

# Isolation and Screening of Azo Dye Reactive Violet 5R Degrading Bacteria from Textile Sludge



A DISSERTATION SUBMITTED TO BRAC UNIVERSITY IN PARTIAL FULLFILLMENT  
OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF SCIENCE IN  
BIOTECHNOLOGY

Submitted by,

Hasan Hasnaeen Ahmed

Student ID: 11236005

24<sup>th</sup> January 2016

Department of Mathematics and Natural Sciences

BRAC University

66, Mohakhali, Dhaka-1212

Bangladesh

## Declaration

I hereby declare that the thesis work titled “Isolation and Screening of Azo Dye Reactive Violet 5R Degrading Bacteria from Textile Sludge” has been written and submitted by me, Hasan Hasnaeen Ahmed (ID-11236005) Department of Mathematics and Natural Sciences under the supervision of Ms. Romana Siddique, Senior Lecturer Department of Mathematics and Natural Sciences without the use of other sources than those mentioned.

It is further asserted that this Bachelor’s Thesis has never been submitted in the same or substantially similar version to any other examinations office. All explanations that have been adopted literally or analogously are marked as such.

(Hasan Hasnaeen Ahmed)

Candidate

CERTIFIED BY

Ms. Romana Siddique

Supervisor

Senior Lecturer

Department of Mathematics and Natural Sciences,

BRAC University

Dhaka, Bangladesh

## **Acknowledgement**

I might want to thank the greater part of the general population who contributed somehow to the work depicted in this study. Above all else, I would like to show endless gratitude towards the Almighty for His endowments that gave me the quality to persevere against all chances and perform well and complete this postulation.

I might want to extend my gratefulness to the numerous individuals including my family members, companions, employees and lab associates, who so liberally added to the work, exhibited in this thesis. Special notice goes to my motivation, Professor Dr. A. A. Ziauddin Ahmad and Professor Naiyyum Choudhury. Without their bolster it would have been difficult to complete my paper and graduate on time. I am everlastingly appreciative to them for his backing and direction.

I am likewise appreciative to my Supervisor, Ms. Romana Siddique, Senior Lecturer, Department of Mathematics and Natural sciences, BRAC University, particularly to share her significant time and information about this thesis. I am enchanted to have her as my aide and I can't express gratitude toward her enough for the challenges she confronted for me. So also, significant appreciation goes to Associate Professor Dr. Mahboob Hossain and Assistant Professor Jebunnesa Chowdhury, who helped by drawing in me with new thoughts, and requesting a high caliber of work from every one of my attempts.

I might likewise want to take this chance to express my sincerest appreciation to the Laboratory Officer Asma Binte Afzal, Teaching Assistants Nahreen Mirza, Shaan Mahameed for their unremitting help; determined backing and interminable consolation that has made this study a lovely adventure.

To wrap things up, I would like to thank my Friends Ilma Rahman, Shagoofa Rakshanda, Fahim Ahmed Alif and Ahmed Abdullah Fahim for being very supportive for the last 4 years.

## Table of Contents

<b>contents</b>	<b>Page number</b>
Abstract	i
List of figures	ii
List of abbreviations	iii
List of tables	iv
<b>Chapter 1: Introduction</b>	1
1.1 Background	2
1.2 Commercial use of dye	3
1.3 Environmental pollution	4
1.4 Biodegradation as an alternative treatment	5
1.5 Mechanism of biodegradation of dyes	6
1.6 Enzymes in azo dye degradation	6
1.6.1 Laccases	7
1.6.2 Degradation of azo dyes by azo reductases	8
1.7 Literature review	9
1.7.1 Historical background	9
1.7.2 Development of a biological process	10
1.8 Objectives	11
<b>Chapter 2: materials and methods used</b>	12
2.1 Materials	13
2.1.1 Sludge samples	13
2.1.2 Media	13
2.1.2.1 SM (salt media) broth media	13
2.1.2.2 Starch agar	13
2.1.2.3 Nitrate broth	13
2.2 Methods	14
2.2.1 Sample collection	14

2.2.2 Inoculum development	14
2.2.3 Observation	14
2.2.3.1 Table of absorbance for RV 5R	15
2.2.3.2 Decolorization of dyes after inoculation and incubation	17
2.2.4 Screening and isolation	21
2.2.5 Biochemical Identification	25
2.2.5.1 Starch hydrolysis	25
2.2.5.2 triple sugar iron test	25
2.2.5.3 Gram staining	26
2.2.5.4 Simmon's citrate	26
2.2.5.5 Oxidase test	27
2.2.5.6 Catalase test	27
2.2.5.7 Nitrate reduction test	27
2.2.5.7.1 Nitrate broth media	28
2.2.5.8 MRVP	28
2.2.5.9 Indole test	28
2.2.5.10 Urease test	28
2.2.5.11 Growth at 6.5% NaCl solution	29
2.2.5.12 Acid production from carbohydrates	29
2.2.5.13 Decolorization	29
2.2.5.15 ABIS microbiology software	30
<b>Chapter 3: Results and observations</b>	31
3.1 Biochemical tests	32
3.1.1 Methyl red and Vogues Proskauer (MRVP)	32
3.1.2 Catalase test results	33
3.1.3 Oxidase test	35
3.1.4 Nitrate reduction test	36
3.1.5 Simmon's citrate test	37
3.1.6 Triple sugar iron test	37
3.1.7 Motility Indole Urease test	40
3.1.8 Starch hydrolysis	42
3.1.9 Gram staining results	44
3.2 Absorbance readings	46
3.2.1 Further verification	49
3.3 Decolorization	50
3.4 Final identification results	52

<b>Chapter 4: Discussion</b>	59
4. Discussion	60
<b>Chapter 5: Conclusion</b>	62
5. Conclusion	63
<b>Chapter 6: References</b>	65
6. references from journals	66
<b>Appendices</b>	73

