal of unwanted coloring materials from fibers. Currently, hydrogen peroxide and peracetic acid are used as bleaching agents to enhance the whiteness of fibers.

Mercerization: In this process, cold or hot caustic soda (NaOH) is used to improve the physical and chemical properties of fibers namely lusture, strength, dyes affinity and fiber's appearance.

Dyeing and printing: At this stage, a variety of auxiliary chemicals are used to improve the attachment of dye molecules with fibers. Dyeing is the major process in textile production industries for the addition of colors to fabrics. Different dyes such as azoic dye, vat dye, reactive acid dye, sulfur dye, basic dye, direct dye, pigments and metal complex dyes are extensively used in TIs worldwide (Kishor et al., 2021a). Phthalates, dyes, metals, solvents, formaldehyde and urea are commonly used at printing stage.

Finishing: It is the final stage of textile production process in which different types of protection and maintenance chemicals such as biocides, synthetic organic or inorganic chemicals are used to improve and maintain the specific properties of fibers like stain proofing, softening, waterproofing, flame retardance and protection from microbial activities as well as UV damage.

3.3. Wastewater generation and its characteristics

TIs discharged a large volume of highly colored and potentially toxic wastewater into the environment. In India, ~ 830 million m^3/year of groundwater is used and ~ 640 million m^3/year of wastewater is discharged while in China, ~ 8.65×10^9 m^3/year of groundwater is used and ~ 1.84×10^9 m^3/year of wastewater is discharged by the TIs (Jegatheesan et al., 2016). China, United States and United Kingdom annually discharge ~26.0, 12.4 and 1.0 million tons of textile waste, respectively (Statista, 2016). In TIs, dyeing and washing stages are the major sources of wastewater generation. TIs generally consumes ~ 1.6 million liters of groundwater to produce 8000 kg of textile fabric per day, out of which ~ 30-40% water is used in dyeing process, 60-70% at washing stage and ~ 10-50% of unused dyes are released into the aquatic resources along with the generated wastewater (Kishor et al., 2021a). The printing, bleaching, scouring and finishing processes in TIs also generate up to 1 to 10 million litters wastewater per day. According to the World Bank, ~ 17-20% of wastewater is released into the environment from the dyeing and finishing processes (Khandare and Govindwar, 2015). More than 2000-22000 kg of highly toxic acidic dyes are discharged from the washing process of polyamide textiles and ~ 2000-22000 kg of direct dyes are released from the washing stage of cotton manufacturing industries (KEMI, 2014). The physico-chemical characteristics of TWW are given in **Table 3.1.**

Table 3.1: Physico-chemical characteristics of textile wastewater.

S. No.	Characteristic	Permissible limit		Recorded values
		CPCB (2013)	NEQS	
1.	pH	5.5-9.0	6-10	9.7 ± 0.1
2.	Temperature (°C)	40 ºC	40	40 ± 1
3.	EC (us/m)	0.85	NG	5.93 ± 0.65
4.	Color	Colorless	NG	Dark blueish
5.	BOD (mg/L)	30	80	694.33 ± 3.05
6.	COD (mg/L)	250	150	1733 ± 5.29
7.	TOC (mg/L)	NG	NG	3762 ± 9.84
8.	TS (mg/L)	NG	NG	7076 ± 4.58
9.	TDS (mg/L)	2100	3500	7165 ± 6.24
10.	TSS (mg/L)	100	150	464 ± 2.64
11.	Sulfate (mg/L)	1000	600	1575 ± 7
12.	Chloride (mg/L)	1000	1000	1691.66 ± 5.03
13.	Nitrogen (mg/L)	NG	40	9.63 ± 0.15
14.	Phosphate (mg/L)	5.0	NG	8.2 ± 0.1
15.	Phenol (mg/L)	0.1	0.1	1.348 ± 0.09
16.	Surfactant (mg/L)	NG	NG	11.46 ± 0.25
17.	Heavy metals (mg/L)			
18.	Cadmium (mg/L)	2.0	0.1	0.84 ± 0.11
19.	Chromium (mg/L)	2.0	0.1	1.54 ± 0.24
20.	Lead (mg/L)	0.1	NG	0.184 ± 0.08
21.	Nickel (mg/L)	3.0	1.0	1.32 ± 0.009

Foot Note: All the values are means of triplicates (n =3) ± SD.
*Except pH, all the parameters are expressed in “mg/L”, but the conductivity is expressed in “µmho/cm”.
*EC: Electrical Conductivity; BOD: Biochemical Oxygen Demand; COD: Chemical Oxygen Demand; TDS: Total Dissolved Solids; TSS: Total Suspended Solids; TOC: Total Organic Carbon; TS: Total solids; Alk.: alkalinity

In addition, more than thousands of dyes are also discharged from TIs along with the wastewater. TWW is characterized by high dye content, pH, BOD, COD, TDS, TS, SS, TOC, TSS, chloride and phosphate (Tara et al., 2020; Kishor et al., 2021b). TWW contains sulphate, nitrate, phosphate, chloride, electrical conductivity, turbidity, alkalinity, salt, acid, base and mordant (Kaur et al., 2018; Cao et al., 2019; Khan et al., 2021). It also contains RCPs (dyes), surfactant, VOC, chlorobenzene, phenol, dioxin, bleaching, fixing and finishing agents along with various metals like Cr, Cd, Pb, Sb, As, Cu, Ni and Zn (Khandare and Govindwar, 2015). However, the nature and chemical composition of TWW is largely depend on the chemicals used as well as processes adopted by the TIs.

3.4. RCPs/dyes in TWW

Globally, ~280,000 tons of different synthetic dyes (RCPs) are discharged from TIs into the environment (Samuchiwal et al., 2021). Dyes have high fastness, low cost, easy to use, various recalcitrant color and high stability to light, temperature, detergents, chemicals and microbial degradation (Kishor et al., 2021c). Dyes are used to add color to cloth, paper, leather and other objects. Dyes are aromatic, heterocyclic, recalcitrant and possess different chromophore groups such as azo (-N=N-), nitro (-N=O), carbonyl (-C=O), quinoid groups and auxochromes groups like hydroxyl (-OH), carboxyl (-COOH), amine (-NH_2) and sulphonate (-SO_3H) (Kishor et al., 2021d; Srivastava and Bandu, 2022). Dyes such as MO, CR, MB, AB, reactive dye, direct red, remazol red, scarlet, malachite green, acid orange and remazol brilliant blue R are extensively used in TIs (Noman et al., 2020). These dyes are highly resistant to light, water, chemicals, detergents and microbial activities. Dyes are mainly categorized into two groups: natural and man-made or synthetic dyes. Natural dyes are environmentally friendly but are little used in dyeing process, whereas synthetic dyes are used extensively, but unfortunately are not eco-friendly due to their complex and stable structure, resistant for microbial degradation, hazardous effects on environment and living organisms (Ceretta et al., 2020). Dyes are used in textile, leather, paints, photographs, dyeing, paper, food, cosmetic, plastic, prints, carpet and medicine industries. Based on their, application (**Fig. 3.1**), particle charge and molecular structure (**Fig. 3.2**), dyes can be classified into different groups.

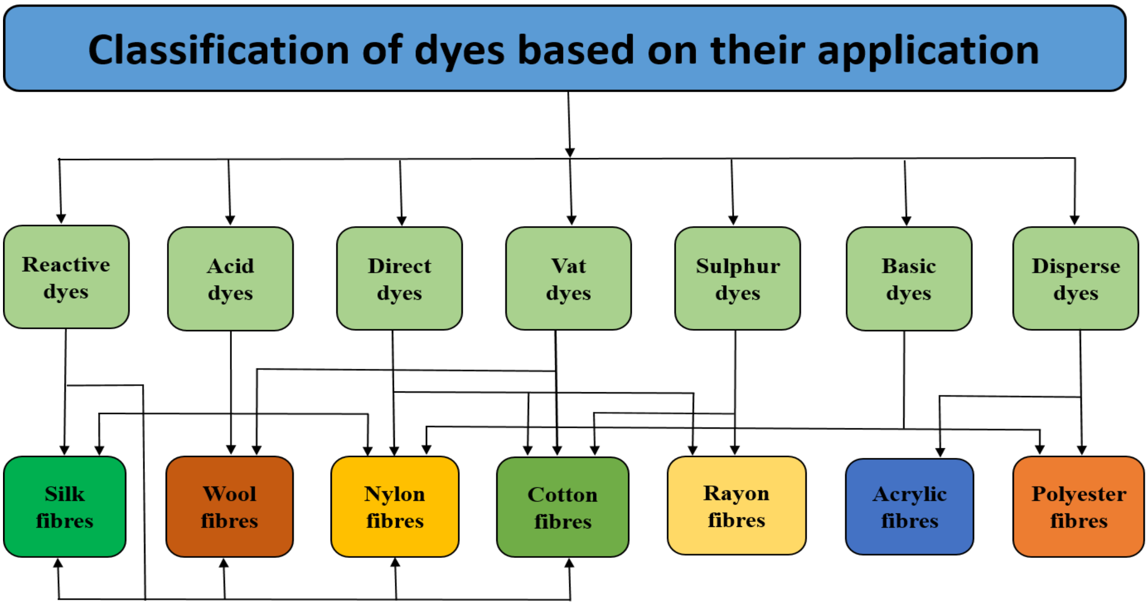
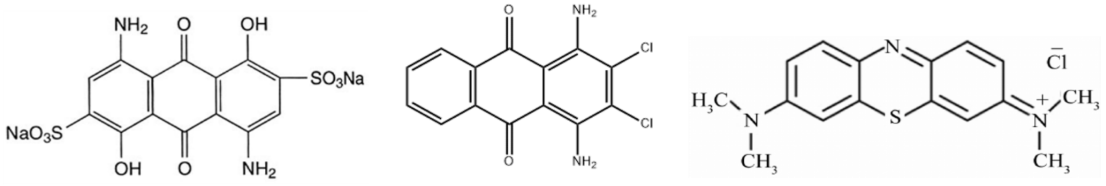


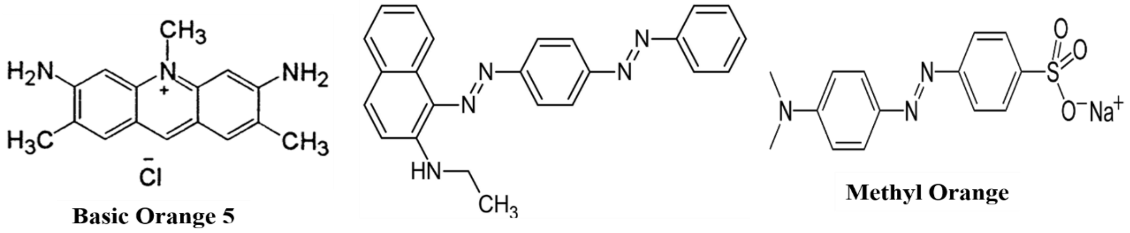
Fig. 3.1: Different classes of recalcitrant coloring pollutants (RCPs/dyes) based on fabric used.

Based on the molecular structure, textile dyes are classified into azo dyes (CR, MO and reactive black 5), indigoid dyes (acid blue 71 and vat red 6), anthraquinone dyes (alizarin, reactive blue 4 and acid blue 25), triarylmethane dyes (basic red 9, malachite green and CV) and nitro and nitroso dyes (naphthol yellow S and mordant green 4).

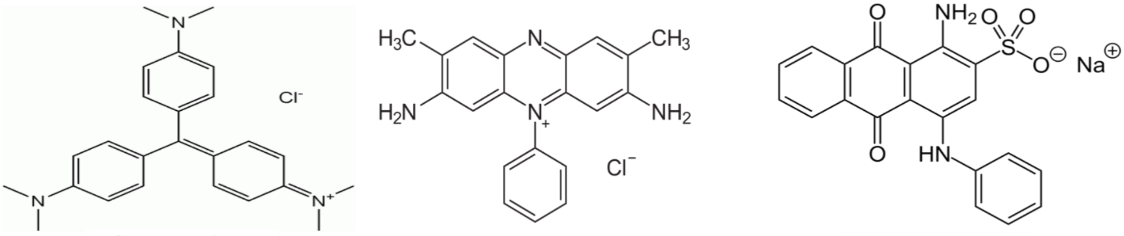
Based on charge, dyes are classified into the anionic dyes (MO, direct and acid dyes), cationic dyes (MB and CV) and neutral dyes (dispersed dyes). Among these dyes, azo dyes are the largest and most versatile group of synthetic dyes accounting ~ 80% of total dyes used in TIs. For example, MO is a synthetic, sulfonated and high water-soluble anionic dye. It’s widely used in dyeing, leather, textile and pulp paper industries (Kishor et al., 2021d). MB dye is used in dyeing of cotton, wool, leather and paper (Kishor et al., 2021c). According to (KEMI, 2014), ~ 3500 different synthetic dyes are used in various industries, out of which, ~ 84% belong to the sulphonate azo dye group.



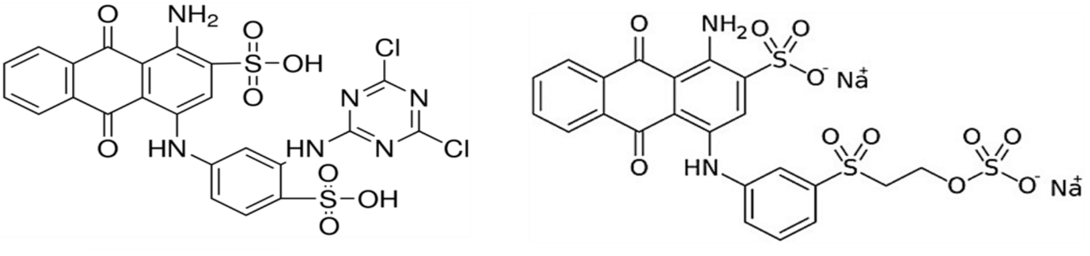
Acid Blue 45 **Disperse Dye** **Methylene Blue**



Basic Orange 5 **Sudan Red 7B** **Methyl Orange**



Crystal Violet **Safranin** **Acid Blue 25**



Reactive Blue 4 **Reactive Blue 19**

Fig. 3.2: The chemical structure of different recalcitrant coloring pollutants (RCPs/dyes) used in textile industries.

3.5. Ecotoxicological and health concerns of TWW pollutants

TIs are generating huge quantity of dark-colored wastewater, which acts as a major source of environmental pollution. TWW contains high BOD, COD, suspended solids, various toxic metals and a complex mixture of RCPs, acid, base, salts and many auxiliary chemicals making it more complex and highly toxic in nature (Cao et al., 2019; Kishor et al., 2021a). TWW is the most polluting wastewater than other wastewaters. ~ 72 different types of highly toxic pollutants were reported in TWW, of which ~ 30 pollutants are resistant to microbial degradation leading to severe environmental and health hazards (KEMI, 2014; Kishor et al., 2021a). The applications and toxicological effects of different chemicals used in TIs are given in **Table 3.2**. Various toxic metals such as Cr, Sb, Cu, Zn, Pb, Cd, and Ni are reported in TWW. Metals are well reported to reduce seed germination, root length, shoot length and biomass production in plants and decrease in microbial activity/diversity (Haq et al., 2018; Kishor et al., 2021b). These metals are also known to accumulate in living tissues through food chain, posing severe effects on human and animal health such as diarrhea, liver, neuromuscular, hemorrhage, dermatitis, central nervous system disorder and kidney malfunctioning (Kumar et al., 2019). TWW causes coloration of water leading to the reduction in the penetration power of sunlight, decrease in photosynthetic activity of aquatic plants as well as dissolved oxygen content resulting in anoxic conditions and thus, affecting the normal life of aquatic fauna and flora both (Kaur et al., 2018; Kishor et al., 2021c).

The nonylphenol ethoxylates (NPEs) used as a surfactant at washing stage for scouring fibers is well reported as an endocrine-disrupting chemical and can lead to the hormonal imbalance in living beings (Environment Agency, 2008). TWW causes allergic reactions, carcinogenic, mutagenic and cytotoxic effects in plant, rat, fish, mollusc, microbe and mammalian cells (Chandanshive et al., 2020) (**Fig. 3.3**). It also affects various organs like liver, kidney, brain, nervous system and reproductive system in humans and animals (Khan et al., 2021). Azo dyes and their various degradation products are reported to cause severe health hazards such as haemorrhage, nausea and ulceration of skin and also cause cancers of spleen and urinary blander in human and mammalian cells (Paździor et al., 2019). Various additives, dioxin, detergents, pesticide, phenol, surfactants, mordants, fasteners, salts, formaldehyde and finishing chemicals are also reported as lethal to human/animal health (Cai et al., 2020).

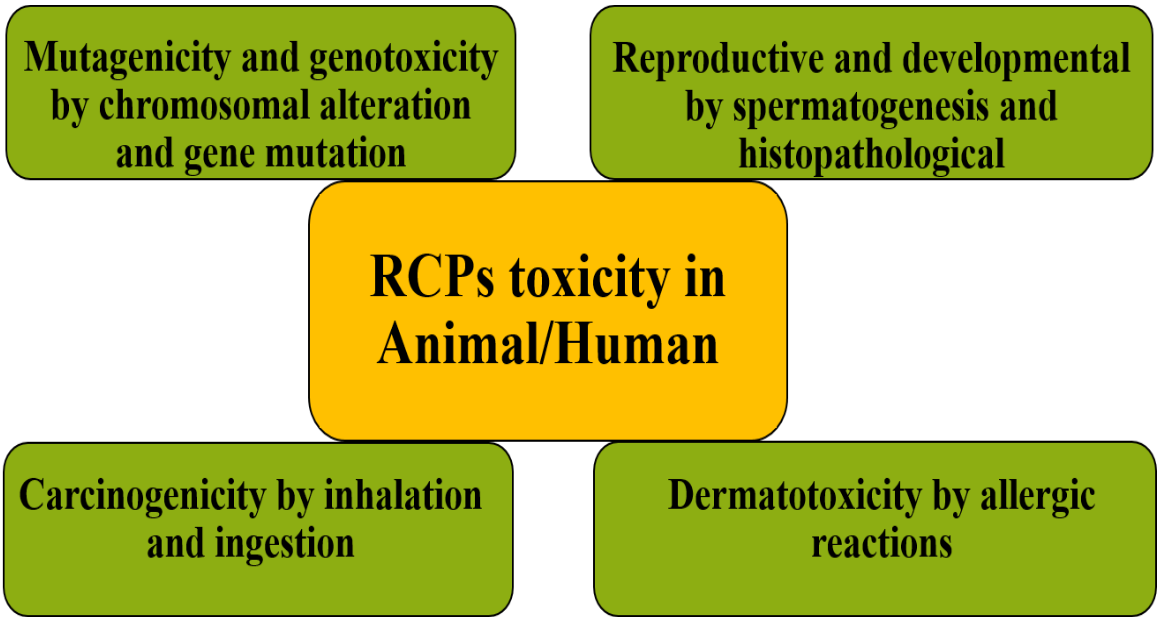


Fig. 3.3: Toxic effects of recalcitrant coloring pollutants (RCPs)/dyes in living beings.

Malachite green (MG) is reported as an RCP, which severely affects human beings due to the reduced food intake, growth, fertility rate as well as damaged kidney, spleen, heart and liver. It also causes increase in WBCs count, anemia, decrease in RBC count (dyescrasia), inflicts lesions on eyes, skin, lungs and bones (Kishor et al., 2021a). Because of these toxic effects, MG dye has been banned in many countries and not allowed by US Food and Drug Administration, but it is still used in several countries (Noman et al., 2020). Tartrazine is a type of azo dye, which is used in food, pharmaceuticals, textile and cosmetic industries and causes various health problems like allergy, asthma, skin eczema, immunosuppression, hypersensitivity, carcinogenic and mutagenic effects in living organisms (Srivastva and Bandhu, 2022). Acid black 210 and its metabolite (4-nitroaniline) are characterized as a human carcinogen (Kishor et al., 2021a). AB dye is widely used in TI, which intercalates with the helical structure of DNA, duplex RNA and partitioned to the lipid membrane of the cells (Haq et al., 2018). Basic red 9 is a triarylmethane dye used in textile, cosmetic, leather, printing, paper and ink industries and reported as a potential carcinogen, allergen, mutagen and skin irritating agent and also develop tumors in blander, liver and mammary glands (Kaur et al., 2018). Bharagava et al. (2018) reported that CV dye is persistent in nature and known for its carcinogenic, clastogenic nature and tumor growth-promoting effect in fishes.

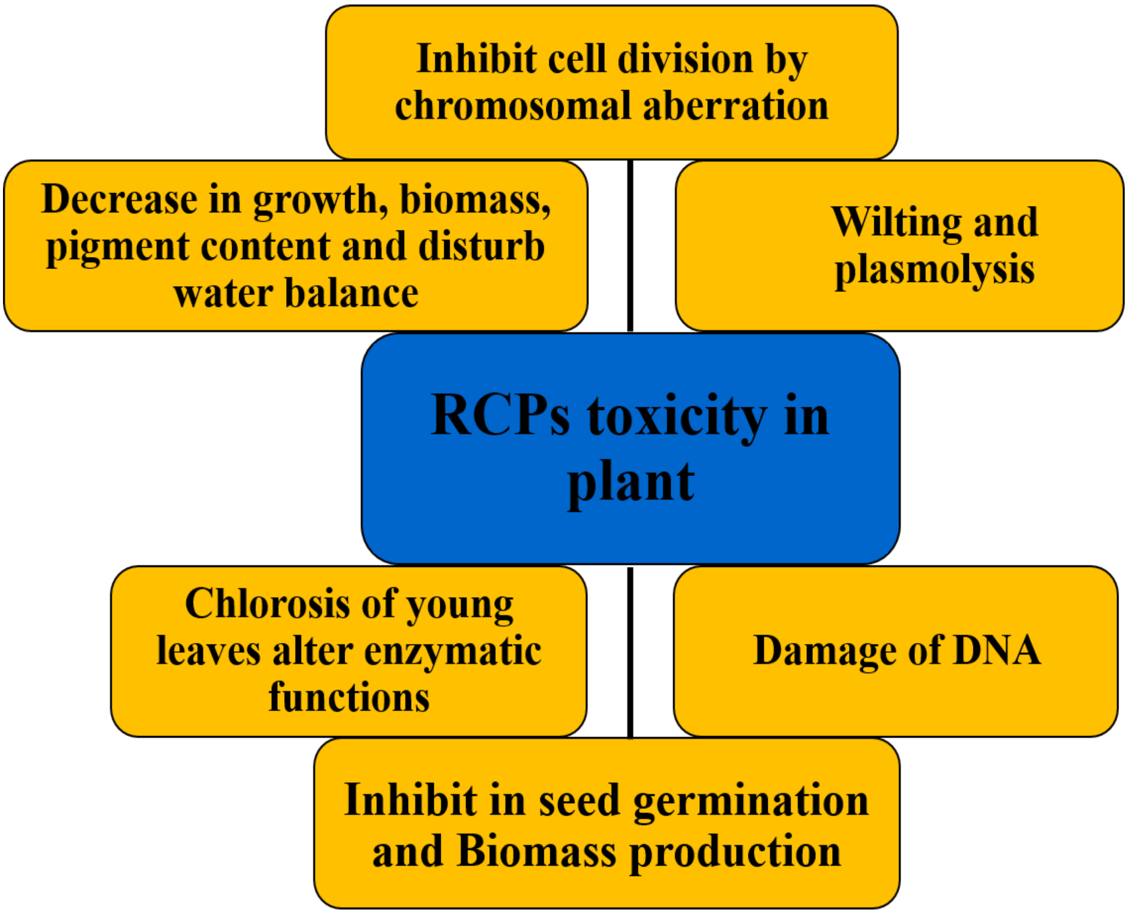


Fig. 3.4: Toxicity profile of recalcitrant coloring pollutants (RCPs)/dyes in plant.

According to the International Agency for Research for Cancer (IARC), benzidine dye acts as a powerful carcinogen for living beings. It is banned for more than 20 years before due to its carcinogenic nature, but it is still regularly used in many countries (Joint Research Centre (JRC), 2014). Reactive orange and CR dyes are used in dyeing industries and reported to causes serious threats to all forms of life (Kishor et al., 2021b). MB dye is reported to increase blood pressure, hypertension and mycocardardial depression. According to the National Occupational Exposure Survey of USA, around 69563 workers have been potentially exposed to MB dye at their working place from 1981 to 1983 (Kishor et al., 2021c). It is also highly toxic to seed germination rate and biomass production of plants (Kishor et al., 2021c) (**Fig. 3.4**). Some dyes like erythrosine and xanthene are reported to cause allergic, neurotoxic, carcinogenic, xenoestrogenic and DNA damage in animals/humans (Afrin et al., 2021). MO dye is used in dyeing, leather, textile and pulp paper industries and causes hypersensitivity, allergies, dermatitis and intestinal cancer in living beings.

Table 3.2: Application and toxicity of different chemicals used in textile industries at various stages.

Chemicals	**Applications**	**Toxicity**
Chlorinated solvents e.g. Trichloroethane (TCE)	Applied in scouring of fabric (removal of impurities from fibers)	Affect central nervous system, liver, kidneys and atmospheric activities (ozone-depletion)
Carbon disulphide (CS_2)	Applied in manufacturing of viscose rayon fibers	Neurological, gastrointestinal and reproductive toxicity, leukaemia, and kidney diseases
Alkylphenols, nonylphenol ethoxylates and nonylphenols	Applied as scouring agents (wool) and dye-dispersing agents for dyes	Toxic to aquatic life and disrupt endocrine system, cause breast cancer and neuro-developmental delays in children
Chlorobenzenes (e.g. hexachlorobenzene (HCB))	Applied as dyeing carrier, dyestuff and in preparation of azo pigments	Carcinogenic in human as per International Agency for Research on Cancer (IARC), affect liver, endocrine and thyroid
Phthalates (di-isononyl phthalate and butyl benzyl phthalate)	Applied as printing, dyeing and coating agent in textiles	Carcinogenic, endocrine disrupters and aquatic toxicant, affect and impair fertility
Heavy metals like antimony, cadmium, lead, mercury, arsenic, nickel and chromium	Applied in preparation of pigments and dyes applied in coloring of textiles	Carcinogenic, mutagenic, reproductive disorders, damage blood cells, kidney and liver
Azo dyes (e.g. methyl nitro, orange, remazol, reactive dyes etc.)	Applied in dyeing for cotton, viscose, silk, wool and other fibers	Carcinogenic, mutagenic, allergic, aquatic toxicant and respiratory diseases asthma
Organotin compounds (DBT: dibutyltin, TBT: tributyltin, and TPhT: triphenyltin)	Applied as biocide to prevent heavy-duty textiles and reduce body odor	Affect immune and reproductive systems and mucous membrane irritation, muscular weakness and breathing problems
Perfluorinated chemicals (PFCs): perfluorooctane sulphonate (PFOS), a banned persistent organic pollutant (POP) in Stockholm Convention	Applied to make the textiles both water and stain-proof	Carcinogenic and toxic to immune, endocrine and attention deficit hyperactivity disorder (ADHD), alter thyroid hormone and increased total and non-HDL (bad) cholesterol levels S
Formaldehyde	Applied to make softer, water-resistant and prevent shrinking of cloth	Human carcinogenic as per IARC and U.S. Environmental Protection Agency (EPA), allergies and irritate mucous membrane
Volatile organic compounds like toluene, methyl isobutyl ketone, xylene, methyl ethyl ketone, dichloromethane, 1,1,1-trichloroethane)	Applied in preparation of solvent-based inks or pastes used in printing and drying of textiles	Staggering, weakness and personality changes and numbness in the fingers and repeated exposure to moderate to high amount may cause kidney and liver damage

Short-chain chlorinated paraffins (SCCPs): classified as POPs under Stockholm Convention	Applied as a flame retardant, waterproof and rot-proof to heavy textiles, like military tents	Endocrine disruptor, affect kidney, liver and thyroid gland and carcinogenic as per IARC)
Chlorophenols like pentachlorophenol (PCP)): cauterized as group B2 probable human carcinogen as per USEPA	Applied as a preservative (fungicide) on heavy-duty textiles and fibers	Carcinogenic, affect the immune, cardiovascular and nervous system, kidney, blood, liver and eyes and cause dermatitis
Polybrominated biphenyls, polybrominated diphenyl ethers, and hexabromocyclododecane e.g. hexabromobiphenyls	Applied to make textiles less flammable to prevent burning	Carcinogens, thyroid effects, endocrine disruption and classified as POPs under United Nation Environment Programme (UNEP)
Dioxins	Applied as a preservative agent for cotton and other fibers during sea transit	Cancer in Lung and liver, diabetes, cardiovascular disease and thyroid hormones and altered immunologic response
Organophosphorus compounds e.g. tributylphosphate (TBP)	Applied as a strong wetting agent and pigments preparation	Eye, nose, skin and mucous membranes irritation and also causes bladder cancer in rats
Silver and nanosilver compounds	Used as antimicrobial agents in textile industries	Bacterial resistance, ecotoxicity, affect lungs and neuronal cells

It's well reported to causes high toxicity, carcinogenic, mutagenic and teratogenic effects in humans and animals (Ayed et al., 2010). It also reduced soil fertility, crop yield and biodiversity by causing salinity and soil pollution (Kishor et al., 2021d). According to KEMI (2014), ~ 3500 highly toxic chemicals are commonly used in TI during the production processes. Among these, ~ 1000 are registered under REACH. It is estimated that ~ 10% of these textile chemicals are potential toxic for human health and ~ 5% of these substances are highly toxic to environment.

3.6. Various methods reported for the treatment of TWW

Several physico-chemical, biological and advanced oxidation methods have been reported by various researchers for the treatment of TWW. These approaches can be used either individually or in combination to improve/accelerate the treatment efficiency of TWW.

3.6.1. Physico-chemical methods

The physico-chemical methods like adsorption and coagulation/flocculation are reported effective for the color removal from TWW (Cao et al., 2019; Kishor et al., 2021a). The adsorption method transforms the pollutants from one phase to another phase whereas, coagulation/flocculation methods decolorize the selective dyes like sulfur and dispersive dyes, but are not effective in the decolorization of acid, reactive, direct and vat dyes from

TWW (Kumar et al., 2019). Thus, these treatment methods are not sufficient in many ways. For example, these methods are highly expensive, time-consuming, less applicable and produced huge amounts of highly toxic sludge as a secondary pollutant, which causes serious contamination in environment and pose severe effects on human/animals health (Cao et al., 2019; Cai et al., 2020).

The major advantages of adsorption and coagulation/flocculation methods includes its easy to use, well-proven, utilize readily available chemicals, easy operating conditions and good color removal efficiency while disadvantages are high cost, pH sensitive, inability to destroy and generate large amounts of waste materials (sludge) (Paździor et al., 2019).

3.6.2. Biological treatment (BT) methods

BT are green, eco-friendly and inexpensive treatment methods that can be used effectively in treatment of industrial wastewaters. Due to their genetic diversity and versatility, these are the most effective and alternatives for the degradation and mineralization of TWW pollutants (Paździor et al., 2019). BT process can be carried out under aerobic, anaerobic or facultative anaerobic conditions by using different categories of microorganisms or their enzymatic machinery (Kishor et al., 2021a). The microorganisms such as bacteria, fungi, yeast and algae have been reported by various researchers in treatment of TWW (Afrin et al., 2021). These microorganisms can degrade and mineralize an array of wastewater pollutants by using their different metabolic pathways and biosorption processes (Buntic et al., 2017; Kishor et al., 2021b). Further, these are also capable to reduce BOD, COD, TDS, TSS, TOC, turbidity and detoxify various metals from the synthetic and real TWW (Bilinska et al., 2019).

Tables 3.3: Different microbial agents used in treatment of textile wastewater by various workers.

Strain name	Wastewater/ dye	Optimized conditions	Treatment efficiency	Reference
Bacteria culture				
Bacillus cohnni RKS9	Textile wastewater and Congo red dye	pH 7.2, 32 °C, 100 mg/L, static and 100 rpm 48 and 12h	Color (93.2%) & (99%)	Kishor et al., 2021b
Pseudomonas putida	Textile wastewater	pH 7, 35 °C, 80 rpm, aerobic and 90h	Color (87%)	Sen et al., 2020
Halomonas sp.	Azo dye	30 °C and 24h	-	Herrera-Garcia et al., 2019
Pseudomonas aeruginosa Gb30	Reactive Black 5 dye	pH 8, 37 °C, 50 mg/L and 0.629 mg/L static and 24h	Color (35%)	Louati et al., 2020

Bacillus sp. KM201428	Reactive Black 5 dye	3.9 mg/L, pH 9, 25 °C and 120h	Color (97%)	Wanyonyi et al., 2019
Aeromonas hydrophila	Crystal violet dye	pH 7, 35 °C, 50 mg/L, static and 8h	Color (99%)	Bhargava et al. 2018
Arthrobacter soli BS5	Reactive Black 5 dye	pH 5-9, 37 °C, 50 mg/L and 120h	Color (98%)	Khan and Malik 2017
Proteus mirabilis LAG	Reactive blue 13 dye	pH 7, 35 °C, static and 5h	Color (84%)	Holkar et al., 2016
Fungi/Yeast culture				
Aspergillus strain	Azo dyes mixture	pH 7, 35 ºC, 100 mg/L, static and 210 min	Color (86%)	Ameen et al., 2021
Trametes versicolor	Reactive blue 19 dye	pH 4, 50 ºC, 200 mg/L, shaking (120 rpm) and 210 min	Color (85%)	Dauda and Erkurt 2019
Oudemansiella canarii	Congo red dye	pH 5.5, 30 °C, 50 mg/L and 24h	Color (80%)	lark et al., 2019
Pichia Kudriavzevii CR-Y103	Reactive orange dye	pH 6, 30 °C, 50 mg/L shaking and 24h	Color (100%)	Rosu et al., 2018
Aspergillus bombycis	Reactive red 31 dye	pH 6, 35 °C, static and 12h	Color (94%)	Khan and Fulekar 2017
Pichia occidentalis G1	Acid red B dye	pH 5, 30 °C, 50 mg/L, shaking and 16h	Color (98%)	Song et al., 2017
Diaporthe sp.	Methyl Violet dye	100 mg/L and 24h	Color (84.87%)	Ting et al., 2016
Algal/microalgae culture				
Scenedesmus	Methylene Blue dye	pH 9, 30 °C 200 mg/L and 120 rpm	Color (87.69%)	Afshariani and Roosta 2019
Chlorella Vulgaris	Dye effluent dye	pH 8, 30 °C and 10d	Color (100%)	Devaraja et al., 2017
Chlorella vulgaris PSBDU06	Indigo blue dye	pH 5 and 24h	Color (49.03)	Revathi et al., 2017
Chlorella pyrenoidosa NCIM 2738	Reactive red dye	pH 3, 25 °C, 50 mg/L and 30 min	COD (82.73%)	Sinha et al., 2016

Among these, bacteria have strong potential for the treatment of TWW because these are easy to be cultured, fast life phase and capable to grow on various substances than other microbes (Buntic et al., 2017; Bharagava et al., 2018). Further, bacteria can degrade or convert many toxic compounds into non-toxic products (Kishor et al., 2021a). For example, many bacterial species like *Arthrobacter, Pseudomonas*, *Bacillus*, *Aeromonas hydrophila* and *Lysinibacillus* are reported capable to remove dyes, dissolved solids and heavy metals as well as reduce their toxicity up to a significant level (Kumar et al., 2019; Samuchiwal et al., 2021).

Further, for the effective treatment of TWW, various workers have reported that microbial consortium is more effective than axenic cultures (Kurade et al., 2012). In microbial consortium, individual strains may attack dyes molecules at different positions (Paździor et al., 2019). Moreover, the degradation products, which appeared due to the metabolic activity of one strain, may be utilized as substrate by another strain (Paździor et al., 2019). In a study, a bacterial consortium was found to degrade and decolorize the textile dyes more rapidly than a single bacterial culture (Kishor et al., 2021a). Recently, some researchers have developed bacteria-yeast, fungal-yeast, bacteria-fungal and bacteria-algal consortia that were found effective to remove color and COD significantly (Kumar et al., 2019; Paździor et al., 2019). The main advantages of BT processes are green, eco-friendly, low cost, no sludge generation, complete mineralization and globally acceptable, but also has disadvantages like long time requirement and ineffective against toxic compounds (Kishor et al., 2021a).

3.6.3. Enzymatic treatment

Many enzymes are reported to involve in the degradation of industrial wastewater pollutants as well as remediation of contaminated sites (Haq et al., 2018; Kishor et al., 2021a). A variety of microorganisms are capable to produce different types of extra-and intracellular enzymes during the degradation processes. Enzymes such as azoreductase, laccase, peroxidases and polyphenol oxidases are well reported to degrade the industrial wastewater pollutants (Singh et al., 2015). Azoreductase and laccase are the most effective enzymes in the remediation of recalcitrant azo dyes and wastewater than other enzymes (Kishor et al., 2021a).

Table 3.4: Different enzymes used by various researchers in treatment of textile wastewater/dyes.

Enzymes	**Strains**	**Dyes/Textile wastewater**	**Treatment efficiency**	**Time (h/d)**	**References**
Lignin peroxidase (LiP)	*Bacillus albus*	Methylene blue	Color (99%)	6h	Kishor et al., 2021c
Manganese peroxidase (MnP)	*Pseudomonas aeruginosa*	Methyl orange	Color (99%)		Kishor et al., 2021d
LiP, MnP, laccase & azoreductase	*Bacillus cohnni*	Congo red & Textile wastewater	Color (99%) & Color (93%)	12 & 48	Kishor et al., 2021b
Laccase	*Trametes versicolor*	Reactive blue 19	Color (85%)	210 min	Dauda and Erkurt 2020

Laccase	*Pleurotus ostreatus* HAUCC 162	Textile dyes	Color (91.5%)	24h	Zhou et al., 2019
Azoreductase	*Halomonas* sp. GT	Acid Brilliant Blue GR	Color (100%)	96h	Tian et al., 2019
Laccase	*Phomopsis* sp.	Textile wastewater	Color (99%)	2.5h	Navada and Kulal 2019
LiP	*Serratia liquefaciens*	Azure B	Color (90%)	48h	Haq et al., 2018
Laccase	*Pleurotus pulmonarius*	Malachite green	Color (68.6%)	36h	Lallawmsanga et al., 2018
LiP and laccase	*Aeromonas hydrophila*	Crystal violet	Color (99 %)	8h	Bharagava et al., 2018
Azoreductase, NADH-DCIP reductase & laccase	*Bacillus circulans* BWL1061	Methyl orange	Color (99.22%)	4h	Liu et al., 2017
Laccase	*Ganoderma lucidum* BCRC 3612	Acid orange 7	Color (90%)	14d	Lai et al., 2017
Laccase & peroxidase	*Bacillus aryabhattai* DC100	Coomassie Brilliant Blue G-250 and Indigo Carmine	Color (100%)	72h	Paz et al., 2017
Azoreductase	*Alcaligenes* sp.	Congo red	Color (98.76%)	48h	D’Souza et al., 2017
Laccase, LiP, azoreductase & NADH-DCIP reductase	*Aspergillus* sp. NCIM-1146 and *Providencia* sp. HSL1	Textile effluent	Color (92%)	30h	Lade et al., 2016

Azoreductase (EC 1.7.1.6) is a major class of azo dye degrading enzyme, which is considered as flavoproteins and classified as flavin-dependent and flavin-independent azoreductase (Singh et al., 2015). Flavin-dependent azoreductase are again classified based on the electron donors such as NADH, NADPH or both. Azoreductase has great potential to degrade and decolorize various dyes by catalyzing the cleavage of azo linkage (-N=N-) under aerobic or anaerobic conditions (Kumar et al., 2019). During the decolorization of azo dyes, the reducing agents such as FADPH, NADPH and NADH act as electron donor and also participate in the breaking of azo linkage at both extra and intracellular sites of bacterial cell membrane (Kishor et al., 2021b). The degraded aromatic products are highly stable under anaerobic conditions and thus, further mineralization of these colorless aromatics products takes place under aerobic conditions (Srivastava and Bandhu, 2022). For instance, *Halomonas*, *Bacillus cohnni*, *Bacillus circulans* and *Bjerkandera adusta* are capable to degrade dyes and TWW by producing azoreductase enzyme (Buntic et al., 2017; Kishor et al., 2021b; Srivastava and Bandhu, 2022).

Laccase (EC1.10.3.2) is a promising multicopper oxidase enzyme, which is most effective in treatment of different dyes such as CV, methyl violet, cotton blue and TWW (Kishor et al., 2021b). It is non-specific in nature and neither uses oxygen as electron accepter, nor need co-factor (Singh et al., 2015). ABTS (2, 2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) is a low molecular weight compound and acts as a redox mediator for laccase enzymes (Haq et al., 2018; Yadav et al., 2022). The presence of this redox mediator enhances the treatment efficiency of enzymes many folds. Kishor et al. (2021b) reported that laccase produced by *Bacillus cohnni* during the degradation of TWW pollutants.

Further, other enzymes like lignin peroxidase (LiP), veratryl alcohol oxidase, tyrosinase, NADH-DCIP reductase and polyphenol oxidase are also reported to play an important role in the degradation of TWW pollutants (Singh et al., 2015; Buntic et al., 2017). In addition, the utilization of different enzyme combinations offers a significant advantage over the use of a single enzyme. However, the enzymatic degradation of TWW pollutants has often seen as an inexpensive viable option and expanding technology due to its cost-effectiveness and environmental compatibility. The main advantages of enzymatic treatment are eco-friendly and possibility to complete remediation of pollutants whereas disadvantages include long time requirement, sensitive to temperature, pH, inactive against toxic compounds and not applicable at large scale (Kishor et al., 2021a).

3.6.4. Microbial degradation mechanism of RCPs/dye

Various researchers have reported that many bacterial species are capable to degrade and mineralize RCPs/dyes by producing the catabolic enzymes (Singh et al., 2015; Kishor et al., 2021b). For example, the newly isolated *Enterococcus faecalis* YZ 66 strain showed high potential for degradation of direct red 81 (DR81) dye (Sahasrabudhe et al., 2014). *E. faecalis* is a gram-positive and facultative anaerobe capable to survive and grow under extreme conditions. Bacterial degradation of azo dye DR81 is initiated by the reductive cleavage of azo bond (-N=N-) by producing azoreductase enzyme in the presence of redox mediator under anaerobic environment (**Fig 3.5A**). It results in the generation of various low molecular weight, colorless and aromatic amines such as sodium-4-aminobenzenesulfonate, 1,4-benzenediamine and 7-benzylamino-3-dibenzyl-1-4-hydroxy naphthalene-2-sulfonic acid. Further, the degradation of sodium-4-aminobenzenesulfonate takes place by deamination process resulting sodium benzenesulfonate whereas 7-

benzylamino-3-dibenzyl-1-4-hydroxy naphthalene-2-sulfonic acid is degraded by laccase enzyme into 1-phenylmethanamine-ethene and 8-aminonaphthol and finally these get converted into naphthalene. (A).

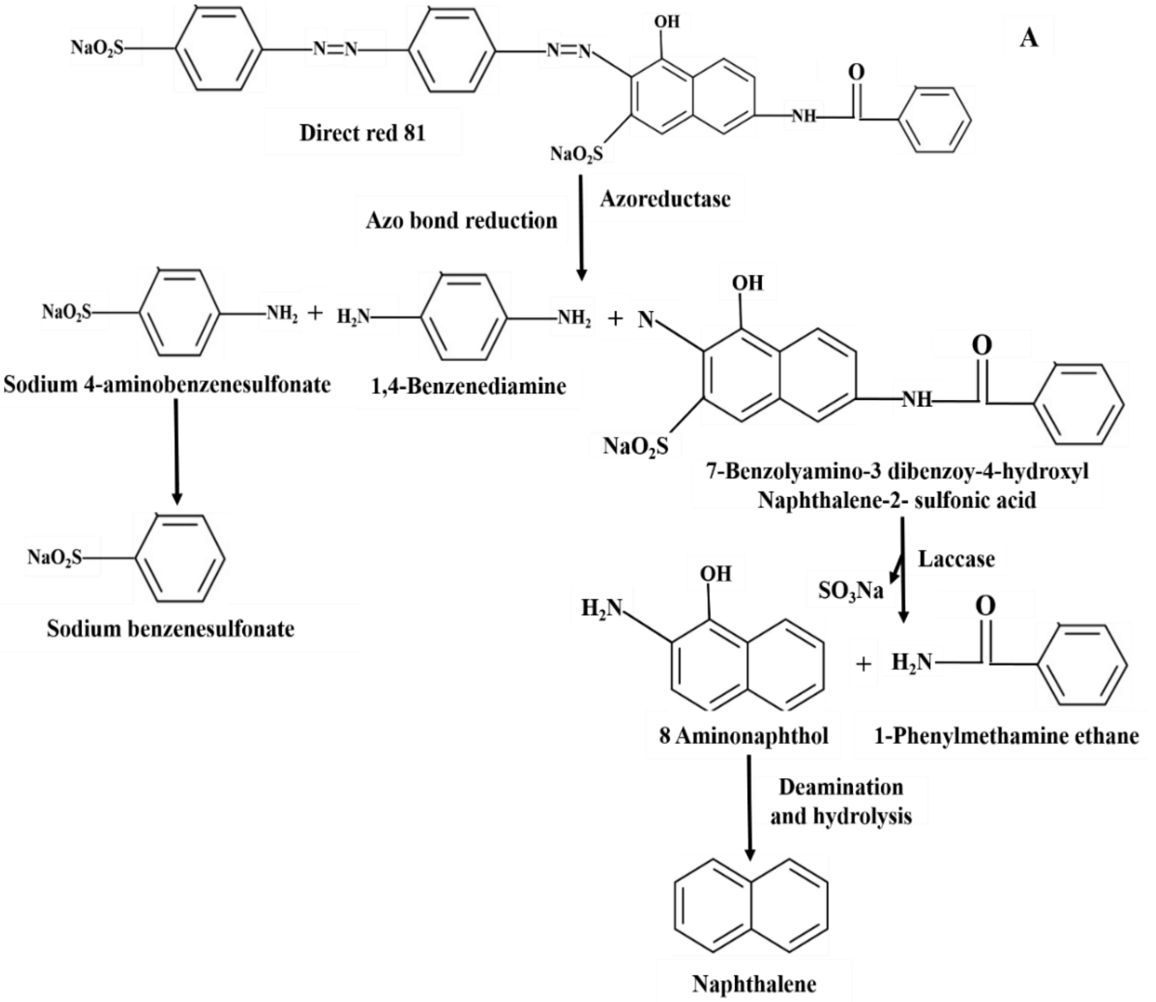


Fig 3.5: Degradation pathway (A) of direct red 81 dye by *Enterococcus faecalis* YZ 66.