## Abstract

Azo dyes account for the majorly produced synthetic dye substances in industries. They are extensively used in the textile, leather, pharmaceutical and cosmetics industries, posing a threat to all life forms. Harmful chemicals are consistently used in the dyeing process. These include dioxin, toxic heavy metals such as chrome, copper, zinc and formaldehyde. These are proven to be potential carcinogens. The dyes also adsorb and reflect the sunlight entering the water, thereby interfering with the aquatic species growth and hindering photosynthesis. The solution to these remedies are generally available within the environment. There are certain microorganisms which have the ability to degrade these azo dyes. In this study three bacterial strains, *Streptococcus equi subsp. zooepidemicus*, *Brevibacillus centrosporus*, *Paenibacillus azoreducens*, have been extracted from the sludge samples collected from Ridesha Textile Industry, Bhaluka. ABIS microbiology software (Advanced Bacterial Identification Software) was used to justify and determine the identity of these bacteria with the aid of results obtained from the biochemical tests that were undertaken. They have remarkably reacted to the azo dye Reactive Violet 5R. They have decolorized this dye with each of them producing unique results. The decolorization rate differed for every organism inoculated into the azo dye, reactive violet 5R. This decolorization rate was also different for the various concentrations of the same dye. *Brevibacillus centrosporus* displayed a decolorization rate of 94.55%, 90.79%, 91.17% when inoculated and incubated in an SM broth containing the azo dye reactive violet 5R at 1% (v/v), 3% (v/v), 5% (v/v) concentrations for a consecutive 5 days. *Paenibacillus azoreducens* projected a decolorization rate of 85.63%, 86.48%, 38.81% for the respective concentrations, 1% (v/v), 3% (v/v), and 5% (v/v) of the azo dye reactive violet 5R. *Streptococcus equi subsp. zooepidemicus* produced intriguing results where the decolorization rates were 67.78%, 21.69%, 40.10% for the respective concentrations 1% (v/v), 3% (v/v) and 5% (v/v) of the azo dye reactive violet 5R, but the rate of growth of this organism in the SM broth media was much higher as proven by the absorbance rates achieved in the consecutive 5 days of inoculation and incubation. The organisms obtained after conducting this study can be used as a biological consortia for the

treatment of the textile effluents. In the future, the genes responsible for the dye degrading characteristics can be isolated from their hosts and with the help of recombinant technology it

### List of Figures:

<b>Figure title</b>	<b>Page number</b>
Fig 1.1a) structure of azo dye reactive violet 5R	2
Fig 1.1b) dyes being dumped into the rivers	3
Fig 1.3a) dyes being dumped through tunnels	4
Fig 1.3b) dyes polluting the rivers and lakes.	5
Fig 2.2.3a): Reactive violet 5R, 1% concentration	17
Fig 2.2.3b) decolorization of reactive violet 5R, 1%, number 1 sample after 5 days	18
Fig 2.2.3c) decolorization of reactive violet 5R, 1%, number 1 sample after 5 days	18
Fig 2.2.3d) reactive violet 5R, 5%, control	19
Fig 2.2.3e) decolorization of reactive violet 5R, 5%, sample number 1 after 5 days	19
Fig 2.2.3f) no visible changes of reactive violet 5R, 5%, sample number 2 after 5 days	19
Fig 2.2.3g) reactive violet 5R, 10%, concentration	20
Fig 2.2.3h) reactive violet 5R, 10%, sample number 1	20
Fig 2.2.3i) no visible change in reactive violet 5R, 10% sample 2 after 5 days	20



Fig 2.2.4a) colonies observed in $10^{-4}$ for Reactive violet 5R, 1%, sample no. 1 and $10^{-5}$ for reactive violet 5R, 1% sample no. 1	22
Fig 2.2.4b) colonies observed in $10^{-4}$ for reactive violet 5R, 1% for sample no. 2 and $10^{-5}$ reactive violet 5R, 5 % sample no. 2	23
Fig 2.2.4c) colonies observed in $10^{-5}$ reactive violet 5R, 10% sample no.1 and $10^0$ reactive violet 5R, 1% for sample no. 2	23
Fig 2.2.4 d) colonies observed in $10^{-3}$ for reactive violet 5R, 1% for sample no. 2 and $10^{-4}$ reactive violet 5R, 10% for sample no. 1	24
Fig 2.2.4e) colonies observed in $10^{-4}$ reactive violet 5R, 5% for sample no. 1 and $10^{-5}$ reactive violet 5R, 5% for sample no. 1	24
Fig 2.2.4f) colonies observed in $10^{-5}$ reactive violet 5R, 10% for sample no.1 and $10^{-4}$ reactive violet 5R, 10% for sample 1	25
Fig 3.1.1a) methyl red and vogues proskauer samples incubated for 24 hours at 37 degrees	32
Fig 3.1.1b) methyl red samples and vogues proskauer samples after adding the barrit reagent	33
Fig3.1.2a) 1% (ii)D, 1% (ii) A, 10% (ii) c, all positive.	33
Fig3.1.2b) 10% (i)A, 10%(ii) D, 10% (ii) E, all positive	33
Fig 3.1.2c) 5% (i) D, 1% (i) D, 10% (i) B, 5% (i) E, all positive	34
Fig 3.1.2d) 1% (ii) D, 1% (ii) A, 10% (ii) C, all positive	34
Fig 3.1.2e) 1% (ii) C, 1% (ii) B, 5% (ii) D, all positive except 5% (ii) D.	34
Fig 3.1.2f) 5% (i) B, 5% (ii) E, 1% (i) C, all positive except 1% (i) C	34

Fig 3.1.3a) slightly positive 5%(ii) E (oxidase test)	35
Fig 3.1.3b) all negative (oxidase test)	35
Fig 3.1.4a) positive test results(nitrate reduction test)	36
Fig 3.1.4b) all negative (nitrate reduction test)	36
Fig 3.1.5 a) positive tested samples ( 5%(i) C, 5% (i) E, 10% (ii) D.	37
Fig 3.1.6a) all negative, Hydrogen sulfide slightly positive.	38
Fig 3.1.6b) all negative, slant red.	39
Fig 3.1.6c) slant yellow, all negative	40
Fig 3.1.7a) 10% (ii) E, urease positive, indole negative, non, motile	41
Fig 3.1.7b) 5% (i) A, motile, rest negative	41
Fig 3.1.7c) 1% (ii) D, motile, rest negative	41
Fig 3.1.8a) all positive (starch hydrolysis)	42
Fig 3.1.8b) all negative(starch hydrolysis)	43
Fig 3.1.9a) A gram positive, rod	45
Fig 3.1.9b) gram negative rod	45
Fig 3.1.9c) gram positive cocci	46
Fig 3.2.1a) the results for the biochemical test	49
Fig 3.3a) decolorization of RV 5R by the organism 10% (ii) E after 5 days	50
Fig 3.3b) decolorization of RV 5R by the organism 5% (i) A after 5 days	50
Fig 3.3a) decolorization of RV 5R by the organism 15% (ii) D after 5 days	51

Fig 3.4a) 10%(ii)E <i>Streptococcus equi subsp. zooepidemicus</i>	52
Fig 3.4b) 5% (i) A <i>brevibacillus centrosporus</i>	53
Fig 3.4 c) 5%(ii) D <i>Paenibacillus azoreducens</i>	53
Fig 3.4 d) 10% (i) D <i>Bacillus simplex</i>	54
Fig 3.4 e) 1% (i) C <i>Bacillus firmus</i>	54
Fig 3.4 f) 5% (i) C <i>Bacillus megaterium</i>	55
Fig 3.4g) 10% (i)B <i>Bacillus simplex</i> )	55
Fig 3.4 h) 10% (i) A <i>Bacillus Nealsonii</i>	56
Fig 3.4 i) 10% (i) C <i>Bacillus firmus</i>	56
Fig 3.4.1a) decolorization rates of 1% (v/v), 3% (v/v), 5% (v/v) of RV 5R by the organism (5% (i) A) ( <i>brevibacillus centrosporus</i> )	57
Fig 3.4.1 b) decolorization rates of 1% (v/v), 3% (v/v), 5% (v/v) of RV 5R by the organism (5%(ii)D) ( <i>Paenibacillus azoreducens</i> )	57
Fig 3.4.1c) decolorization rates of 1% (v/v), 3% (v/v), 5% (v/v) of RV 5R by the organism <i>Streptococcus equi subsp. zooepidemicus</i>	58

## List of abbreviations

<b>Abbreviations</b>	<b>Descriptions</b>
SM	Salt media
<i>et al</i>	And others
NA	Nutrient agar
mg	Milligram
sp.	Species
ml	Milliliter
RV5	Reactive violet 5r
MR	Methyl red
VP	Vogues proskauer
Rpm	Rotations per minute
v/v	Volume by volume
g/l	Gram per liter
nm	nanometer

**List of tables:**

	<b>Page number</b>
Table 2.2.3.1: table of absorbance for consecutive 5 days.	15
Table 2.2.4.1) the colonies to be further analyzed with the help of biochemical tests.	21
Table 3.1.6.1 triple sugar iron test results	37
Table 3.1.7.1 motility indole urease test results	40
Table 3.1.8.1: starch hydrolysis results	43
Table 3.1.9.1: gram staining results	44
Table 3.2.a organisms and their initial absorbance readings	46
Table 3.2.b five days of absorbance readings of the selected organisms at different concentrations	48
Table 3.2.1 results for the acid production from carbohydrates	49



# CHAPTER ONE: Introduction

## **Introduction:**

### **1.1 Background**

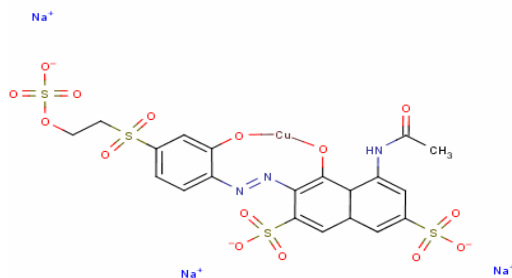


Fig 1.1a) structure of azo dye reactive violet 5r

The earth consists of one-third of land and two thirds of water. For centuries civilizations have relied on the ocean and its water bodies for various sorts of purposes. Wastes are often seen to be disposed in water bodies such as lakes, ponds, rivers. Most of these wastes come from factories, industries, tanneries etc

Dyes or the colors used in various different industries are one of the primary elements responsible in polluting the water bodies. The chemical structure of these coloured dyes are characterized by highly substituted aromatic rings joined by one or more azo groups(-N=N-). These substituted ring structures make these molecules recalcitrant. This is why it cannot be degraded by conventional wastewater treatment processes. (Thakur et al., 2012) One such azo dye is the Reactive Violet 5R with the chemical name trisodium;(3Z)-5-acetamido-3-[[2-hydroxy-4-(2-sulfonatoxyethylsulfonyl)phenyl]hydrazinylidene]-4-oxo-4a,8a-dihydronaphthalene-2,7-disulfonate;copper with the molecular formula,  $C_{20}H_{16}N_3Na_3O_{15}S_4$





Fig 1.1b) dyes being dumped into the rivers

## 1.2 Commercial use of dyes

Vast quantities of dyes have been synthesized until now, and dye manufacturing has become a significant part of the chemical industry. Over 10,000 different dyes with an annual production of over  $7 \times 10^5$  metric tons worldwide are commercially available (Bheemaraddi et al., 2014). Dyes are an important source in the textile industries along with paper production, food technology, agricultural research, light harvesting arrays, photochemical cells, hair colouring and cosmetics (Khan et al., 2014). Poly-aromatic molecules which are also known as Synthetic dyes give a permanent color to materials like textile fabrics. (Singh et al., 2014). Azo dyes are one such diverse group of synthetic organic compounds that account for the majority of all textile dyestuffs produced. They are the most extensively used in a number of industries such as textile dyeing, paper, food, leather, cosmetics, and pharmaceutical industries (Bheemaraddi et al., 2014).

### 1.3 Environmental pollutions

The amount of dye lost depends upon the class of dye application. This varies from 2% loss while using basic dyes to 50% loss in certain reactive sulfonated dyes which eventually leads to severe contamination of surface and ground waters in the vicinity of dyeing industries (O'Neill, et al., 1999). Improper textile dye effluent disposal in aqueous ecosystems leads to the reduction in sunlight penetration which in turn decreases photosynthetic activity, dissolved oxygen concentration, water quality and depicts acute toxic effects on aquatic flora and fauna, causing severe environmental problems worldwide (Vandevivere et al, 1998). Azo dyes also create a negative impact by causing an effect on the total organic carbon (TOC), biological oxygen demand (BOD), and chemical oxygen demand (COD) (Saratale et al., 2009;) Many synthetic azo dyes and their metabolites are toxic, carcinogenic, and mutagenic (Myslak et al.,1988).Moreover, they can cause human health disorders through direct or indirect exposure such as nausea, hemorrhage, ulceration of the skin and mucous membranes, and severe damage to kidneys, the reproductive system, liver, brain, and central nervous system.