Kishor et al. (2021d) isolated a potential bacteria (*P. aeruginosa*) form TWW and sludge sample, which showed high efficiency in degradation of MO dye. During degradation, in first step, the demethylation of MO dye takes place leading to the formation of 4-[(4-aminophenyl) diazenyl] benzene sulfonate (A). In second step, product (A) was converted into 4, 2-((dihydroxymethyl) hyrazono-4) 5-benzene sulfonate (B) due to the cleavage of azo group and aromatic ring. In third step, demethylation and dihydroxylation of product (B) takes places leading to the formation of 4-(triazan-2-yl) benzene sulfonic (C). In last step, product C get finally degraded and mineralized into simpler molecules (i.e. water and carbon dioxide) **Fig. 3.6B**.

Furthermore, crystal violet (CV) a triphenylmethane dye, which is used in TIs, was effectively degraded by a bacterium i.e. *Aeromonas hydrophila* (*A. hydrophila*) isolated from textile wastewater (Bharagava et al., 2018). The degradation pathway of CV dye by the isolated bacterium *A. hydrophila* is shown in **Figure 3.6C** in which it was first transformed into phenol-2-6-bis (1,1-dimethylethyl) followed by further conversion into 2,6-dihydroxyacetophenone and finally into benzene as final product.

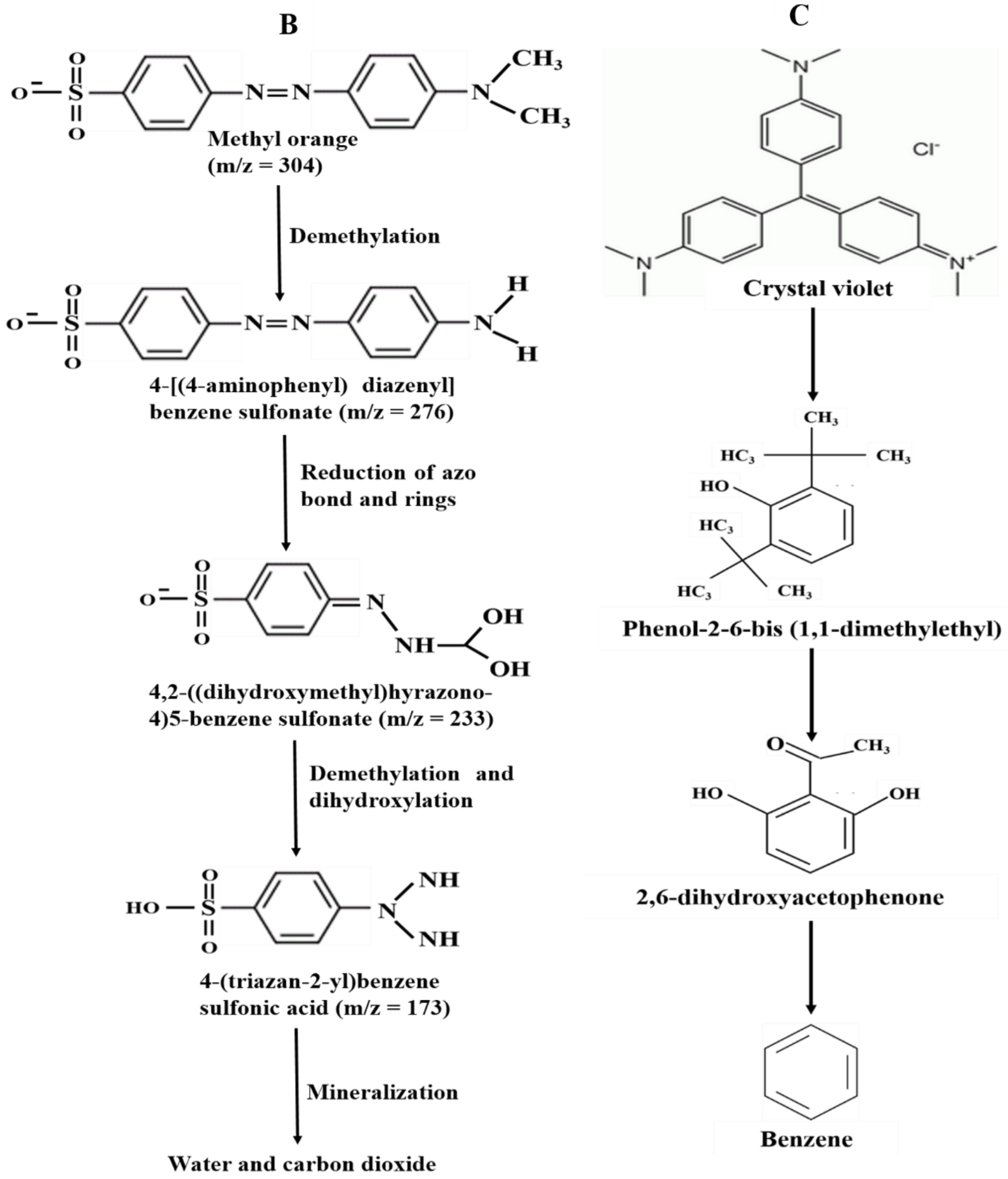


Fig 3.6: Degradation mechanism of methyl orange (B) and crystal violet dye (C) by isolated *P. aeruginosa* and *A. hydrophila*.

3.6.5. Microbial fuel cells (MFCs)

MFCs represent a novel, advance and sustainable approach for the treatment of TWW along with the power generation and CO_2 emission reduction (Oon et al., 2020). MFCs seem to be effective technology for the degradation of TWW pollutants. Many electrodes are investigated in MFCs for the effective treatment of wastewater. Out of the used electrodes, oxygen (O_2) and platinum (Pt) was found as most suitable electron acceptor in MFCs system, but these have many limitations like high cost, poor kinetics of oxygen reduction reaction (ORR) and create toxicity to the environment (Sun et al., 2020; Kishor et al., 2021a). Thus, various researchers have used different electrodes such as nickel, manganese and copper for the oxygen reduction reactions (ORRs) in MFCs (Kishor et al., 2021a; Yadav et al., 2022).

Miran et al. (2018) used a sulfate-reducing mixed communities comprising of *Proteobacteria*, *Desulfovibrio* and *Deltaproteo bacteria* have a great potential to decolorize 89.4%, 48.2% and 52.7%, respectively of Acid red 114 dyes with generation of electrical energy upto 258 ± 10 mW/m^2. The major advantages of MFCs are the complete mineralization of pollutants, electricity generation, reduced sludge generation and CO_2 emission (IIamathi and Jayapriya, 2017) while the major disadvantages are the high operation cost, energy recovery, system development and it can’t be applicable at extremely low temperature because the microbial activities are very slow at lower temperature.

3.6.6. Plant treatment

Phytoremediation method uses different class of plants for the treatment of harmful pollutants (Srivastava and Bandhu, 2022). Plants are well reported to remediate dyes, dissolve solids and heavy metals from TWW (Chandanshive et al., 2020). Further, many plant species has potential to convert/degrade toxic recalcitrant and emerging pollutants into non-toxic products by adsorption, accumulation and degradation process (Kadam et al., 2018). Many plants act as host for aerobic and anaerobic microorganisms, supplying chemical nutrients, providing shelter and oxygen (Kumar et al., 2019; Kishor et al., 2021a). Plants in stress conditions can activate efficient enzymatic machinery, which can take up hazardous chemicals as substrates and completely degraded into non-toxic products (Paździor et al., 2019).

Table 3.5: Different plants used in remediation/treatment of textile wastewater/dyes.

Plant	**Textile Wastewater & dye**	**Treatment efficiency**	**Time (h/d)**	**References**
Vetiveria zizanioides	Remazol red and Textile wastewater	Color (93%) & ADMI (74%)	48h & 72h	Chandanshive et al., 2020
Bacopa monnieri	Reactive 9 and Direct 5	100%	14d	Shanmugam et al., 2020
Hyacinth	Textile effluent	Color (83%) & COD (89%)	7d	Safauldeen et al., 2019
Phragmites australis and *Typha domingensis*	Textile effluent	Color (97%) & COD (87%)	8d	Tara et al., 2019
Portulaca grandiflora	Textile wastewater	Color (59%)	30d	Chandanshive et al., 2018
Gaillardia grandiflora	Textile wastewater	Color (73%)	30d	Chandanshive et al., 2018
Azolla pinnata	Methylene blue	Color (85%)	24h	Al-Baldawi et al., 2018
Fimbristylis dichotoma and *Ammannia baccifera*	Textile effluent and Methyl orange	Color (95%)	48h	Kadam et al., 2018
Typha angustifolia and *Paspalum scrobiculatum*	Congo red and textile industry effluent	Color (94%) & COD (70%)	48h & 96h	Chandanshive et al., 2017
Scirpus grossus	Methylene blue	86%	72d	Almaamary et al., 2017
Typha angustifolia	Congo Red and textile industry effluent	Color (80%) & COD (65%)	48h & 96h	Chandanshive et al., 2017
Ipomoea aquatica	Brown 5R and textile wastewater	Color (94%) & COD (87%)	72h & 72 h	Rane et al., 2016
Salvinia molesta	Textile effluent and Rubine GFL	Color (97%)	72h	Chandanshive et al., 2016
Physalis minima	Reactive black 8	Color (76%)	120h	Jha et al., 2015
Alternanthera philoxeroides	Remazol red	Color (100%)	72h	Rane et al., 2015
Blumea malcolmii Hook	Brilliant Blue R	Color (98%)	24h	Kagalkar et al., 2015
Lemna minor	Triarylmethane	Color (88%)		Torbati, 2015
Bacillus pumilus and *Pogonatherum crinitum*	Textile wastewater	BOD (78%) & COD (70%)	12d	Watharkar et al., 2015

Many plants such as *Glandularia pulchella*, *Vetiveria zizanioides*, *Physalis minima*, *Scirpus grossus*, *Ipomoea aquatic* and *Azolla pinnata* are used to degrade RCPs from TWW (Tara et al., 2019; Chandanshive et al., 2020) (**Table 3.5**). Further, a mixed culture of fungi and bacteria with *in vitro* development of plants system was seems to be more effective in the decolorization of TWW (Kadam et al., 2018; Chandansive et al., 2020). Recently, the combination of plant systems of *Typha angustifolia-Paspalum scrobiculatum*, *Fimbristylis dichotoma-Ammannia baccifera* were capable to remove MO dye along with a significant reduction in ADMI, BOD, TDS, COD and TSS from TWW than single plants (Tara et al., 2019; Kumar et al., 2019; Kishor et al., 2021a).

It has some major advantages like eco-friendly, solar-driven approach, cost-effective, low maintenance, negligible nutrient used, possible recovery and reuse of valuable metals, applicable for laboratories, pilot and field study while disadvantages includes slow process, difficulty to achieve acceptable levels of decontamination, toxic metals leached into groundwater and possibility of food chain contamination.

3.6.7. Constructed wetland (CW)

CW is a man-made system utilizing the natural ability of plants to remediate/treat pollutants. CW employed natural substances including wetland vegetation, bedding materials and its associated microbial flora for the remediation of RCPs (Paździor et al., 2019; Kishor et al., 2021a). Although, the bedding materials like gravel, zeolite rock and sand are used to enhance the treatment efficiency (Kadam et al., 2018). Different macrophytes like *Phragmites, australis, Typha angustifolia, Panicum elepantipes* and *Myriophyllum spicatus* are applied in CW treatment process (Hussain et al., 2019; Oon et al., 20220). The wetland plants provide habitat and nutrients potential to rhizo- and endophytic microorganisms while, microorganisms help their host plants to gain more biomass by reducing the contaminant stress, performing plant growth promoting services and accelerate treatment performance (Hussein and Scholz, 2018).

CW is two types i.e. horizontal sub-surface flow and vertical sub-surface flow, which functions under both aerobic as well as anaerobic conditions (Kishor et al., 2021a). In aerobic conditions, organic load is removed adequately, but color is not reduced significantly; where as in anaerobic condition, the situation is reversed where color is removed effectively, but organic compounds are not degraded effectively (Kadam et al., 2018; Hussein and Scholz, 2018; Oon et al., 2020). For instance, vertical-flow pilot-scale constructed wetland augmented with bacterial endophytes was found effective to remove and reduce color, dissolved solids and heavy metals from the real TWW (Hussein and Scholz, 2018). Horizontal-flow and vertical-flow pilot-scale constructed wetland planted with *Phragmites australis* was found effective to remove (89%) of COD, (91%) of BOD and (96%) of TDS from textile bleaching effluent (Hussain et al., 2019).

In present time, the CWs developed with microbial fuel cell (CW-MFC) were able to decolorize 96% of acid red 18, 67% of acid orange 7 and 60% of CR dye along with the generation of bioelectricity (Oon et al., 2020). The major advantages of CWs are eco-friendly, efficient color removal, self-regeneration, no energy requirement, recharging ground water, providing excellent habitat for aquatic and wildlife, no biosolids and sludge generation and disadvantages like require large land area, costly to design and construction, facilitate mosquito breeding, high monitoring requirement, require skill and management, require long period for vegetation and establishment for optimum treatment efficiency (Hussain et al., 2019).

3.7. Advanced oxidation processes (AOPs)

AOPs are considered as emerging, fast and competitive methods for the removal of refractory contaminates. AOPs were first evaluated for the treatment of drinking water in 1980s and became widely applicable in the treatment of industrial wastewaters (Paździor et al., 2019). In AOPs, different oxidizing agents such as O_3, H_2O_2 and many catalysts such as Fe_2O_3, ZnO, CdS, TiO_2, GaP, and ZnS as well as high energy radiation like UV light are used (Kaur et al., 2018; Kumar et al., 2019; Noman et al., 2020).

In AOPs, the highly oxidative and non-reactive species such as hydroxyl (OH•) and sulfate radicals (SO_4^-) are produced, which act as mediator in electron transfer, hydrogen abstraction and radical addition reactions (Paździor et al., 2019). Thus, AOPs are effective to degrade or transform many refractory compounds into non-toxic compounds. Currently, many AOPs including ozonation, photo-fenton, photo-catalytic, sono-catalytic, electro-coagulation and electro-oxidation process are widely applied in treatment of various RCPs/dyes, dissolved solids and heavy metals from TWW (Kaur et al., 2018; Kishor et al., 2021a). But, these are expensive, high chemical demanding, use complicated procedures, applied high electrical energy secondary pollutant (Kaur et al., 2018; Kumar et al., 2019).

Table 3.6: Various advanced oxidation processes (AOPs) used in treatment of textile wastewater/dyes.

Advanced oxidation processes	**Textile wastewater/dyes**	**Treatment efficiency (%)**	**References**
Nanofiltration (NF) process ($Al2O3/ZrO2$, $TiO2$ & NF200)	Hot textile wastewater	Color (82%), COD (90%), TDS (76%) & turbidity (82%)	Ağtaş et al., 2020
Membrane treatment (h-bio CHFM) process (Hydroxyapatite)	Textile wastewater	Color (99%), COD (80%), turbidity (99%) & Heavy metals (100%)	Hubadillah et al., 2020
Photo-fenton (PF) process ($ZnAl_2O_4/BiPO_4$)	Methylene blue & wastewater	Color (83%) & COD (76%)	Tian et al., 2020
Photocatalytic process (PPy or ZnO)	Direct black 22	Color (83%)	Ceretta et al., 2020
Sonophotocatalytic process (US/UV/ZnO/PS)	Acid blue 113 & wastewater	COD (96%) & TDS (97%)	Asgari et al., 2020
Ozonation (Zn-S)	Direct black 22	Color (76%)	Hien et al., 2020
Electrochemical oxidation (EO) (Al and Fe)	Real textile wastewater	Color (94.9%), turbidity (83%) & TOC (42%)	Bener et al., 2019
Ozonation (Chlorine or bromine)	Textile wastewater	Color (58%)	Oktem et al., 2019
Photocatalytic process (TiO_2)	Methylene blue	Color (42%)	Fazal et al., 2019
Advance oxidation process (APO) (H_2O_2/UV)	Textile wastewater	Color (95%), TOC (93%) & salts (95%)	Rosa et al., 2019
Electrocoagulation (EC) (Pt-CO and NTU)	Dyeing textile wastewater	Color (86%), COD (82%) & turbidity (59%)	Nunez et al., 2019
Photocatalytic process ($CuO/Cu(OH)_2$)	Reactive green 19A & wastewater	Color (98%), COD (84%) & TOC (80%)	Saratale et al., 2018
Electrochemical oxidation (EO) (Ti/RuO_2)	Real textile wastewater	Color (94%) & COD (86%)	Kaur et al., 2018
Electrochemical oxidation (EO) process ($RuO_2/IrO_2/TiO_2$)	Reactive Black 5	Color (100%)	Jager et al., 2018
Heterogeneous photocatalytic process (HT/Fe/TiO2)	Textile wastewater	Color (96%)	Arcanjo et al., 2018
Chemical coagulation-Electro-oxidation (CC-EO) ($Al_2(SO_4)_3$)	Textile wastewater	Color (100%), COD (93%) & TOC (75%)	GilPavas et al., 2018
Electrochemical degradation process (Ti/RuO_2 & Sb_2O_3)	Reactive Black 5	Color (99%) and COD (73%)	Viana et al., 2018
Advanced oxidation process (AOP) (O_3,O_3/H_2O_2, O_3/UV & $O_3/UV/H_2O_2$)	Textile dyes	Color (90%)	Bilinska et al., 2017

3.7.1. Ozonation

Ozonation is a chemical oxidation process used to remove environmental pollutants. Ozone (O_3) is a strong oxidizing agent and non-selective in nature that can decompose various RCPs of TWW (Kumar et al., 2019) (**Table 3.6**). It produces highly reactive species like hydroxyl and other radicals, which rapidly decolorize and detoxify RCPs, dissolve solids and heavy metals (Oktem et al., 2019; Hien et al., 2019). Many studies have reported that ozonation can effectively decolorize and remove a variety of RCPs, TOC, BOD, COD, TDS, TS, and Sb, Cr, Cd, and Pb from TWW (Wang et al., 2019; Hien et al., 2019). The major advantages of ozonation process are fast color removal with no alterations in volume while disadvantages are: it is applicable in gaseous state, short half-life (20 min), sensitive to pH and sludge disposal problems (Noman et al., 2019).

3.7.2. Photo-Fenton process

Photo-Fenton process ($UV/Fe^{2+}/H_2O_2$) has emerged as a most effective method for industrial wastewater treatment (Kishor et al., 2021a). It is able to remove RCPs, COD, TOC, BOD and TDS as well as various metals from wastewater (GilPavas et al., 2018; Paździor et al., 2019). But, this method also has some drawbacks like: (1) TWW is alkaline in nature, but PF process is effective in acidic condition. (2) colored pollutants reduce light penetration power. (3) ferric-organic complexes can be formed and thus, decreases radical generation efficiency. (4) high load of inorganic ions such as SO_4^{2-}, CO_3^-, and Cl^- may result the formation of inorganic ion-ferric complexes leading to decrease in radical generation (Bilinska et al., 2016). These drawbacks can be minimized by the addition of oxalic acid to FP treatment process.

Ferrioxalate complexes block the production of stable complexes between ferric ions and organic species (Arcanjo et al., 2018; Paździor et al., 2019). These also increase the quantum yield, enhance regeneration of ferric ions and thus, produce a large amount of hydroxyl radicals. PF/Ferrioxalate process is capable for the treatment of synthetic cotton-textile wastewater (Arcanjo et al., 2018). The addition of oxalic acid to FP process is limited with the iron precipitation and also promotes the degradation of TWW. This method has major advantages such as efficient in color and COD removal, degradation of RCPs and more effective than fenton processes at lower dose of ferrous sulfate due to additional benefit of UV radiation and disadvantages like high operation cost, less catalytic power and ineffective in case of copper phthalocyanine dye (Kumar et al., 2019).

3.7.3. Photo-catalytic treatment

It is an emerging approach with a great potential for the treatment of recalcitrant contaminates. In UV-TiO_2 process, the photoactivation of semicacondutores is initiated by irradiation with electron-hole pairs appearing as a result of band gap excitation (Kaur et al., 2018; Fazal et al., 2019). The oxidation and reduction processes are possible at or near the surface of the photo excited particles. The light generated possible hole may react with electron donors to generate hydroxyl radical (Kumar et al., 2019). Hydroxyl radicals can oxidize organic compounds into non-toxic. In this process, various semiconductor catalysts such as TiO_2, CdS, ZnO, CuNPs, GaP, SA/TiO_2, Fe_2O_3, TiO_2/UV, α-Bi_2O_3-ZiO, $S_2O_8^{-2}$/Fe^{2+} and ZnS are used and generate highly reactive species such as H_2O_2, O_3, $O\dot{H}$, and O^{-2} (Kumar et al., 2019; Kishor et al., 2021a). These species are highly effective in mineralizing and detoxifying various RCPs and refractory pollutants from TWW.

Among various semiconductors, TiO_2 is widely used in wastewater treatment (Kaur et al., 2019). For example, Fazal et al. (2019) reported a biochar-TiO_2 composite for the treatment of wastewater with 99.20% photodegradation efficiency of dye-simulate wastewater. Saratale et al. (2018), synthesized CuO/$Cu(OH)_2$ nanostructures that able to decolorize reactive green 19A dye and TWW. The major advantages of photo-catalytic treatment are short time consuming, little or no chemicals consumption, efficient for RCPs, high stability and COD reduction while disadvantages are the high treatment cost, limited applications subjected to light, catalysts fouling, formation of by-products and problem of fine catalyst separation from the treated effluent (slurry reactors) (Kishor et al., 2021a).

3.7.4. Sono-catalytic process

Sonolysis is a simple chemical method used in wastewater treatment. But, it is limited by poor elimination efficiency, high cost and not environmentally safe (Paździor et al., 2019). It is widely used to remove and detoxify toxic RCPs from TWW. Many semiconductors such as CdSe, CdS/TiO_2, and $KNbO_3$ have been successfully used as catalyst to reduce organic loads, suspended solids and dye content from TWW (Kumar et al., 2019; Kishor et al., 2021a). In recent years, many nano-composites such as Fe_3O_4-graphene/ZnO/SiO_2, CdSe/GQDs and TiO_2-BC have been used to remove MB, MO, rhodamine B and reactive blue 69 dye, dissolve solids and inorganic refractory chemicals from TWW (Kumar et al., 2019; Asgari et al., 2020). Asgari et al. (2020) developed a sono-photolytic-activated ZnO/persulfate (US/UVZnO/PS) composite to remove and mineralize real TWW.

The major advantages of this process are the short time requirement, efficient to toxic or non-biodegradable compounds while disadvantages are high treatment cost and require high amount of dissolved oxygen (Paździor et al., 2019).

3.7.5. Electro-coagulation (EC) treatment

EC is an electrochemical process that broadly used in treatment of RCPs due to its high efficiency. It is an efficient method to treat RCPs, heavy metals, phenols, surfactants, pesticides (Srivastava and Bandhu, 2022). The EC setup consists of cathode and anode electrode that are connected to the external monopolar or bipolar power supply. In EC process, electrical energy is used to dissolve iron (Fe) and aluminum (Al) to remove TWW (Paździor et al., 2019; Yadav et al., 2022). At cathode, hydroxide ions are formed, which helps to remove flocculants from wastewater and metal ions generated from the sacrificial anode act as destabilizing agents and neutralize the electric charge of contaminates resulting in the removal of pollutants from wastewaters (Kumar et al., 2019). Different electrodes such as iron, stainless steel, aluminum, mild steel and graphite can be used in EC treatment process in single as well as in different combinations for the effective degradation of various toxic RCPs from synthetic and real TWW (Paździor et al., 2019; Bener et al., 2019).

But, iron and aluminum are most widely used as electrode materials in EC process. For instance, the use of aluminum in TWW treatment significantly removes color and reduces BOD, TOC, COD and TSS (Bilinska et al., 2016). Recently, EC process was found to be effective to remove 94.9% color, 83.5% turbidity, 64.7% TSS, 42.2% TOC and 18.6% COD from treated wastewater (Bener et al., 2019). EC treatment method has some major advantages like no chemical requirement, short treatment time, smooth operation and requirement of a low dose of colloidal particles and disadvantage like high operation cost, cathode passivation and generation of toxic sludge as secondary pollutant (Bener et al., 2019; Paździor et al., 2019).

3.7.6. Electro-chemical oxidation (EO)

EO provides an alternative way for the treatment of toxic or non-biodegradable wastewater like TWW. It produces highly reactive species (OH•), which broadly remove RCPs, SS and toxic metals from TWW (Kaur et al., 2018; Kishor et al., 2021a). The anodic oxidation process applied to remove color, toxic chemicals along with the reduction in COD, BOD, and TDS from wastewaters (Paździor et al., 2019). For example, Abdessamad et al. (2015) achieved 100% COD removal from TWW by using anodic oxidation process.

EO uses many electrodes of substances like Ti/IrO_2, Ti/SnO_2, Ti/PBO_2, BDD, graphite and PbO_2, Ti/RuO_2 and SnO_2 as anode in treatment of TWW (Kumar et al., 2019; Paździor et al., 2019). These electrodes have potential for the oxidation of pollutants and high oxygen over potential (1.9V). BDD appears more effective electrode in the removal of TWW due to it has high stability, generate high potential O_2 overvoltage (2.7V) and inactive surfaces with less adsorption capacity (Arcanjo et al., 2019).

Ti/RuO_2 is a potentially stable and has high chemical and mechanical strength, high oxygen over potential (≈2.0V) and produces strong oxidants such as HOCl, Cl_2 and ClO^- etc. It is highly efficient to effectively treat dyestuffs, reduces dissolved and suspended organic and inorganic pollutants from TWW (Kaur et al., 2018; GilPavas et al., 2018). EO method has major advantages such as high decolorization efficiency, efficient for persistent or toxic pollutants, no additional chemical requirement while disadvantages like high operation cost, toxic metabolites generation and steam stripping.

3.7.7. Membrane technologies (MTs)

MTs use various classes of permeable membranes in treatment of industrial wastewaters (Noman et al., 2020). Many emerging membranes such as RO, NF, MF and UF are widely utilized in separation and desalination of dyeing and TWW (Kumar et al., 2019; Kishor et al., 2021a). All these membranes are found effective in removal of color, organic salts and suspended impurities from TWW (Dasgupta et al., 2015). Sahinkaya et al. (2018) have used RO technique, which showed 94% decolorization of TWW. NF is more effective method as compared to RO treatment because it requires lower pressure (Dasgupta et al., 2015). For instance, UH004, PA6DT-C and polyamide NF are capable to decolorize MB, direct, reactive blue 2 dyes, reactive black 5, reactive blue 15, reactive orange 16, reactive yellow 145 and reactive red 194 from synthetic wastewaters (Dasgupta et al., 2015; Rondon et al., 2015).

Currently, pilot scale ceramic UF/NF was able to remove 90.1% of COD, 82.2% of color, 82% of total hardness and 76.8% of TOC from real disperse and reactive printing wastewater (Ağtaş et al., 2020). Further, Hubadillah et al. (2020) developed a potential hydroxyapatite (HAp)-based bio-ceramic hollow fiber membranes, which remove 99.9% of color, 80.1% of COD, 99.4% of turbidity, 30.1% of conductivity and 100% of metals from TWW. MTs has some major advantages like no chemical requirement, effective in color removal, complete water/wastewater purification and produce a high-quality treated wastewater while disadvantages are membrane fouling, clogging, scaling and cleaning, pre-treatment requirement, high pressure requirement, concentrated sludge production and high cost of membrane replacement (Ağtaş et al., 2020).

3.7.8. Membrane bioreactors (MBRs)

MBRs are a combination of biological approach and membrane filtration, which is widely used in treatment of wastewaters (Yurtsever et al., 2017). In MBR, the microbial communities play a crucial role in degradation and mineralization of various RCPs present in TWW whereas, membranes separate the microorganisms, macro-molecules as well as allow the water and dissolved species to pass through (Kishor et al., 2021a). MBR is a simple, cost-effective and reliable method, which produce high quality recyclable treated water. It has a strong potential to remove high nutrient load, organic chemicals and RCPs. But, its major disadvantage is the fouling of membrane in bioreactor i.e. deposition of cells on the membrane, exertion of extracellular polymeric substances (EPS) and soluble microbial products (SMP), which largely depends on the density of microbial cells and microbial population structure (Rondon et al., 2015).

Enhanced membrane bioreactor (e-MBR) in combination with two anoxic bioreactors (ARs), aerated bioreactor (AMBR), UV-unit and a granular activated carbon (GAC) filter was found effective in removal of ~99% COD, 95% color, 73% phosphorus and 97% nitrogen from TWW (Rondon et al., 2015). Yurtsever et al. (2017) used a microbial community of anaerobic (AnMBR) and aerobic bioreactors (AeMBR) technology to remove RCPs. The aerobic process is restricted by the low aerobic biodegradability, while microbial anaerobic process is efficient for TWW decolorization, but it produced highly toxic intermediate products (Kishor et al., 2021a).

In this context, a novel anaerobic-aerobic algal-bacterial photobioreactor was developed for the treatment of TWW. This technology was capable to decolorize 99.1% of disperse orange 3 and 96.3% disperse blue 1. In photobioreactor, the symbiotic interaction is based on the mutualistic exchange of CO_2 and O_2 between bacteria and microalgae. Furthermore, the pollutants adsorption onto microalgae cell wall can facilitate the dye mineralization whereas; bacteria can improve algal growth by producing growth-promoting factors (Kishor et al., 2021a).

MBRs has major advantages including reliable, small footprint, biogas production, high quality of treated effluent, lower sludge generation, higher nutrient removal, high degradation rate of organic and inorganic pollutants while disadvantages like aeration limitations, stress on sludge in external MBR, membrane fouling and higher operation cost (Kishor et al., 2021a).

3.8. Combined treatment methods (CTMs)

CTMs use physical, chemical and biological methods in combination that results in better degradation and mineralization of TWW as compared to the single methods (Paździor, et al., 2019). TWW is a complex matrix and contains various RCPs, surfactants, salts, heavy metals and disinfectants (Kishor et al., 2021a). The reported treatment methods are not always effective and also have some serious disadvantages. For example, AOPs are not feasible due to the high cost, incomplete mineralization and generation of large amount of toxic products (sludge) (Kumar et al., 2019). Beside this, BT takes long time for the complete mineralization of pollutants and ineffective against complex and recalcitrant pollutants (Kishor et al., 2021a). Therefore, CTMs showed better degradation efficiency of TWW. For example, the combination of AOP and biological process seems to be an alternative approach to treat TWW. In this process, AOP breakdown the complex structure of pollutants by free radical attack, generating products, which are more biodegradable and then biological process further degrade and mineralize such products into the small, simple and non-toxic metabolites by involving oxidoreductive enzymes (Waghmode et al., 2019).

Table 3.7: Combined treatment approaches for remediation of textile wastewater/dyes.

Combined treatment approaches	**Textile wastewater and dyes**	**Treatment efficiency**	**References**
Ultrafiltration + Nanofiltration (UF-NF) treatment	Real textile printing wastewater	Color (83.5%), COD (89%) & TOC (86.4%)	Agtas et al., 2020
Biological + Photocatalysis treatment	Real textile wastewater	Color (95.7%) & TOC (99.8%)	Ceretta et al., 2020
Plasma oxidation (PO) + Microbial fuel cell (MFC)	Methylene blue	Color (97.7%)	Sun et al., 2019
Ozonation + regenerated granular activated carbon	Textile wastewater	----	Wang et al., 2019
Adsorption + photocatalysis	Textile wastewater	Color (99.20 %)	Fazal et al., 2020
Electrocoagulation (EC) + ozonation (O_3) treatment	Textile wastewater reuse	Color (95%)	Bilinska et al., 2019
Fenton + Ultrafiltration (UF) treatment	Textile wastewater	Color (99%)	Buthiyappan et al., 2019
Coagulation-flocculation (C-F) + Fenton or photo-fenton	Textile wastewater	Color (87%)	GilPavas et al., 2017
Forward osmosis (FO) + Coagulation/ flocculation (CF)	Textile wastewater	Color (95%)	Han et al., 2016
Photo-assisted electrochemical + simultaneous chlorine photolysis treatment	Textile wastewater	COD (86%) & TOC (92%)	De Mello Florencio et al., 2016
Biological + Chemical treatment	Textile wastewater	Color (92%) & COD (87%)	Hayat et al., 2015
SBR (sequencing batch reactor) + Fenton process as post treatment	Azo dye AR18	Color (100%) & COD (97%)	Azizi et al., 2015
Combined anaerobic-Ozonation process	Textile wastewater	Color (100%) & COD (90%)	Punzi et al., 2015

A combination of ceramic UT-NF system was found effective in removal of 83.5% color, 89% COD, 86.4% TOC and 68% hardness from real printing wastewater (Ağtaş et al., 2020). Sun et al. (2020) evaluated a combined system of plasma oxidation and MFCs and found effective to mineralize 97.7% of MB dye with the generation of electrical energy upto 519 mW m^{-2}. Different physico-chemical, AOPs and biological methods used in combination for the treatment of TWW are summarized in **Table 3.7**. The major advantages of CTMs are fast treatment efficiency, efficient for toxic or non-biodegradable pollutants and maximum removal of contaminants while disadvantages are the high treatment cost and sludge generation.

3.9. Possible recycling and reuse of treated TWW

Water is an essential compound to sustain all living creatures on the planet. ~ 97% of the water resources are salty, which are not drinkable. More than 2% of the world’s water is tied up in glaciers and ice caps and only less than 1% water is potable. Currently, the level of potable water is decreasing day by day due to the continuous increase in water pollution and overuse of water in industries. Industries use a large volume of potable water in different production process (Bilinska et al., 2019). For example, ~1.6 million L of fresh water is used in TIs to produce 8000 kg of textiles and discharge as a wastewater along with RCPs, salts, VOCs, phenols, surfactants and toxic metals into natural water bodies (Kumar et al., 2019; Kishor et al., 2021b). Further, 20% of the world’s population is suffering from water deficiency and 40% from contaminated water (Núñez et al., 2019).

To solve such issues, many techniques are developed for adequate treatment of wastewaters that allows the reuse of treated water with many economic and environmental benefits. For example, EC process is effectively decolorizes TWW and allows the treated wastewater for recycling and reuse in the wool dyeing process and agricultural irrigation (Watharkar et al., 2018). UF/NF ceramic membrane process has potential to recover and reuse hot TWW that significantly reduces water consumption and electricity charges (Ağtaş et al., 2020). Rosa et al. (2020) applied AOPs for the recycling of TWW of ~22.47%. These treatment technologies reduce water consumption, chemicals and treatment cost along with the environmental threats, public health concern as well as improved the industry image before society.

3.10. Various analytical techniques used to detect and characterize TWW pollutants

TWW is well reported to contain a variety of RCPs, out of which some are degraded and mineralized while some get converted into metabolic products (Kishor et al., 2021a). These compounds are required to be characterized and identified by various analytical techniques to understand their nature for the environment sustainability. UV–vis spectroscopy is the primary technique used to measure the decolorization of TWW. American Dye Manufacturers Institute (ADMI) tristimulus filter method is used to calculate the decolorization of real and synthetic TWW (Kurade et al., 2012). FT-IR analysis is used to identify the functional groups present in parent compounds and their metabolites produced during the treatment process (Kishor et al., 2021b).

Table 3.8: Various analytical techniques used to detect and characterize the textile wastewater pollutants.

Analytical techniques	Metabolic products identified	RCPs/Dyes
UV-vis, FT-IR and GC-MS,	Phenol, 2, 6-bis (1,1-dimethylethyl) and 2, 6-dihydroxyacetophenone and benzene	Crystal Violet
HPLC and GC-MS	1 and 2-[(3-diazenylphenyl) sulfony 1] ethanesulgonate 8-[(4-chloro-1,3,5-triazin-2-yl)amino] naphthalene-1ol and benzene-oxosulfane oxide	Remazol Red
UV-vis, FT-IR, HPLC and LC-MS	1,1-biphenyl-4,4-diyldidiazene, sodium 4-amino-3-hyroxynapthalene-1-sulfonate and 1-aminonaphthalen-2naphthalen-2-ol	Congo Red
UV-vis, FT-IR, HPLC and LC-MS	Sodium 4-(phenydiazenyl)benzenesulfonate, 4-(phenyldiazenyl) benzenesulfonate and 4-sulfanylphenol	Methyl Orange
HPLC and GC-MS	L-proline, N-valeryldecyl ester, 3,5 di-tert-butyl-4-trimethylsiloxytoulene and 1,2-benzenedicarboxylic acid and diisooctyl ester	Dt. T Blue GLL
LC-ESI-MS/MS	Phthalic acid (product) and 4-hydroxy-2-oxovaleric acid	Direct Black G
UV-vis, FT-IR and LC-MS	N, N-dimethyl p-phenylenediamine, 4-(dimethylamino) phenol, 4-diazenylbenzene sulfonic acid, and 4-amino sulfonic acid	Methyl Orange
UV-vis, FT-IR, HPLC and LC-MS	Biphenyl diamine and 1,20-diaminonapthalene-4-sulfonic acid	Congo Red
UV-vis, FT-IR, TLC and GC-MS	1,2,7 triamino-8-hydoxy-3,6- naphthalinedisufonate, 1-sulphonic,2-(4-aminobenzenesulphonly and oxalic acid	Reactive Black 5
UV-vis, FT-IR, HPLC and GC-MS	1,3,5-triazine 2,4-diol, naphthalene 2-diazonium 1,5-disufonic acid sodium 1-naphthol-3-sulfonate, naphthalene diazonium and naphthalene	Reactive Orange 4
UV-vis, FT-IR, HPTLC and GC-HRMS	Naphthalene-1- yldiazene, naphthalene, 1-(2-methylphenyl)-2phenyldiazene, and diphenyldiazene	Solvent Red 24
UV-vis, FT-IR, HPLC and GC-MS	sodium-4-aminobenzenesulfonate, 1,4-benzenediamine and 7-benzylamino-3-dibenzyl-1-4-hydroxy naphthalene-2-sulfonic acid, 1-phenylmethanamine-ethene and 8-aminonaphthol, naphthalene	Direct Red 81
FT-IR, HPLC, HPTLC and GC-MS	4(5-hydoxy, 4-amino cyclopentane) sulfobenzene and 4(5-hydroxy cyclopentane) sulfobenzene	Reactive Yellow-84A
UV-vis, FT-IR, HPLC and GC-MS	Methanesulfinic acid, 4-[(6-amino-4-chloro-1,2,3,4-tertrahydro-1,3,5-triazin-2-yl) amino] decahydronaphthalene-2,7-disulfonate, 4-[(4-chloro-1,3,5,-tiazin-2-yl) amino] naphthalene-2,7-disulfonate], and 4-chloro-1,3,5-tiazin-2-amine, 1,3,5-triazine	Remazol Red

UV-vis, FT-IR, GC-MS and 1H NMR	[2-amino-8- (2- (4- (6- (7-amino-3,6,8-trihydroxynaphthalene-1-ylamino) pyridine-2-ylamino) phenylamino) pyrimidin-4ylamino) naphthalene-1,3,6,-triol], and 8-(4,6-dichloro-1,3,5-triazin-2-ylamino)-2diazenylnaphthalene-1,3,6-triol	Reactive Red
UV-vis, FT-IR, HP-LC and GC-MS	N-ethyl-4-[(2- methyl-4-nitrophenyl) diazenyl] aniline, 4-[(2-methyl-4-nitrophenyl) diazenyl] phenol, 1-(2-methyl-4-nitrophenyl)-2-phenyl diazene, 2-methyl 4-nitroaniline	Textile wastewater
UV-vis, FT-IR, HP-LC and GC-MS	[GG-A], {ethyl [(4-oxo-3-4-dihydroquinolin-2-yl) methyl] amino} acetaldehyde [Rt-19.383-MW, 244, m/z – 244]	Scarlet RR
HPTLC, HPLC, FT-IR and GC-MS	5-Sulfone diazonium, 4-methyl-2-m-tolyamino-cyclopentanol	Golden Yellow HER
UV-vis, FT-IR, HPLC and 1H NMR	Methyl metanilic acid, 4-aminobenzoic acid and Benzoic acid	Remazol Orange
UV-vis, HPLC and GC-MS	6-(acetylamino) naphthalene-2-sufonic acid, 2-(4-aminophenyl) ethanesulfonic acid, aniline	Reactive orange 16

HPLC is employed for the detection, identification and quantification of organic compounds present in TWW (Kurade et al., 2012). The GC-MS and LC-MS/MS techniques are used to characterize and identify the organic compounds and their metabolites (Kishor et al., 2021b). In addition, the nuclear magnetic resonance (NMR) a powerful analytical technique is used to confirm the presence and position of protons in organic compounds and their metabolites. However, the details of different techniques used by various researchers for the characterization and identification of TWW pollutants as well as their metabolites are listed in **Table 3.8**.

3.11. Challenges and Key Issues

TI is facing many serious challenges from the public and government sector like increased cost of raw textile materials due to the stringent environmental regulations, increasing demand of various types of textile fabrics, lack of advanced processing techniques and waste treatment technologies in developing countries, lack of specific dedicated industrial areas for the positioning of TI, poor capacity utilization leading to the higher financial cost and over heads charges and the lack of financial support from the Government.

The mitigation of these challenges requires large scale financial supports from the Government sector for proper functioning of TI, especially for small scale industries. TI should also use eco-friendly/natural coloring/auxiliaries agents of biological origin instead of synthetic agents as it may be helpful in the reduction of treatment cost for environmental cleanup. TI should adopt recycling/reuse of treated wastewater to minimize the use of fresh ground water for economic and environmental benefits. There is a need to revisit the textile processing industries to ensure the sustainability in the core of industries as these are the key driver of many nations' economy.

Chapter 4

Physico-chemical analysis of textile wastewater (TWW) collected from textile industry

Physico-chemical analysis of textile wastewater (TWW) collected from textile industry

4. Introduction

A large volume of groundwater and a wide range of synthetic chemicals are used in textile industries (TIs) at various stages during the textile production processes (Kishor et al., 2021a). Thus, TIs discharge a large volume of highly polluted wastewater (TWW) into the environment (Kumar et al., 2019). Textile wastewater (TWW) is complex in nature and causes serious threats to our natural resources (water/soil) available on the earth (Cao et al., 2019; Kishor et al., 2021a). TWW have dark color, high pH, temperature, biochemical oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS), total suspended solids (TSS), total organic carbon (TOC), phenol, chloride, nitrate, sulphate and phosphate (Khan and Malik, 2017; Kishor et al., 2021b). TWW also contains a variety of highly toxic organic and inorganic chemicals (Bilinska et al., 2019; Kishor et al., 2021b). Therefore, the characteristics of TWW may vary from industry to industry, raw materials and chemicals, types of final products and production processes adopted by TIs (Kaur et al., 2018; Kishor et al., 2021a).

TWW is well reported for its carcinogenicity, mutagenicity, neurotoxicity, genotoxicity and allergenic effects in animals and humans (Noman et al., 2020). TWW causes detrimental effects on water quality by the coloration of water, reduced sunlight penetration, decrease in photosynthetic activity of aquatic plants, dissolved oxygen content and increase in BOD and COD levels leading to an anoxic conditions that adversely affect fauna/flora and microbial diversity (Cao et al., 2019; Samuchiwal et al., 2021). It reduces soil fertility, crop yield and biodiversity by causing soil salinity and soil pollution (Chandanshive et al., 2020). TWW also reduces seed germination, root length, shoot length and biomass production of plants (Haq et al., 2018; Kishor et al., 2021b). However, the adequate treatment of TWW is required before its final discharge into the environment for the protection of public health and environment. Moreover, TIs are heavily suffering from the negative impact caused by its highly toxic wastewater that causes serious damage to the receiving environment and enormous pressure from the pollution controlling authorities to regulate and minimize the load of pollution parameters in discharged TWW. Therefore, the present study was aimed to characterize the TWW before and after treatment for various physico-chemical parameters to know their pollution profile for environmental safety.