Fig 1.3a) dyes being dumped through tunnels



Fig 1.3b) dyes polluting the rivers and lakes.

#### **1.4 Biodegradation as an alternative treatment**

Various physicochemical methods have been used for the removal of dyes from wastewater (Wang et al, 2009). These involve advanced processes like adsorption, chemical precipitation and flocculation, photolysis, chemical oxidation and reduction, electrochemical treatment, and ion pair extraction. Due to the high cost, low efficiency, limited versatility, interference by other wastewater constituents, and the handling of the waste generated these methods have been found to be less effective (Kaushik et al, 2009). Contrarily, biological processes provide an alternative to existing technologies because they are more cost-effective, environmental friendly and do not produce large quantities of sludge. Many microorganisms belonging to the different taxonomic groups of bacteria, fungi, actinomycetes, and algae have been reported for their ability to decolorize azo dyes (Asad et al., 2007). Pure fungal cultures have been used to develop bioprocesses for the mineralization of azo dyes, but their long growth cycle and moderate decolorization rate limits their decolorizing efficacy (Moosvi S et al., 2007;) In contrast, bacterial

decolorization is relatively faster. Despite of their success in degrading azo dyes, comprehensive solutions for sulfonated azo dyes removal are have not yet been achieved. This calls for continued search for new organisms and technologies.

### **1.5 Mechanism of biodegradation of dyes**

The bacterial reduction of the azo dye is usually nonspecific and bacterial decolorization is normally faster. A wide range of aerobic and anaerobic bacteria have been extensively reported as degraders of azo dyes. These involve *Bacillus subtilis*, *Pseudomonas sp*, *Escherichia coli*, *Rhabdobacter sp*, *Enterococcus sp*, *Staphylococcus sp*, *Xenophilus sp*, *Corneybaterium sp*, *Clostridium sp.*, *Micrococcus dermacoccus sp*, *Acinetobacter sp*, *Geobacillus*, *Lactobacillus*, *Rhizobium*, *Proteus sp*, *Morganella sp*, *Aeromonas sp*, *Alcaligenes ap*, and *Klebsiella sp* (Stolz., 2001; Pearce et al., 2003; Olukanni et al., 2006; Vijaykumar et al., 2007; Hsueh and Chen, 2008; Lin and Leu, 2008). Some strains of aerobic bacteria use azo dyes as sole source of carbon and nitrogen (Coughlin et al., 2002); others only reduce the azo group by special oxygen-tolerant azo reductases.

A group of investigators investigated the ability of bacteria in metabolism of azo dyes. Under aerobic condition azo dyes cannot be readily metabolized, and as a result of metabolic pathways it is degraded into intermediate compounds but not mineralized. It can be completely degraded under coupled aerobic anaerobic degradation (McMulan et al., 2001). In anaerobic condition, the azo bond undergoes cleavage to generate aromatic amines and it was mineralized by nonspecific enzymes through ring cleavage under aerobic condition. Therefore, coupled anaerobic treatment followed by aerobic treatment can be an efficient degradation method of azo dyes (Feigel et al., 1993). It has been witnessed that bacterial strains showed good growth when cultured in aerobic or agitation culture but color removal was obtained with a high efficiency when cultured in anoxic or anaerobic condition. (Chen et al., 2003) Mixed bacterial culture can give a better degradation rate than the individual strain.

### **1.6 Enzymes in azo-dye degradation**

Microbial degradation of azo dyes is mediated by enzymes. The predominant enzymes are azoreductase, laccases, lignin peroxidase, manganese peroxidase, and hydroxylases. Laccase and

azoreductase have been shown to degenerate azo dyes (Rodriguez et al., 1999; Reyes et al., 1999). Dye molecules display a high structural variety, they are degraded only by few enzymes. Enzymatic processes are very promising for the decolorization of synthetic azo dyes. Wide variety of microorganisms excrete different active enzymes like laccases, phenolic oxidases, peroxidases and variety of azo-dye reductases enzymes.

### 1.6.1 Laccases

Laccase enzymes degrade the azo dye through a non-specific free radical mechanism to form phenolic compounds and thereby prevent the formation of toxic aromatic amines (Chivukula et al., 1995). The mechanism of laccase involves the removal of an H<sup>+</sup> atom from the hydroxyl and amino groups of the ortho and para substituted phenolic substrates and aromatic amines. (Chivukula et al., 1995; Kirby et al., 2000; Peralta et al., 2003; Blaquez et al., 2004; Novotny et al., 2004). These enzymes are multicopper phenol oxidases. They decolorize azo dyes through a highly nonspecific free radical mechanism forming phenolic compounds, thereby avoiding the formation of toxic aromatic amines (Chivukula et al., 1995; Wong and Yu, 1999). Laccase oxidizes the phenolic group of the azo dyes frequently, this is preceded by a nucleophilic attack by water on the phenolic ring carbon bearing the azo linkages (Susana et al., 2005). During this reaction, cross-coupling of the reactive species results in the formation of C-C and C-O bonds between phenolic molecules and of C-N and N-N bonds between aromatic amines (Andrea et al., 2005). Laccase preparations obtained from *Pleurotus ostreatus*, *Schizophyllum commune*, *Sclerotium rolfisii* and *Neurospora crassa*, increased up to 25% the decolorization of individual commercial triarylmethane, anthraquinonic, and indigoid textile dyes (Abadulla et al., 2000). Laccase enzyme is a blue copper oxidase that catalyzes the four electron reduction of molecular oxygen to water. These enzymes are mainly obtained from lignin degrading fungi such as *Trametes versicolor* and *T.villoa* as well as fungi like *Fusarium soloni* and *Cladospora cladosporioides*. (abedin et.al,2008) *B. stearothermophilus*, *Pseudomonas aeruginosa*, and *Mycoplasma pneumoniae* (Yasuhko et. al., 2001). Fungi usually degrade dyes by exo enzymes like peroxidases (Duran et al., 2002) and phenol oxidases (Glenn et al., 1986). The ligninolytic fungi like *P. chrysosporium* produces lignin peroxidase (LiP) (Glenn et al., 1983; Tien et al., 1983) and manganese peroxidase (MnP) (Wariishi et al., 1988). Several reports have shown that LiP or MnP are directly involved in the degradation of various dyes (Paszczynski et al., 1991;

Pasti et al., 1992; Goszczynski et al., 1994;). Manganese peroxidase was reported as the main enzyme involved in dye decolorization by *Phanerochaete chrysosporium* (Chagas and Durrant, 2001) and lignin peroxidase for *Bjerkandera adusta* (Robinson et al., 2001). Some non-white-rot fungi that can successfully decolorize dyes have also been reported by Bumpus (2004).