4.1. Materials and methods

4.1.1. Chemicals

The chemicals used in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals were of analytical grade and highest purity.

4.1.2. Collection of textile wastewater from textile industry

The TWW samples were collected in pre-sterilized clean containers (capacity 20 l; Tarson Production Pvt. Ltd., USA) from the outlet of a Handloom Bhandar located at Unnao (26.48^{o} N, 80.43^{o} E), Uttar Pradesh (UP), India (**Fig. 4.1**).

Unnao is the largest industrial city in the UP and famous for its industrial sectors worldwide (Bharagava et al., 2018). Textile is one of the biggest industries in Unnao. The collected TWW samples were store at 4 °C in laboratory and employed for the analysis of physio-chemical parameters, isolation of bacteria, biodegradation studies, recalcitrant coloring pollutants (RCPs) and their metabolites characterization and toxicity evaluation.

Fig 4.1: Collection of textile wastewater from the outlet of a Handloom Bhandar, Unnao, Uttar Pradesh, India.

4.1.3. Physico-chemical analysis of collected textile wastewater sample

The physico-chemical analysis of TWW was performed in triplicates as per the standard protocols outlined in the "Standard Methods for Examination of Water and Wastewater" (APHA 2012). TWW before and after treatment was characterized for pH, temperature, color (American Dye Manufaturing Institute (ADMI), TDS, electrical conductivity (EC), COD, BOD, TSS, total organic carbon (TOC), total solids (TS), phenol, chloride, sulphate, surfactant, phosphate, nitrogen and metal ions (chromium (Cr), zinc (Zn), cadmium (Cd), nicekle (Ni), arsenic (As), lead (Pb)). The pH and temperature were confirmed by using a digital desktop pH meter (Systronics 361, India) and glass thermometer. The color (ADMI) was monitored by ADMI 3WL tristimulus filter method, BOD by 5-day method, COD by open reflux method, respectively. The TDS, TSS, TS and nitrogen was measured by gravimetric method and TOC-V_{csn} analyzer (Shimadzu, Japan). The phenol was measured by 4-aminoantipyerene method, chloride by iodometric method, sulphate by $BaCl_2$ precipitation method and phosphate by Vanadomolybdo-phosphoric acid colorimetric method, respectively.

Further, different metal ions like Ni, Cr, As Cd, Fe and Pb were detected and quantified by Atomic Absorption Spectrophotometric (AAS) after acid digestion method. All the physico-chemical parameters of this study were analyzed at Indian Institute of Toxicology research (CSIR-IITR), Lucknow (UP) India.

4.1.3.1.pH

The negative log of the hydrogen ion concentration is called pH ($pH= - \log_{10} [H^+]$). pH is an important quality parameter for both the water and wastewater. pH plays an important role in the wastewater treatment process as it has a direct influence on wastewater treatability-regardless of whether treatment is physical/chemical or biological. In present study, the pH of TWW collected from Handloom Bhandar was analyzed by a digital desktop pH meter (Systronics 361, India) according to the manufacturer instructions.

4.1.3.2.Temperature

Temperature is a very important parameter because of its effect on chemical reactions on reaction rates, aquatic life and the solubility of essential gases such as oxygen in the water. The temperature (ºC) of TWW collected from Handloom Bhandar was determined by a Lab Pro Laboratory Analog chemical Thermometer (Lab Pro Inc., CA, USA).

4.1.3.3.Electrical conductivity (EC)

EC is a measure of how well a material can conduct electricity. This ability is directly related to the concentration of ions present in water or wastewaters. These conductive ions come from dissolved salts and inorganic materials such as alkalis, chlorides, sulfides and carbonate compounds. Compounds that dissolve into ions are also known as electrolytes. The more ions that are present, the higher the conductivity of water. Likewise, the fewer ions that are in the water or wastewater, the less conductive it is. EC is usually measured in micro-or millisiemens per centimeter (µS/cm or mS/cm^{-1}). The EC of TWW was determined by an electrochemical method using an HQ14D Portable Conductivity Meter (Hach Company, Colorado, USA).

4.1.3.4. Biochemical oxygen demand (BOD)

BOD is the most widely used test to know the index of organic pollution present in water or wastewater. BOD is the amount of dissolved oxygen (DO) need to break down biodegradable organic compounds present in wastewater by biological agents (APHA, 2012). The sufficient oxygen is available, aerobic biological decomposition (i.e. stabilization of organic waste) by microorganisms will continue until all waste is completely consumed. It is also known as "BOD_5" since it is based on the accurate measurement of DO at the beginning and end of a five-day period. The sample was incubated in dark placed to prevent the photosynthetic activity. The change in concentration of DO over five days represents the "oxygen demand" for respiration by the microorganisms in sample. If wastewater have high BOD, it means the wastewater contains too much bio-degradable organic compounds, which damage receiving water bodies. Therefore, the determination of BOD is very important for water and wastewater.

For this, 300 mL of untreated and bacteria TWW was collected in BOD bottles and incubated in BOD incubator for five days at 30 ºC. Afterward, the BOD bottles were taken and added 2.0 mL of manganese sulphate ($MnSO_4$), alkali azide and concentration sulphuric acid (H_2SO_4). Shake the bottles, taken 200 mL sample and titrate with 0.025N sodium sulphate. Starch solution was added as an indicator (gives purple color) and the end point color changes from purple to colorless. BOD bottle with aerated water and without wastewater sample was taken as blank. The calculation of BOD was done according to the following equation:

$$\mathbf{BOD_5\ (mg/L)} = \text{Blank} - \text{titrate sample value} \times 300\ /\ \text{volume of sample}$$

4.1.3.5. Chemical oxygen demand (COD)

COD test is used to determine the quality of water or wastewater. COD test is faster, more accurate and have ability to oxidize organic and inorganic matters as compared to BOD test. COD test is also able to remove toxicity and non-biodegradable matter present in wastewater. The high value of COD indicates the higher organic and inorganic load present in wastewater. The organic and inorganic matter present in wastewater is oxidized by potassium dichromate ($K_2Cr_2O_7$) in presence of silver sulphate (Ag_2SO_4), sulfuric acid (H_2SO_4) and mercuric chloride ($HgCl_2$).

For COD determination, 20 mL of untreated and treated TWW sample were taken, diluted to 50 mL in 250 mL refluxing flask and added 1.0 mL $HgSO_4$. Some glass beads and 5 mL sulphuric acid was added very slowly with proper mixing to dissolve $HgSO_4$. Afterward, cooled while mixing the sample to avoid the loss of volatile materials and then added 25 mL of 0.0417 M $K_2Cr_2O_7$ solution and mixed well. Attached the flask to a condenser and turned on cooling water. Remaining sulphuric acid (70 mL) was added through the open end of the condenser, continued swirling and mixing while adding the sulphuric acid. The open end of condenser was covered with a small beaker to prevent foreign material from entering refluxing mixture and refluxed for 2h.

After cooling, the samples were titrated with 0.1N ferrous ammonium sulphate (2-3 drops) and ferrion indicator. The end point was observed as reddish brown appeared. Similarly, refluxed and titrated a blank containing the reagents and a volume of DW equal to that of the sample. After digestion, the remaining unreduced ($K_2Cr_2O_7$) is titrated with ferrous ammonium sulfate to determine the amount of ($K_2Cr_2O_7$) consumed and the oxidizable organic matter is calculated in terms of oxygen equivalent. COD of the TWW was calculated by the following equation:

COD (mg/L) = Blank – titrate sample × N of FAS × 8000 / volume of sample

4.1.3.6. Total solids (TS)

Total solids is defined as the total amount of substances either dissolved, suspended or settleable in wastewater and remains as residue upon evaporation and subsequent drying at a defined temperature (103-105 ºC). The TS includes both total dissolved solids (TDS) and total suspended solids (TSS).

For TS determination, firstly heated the clean evaporating dish at 103 to 105 ºC for 1h and then cooled in a desiccator and weighed immediately. Further, 20 mL of well mixed TWW sample was taken in a pre-weighed evaporating dish and then evaporated to dryness in a drying oven at approximately 2 ºC below boiling to prevent splattering. Dried evaporated sample for at least 1h in an oven at 103 to 105 ºC, cooled the evaporating dish in a desiccator to balance temperature and then weighed. Repeated cycles of drying, cooling, desiccating and weighing were performed till a constant weight was obtained, or until weight change was less than 4% of the previous weight or 0.5 mg. The TS in the TWW was calculated by the following equation:

Total solids (mg/L) = Final weight – Initial weight × 1000 × 1000 / Volume of sample

4.1.3.7. Total dissolved solids (TDS)

TDS is the term used to describe the inorganic and small amount of organic matter present in water or wastewater. TDS includes nitrate, chloride, calcium, phosphorus, iron, sulfur and other ions particles that are passed through the filter with pore size (0.002μm).

For determination of TDS, firstly heated the clean dish at 103 to 105 ºC for 1h and then cooled in a desiccator and weighed immediately. Further, 20 mL well mixed TWW sample was taken in a pre-weighed evaporating dish and evaporated to dryness in a drying oven at 2 ºC. Afterward, the dried and evaporated TWW sample for at least 1h in an oven at 103 to 105 ºC, cooled the evaporating dish in desiccators to balance the temperature and weighed immediately. Repeated cycles of drying, cooling, desiccating and weighing were performed till a constant weight was obtained, or until weight change was less than 4% of previous weight or 0.5 mg. The TDS of TWW was calculated by the following equation:

TDS (mg/L) = Final weight – Initial weight × 1000 × 1000 / Volume of sample

4.1.3.8. Total suspended solids (TDS)

The difference between the total solids and total dissolved solids is suspended solids.

TSS (mg/L) = TS-TDS

4.1.3.9. Phenol

Phenol is defined as a hydroxy derivative of benzene and its condensed nuclei may occur in domestic and industrial wastewaters. Steam-distillable phenols react with 4-Aminoantipyrine at pH 7.9 ± 0.1 in the presence of potassium ferricyanide to form a colored antipyrine dye. This dye is extracted from aqueous solution with $CHCl_3$ and the absorbance is measured at 460 nm. This method covers the phenol concentration ranging from 1.0 μg/L to over 250 μg/L with a sensitivity of 1 μg/L.

For determination of phenol, TWW sample (pH 2-3) was taken in a distillation flask and removed oil and grease by transferring it in a separator funnel with 25 mL of chloroform and then added four drops of orthophosphoric acid and three drops of methyl orange as indicator. Distilled the solution and placed 500 mL distillate in a 1000 ml flask. Prepared a 500 mL blank and a series of 500 mL of phenol standards (5, 10, 20, 30, 40, and 50 μg phenol). The treated samples, blank and standards are as follows: added 12.0 mL 0.5 N NH_4OH and immediately adjusted pH to 7.9 ± 0.1 with phosphate buffer (10 mL) and transferred it to a 1000 ml separating funnel. Added 3 ml of 4-aminoantipyrine solution, mixed well followed by addition of 3 mL $K_3Fe(CN)_6$ solution. Mixed the solution well and let color to develop for 15 min. Extracted immediately with 50 ml of chloroform each time. Shaken the separating funnel many times (10 times), let $CHCl_3$ settle, shaken again and let the $CHCl_3$ to settle again. Filtered each $CHCl_3$ extract through filter paper or fritted glass funnels containing a 5 g layer of anhydrous Na_2SO_4. The dried extract was collected in clean test tubes or cells for absorbance measurements. Read absorbance of samples and standard against the blank at 460 nm. Constructed calibration curve by plotting absorbance against the micrograms of phenol concentration and calculated the amount of phenol in samples using this curve. The concentration of phenol in TWW was calculated by the following equation:

$$\textbf{Phenol (mg/L)} = (A \times 100) / B$$

[Where, A = phenol in sample (mg) from calibration curve, B = volume of sample (mL)].

4.1.3.10. Chloride

Chloride was determined in wastewater or water by titration against silver nitrate using potassium chromate as an indicator. Silver nitrate reacts with chloride ions present in water and produces white soluble precipitate of silver chloride. At the end point when all the chloride gets precipitated then free silver ions reacts with chromate from reddish brown color of silver chromate. Blank was taken with distilled water with same procedure. The chloride in TWW was calculated by the following equation:

$$\textbf{Chloride (mg/L)} = (\text{Sample} - \text{Blank}) \times \text{N of AgNO3} \times 35.45 \times 1000 / \text{Volume of sample}$$

4.1.3.11. Sulphate

Sulphur (sulphate) is an essential component of all living organisms for protein synthesis. The death of animals, microbes and plants and decomposed by microbes into hydrogen sulphide in anaerobic condition and sulphur in aerobic condition. Sulphur is oxidized into sulphate by bacteria and algae in presence of dissolved oxygen, which utilized as a nutrient for their growth by microbes and plants and passed into animals when they feed. The hydrogen sulphide is highly toxic, corrosive and foul smelling. It also inhibits growth and metabolic activity of organisms by reacting, precipitate and making unavailable various trace nutrients.

For determination of sulphate, 50 mL of TWW (pH 4.5-5.0) was collected in flask and added hydrochloric acid (2.0 mL). Placed at hot plate for boiling and stirring gently. The $BaCl_2$ was slowly added until precipitation appeared to be completed and added about 2 mL in excess. The digest was precipitated at 80-90 ºC for overnight. The samples were filtered with ashless filter paper and washed with warm distilled water until washings are free of chloride indicated by testing with $AgNO_3$-HNO_3 reagent. Added a few drops of silicone fluid to the suspension before filtering, to prevent adherence of precipitate to holder. Dried, filter and precipitated in a conventional oven at a temperature of 103 to 105 ºC. Cooled in a desiccator and weighed. The concentration of sulphate in TWW was calculated by the following equation:

Sulphate (mg/L) = ($BaSO_4 \times 411.5$) / Volume of sample (mL)

4.1.3.12. Phosphate

Phosphate is an essential to living organisms for deoxyribonucleic acid (DNA), adenosine triphosphate (ATP) and protein. The organisms die, the phosphorus return into the soil through decomposition by the microorganisms. It picked up by ground water or rainfall and swept into rivers and lakes. In accesses amount, it damage aquatic life by excessive growth of algae (algal bloom), reduced sunlight penetration, decrease in photosynthetic activity of other aquatic plants, dissolved oxygen content and increase in BOD and COD levels leading to eutrophication. Therefore, there is need to confirm the phosphate concentration in untreated and treated textile wastewater for environmental safety.

In this respect, TWW sample was taken and diluted to 100 mL with distilled water and added 4.0 mL molybdate reagent I and 0.5 mL (10 drops) stannous chloride reagent I. After 10 min, but before 12 min, measured color photometrically at 690 nm and compared with a calibration curve using distilled water blank. The concentration of phosphate in TWW was calculated by the following equation:

Phosphate (mg/L) = P (104.5 mL final volume) / Volume of sample (mL) × 1000

4.1.3.13. Nitrate

Nitrate is present in water or wastewaters by agricultural, domestic and industrial activities. It is highly toxic to public health and environment. It damage aquatic life by excessive growth of algae (algal bloom), reduced sunlight penetration, decrease in photosynthetic activity of other aquatic plants, dissolved oxygen content and increase in BOD and COD levels leading to eutrophication. The U.S. Public Health Service (USPHS) has designated the safe limit of nitrogen in nitrates to be 10 mg/L. Nitrates in drinking water are particularly dangerous to small children, infants and fetuses. Therefore, there is need to confirm the nitrate concentration in untreated and treated textile wastewater for environmental safety.

For determination of nitrate, TWW sample was taken in flask and added 1.0 mL of sulfanilic acid and mixed thoroughly. In a dry 10 mL graduated cylinder, measure 1.0 mL of Zn/NaCl and swirl the flask for 7 min. Filtered with a vacuum flask after seven minutes and rinse the flask well with DW and pour water sample back into the flask, color development: added 1.0 mL of naphthylethylenediamine reagent to the filtered sample and mixed. Properly, added 1.0 mL of 2M sodium acetate solution and mixed. Properly, allow 5 min for color development, spectrophotometric Measurement: measure the color intensity with the spectrophotometer set at 550 nm.

4.1.4. Heavy metals analysis of collected textile wastewater

The analysis of heavy metals in untreated and treated TWW were done by nitric-perchloric acid digestion method as per the standard protocols of APHA (2012). Briefly, 10 mL of digestion solution (HNO_3 + $HClO_4$) was added in 20 mL of filtered (Whatman) TWW. The samples were kept for digestion at 85 ºC to oxidize the oxidizable matter until the dense white fumes comes out followed by the formation of white precipitates, an indication of complete digestion. After cooling, the samples were dissolved in 10 mL of double distilled water, filtered through Whatman no. 42 filter paper and transferred to a volumetric flask. The obtained samples were dissolved in double distilled water and used for heavy metals analysis. The heavy metals analysis was done by the inductively coupled plasma spectrophotometer (Thermo Electron; Model IRIS Intrepid II XDL, USA). The concentration of heavy metals in TWW collected from Handloom Bhandar was calculated by the following equation:

Heavy metals (mg/L) = (Observed concentration - Blank) × Dilution factor

4.1.5. Statistical analysis

All the experiments were carried out in triplicates to minimize the experimental errors and the results obtained from each set of experiments were expressed as mean and standard deviation.

4.2. Results and discussion

In many developing countries, TIs discharge a large volume of dark-colored highly polluted wastewater into the environment (Kishor et al., 2021a). Therefore, the quality of real untreated and bacteria treated TWW is very important for the disposal point of view. In present work, the untreated TWW showed dark color (ADMI 1354) with high values of pH (9.56), temperature (39 ºC), EC (6.36 us/m), COD (1746 mg/L), BOD (699 mg/L), TOC (3801 mg/L), TDS (7203 mg/L), TSS (501 mg/L), TS (7101 mg/L), phenol (2.27 mg/L), nitrogen (11.13 mg/L), surfactant (9.80 mg/L), chloride (1731 mg/L), sulphate (1605 mg/L) and phosphate (9.33 mg/L). It also showed high values of Cr (1.70 mg/L), Ni (4.23 mg/L), Cd (1.10 mg/L), As (2.55 mg/L), Fe (3.15 mg/L) and Pb (0.31 mg/L). All the values of various physicochemical parameters are found to be higher than permissible limit set by the Central Pollution Control Board 2013 (CPCB 2013) and the National Environment Quality Standard (Hussain et al., 2019).

Table 4.1. Physico-chemical characteristics of textile wastewater before and after bacterial treatment.

Physico-chemical characteristics	Untreated TWW	Bacteria treated TWW	Removal (%)	CPCB (2013)
Temperature (°C)	39 ± 2.64	30 ± 1.56	----	40 ⁰C
pH	9.56 ± 0.30	7.13 ± 0.25	----	5.5-9
Color (ADMI)	1354 ± 35.6	15 ± 3.02	99	NG
EC (us/m)	6.36 ± 1.00	1.03 ± 0.40	83.80	0.85
COD (mg/L)	1746 ± 11.01	196 ± 6.24	88.77	250
BOD (mg/L)	699 ± 8.50	42 ± 5.56	93.99	30
TOC (mg/L)	3801 ± 44.61	1044 ± 12.01	72.53	NG
TS (mg/L)	7101 ± 16.80	1996 ± 8.62	71.89	NG
TSS (mg/L)	501 ± 21.82	118 ± 15.53	76.44	100
TDS (mg/L)	7203 ± 17.34	2027 ± 18.68	71.85	2100
Surfactant	9.80 ± 0.65	1.09 ± 0.15	88.88	NG
Phenol (mg/L)	2.27 ± 0.33	0.32 ± 0.02	85.90	1.0
Chloride (mg/L)	1731 ± 22.03	1128 ± 17.05	34.83	250
Phosphate (mg/L)	9.33 ± 0.55	4.96 ± 0.51	46.83	5.0
Nitrogen (mg/L)	11.13 ± 1.07	3.83 ± 0.40	65.58	10
Sulphate (mg/L)	1605 ± 25.94	946 ± 10.59	41.05	1000
Chromium (Cr) (mg/L)	1.70 ± 0.35	0.47 ± 0.09	72.35	2.0
Lead (Pb) (mg/L)	0.31 ± 0.15	0.05 ± 0.01	83.87	0.1
Cadmium (Cd) (mg/L)	1.10 ± 0.27	0.23 ± 0.02	79.09	2.0
Iron (Fe) (mg/L)	3.15 ± 0.27	2.12 ± 0.19	32.69	3.0
Arsenic (As) (mg/L)	2.55 ± 0.39	0.87 ± 0.05	65.88	0.2
Nickel (Ni) (mg/L)	4.23 ± 0.77	0.71 ± 0.08	83.21	3.0

EC: Electrical conductivity; TOC: Total organic carbon; BOD: Biochemical oxygen demand; COD: Chemical oxygen demand; TS: total solids; TDS: total dissolved solids; TSS: Total suspended solids. All the values are mean of three replicates ± SD.

The high color content in TWW might be due to the use of different RCPs/dyes at dyeing and printing stages in TIs (Kaur et al., 2018). The high pH and EC might be due to the use of salts, detergents and soaps in scouring and mercerization stages. The high values of COD, BOD and TOC might be due to the presence of various recalcitrant coloring pollutants (RCPs), organic compounds and dissolved minerals (Kurade et al., 2012). The high TDS, TSS and TS values are probably caused by the presence of dissolved organic and inorganic substances. The phenol in TWW might be due to the use of phenolic compounds (Pentachlorophenol and phthalates) in TIs (Bilinska et al., 2019). The chloride, nitrogen and sulphate could be attributed to the use of chlorobenzenes, sodium chloride, trichloroethane, hydrochloric acid and sodium sulphate and carbon disulphide (Khandare and Govindwar, 2015). The phosphate might be due to the presence of organophosphorus compounds (tributylphosphate). In addition, the high concentration of different heavy metals might be associated with the various RCPs and additive compounds (GilPavas et al., 2018).

If TWW is discharged without adequate treatment, it may cause serious effects on environment (water/soil) and public health. For example, the color normally reduced sunlight penetration, photosynthetic activity and give intensive coloration, leading to eutrophication and perturbations (Kishor et al., 2021b). The high values of BOD, COD and TOC pose severe threats in flora and fauna due to decrease in dissolved oxygen content (DOC) of receiving aquatic resources (Kumar et al., 2019). The TDS, TSS and TS cause water and soil pollution due to the alteration in ion composition and salinity. The phenolic compounds are well reported to be carcinogenic, endocrine disrupters and impair fertility in living organisms (Bharagava et al., 2018). The nitrate, iron, chloride, sulphate and phosphate damage the aquatic life (plants, animals and microbial diversity) due to the eutrophication process (Cao et al., 2019). The Cr, Cd, As and Pb are well reported to cause high toxicity, genotoxic and carcinogenic threats in animals and humans (Kaur et al., 2018). However, the treatment of TWW is urgently needed for the safety of public health and environment.

But after bacterial treatment, the quality of TWW improved significantly with 99%, 83.80%, 88.77%, 93.99%, 72.53%, 71.85%, 76.44%, 71.89% 85.90%, 65.58%, 34.83%, 41.05% and 46.83% reduction in color (ADMI), EC, COD, BOD, TOC, TDS, TSS, TS, phenol, nitrate, chloride, sulphate and phosphate, respectively. The developed bacterial consortium also removed Cr (72.35%), Cd (79.09%), Ni (83.21%), As (65.88%), Fe (32.69%) and Pb (83.87%). The dark-color turns into the colorless/transparent due to the degradation of RCPs by developed bacterial consortium. The reduction in EC, COD, BOD, TOC, TDS, TSS, TS, phenol, nitrate, chloride, sulphate and phosphate from TWW might be due to the utilization of pollutants by bacteria as carbon and nitrogen source for their growth and metabolic activity (Bilinska et al., 2019).

The reduction in Cr, Zn, Cd, Ni, As and Pb might be associated with the bioaccumulation inside the bacterial cells or binding with the extracellular (lipopolysaccharide) membrane (Kishor et al., 2021b). Similarly, Chandanshive et al.

(2017) reported 94% of color, 76% of COD, 70% of BOD, 75% of TDS and 47% of TSS reduction from the real textile wastewater by a consortium-TP after 96h. *Bacillus cohnni* was also found capable to remove 77% of COD, 86% of BOD, 66% of TDS, 60% of TSS, 67% of TOC, 66% of TS, 68% of sulphate and 20% of phenol from real TIWW within 48h (Kishor et al., 2021b). These results indicated that RKS-TEX-267 can be used effectively in treatment of textile wastewater pollutants.

4.3. Conclusion

The TWW is highly toxic to environment and public health. In present work, the various physico-chemical parameters of TWW collected from a Handloom Bhandar situated at Unnao, U.P., India was analyzed to know the pollution profile. For untreated TWW, the recorded values of all physico-chemical parameters were found to be beyond the recommended standard discharged limits for industrial wastewaters. Therefore, TWW is not suitable to discharge into the environment because it may cause serious threats to environment and living beings. However, its adequate treatment is required for the environment and public health protection.

But after bacterial treatment, TWW showed an appreciable reduction in all the pollution parameters. The reduction in values of all pollution parameters from TWW might be due to the utilization of pollutants as a carbon and nitrogen source by bacterial strains for their growth and metabolism. Therefore, the newly developed bacterial consortium RKS-TEX267 showed high potential for the treatment of textile wastewater pollutants.

Chapter 5

Isolation, screening and characterization of bacteria capable for the degradation of recalcitrant colouring pollutants (RCPs) in textile wastewater

Isolation, screening and characterization of bacteria capable for the degradation of recalcitrant colouring pollutants (RCPs) in textile wastewater

5. Introduction

Textile industries (TIs) play a major role by contributing global economy in many developing countries (Bilinska et al., 2019). But unfortunately, these are also the major sources of environmental pollution as these produces a large volume of highly toxic wastewater having dark color, high pH, BOD, COD, TOC, TDS, TSS, sulphate, nitrate, phosphate and phenols (Kishor et al., 2021b). It also contains a variety of recalcitrant coloring pollutants (RCPs) and toxic metal ions, which cause serious threats to environment and living beings (Bilinska et al., 2019; Kishor et al., 2021b). TWW reduces sunlight penetration in aquatic bodies, which in turn decreases photosynthetic activity and dissolved oxygen concentration, thus ultimately damaging the aquatic life (flora/fauna) (Kumar et al., 2019). It causes negative impact on soil property and microbial diversity by bioaccumulation of RCPs and metal ions (Noman et al., 2020). RCPs are also toxic to animals and plants (Kishor et al., 2021d). Moreover, it has been also reported to cause a variety of severe toxic effects in living beings (Bilinska et al., 2019). Therefore, it becomes necessary to adequately treat and detoxify the RCPs from TWW to protect the environment and public health.

Several physico-chemical and advanced oxidation processes (AOPs) are reported to treat TWW. But, these are not suitable due to high expensive, environmental destruction as well as cause secondary pollution (sludge) (Bilinska et al., 2019; Kishor et al., 2021a). Currently, microbial treatment seems to be an alternative approach to effectively degrade/detoxify the RCPs in TWW. For example, several microbial strains such as *Pseudomonas, Sphingomonas, Caulobactor, Aeromonas, Serratia, Pigmentiphaga, Bacillus* and *Shewanella* have been reported effective in treatment of RCPs and industrial wastewaters (Kumar et al., 2019; Cao et al., 2019; Kishor et al., 2021b). Microorganisms can degrade, decolorize, detoxify and mineralize various pollutants by using different metabolic pathways (Cao et al., 2019; Kishor et al., 2021b). In addition, search for the pollutants degrading/detoxifying novel microbes could pave the way towards the sustainable treatment and management of industrial wastewaters. Therefore, the present study was aimed to isolate, screen and characterize the potential bacterial strains capable for the degradation of RCPs from TWW for environmental safety.

5.1. Material and methods

5.1.1. Chemicals

Methylene blue (MB), methyl orange (MO), congo red (CR), phenol red (PR) and guaiacol were procured from Sigma-Aldrich, USA. Nutrient agar (NA) medium (g/L, HM peptone B # 1.5; yeast extract 1.5; sodium chloride 5.0; peptone 5.0; agar 15.0) was used for the isolation, screening and characterization of potential bacterial strains capable for the degradation of TWW. Whatman® filter papers 1.2 µm (Whatman, England, UK) and membrane filter 0.22 µm (Millipore Ltd, Bedford., MA) were used to filter TWW and dyes. All the chemicals are of the highest purify and analytical grade.

5.1.2. Isolation and purification of bacterial strains from TWW and sludge samples

TWW and sludge samples were used for the isolation of bacterial strains for efficient degradation of RCPs from TWW (Bharagava et al., 2018; Kishor et al., 2021b). Briefly, 5.0 gm of sludge and 10 mL of TWW samples were added in 85 mL of nutrient broth (NB) amended with 100 mg/L of MB, MO, CR and PR dye and placed in incubator shaker (LI-BODS-10, LABARD) under 100 rpm at 30±2 °C for 15 days. Afterward, 15 mL (v/v) of acclimatized culture was collected and added in 85 mL of fresh NB and incubated at 100 rpm in incubator shaker at 30±2 ºC. After 4 days, 1.0 mL (v/v) sample was serially diluted and 40 µL of this diluted suspension was spread on NA plates. The plates were placed at 30±2 ºC in BOD incubator. After two days, the morphologically different bacterial colonies developed on NA plates were selected, picked up and purified by the repeated streak plate technique.

5.1.3. Screening of potential bacterial strains for the degradation of RCPs

For the effective degradation of RCPs, all the bacterial isolates were screened for laccase, lignin peroxidase (LiP) and manganese peroxidase (MnP) enzyme activity and MO, CR and MB dye decolorization (Kishor et al., 2021b). For enzyme activity, guaiacol, MB and PR dye were used as an indicator for laccase, LiP and MnP enzyme’s activity, respectively (Buntic et al., 2017). Briefly, a loopful culture of isolated bacterial strains (RKS1-RKS8) were streaked on 50 µL (1M) of guaiacol and 100 mg/L of MB and PR amended NA plates and incubated at 30±2 ºC. After five days, only six bacterial strains (RKS2, RKS3, RKS4, RKS6, RKS7 and RKS8) showed a clear decolorization zone around the bacterial colonies on NA plates.

MnP, LiP and laccase enzyme-producing bacterial strains were further screened on the basis of MB, MO and CR dye decolorization efficiency. Briefly, a loopful culture of all isolated strains were inoculated in 10 mL NB broth and placed at 30± ºC under 100 rpm in BOD incubator shaker. After 24h, 1.0 mL (v/v) culture was transferred in 100 mg/L of MB, MO and CR dye-containing NB broth (pH 7.0) and incubated at 30±2 ºC, 100 rpm and static conditions in a BOD incubator shaker. After 24h, 4 mL (v/v) aliquot was taken from flaks and centrifuged (8,000 × g at 4 ºC for 10 min) in a refrigerated centrifuge (REMI Instruments Pvt. Ltd., India) to separate the biomass. The supernatant was used to measured decolorization of MB, MO and CR at λ_{max} = 668, 464 and 497 nm by double-beam UV-VIS spectrophotometer (Labtronics, Pvt. Ltd., India). NB broth containing MO, CR and MB dye without bacterial culture was considered as control. The % decolorization was calculated and maximum dye decolorizing bacterial isolates were selected for further studies.

$$\text{Decolorization (\%)} = \frac{\text{Absorbance (R1)} - \text{Absorbance (R2)}}{\text{Absorbance (R1)}} \times 100$$

Where R_1 is initial absorbance, R_2 is final absorbance of MB, MO and CR dye decolorization.

5.1.4. Characterization and identification of potential bacterial isolates

Based on the enzymatic activity and dye decolorization efficiency, only three potential bacterial strains (RKS2, RKS6 & RKS7) were selected. The isolated bacterial strains were further characterized by cellular morphology and biochemical tests. Bacterial colonies were grown on NA plates and studied based on their color, shape and size etc.

5.1.4.1. Morphological characterization

The cellular morphology of isolated bacterial strains (RKS2, RKS6 & RKS7) was confirmed by the Grams staining test (Aaneja, 2007).

Gram Staining

Principle

Gram staining (GS) was discovered by Danish Bacteriologist Hans Christian Gram in 1884. GS is a basic technique used to classify the bacteria into gram-positive and gram-negative. The bacterial smear is stained with primary stain (crystal violet) and fixed by mordant stain (iodine) to increase the binding capacity of the primary stain. Alcohol or acetone is used as a decoloring agent to remove primary stain from cells, then safranin (counterstain) is used to provide color contrast in cells. The gram-positive bacterial cell wall is a thick layer made of peptidoglycan (protein-sugar complexes (50-90%)) and lipid content (10%). The decoloring agent causes dehydration and shrinks of the thick cell wall, thus closing the pores of the cell wall and preventing the stain from exiting the cells. As a result stains appeared blue or purple in color whereas gram-negative bacteria have a thin layer of peptidoglycan (10%) and high lipid content. The CV-iodine complex is washed from the cell wall due to the dissolved lipid of cell wall by decolorizing agents and the stain appeared as red or pink in color.

Reagents and chemicals

Ammonium oxalate-crystal violet stain

Solution A

Crystal violet : 10 gm
Ethanol (95%) : 100 mL

Mixed and dissolved

Solution B

Ammonium oxalate : 1 gm
Distilled water : 100 mL

For use, mixed 2 mL of solution A and 80 mL of solution B

Solution A + Solution B = Crystal violet

Lugol's iodine

Iodine : 5 gm
Potassium iodide (KI) : 10 gm
Distilled water : 100 mL

Dissolved the iodide and iodine in some of the water and adjusted to 100 mL with distilled water.

Ethyl alcohol 95%

95 ml ethyl alcohol + 5 ml distilled water

Safranin

Safranin : 2.5 gm
Ethyl alcohol 95% : 10 mL

These were added in 100 ml distilled water

Procedure

A loopful culture of overnight grown bacterial strains was taken and a thin smear on the clean slides was made. The slides were air dried and fixed. Afterward, the slides were poured with crystal violet stain and kept for one min and then washed with distilled water. Now, iodine solution was applied for half min and after then the iodine solution was drained off. The smear was decolorized with a few drops of acetone (10-15 sec) and washed thoroughly with water. The slides were counterstained with safranin for a half min, washed again and stand slide on end to drain or blot dry and then observed under microscope.

Interpretation

Blue or Violet color: **Gram-positive**

Red or Pink color: **Gram-negative**

5.1.4.2. Biochemical characterization

The biochemical characterization of isolated bacterial strains were performed based on the various biochemical tests such as catalase, citrate utilization, starch hydrolysis, MR-VP test, motility test, indole production and urease test as per standard procedures of Bergey's Manual of Determinative Bacteriology (Whitman et al. 2012).

Catalase test

Principle

Catalase test is a basic tool used to differentiate catalase and non-catalase enzyme-producing bacteria. Hydrogen peroxide (H_2O_2) is lethal to cells. Several bacteria can produce catalase enzymes, which break down the H_2O_2 into O_2 (bubbles) and H_2O and non-catalase-producing bacteria not/less produce bubbles. The bacteria thereby protect themselves from the lethal effect of H_2O_2, which is used as an end product of aerobic metabolism.

$$H_2O_2 \xrightarrow{\text{Catalase}} H_2O + O_2 \text{ (Bubbles)}$$

Procedure

A loopful culture of isolated bacterial strains was collected and formed a smear on the clean slide. Afterward, 0.5 mL of H_2O_2 (3%) was dropped on the bacterial smear and observed for the production of bubbles (O_2) within a few seconds.

Interpretation

Observed gas bubbles (O_2): **catalase positive**

Gas bubbles (O_2) not observed: **catalase negative**

Citrate utilization test

Principle

The citrate media was discovered by Koser in 1920s. It is used to differentiate the *Escherichia coli* (Enterobacteriaceae) and *Enterobacter* (aerogenes) based on the utilization of ammonium salt as a nitrogen source and citrate as a carbon source. Later on, Simmon modified this medium by adding bromothymol blue and agar. Microbial strains use sodium citrate and ammonium dihydrogen phosphate as a carbon and nitrogen sources. The utilization of these salts causes the medium to become alkaline, thus the color of medium changes from green to blue.

Procedure

Simmon's citrate agar media was prepared, sterilized by autoclaving at 121 °C for 15 minutes. Afterward, the media was poured into test tubes and allowed to solidify in slanting position. One loopful culture of isolated bacterial strains were streaked on test tube and incubated at 30± ºC. After 48h, the color of the medium was changed from green to blue indicating that the test is positive and if remains green, then test is negative**.**

Interpretation

The blue color of media***:* Citrate utilized**

The original green color of medium: **Citrate not utilized**

Urease test

Principle

The microbial strains were streaked on a urea agar medium. If stains produce urease enzyme, which break downs urea into NH_3 and CO_2 by hydrolysis. The medium becomes alkaline as shown by change in color of the indicator to red-pink.

$$(NH_2)_2CO + H_2O \longrightarrow CO_2 + 2NH_3$$

Procedure

Urea agar media was prepared, sterilized by autoclave at 121 ^{0}C for 15 minutes. Afterward, the media was poured into test tubes and allowed to solidify in slanting position. One loopful culture of isolated bacterial strains were streaked on the slant in test tube and incubated at 30±2 ºC. After 48h, the color of the medium was changed from oraganish yellow to pink indicating the test is positive and if remains oraganish yellow, the test is negative.

Interpretation

Pink color of media: **Urea Utilized**

Oraganish yellow color of medium**: Urea not utilized**

Starch hydrolysis test

In this test, the bacterial strains are grown on the starch agar medium. If bacteria can hydrolyze starch, they showed a clear zone around bacterial growth on addition of iodine solution while the strains not hydrolyzing starch, not show clear zone around the bacterial growth.

Procedure

A loopful culture of isolated bacterial strains were collected and made a single streak inoculation on starch agar plates. The plates were incubated at 30±2 ºC for 48h. Afterward, the iodine solution was flooded on the surface of plates for 30 seconds. If a clear zone was observed around the bacterial growth, it indicated that the bacteria were able to hydrolyze starch and if a clear zone around the bacterial growth was not observed, it indicated that bacteria were not able to hydrolyze starch.

Interpretation

The clear zone developed around the bacterial growth: **Starch Utilized**

The clear zone not developed around the bacterial growth: **Starch not utilized**

Indole test

Indole test is a type of IMViC test used to determine the ability of bacteria to convert tryptophan into Indole. It might be due to the production of tryptophanase enzyme.

Procedure

Tryptone broth was prepared, sterilized in autoclave at 121 ºC for 20 min and poured into test tubes. One loopful culture of isolated bacterial strains were inoculated in tryptone broth and incubated at 30±2 ºC for 48h. Afterward, 1 mL of Kovacs reagent were added and tubes were shaken gently from 10-15 min. The tubes were allowed to stand for a few min to permit the reagent to the top and observed cherry color layer in the top layer.

Interpretation

The developed cherry color layer in top layer: **Positive test**

The no color change**: Negative test**

Methyl red (MR) and Voges Proskauer (VP) test

MR and VP tests are used to differentiate facultative anaerobic enteric bacteria that produce strong acid (acetic acid) and neutral product (acetoin) as end product. In MR test, the microbial strains were inoculated in glucose-containing broth, if microbes can convert glucose into stable acid and thus, the color of medium changes from yellow to red. In VP test, the microbial strains were inoculated in glucose-containing broth, if the microbes have ability to convert glucose into neutral product (acetoin) as an end product and thus, color of medium changes from yellow to ruby pink (red).

Procedure

MR-VP broth media (pH 6.9) was prepared and sterilized in autoclaved at 121 ºC for 20 min. The selected bacterial strains were inoculated in test tubes and incubated at 30±2 ºC for 48h. In MR test, 5 drops of MR solution (0.02%) was added in test tubes and the observed change in color of medium. If the observed red color indicating positive test and observed yellowish color is indicating the negative test. In the VP test, 12 drops of VP reagent first (Naphthol) and 2-3 drops of VP reagent second (potassium hydroxide (40%)) were added in test tubes and shake the tubes gently for 30 seconds with the caps off to expose the media to oxygen and kept for 30 min. The developed crimson-to ruby pink (red) color is indication of positive test while no change in color is indicating negative test.

Interpretation

For the MR test

The developed red color: **Positive test**

The no color (yellow) change: **Negative test**

For the VP test

The developed ruby pink (red) color: **Positive test**

The no color (yellow) change: **Negative test**

Motility test

Motility test was utilized to differentiate the motile and non-motile bacteria. Semi-solid agar medium was used to confirm motility, which allowed motile bacteria to move readily through them causing cloudiness. The isolated bacterial strains were stabbed into the center of semi-solid agar and if the observed a diffuse zone of growth extending out from the line of stabbed streaking is indicating the positive test.

Procedure

The semisolid agar medium was prepared and sterilized in autoclaved at 121 ^{0}C for 20 min. Bacterial strains were stabbed to a depth in middle of the tubes and incubated at 30±2 ^{0}C for 4-7d. They observed a diffused zone around the line of inoculation. The diffused and hazy growth was observed, indicating positive response while if the observed sharply margins and clearly transparent, indicating the negative response.

Interpretation

The developed diffused and hazy zone: **Positive response**

The observed sharply margins and transparent: **Negative response**

5.1.4.3. Molecular characterization

The molecular identification of isolated bacterial strains were done by 16S rRNA gene sequence analysis. The genomic DNA was prepared form the overnight grown cultures according to method described by (Bharagava et al., 2018). Approx. 5 µL of template

DNA was taken for the amplification of 16S rRNA gene and universal primers 27F (5'-AGAGTTTGATCCTGGCTCACG-3') and 1492R (5'-TACGGTTACCTTGTTACGACTT-3') (Narde et al., 2004). 100 ng of DNA template, lX of PCR buffer, 3.0 mM of $MgCl_2$, 200 μM of dNTP each, 25 pmol of primer and 2.5 units of DNA polymerase template was added in reaction mixture followed by denaturation, annealing and extension at 94 ºC for 1 min, 45 ºC and 72 ºC (30 cycles), respectively.

A gel extraction kit (Merk Biosciences, Bangalore., India) was used to purify the PCR amplified products, which was further sequenced by Biokart India Pvt, Ltd, (India). The obtained nucleotide sequences were analyzed at National Centre for Biotechnological Information (NCBI, USA) server using BLAST software available online at https://blast.ncbi.nlm.nih.gov/Blast.cgi and the corresponding sequences were downloaded and aligned using the Clustal-X program. The phylogenetic tree was constructed by the neighbor-joining method using MEGA software (v7.0) online available at www.megasoftware.net. In addition, the 16S rRNA sequences were also submitted to the GenBank database to receive the accession numbers for the isolated bacterial strains.

5.2. Results and discussion

5.2.1. Characteristics of isolated bacterial strains

In present work, a total of eight morphologically distinct bacterial colonies (RKS1-RKS8) were isolated from TWW and sludge samples as shown in **Fig. 5.1**. All the isolated bacterial strains were selected, picked up and purified by repeated streaking method as shown in **Fig. 5.2**.

Further, all the isolated bacterial strains (RKS1-RKS8) were screened based on the LiP, MnP and laccase enzyme activity and CR, MB and MO dye decolorization efficiency. Out of eight bacterial strains, only five bacterial strains i.e. RKS2, RKS4, RKS6, RKS7 and RKS8 were found capable to produce a clear decolorization zone around the bacterial colonies on the guaiacol and dye amended plates within seven days **(Fig.5.3)**. The bacterial strains RKS2 and RKS4 were produced a clear white decolorization zone around colonies grown on MB dye amended NA plates. The strain RKS6 was produced a dark reddish decolorization zone around the colonies grown on guaiacol amended NA agar plate. The bacterial strains RKS7 and RKS8 were produced a clear yellow decolorization zone around colonies grown on PR dye amended NA plates. The production of dark reddish zone, white zone and yellow decolorization zone around colonies indicated the laccase, LiP and MnP enzyme activity **(Fig.5.3)** and were selected for further study.

In addition, these bacterial strains were further screened based on their CR, MO and MB dye decolorization efficiency and only three bacterial strains (RKS2, RKS6 & RKS7) showed the maximum dye decolorization efficiency. RKS2 showed 98% decolorization of MB dye, RKS6 showed 91% decolorization of CR dye and RKS7 showed 94% decolorization of MO dye and selected for further studies (**Fig. 5.4**).

5.2.2. Morphological characteristics

Based on the enzyme activity and dye decolorization efficiency, only three potential bacterial strains (RKS2, RKS6 & RKS7) were finally selected and identified on the basis of various morphological tests. The morphological characters examined in the present study were: Gram staining, shape, pigmentation, surface texture, margin, elevation and motility. The morphological characteristics of the isolated bacterial strains RKS2, RKS6 & RKS7 are listed in **Table 5.1**. The bacterium (RKS2) appeared as a white, circular and non-translucent colony on NA agar plates. It was a gram-positive, rod-shaped and non-motile bacterium. A bacterium (RKS6) appeared as milky white colonies on NA plate. It was a gram-positive, rod-shaped and non-motile bacterium. A bacterium (RKS7) appeared as milky white, concave and smooth colonies on NA plates. It was a gram-positive, motile and rod-shaped bacterium. The morhological characteristics of isolated bacterial strains are shown in **Fig. 5.5**.

5.2.3. Biochemical characteristics of bacterial strains

All the three potential bacterial strains i.e. RKS2, RKS6 & RKS7 were also characterized by biochemical tests. The biochemical characteristics of the isolated bacterial strains are listed in **Table 5.1**. The bacterium RKS13-2 showed positive reaction for catalase and VP test whereas negative for starch utilization, citrate utilization, indole production, H_2S production and urease test. RKS12 showed positive test for catalase, VP, starch utilization and citrate utilization and negative reaction for urease test, indole production and H_2S production. The bacterium RKS7 showed positive test for urease, citrate utilization, starch utilization, catalase and MR test and negative test for VP, indole production and H_2S production. The biochemical characteristics of isolated bacterial strains are shown in **Fig. 5.6 & 5.7**.

5.2.4. Molecular characteristics of isolated bacterial strains

The isolated bacterial strains were further characterized by 16S rRNA gene sequencing analysis. According to 16S rRNA gene sequencing analysis, the bacterial strains RKS2, RKS6 and RKS7 showed the highest similarity with *Bacillus Albus*, *Bacillus Megaterium* and *Bacillus Paramycoides*, respectively. The phylogenetic tree of RKS2, RKS6 and RKS7 were shown in **Fig. 5.8 & 5.9**. Thus, the isolated bacterial strains RKS2, RKS6 and RKS7 were identified as *B. Albus, B. Megaterium* and *B. Paramycoides* with accession numbers MW407057, OK001869 and OK001866, respectively.

Table 5.1. Morphological, biochemical and molecular characteristics of isolated bacterial strains.

Strains	Morphological characteristics	Biochemical characteristics	Molecular identification
RKS2	Milky white, circular, non-translucent, gram +ve, rod-shaped and non-motile bacterium.	Catalase (+ve), VP (+ve), starch utilization (-ve), citrate, utilization (-ve), urease test (-ve), indole production (-ve) MR (-ve) and H_2S production (-ve).	*Bacillus Albus* (MW407057)
RKS6	Concave, milky white, smooth, gram +ve, rod-shaped and non-motile bacterium.	Catalase (+ve), VP (+ve), starch utilization (+ve), citrate utilization (+ve), urease test (-ve), indole production (-ve), MR (-ve) and H_2S production (-ve).	*Bacillus Megaterium* (OK001869) *Bacillus*
RKS7	Milky white, concave, smooth, gram +ve, motile and rod-shaped bacterium.	Catalase (+ve), VP (-ve), starch utilization (+ve), citrate utilization (+ve), urease test (+ve), indole production (-ve), MR (+ve) and H_2S production (-ve).	*Bacillus Paramycoides* (OK001866)

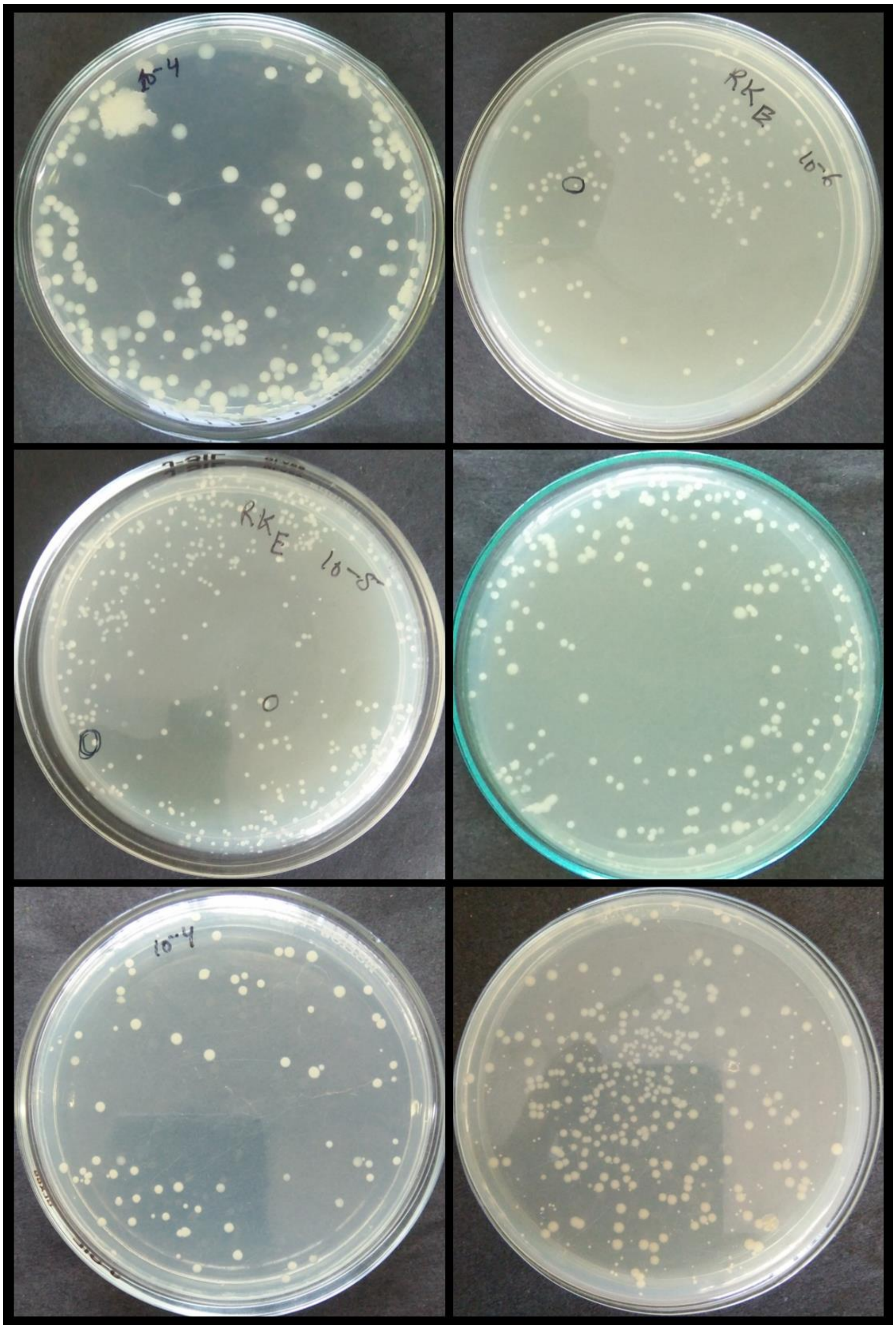


Fig. 5.1: Isolated bacterial colonies on nutrient agar plates from textile wastewater and sludge samples.

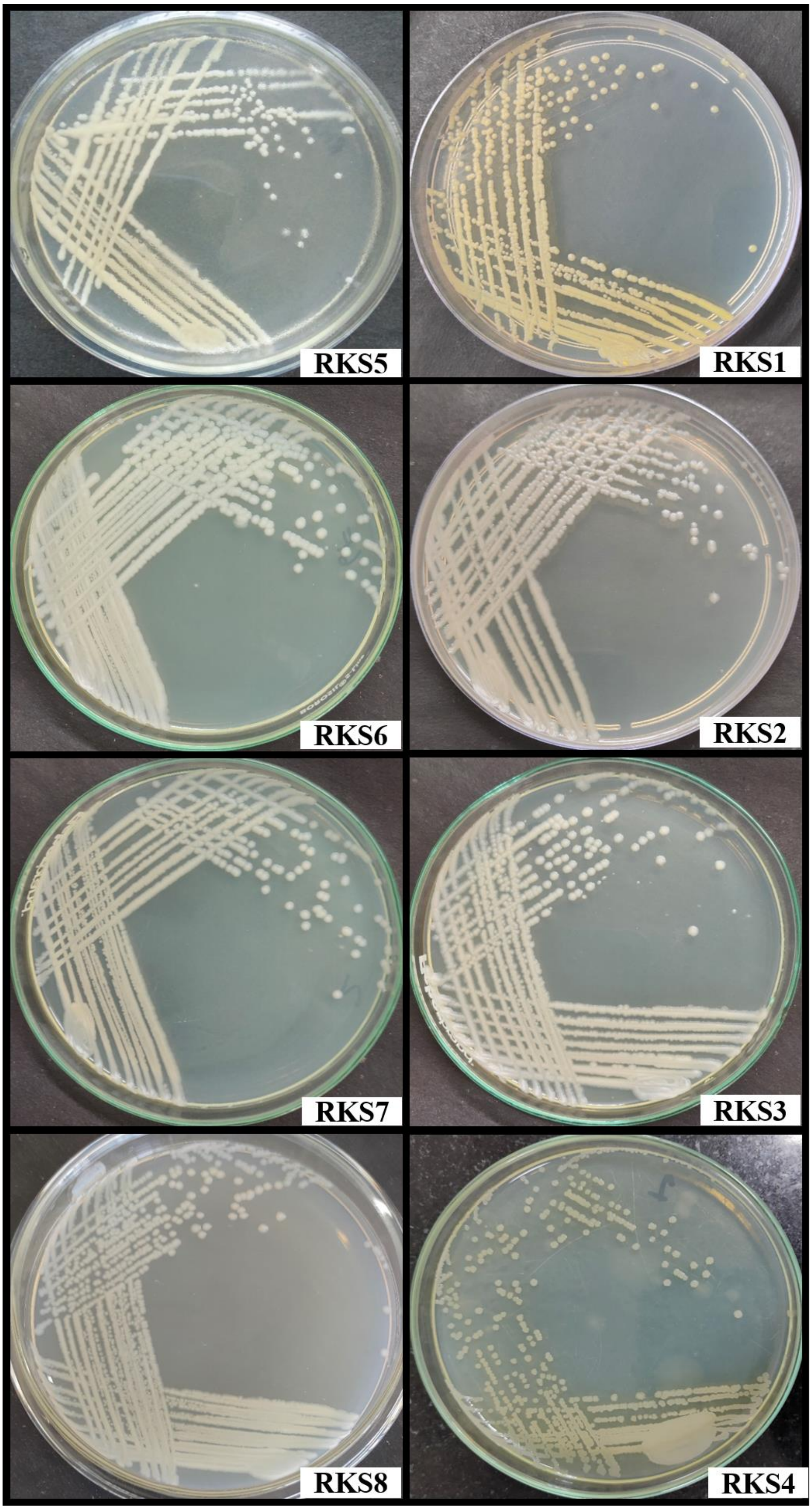


Fig. 5.2: Purified isolated bacterial colonies (RKS1-RKS8) on nutrient agar plates.

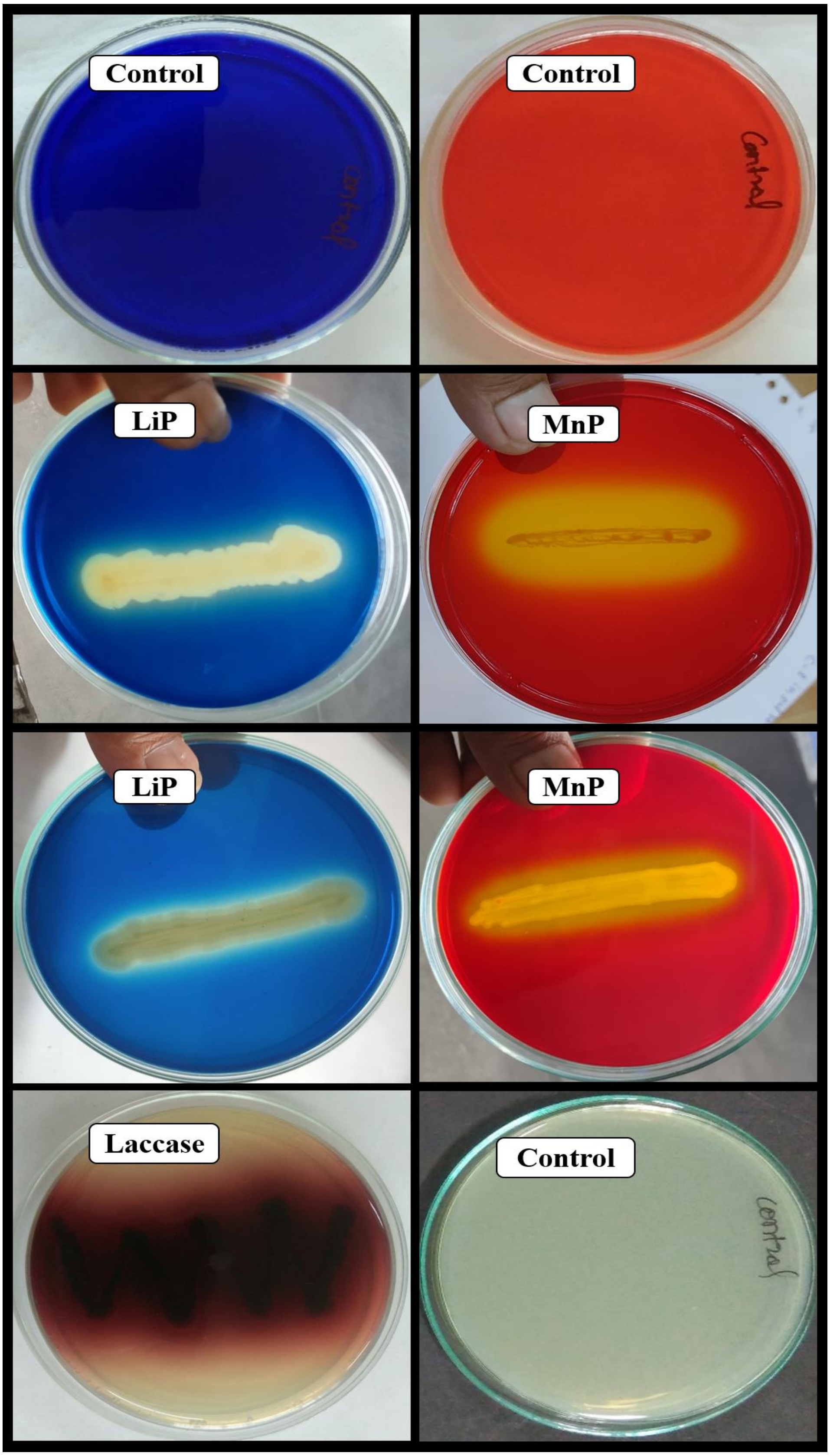


Fig. 5.3: Maganese peroxidase (MnP = RKS7 and RKS8), lignin peroxidase (LiP = RKS2 and RKS4) and laccase (RKS6) enzyme activity of isolated bacterial strains.

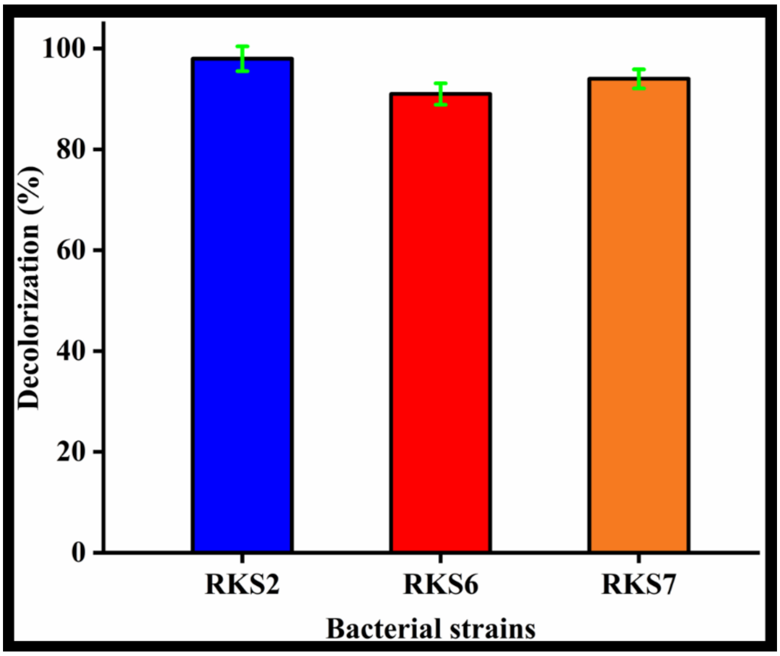


Fig. 5.4: Dyes (methylene blue, congo red & methyl orange dye) decolorization efficiency of isolated bacterial (RKS2, RKS6 & RKS7) strains.

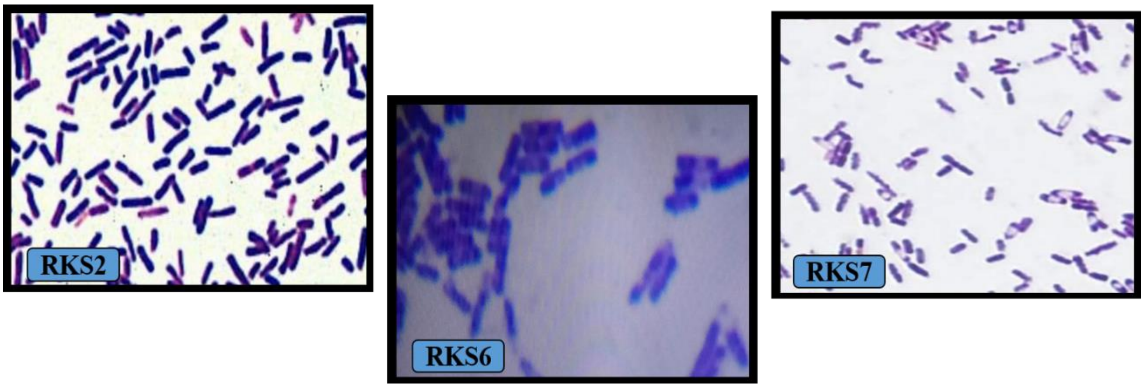


Fig. 5.5: Gram staining of isolated bacterial (RKS2, RKS6 & RKS7) strains.

Fig. 5.6: Catalase test of isolated bacterial (RKS2, RKS6 & RKS7) strains.

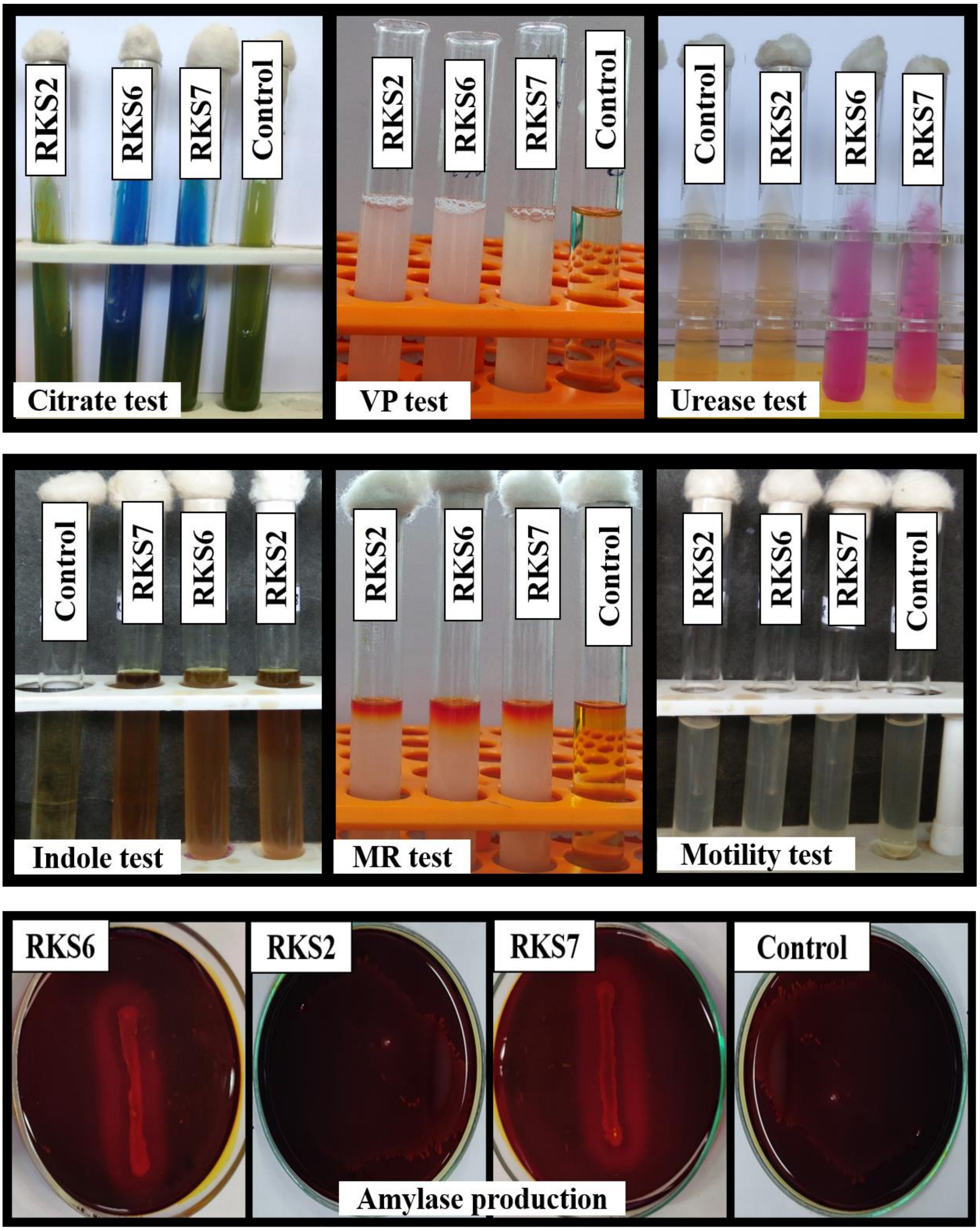


Fig. 5.7: Biochemical characteristics of isolated bacterial (RKS2, RKS6 & RKS7) strains.

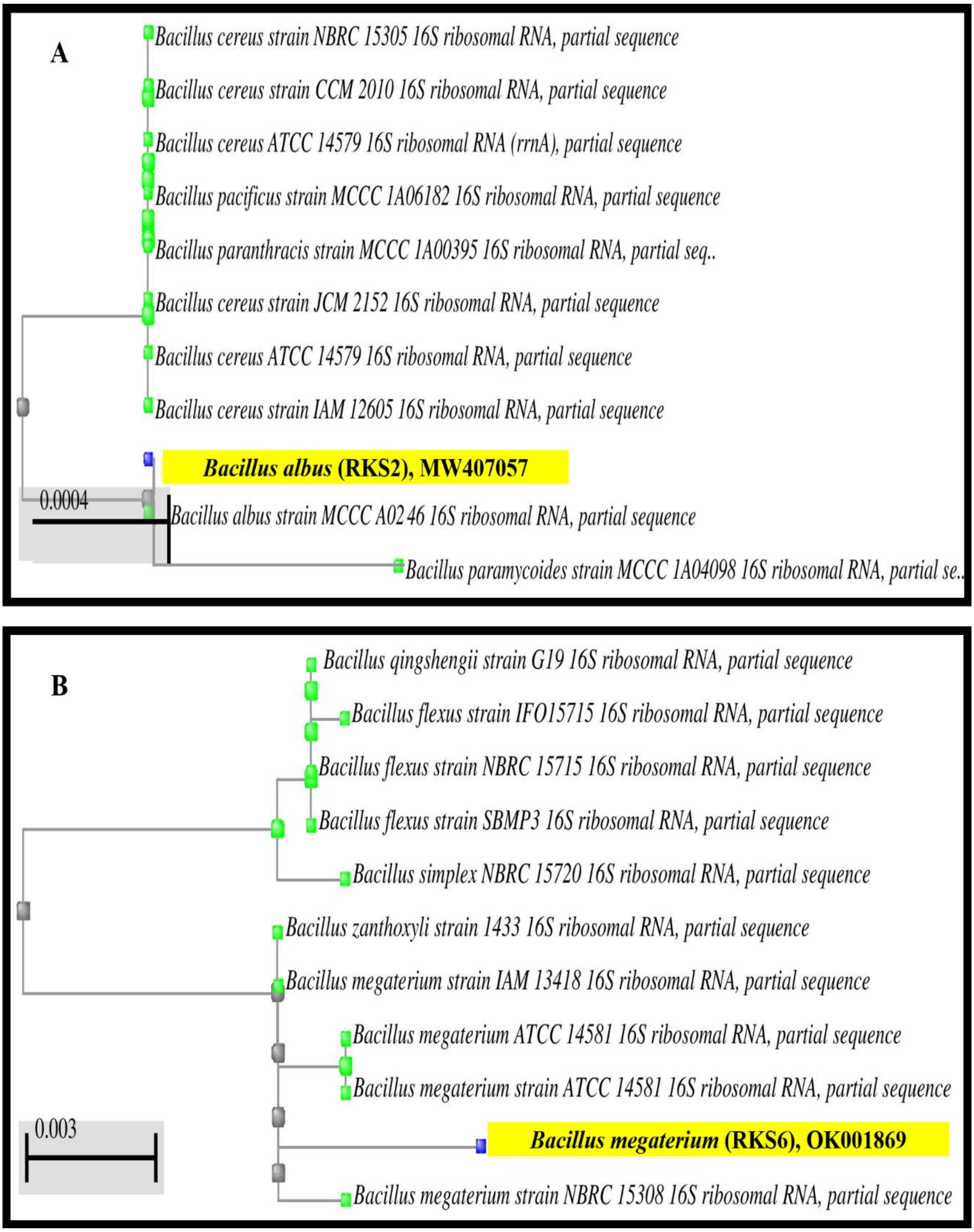


Fig. 5.8: Phylogenetic tree showing the relationship of isolated bacterial strains, *Bacillus albus* RKS2 (A) and *Bacillus megaterium* RKS6 (B) with its neighboring bacterial species.

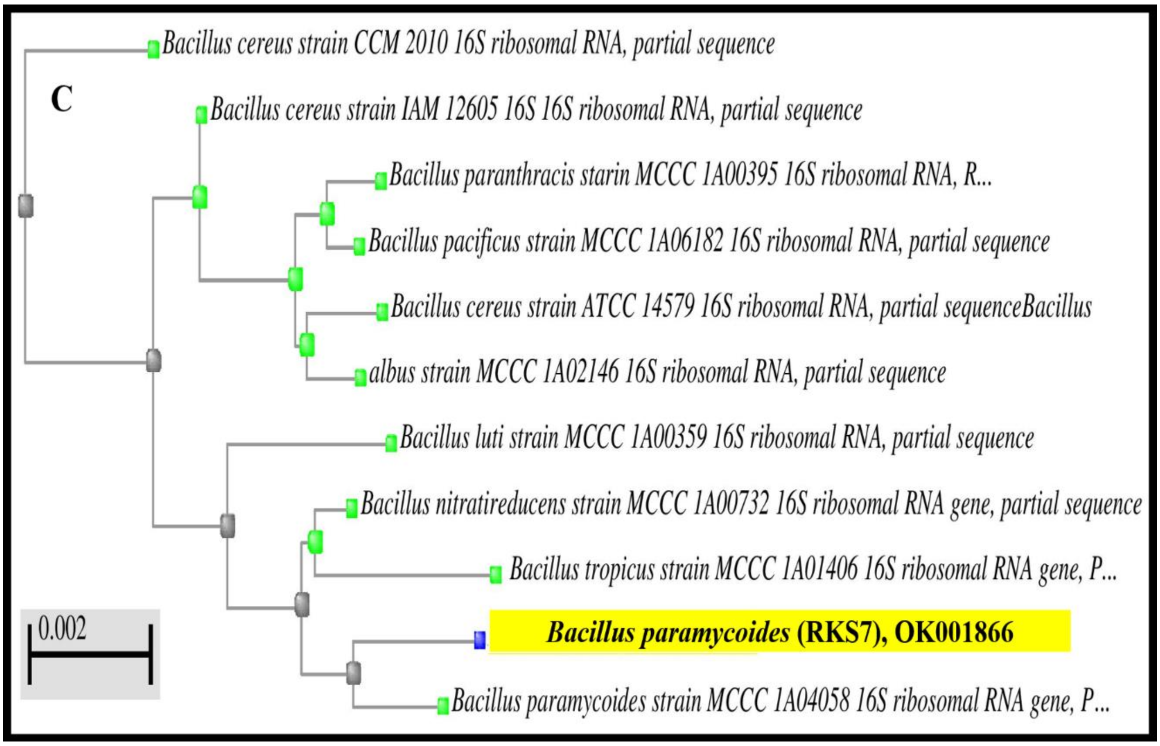


Fig. 5.9: Phylogenetic tree showing the relationship of isolated bacterial strains, *Bacillus paramycoides* RKS7 (C) with its neighboring bacterial species.

.

5.3. Conclusion

The present study was aimed to isolate, screen and characterize bacterial strains capable for the degradation of RCPs in TWW. In present study, a total of eight (08) bacterial strains were isolated from collected TWW and sludge samples and purified by the repeated streaking method. These bacterial strains (RKS1-RKS8) were screened for the ability of enzyme activity and dyes decolorization efficiency. Out of eight (08) bacterial strains, only five bacterial strains i.e. RKS2, RKS4, RKS6, RKS7 & RKS8 showed LiP, MnP and laccase enzyme activity. Further, only three bacterial strains showed maximum dyes decolorization efficiency such as RKS2 showed 98% decolorization of MB dye, RKS6 showed 91% decolorization of CR dye and RKS7 showed 94% decolorization of MO dye. In addition, these bacterial strains were also identified as *Bacillus Albus* (RKS2), *Bacillus Megaterium* (RKS6) and *Bacilus Paramycoides* (RKS7), respectively on the basis of 16S rRNA gene sequence analysis. Overall, on the basis of bioremediation potential, these bacteria can be used either alone or in combination for the degradation and detoxification of RCPs from TWW.

Chapter 6

Development and optimization of a potential bacterial consortium for the effective degradation of recalcitrant colouring pollutants (RCPs) in textile wastewater

Development and optimization of a potential bacterial consortium for the effective degradation of recalcitrant colouring pollutants (RCPs) in textile wastewater

6. Introduction

Textile wastewater (TWW) is well reported to causes severe threats on environment and living beings due to presence of various toxic RCPs, organic and inorganic pollutants (Kumar et al., 2019). TWW is characterized by high pH, dark color, COD, BOD, TOC, TSS and TDS, which extremely damage our natural resources (soil/water) worldwide. For this, various physical-chemical and advance oxidation processes (AOPs) are reported in treatment of industrial wastewaters (Bilinska et al., 2019). But, these methods are not suitable at large scale due to their high cost, incomplete mineralization and generation of various highly toxic intermediates (Zhuang et al., 2019).

Therefore, there is a need to replace various physical-chemical and AOPs by the cost effective, eco-friendly and effective treatment methods to efficiently remediate the TWW. Biological treatment (BT) is an eco-friendly, cost-effective and efficient method, which used metabolic and enzymatic ability of microbes and plants to degrade TWW (Kishor et al., 2021b). There are various studies reported on the microbial degradation of RCPs from TWW (Haq et al., 2018; Cao et al., 2019; Kishor et al., 2021b). However, there is a scarcity of literature available on the use of a microbial consortium, especially bacterial consortia for the treatment of RCPs from TWW. TWW is very complex in nature and contains a mixture of RCPs, organic compounds and metal ions and thus, single/pure culture of any biological agents are not efficient to remediate TWW.

However, the consortium treatment is more suitable and effective tool to degrade RCPs than pure culture (Kurade et al., 2012). The consortium treatment has high potential to remediate mixture of RCPs, organic and inorganic compounds from TWW due to the synergistic role of bacterial populations and their catabolic enzymes (Kurade et al. 2012). Bacteria play a major role in the degradation and decolorization of RCPs from TWW (Kishor et al., 2021b). Therefore, it requires some specific conditions that favor the optimum growth for the effective degradation of RCPs. Environmental and nutritional factors, as well as the size of inoculum and agitation rate, are the major process parameters in the microbial remediation (Buntic et al., 2017; Kishor et al., 2021b). These factors are directly or indirectly influence the microbial enzymatic and metabolic activities during the degradation of RCPs from TWW.

Therefore, the present study was aimed to develop a new bacterial consortium for the effective remediation of RCPs from TWW. Further, the effects of various environmental (pH and temperature) and nutritional (carbon and nitrogen sources) factor, as well as inoculum concentration and shaking/static condition on the degradation of RCPs from TWW by the newly developed bacterial consortium, was studied to achieve the better treatment efficiency.

6.1. Materials and methods

6.1.1. Chemicals and media's

All the chemicals are of the highest purify and analytical grade. Nutrient agar (NA) medium (g/L, yeast extract 1.0; NaCl 5.0; peptone 5.0; agar 15.0) was used for the development and optimization of bacterial consortium for the treatment of RCPs from TWW. The collected TWW sample was used for degradation studies. Whatman® Grade GF/C filter papers (pore size 1.2 μm) (Whatman, England, UK) were used for the filtration of TWW.

6.1.2. Development and optimization of a bacterial consortium

6.1.2.1. Biointeraction study of isolated bacterial strains

Based on the performance of monocultures (RKS2, RKS6 and RKS7) in degradation of RCPs, a potential bacterial consortium RKS-TEX267 was developed. For this, a biointeraction study was carried out before the development of bacterial consortium for the effective degradation of RCPs from TWW. Briefly, one loopful culture of selected bacterial strains was inoculated separately (aseptically) in 10 mL sterilized NB and placed in BOD incubator shaker. After 24h, one bacterial strain (50 μL) was spreaded on NA agar plate and other two strains (20 μL) were inoculated on the wells made on plates. The plates were incubated in BOD incubator at 30±2 ºC for 24h and no inhibition zone was observed. The result indicated that all the selected bacterial strains were able to grown with each other without forming any inconsistency or inhibition zone.

6.1.2.2. Development of a potential bacterial consortium

Based on the biointeraction study of each selected bacterial strains, a new bacterial consortium RKS-TEX267 was developed. Briefly, one loopful culture of selected bacterial strains (RKS2, RKS6 and RKS7) were inoculated separately (aseptically) in 15 mL sterilized NB and placed in BOD incubator shaker (100 rpm) for 24h at 30±2 ºC. Afterward, 5.0 mL of each bacterial cultures (RKS2, RKS6 and RKS7) were inoculated in 30 mL fresh sterilized NB and further incubated in BOD incubator shaker at same conditions for 24h. The developed bacterial consortium (RKS-TEX267) was used in further studies.

6.1.2.3. Optimization of the newly developed bacterial consortium at various environmental and nutritional parameters

Different environmental and nutritional parameters (pH, incubation temperature, inoculum size, salt concentration, shaking and static as well as carbon and nitrogen sources), are directly or indirectly influence the microbial growth, reproduction, enzymatic machinery and metabolic activity (Buntic et al., 2017; Kishor et al., 2021c). Therefore, these parameters were optimized to achieve more effective decolorization of RCPs by developed bacterial consortium (RKS-TEX267). All the decolorization experiments were done in triplicates.

6.1.2.3.1. Optimization of inoculum size

For the optimization of inoculum size, briefly, different inoculum size (2-10%) of the developed bacterial consortium RKS-TEX267 was added in 98-90 ml real undiluted filter (Whatman) sterilized TWW (pH 4-10) and flasks were incubated in incubator shaker at 30±2 ºC. After 24h, an aliquot (4 mL) was taken and centrifuged (10,000 x g for 10 min at 4 ºC) to separate the cells biomass. The decolorization was analysed according to the American Dye Manufacturing Institute (ADMI 3WL) tristimulus filter method (Kurade et al., 2012) and % measured by following the equation as below:

$$\text{ADMI removal (\%)} = (\text{ADMIR}_0) – (\text{ADMIR}_t) / (\text{ADMIR}_0) \times 100$$

Where, ADMIR_0 is the initial ADMI value and ADMIRt is the final value

6.1.2.3.2. Optimization of pH

For the optimization of pH, briefly, 10% (v/v) of the bacterial consortium was added in 90 mL real undiluted filter (Whatman) sterilized TWW (pH 4-10) and flasks were incubated in an incubator shaker at 30±2 ºC. After 24h, an aliquot (4 mL) was taken and centrifuged (10,000 x g for 10 min at 4 ºC) to separate the cells biomass. The % decolorization was monitored as discussed above in section 6.2.2.3.1.

6.1.2.3.3. Optimization of temperature

For the optimization of temperature, briefly, 10% (v/v) of the newly developed bacterial consortium RKS-TEX267 was added in 90 mL real undiluted filter (Whatman) sterilized TWW (pH 7) and flasks were incubated in incubator shaker at (15-45±2 ºC). After 24h, an aliquot (4 mL) was taken and centrifuged (10,000 x g for 10 min at 4 ºC) to separate the cells biomass. The % decolorization was monitored as discussed above in section 6.2.2.3.1.

6.1.2.3.4. Optimization of carbon and nitrogen sources

For the optimization of carbon and nitrogen sources, 10% (v/v) of the bacterial consortium was added in 90 mL real undiluted filter (Whatman) sterilized TWW (pH 7) supplemented with 1% (w/v) of different carbon sources (sucrose, glucose, lactose, maltose and starch) and nitrogen sources (beef extract, peptone, yeast extract, urea and ammonium sulphate) and flasks were incubated in incubator shaker at 30±2 ºC. After 24h, an aliquot (4 mL) was taken and centrifuged (10,000 x g for 10 min at 4 °C) to separate the cells biomass. The % decolorization was monitored as discussed above in section 6.2.2.3.1.

6.1.2.3.5. Optimization of salt concentrations

For the optimization of salt concentrations, 10% (v/v) of bacterial consortium was added in 90 mL real undiluted filter (Whatman) sterilized TWW (pH 7) supplemented with 0-5% of NaCI concentrations and flasks were incubated in incubator shaker at 30±2 ºC. After 24h, an aliquot (4 mL) was taken and centrifuged (10,000 x g for 10 min at 4 ºC) to separate the cells biomass. The % decolorization was monitored as discussed above in section 6.2.2.3.1.

6.1.2.3.6. Optimization of shaking and static condition

For the optimization of shaking and static condition, 10% (v/v) of bacterial consortium was added in 90 mL real undiluted filter (Whatman) sterilized TWW (pH 7) and flasks were incubated in incubator shaker (shaking (100 rpm) and static condition) at 30±2 ºC. After 24h, an aliquot (4 mL) was taken and centrifuged (10,000 x g for 10 min at 4 ºC) to separate the cells biomass. The % decolorization was monitored as discussed above in section 6.2.2.3.1.

6.1.3. Degradation of RCPs from TWW by the newly developed bacterial consortium RKS-TEX267 at optimized conditions

The degradation and decolorization of RCPs present in real undiluted TWW by developed bacterial consortium was performed in conical flasks (250 mL) at optimized conditions. Briefly, 10 mL (v/v) of selected single bacterial cultures and developed bacterial consortium was added in 90 mL real undiluted filter (Whatman) sterilized TWW (pH 7.0) and flasks were incubated in BOD incubator shaker at optimized conditions. After 6, 12, 18 and 24h, an aliquot (4.0 mL) were taken from flasks and centrifuged (10,000 x g for 10 min at 4 ºC) in a refrigerated centrifuge (Hettich, Universal 320 R Germany) to separate the cells biomass. The decolorization study was performed in triplicates and the uninoculated flasks containing RCPs were considered as a control. The decolorization was analysed according to the American Dye Manufacturing Institute

(ADMI 3WL) tristimulus filter method (Kurade et al., 2012) and % decolorization was measured by following the equation as below:

$$\text{ADMI removal (\%)} = (\text{ADMIR}_0) - (\text{ADMIR}_t) / (\text{ADMIR}_0) \times 100$$

Where, ADMIR_0 is the initial ADMI value and ADMIRt is the final value.

6.1.4. Statistical analysis

All the data collected from the all sets of experiments was employed to test the variability and validity of the results and expressed as the means and ± standard deviation.

6.2. Results and discussion

In present study, a potential bacterial consortium RKS-TEX267 was developed using three potential pollutants degrading bacterial strains, *Bacillus albus* (RKS2), *Bacillus megaterium* (RKS6) and *Bacillus paramycoides* (RKS7) on the basis of compatibility test among them. Results indicated that all the selected bacterial strains were able to grown with each other without forming any inhibition zone as shown in **Fig. 6.1**. Growth curve of isolated bacterial strains RKS2 (A), RKS6 (B) and RKS7 (C) as shown in **Fig. 6.1**. Therefore, all the selected bacterial strains were used for the development of new bacterial consortium RKS-TEX267 and the used for the degradation of TWW.

6.2.1. Effect of various environmental and nutritional parameters on the degradation of RCPs by the bacterial consortium

6.2.1.1. Effects of inoculum size

In present work, different inoculum size (2%, 4%, 6%, 8% and 10%) was used for the effective degradation of RCPs by developed bacterial consortium (**Fig. 6.3A**). The 19%, 43%, 75% and 88% decolorization was recorded at 2%, 4%, 6% and 8% within 24h, respectively. At 10% inoculum size, 98% decolorization efficiency was recorded at same conditions. However, 10% inoculum size of developed bacterial consortium was optimum for the maximum decolorization of RCPs. The decolorization efficiency increases with increases in inoculum size. It might be attributed to the increased number of bacterial cells involved in RCPs degradation. Similarly, 10% inoculum size of consortium (bacterial-algal) was reported optimum for the maximum decolorization (99%) of textile dyes (Ayed et al., 2021). Afrin et al. (2022) reported 97.4% decolorization of textile dyes by bacterial consortium at 10% inoculum size.

6.2.1.2. Effect of pH

In present work, the developed bacterial consortium was able to decolorize RCPs at different pH values (**Fig. 6.3B**). At pH below and above 7, the decolorization potential of consortium decreased. But, the highest decolorization (96%) was recorded at pH 7 and minimum decolorization (15% & 21%) was recorded at pH 4 and 10. The consortium also showed 57%, 81%, 86% and 67% decolorization at pH 5, 6, 8 and 9, respectively. The study indicated that neutral pH may be optimum for the growth of bacterial consortium. The minimum decolorization recorded at lower and high pH might be due to the inhibition in enzymatic and metabolic activity of consortium. Similarly, Haque et al. (2021) recorded 97% decolorization of methyl orange by a biofilm-forming consortium at pH 7. Afrin et al. (2022) also found 97.4% decolorization of textile wastewater by the bacterial consortium at pH 7.

6.2.1.3. Effect of temperature

Temperature highly influences the microbial growth, reproduction, enzymatic and metabolic activity (Afrin et al., 2022). In present study, with increase and decrease in temperature from 30±2 ºC, the decolorization efficiency of bacterial consortium was decreased. The bacterial consortium showed 97% decolorization at 30±2 ºC within 24h. The 56%, 85%, 88% and 66% decolorization were recorded at 20±2 ºC, 25±ºC, 35±2 ºC and 40±2 ºC, respectively. Only 18% and 24% decolorization was attained at 15±2 ºC and 45±2 ºC, respectively. The decolorization profile of RCPs by developed bacterial consortium at different temperatures is shown in **Fig. 6.3C.** Thus, the optimum temperature for effective treatment was recorded to be 30±2 ºC. But at 15±2 ºC and 45±2 ºC, the decolorization efficiency was drastically decreased due to the loss of cell sustainability and enzymatic machinery (Buntic et al., 2017). Similarly, Kishor et al. (2021c) recorded 99% decolorization of methylene blue (MB) dye at 30±2 ºC. The highest decolorization of crystal violet (CV) and safranin dye was reported at 30±2 ºC (Buntic et al., 2017).

6.2.1.4. Effect of carbon and nitrogen sources

In present study, 1% of different carbon sources (sucrose, glucose, lactose, maltose and starch) and nitrogen sources (beef extract, peptone, yeast extract, urea and ammonium sulphate) were used to achieve efficient decolorization of RCPs by developed bacterial consortium (**Fig. 6.3D & 6.3E**). The maximum decolorization (97%) was recorded in presence of glucose. In contrast, the developed bacterial consortium showed 92%, 89%, 84% and 75% decolorization of RCPs in presence of sucrose, lactose, maltose and starch, respectively (**Fig. 6.3D**). The bacteria used glucose as energy and electron donor sources resulting in enhanced decolorization of RCPs. Samuchiwal et al. (2021) also reported maximum decolorization (73%) of textile effluent in presence of glucose by microbial consortium.

In case of nitrogen sources, the presence of yeast extract showed the maximum (98%) decolorization of RCPs within 24h, while the presence of peptone, beef extract, urea and ammonium sulphate showed 91%, 73%, 58% and 47% decolorization, respectively (**Fig. 6.3E**). Similarly, in presence of yeast extract, 73% decolorization of textile effluent wastewater was reported by Samuchiwal et al. (2021).

6.2.1.5. Effects of salt concentrations

TWW normally contains high concentration of salts, which inhibit microbial growth, reproduction, enzymatic and metabolite activity (Ali et al., 2021). Therefore, the effect of different concentrations of salt (0-5% NaCI) on decolorization efficiency of bacterial consortium is shown in **Fig. 6.3F**. The decolorization of TWW by the bacterial consortium was decreased with an increase in salt concentrations (up to 5% NaCI). At 0-1% salt concentration, 96% decolorization of RCPs was attained within 24h. At 2%, 3%, 4% and 5% concentrations, 89%, 76%, 55% and 38% decolorization was recorded. Similarly, Ali et al. (2021) reported 95% degradation of textile wastewater in presence of 1% (NaCl).