### **1.6.2 Degradation of azo dyes by azo reductases**

Azo reductases are membrane bound enzyme that catalyzes the reaction only in presence of reducing equivalents like FADH and NADH (Robinson et al., 2001). Therefore the reduction process takes place in bacterial cells with intact cell membranes (Russ et al., 2000). In Gram negative bacteria, enzyme can make direct contact with either the azo dye substrate or a redox mediator at the cell surface (Myers and Myers, 1992). In addition, some low molecular weight redox mediator compounds can act as electron shuttles between the azo dye and an NADH - dependent azo reductase that is situated in the outer membrane (Gingell and Walker,1971). These enzymes are oxygen sensitive, so in extracellular environment, this reduction mechanism will be inhibited by oxygen. It was reported that the membrane-bound and the cytoplasmic azo reductases are two different enzyme systems. (Kudlich et al., 1997). The whole mechanism for the redox reaction was under anaerobic condition, whereas the redox mediators depended on cytoplasmic reducing enzymes to supply electrons. (Yoo et al., 2001). The direct enzymatic reaction of an azo reductase, may be a dehydrogenase enzyme that is synthesized throughout the cytoplasm (Bragger et al., 1997).

## 1.7 literature review

### 1.7.1 Historical background

It was reported that the inefficiency in dyeing processes has resulted in 10-15% of unused dyestuff entering the wastewater directly (Zollinger, 1987; Spadarry et al., 1994). Presence of color in textile effluent gives a clear-cut sign of water being polluted, and release of this highly colored and complex effluent can damage directly the receiving water. Furthermore, it is difficult to degrade the mixtures of the wastewater from textile industry by conventional biological treatment processes, because their ratio of BOD/COD is less than 0.3 (Chun and Yizhong, 1999). In some cases, traditional biological procedures were combined with physical or chemical treatment processes to achieve better decolorization (Vandevivere et al., 1998), but chemical or physical - chemical methods are generally costly, less efficient and of limited applicability, and produce wastes, which are difficult to dispose of.

Biodegradation of commercially available textile dyes namely Reactive Orange – M2R, Reactive Blue – MR, Reactive Yellow – M4G and Reactive Black-B were studied against five bacterial isolates which have been isolated from the dye effluent sample by Pour plate method and percentage decolorization. Three different bacteria were isolated from the textile dye effluent. Based on preliminary tests, plating on selective media and biochemical tests, they were identified as *Bacillus sp.*, *Escherichia coli* and *Pseudomonas fluorescens*.

A research was executed where eighteen textile effluent adapted bacterial isolates belonging to the genera, *Bacillus*, *Acinetobacter*, *Staphylococcus*, *Legionella* and *Pseudomonas* were investigated for the potential of textile effluent adapted bacteria in decolorizing it. *Bacillus* and *Legionella* were found to have use in effluent treatment. (Olukanni et al., 2005). It was further analyzed to check the ability of *Staphylococcus aureus*, *Bacterioides fragilis*, *Bacillus subtilis*, *Bacillus cereus*, *Clostridium perfringens*, *Escherichia coli* and *Peptostreptococcus sp.* to reduce and stabilize textile effluents containing predominantly Indigo Blue.

Simultaneously in another research which was carried out showed, five different bacterial isolates from the textile dye effluent sample. These were identified as *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae* and *Escherichia coli* (Manivannan et al., 2011). Based on preliminary tests, plating on selective media and biochemical tests, they were identified as *Bacillus* sp *Escherichia coli* and *Pseudomonas fluorescens*.

### **1.7.2 Development of a biological process**

As a viable alternative, biological processes have been developed and received increasing interest due to their cost efficiency, ability to produce less sludge, and environmental benignity (Banat et al., 1996). Therefore, to develop a practical bioprocess for treating dye-containing wastewater is of great significance. The effectiveness of microbial decolorization depends on the adaptability and the activity of selected microorganisms. Attempt to degrade this complex compound was made in early 90s. But henceforth the success rate was too low.

In recent times however, photocatalytic experiments were introduced and performed in a batch system (pyrex vessel) with a total capacity of 1 L (batch mode). The total volume of the dye solution in the system was 800 mL. The reactor containing reaction solution (mixture of dye solution and photocatalyst powder) was placed on a magnetic stirrer to provide appropriate mixing. A medium-pressure mercury lamp (UV-C, maximum emission at 247.3 nm) with a power of 150 W and 66 mm length was used as the light source. The UV lamp was placed centrally and parallel to the length of the cylinder in the reactor, inside a quartz tube. (Karimi L, et al., 2011). The reactor was covered with an aluminum foil sheet to prevent UV emission around it. Due to the production of heat by the light source, the temperature of the solution was maintained constant at  $21\pm 3^{\circ}\text{C}$  in all experiments by cooling water around the vessel.

In order to perform the photocatalytic experiments, the working solution, containing 50 mg/L of dye and 0.2 g/L of  $\text{TiO}_2$  was prepared using tap water. Prior to the photocatalytic process, the suspensions were magnetically stirred in the dark for 30 min to reach adsorption-desorption equilibrium between the dye and  $\text{TiO}_2$ . Samples (5 mL) were taken at specific time intervals, centrifuged at 6000 rpm for 15 min and then filtered through a  $0.45\ \mu\text{m}$  syringe filter in order to



separate TiO<sub>2</sub> particles (Mahvi AH et al., 2009). Measurement of absorbance was carried out at maximum wavelength of RB5 ( $\lambda_{\text{max}} = 597 \text{ nm}$ ). UV–vis spectra of samples were recorded between 200 and 800 nm. Photocatalytic degradation of different concentrations of RB5, namely, 100, 200, 300 and 500 mg/L was also examined.