6.2.1.6. Effect of shaking and static condition

In present study, the decolorization of RCPs by bacterial consortium was performed at shaking (100 rpm) and static condition is shown in **Fig. 6.4**. In shaking condition, the consortium showed only 44% decolorization within 24h whereas in static condition, consortium showed 97% decolorization within same condition. Results indicated that the facultative anaerobic condition was optimum for the effective degradation of RCPs. Similarly, Afrin et al. (2022) reported 97.4% decolorization of textile dyes by bacterial consortium in static condition.

6.3. Degradation of RCPs from TWW by the newly developed bacterial consortium RKS-TEX267 at optimized conditions

TWW contains various RCPs that cause serious threats in environment and public health (Cao et al., 2019; Ali et al., 2021). These are also highly toxic to plants growth and biomass production (Kishor et al., 2021b). Therefore, the bacterial consortium was developed for the effective degradation of RCPs. In present study, the bacterial strains *Bacillus albus, Bacillus megaterium* and *Bacillus paramycoides* were used individually and their consortium for the effective degradation of RCPs at optimized conditions. The *B. albus, B. megaterium* and *B. paramycoides* showed 72.42%, 58.57% and 52.79% decolorization of RCPs within 24h at optimized conditions, respectively whereas, the bacterial consortium showed more than 99% decolorization of RCPs at same conditions as shown in **Fig. 5A.**

Further, the results also indicated that developed bacterial consortium showed faster, higher and more efficient decolorization than individual cultures. It might be occurred due to the synergistic role of bacterial strains in degradation of RCPs. Similarly, Ali et al. (2021) used a consortium than single cultures for the effective treatment of textile wastewater. The bacterial consortium of *Pseudoarthrobacter, Sphingomonas* and *Gardonia stentrophomonas* showed effective decolorization of reactive black 5 dye than pure culture at optimized conditions (Eskandari et al., 2019). No decolorization was recorded in control. After decolorization, 4.0 mL sample was taken, centrifuged and biomass was washed with ethanol and colorless biomass was obtained indicating that decolorization was occurred due to the degradation, not by adsorption.

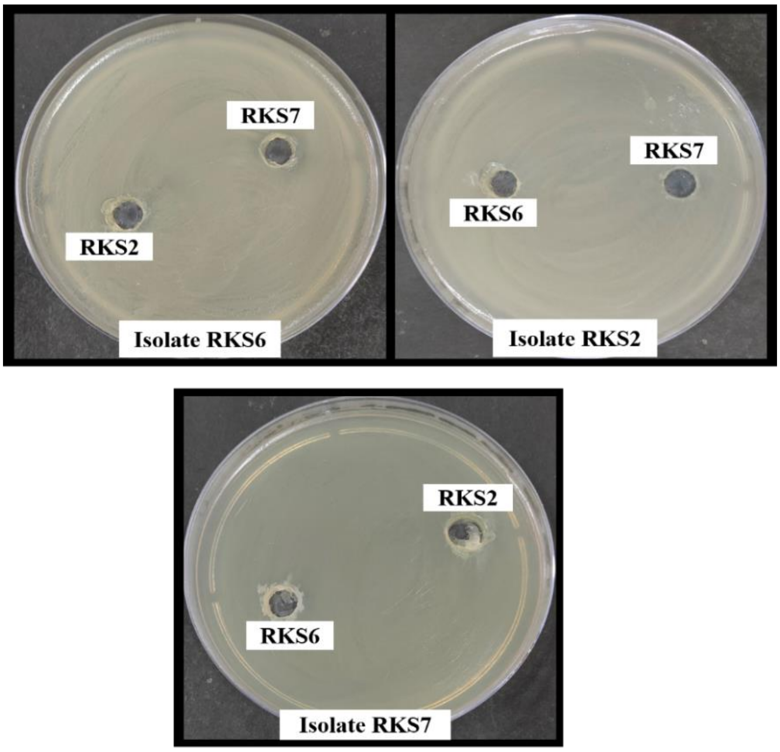


Fig. 6.1: Biointeraction study among the selected bacterial strains (RKS2, RKS6 and RKS7).

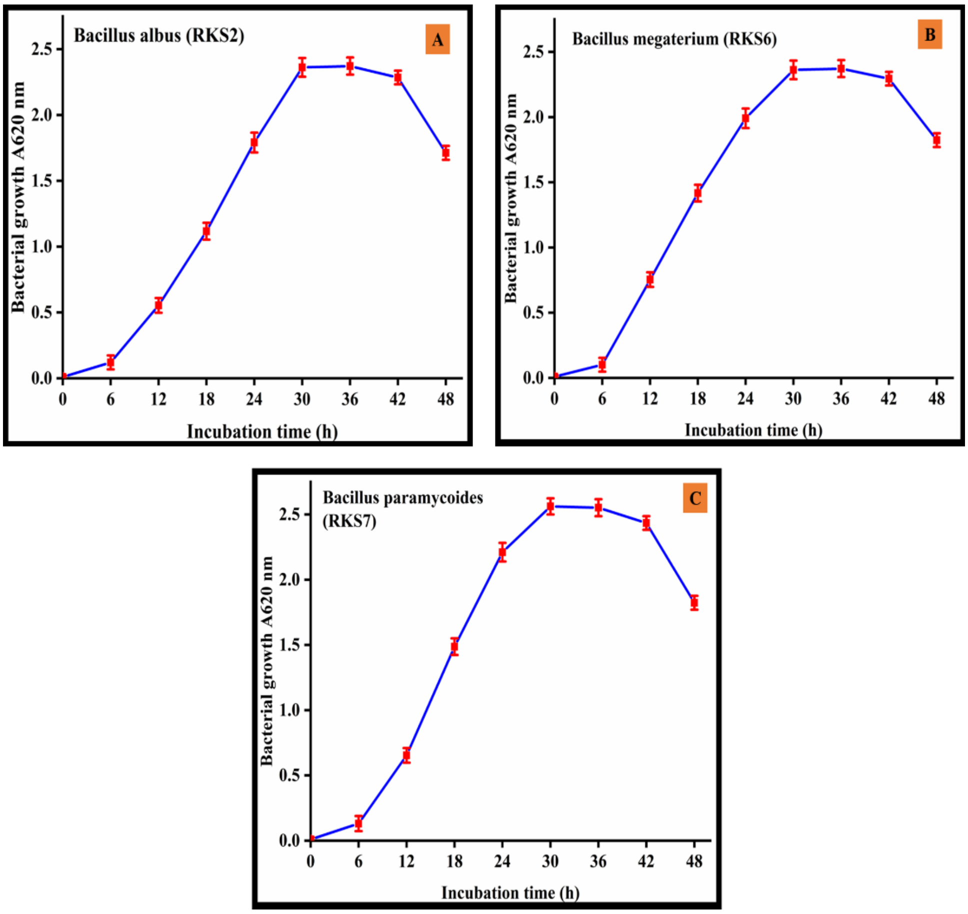


Fig. 6.2. Growth curve of isolated bacterial strains RKS2 (A), RKS6 (B) and RKS7 (C).

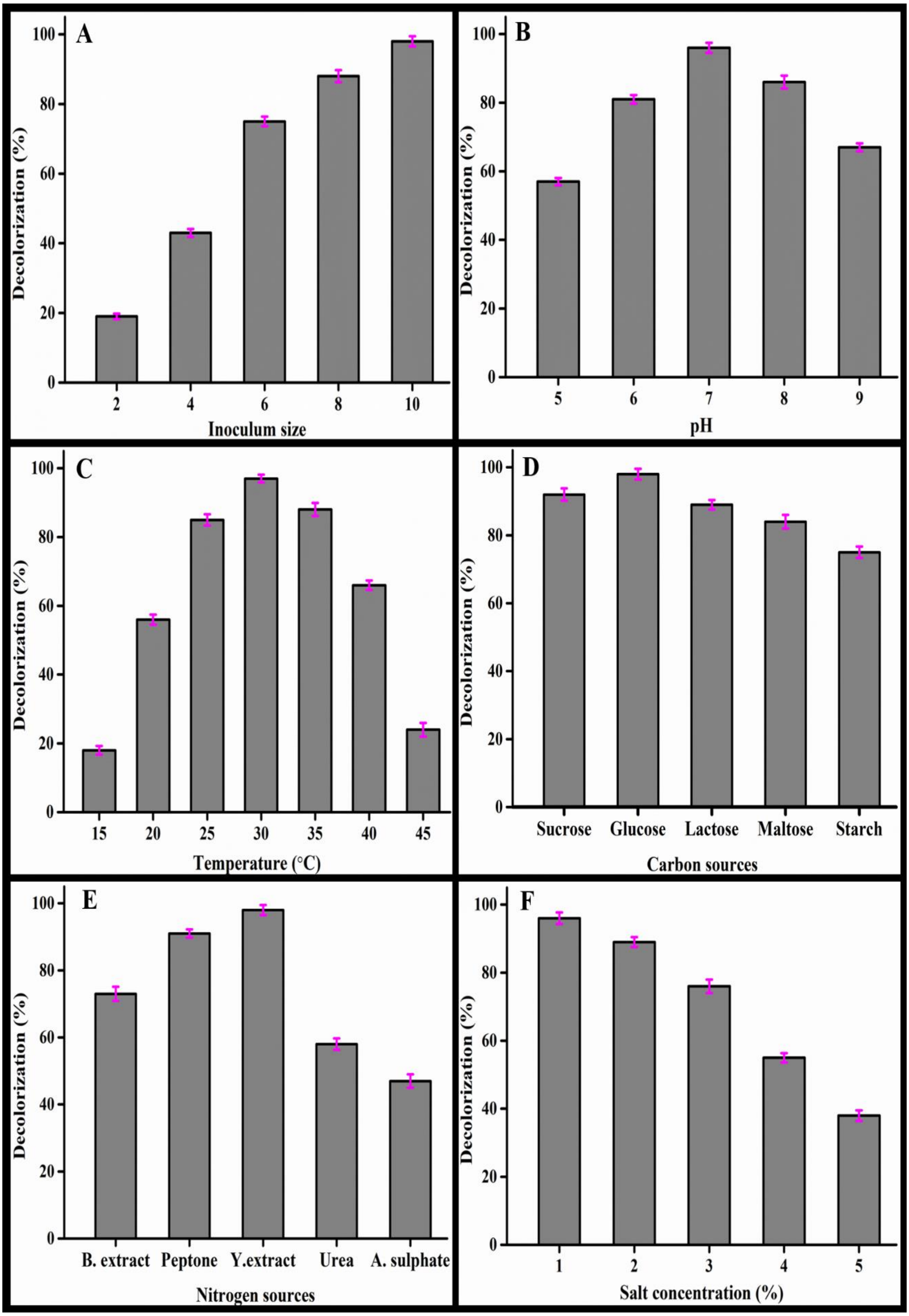


Fig. 6.3: Effect of inoculum size (A), pH (B), temperature ºC (C), carbon sources (D), nitrogen sources (E) and salt concentration (F) on bacterial treatment process.

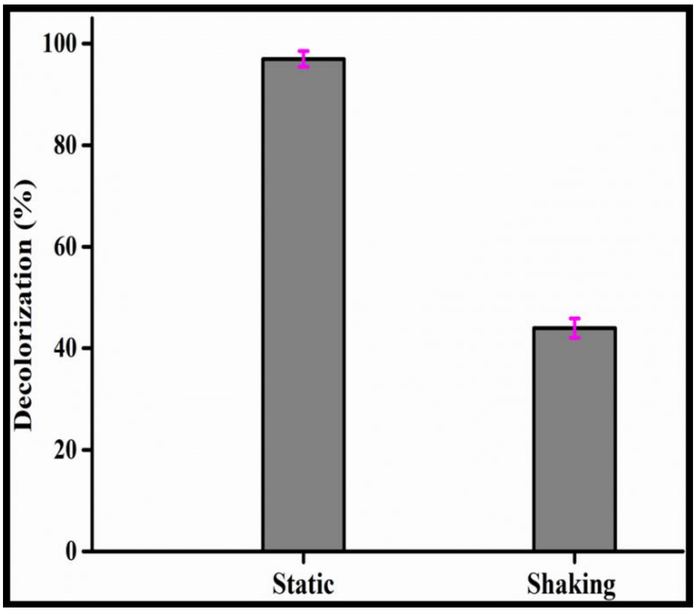


Fig. 6.4: Effect of static and shaking condition on bacterial treatment process.

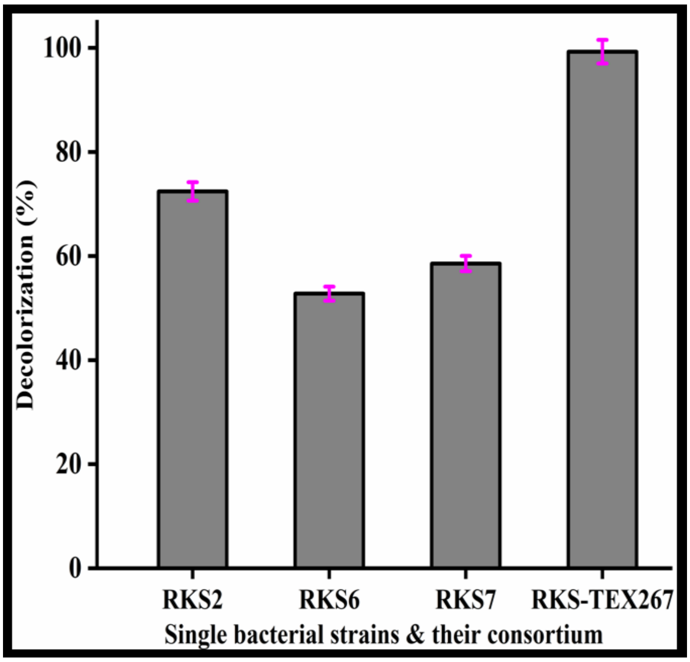


Fig. 6.5: Decolorization of textile wastewater by axenic bacterial cultures and their consortium RKS-267.

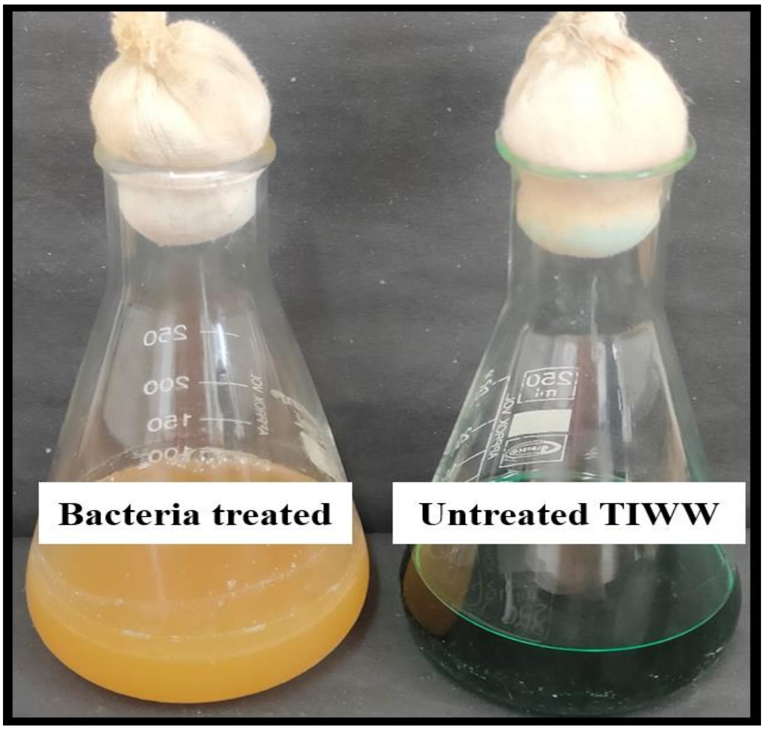


Fig. 6.6: Untreated and treated textile wastewater by axenic bacterial cultures and their consortium RKS-TEX267.

6.4. Conclusion

The present study was aimed to develop a new potential bacterial consortium for the effective degradation of RCPs from the real TWW. In present study, a new bacterial consortium RKS-TEX267 was developed comprising three potential pollutants degrading bacterial strains, *B. albus* (RKS2), *B. megaterium* (RKS6) and *B. paramycoides* (RKS7). These bacterial strains are compatible to each other and able to work together during degradation study. The consortium RKS-TEX267 was able to decolorize 99.28% of TWW as compared to individual bacterial strains, *B. albus* RKS2 (72.42%), *B. megaterium* RKS6 (58.57%) and *B. paramycoides* RKS7 (52.79) at optimized conditions. Further, the optimized conditions were found to be pH 7, temperature 30±2 °C, inoculum size 10%, salt concentration and 1%, static condition. Further, the best carbon and nitrogen sources were found as glucose and yeast extract. Overall, the developed and optimized bacterial consortium RKS-TEX267 can be effective in the degradation and detoxification of RCPs in real TWW.

Chapter7

Detection and characterization of recalcitrant colouring pollutants (RCPs) and metabolites in untreated and bacteria treated TWW using FT-IR and GC-MS analysis

Detection and characterization of recalcitrant colouring pollutants (RCPs) and metabolites in untreated and bacteria treated TWW using FT-IR and GC-MS analysis

7. Introduction

A wide variety of chemicals and large amount of freshwater is used in sizing, bleaching, dyeing, printing, washing and finishing stages during the textile production processes (Blinska et al., 2019). Textile wastewater (TWW) contains a variety of highly toxic recalcitrant coloring pollutants (RCPs), dissolved solids and toxic metals (Khan and Malik, 2017). TWW causes direct pollution in aquatic bodies and soils ecologies and indirect pollution in groundwater. However, the treatment of these pollutants from our natural environment is an absolute requirement to promote the sustainable development of our society with low environmental impact. RCPs (dyes) are defined as synthetic, recalcitrant, highly toxic and heterocyclic aromatic compounds, which widely used in dyeing, tannery, plastic, cosmetics, food, textile, paper and medicinal industries to add color to products (Kishor et al., 2021a). These dyes are highly resistant to light, water, chemicals, detergents and microbial activities.

Globally, thousands of synthetic RCPs/dyes are used in TIs during the dyeing process. More than 7×10^7 tons of RCPs are produced and ~280,000 tons of RCPs are released into water bodies annually (Bilinska et al., 2019; Kumar et al., 2019). RCPs such as methyl orange (MO), congo red (CR), methylene blue (MB), azure B (AB), reactive dye, direct red, scarlet, malachite green (MG) and acid orange dyes are used in TIs (Kishor et al., 2021a; Afrin et al., 2021). RCPs are the major sources of pollution in water/soil resources (Cao et al., 2019). These pollutants are resistant and not easily degraded by biological, chemical, or physical means and persistent in environment for a long time. RCPs cause serious threats in soil and water ecologies along with severe threats to living beings in different ways (Srivastava and Bandhu, 2022).

Hence, the information about the nature and characteristics of RCPs present in TWW is urgently required to understand their mechanism of toxicity and to protect environment and public health.

Therefore, the present study was aimed to characterize and identify the unknown RCPs and metabolites present in untreated and treated TWW. For this, the liquid-liquid extraction (LLE) method was used to extract RCPs and their metabolites from TWW. RCPs and their metabolites were identified by Fourier Transform-Infrared Spectroscopy (FT-IR) and Gas Chromatography-Mass Spectrometry (GC-MS) analysis.

7.1. Materials and methods

7.1.1. Chemicals

All the required chemicals, reagents and solvents used in the experiments were of the highest purity (purity ≥ 99%) and analytical grade. The chemicals, reagents and solvents were purchased from Sigma-Aldrich (St. Louis, MO, USA). Whatman® Grade GF/C filter papers (pore size 1.2 μm) (Whatman, England, UK) were used to filter collected TWW. After filtration, the TWW sample was used for the detection and characterization of RCPs and their metabolites to know their nature and characteristics by FT-IR and GC-MS analysis.

7.1.2. Sample preparation and characterization of RCPs and metabolites in untreated and treated TWW by FT-IR analysis

After and before bacterial treatment, 20 mL (v/v) aliquot was collected, centrifuged and supernatant was oven-dried at 85 ⁰C for overnight. The dried residues were mixed with 400 mg of potassium bromide (KBr) in a ratio of 5:95 (w/w), samples were finely ground and fused into a thin pellet prepared under vacuum condition using a PCI hydraulic press with capacity of 15 tons and fixed in the sample holder for analysis. The FT-IR analysis was done on a Nicolet ™ 6700 Thermo Scientific, USA spectrometer from 400-4000 cm^{-1} with a 16 scan speed. Scanning was performed to obtain the spectrum and measured in the ambient air against the pure KBr as a background spectrum. The data processing was performed using the software OMNIC™ (v7.4). The assignment of the absorption peaks observed in the FT-IR spectrum was done on the basis of those outlined in the "Introduction to Organic Spectroscopy" (Lambert 1987).

7.1.3. Sample preparation and characterization of RCPs and their metabolites in untreated and treated TWW by GC-MS analysis

100 mL (v/v) of untreated and bacteria treated TWW samples were collected and centrifuged at 8000 x g for 10 min at 4 ⁰C to separate cell biomass and other suspended particles. The obtained supernatant was acidified (pH ≤ 2.0) with 1N hydrochloric acid and extracts used to extract the RCPs and their metabolites by using the equal volume of ethyl acetate (HPLC grade) following the LLE method (Bharagava et al., 2018). The solvent layer containing RCPs and their metabolites were filtered (Whatman), dried over sodium sulphate (Na_2SO_4) and evaporated in a Rotavapor (Rotavapor RE 120, Buchi, Flawil, Sweden) at 45 ⁰C. Afterward, the dry residues were dissolved in HPLC grade methanol (4.0 mL), filtered through a (0.22 μm) membrane filter (Millipore Ltd, Bedford., MA) and used in GC-MS analysis.

Further, obtained extract (5.0 µL) was injected into a PE-5MS column (0.18 mm diameter, 20 m long, 0.18 mm film thickness). The column temperature was kept at 80 ºC (2 min), 50-280 ºC (10 ºC/min) and hold time (7 min). Helium gas was employed as carrier gas and the flow rate was programmed at 1.0 mL/min. The temperature of injection port and MS transfer line were set at 280 ºC and 290 ºC, respectively. The RCPs and their metabolites were characterized on the basis of RT (Retention Time), fragmentation pattern, and mass spectra by using the NIST Library (National Institute of Standards and Technology) (version 1.10 beta, Shimadzu).

7.2. Results and discussion

7.2.1. Characteristics of RCPs and their metabolites in untreated and treated TWW by FT-IR and GC-MS analysis

A variety of organic chemicals (RCPs) are used in textile industry during textile production processes and discharged into the environment. These chemicals are recalcitrant in nature and persist in environment for a long time, which causes serious threats to environment and living beings. Therefore, the analysis of these compounds are required to known their nature and characteristics for environmental safety.

7.2.1.1. FT-IR analysis

The assignment of absorption peaks observed in FT-IR spectrum was done on the basis of those outlined in the "Introduction to Organic Spectroscopy" (Lambert 1987) to know the relevant chemical bonds and functional groups in RCPs and their metabolites. FT-IR spectrum of RCPs and their metabolites showed different peaks as shown in **Fig. 7.1A & 7.1B**. In RCPs, the peak appeared at 3444.4 cm^{-1} showed the presence of N-H stretching of aromatic amines and amides (**Fig. 7.1A**). The peaks were recorded at 1634.6 cm^{-1} for C=O and NH_2 deformation in primary amines, 1354.3 cm^{-1} for NO_2 sym stretching of nitro compounds and 1127.3 cm^{-1} for C=S stretching in thiocarbonyl compounds, respectively. The peaks appeared at 1425.0 cm^{-1} and 870.3 cm^{-1} for O-H and CH_2 bending, respectively. The peaks appeared at 814.7, 692.5 and 622.2 cm^{-1} for C-H, C-C-CHO and O-H deformation that representing 1,2,4 trisubst benzenes, aldehydes compounds and phenolic compounds, respectively. Different functional groups present in RCPs and their metabolites as shown in **Table 7.1**.

After treatment, different metabolites were produced, which appeared at 3415.7, 2962.4, 1661.3 and 1596.5 cm^{-1} for O-H, C-H, C=N and NH_2 stretching (**Fig. 7.1B**), respectively. The peaks were recorded at 1407.8, 1308.2, 1131.2 and 622.9 cm^{-1} for O-H, N=N-O, C=S and O-H deformation. The results indicated that RKS-TEX267 may degrade RCPs into the various metabolites.

Table 7.1: Different functional groups identified in RCPs and their metabolites by FT-IR analysis.

Obseved frequencies (cm-1)	**Recorded peaks**	
	Recalcitrant coloring pollutants (RCPs)	**Metabolites**
3444.4	N-H stretching	---
3415.7	---	O-H stretching
2962.4	---	C-H stretching
1661.3	---	C=N stretching
1634.6	C=O & N-H stretching	---
1596.5	---	NH_2 deformation
1425.0	O-H	---
1407.8	---	O-H stretching
1354.3	NO_2 sym stretching	---
1308.2	---	N=N-O deformation
1131.2	---	C=S stretching
1127.3	C=S stretching	---
870.3	O-H & CH_2 bending	---
814.7	C-H stretching	---
692.5	C-C-CHO deformation	---
622.9	---	O-H stretching
622.2	O-H stretching	---

7.2.1.2. GC-MS analysis

In present study, various RCPs were found in untreated TWW by GC-MS analysis as shown in **Fig. 7.2 & Table 7.2.** Most of the RCPs are highly toxic, persistent and aromatic in nature. In GC-MS analysis, various peaks recorded at RT 7.64, 10.31, 13.23, 16.47, 18.61, 19.42, 21.82, 25.37, 27.57, 30.59, 33.09 and 35.52 corresponded to the presence of 1-Ethoxy-2-propanol, O-Ethyl hydroxylamine, Dodecamethylcyclo-hexasiloxane, 1,2-Benzendicarboxylic acid, Carbolic acid (Phenol), Pentachlorophenol, Tetracosamethylcyclo dodecasiloxane, 2-Octen-1-ol, 3,7-dimethyl-, isobutyrate, Phthalic acid, 2-chloropropyl isobutyl ester, Stearic acid, TMS derivative, 1-Monomyristin and 2,6-Dichloro-4-(1,1 dimethyl ethyl) phenol, respectively. Many other peaks also recorded at RT 37.32, 38.98, 41.54, 45.74 and 50.63 corresponding to the presence of Glycerol monostearate, Dotriacontane, 1-iodo-, Heptadecane, Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite and Tris(2,4-di-tert-butylphenyl) phosphate, respectively (**Fig. 7.2A**).

The phenolic compounds such as Carbolic acid (Phenol), Pentachlorophenol and Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite are used to prepare dyes, antimicrobial agents, impregnation agents, phase change, protective agents and antifouling paint ingredients in TIs (Kaur et al., 2018; Kishor et al., 2021a). Phenolic compounds are well reported as carcinogenic and damage liver, red blood cells and kidney. These also inhibit root, hypocotyl growth, water and minerals uptake as well as photosynthesis and enzymatic activity in plants (Chandansive et al., 2020). The dyes components like 2-octen-1-ol, 3,7-dimethyl-, Isobutyrate, Tetracosamethylcyclo dodecasiloxane and 2,6-Dichloro-4-(1,1 dimethyl ethyl) phenol are used in dyeing and printing stages during the textile production process (Kishor et al., 2021b). Dyes are highly toxic to all forms of life and aquatic life due to reduction in photosynthetic activity, dissolved oxygen concentration and increase in BOD and COD levels (Kumar et al., 2019). Phthalic acid like 1,2-benzendicarboxylic acid and Phthalic acid, 2-chloropropyl isobutyl ester are used as an adhesive, printing ink, detergent and surfactant agent in TIs (Bilinska et al., 2019).

Phthalic acid is reported as a carcinogenic, aquatic toxicant and endocrine-disrupting agent as well as causes malformations, fetal death and reproductive toxicity to living beings (Kishor et al., 2021a). The O-Ethyl hydroxylamine and 1-Ethoxy-2-propanol are used as antibacterial and solvent agents in printing and writing inks during the printing process. These compounds are highly toxic to public health and environment. In addition, the Dodecamethylcyclo-hexasiloxane is used as a cleaning agent, Stearic acid as a surfactant and softening agent, 1-Monomyristin as an antimicrobial agent, Glycerol monostearate as an emulsifier agent, Dotriacontane, 1-iodo- as a boiling solvent and Tris (2,4-di-tert-butylphenyl) phosphate as a stabilizing agent in TIs (Kaur et al., 2018; Kishor et al., 2021b). All these compounds are well reported to cause severe threats to the environment and living organisms (Kaur et al., 2018; Kumar et al., 2019).

Table 7.2: Different recalcitrant coloring pollutants (RCPs) identified in untreated TWW by GC-MS analysis.

Recalcitrant coloring pollutants (RCPs)	**Retention Time**	**Ecotoxicological effects**	**Application**
1-Ethoxy-2-propanol	7.64	Causes eye, skin & respiratory tract irritation	Used as an printing & writing inks
O-Ethyl hydroxylamine	10.31	Damage soil & aquatic life	Antibacterial agent
Dodecamethylcyclo-hexasiloxane	13.23	Toxic to aquatic ecosystem	Used as a cleaning agent
1,2-Benzendicarboxylic acid	16.47	Carcinogenic, endocrine disrupter & aquatic toxicants	Applied as adhesives &

			printing inks
Carbolic acid (Phenol)	18.61	Damage liver & kidney	Impregnation agent for textile
Pentachlorophenol	19.42	Toxic to cardiovascular, blood, liver & eyes	Antimicrobial agent
Tetracosamethylcyclo dodecasiloxane	21.82	Toxic to environment (water/soil)	Fluorescent dye component
2-Octen-1-ol, 3,7-dimethyl-, isobutyrate	25.37	Not found	Dye component
Phthalic acid, 2-chloropropyl isobutyl ester	27.57	Causes fetal death, malformations & reproductive toxicity	Used as an surfactant & detergent in dyeing
Stearic acid, TMS derivative	30.59	Causes gastrointestinal irritation with vomiting & diarrhea	Applied as a softening agent
1-Monomyristin	33.09	Not determined	Antimicrobial agent
2,6-Dichloro-4-(1,1 dimethyl ethyl) phenol	35.52	Toxic to aquatic life	Dye component
Glycerol monostearate	37.32	Causes flatulence & abdominal cramps	Emulsifier agent
Dotriacontane, 1-iodo-	38.98	Not determined	Boiling solvent
Heptadecane	41.54	Toxic to public health and environment	Appropriate suspended solvent
Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite	45.74	Hazards to living beings & environment	Phase change & protective agent
Tris(2,4-di-tert-butylphenyl) phosphate	50.63	Causes cytotoxic, mutagenic & endocrine disrupting threats	Stabilizer agent

After bacterial treatment, different degraded metabolites were identified at RT 7.74, 11.11, 13.01, 15.99, 21.71, 24.60, 29.79 and 39.42 indicating the presence of 2(3H)-Thiazolone, 4-methyl, Pentan-3-ol, trimethylsilyl ether, D-(-)-Erythrose, tris (trimethylsilyl) ether, trimethylsilyloxime, Methyl salicylate, TMS derivative, Hexadecane, 2,6,11,15-tetramethyl, 5-Stannaspiro[4.4]nona-1,3-diene, 6,6,9,9-tetrakis (trimethylsilyl), Dichloroacetic acid, heptadecyl ester and Octadecane, 3-ethyl-5-(2-ethylbutyl), respectively (**Fig. 7.2B**). In addition, many other peaks were also recorded at RT 42.05, 45.75 and 33.15/35.58 corresponding to 9-Desoxo-9x-hydroxy-7-ketoingol, Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite and Octatriacontyl pentafluoropropionate, respectively. The study indicated that RCPs are decolorized, degraded and transformed into new metabolites by developed consortium (**Table 7.3**). But, only one compound i.e. Phenol, 2,4-bis(1,1-dimethylethyl), phosphite was not degraded by consortium. The GC-MS spectrum of RCPs (A) and their metabolites (B) as shown in **Fig. 7.2**.

Table 7.3: Different metabolites identified in treated textile wastewater by GC-MS analysis.

Degraded metabolites	**Retention Time**	**Mol. Wt. (g/mol)**	**Remark**
2(3H)-Thiazolone, 4-methyl	7.74	143.2	Transformed product
Pentan-3-ol, trimethylsilyl ether	11.11	160.3	Degraded product
D-(-)-Erythrose, tris (trimethylsilyl) ether, trimethylsilyloxime	13.01	308.6	Transformed product
Methyl salicylate, TMS derivative	15.99	152.1	Degraded product
Hexadecane, 2,6,11,15-tetramethyl	21.71	282.5	Transformed product
5-Stannaspiro[4.4]nona-1,3-diene, 6,6,9,9-tetrakis (trimethylsilyl)	24.60	120.19	Degraded product
Dichloroacetic acid and heptadecyl ester	29.79	367.4	Degraded product
Octatriacontyl pentafluoropropionate	33.15/35.58	697.0	Transformed product
Octadecane, 3-ethyl-5-(2-ethylbutyl)	39.42	366.7	Degraded product
9-Desoxo-9x-hydroxy-7-ketoingol	42.05	534.5	Degraded product
Phenol, 2,4-bis (1,1-dimethylethyl), phosphite	45.75	646.9	Not degraded

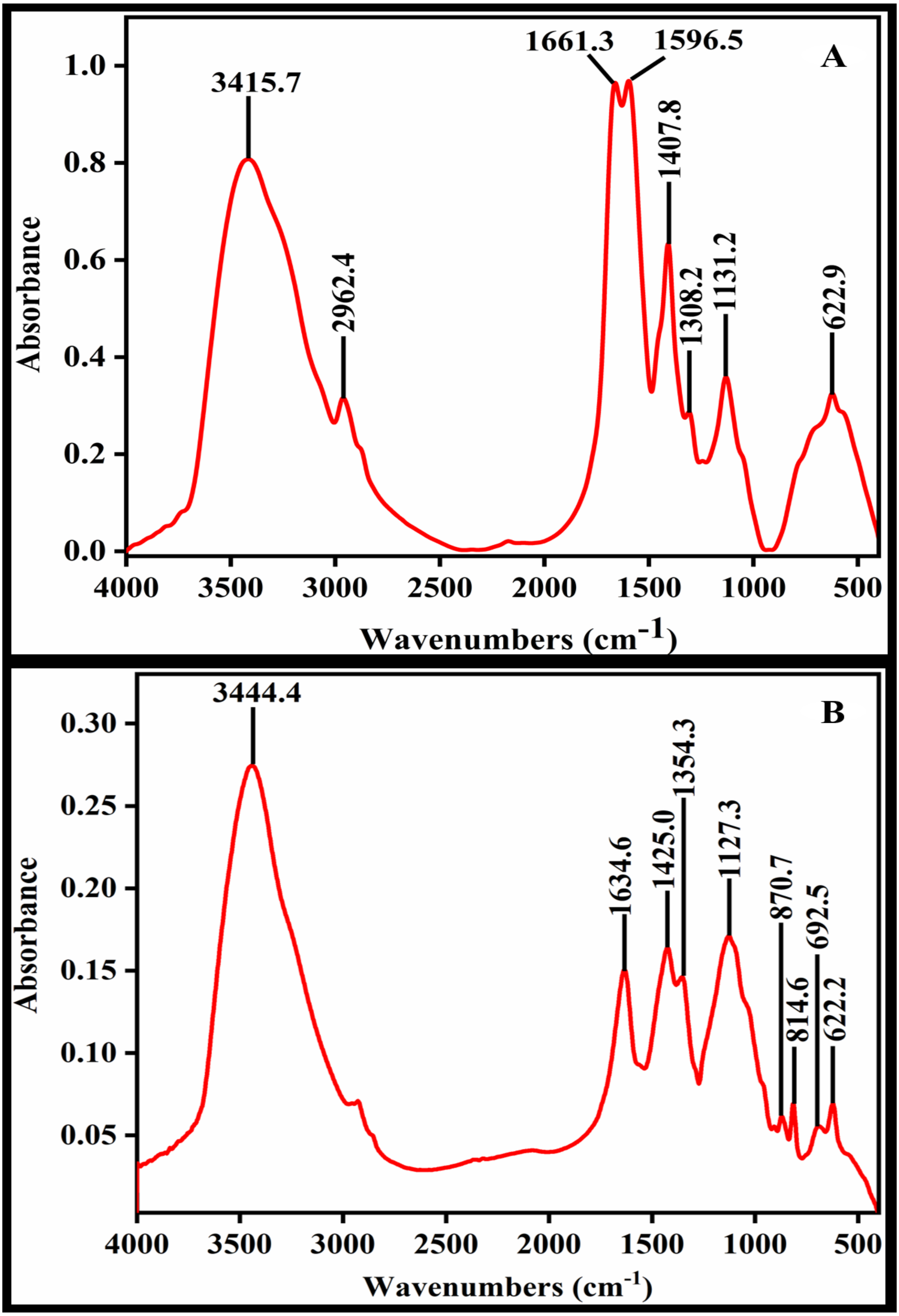


Fig. 7.1: FT-IR analysis of RCPs (A) and their metabolites (B).

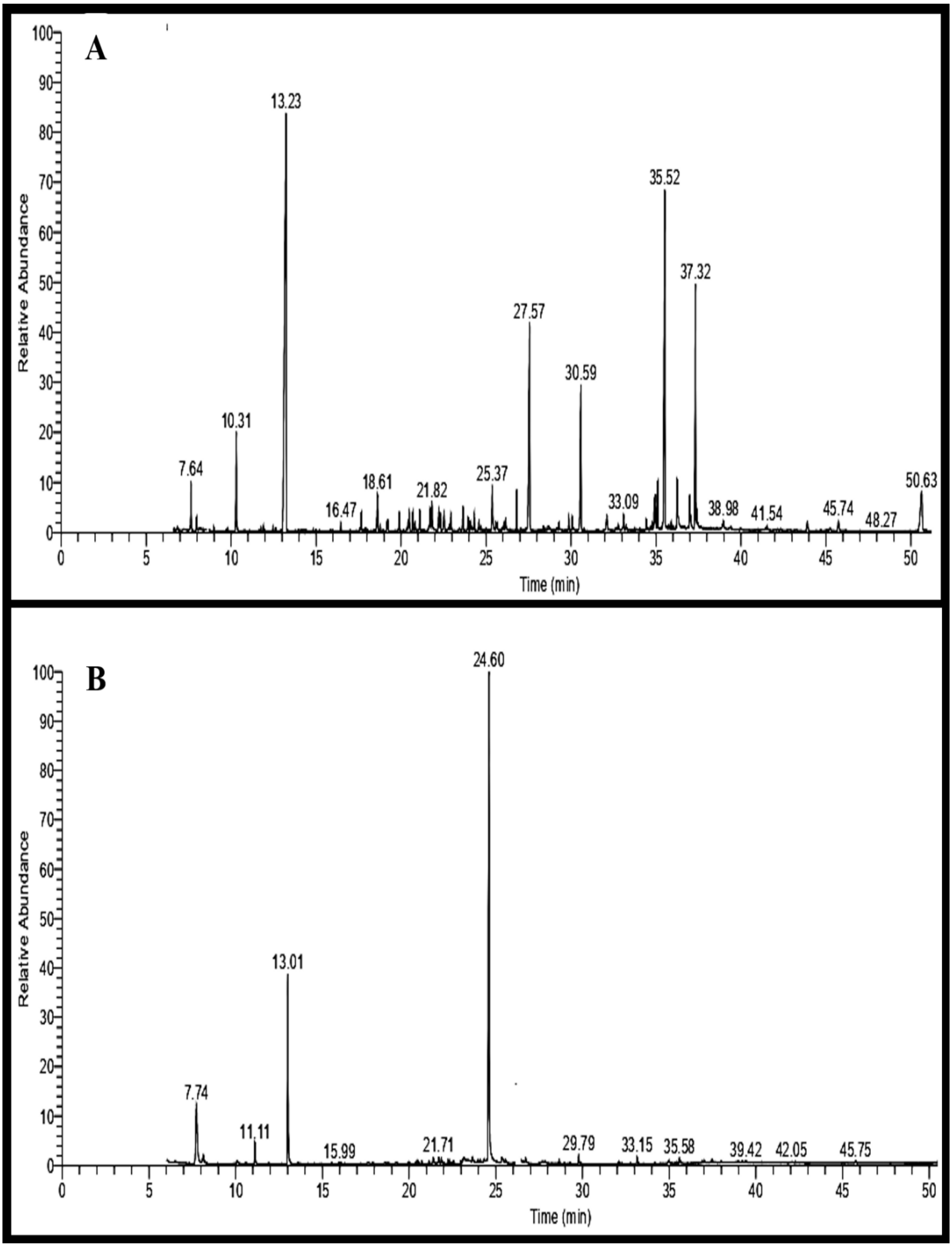


Fig. 7.2: GC-MS chromatogram of RCPs (A) and their metabolites (B).

7.3. Conclusion

The present study was aimed to characterize the RCPs and their metabolites present in untreated and treated TWW. In present study, the untreated and treated TWW contains a variety of RCPs and their metabolites were characterized by FT-IR and GC-MS analysis. Most of the RCPs are EDCs, mutagens, carcinogens, allergens and aquatic toxicants, which cause serious threats to aquatic life (fauna/flora) and soil biodiversity. Hence, the discharge of this highly polluted and toxic wastewater in the aquatic bodies is not suitable with reference to the environment and public health. After bacterial treatment, various RCPs are decolorized, degraded and transformed into new metabolites by newly developed bacterial consortium RKS-TEX267. Thus, the newly developed bacterial consortium RKS-TEX267 showed a high potential to degrade and decolorize the TWW.

Chapter 8

Detection and characterization of enzyme responsible for the degradation anddetoxification of recalcitrant colouring pollutants (RCPs)

Detection and characterization of enzyme responsible for the degradation and detoxification of recalcitrant colouring pollutants (RCPs)

8. Introduction

Textile industries (TIs) play a major role by contributing global economy in developing countries (Bilinsika et al., 2019). But unfortunately, these are also a major contributor of environmental pollution and health hazards (Chandanshive et al., 2020). The wastewater discharged from TIs is very complex matrix and contains various toxic recalcitrant coloring pollutants (RCPs/dyes), dissolved solids, organic pollutants and metal ions (Kaur et al., 2018). These pollutants are transported to a long distance along with the wastewater, persist in environment (water/soil) for a long time (Bilinsika et al., 2019). Textile wastewater (TWW) is highly toxic to environment and living organisms due to ecotoxicological effects and bioaccumulation in terrestrial and aquatic life (Khandare and Govindwar, 2015). Thus, it is necessary to adequately treat the TWW before its final discharge into the water bodies to protect the environment and public health.

Different physico-chemical methods, membrane treatment technologies (MTTs) and advanced oxidation processes (AOPs) were reported for the treatment of wastewater pollutants (Kaur et al., 2018; Bilinska et al., 2019). These approaches are required high amount of chemicals, expensive and environmentally destructive chemicals and also cause secondary pollution by generating toxic intermediates (sludge) (Kumar et al., 2019). Therefore, an environmental and economically feasible wastewater treatment option with high treatment efficiency will be more preferable and acceptable to TWW.

Currently, the biological treatment is an eco-friendly, sustainable, cost-effective and efficient method to remediate TWW (Haq et al., 2018; Ceretta et al., 2020). It utilizes the metabolic and enzymatic ability of microbes and plants to degrade the RCPs (Kishor et al., 2021b; Srivastava and Bandhu, 2022). Among these, the use of degradative enzymes in bioremediation of environmental pollutants is an emerging area of research. A variety of enzymes (lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase) are produced by microorganisms that can decolorize, degrade, detoxify and transform a wide range of organic and inorganic textile pollutants (Kishor et al., 2021a; Singh et al. 2015).

Enzymes have a high potential to remediate environmental pollutants due to their high specificity to a broad range of substrates (pollutants) under extreme conditions that microbes cannot thrive, high effectiveness at low pollutant concentration, high activity in the presence of inhibitors of microbial metabolism and high mobility (small size) than microorganisms. Hence, the enzyme treatment is an effective approach for the degradation of RCPs from real TWW. Therefore, the present study was aimed to detect and characterize the enzymes responsible for the degradation of RCPs from real TWW.

8.1. Materials and methods

8.1.1. Chemicals

All the required chemicals, reagents and solvents used in the experiments are of highest purity (purity ≥ 99%) and analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA). Nutrient agar (NA) medium (g/L, HM peptone B # 1.5; yeast extract 1.5; sodium chloride 5.0; peptone 5.0; agar 15.0) was used to detect and characterize the degradative enzyme for the degradation of TWW. Whatman® Grade GF/C filter papers (pore size 1.2 µm) (Whatman, England, UK) were used for the filtration of TWW.