A combined biological photochemical process was undertaken. To carry out these experiments, synthetic medium was treated with *Candida tropicalis* JKS2 cells for 24 h and after centrifugation and filtration, fed into the reactor. At different time intervals, samples were withdrawn, centrifuged and filtered through a 0.45  $\mu\text{m}$  membrane filter following which UV–vis scan spectroscopy and COD measurements were carried out. COD measurements (for both biological and photocatalytic steps of the process) were performed following the closed reflux, colorimetric method using a COD reactor, according to standard procedures

### **1.8 Objectives**

The microorganisms involved in dye degrading are mostly fungi (Barr et al., 1994) and bacteria (Banet et al., 1997 Biological treatment may involve both aerobic and anaerobic degradation by microorganisms.). Microbial treatment is cost effective and is receiving wide attention (Van der Zee et al., 2005). The study conducted provides substantial evidence of the efficiency of the organisms to be decolorize the dye azo dye reactive violet 5R. This will eventually lead to the treatment of waste effluents along with the elimination of a dye's carcinogenic properties.

# CHAPTER TWO: MATERIALS AND METHODS

## **2. Materials and methods used**

This research work was carried out at the microbiology and Biotechnology Laboratory of the Mathematics and Natural science department

### **2.1 Materials**

#### **2.1.1 Sludge samples**

The samples were sludge extracts taken from Ridesha Textile Industries, Bhaluka. This was the source of microbial cultures which are later extracted and identified.

#### **2.1.2 Media**

##### **2.1.2.1 SM (salt media) broth media**

SM broth media consists of glucose (1g/liter), peptone (10g/liter), yeast extract (1g/liter) potassium dihydrogen phosphate (1.9g/liter) dipotassium hydrogen phosphate (0.6g/liter). All the components were added and mixed up in 1 liter beakers via measuring cylinders. The following amount was added in this concentration until 1.8 liters of SM broth was made. The pH levels varied from 6.9 to 7.1. These were later autoclaved and then the dye reactive violet 5r was added and left in the shaking incubator for 2 weeks at a temperature of 37 degrees Celsius.

##### **2.1.2.2 Starch agar**

Starch agar media is composed of beef extract 3g/liter, soluble starch 10g/liter, and nutrient agar 15g/liter.

##### **2.1.2.3 Nitrate broth**

Nitrate broth consists of sulfonic acid (solution A)-5 drops, alpha-naphthylamine (solution B)- 5 drops, zinc powder- very small amount only added to the ones which did not turn red after adding both the solutions respectively

## **2.2 Methods**

### **2.2.1 Sample collection**

Sludge sample was collected from Ridesha Textile Industries, Bhaluka. Dye samples were collected from Orient chemicals limited, Dhaka.

### **2.2.2 Inoculum development**

The SM broth was prepared at a volume of 1.8 liters and then it was distributed into 9 conical flasks at an equal volume of 200ml. After preparation of SM broth media, the following dyes were added at 3 different concentrations, 1% (v/v), 5% (v/v) and 10% (v/v). The soil samples were diluted with water. 5 grams of soil sample was weighed out in a top pan balance and added to 50ml of water in a 100ml beaker. This was mixed thoroughly.

The next step was to collect a volume of 1000 microliters of soil sample solution from the beaker and add it to the 9 conical flasks. This was mixed thoroughly. The volume to volume ratio was 1:200

### **2.2.3 Observation**

The 9 conical flasks were placed in the shaking incubator and the optical density was measured for 5 days at 37° C. Color changes were observed during this 10 days. Their absorbance readings were recorded.

### 2.2.3.1 Table of absorbance for reactive violet 5R

The samples were centrifuged at a speed of 3000 rpm for 5 minutes. The next task was to extract the supernatant. After that the absorbance readings for the supernatant was measured with the help of a spectrophotometer at a wavelength of 410 nanometers

#### Day 0

Concentration of RV 5R	Absorbance of control	Absorbance at 410nm	
		Sample no. 1	Sample no.2
1%	0	0.019	0.005
5%	0	0.027	-0.034
10%	0	0.081	0.008

#### Day 1

Concentration of RV 5R	Absorbance of control	Absorbance at 410nm	
		Sample no.1	Sample no.2
1%	0	-0.032	-0.081
5%	0	0.307	0.051
10%	0	0.116	0.022

**Day 2**

<b>Concentration of RV 5R</b>	<b>Control</b>	<b>Absorbance at 410nm</b>	
		<b>Sample no. 1</b>	<b>Sample no.2</b>
1%	0	-0.384	-0.384
5%	0	-0.104	0.074
10%	0	0.445	0.210

**Day 3**

<b>Concentration of RV 5R</b>	<b>Absorbance of control</b>	<b>Absorbance at 410nm</b>	
		<b>Sample no.1</b>	<b>Sample no.2</b>
1%	0	-0.145	-0.123
5%	0	-0.485	0.572
10%	0	0.865	0.116

**Day 4**

<b>Concentration of RV 5R</b>	<b>Absorbance of control</b>	<b>Absorbance at 410nm</b>	
		<b>Sample no.1</b>	<b>Sample no.2</b>
1%	0	0.049	0.035
5%	0	-0.313	-0.304
10%	0	-0.300	-0.341

## Day 5

Concentration of RV 5R	Absorbance of control	Absorbance at 410nm	
		Sample no.1	Sample no.2
1%	0	-0.062	-0.099
5%	0	-0.173	0.166
10%	0	0.616	0.008

Table 2.2.3.1: table of absorbance for consecutive 5 days.