8.2. Preparation of cell-free extract

For the expression of enzymes and preparation of cell-free extract, 10% of the pre-cultures of selected bacterial strains (RKS2, RKS6 and RKS7) were inoculated in real TWW (undiluted, 90 mL, pH 7.0) in conical flasks (250 mL) and incubated at 30±2 ºC under static condition in incubator shaker (LI-BODS-10, LABARD). After 6, 12, 18, 24 and 30h, aliquot (10 mL) was taken and centrifuged at 5000 x g for 10 min at 4 ºC to separate biomass. The obtained biomass was suspended in 50 mM, pH 7.4 of potassium phosphate buffer for the sonication and keeping sonifier output at 40 amps and providing 12 stockers each of 40 s within two min interval at 4 ºC. The homogenate was centrifuged at 5000 × g for 10 min at 4 ºC to obtain a supernatant, which was considered as crude enzyme extract and used for the determination of enzyme activity.

8.2.1. Enzyme assays

The activity of laccase, MnP and LiP enzyme was monitored by a spectrophotometer. For laccase enzyme activity, 10 mM, pH 5 of acetate buffer (2.5 mL), 2.0 mM of guaiacol (1.0 mL) and enzyme extract (0.5 mL) was inoculated in reaction sample and placed at 25±2 ºC for 2h (Bharagava et al., 2018) . The activity was monitored at 420 nm.

For LiP enzyme activity, the 125 mM, pH 3.0 of sodium tartrate buffer (1.5 mL), 0.160 mM of AB dye (0.5 mL), crude enzyme extract (1.5 mL) and 2 mM of H_2O_2 (0.5 mL) was added in reaction mixture. H_2O_2 was added in reaction sample to start the reaction and incubated at 25±2 ºC for 20 min. The activity was measured at 310 nm (Haq et al., 2018).

MnP enzyme activity was done according to Kishor et al. (2021b). Briefly, 1.0 mL of PR (0.1 mM), 1.0 mL of enzyme extract, 0.5 mL of $MnSO_4$ and 1.5 mL of phosphate buffer (pH 7.0) were added in reaction sample. The reaction was started by adding 0.5

mL of H_2O_2 (1 mM) and stopped by adding 40 µL of NaOH (5M) to reaction sample. After every 4 min, the sample was collected and enzyme activity was monitored at 610 nm.

The enzyme activity assays were done in triplicates and blank was prepared with distilled water except enzyme. One unit of enzyme activity is defined as the amount of active enzyme needed to oxidized one µmol of substrate/min.

8.2.2. SDS-PAGE analysis

Denaturing sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out on Polyacrylamide Gel Electrophoresis unit (GX-SCZ2, Genetix Biotech Asia Pvt. Ltd) by using 10% polyacrylamide in gel. The samples were loaded in duplicates and the concentration of enzyme extract was calculated by comparing with the standard curve at 260 nm absorbance. The composition of separating gel and stacking gel has been discussed below:

Materials

- Casting gel unit for electrophoresis
- Siliconized Pasteur pipettes
- Syringes equipped with blunt, stub nosed, needles
- Vacuum chamber for degassing gels
- Micropipettes (10-300 µL)
- Stock 30 % T: 0.8 % C Acrylamide monomer
- 1.5 M Tris-HCl buffer, pH 8.8
- 10 % (w/v) SDS
- 10% (w/v) Ammonium persulfate

Separation gel

- 10 mL of Acrylamide monomer
- 2.6 mL of Tris-HCl Buffer, pH 8.8
- 0.1 mL of 10 % (w/v) SDS
- 3.8 mL of H2O

Stacking gel

- 67 mL of Acrylamide monomer

- 1.25 mL of Tris buffer, pH 8.8
- 0.05 mL of 10 % (w/v) SDS
- 2.975 mL of H2O

Procedure

1. Assemble the slab gel unit with the glass sandwich set in the casting mode with 1.5 mm space.
2. Prepare a separating gel in a separate small beaker.
3. Add separating gel to a side arm flask, stopper the flask and attach to a vacuum pump equipped with a cold trap. Turn on the vacuum and degas the solution for ~10 min. During this period, gently swirl the solutions in the flask.
4. Exit the vacuum, open the flask and add 100 μL of ammonium persulfate and 10 μL of TEMED to the solution.
5. Add stopper to the flask and degas for additional 2 min with gentle swirl to mix the solutions.
6. Transfer appropriate amount of degassed solution to casting chamber without any air bubble formation.
7. Immediately fill in water to the top of separating gel for preventing the formation of the meniscus.
8. Let it settle for 20-30 min to gelate.
9. Prepare a stacking gel as separating gel preparation method and add 0.05 mL of ammonium persulfate and 0.005 mL of TEMED.
10. Pour the stacking gel onto the separating gel.
11. Insert the well-forming comb without trapping air and wait for 20-30 min to let it gelate.
12. Take out the comb after complete gelation.
13. The prepared samples were mixed with sample buffer and were heated in the boiling water for 5-10 min.
14. Now load the samples into wells and protein markers into the first lane. Now cover the top and connect the anodes.
15. Set an appropriate volt and run the electrophoresis.
16. After completion of total running time, stop SDS-PAGE running when the down most sign of protein marker reaches the foot line of the glass plate.

On completion of electrophoreses, the protein bands on the gel were stained with

Coomassie brilliant blue R-250 dye and destained with a destaining solution and left for overnight. The molecular weight was estimated by comparing with standard protein ladder.

8.2.3. Statistical analysis

All the laboratory experiments were performed in triplicates (n = 3) to confirm the variability and validity of the results expressed as mean ± SD.

8.3. Results and discussion

8.3.1. Enzyme activity and characteristics

Different catalytic enzymes present in microorganisms are only responsible for the degradation and mineralization of industrial wastewater pollutants (Haq et al., 2018; Kumar et al., 2019). The ligninolytic enzymes (laccase, LiP and MnP) can degrade and mineralize various environmental pollutants into water and carbon dioxide (Bilinska et al., 2019; Kishor et al., 2021a). These enzyme also reported to degrade and detoxify various RCPs CR, MB, MO, AB and reactive black dye and TWW (Singh et al., 2015; Yadav et al., 2022).

In present work, the LiP, MnP and laccase enzymes were detected during the decolorization of TWW by developed bacterial consortium RKS-TEX267. LiP (EC 1.1.14) is a heme-containing enzyme, which catalyzes hydrogen peroxide-dependent oxidative degradation of many recalcitrant pollutants. The oxidative action produces radical cations through one electron oxidation leading to side-chain cleavage, rearrangement and demethylation (Singh et al., 2015). LiP enzyme have ability to degrade many RCPs, phthalates and polycyclic aromatic hydrocarbons (Kishor et al., 2021a). LiP enzyme is well reported to degrade phenolic and non-phenolic compounds from industrial wastewater (Bharagava et al., 2018; Haq et al., 2018).

MnP (EC 1.11.1.13) is a ligninolytic enzyme that has a wide potential to oxidize or decompose pollutants into non-toxic and inorganic compounds (Kishor et al., 2021d). MnP enzyme can decolorize, degrade, detoxify and mineralize the textile wastewater pollutants (Kishor et al., 2021d; Yadav et al., 2022). For example, MnP enzyme have a high potential to degrade and detoxify the various RCPs like orange IV, crystal violet, safranin dye and TWW pollutants (Buntic et al., 2017; Cao et al., 2019; Kishor et al., 2021b).

Laccase (EC1.10.3.2) is a promising multicopper oxidase enzyme, which is most effective in treatment of various RCPs and wastewater pollutants (Kishor et al., 2021b). It is non-specific in nature and neither use oxygen as electron accepter, nor need co-factor

(Singh et al., 2015). ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) is a low molecular weight compound and acts as a redox mediator for laccase enzymes. The presence of this redox mediator enhances the treatment efficiency of enzymes many folds (Kishor et al., 2021a). The laccase enzyme is well known to degrade and detoxify many RCPs and environmental contaminants.

During treatment study, the strain of *Bacillus albus* (RKS2) showed LiP, *Bacillus paramycoides* (RKS7) showed laccase, *Bacillus megaterium* (RKS6) showed MnP enzyme activity. In present study, it was recorded that laccase, LiP and MnP enzyme activity increased up to 13.43, 7.54 and 9.67 IU / mL till 24h, and after then steadily decreased. The results of LiP, MnP and laccase enzyme activity during the decolorization as shown in **Fig. 8.1**. Similarly, Kishor et al. (2021b) reported that *Bacillus cohnni* produced LiP, MnP and laccase enzyme, which involve in decolorization of congo red dye and TWW within 12-48 h. A bacterial yeast consortium produced laccase and oxidative enzymes, which able to decolorize and degrade textile dye and textile industry effluent within 18-48h (Kurade et al., 2012).

Further, MnP, LiP and laccase enzymes were characterized by SDS-PAGE analysis and found to have molecular weight of protein around 126 kDa, 58 kDa and 97 kDa in crude extract of selected bacterial strains as shown in **Fig. 8.2.** Similarly, Zhang et al. (2013), also purified MnP enzyme (45 kDa) produced by *Cerrena unicolor* BBP6 during the decolorization of MO dye and denim bleaching wastewater. Haq et al. (2018) purified LiP (28 kDa) from *Serratia liquefaciens* during the decolorization of Azure B dye. Bharagava et al. (2018) also purified 45 kDa LiP and 60 kDa from *A. hydrophila* during the decolorization of crystal violet dye. Therefore, the synergistic role of LiP, MnP and laccase enzymes may be the key potential for the effective degradation of RCPs from TWW.

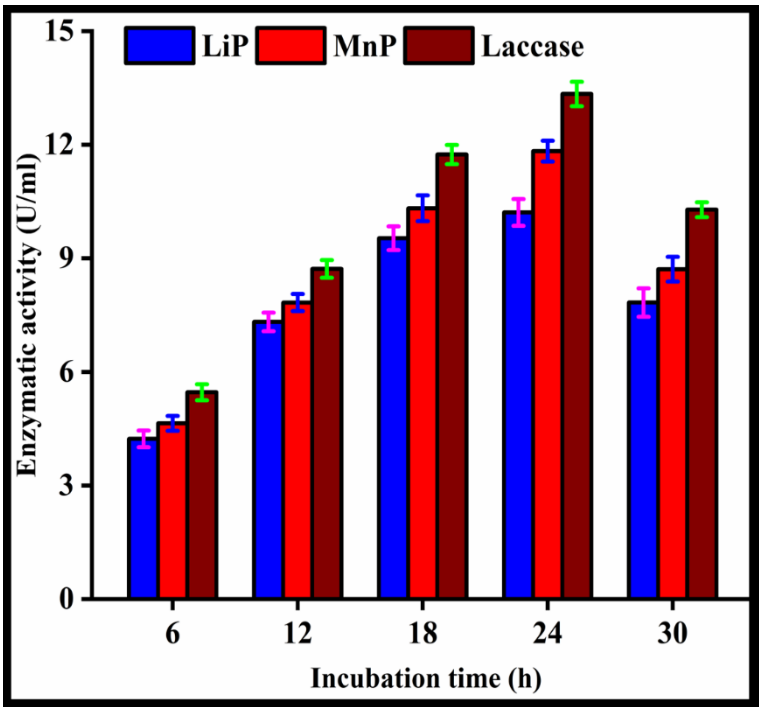


Fig. 8.1: Activity of LiP, laccase and MnP enzyme produced by bacterial consortium RKS-TEX267 during decolorization of RCPs.

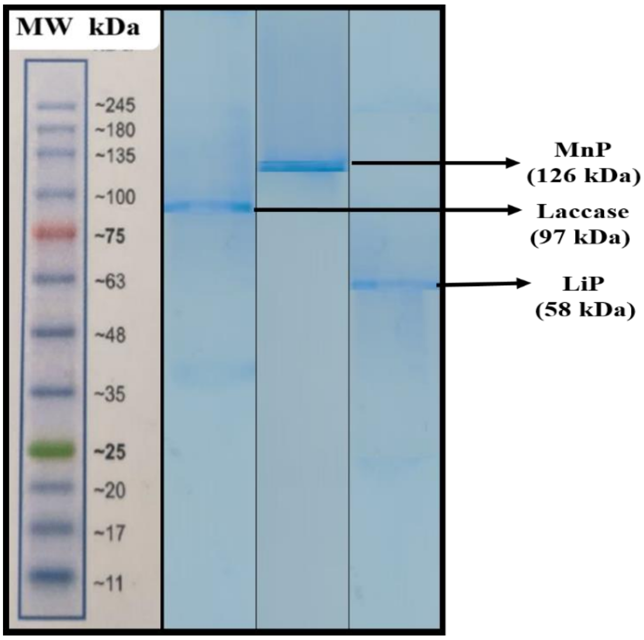


Fig: **8.2:** SDS-PAGE analysis of LiP, MnP and laccase enzyme produced during the decolorization of TWW by bacterial consortium RKS-TEX267.

8.4. Conclusion

The present study was aimed to detect and characterize the enzymes responsible for the degradation of RCPs in TWW. The ligninolytic enzyme (laccase, LiP and MnP) can degrade and mineralize various environmental pollutants into water and carbon dioxide. In present study, *Bacillus albus* (RKS2) showed LiP, *Bacillus megaterium* (RKS6) showed MnP and *Bacillus paramycoides* (RKS7) showed laccase enzyme activity during the treatment of TWW, which were characterized by the SDS-PAGE analysis and found to have molecular weight of protein around 58 kDa, 126 kDa and 97 kDa in crude extract of the LiP, MnP and laccase enzyme, respectively. Overall, these selected bacterial strains were able to degrade the RCPs present in TWW.

Chapter 9

Toxicity evaluation of textile wastewater before and after bacterial treatment byusing terrestrial /aquatic test models for environmental safety

Toxicity evaluation of textile wastewater before and after bacterial treatment by using terrestrial /aquatic test models for environmental safety

9. Introduction

Textile industries (TIs) utilized large volumes of potable water and wide range of synthetic chemicals at various stages during the production of textile products (Kishor et al., 2021a). Globally, ~ 7×10^7 tons of different dyes are produced, more than 10,000 tons of dyes are used in TIs and ~280,000 tons of dyes are released into the environment during dyeing process (Bilinska et al., 2019; Kishor et al., 2021b). Around 70% of dyes are not fixed to fibers and get discharged into water bodies along with the wastewater (Kaur et al., 2018). The wastewater discharged from TIs is very complex and has various toxic recalcitrant coloring pollutants (RCPs/dyes), dissolved solids, color content, organic pollutants and metal ions (Khandare and Govindware, 2015; Kaur et al., 2018). They persist in environment for long time and cause severe threats to water/soil ecosystem and living organisms (Srivastava and Bandhu, 2021; Yadav et al., 2022).

In aquatic ecosystem, TWW causes detrimental impacts on water quality by the coloration of water, reduced sunlight penetration, photosynthetic activity of aquatic plants and dissolved oxygen content and thus, increased in BOD and COD levels leading to anoxic conditions that adversely affect aquatic life (fauna/flora) and microbial diversity (Cao et al., 2019; Kumar et al., 2019). In soil ecosystem, it reduced soil fertility, crop yield and biodiversity by the causing soil salinity and soil pollution (Chandanshive et al., 2017; Noman et al., 2020). In many developing countries, TWW is commonly used for the irrigation of agricultural land (soil pollution). TWW also reduce seed germination, root length, shoot length and biomass production of plants (Haq et al., 2018; Kishor et al., 2021c). This uncontrolled and illegal practice paves a way for the bioaccumulation of toxic RCPs at sequentially higher trophic levels in food chain *via* consumption by humans/animals and thus, resulting in severe health threats such as diarrhea, liver, neuromuscular, hemorrhage, dermatitis, central nervous system disorder and kidney malfunctioning (Kumar et al., 2019; Kishor et al., 2021a; Yadav et al., 2022). Hence, it is essential to study the toxicity of untreated and bacteria treated TWW on routinely growing agricultural crops.

Therefore, the present study was aimed to evaluate the toxicity of TWW before and after treatment with newly developed bacterial consortium RKS-TEX267. The Mung bean (*Vigna radiata*) and Black gram (*Vigna mungo*) were used as a terrestrial model to evaluate the toxicity of untreated and bacteria treated TWW.

9.1. Materials and methods

9.1.1. Chemicals and materials

All chemicals and reagents used in experiments were of the highest purity (purity ≥ 99%) and analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA). Whatman® Grade GF/C filter papers (pore size 1.2 µm) (Whatman, England, UK) were used to filter untreated TWW. The seeds of *V. radiata* and *V. mungo* were purchased from the local market of Lucknow, Uttar Pradesh., India and used to study the toxicity effects of untreated and bacteria treated TWW.

9.1.2. Toxicity of untreated and bacterial treated TWW on the seed germination and seedling growth of *Vigna radiata* and *Vigna mungo*

The utilization of higher plants in toxicity assessment of industrial wastewater pollutants is an attractive tool to monitor the ecotoxicological threats. In this respect, the phytotoxicity assay is widely reported to evaluate the toxicity of industrial wastewater pollutants (Chandanshive et al., 2020). In present study, *V. radiata* and *V. mungo* were used as a terrestrial model to evaluate toxicity of untreated and treated TWW by developed bacterial consortium RKS-TEX267. These two seeds are widely used in Indian Agriculture (Kishor et al., 2021b). According to "OECD Guideline for the Testing of Chemicals" the *V. radiata* and *V. mungo* have high sensitivity to toxic wastewaters pollutants and compatibility with the environmental conditions within a specific time frame (Chandanshive et al., 2020).

For toxicity evaluation, briefly, 500 mL (v/v) of bacteria treated TWW was collected and centrifuged at 8000 × g for 15 min at 4 ºC to remove bacterial biomass and suspended particles. Ten uniform healthy seeds of *V. radiata* and *V. mungo* were selected, sterilized with mercuric chloride (2.0%) for two min to avoid fungal contamination followed by three times washing with deionized water. All seeds were placed in sterilized Petri-plates between filter paper (Whatman, England, UK) and irrigated with 5 mL of groundwater (control) and with the same volume of untreated and bacteria treated TWW. Afterward, the plates were incubated in BOD incubator at 28±2 ºC for 7d (Bharagava et al., 2018). The toxicity was evaluated in terms of seed germination, root length, shoot length and biomass production. Groundwater was employed as a control and toxicity test was done in triplicates at room temperature. The germination (%) was measured by the followed equation:

$$\text{Germination (\%)} = \text{No. of seeds germinated} / \text{No. of seeds} \times 100$$

9.1.3. Statistical analysis

All the laboratory experiments were performed in triplicates (n = 3) to confirm the variability and validity of the results expressed as mean ± SD.

9.2. Result and discussion

Unnao is a heavily industrialized city of Uttar Pradesh, India. It discharges a large volume of wastewater from textile industries into the environment. TWW causes harmful impacts on the nearby flora and fauna (Bharagava et al., 2018). TWW is highly toxic to plant growth and biomass production (Kishor et al., 2021b). Therefore, it is essential to study the toxicity of untreated and bacteria treated TWW on *V. radiata* and *V. mungo*.

The seed germination experiment was carried out to explore the toxic effects of untreated and bacteria treated TWW on plant growth and biomass production. In present study, the seeds irrigated with untreated TWW, showed only 40% germination in *V. radiata* and *V. mungo* **(Table 9.1).** Untreated TWW also showed (92.6% and 94.8%), (93.7% and 93.6%) and (82.6% and 89.4%) inhibition in root length, shoot length and biomass production of *V. radiata* and *V. mungo*, respectively as compared to control. The reduction in seed germination, root length, shoot length and biomass production in plants irrigated with untreated TWW indicated that TWW is highly toxic to plants growth and biomass production.

Table: 9.1. Toxicity of untreated and bacteria treated textile wastewater on *Vigna radiata* and *Vigna mungo*.

	No. of Seeds	**No. of Seeds Ger.**	**Ger. (%)**	**Root length (cm)**	**Shoot length (cm)**	**Biomass (gm)**
Vigna radiata						
Control	10	10	100%	3.53 ± 0.40	7.26 ± 0.61	2.93 ± 0.15
BT-TWW	10	10	100%	2.96 ± 0.30	6.13 ± 0.45	2.26 ± 0.20
UT-TWW	10	4	40%	0.26 ± 0.14	0.46 ± 0.20	0.51 ± 0.41
Vigna mungo						
Control	10	10	100%	4.23 ± 0.28	8.24 ± 0.35	3.24 ± 0.51
BT-TWW	10	9	90%	3.93 ± 0.25	7.86 ± 0.47	2.77 ± 0.47
UT-TWW	10	4	40%	0.22 ± 0.13	0.53 ± 0.17	0.45 ± 0.06

UT-TWW: Untreated textile wastewater; BT-TWW: Bacterial treated textile wastewater; Ger.: Germinated and germination. All the values are means of triplicates (n =3) ± SD.

But, when seeds were irrigated with bacteria treated TWW, 100% and 90% germination in *V. radiata* and *V. mungo*, respectively were recorded (**Table 9.1**). The bacteria treated TWW also showed significantly higher improvement in root length (83.9% and 92.9%), shoot length (84.4% and 95.4%) and biomass production (77.1% and 85.5%) as compared to untreated TWW **(Fig. 9.1).** Results indicated that TWW may be degraded into less/non-toxic metabolites by the developed bacterial consortium RKS-TEX267. Similarly, Kishor et al. (2021b) also reported that bacteria treated textile industry wastewater was less toxic to plant as compared to the untreated textile wastewater. The consortium treated textile industry effluent was reported as less toxic to plant than untreated textile effluent (Buntic et al., 2017).

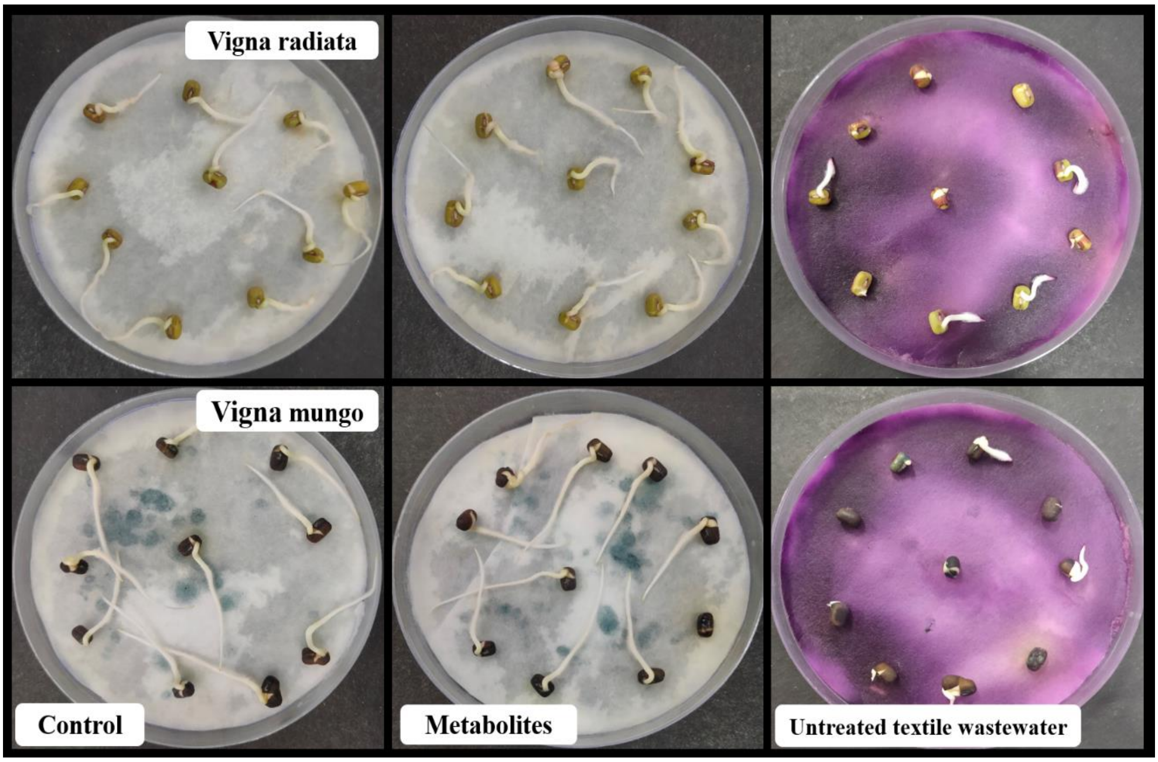


Fig: 9.1: Toxic effects of RCPs and their metabolites on *Vigna radiata* and *Vigna mungo* after two days.

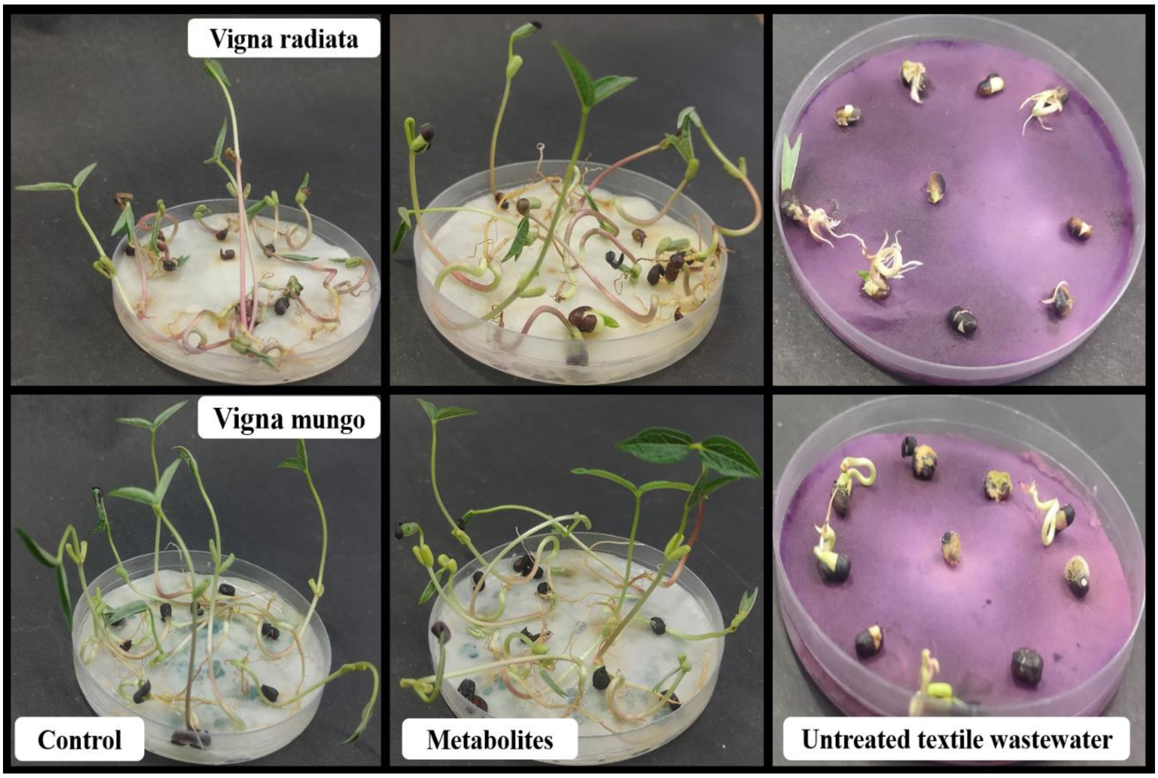


Fig: 9.2: Toxic effects of RCPs and their metabolites on *Vigna radiata* and *Vigna mungo* after seven days.

9.3. Conclusion

The present study was aimed to evaluate the toxicity of textile wastewater before and after treatment with newly developed bacterial consortium RKS-TEX267. In present study, the Untreated TWW showed (40%), (92.6% and 94.8%), (93.7% and 93.6%) and (82.6% and 89.4%) inhibition in germination, root length, shoot length and biomass production of *V. radiata* and *V. mungo,* respectively as compared to control. It clearly indicated that untreated TWW is highly toxic to plants growth and biomass production. But treated TWW showed significantly higher improvement in germination (100% and 90%), root length (83.9% and 92.9%), shoot length (84.4% and 95.4%) and biomass production (77.1% and 85.5%) as compared to untreated TWW. Results indicated that TWW may be degraded into less/non-toxic metabolites by bacterial consortium RKS-TEX267 and treated TWW may be used as a liquid fertilizer to irrigate agricultural crops.

Chapter 10

Summary and Conclusions

10. Summary and Conclusions

Textile industries (TIs) play a major role by contributing to the global economy in many developing countries. TIs contribute textile trade, world export, market value and employment for urban and rural peoples. It also shares a total 13 % of exports and 5th largest source of foreign currency. But unfortunately, these are also the major sources of environmental pollution because these discharge a large volume of highly toxic wastewater having dark color, high pH, biochemical oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS), total suspended solids (TSS), sulphate, nitrate, phosphate and phenols. TWW also contains a variety of recalcitrant coloring pollutants (RCPs/dyes), organic compounds and heavy metals, which are used in TIs during textile production processes. Various environmental protection agencies and pollution control authorities have prioritized several chemicals as highly toxic and hazardous to nature and restricted their use in TIs. These chemicals are not fully attached to fibers and get discharged into water bodies along with the wastewater. These persist in environment for long time and cause serious threats to the water and soil ecosystem along with severe toxic effects in animal and human. However, the adequate degradation and detoxification of RCPs in real TWW is required to protect the environment and public health.

Biological treatment is a sustainable, cost-effective and eco-friendly method to effectively degrade and detoxify industrial wastewater pollutants. Therefore, in present study, a total of eight (08) morphologically distinct bacterial colonies (RKS1-RKS8) were isolated from TWW and sludge samples collected from a Handloom Bhandar, Unnao, (UP), India. Further, all the isolated purified bacterial strains (RKS1-RKS8) were screened based on the lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase enzyme activity and congo red (CR), methylene blue (MB) and methyl orange (MO) dye decolorization efficiency. Out of eight (08) bacterial strains, only five bacterial strains i.e. RKS2, RKS4, RKS6, RKS7 and RKS8 were capable to produce a clear decolorization zone around the bacterial colonies on guaiacol, methylene blue and phenol red dye amended plates indicating the laccase, LiP and MnP enzyme activity.

In addition, these bacterial strains were further screened based on the CR, MO and MB dye decolorization efficiency and only three bacterial strains (RKS2, RKS6 & RKS7) showed the maximum dye decolorization efficiency. RKS2 showed 98% decolorization of MB dye, RKS6 showed 91% decolorization of CR dye and RKS7 showed 94% decolorization of MO dye. Thus, it was confirmed that the isolated bacterial strains are highly effective in degradation and mineralization of RCPs from TWW.

The isolated bacterial strains were further characterized to confirm their identity based on the morphological, biochemical and molecular identification tests. For morphological tests, the bacterium (RKS2) appeared as a white, circular and non-translucent colony on NA agar plates. It was a gram-positive, rod-shaped and non-motile bacterium. A bacterium (RKS6) appeared as milky white colonies on NA plate. It was a gram-positive, rod-shaped and non-motile bacterium. A bacterium (RKS7) appeared as milky white, concave and smooth colonies on NA plates. It was a gram-positive, motile and rod-shaped bacterium. For biochemical tests, the bacterium RKS2 showed positive reaction for catalase and VP test whereas negative for starch utilization, citrate utilization, indole production, H_2S production and urease test. RKS6 showed positive test for catalase, VP, starch utilization and citrate utilization and negative reaction for urease test, indole production and H_2S production. The bacterium RKS7 showed positive tests for urease, citrate utilization, starch utilization, catalase and MR test and negative test for VP, indole production and H_2S production.

For molecular identification, according to the 16S rRNA gene sequencing analysis, the bacterial strains RKS2, RKS6 and RKS7 showed the highest similarity with *Bacillus albus, Bacillus megaterium* and *Bacillus paramycoides,* respectively. Thus, the isolated bacterial strains RKS2, RKS6 and RKS7 were identified as *B. albus*, *B. megaterium* and *B. paramycoides* with GenBank accession numbers MW407057, OK001869 and OK001866, respectively.

A new bacterial consortium RKS-TEX267 was developed using these three potential pollutants degrading bacterial strains, *Bacillus albus* (RKS2), *Bacillus megaterium* (RKS6) and *Bacillus paramycoides* (RKS7) on the basis of performance of the single cultures in treatment of TWW. For development of bacterial consortium, the selected bacterial strains were confirmed for their compatibility test/ bio-interaction study. Results indicated that all the selected bacterial strains were able to grow with each other without forming any inhibition zone.

Afterward, the newly developed bacterial consortium RKS-TEX267 was optimized at various environmental and nutritional parameters as well as inoculum concentration and agitation rate for the effective degradation of RCPs from TWW. Results revealed that the newly developed bacterial consortium RKS-TEX267 showed 99.28% decolorization of RCPs at optimized conditions within 24h. The optimized conditions were found to be pH 7, temperature 30±2 °C, inoculum size 10%, salt concentration 1%, static condition and best carbon and nitrogen sources were found glucose and yeast extract for the effective degradation RCPs in real TWW.

The untreated real TWW showed dark color (ADMI 1354) with high values of pH (9.56), temperature (39 ºC), EC (6.36 us/m), COD (1746 mg/L), BOD (699 mg/L), TOC (3801 mg/L), TDS (7203 mg/L), TSS (501 mg/L), TS (7101 mg/L), phenol (2.27 mg/L), nitrogen (11.13 mg/L), surfactant (9.80 mg/L), chloride (1731 mg/L), sulphate (1605 mg/L) and phosphate (9.33 mg/L). It also showed high values of Cr (1.70 mg/L), Ni (4.23 mg/L), Cd (1.10 mg/L), As (2.55 mg/L), Fe (3.15 mg/L) and Pb (0.31 mg/L). All the values of various physicochemical parameters are found to be higher than permissible limit for the Central Pollution Control Board (CPCB 2013) and the National Environment Quality Standard.

After bacterial treatment, the quality of TWW improved significantly with 99%, 83.80%, 88.77%, 93.99%, 72.53%, 71.85%, 76.44%, 71.89% 85.90%, 65.58%, 34.83%, 41.05% and 46.83% reduction in color (ADMI), EC, COD, BOD, TOC, TDS, TSS, TS, phenol, nitrogen, chloride, sulphate and phosphate, respectively. The developed bacterial consortium RKS-TEX267 also removed Cr (72.35%), Cd (79.09%), Ni (83.21%), As (65.88%), Fe (32.69%) and Pb (83.87%) from real TWW.

Fourier Transform Infrared (FT-IR) and Gas Chromatography-Mass Spectrometry (GC-MS) analysis were used to identify the RCPs and their metabolites produced during the treatment process. FT-IR results showed various functional groups present in RCPs and after treatment, these functional groups convert/transform into new metabolites. Further, GC-MS results revealed that most of the RCPs present in real untreated TWW were degraded and transformed into metabolites by the newly developed bacterial consortium RKS-TEX267 at optimized conditions. Thus, results indicated that the developed bacterial consortium RKS-TEX267 can be used effectively in treatment of industrial wastewater pollutants.

Further, the degradation of RCPs are only possible due to the ligninolytic enzymes present in microorganisms. Laccase, LiP and MnP enzyme was detected during the decolorization of RCPs. Results revealed that the selected bacterial strains, *Bacillus albus* (RKS2) showed LiP, *Bacillus megaterium* (RKS7) showed MnP and *Bacillus paramycoides* (RKS7) showed laccase enzyme as confirmed by the quantitative analysis. Further, MnP, LiP and laccase enzymes were characterized by SDS-PAGE analysis and found to have molecular weight of around 126 kDa, 58 kDa and 97 kDa, respectively in the crude enzymes extract. Overall, the synergistic role of LiP, MnP and laccase enzymes may be key potential for the effective degradation of RCPs from real TWW.

In addition, the toxicity test of TWW before and after bacterial treatment was performed by using *Vigna radiata* and *Vigna mungo* as a terrestrial model. Results revealed that the untreated TWW was highly toxic in nature as it showed inhibitory effects on seed germination, root length, shoot length and biomass production of *Vgna radiata* and *Vigna mungo*. The treated TWW showed significant improvement in seed germination (100-90%), root length, shoot length and biomass production as compared to untreated TWW. Results indicated that TWW may be degraded into less/non-toxic metabolites by developed bacterial consortium RKS-TEX267. Thus, the bacteria treated TWW could be used as a liquid fertilizer for the irrigation of agricultural crops.

Overall, the present study concludes that the newly developed bacterial consortium RKS-TEX267 comprising *Bacillus albus* (RKS2), *Bacillus megaterium* (RKS6), *Bacillus paramycoides* (RKS7) were more effective in degradation and detoxification of RCPs from real TWW. This study was perhaps the first attempt on the development of a new bacterial consortium with identified potential bacterial strains and its application in degradation and detoxification of RCPs from real undiluted TWW. Therefore, this study can be useful to develop a bacteria-based treatment process for the degradation of industrial wastewater pollutants for environmental safety and to promote the sustainable development of our society with less environmental impacts.

Chapter 11

Bibliography

References

Abdessamad, N., Akrout, H., Bousselmi, L., 2015. Anodic oxidation of textile wastewaters on boron-doped diamond electrodes. Environ. Technol**.** 36(24), 3201-3209.

Afrin, S., Shuvo, H.R., Sultana, B., Islam, F., Rus'd, A.A., Begum, S., Hossain, M.N., 2021. The degradation of textile industry dyes using the effective bacterial consortium. Heliyon 7(10), 08102.

Afshariani, F., Roosta, A., 2019. Experimental study and mathematical modeling of biosorption of methylene blue from aqueous solution in a packed bed of microalgae Scenedesmus. J. Clean. Prod. 225, 133-142.

Ağtaş, M., Yılmaz, Ö., Dilaver, M., Alp, K., Koyuncu, I., 2020. Hot water recovery and reuse in textile sector with pilot scale ceramic ultrafiltration/nanofiltration membrane system. J. Clean. Prod. 256, 120359.

Al-Baldawi, I.A., Abdullah, S.R.S., Anuar, N., Hasan, H.A., 2018. Phytotransformation of methylene blue from water using aquatic plant (*Azolla pinnata*). Environ. Technol. Innov. 11, 15-22.

Ali, S.S., Al-Tohamy, R., Koutra, E., Kornaros, M., Khalil, M., Elsamahy, T., El-Shetehy, M., Sun, J., 2021. Coupling azo dye degradation and biodiesel production by manganese-dependent peroxidase producing *oleaginous* yeasts isolated from wood-feeding termite gut symbionts. Biotechnol. Biofuels 14(1), 1-25.

Almaamary, E.A.S., Abdullah, S.R.S., Hasan, H.A., Rahim, R.A.A., Idris, M., 2017. Treatment of methylene blue in wastewater using *Scirpus grossus.* Malaysian J. Anal. Sci. 21(1), 182-187.

Ameen, F., Dawoud, T.M., Alshehrei, F., Alsamhary, K., Almansob, A., 2021. Decolorization of acid blue 29, disperse red 1 and congo red by different indigenous fungal strains. Chemosphere 271, 129532.

APHA. 2012. Standard Methods for the Examination of Water and Wastewater, twenty first ed. American Public Health Association, Washington, D.C.

Arcanjo, G.S., Mounteer, A.H., Bellato, C.R., da Silva, L.M.M., Dias, S.H.B., da Silva, P.R., 2018. Heterogeneous photocatalysis using TiO_2 modified with hydrotalcite and iron oxide under UV–visible irradiation for color and toxicity reduction in secondary textile mill effluent. J. Environ. Manage. 211, 154-163.

Asgari, G., Shabanloo, A., Salari, M., Eslami, F., 2020. Sonophotocatalytic treatment of AB113 dye and real textile wastewater using ZnO/persulfate: Modeling by response surface methodology and artificial neural network. Environ. Res. 184, 109367.

Ayed, L., Khelifi, E., Jannet, H.B., Miladi, H., Cheref, A., Achour, S., Bakhrouf, A., 2010. Response surface methodology for decolorization of azo dye Methyl Orange by bacterial consortium: Produced enzymes and metabolites characterization. Chem. Eng. J. 165(1), 200-208.

Ayed, L., Ladhari, N., El Mzoughi, R., Chaieb, K., 2021. Decolorization and phytotoxicity reduction of reactive blue 40 dye in real textile wastewater by active consortium: Anaerobic/aerobic algal-bacterial-probiotic bioreactor. J. Microbiol. Methods 181, 106129.

Azizi, A., Moghaddam, M.A., Maknoon, R., Kowsari, E., 2015. Innovative combined technique for high concentration of azo dye AR18 wastewater treatment using modified SBR and enhanced Fenton process as post treatment. Process Saf. Environ. Prot. 255-264.

Bener, S., Bulca, Ö., Palas, B., Tekin, G., Atalay, S., Ersöz, G., 2019. Electrocoagulation process for the treatment of real textile wastewater: Effect of operative conditions on the organic carbon removal and kinetic study. Process Saf. Environ. Prot. 129, 47-54.

Bharagava, R.N., Mani, S., Mulla, S.I., Saratale, G.D., 2018. Degradation and decolourization potential of an ligninolytic enzyme producing *Aeromonas hydrophila* for crystal violet dye and its phytotoxicity evaluation. Ecotoxicol. Environ. Saf. 156, 166-175.

Bilińska, L., Blus, K., Gmurek, M., Ledakowicz, S., 2019. Coupling of electrocoagulation and ozone treatment for textile wastewater reuse. Chem. Eng. J. 358, 992-1001.

Bilińska, L., Gmurek, M., Ledakowicz, S., 2017. Textile wastewater treatment by AOPs for brine reuse. Process Saf. Environ. Prot. 109, 420-428.

Buntić, A.V., Pavlović, M.D., Antonović, D.G., Šiler-Marinković, S.S., Dimitrijević-Branković, S.I., 2017. A treatment of wastewater containing basic dyes by the use of new strain *Streptomyces microflavus* CKS6. J. Clean. Prod. 148, .347-354.

Buthiyappan, A., Raja Ehsan Shah, R.S.S., Asghar, A., Abdul Raman, A.A., Daud, M.A.W., Ibrahim, S., Tezel, F.H., 2019. Textile wastewater treatment efficiency by Fenton oxidation with integration of membrane separation system. Chem Eng. Commun. 206(4), 541-557.

Cai, H., Liang, J., Ning, X.A., Lai, X., Li, Y., 2020. Algal toxicity induced by effluents from textile-dyeing wastewater treatment plants. J. Environ. Sci. 91, 199-208.

Cao, J., Sanganyado, E., Liu, W., Zhang, W., Liu, Y., 2019. Decolorization and detoxification of Direct Blue 2B by indigenous bacterial consortium. J. Environ. Manage. 242, 229-237.

Ceretta, M.B., Vieira, Y., Wolski, E.A., Foletto, E.L., Silvestri, S., 2020. Biological degradation coupled to photocatalysis by ZnO/polypyrrole composite for the treatment of real textile wastewater. J. Water Process. Eng. 35, 101230.

Chandanshive, V., Kadam, S., Rane, N., Jeon, B.H., Jadhav, J., Govindwar, S., 2020. In situ textile wastewater treatment in high rate transpiration system furrows planted with aquatic macrophytes and floating phytobeds. Chemosphere 252, 126513.

Chandanshive, V.V., Kadam, S.K., Khandare, R.V., Kurade, M.B., Jeon, B.H., Jadhav, J.P., Govindwar, S.P., 2018. In situ phytoremediation of dyes from textile wastewater using garden ornamental plants, effect on soil quality and plant growth. Chemosphere 210, 968-976.

Chandanshive, V.V., Rane, N.R., Gholave, A.R., Patil, S.M., Jeon, B.H., Govindwar, S.P., 2016. Efficient decolorization and detoxification of textile industry effluent by *Salvinia molesta* in lagoon treatment. Environ. Res. 150, 88-96.

Chandanshive, V.V., Rane, N.R., Tamboli, A.S., Gholave, A.R., Khandare, R.V., Govindwar, S.P., 2017. Co-plantation of aquatic macrophytes *Typha angustifolia* and *Paspalum scrobiculatum* for effective treatment of textile industry effluent. J. Hazard. Mater. 338, 47-56.

CPCB (Central Pollution Control Board) 2013. Pollution control acts, rules and notifications issued thereunder. Pollution Control Law Series: PCLS/02/2013, MOEF, GOI, Delhi.

D’Souza, E., Fulke, A.B., Mulani, N., Ram, A., Asodekar, M., Narkhede, N. and Gajbhiye, S.N., 2017. Decolorization of Congo red mediated by marine *Alcaligenes* species isolated from Indian West coast sediments. Environ. Earth Sci. 76(20), 1-13.

Dasgupta, J., Sikder, J., Chakraborty, S., Curcio, S., Drioli, E., 2015. Remediation of textile effluents by membrane based treatment techniques: a state of the art review. J. Environ. Manage. 147, 55-72.

Dauda, M.Y., Erkurt, E.A., 2020. Investigation of reactive Blue 19 biodegradation and byproducts toxicity assessment using crude laccase extract from *Trametes versicolor*. J. Hazard. Mater. 393, 121555.

de Mello Florêncio, T., de Araújo, K.S., Antonelli, R., de Toledo Fornazari, A.L., da Cunha, P.C.R., da Silva Bontempo, L.H., de Jesus Motheo, A., Granato, A.C., Malpass, G.R.P., 2016. Photo-assisted electrochemical degradation of simulated textile effluent coupled with simultaneous chlorine photolysis. Environ. Sci. Pollut. Res, 23(19), 19292-19301.

Devaraja, S., Bharath, M., Deepak, K., Suganya, B., Vishal, B.S., Swaminathan, D., Meyyappan, N., 2017. Studies on the effect of red, blue and white LED lights on the productivity of *Chlorella vulgaris* to treat dye industry effluent. Adv. Biotech. Micro. 6(2), 27-32.

Environment Agency, United Kingdom, Potential environmental risks arising from the use of alkyl phenol ethoxylates in textiles. Revised draft of June 2008.

Eskandari, F., Shahnavaz, B., Mashreghi, M., 2019. Optimization of complete RB-5 azo dye decolorization using novel cold-adapted and mesophilic bacterial consortia. J. Environ. Manage. 241, 91-98.

Fazal, T., Razzaq, A., Javed, F., Hafeez, A., Rashid, N., Amjad, U.S., Rehman, M.S.U., Faisal, A., Rehman, F., 2020. Integrating adsorption and photocatalysis: A cost effective strategy for textile wastewater treatment using hybrid biochar-TiO2 composite. J. Hazard. Mater. 390, 121623.

GilPavas, E., Dobrosz-Gómez, I., Gómez-García, M.Á., 2017. Coagulation-flocculation sequential with Fenton or Photo-Fenton processes as an alternative for the industrial textile wastewater treatment. J. Environ. Manage. 191, 189-197.

GilPavas, E., Dobrosz-Gómez, I., Gómez-García, M.Á., 2018. Optimization of sequential chemical coagulation-electro-oxidation process for the treatment of an industrial textile wastewater. J. Water Process. Eng. 22, 73-79.

Han, G., Liang, C.Z., Chung, T.S., Weber, M., Staudt, C., Maletzko, C., 2016. Combination of forward osmosis (FO) process with coagulation/flocculation (CF) for potential treatment of textile wastewater. Water Res. 91, .361-370.

Haq, I., Raj, A., 2018. Biodegradation of Azure-B dye by *Serratia liquefaciens* and its validation by phytotoxicity, genotoxicity and cytotoxicity studies. Chemosphere 196, 58-68.

Haque, M.M., Haque, M.A., Mosharaf, M.K., Marcus, P.K., 2021. Decolorization, degradation and detoxification of carcinogenic sulfonated azo dye methyl orange by newly developed biofilm consortia. Saudi J. Biol. Sci. 28(1), 793-804.

Hayat, H., Mahmood, Q., Pervez, A., Bhatti, Z.A., Baig, S.A., 2015. Comparative decolorization of dyes in textile wastewater using biological and chemical treatment. Sep. Purif. Technol. 154, 149-153.

Herrera-González, A.M., Caldera-Villalobos, M., Peláez-Cid, A.A., 2019. Adsorption of textile dyes using an activated carbon and crosslinked polyvinyl phosphonic acid composite. J. Environ. Manage. 234, 237-244.

Hien, N.T., Nguyen, L.H., Van, H.T., Nguyen, T.D., Nguyen, T.H.V., Chu, T.H.H., Nguyen, T.V., Vu, X.H., Aziz, K.H.H., 2020. Heterogeneous catalyst ozonation of Direct Black 22 from aqueous solution in the presence of metal slags originating from industrial solid wastes. Sep. Purif. Technol. 233, 115961.

Holkar, C.R., Jadhav, A.J., Pinjari, D.V., Mahamuni, N.M., Pandit, A.B., 2016. A critical review on textile wastewater treatments: possible approaches. J. Environ. Manage. 182, 351-366.

Hubadillah, S.K., Othman, M.H.D., Tai, Z.S., Jamalludin, M.R., Yusuf, N.K., Ahmad, A., Rahman, M.A., Jaafar, J., Kadir, S.H.S.A., Harun, Z., 2020. Novel hydroxyapatite-based bio-ceramic hollow fiber membrane derived from waste cow bone for textile wastewater treatment. Chem. Eng. J. 379, 122396.

Hussain, Z., Arslan, M., Shabir, G., Malik, M.H., Mohsin, M., Iqbal, S., Afzal, M., 2019. Remediation of textile bleaching effluent by bacterial augmented horizontal flow and vertical flow constructed wetlands: a comparison at pilot scale. Sci. Total Environ. 685, 370-379.

Hussein, A., Scholz, M., 2018. Treatment of artificial wastewater containing two azo textile dyes by vertical-flow constructed wetlands. Environ. Sci. Pollut. Res. 25(7), 6870-6889.

Iark, D., dos Reis Buzzo, A.J., Garcia, J.A.A., Côrrea, V.G., Helm, C.V., Corrêa, R.C.G., Peralta, R.A., Moreira, R.D.F.P.M., Bracht, A., Peralta, R.M., 2019. Enzymatic degradation and detoxification of azo dye Congo red by a new laccase from *Oudemansiella canarii*. Bioresour. Technol. 289, 121655.

Ilamathi, R., Jayapriya, J., 2018. Microbial fuel cells for dye decolorization. Environ. Chem. Lett. 16(1), 239-250.

Jager, D., Kupka, D., Vaclavikova, M., Ivanicova, L., Gallios, G., 2018. Degradation of Reactive Black 5 by electrochemical oxidation. Chemosphere 190, 405-416.

Jegatheesan, V., Pramanik, B.K., Chen, J., Navaratna, D., Chang, C.Y., Shu, L., 2016. Treatment of textile wastewater with membrane bioreactor: a critical review. Bioresour. Technol. 204, 202-212.

Jha, P., Modi, N., Jobby, R., Desai, N., 2015. Differential expression of antioxidant enzymes during degradation of azo dye reactive black 8 in hairy roots of *Physalis minima* L. Int. J. Phytoremediation 17(4), 305-312.

Joint Research Centre (JRC) Environmental Improvement Potential of Textiles (IMPRO Textiles) http://ipts.jrc.ec.europa.eu/publications/pub.cfm?id=6960 (2014).

Kadam, S.K., Watharkar, A.D., Chandanshive, V.V., Khandare, R.V., Jeon, B.H., Jadhav, J.P., Govindwar, S.P., 2018. Co-planted floating phyto-bed along with microbial fuel cell for enhanced textile effluent treatment. J. Clean. Prod. 203, 788-798.

Kagalkar, A.N., Khandare, R.V., Govindwar, S.P., 2015. Textile dye degradation potential of plant laccase significantly enhances upon augmentation with redox mediators. RSC advances 5(98), 80505-80517.

Kaur, P., Kushwaha, J.P., Sangal, V.K., 2018. Electrocatalytic oxidative treatment of real textile wastewater in continuous reactor: degradation pathway and disposability study. J. Hazard. Mater. 346, 242-252.

KEMI (Swedish Chemical Agency), Chemicals in textiles-risks to human health and the environment. Report from a government assignment. (2014) Report No 6/14.

Khan, R., Fulekar, M.H., 2017. Mineralization of a sulfonated textile dye Reactive Red 31 from simulated wastewater using pellets of *Aspergillus bombycis*. Bioresour. Bioprocess 4(1), 1-11.

Khan, S., Bhardwaj, U., Iqbal, H.M., Joshi, N., 2021. Synergistic role of bacterial consortium to biodegrade toxic dyes containing wastewater and its simultaneous reuse as an added value. Chemosphere 284, 131273.

Khan, S., Malik, A., 2018. Toxicity evaluation of textile effluents and role of native soil bacterium in biodegradation of a textile dye. Environ. Sci. Pollut. Res. 25(5), 4446-4458.

Khandare, R.V., Govindwar, S.P., 2015. Phytoremediation of textile dyes and effluents: Current scenario and future prospects. Biotechnol. Adv. 33(8), 1697-1714.

Kishor, R., Purchase, D., Saratale, G.D., Ferreira, L.F.R., Bilal, M., Iqbal, H.M., Bharagava, R.N., 2021b. Environment friendly degradation and detoxification of

Congo red dye and textile industry wastewater by a newly isolated *Bacillus cohnni* (RKS9). Environ. Technol. Innov. 22, 101425.

Kishor, R., Purchase, D., Saratale, G.D., Ferreira, L.F.R., Hussain, C.M., Mulla, S.I., Bharagava, R.N., 2021d. Degradation mechanism and toxicity reduction of methyl orange dye by a newly isolated bacterium *Pseudomonas aeruginosa* MZ520730. J. Water Process. Eng. 43, 102300.

Kishor, R., Purchase, D., Saratale, G.D., Saratale, R.G., Ferreira, L.F.R., Bilal, M., Chandra, R., Bharagava, R.N., 2021a. Ecotoxicological and health concerns of persistent coloring pollutants of textile industry wastewater and treatment approaches for environmental safety. J. Environ. Chem. Eng. 9(2), 105012.

Kishor, R., Saratale, G.D., Saratale, R.G., Ferreira, L.F.R., Bilal, M., Iqbal, H.M., Bharagava, R.N., 2021c. Efficient degradation and detoxification of methylene blue dye by a newly isolated ligninolytic enzyme producing bacterium *Bacillus Albus* MW407057. Colloids and Surfaces B: Biointerfaces, 206, 111947.

Kumar, A., Sharma, G., Naushad, M., Ala'a, H., García-Peñas, A., Mola, G.T., Si, C., Stadler, F.J., 2020. Bio-inspired and biomaterials-based hybrid photocatalysts for environmental detoxification: A review. Chem. Eng. J. 382, 122937.

Kurade, M.B., Waghmode, T.R., Kagalkar, A.N., Govindwar, S.P., 2012. Decolorization of textile industry effluent containing disperse dye Scarlet RR by a newly developed bacterial-yeast consortium BL-GG. Chem. Eng. J. 184, 33-41.

Lai, C.Y., Wu, C.H., Meng, C.T., Lin, C.W., 2017. Decolorization of azo dye and generation of electricity by microbial fuel cell with laccase-producing white-rot fungus on cathode. Appl. Energy 188, 392-398.

Lallawmsanga, Leo, V.V., Passari, A.K., Muniraj, I.K., Uthandi, S., Hashem, A., Abd_Allah, E.F., Alqarawi, A.A., Singh, B.P., 2019. Elevated levels of laccase synthesis by *Pleurotus pulmonarius* BPSM10 and its potential as a dye decolorizing agent. Saudi J. Biol. Sci. 26(3), 464-468.

Liu, W., Liu, C., Liu, L., You, Y., Jiang, J., Zhou, Z., Dong, Z., 2017. Simultaneous decolorization of sulfonated azo dyes and reduction of hexavalent chromium under high salt condition by a newly isolated salt-tolerant strain *Bacillus circulans* BWL1061. Process Saf. Environ. Prot. 141, 9-16.

Louati, I., Elloumi-Mseddi, J., Cheikhrouhou, W., Hadrich, B., Nasri, M., Aifa, S., Woodward, S., Mechichi, T., 2020. Simultaneous cleanup of Reactive Black 5 and cadmium by a desert soil bacterium. Ecotoxicol. Environ. Saf. 190, 110103.

Miran, W., Jang, J., Nawaz, M., Shahzad, A., Lee, D.S., 2018. Sulfate-reducing mixed communities with the ability to generate bioelectricity and degrade textile diazo dye in microbial fuel cells. J. Hazard. Mater. 352, 70-79.

Narde, G.K., Kapley, A., Purohit, H.J., 2004. Isolation and characterization of *Citrobacter* strain HPC255 for broad-range substrate specificity for chlorophenols. Curr. Microbiol. 48 419-423.

Navada, K.K., Kulal, A., 2020. Enhanced production of laccase from gamma irradiated endophytic fungus: A study on biotransformation kinetics of aniline blue and textile effluent decolourisation. J. Environ. Chem. Eng. 8(2), 103550.

Noman, M., Shahid, M., Ahmed, T., Niazi, M.B.K., Hussain, S., Song, F., Manzoor, I., 2020. Use of biogenic copper nanoparticles synthesized from a native *Escherichia* sp. as photocatalysts for azo dye degradation and treatment of textile effluents. Environ. Pollut, 257, 113514.

Núñez, J., Yeber, M., Cisternas, N., Thibaut, R., Medina, P., Carrasco, C., 2019. Application of electrocoagulation for the efficient pollutants removal to reuse the treated wastewater in the dyeing process of the textile industry. J. Hazard. Mater. 371, 705-711.

Oktem, Y.A., Yuzer, B., Aydin, M.I., Okten, H.E., Meric, S., Selcuk, H., 2019. Chloride or sulfate? Consequences for ozonation of textile wastewater. J. Environ. Manage. 247, 749-755.

Oon, Y.L., Ong, S.A., Ho, L.N., Wong, Y.S., Dahalan, F.A., Oon, Y.S., Teoh, T.P., Lehl, H.K., Thung, W.E., 2020. Constructed wetland–microbial fuel cell for azo dyes degradation and energy recovery: Influence of molecular structure, kinetics, mechanisms and degradation pathways. Sci. Total Environ. 720, 137370.

Paz, A., Carballo, J., Pérez, M.J., Domínguez, J.M., 2017. Biological treatment of model dyes and textile wastewaters. Chemosphere 181, 168-177.

Paździor, K., Bilińska, L., Ledakowicz, S., 2019. A review of the existing and emerging technologies in the combination of AOPs and biological processes in industrial textile wastewater treatment. Chem. Eng. J. 376, 120597.

Punzi, M., Nilsson, F., Anbalagan, A., Svensson, B.M., Jönsson, K., Mattiasson, B., Jonstrup, M., 2015. Combined anaerobic–ozonation process for treatment of textile wastewater: removal of acute toxicity and mutagenicity. J. Hazard. Mater. 292, 52-60.

Rane, N.R., Chandanshive, V.V., Watharkar, A.D., Khandare, R.V., Patil, T.S., Pawar, P.K., Govindwar, S.P., 2015. Phytoremediation of sulfonated Remazol Red dye and textile effluents by *Alternanthera philoxeroides*: an anatomical, enzymatic and pilot scale study. Water Res. 83, 271-281.

Rane, N.R., Patil, S.M., Chandanshive, V.V., Kadam, S.K., Khandare, R.V., Jadhav, J.P., Govindwar, S.P., 2016. *Ipomoea hederifolia* rooted soil bed and *Ipomoea aquatica* rhizofiltration coupled phytoreactors for efficient treatment of textile wastewater. Water Res. 96, 1-11.

Revathi, S., Kumar, S.M., Santhanam, P., Kumar, S.D., Son, N., Kim, M.K., 2017. Bioremoval of the indigo blue dye by immobilized microalga *Chlorella vulgaris* (PSBDU06). J. Sci. Ind. Res. 76(1), 50-56

Rondon, H., El-Cheikh, W., Boluarte, I.A.R., Chang, C.Y., Bagshaw, S., Farago, L., Jegatheesan, V., Shu, L., 2015. Application of enhanced membrane bioreactor (eMBR) to treat dye wastewater. Bioresour. Technol. 183, 78-85.

Rosa, J.M., Tambourgi, E.B., Vanalle, R.M., Gamarra, F.M.C., Santana, J.C.C., Araújo, M.C., 2020. Application of continuous H2O2/UV advanced oxidative process as an option to reduce the consumption of inputs, costs and environmental impacts of textile effluents. J. Clean. Prod. 246, 119012.

Rosu, C.M., Avadanei, M., Gherghel, D., Mihasan, M., Mihai, C., Trifan, A., Miron, A., Vochita, G., 2018. Biodegradation and detoxification efficiency of azo-dye reactive orange 16 by *Pichia kudriavzevii* CR-Y103. Water Air Soil Pollut. 229(1), 1-18.

Safauldeen, S.H., Abu Hasan, H., Abdullah, S.R.S., 2019. Phytoremediation efficiency of water hyacinth for batik textile effluent treatment. J. Ecol. Eng. 20(9).

Sahasrabudhe, M.M., Saratale, R.G., Saratale, G.D., Pathade, G.R., 2014. Decolorization and detoxification of sulfonated toxic diazo dye CI Direct Red 81 by *Enterococcus faecalis* YZ 66. J. Environ. Health Sci**.** 12(1), 1-13.

Sahinkaya, E., Sahin, A., Yurtsever, A., Kitis, M., 2018. Concentrate minimization and water recovery enhancement using pellet precipitator in a reverse osmosis process treating textile wastewater. J. Environ. Manage. 222, 420-427.

Samuchiwal, S., Gola, D., Malik, A., 2021. Decolourization of textile effluent using native microbial consortium enriched from textile industry effluent. J. Hazard. Mater. 402, 123835.

Samuchiwal, S., Gola, D., Malik, A., 2021. Decolourization of textile effluent using native microbial consortium enriched from textile industry effluent. J. Hazard. Mater. 402, 123835.

Saratale, R.G., Ghodake, G.S., Shinde, S.K., Cho, S.K., Saratale, G.D., Pugazhendhi, A., Bharagava, R.N., 2018. Photocatalytic activity of CuO/Cu (OH) 2 nanostructures in the degradation of Reactive Green 19A and textile effluent, phytotoxicity studies and their biogenic properties (antibacterial and anticancer). J. Environ. Manage. 223, 1086-1097.

Saratale, R.G., Saratale, G.D., Govindwar, S.P., Kim, D.S., 2015. Exploiting the efficacy of *Lysinibacillus* sp. RGS for decolorization and detoxification of industrial dyes, textile effluent and bioreactor studies. J. Environ. Sci. Health, Part A, 50(2), 176-192.

Satish, K.R., 2018. Indian textile industry: Opportunities, challenges and suggestions. Trends Textile Eng. Fashion Technol. 2, 1-5.

Sen, S.K., Patra, P., Das, C.R., Raut, S., Raut, S., 2020. Pilot-scale evaluation of bio-decolorization and biodegradation of reactive textile wastewater: an impact on its use in irrigation of wheat crop. Water Resour. Ind. 21, 100106.

Shanmugam, L., Ahire, M., Nikam, T., 2020. *Bacopa monnieri* (L.) Pennell, a potential plant species for degradation of textile azo dyes. Environ. Sci. Pollu. Res. 27(9), 9349-9363.

Sharma, R., Malaviya, P., 2021. Constructed wetlands for textile wastewater remediation: A review on concept, pollutant removal mechanisms, and integrated technologies for efficiency enhancement. Chemosphere 133358.

Singh, R.L., Singh, P.K., Singh, R.P., 2015. Enzymatic decolorization and degradation of azo dyes–A review. Int. Biodeterior. Biodegr. 104, 21-31.

Sinha, S., Singh, R., Chaurasia, A.K., Nigam, S., 2016. Self-sustainable *Chlorella pyrenoidosa* strain NCIM 2738 based photobioreactor for removal of Direct Red-31 dye along with other industrial pollutants to improve the water-quality. J. Hazard. Mater. 306, 386-394.

Song, L., Shao, Y., Ning, S., Tan, L., 2017. Performance of a newly isolated salt-tolerant yeast strain *Pichia occidentalis* G1 for degrading and detoxifying azo dyes. Bioresour. Technol. 233, 21-29.

Srivastava, A., Bandhu, S., 2022. Biotechnological advancements and challenges in textile effluents management for a sustainable bioeconomy: Indian case studies. CSCEE, 100186.

Statista, 2016. Worldwide production volume of chemical and fibers from (1975 to 2014) The statistics portal. http://www.statista.com/statistics/263154/worldwide-production-volume-of-textilefibers- since-1975/. Accessed 25 May 2016 sustainable-textiles/valuing-our-clothes%20. Accessed 8 Sept 2016.

Sun, Y., Cheng, S., Lin, Z., Yang, J., Li, C., Gu, R., 2020. Combination of plasma oxidation process with microbial fuel cell for mineralizing methylene blue with high energy efficiency. J. Hazard. Mater. 384, 121307.

Tara, N., Arslan, M., Hussain, Z., Iqbal, M., Khan, Q.M., Afzal, M., 2019. On-site performance of floating treatment wetland macrocosms augmented with dye-degrading bacteria for the remediation of textile industry wastewater. J. Clean. Prod. 217, 541-548.

Tian, Q., Ran, M., Fang, G., Ding, L., Pan, A., Shen, K., Deng, Y., 2020. ZnAl2O4/BiPO4 composites as a heterogeneous catalyst for photo-Fenton treatment of textile and pulping wastewater. Sep. Purif. Technol. 239, 116574.

Ting, A.S.Y., Lee, M.V.J., Chow, Y.Y., Cheong, S.L., 2016. Novel exploration of endophytic *Diaporthe* sp. for the biosorption and biodegradation of triphenylmethane dyes. Wat. Air Soil Poll. 227(4), 1-8.

Torbati, S., 2015. Feasibility and assessment of the phytoremediation potential of duckweed for triarylmethane dye degradation with the emphasis on some physiological responses and effect of operational parameters. Turk. J. Biol. 39(3), 438-446.

Viana, D.F., Salazar-Banda, G.R., Leite, M.S., 2018. Electrochemical degradation of Reactive Black 5 with surface response and artificial neural networks optimization models. Sep. Purif. Technol. 53(16), 2647-2661.

Vieira, G.A.L., Cabral, L., Otero, I.V.R., Ferro, M., de Faria, A.U., de Oliveira, V.M., Bacci, M., Sette, L.D., 2021. Marine associated microbial consortium applied to RBBR textile dye detoxification and decolorization: Combined approach and metatranscriptomic analysis. Chemosphere 267, 129190.

Waghmode, T.R., Kurade, M.B., Sapkal, R.T., Bhosale, C.H., Jeon, B.H., Govindwar, S.P., 2019. Sequential photocatalysis and biological treatment for the enhanced degradation of the persistent azo dye methyl red. J. Hazard. Mater. 371, 115-122.

Wang, T.N., Lu, L., Wang, J.Y., Xu, T.F., Li, J. and Zhao, M., 2015. Enhanced expression of an industry applicable CotA laccase from *Bacillus subtilis* in *Pichia pastoris* by non-repressing carbon sources together with pH adjustment: recombinant enzyme characterization and dye decolorization. Process Biochem**.** 50(1), 97-103.

Wang, W.L., Hu, H.Y., Liu, X., Shi, H.X., Zhou, T.H., Wang, C., Huo, Z.Y., Wu, Q.Y., 2019. Combination of catalytic ozonation by regenerated granular activated carbon (rGAC) and biological activated carbon in the advanced treatment of textile wastewater for reclamation. Chemosphere 231, 369-377.

Wang, W.L., Hu, H.Y., Liu, X., Shi, H.X., Zhou, T.H., Wang, C., Huo, Z.Y., Wu, Q.Y., 2019. Combination of catalytic ozonation by regenerated granular activated carbon (rGAC) and biological activated carbon in the advanced treatment of textile wastewater for reclamation. Chemosphere 231, 369-377.

Wanyonyi, W.C., Onyari, J.M., Shiundu, P.M., Mulaa, F.J., 2019. Effective biotransformation of reactive black 5 dye using crude protease from *Bacillus cereus* strain KM201428. Energy Procedia 157, 815-824.

Watharkar, A.D., Kadam, S.K., Khandare, R.V., Kolekar, P.D., Jeon, B.H., Jadhav, J.P., Govindwar, S.P., 2018. *Asparagus densiflorus* in a vertical subsurface flow phytoreactor for treatment of real textile effluent: a lab to land approach for in situ soil remediation. Ecotoxicol. Environ. Saf. 161, 70-77.

Watharkar, A.D., Khandare, R.V., Waghmare, P.R., Jagadale, A.D., Govindwar, S.P., Jadhav, J.P., 2015. Treatment of textile effluent in a developed phytoreactor with immobilized bacterial augmentation and subsequent toxicity studies on *Etheostoma olmstedi* fish. J. Hazard. Mater. 283, 698-704.

Yadav, A., Kumar, P., Rawat, D., Garg, S., Mukherjee, P., Farooqi, F., Roy, A., Sundaram, S., Sharma, R.S., Mishra, V., 2022. Microbial fuel cells for mineralization and decolorization of azo dyes: Recent advances in design and materials. Sci. Total Environ. 154038.

Yurtsever, A., Calimlioglu, B., Sahinkaya, E., 2017. Impact of SRT on the efficiency and microbial community of sequential anaerobic and aerobic membrane bioreactors for the treatment of textile industry wastewater. Chem. Eng. J. 314, 378-387.

Zhang, H., Zhang, J., Zhang, X., Geng, A., 2018. Purification and characterization of a novel manganese peroxidase from white-rot fungus *Cerrena unicolor* BBP6 and its application in dye decolorization and denim bleaching. Process Biochem. 66, 222-229.

Zhuang, M., Sanganyado, E., Zhang, X., Xu, L., Zhu, J., Liu, W., Song, H., 2020. Azo dye degrading bacteria tolerant to extreme conditions inhabit nearshore ecosystems: optimization and degradation pathways. J. Environ. Manage. 261, 110222.

Publications

Review/Research papers

1. **Kishor, R.,** Raj, A., Bharagava, R.N., 2022. Synergistic role of bacterial consortium (RKS-AMP) for treatment of recalcitrant coloring pollutants of textile industry wastewater. J. Water Process. Eng. 47, 102700.

2. **Kishor, R.,** Purchase, D., Saratale, G.D., Saratale, R.G., Ferreira, L.F.R., Bilal, M., Chandra, R., Bharagava, R.N., 2021. Ecotoxicological and health concerns of persistent coloring pollutants of textile industry wastewater and treatment approaches for environmental safety. J. Environ. Chem. Eng. 9(2), 105012.

3. **Kishor, R.,** Purchase, D., Saratale, G.D., Ferreira, L.F.R., Bilal, M., Iqbal, H.M., Bharagava, R.N., 2021. Environment friendly degradation and detoxification of Congo red dye and textile industry wastewater by a newly isolated *Bacillus cohnni* (RKS9). Environ. Technol. Innov. 22, 101425.

4. **Kishor, R.,** Saratale, G.D., Saratale, R.G., Ferreira, L.F.R., Bilal, M., Iqbal, H.M., Bharagava, R.N., 2021. Efficient degradation and detoxification of methylene blue dye by a newly isolated ligninolytic enzyme producing bacterium *Bacillus Albus* MW407057. Colloids Surf. B: Biointerfaces 206, 111947.

5. **Kishor, R.,** Purchase, D., Saratale, G.D., Ferreira, L.F.R., Hussain, C.M., Mulla, S.I., Bharagava, R.N., 2021. Degradation mechanism and toxicity reduction of methyl orange dye by a newly isolated bacterium *Pseudomonas aeruginosa* MZ520730. J. Water Process. Eng. 43, 102300.

6. Saxena, G., **Kishor, R.,** Purchase, D. and Bharagava, R.N., 2019. Chandra R, Dubey NK, Kumar V: Phytoremediation of environmental pollutants. Environ. Earth Sci. 78, 418.

Book chapters

1. **Kishor, R.,** Bharagava, R.N., Saxena, G., 2018. Industrial wastewaters: the major sources of dye contamination in the environment, ecotoxicological effects, and bioremediation approaches. In book: Recent Advances in Environmental Management by Bharagava, R.N. (Ed.). CRC Press/Taylor & Francis Group, Boca Raton 1-25.

2. Saxena, G., **Kishor, R.,** Saratale, G.D., Bharagava, R.N., 2020. Genetically modified organisms (GMOs) and their potential in environmental management: constraints, prospects and challenges. In book: Bioremediation of industrial waste for environmental safety - Vol. 11: Biological Agents and Methods for Industrial Waste Management by R.N. Bharagava and G. Saxena (Eds.). Springer Nature, Singapore. 1-19,

3. Saxena, G., **Kishor, R.**, Bharagava, R.N., 2020. Application of microbial enzymes in degradation and detoxification of organic and inorganic pollutants. In book: Bioremediation of industrial waste for environmental safety. Vol. 1: Industrial Waste and its Management by G. Saxena and R.N. Bharagava (Eds.). Springer Nature, Singapore 41-51.

4. **Kishor, R.,** Purchase, D., Ferreira, L.F., Mulla, S.I., Bilal, M., Bharagava, R.N. 2020. Environmental and health hazards of textile industry wastewater pollutants and its treatment approaches. In book: Handbook of Environmental Materials Management by Hussain, C.M. (Ed.). Springer Nature, Switzerland 1-24.

5. Saxena, G., **Kishor, R.,** Zainith, S., Bharagava, R.N., 2021. Environmental contamination, toxicity profile and bioremediation technologies for treatment and detoxification of textile effluent. In book: Bioremediation for Environmental Sustainability by Saxena, G. Kumar, V. and Shah M.P. (Eds). Elsevier, United States of America (USA) 415-434.

6. Zainith, S., Saxena, G., **Kishor, R.**, Bharagava, R.N., 2021. Application of microalgae in industrial effluent treatment, contaminants removal, and biodiesel production: Opportunities, challenges, and future prospects. In book: Bioremediation for Environmental Sustainability by Saxena, G. Kumar, V. and Shah M.P. (Eds). Elsevier, United States of America (USA) 481-517.

7. Saxena, G., **Kishor, R.,** Bharagava, R.N., Das, P., Gupta, P.K., Kumar, N., 2021. Emerging green technologies for biological treatment of leather tannery chemicals and wastewater. In book: Bioremediation for Environmental Sustainability by Saxena, G. Kumar, V. and Shah M.P. (Eds). Elsevier, United States of America (USA) 435-457.

8. **Kishor, R.,** Bharagava, R.N., Ferreira, L.F.R., Bilal, M., Purchase, D., 2021. Molecular techniques used to identify perfluorooctanoic acid degrading microbes and their application in a wastewater treatment reactor/plant. In book: Wastewater Treatment Reactors by Shah M.P. and Rodriguez-Couto (Eds). Elsevier, USA 253-271.
9. Singh, A., **Kishor, R.,** Bharagava R.N., Yadav, B.C., Existing and emerging treatment technologies for the degradation and detoxification of textile industry wastewater for the environmental safety. In book: Bioremediation: Green Approaches for a Clean and Sustainable Environment. CRC Press/Taylor & Francis Group.
10. **Kishor, R.,** Singh, A., More, N.K., and Bharagava R.N., A Sustainable Approach to the Degradation and Detoxification of Textile Industry Wastewater for Environmental Safety. In book: Bioremediation: Green Approaches for a Clean and Sustainable Environment. CRC Press/Taylor & Francis Group.

Paper and Chapters communicated

1. **Kishor, R.,** and Bharagava R.N. Biotreatment and production of value added products from waste algal biomass. In book: Bio-based Materials & Waste for Energy Generation & Resources Management by Hussain C.M. and Bharagava R.N. (Ed.). Elsevier, Newark NJ, USA.
2. Singh, A., **Kishor, R.,** Bharagava, R.N., Yadav, B.C., Heterostructure based ZnO/NiO functional nanomaterial for liquified petroleum gas (LPG) sensing and photocatalytic activity at ambient room temperature. J. Environ. Chem. Eng.

Paper Presented

1. **Roop Kishor** and Ram Naresh Bharagava. Isolation and characterization of bacteria capable for the degradation and decolorization of textile industry wastewater for environmental safety. In: **International Conference on Environmental sustainability: Innovations, Translations Dimensions and Way Forward,** organized by **“Department of Energy and Environment’’,** Babasaheb Bhimrao Ambedkar University (BBAU), Lucknow, Uttar Pradesh, India **(Poster Presentation).**

2. **Roop Kishor** and Ram Naresh Bharagava. Development of bacterial consortium for the degradation and detoxification of textile industry wastewater. In: ‘‘**Integrated Approaches in Science & Technology for Sustainable Future**’’, held on **February 28th to March 01th, 2022,** organized by ‘‘**Faculty of Sciences and Faculty of Life Sciences**’’, **J. C. Bose University of Science and Technology, Fridabad, Haryana,** India **(Poster Presentation).**

Conferences and Workshop

1. **Attended and successfully** completed a international conference on ‘‘**Integrated Approaches in Science & Technology for Sustainable Future**’’, organized by ‘‘**Faculty of Sciences and Faculty of Life Sciences**’’, **J. C. Bose University of Science and Technology, Fridabad, Haryana,** India from **February 28 to March 01, 2022.**
2. **Attended and successfully** completed a international conference program on **“Environmental sustainability: Innovations, Translations Dimensions and Way Forward** organized by **“Department of Energy and Environment’’, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh**, India from **February 11 to February 12, 2020.**
3. **Attended and successfully** completed a Integrated Workshop program on **“Publication Ethics and Patenting’’** organized by **“Department of Energy and Environment’’, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh,** India from **February 10, 2020.**
4. **Attended and successfully** completed a Workshop program on **“Basic of Flow Cytometry and its Application in Biomedical Sciences** organized by **“Department of Biotechnology’’ Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh**, India from **March 05 to 7March, 2020.**

Reprints

Journal of Water Process Engineering 47 (2022) 102700

Contents lists available at ScienceDirect

Journal of Water Process Engineering

journal homepage: www.elsevier.com/locate/jwpe

Synergistic role of bacterial consortium (RKS-AMP) for treatment of recalcitrant coloring pollutants of textile industry wastewater

Roop Kishor [a], Abhay Raj [b], Ram Naresh Bharagava [a,*]

[a] Laboratory of Bioremediation and Metagenomics Research (LBMR), Department of Environmental Microbiology (DEM), Babasaheb Bhimrao Ambedkar University (A Central University), Vidya Vihar, Raebareli Road, Lucknow 226 025, U.P., India.

[b] Environmental Microbiology Section, CSIR-Indian Institute of Toxicology Research (CSIR-IITR), Post Box 80, M.G. Marg, Lucknow 226 001, Uttar Pradesh, India

ARTICLE INFO

Keywords:
Recalcitrant coloring pollutants
Textile industry wastewater
Bacterial consortium
Degradation
Ligninolytic enzyme
FT-IR analysis
GC–MS analysis
Toxicity

ABSTRACT

In this study, a bacterial consortium (RKS-AMP) was developed, which showed 99.28% decolorization of RCPs with significant reduction in COD (88.77%), BOD (93.99%), TOC (72.53%), phenol (85.90%) within 15 h at 30 ± 2 °C, pH 7, inoculum size (10%), salt concentration (1%), static condition in presence of glucose and yeast extract as carbon and nitrogen source, respectively. It also removed 72.35% of (Cr), 79.09% of (Cd), 65.88% (As) and Pb (83.87%) at same conditions. During RCPs treatment, lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase enzyme activities were also recorded indicating that these enzymes are involved in RCPs degradation. The RCPs present in TIWW and their metabolites produced during the treatment process were identified by FT-IR and GC–MS analysis. In addition, the toxicity studies showed that metabolites of RCPs allowed 100–90% seed germination with significant improvement in root length, shoot length and biomass production in *Vigna radiata* and *Vigna mungo* indicating that the developed bacterial consortium (RKS-AMP) was effective in degradation and detoxification of RCPs. Thus, this newly developed bacterial consortium (RKS-AMP) can be used effectively in degradation and detoxification of textile industry wastewater pollutants.

1. Introduction

Textile industries (TIs) play a major role by contributing to global economy in developing countries [1,2]. Worldwide, TIs contribute ~5% of total textile trade, ~5% of total export, ~$2000 billion of market value and around 120 million employments for urban and rural peoples [2,3]. But, TIs discharge a large volume of highly polluted wastewater into the environment. In China and India, around 1.84×10^9 m^3 and 640 million m^3 wastewater is discharged from TIs annually [4,5]. Textile industry wastewater (TIWW) is characterized by dark color, high pH, biochemical oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS), total suspended solids (TSS) and total organic carbon (TOC) [1,2,6]. In addition, TIWW has various recalcitrant coloring pollutants (RCPs/dyes), organic chemicals and toxic metal ions [1,3]. Among these, RCPs are the major source of environmental pollution and health hazards [1,4].

More than thousands of RCPs are used in TIs. ~70% of dyes are not fixed to fibers and 300,000 tons of RCPs are discharged into the environment worldwide [2,4]. RCPs are recalcitrant in nature and transported to a long distance along with wastewater, persist in environment for long time and cause severe threats to water, soil and living organisms [3,4]. In water ecosystem, RCPs reduce sunlight penetration power, photosynthetic activity of aquatic plants and dissolved oxygen (DO) content and thus, increases COD, BOD and TOC level and gives dark color to receiving water bodies, which ultimately damage aquatic life (flora/fauna) [1,6]. In soil ecosystem, these cause detrimental effects on soil property and microbial diversity by the accumulation of RCPs and heavy metals [6,7]. RCPs inhibit seed germination, root length, shoot length and biomass production in plants [2,7]. RCPs cause nausea, haemorrhage and ulceration of skin and also cause cancer of spleen and urinary blander in humans and animals [1,4,7]. TIWW causes allergy, asthma, skin eczema, immunosuppression, hypersensitivity and intercalate with helical structure of DNA, duplex RNA and partitioned to lipid membrane of living cells [4,8]. RCPs also reported as carcinogenic, mutagenic, teratogenic, allergenic and cytotoxic to rat, fish, mollusc, microbe and mammalian cells [2,3]. RCPs damage heart, lung, liver and kidneys in living beings [9,10]. TIWW also reduced food intake, growth, fertility rate and damaged kidney, spleen, brain, nervous system and

---

[*] Corresponding author at: Laboratory of Bioremediation and Metagenomics Research (LBMR), Department of Environmental Microbiology (DEM), Babasaheb Bhimrao Ambedkar University (A Central University), Vidya Vihar, Raebareli Road, Lucknow 226 025, U.P., India.
E-mail addresses: ramnaresh_dem@bbau.ac.in, bharagavarnbbau11@gmail.com (R.N. Bharagava).

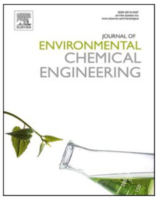
https://doi.org/10.1016/j.jwpe.2022.102700
Received 17 November 2021; Received in revised form 26 February 2022; Accepted 27 February 2022
2214-7144/© 2022 Published by Elsevier Ltd.

Journal of Environmental Chemical Engineering 9 (2021) 105012

Contents lists available at ScienceDirect

Journal of Environmental Chemical Engineering

journal homepage: www.elsevier.com/locate/jece



Review article

Ecotoxicological and health concerns of persistent coloring pollutants of textile industry wastewater and treatment approaches for environmental safety

Roop Kishor[a], Diane Purchase[b], Ganesh Dattatraya Saratale[c], Rijuta Ganesh Saratale[d], Luiz Fernando Romanholo Ferreira[e,f], Muhammad Bilal[g], Ram Chandra[a], Ram Naresh Bharagava[a,*]

[a] *Laboratory of Bioremediation and Metagenomics Research (LBMR), Department of Microbiology (DM), Babasaheb Bhimrao Ambedkar University (A Central University), Vidya Vihar, Raebareli Road, Lucknow 226 025 U.P., India*
[b] *Department of Natural Sciences, Faculty of Science and Technology, Middlesex University, The Burroughs, Hendon, London NW4 4BT, England, UK*
[c] *Department of Food Science and Biotechnology, Dongguk University, Ilsandong-gu, Goyang-si, Seoul, Gyeonggi-do 10326, Republic of Korea*
[d] *Research Institute of Biotechnology and Medical Converged Science, Dongguk University, Ilsandong-gu, Goyang-si, Seoul, Gyeonggido 10326, Republic of Korea*
[e] *Graduate Program in Process Engineering, Tiradentes University (UNIT), Av. Murilo Dantas, 300, Farolândia, 49032-490 Aracaju-Sergipe, Brazil*
[f] *Institute of Technology and Research (ITP), Tiradentes University (UNIT), Av. Murilo Dantas, 300, Farolândia, 49032-490 Aracaju-Sergipe, Brazil*
[g] *School of Life Science and Food Engineering, Huaiyin Institute of Technology, Huaian 223003, China*

ARTICLE INFO

Editor: Dr. GL Dotto

Keywords:
Textile industry
Wastewater
Persistent coloring pollutants
Environmental and health concern
Treatment approaches

ABSTRACT

Textile industry wastewater (TIWW) is considered as one of the worst polluters of our precious water and soil ecologies. It causes carcinogenic, mutagenic, genotoxic, cytotoxic and allergenic threats to living organisms. TIWW contains a variety of persistent coloring pollutants (dyes), formaldehyde, phthalates, phenols, surfactants, perfluorooctanoic acid (PFOA), pentachlorophenol and different heavy metals like lead (Pb), cadmium (Cd), arsenic (As), chromium (Cr), zinc (Zn) and nickel (Ni) etc. TIWW is characterized by high dye content, high pH, chemical oxygen demand (COD), biochemical oxygen demand (BOD), total dissolved solids (TDS), total suspended solids (TSS), total organic carbon (TOC), chlorides and sulphates. Thus, requires adequate treatment before its final discharge into the water bodies to protect public health and environment. The treatment of TIWW is a major challenge as there is no particular economically feasible treatment method capable to adequately treat TIWW. Therefore, there is a need to develop a novel, cost-effective and eco-friendly technology for the effective treatment of TIWW. This review paper emphasizes on the different textile industry processes, wastewater generation, its nature and chemical composition, environmental impacts and health hazards and treatment approaches available for TIWW treatment. It also presents various analytical techniques used to detect and characterize TIWW pollutants and their metabolites, challenges, key issues and future prospectives.

1. Introduction

The textile industries (TIs) are the major sources of the global economy in many countries like China, India, Pakistan, Brazil, Bangladesh and Malaysia, but unfortunately, these are also the major sources of environmental pollution [1,2]. TIs utilize a large volume of potable water and a wide range of synthetic chemicals at different stages during the textile production process [3,4]. TIs generate a large quantity of highly colored wastewater containing a variety of hazardous persistent coloring pollutants (PCPs) that goes into the aquatic resources [5–7]. In major textile countries, the wastewater/effluent is discharged into the rivers, which finally opens into the sea. For example, in India, Kanpur city is a major hub of textile industries that discharges large volumes of wastewater into drains and canals, which opens into the Ganga River and finally, emptying into the Way of Bengal [5].

Thousands of synthetic dyes are used in TIs during the dyeing process. Globally, ~7 × 10^7 tons of different dyes is produced annually and more than 10,000 tons of synthetic dyes are used in TIs [8]. Besides dye molecules, textile industry wastewater (TIWW) also contains a high load of salts, alkalis, binders, dispersants, volatile organic compounds

---

* Corresponding author.
E-mail addresses: bharagavarnbbau11@gmail.com, ramnaresh_dem@bbau.ac.in (R.N. Bharagava).

https://doi.org/10.1016/j.jece.2020.105012
Received 5 October 2020; Received in revised form 23 December 2020; Accepted 29 December 2020
Available online 2 January 2021
2213-3437/Crown Copyright © 2020 Published by Elsevier Ltd. All rights reserved.

Environmental Technology & Innovation 22 (2021) 101425

Contents lists available at ScienceDirect

Environmental Technology & Innovation

journal homepage: www.elsevier.com/locate/eti

Environment friendly degradation and detoxification of Congo red dye and textile industry wastewater by a newly isolated *Bacillus cohnni* (RKS9)

Roop Kishor [a], Diane Purchase [b], Ganesh Dattatraya Saratale [c],
Luiz Fernando Romanholo Ferreira [d,e], Muhammad Bilal [f], Hafiz M.N. Iqbal [g],
Ram Naresh Bharagava [a,*]

[a] *Laboratory of Bioremediation and Metagenomics Research (LBMR), Department of Microbiology (DM), Babasaheb Bhimrao Ambedkar University (A Central University), Vidya Vihar, Raebareli Road, Lucknow 226 025 U.P., India*
[b] *Department of Natural Sciences, Faculty of Science and Technology, Middlesex University, The Burroughs, Hendon, London, NW4 4BT, England, United Kingdom*
[c] *Department of Food Science and Biotechnology, Dongguk University, Seoul, Ilsandong-gu, Goyang-si, Gyeonggi-do, 10326, Republic of Korea*
[d] *Waste and Effluent Treatment Laboratory, Institute of Technology and Research (ITP), Tiradentes University, Farolândia, Aracaju-SE, 49032-490, Brazil*
[e] *Graduate Program in Process Engineering, Tiradentes University (UNIT), Av. Murilo Dantas, 300, Farolândia, 49032-490, Aracaju-Sergipe, Brazil*
[f] *School of Life Science and Food Engineering, Huaiyin Institute of Technology, Huaian 223003, China*
[g] *Tecnologico de Monterrey, School of Engineering and Sciences, Campus Monterrey, Ave. Eugenio Garza Sada 2501, Monterrey, N.L. CP 64849, Mexico*

ARTICLE INFO

Article history:
Received 3 November 2020
Received in revised form 22 January 2021
Accepted 7 February 2021
Available online 11 February 2021

Keywords:
Textile industry wastewater
Congo red dye
Laccase and Lignin peroxidase
Bioremediation
Phytotoxicity

ABSTRACT

Textile industry wastewater (TIWW) is a major source of environmental pollution causing serious threats to all life forms and thus, it must be adequately treated before its final discharge for the safety of environment and public health. In the present study, a potential bacterial strain (RKS9) was isolated from textile (wastewater & sludge) sample for the effective treatment of TIWW resulting in a significant reduction in pollution parameters such as ADMI color (93.87%), COD (77.35%), BOD (86.02%), TDS (66.75%), TOC (67.25%), TSS (60.34%), and phenol (68.55%) within 48 h. This bacterium also decolorized 99% of Congo red dye (100 mg L^{-1}) within 12 h and removed 59.76%, 40.51%, 52.71% and 26.51% cadmium, chromium, lead and nickel, respectively from the TIWW. The activities of azoreductase, laccase, lignin peroxidase (LiP) and manganese peroxidase (MnP) was monitored and metabolites produced during the treatment of dye and TIWW were also analyzed by FT-IR and GC–MS. The phytotoxicity of the untreated and treated TIWW was assessed by seed germination and seedling growth parameters of *Phaseolus mungo* L. and results showed a significant reduction in the toxicity of the treated TIWW, suggesting that the isolated bacterium RKS9 has a remarkable potential to effectively decolorize/detoxify TIWW.