### 2.2.3.2 Decolorization of the dyes after inoculation and incubation



Fig 2.2.3a): Reactive violet 5R, 1% concentration

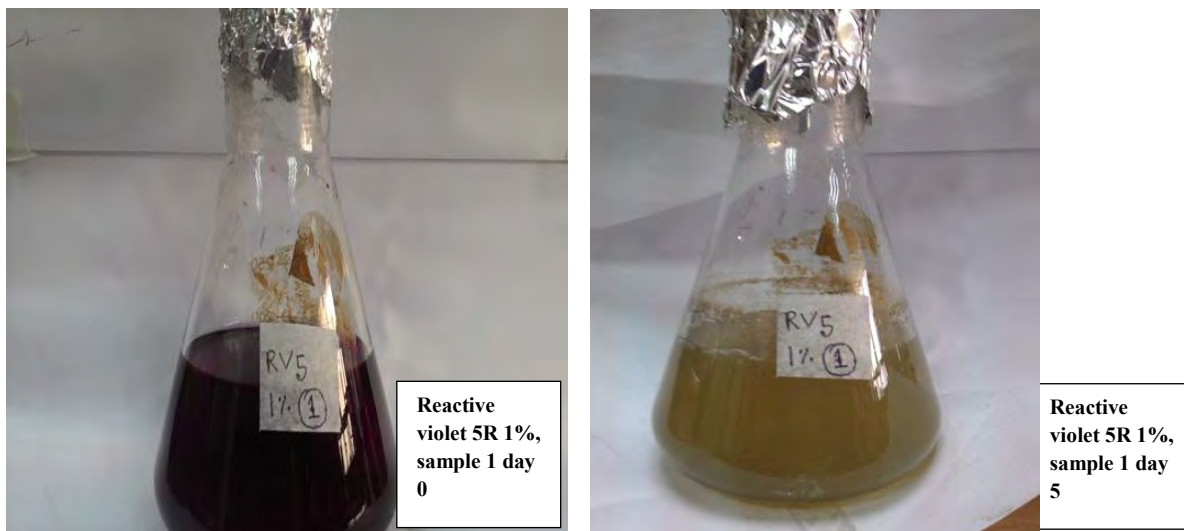


Fig 2.2.3b) decolorization of reactive violet 5R, 1%, number 1 sample after 5 days



Fig 2.2.3c) decolorization of reactive violet 5R, 1%, number 1 sample after 5 days





Fig 2.2.3d) reactive violet 5R, 5%, control



Fig 2.2.3e) decolorization of reactive violet 5R, 5%, sample number 1 after 5 days

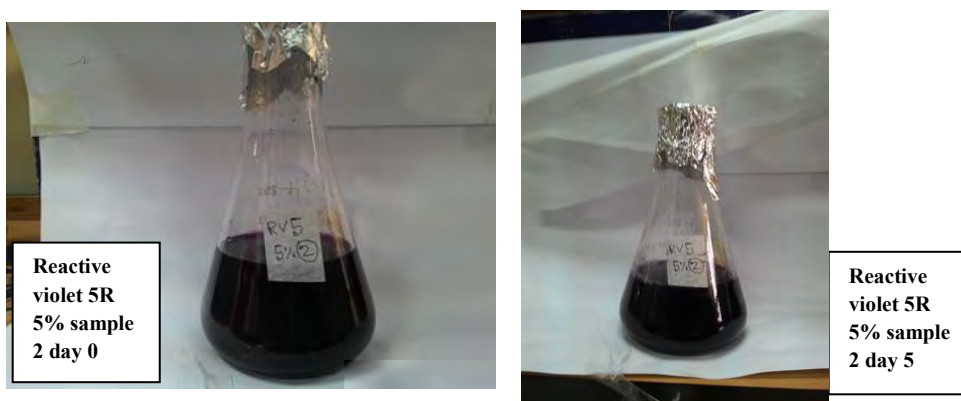


Fig 2.2.3f) no visible changes of reactive violet 5R, 5%, sample number 2 after 5 days

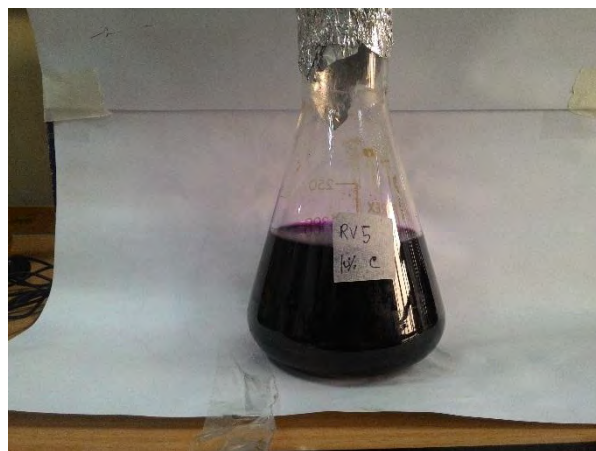


Fig 2.2.3g) reactive violet 5R, 10%, concentration

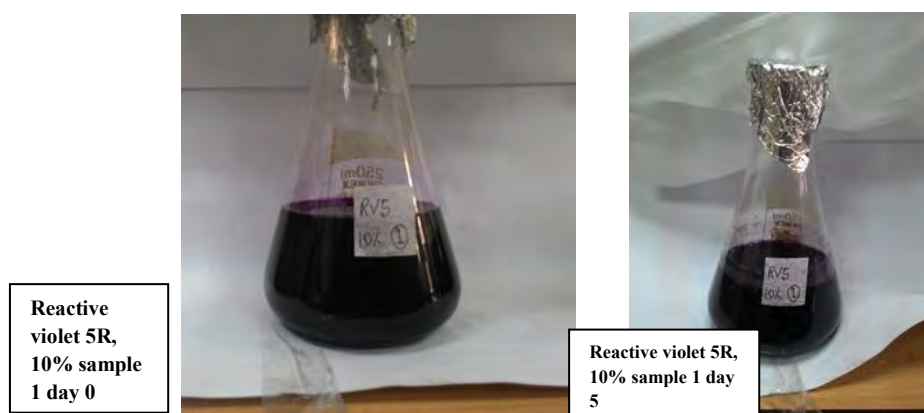


Fig 2.2.3h) no visible change in reactive violet 5R, 10%, sample number 1 after 5 days



Fig 2.2.3i) no visible change in reactive violet 5R, 10% sample 2 after 5 days

## 2.2.4 Screening and isolation

After the above mentioned results were achieved the first step was taken to isolate the microbial samples from the dyes. 50ml of dye sample was taken into falcon tubes and then they were centrifuged. The pellets were taken for further observation whereas the supernatants were discarded. The pellets mostly contained cell samples of the micro-organisms present in the soil sample initially which were introduced into the dye. Then a saline solution was added to the pellets and then they were vortexed. After vortexing a serial dilution process took place, the samples were diluted to the point of  $10^{-4}$  and  $10^{-5}$

After that a screening procedure was carried out judging by the size and morphological characteristics of the colonies. After the following serial dilution was executed and the colonies were observed, each colony was marked individually according to the concentration from which they were obtained and also from the number of the sample. The marked ones are indicated below in the table:

Table 2.2.4.1) the colonies to be further analyzed with the help of biochemical tests.