© 2021 Elsevier B.V. All rights reserved.

---

* Corresponding author.
E-mail addresses: ramnaresh_dem@bbau.ac.in, bharagavarnbbau11@gmail.com (R.N. Bharagava).

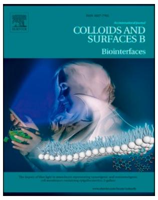
https://doi.org/10.1016/j.eti.2021.101425
2352-1864/© 2021 Elsevier B.V. All rights reserved.

Colloids and Surfaces B: Biointerfaces 206 (2021) 111947

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb



Efficient degradation and detoxification of methylene blue dye by a newly isolated ligninolytic enzyme producing bacterium *Bacillus albus* MW407057

Roop Kishor [a], Ganesh Dattatraya Saratale [b], Rijuta Ganesh Saratale [c], Luiz Fernando Romanholo Ferreira [d,e], Muhammad Bilal [f], Hafiz M.N. Iqbal [g], Ram Naresh Bharagava [a,*]

[a] *Laboratory of Bioremediation and Metagenomics Research (LBMR), Department of Environmental Microbiology (DEM), Babasaheb Bhimrao Ambedkar University (A Central University), Vidya Vihar, Raebareli Road, Lucknow, 226 025, U.P., India*
[b] *Department of Food Science and Biotechnology, Dongguk University, Seoul, Ilsandong-gu, Goyang-si, Gyeonggi-do, 10326, Republic of Korea*
[c] *Research Institute of Biotechnology and Medical Converged Science, Dongguk University-Seoul, Ilsandong-gu, Goyang-si, Gyeonggido 10326, Republic of Korea*
[d] *Graduate Program in Process Engineering, Tiradentes University (UNIT), Av. Murilo Dantas, 300, Farolandia, 49032-490 Aracaju, Sergipe, Brazil*
[e] *Waste and Effluent Treatment Laboratory, Institute of Technology and Research (ITP), Tiradentes University (UNIT), Av. Murilo Dantas, 300, Farolândia, 49032-490 Aracaju, Sergipe, Brazil*
[f] *School of Life Science and Food Engineering, Huaiyin Institute of Technology, Huaian 223003, China*
[g] *Tecnologico de Monterrey, School of Engineering and Sciences, Campus Monterrey, Ave. Eugenio Garza Sada 2501, Monterrey, N.L., CP 64849, Mexico*

ARTICLE INFO

Keywords:
Methylene blue
Lignin peroxidase
Degradation
LC–MS analysis
Phytotoxicity

ABSTRACT

In present work, a LiP enzyme producing bacterium was isolated form textile wastewater and sludge sample and identified as *Bacillus albus* by 16S rRNA gene sequencing analysis. This bacterium decolorized 99.27 % MB dye and removed 83.87 % COD within 6 h at 30 °C, pH 7, 100 rpm and 100 mg/l of dye concentration in presence of glucose and yeast extract as carbon and nitrogen source, respectively. The bacterium also produced LiP enzyme of molecular weight ~48 kDa, characterized by SDS-PAGE analysis. Different metabolites like mono-methylthionine, thionin, (E)-2-(3-Oxopropylidene)-2H-benzo[b][1,4] thiozine-3-carboxylic acid, N-(3,4-dihydroxyphenyl)-N-methylformamide, ethylamine, water and carbon dioxide produced during treatment process were characterized by FT-IR and LC–MS analysis. Further, the toxicity assessment results showed that the toxicity of bacteria treated dye solution was reduced significantly allowing 90 % seed germination indicating that the isolated bacterium *B. albus* has high potential to decolorize and detoxify MB dye for environmental safety.

1. Introduction

The manufacturing of textiles is a complex process, which consists of many stages like sizing, bleaching, dyeing, printing, washing and finishing and the wastewater discharged from textile industries (TIs) is very complex and contains various toxic recalcitrant dyes, dissolved solids, color content and heavy metals [1,2]. Among these, dyes are the major sources of pollution in water resources. Globally, ~280,000 tons of dyes are released into water bodies annually [2–4]. Among these, methylene blue (MB) dye is widely used in dyeing, tannery, plastic, cosmetics, food, textile, paper and medicinal industries to add color to products [5]. MB is a synthetic, recalcitrant and heterocyclic aromatic dye, which widely used in microbiological laboratories to avoid fungal growth and staining agent to classify the microorganisms [2,3]. It is used as an intercalator of nanoporous materials, photoelecrochromatic imaging and characterization of anionic surfactants (active substances [2,5]. It is also used in dyeing of cotton, wool, leather and paper resulting in the discharge of ~70 % unfixed dyes into water bodies like rivers, lakes, streams and ponds [2,6,7].

MB dye containing wastewater causes deadly impacts on water, soil ecologies and living organisms [5,7,8]. In water ecosystem, it reduces the photosynthetic activity and dissolved oxygen (DO) content resulting in an increase in BOD, COD values, which ultimately disturb the aquatic fauna and flora both [1,5]. In soil, it accumulates for long duration resulting an increase in soil salinity and disturbs the microbial communities, which ultimately causes soil pollution [1,2]. It causes carcinogenic, mutagenic and allergic effects to all life forms [1,2]. According to the National Occupational Exposure Survey of USA, around 69,563

---

[*] Corresponding author.
E-mail addresses: bharagavarnbbau11@gmail.com, ramnaresh_dem@bbau.ac.in (R.N. Bharagava).

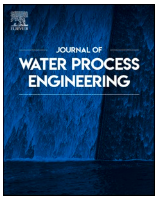
https://doi.org/10.1016/j.colsurfb.2021.111947
Received 11 May 2021; Received in revised form 23 June 2021; Accepted 24 June 2021
Available online 26 June 2021
0927-7765/© 2021 Elsevier B.V. All rights reserved.

Journal of Water Process Engineering 43 (2021) 102300

Contents lists available at ScienceDirect

Journal of Water Process Engineering

journal homepage: www.elsevier.com/locate/jwpe



Degradation mechanism and toxicity reduction of methyl orange dye by a newly isolated bacterium *Pseudomonas aeruginosa* MZ520730

Roop Kishor [a], Diane Purchase [b], Ganesh Dattatraya Saratale [c], Luiz Fernando Romanholo Ferreira [d,e], Chaudhery Mustansar Hussain [f], Sikandar I. Mulla [g], Ram Naresh Bharagava [a,*]

[a] *Laboratory of Bioremediation and Metagenomics Research (LBMR), Department of Environmental Microbiology (DEM), Babasaheb Bhimrao Ambedkar University (A Central University), Vidya Vihar, Raebareli Road, Lucknow 226 025 (U.P.), India*
[b] *Department of Natural Sciences, Faculty of Science and Technology, Middlesex University, The Burroughs, Hendon, London NW4 4BT, England, United Kingdom*
[c] *Department of Food Science and Biotechnology, Dongguk University, Seoul, Ilsandong-gu, Goyang-si, Gyeonggi-do 10326, Republic of Korea*
[d] *Waste and Effluent Treatment Laboratory, Institute of Technology and Research (ITP), Tiradentes University, Farolândia, Aracaju, SE 49032-490, Brazil*
[e] *Graduate Program in Process Engineering, Tiradentes University (UNIT), Av. Murilo Dantas, 300, Farolândia, 49032-490 Aracaju, Sergipe, Brazil*
[f] *Department of Chemistry and Environmental Science, New Jersey Institute of Technology, Newark, NJ 07102, USA*
[g] *Department of Biochemistry, School of Applied Sciences, REVA University, Bangalore, India*

ARTICLE INFO

Keywords:
Methyl orange
Pseudomonas aeruginosa
Manganese peroxidase
Optimization
Biodegradation
Phytotoxicity

ABSTRACT

Methyl orange (MO) dye is recalcitrant in nature, hard to degrade and if released into the soil and aquatic resources could cause serious threats on environment and human health. MO is toxic to plant growth. Bacterial treatment may be a sustainable solution for its degradation and decolourization. In this work, a bacterium (RKS6) was isolated from textile industry wastewater and sludge samples and identified as *Pseudomonas aeruginosa* based on the 16S rRNA gene sequencing analysis. RKS6 showed more than 99% decolorization of MO dye (100 mg/l) and 96% reduction of total organic carbon (TOC) within 12 h, at 30 °C, pH 7 at static conditions. RKS6 also produced MnP enzyme of molecular weight ~53 kDa as characterized by the SDS-PAGE analysis. Further, LC-MS analysis showed that MO dye was degraded into 4-[(4-aminophenyl) diazenyl] benzene sulfonate, 4, 2-((dihydroxymethyl) hyrazono-4) 5-benzene sulfonate, 4-(triazan-2-yl) benzene sulfonic, water and carbon dioxide by RKS6. Toxicity assessment showed that the solution treated by the bacterium allowed 90% seed germination indicating that RKS6 was effective in mineralization and detoxification of MO dye and can be effectively used in industrial wastewater treatment.

1. Introduction

Azo dyes are widely used in dyeing, textile, paint, leather, pulp paper, medicine and cosmetic industries [1,2]. Azo dyes have high fastness, low cost, easy to use, and high stability to light, temperature, detergents, chemicals and microbial degradation in comparison to natural dyes [1–3]. Globally, more than thousand tons of different dyes are used in textile industries (TIs) [4,5] and ~28,000 tons of unfixed dyes are released into the environment annually [5,6]. Azo dyes are the largest and most versatile group of synthetic dyes, which consists one/many azo (-N=N-) bonds and sulfonic (SO^{3-}) bonds, attached to different functional groups such as amino, hydroxyl, methyl, nitro, carboxyl and sulfoxyl [5,7]. Approximately 70% of azo dyes are used in TIs worldwide [5]. Methyl orange (MO) is a synthetic, organic, heterocyclic, sulfonated and high water soluble anionic dye [3,5,7]. MO dye is widely used in dyeing, leather, textile and pulp paper industries [5,7,8]. It's also used as a pH indicator in many research laboratories [7].

If MO dye was discharged without adequate treatment into rivers, wetlands and agriculture fields, it could cause serious effects in the biota and water/soil ecologies [3,7]. For example, even at very low concentration in water OM causes detrimental effects on water quality due to coloration of water that reduces sunlight penetration, decreases photosynthetic activity, dissolved oxygen content, gas solubility and thus increased BOD, COD, TOC and TDS level [3,5,7]. MO is a recalcitrant dye and causes hypersensitivity, allergies, dermatitis and intestinal cancer in living beings [3,5]. MO dye is well reported to causes high

---

* Corresponding author.
E-mail addresses: bharagavarnbbau11@gmail.com, ramnaresh_dem@bbau.ac.in (R.N. Bharagava).

https://doi.org/10.1016/j.jwpe.2021.102300
Received 25 June 2021; Received in revised form 5 August 2021; Accepted 30 August 2021
Available online 9 September 2021
2214-7144/© 2021 Elsevier Ltd. All rights reserved.

Environmental Earth Sciences (2019) 78:418
https://doi.org/10.1007/s12665-019-8454-2

BOOK REVIEW

Chandra R, Dubey NK, Kumar V: Phytoremediation of environmental pollutants

2017 | CRC Press, Taylor & Francis Group, Boca Raton/London/New York | 510 pp, 127 figs | Hardback ISBN 9781138062603, 200 USD, 150.00 GBP | eBook ISBN 9781315161549, 52.16 USD, 37.79 GBP

Gaurav Saxena[1,2] · Roop Kishor[1] · Diane Purchase[3] · Ram Naresh Bharagava[1]

© Springer-Verlag GmbH Germany, part of Springer Nature 2019

This book, *Phytoremediation of Environmental Pollutants* is a very timely and topical contribution in the current scenario of increasing "environmental contamination" and "remediation" to ensure the safest and healthiest environment for living beings. It provides comprehensive information on the principals and practical knowledge of phytoremediation of organic and inorganic pollutants for environmental safety and public health protection. The book describes the physiological, biochemical, microbiological, and molecular aspects of phytoremediation, including the use of genomics, proteomics, transcriptomics, metagenomics, and metabolomics for the development of next-generation phytoremediation phytotechnologies to combat the forthcoming environmental challenges. The book contains 19 chapters focused on the diverse aspects of phytoremediation and is accessible through the internet to readers. In this book, leading experts from different universities, research laboratories, and academic institutions contributed many relevant topics to fill the gaps in our understanding of phytoremediation. In general, the book is excellent and all the chapters are meticulously prepared with fabulous figures and tables to make the information easier to understand and supported with an extensive list of references and URLs for readers interested to learn further details about the subject matter.

The first chapter introduces the basic mechanisms and applications of conventional and novel phytoremediation approaches for organics and heavy metals (HMs). Additionally, the improvement strategies for phytoremediation including the use of chelators and transgenic approaches with pros, cons, and challenges have been described. Further, long-term field trials are also suggested to document time and cost data to testify economic feasibility and commercial success of phytoremediation at the field scale. The characteristics, selection criteria and role of hyperaccumulating plants in the phytoremediation of HM-contaminated sites have been described in depth in Chap. 2. Further, it was concluded that the selection of target plants among known metal hyperaccumulators and exploring new plants for successful phytoremediation is an ongoing challenge. Chapter 3 describes how plants cope with the HM stress through various morphological, biochemical, and physiological adaptation strategies and defense mechanisms during phytoremediation for survival on contaminated sites. The molecular mechanism of HM tolerance, uptake, translocation, and phytoremediation has been well discussed with figures in Chap. 4. Chapter 5 examines the effects of HM accumulation on the plant's internal structure and suggested normalized difference vegetation index (NDVI) as an effective tool to monitor HM stress in remediating plants.

Exploiting plant–microbe interactions could be an excellent strategy to enhance the plant growth and phytoremediation on contaminated sites (Rajkumar et al. 2012). The sources of contamination, toxicity, and mycorrhizal

---

✉ Ram Naresh Bharagava
bharagavarnbbau11@gmail.com

[1] Laboratory for Bioremediation and Metagenomics Research (LBMR), Department of Microbiology (DM), Babasaheb Bhimrao Ambedkar University (A Central University), Vidya Vihar, Raebareli Road, Lucknow, Uttar Pradesh 226 025, India

[2] Environmental Microbiology and Biotechnology Laboratory, School of Environmental Sciences, Jawaharlal Nehru University, New Delhi 110 067, India

[3] Department of Natural Sciences, Faculty of Science and Technology, Middlesex University, The Burroughs, Hendon, London, England NW4 4BT, UK

1 Industrial Wastewaters

The Major Sources of Dye Contamination in the Environment, Ecotoxicological Effects, and Bioremediation Approaches

Roop Kishor, Ram Naresh Bharagava, and Gaurav Saxena

CONTENTS

Chapter 1
Genetically Modified Organisms (GMOs) and Their Potential in Environmental Management: Constraints, Prospects, and Challenges

Gaurav Saxena, Roop Kishor, Ganesh Dattatraya Saratale, and Ram Naresh Bharagava

Abstract Increasing environmental contamination with highly toxic chemicals is warning us to find sustainable technologies to protect the environment and human health, which is a key challenge of the current scenario. A variety of physicochemical technologies are currently being applied presently to decontaminate the environment to safeguard the environment and human health. However, these technologies are costly and chemical-consuming, thus causing secondary pollution and, hence, are not environmental-friendly. As an alternative approach, bioremediation technologies using microbes and plants and their enzymes are currently viewed as eco-friendly and most sustainable technologies due to their self-sustainable and low-cost nature. But sometimes bioremediation technologies are get limited by low degradability/accumulability of microbes and plants, respectively. To overcome these limitations, genetic engineering approaches are highly decisive to design the transgenic microbes and plants for the enhanced biodegradation and biodetoxification of environmental pollutants for sustainable development. Genetically modified organisms (GMOs) offer great potential for environmental remediation, and hence, in this chapter, we focused on the applications of GMOs in the environmental management with risks involved, constraints, and challenges faced by researchers in the release of GMOs for field applications.

Keywords Environmental pollutants · Genetically modified organisms · Environmental remediation · Transgene · Genetic engineering

---

G. Saxena · R. Kishor · R. N. Bharagava (✉)
Laboratory for Bioremediation and Metagenomics Research (LBMR), Department of Microbiology (DM), Babasaheb Bhimrao Ambedkar (Central) University, Lucknow, Uttar Pradesh, India

G. D. Saratale
Department of Food Science and Biotechnology, Dongguk University-Seoul, Goyang-si, Gyeonggi-do, Republic of Korea

© Springer Nature Singapore Pte Ltd. 2020
R. N. Bharagava, G. Saxena (eds.), *Bioremediation of Industrial Waste for Environmental Safety*, https://doi.org/10.1007/978-981-13-3426-9_1

Chapter 3
Application of Microbial Enzymes in Degradation and Detoxification of Organic and Inorganic Pollutants

Gaurav Saxena, Roop Kishor, and Ram Naresh Bharagava

Contents

Abstract Microbial enzymes have been reported to play a diverse role in various industrial applications. Microbial enzymes are also useful in bioremediation of environmental pollutants from industrial wastes due to their high specificity to a broad range of substrates (pollutants), use under extreme conditions that microbe cannot thrive, high effectiveness at low pollutant concentration, high activity in the presence of inhibitors of microbial metabolism, and high mobility (small size) than microorganisms. A variety of enzymes are produced by microorganisms that can be used in the degradation and detoxification of a wide range of organic and inorganic pollutants. This chapter provides an overview on the various microbial enzymes that can be used for the bioremediation of environmental pollutants. In addition, the prospects and challenges in applying microbial enzymes in bioremediation are also discussed in this chapter.

G. Saxena · R. Kishor · R. N. Bharagava (✉)
Laboratory of Bioremediation and Metagenomics Research (LBMR),
Department of Microbiology (DM), Babasaheb Bhimrao Ambedkar University
(A Central University), Lucknow, Uttar Pradesh, India
e-mail: ramnaresh_dem@bbau.ac.in

© Springer Nature Singapore Pte Ltd. 2020
G. Saxena, R. N. Bharagava (eds.), *Bioremediation of Industrial Waste for Environmental Safety*, https://doi.org/10.1007/978-981-13-1891-7_3

Environmental and Health Hazards of Textile Industry Wastewater Pollutants and Its Treatment Approaches

Roop Kishor, Diane Purchase, Luiz Fernando Romanholo Ferreira,
Sikandar I. Mulla, Muhammad Bilal, and Ram Naresh Bharagava

Contents

R. Kishor · R. N. Bharagava (✉)
Laboratory of Bioremediation and Metagenomics Research (LBMR), Department of Microbiology, Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow, India
e-mail: bharagavarnbbau11@gmail.com; ramnarseh_dem@bbau.ac.in

D. Purchase
Department of Natural Sciences, Faculty of Science and Technology, Middlesex University, London, UK

L. F. R. Ferreira
Graduate Program in Process Engineering, Tiradentes University, Murilo Dantas Avenue, Farolândia, Aracaju, Sergipe, Brazil

Institute of Technology and Research, Murilo Dantas Avenue, Farolândia, Aracaju, Sergipe, Brazil

S. I. Mulla
Department of Biochemistry, School of Applied Sciences, REVA University, Bangalore, India

M. Bilal
School of Life Science and Food Engineering, Huaiyin Institute of Technology, Huaian, China

© Springer Nature Switzerland AG 2020
C. M. Hussain (ed.), *Handbook of Environmental Materials Management*,
https://doi.org/10.1007/978-3-319-58538-3_230-1

CHAPTER

17

Environmental contamination, toxicity profile and bioremediation technologies for treatment and detoxification of textile effluent

Gaurav Saxena[1,2], Roop Kishor[3], Surabhi Zainith[3] and Ram Naresh Bharagava[3]

[1] *Laboratory for Microbiology, Department of Microbiology, School of Life and Allied Sciences, Baba Farid Institute of Technology, Dehradun, India*

[2] *Laboratory for Bioremediation and Metagenomics Research (LBMR), Department of Environmental Microbiology (DEM), Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow, India*

[3] *Laboratory for Bioremediation and Metagenomics Research (LBMR), Department of Microbiology (DM), Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow, India*

17.1 Introduction

The manufacturing of textiles uses large volumes of water and a wide range of various chemicals in the dyeing, bleaching, printing, washing, and finishing stages, which are then discharged as effluent to the environment (Kishor et al., 2019; Saxena et al., 2019). Discharged textile effluent generally is characterized by its intensive color, high pH, biological oxygen demand (BOD), chemical oxygen demand (COD), total suspended solid (TSS), total nitrogen, total solid, and toxic metals (Khan and Malik 2017; Kishor et al., 2019). This effluent causes a deadly impact on water bodies due to the reduced penetration of sunlight and decreases the dissolved oxygen in receiving water bodies, thus ultimately affecting the flora/fauna as well as decreasing microbial communities (Bhatia et al., 2017; Bharagava et al., 2018).

Many industries discharge large volumes of dyes containing effluents such as pulp and paper, leather, and food, dyeing, cosmetic, and pharmaceutical (Saxena et al., 2019; Kishor et al., 2019). Various toxic heavy metals including arsenic (As), chromium (Cr), zinc (Zn), cadmium (Cd), lead (Pb), and copper (Cu) are present in textile effluent, which can be regarded as having a nondegradable

Bioremediation for Environmental Sustainability. DOI: https://doi.org/10.1016/B978-0-12-820524-2.00017-1

© 2021 Elsevier Inc. All rights reserved.

20

Application of microalgae in industrial effluent treatment, contaminants removal, and biodiesel production: Opportunities, challenges, and future prospects

Surabhi Zainith[1], **Gaurav Saxena**[2,3], **Roop Kishor**[1] **and Ram Naresh Bharagava**[1]

[1]Laboratory for Bioremediation and Metagenomics Research (LBMR), Department of Microbiology (DM), Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow, India
[2]Laboratory for Bioremediation and Metagenomics Research (LBMR), Department of Environmental Microbiology (DEM), Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow, India
[3]Laboratory for Microbiology, Department of Microbiology, School of Life and Allied Sciences, Baba Farid Institute of Technology, Dehradun, India

20.1 Introduction

With the increase of population, urbanization, and industrialization, issues related to environmental pollution become more severe, resulting in excessive generation of wastes or wastewaters and the release of various toxic contaminants which create harmful effects on the environment (Sood et al., 2012). The increasing pollution load imposes severe risks to the availability and quality of water resources globally. In developing countries like India, water scarcity is a major concern, because the increased population is resulting in large quantities of sewage wastewater. In addition to that, increasing industrialization and excessive use of fertilizers and pesticides in agriculture is resulting in the mixing of contaminated untreated wastewater with the freshwater resources (Chowdhary et al., 2020). The prime source of these pollutants is wastewater or solid wastes, which are released from a wide range of industries like chemical and pharmaceutical, plastics, pulp and paper mills, tannery, distillery, textile mills, refineries, and agriculture (Zainith et al., 2016). Besides this, the domestic and municipal wastes or wastewaters also pose a serious threat to the environment. Agriculture is the largest user of water (87%), while industries and domestic supplies consume 7% and 8%, respectively. According to the World Health Organization (WHO, 2000) and Central Pollution Control Board, India

Bioremediation for Environmental Sustainability. DOI: https://doi.org/10.1016/B978-0-12-820524-2.00020-1
© 2021 Elsevier Inc. All rights reserved.

CHAPTER

18

Emerging green technologies for biological treatment of leather tannery chemicals and wastewater

Gaurav Saxena[1], Roop Kishor[2], Ram Naresh Bharagava[2], Plaban Das[3], Pankaj Kumar Gupta[4] and Naveen Kumar[5]

[1]*Laboratory for Microbiology, Department of Microbiology, School of Life and Allied Sciences, Baba Farid Institute of Technology, Dehradun, India*

[2]*Laboratory for Bioremediation and Metagenomics Research (LBMR), Department of Microbiology (DM), Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow, India*

[3]*Laboratory for Microbiology, Department of Microbiology, Baba Farid Institute of Technology, Dehradun, India*

[4]*Faculty of Environment, University of Waterloo, Waterloo, ON, United States*

[5]*Biotechnology, Bioprocessing & Business Management, School of Life Sciences, The University of Warwick, Coventry, United Kingdom*

18.1 Introduction

Leather industries (LIs) are one of the major contributors in the economy of many developing nations due to ever increasing demand for leather and leather products. Around 2108.94 M mt^2 of leather is annually produced worldwide and the world trade for the leather sector is estimated to be US$ 100 billion per year (FAO, 2008; UNIDO, 2000). Regrettably, LIs are also the major pollution-causing industries in the world. LIs are specialized in processing of hide (skins of large animals such as cows, buffaloes and horses) and skins (skins of small animals such as sheep, goats and calves) for leather production (Saxena et al., 2020a). The prime objective of the tanning process is to convert the hide/skins (a highly putrescible material) into stable and imputrescible products termed as leather that is used for various purposes (Bharagava and Saxena, 2020). Tanning processes are classified into vegetable and chrome-tanning processes depending on the type of tanning reagent (chromium or tannins) used (Aravindhan et al., 2004; ILTIP, 2010). During leather production process, a variety of highly toxic chemicals are used in the tanning process and often discharged along with wastewater into the environment where they create serious ecotoxicological effects in the environment and toxicity in living beings upon exposure (Bharagava and Saxena, 2020;

Bioremediation for Environmental Sustainability. DOI: https://doi.org/10.1016/B978-0-12-820524-2.00018-3

© 2021 Elsevier Inc. All rights reserved.

11

Molecular techniques used to identify perfluorooctanoic acid degrading microbes and their application in a wastewater treatment reactor/plant

Roop Kishor[1], **Ram Naresh Bharagava**[1], **Luiz Fernando Romanholo Ferreira**[2], **Muhammad Bilal**[3] and **Diane Purchase**[4]

[1]Laboratory for Bioremediation and Metagenomics Research (LBMR), Department of Microbiology, Babasaheb Bhimrao Ambedkar University (A Central University), Vidya Vihar, Lucknow, India [2]Institute of Technology and Research, Tiradentes University, Campus Farolandia, Aracaju, Brazil [3]School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai, P.R. China [4]Department of Natural Sciences, Faculty of Science and Technology, Middlesex University, London, United Kingdom

11.1 Introduction

Perfluorooctanoic acid (PFOA) is a synthetic fully fluorinated compound, made of hydrophilic and hydrophobic alkyl chain. The chemical formula of PFOA is $CF_3(CF_2)_6COOH$. PFOA is primarily used as oil, grease, surfactant, surface treatment, and water repellent and it is resistant to heat and acid (Espana et al., 2015; Ong et al., 2017). It is used in textile industries for the manufacturing of many products such as rugs, clothing, carpets, and outdoor equipment (Chen et al., 2013; Chiavola et al., 2019; Longpré et al., 2020). In addition, PFOA is also used in firefighting applications, paper, food packaging, additives, lubricants, paints, and electrical and electronic equipment (Espana et al., 2015; Li et al., 2020).

PFOA is found in many habitats such as drinking water, surface water, groundwater, wastewater treatment plant, soil, sediments, sludge, agricultural food products, marine organisms,

Wastewater Treatment Reactors. DOI: https://doi.org/10.1016/B978-0-12-823991-9.00009-5

© 2021 Elsevier B.V. All rights reserved.

11

A Sustainable Approach to the Degradation and Detoxification of Textile Industry Wastewater for Environmental Safety

Roop Kishor, Arpita Singh, Nandkishor More, and Ram Naresh Bharagava
Babasaheb Bhimrao Ambedkar University (A Central University)

T&F Proofs – Not for Distribution

CONTENTS

11.1 Introduction

Textile industries (TIs) use large volumes of water and a wide range of recalcitrant chemicals in the different stages of production of textiles (Haq et al., 2018; Zhuang et al., 2020; Kishor et al., 2021a). For example, 1.6 million L of water is used for the production of 8,000 kg of fabric per day. Globally, more than 10,000 tons of textile dyes and ~8,000 chemicals are used in TIs (Lade et al., 2016; Kishor et al., 2021a). Approximately 20% of wastewater is discharged from the dyeing and finishing stages, which causes severe threats to public health and environment. Textile industry wastewater (TIWW) is characterized by its high temperature, pH, BOD (biological oxygen demand), COD (chemical oxygen demand), TDS (total dissolved solids), TSS (total suspended solids), DS (dissolved solids) and residual dyes, making it more complex and highly toxic (Chandanshive et al., 2020; Kishor et al., 2021b). Several natural fibres such as jute, cotton, silk and wool and a variety of synthetic fibres such as polyamide, polyester, viscose, nylon and acrylic are used by TIs (Sun et al., 2020).

Dyes are synthetic organic compounds used to add colour to substrates such as cloth, paper, leather and other objects. Dyes are aromatic, heterocyclic and recalcitrant and possess different chromophore groups such as azo (–N=N–), nitro (–N=O), carbonyl (–C=O) and quinoid groups and auxochrome groups such

DOI: 10.1201/9781003181224-11

1

Existing and Emerging Treatment Technologies for the Degradation and Detoxification of Textile Industry Wastewater for the Environmental Safety

Ajeet Singh, Roop Kishor, Ram Naresh Bharagava, and Bal Chandra Yadav
Babasaheb Bhimrao Ambedkar University (A Central University)

CONTENTS

1.1 Introduction

Textile industries (TIs) are spread globally, having a market size of ≈1 trillion dollars, and India contributes to ≈7% of the total world exports. Globally, TIs offer employment to ≈35 million workers and are the fifth largest source of foreign currency (Kaur et al., 2018; Tara et al., 2019; Kishor et al., 2020). India is the second largest exporter of dyes after China. But, unfortunately, TIs are a major source of environmental pollution because they release huge volumes of coloured wastewater into valuable water resources (Bener et al., 2019; Kishor et al., 2021a). Textile production is a complex process, which consists of sizing, desizing, bleaching, scouring, mercerizing, dyeing, printing, washing and finishing stages (Kadam et al., 2018; Sen et al., 2019; Kishor et al., 2021b). These stages use large volumes of freshwater and a large number of different chemicals (Kishor et al., 2020).

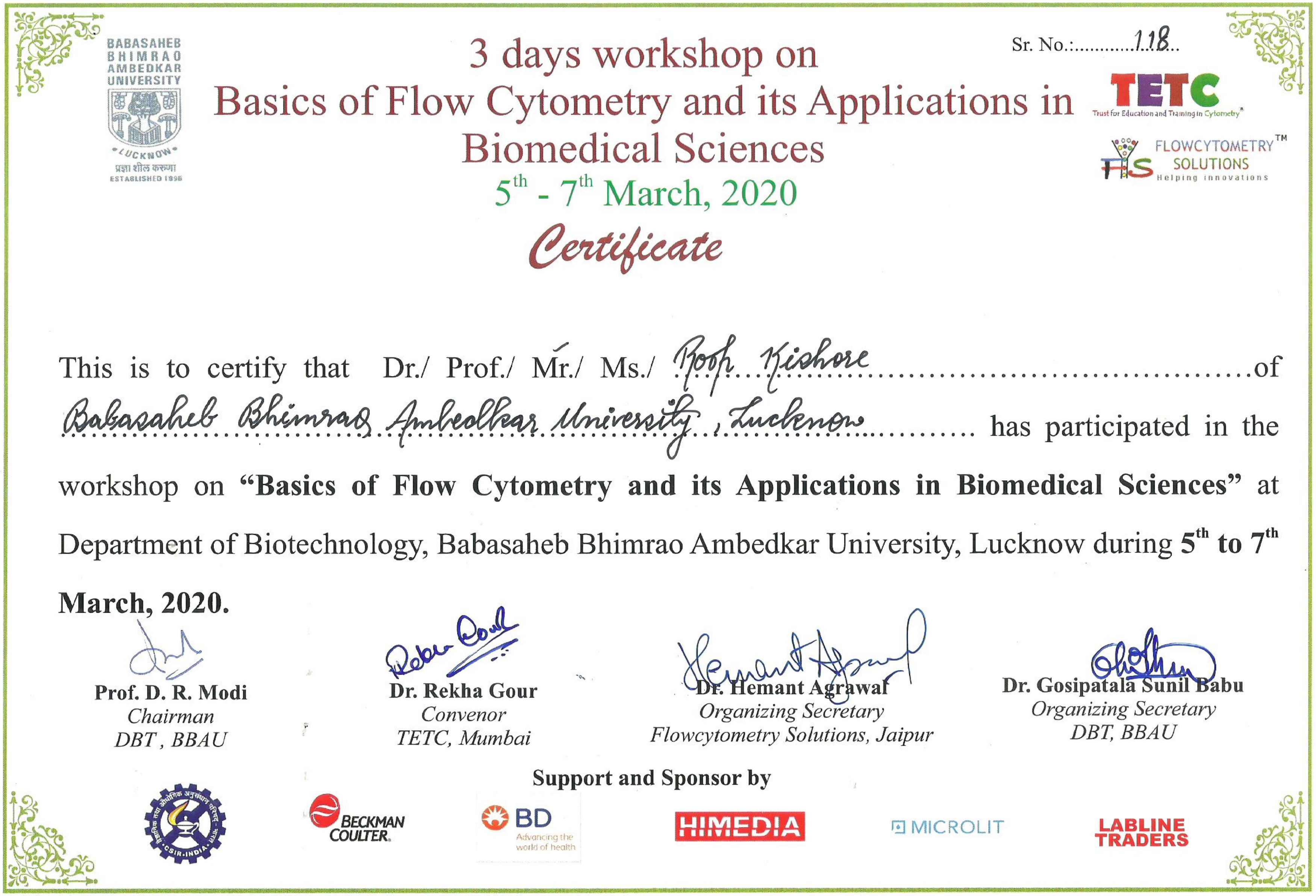
AU: Please check and approve the edit made in the sentence 'Textile industries (TIs) are spread globally…of the total world exports'.

AU: Please check and approve the edit made in the sentence 'Globally, TIs offer employment … the fifth largest source of foreign currency'.

For example, TIs consume ≈1.6 million L of groundwater for the production of 8,000 kg of textile fabrics per day (Khan and Malik, 2017; Kishor et al., 2018) and ≈20% of wastewater is discharged into environment (Kishor et al., 2021a). Several chemicals such as acids, bases, surfactants, salts, dispersants, dyes and finishing agents are used at different stages of textile production (Bener et al., 2019; Kishor et al., 2021c). Among these chemicals, the dyes are the major source of environmental pollution (Chandanshive et al., 2020; Kishor et al., 2021c). Dyes are employed in textile, cosmetic, leather, printing, paper and medicine industries as a colouring agent (Haq et al., 2018; Kishor et al., 2021c). Besides,

T&F Proofs – Not for Distribution

DOI: 10.1201/9781003181224-1



BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
"LUCKNOW"
प्रज्ञा शील करुणा
ESTABLISHED 1996

Sr. No.:............118...

TETC
Trust for Education and Training in Cytometry

FLOWCYTOMETRY™ SOLUTIONS
Helping innovations

3 days workshop on
Basics of Flow Cytometry and its Applications in Biomedical Sciences

5th - 7th March, 2020

Certificate

This is to certify that Dr./ Prof./ Mr./ Ms./ Roop Kishore...................................of Babasaheb Bhimrao Ambedkar University, Lucknow.......... has participated in the workshop on **"Basics of Flow Cytometry and its Applications in Biomedical Sciences"** at Department of Biotechnology, Babasaheb Bhimrao Ambedkar University, Lucknow during **5th to 7th March, 2020.**

Prof. D. R. Modi *Chairman* *DBT , BBAU*	Rekha Gour **Dr. Rekha Gour** *Convenor* *TETC, Mumbai*	Hemant Agrawal **Dr. Hemant Agrawal** *Organizing Secretary* *Flowcytometry Solutions, Jaipur*	**Dr. Gosipatala Sunil Babu** *Organizing Secretary* *DBT, BBAU*

Support and Sponsor by

CSIR-INDIA · BECKMAN COULTER · BD Advancing the world of health · HIMEDIA · MICROLIT · LABLINE TRADERS

Integrated Workshop on Publication Ethics and Patenting

10 February, 2020

Organized By

BABASAHEB
BHIMRAO
AMBEDKAR
UNIVERSITY
LUCKNOW
प्रज्ञा शील करुणा
ESTABLISHED 1996

Department of Energy and Environment, School of Environmental Sciences
Babasaheb Bhimrao Ambedkar University
(A Central University)
Vidya Vihar, Raebareli Road, Lucknow-226 025

In Collaboration With

Society for Environmental Sustainability
Society for Science of Climate Change and Environmental Sustainability
CEM IUCN Commission on Ecosystem Management
Springer SPRINGER NATURE
SERB INDIA Science and Engineering Research Board Department of Science & Technology Government of India
DST CPR DST CENTRE FOR POLICY RESEARCH

Certificate

This is to certify that ~~Prof./Dr.~~/Mr./~~Ms.~~ .... Roop Kishor .... from .... DM, BBA University, Lucknow .... has participated ~~as Chairperson/Co-Chairperson/delivered Plenary Lecture/Lead Talk/ Invited Talk~~ in Integrated Workshop on **"Publication Ethics and Patenting"** held on 10 February 2020 on the title .... — ....

....

Naveen Arora
Prof. Naveen Kumar Arora
Convener & Organizing Secretary
Head, Department of Energy and Environment
BBAU

RPSingh
Prof. Rana Pratap Singh
President,
Society for Environmental Sustainability
Dean, Academic Affairs, BBAU

SK Dwivedi
Prof. S.K. Dwivedi
Vice President,
Society for Environmental Sustainability
Dean, School of Environmental Sciences, BBAU

International Conference on Environmental Sustainability : Innovations, Translational Dimensions and Way Forward

11 - 12 February, 2020

Organized By

Department of Energy and Environment
School of Environmental Sciences
Babasaheb Bhimrao Ambedkar University
(A Central University) NAAC 'A' Accredited
Vidya Vihar, Raebareli Road, Lucknow-226 025

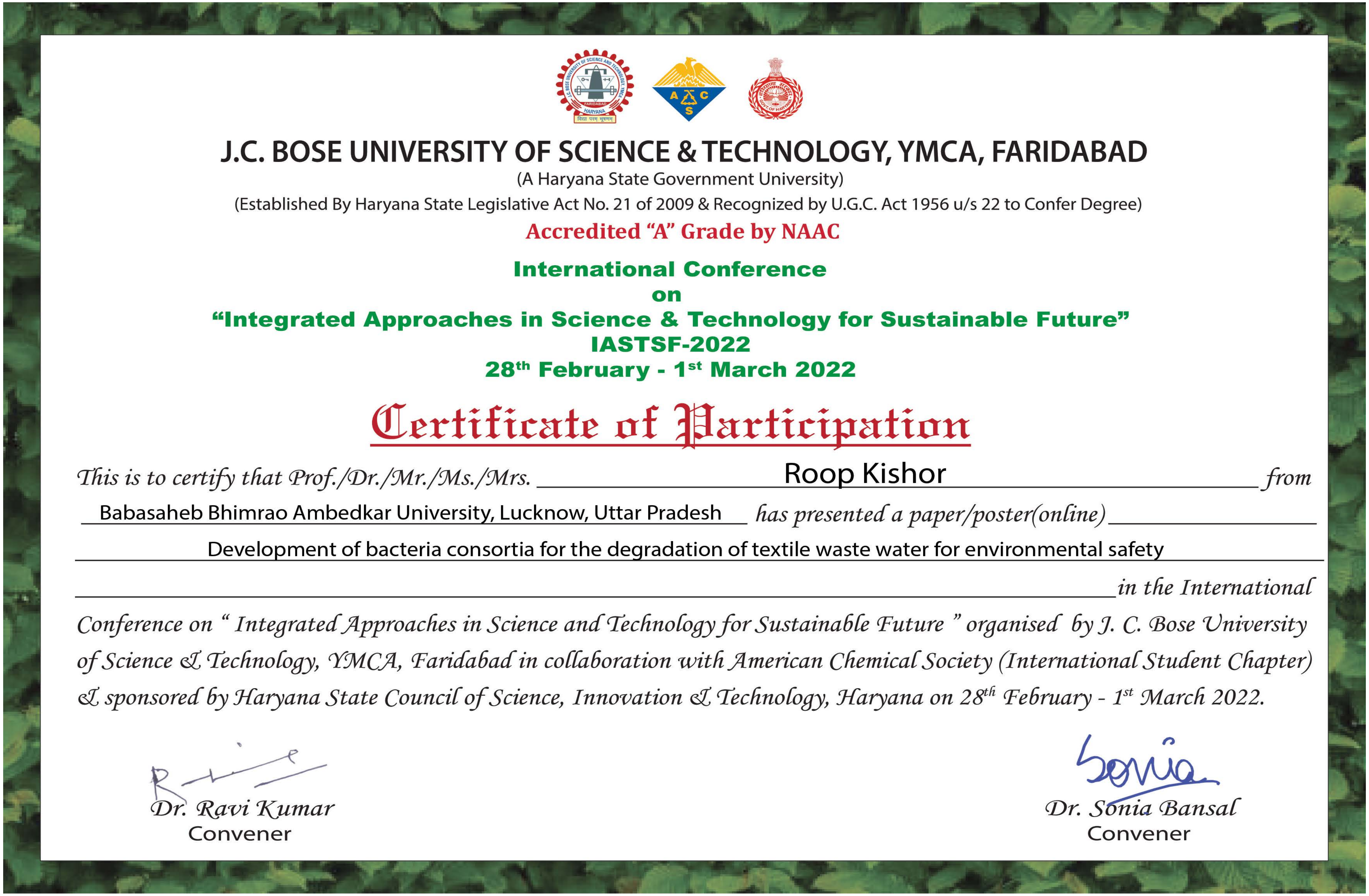
In Collaboration With

Society for Environmental Sustainability · Society for Science of Climate Change and Environmental Sustainability · CEM IUCN Commission on Ecosystem Management · Springer SPRINGER NATURE · SERB INDIA Science and Engineering Research Board Department of Science & Technology Government of India · DST CPR DST CENTRE FOR POLICY RESEARCH

Certificate

This is to certify that ~~Prof.~~/Dr./Mr./~~Ms~~. Roop Kishor from Dept of Microbiology, BBA University, Lucknow has participated ~~as Chairperson/Co-Chairperson/ delivered Plenary Lecture/Lead Talk/Invited Talk/Oral~~/Poster Presentation in **International Conference on "Environmental Sustainability: Innovations, Translational Dimensions and Way Forward"** held on 11-12 February 2020 on the title Isolation & characterization of bacteria capable of degradation & decolourization of textile industry wastewater for environmental safety.

Naveen Arora	RPSingh	Dwivedi
Prof. Naveen Kumar Arora Convener & Organizing Secretary Head, Department of Energy and Environment BBAU	**Prof. Rana Pratap Singh** President, Society for Environmental Sustainability Dean, Academic Affairs, BBAU	**Prof. S.K. Dwivedi** Vice President, Society for Environmental Sustainability Dean, School of Environmental Sciences, BBAU



J.C. BOSE UNIVERSITY OF SCIENCE & TECHNOLOGY, YMCA, FARIDABAD

(A Haryana State Government University)
(Established By Haryana State Legislative Act No. 21 of 2009 & Recognized by U.G.C. Act 1956 u/s 22 to Confer Degree)
Accredited “A” Grade by NAAC

International Conference
on
“Integrated Approaches in Science & Technology for Sustainable Future”
IASTSF-2022
28th February - 1st March 2022

Certificate of Participation

This is to certify that Prof./Dr./Mr./Ms./Mrs. Roop Kishor *from* Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh *has presented a paper/poster(online)* Development of bacteria consortia for the degradation of textile waste water for environmental safety *in the International Conference on “ Integrated Approaches in Science and Technology for Sustainable Future ” organised by J. C. Bose University of Science & Technology, YMCA, Faridabad in collaboration with American Chemical Society (International Student Chapter) & sponsored by Haryana State Council of Science, Innovation & Technology, Haryana on 28th February - 1st March 2022.*

Dr. Ravi Kumar
Convener

Sonia
Dr. Sonia Bansal
Convener

Biographical Sketch

Mr. Roop Kishor was born in 1995 and completed school education (2012) from Government Schools at Gawan, Bhimnagar, Uttar Pradesh (UP), India. He received B.Sc. degree (2015) in Microbiology from University is Institute of Foreign Trade and Management, Moradabad, Uttar Pradesh (UP), India and then moved to Babasaheb Bhimrao Ambedkar (A Central) University, Lucknow (UP,) India where completed M.Sc. degree (2017) in Environmental Microbiology. Because of his keen interest in environmental protection, his joined Babasaheb Bhimrao Ambedkar (A Central) University, Lucknow, (UP), India to further pursue doctoral research work in Environmental Microbiology at the Laboratory for Bioremediation and Metagenomics Research (LBMR), Department of Microbiology (DEM). His area of research includes Environmental Microbiology, Toxicology, Bioremediation of Environmental Contaminants/Wastewater and Metagenomics. He has been qualified (2017) National Eligibility Test (NET) and also selected as a Junior Research Fellowship (JRF) of Department of Science and Technology (DST), Government of India (GOI), India. He also holds the life memberships of the Association of Microbiologists of India (AMI), and Indian Science Congress Association (ISCA).