Rv5% (i) D
Rv10% (i)D
Rv10%(i) B
Rv10%(ii)C
Rv5%(ii)D
Rv5%(ii)E
Rv10%(ii)E
Rv1%(ii)A
Rv1%(ii)B
Rv10%(i)A
Rv1%(ii)D
Rv5%(i)B
Rv1%(ii)C
Rv5%(i)E
Rv10%(i)D

Rv10%(ii)D
Rv1%(i)D
Rv5%(i)A
Rv5%(i)C
Rv1%(i)C

Then these were individually transferred into nutrient broth test tubes. These were then left to inoculate for one whole day at a temperature of 37° C. By the appearance of a certain level of turbidity it is assumed that bacterial growth has taken place. This step was done just to enrich the cultures. Then these samples were individually inoculated into a different sterilized petri dishes containing nutrient agar based on their morphological characteristics. Nutrient agar is an excellent media for the growth of organisms. The following results are shown in the pictures.

They were left to incubate for a day at a temperature of 37° C. These samples were later on used for the following biochemical tests listed previously. The following results were obtained which later on helped to determine the genus of the microorganisms present in the sample.



Fig 2.2.4a) colonies observed in  $10^{-4}$  for Reactive violet 5R, 1%, sample no. 1 and  $10^{-5}$  for reactive violet 5, 1% sample no. 1



Fig 2.2.4b) colonies observed in  $10^{-4}$  for reactive violet 5R, 1% for sample no. 2 and  $10^{-5}$  reactive violet 5, 5 % sample no. 2

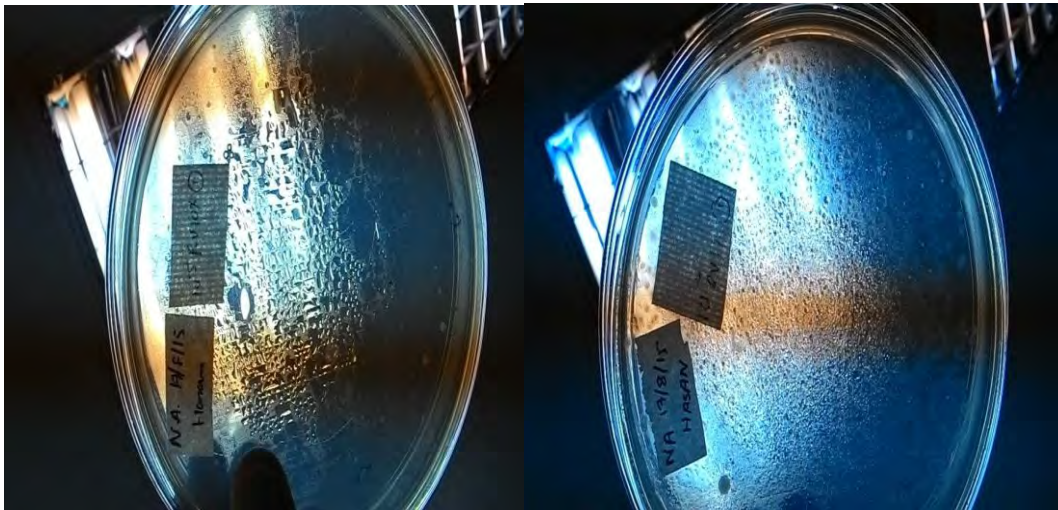


Fig 2.2.4c) colonies observed in  $10^{-5}$  reactive violet 5R, 10% sample no.1 and  $10^0$  reactive violet 5, 1% for sample no. 2



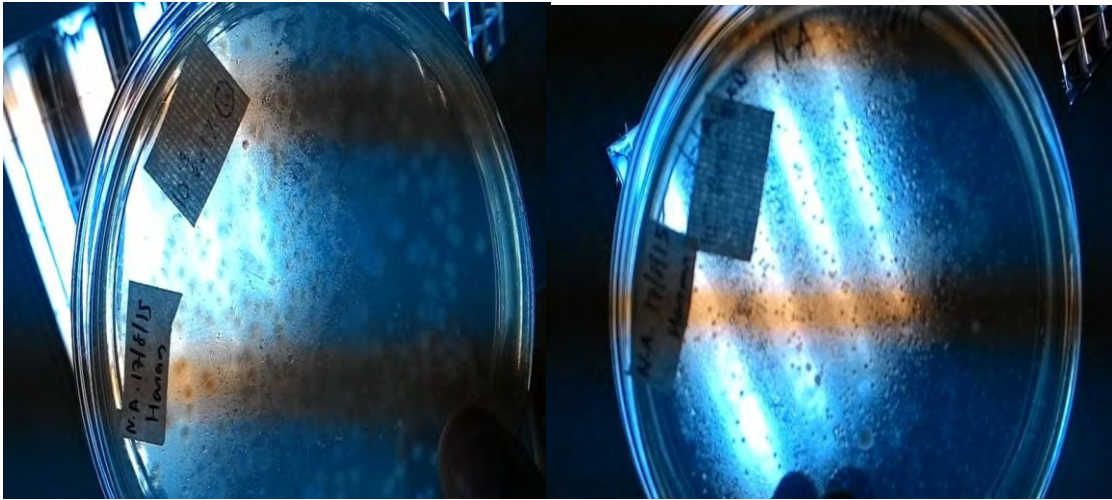


Fig 2.2.4 d) colonies observed in  $10^{-3}$  for reactive violet 5R, 1% for sample no. 2 and  $10^{-4}$  reactive violet 5, 10% for sample no. 1

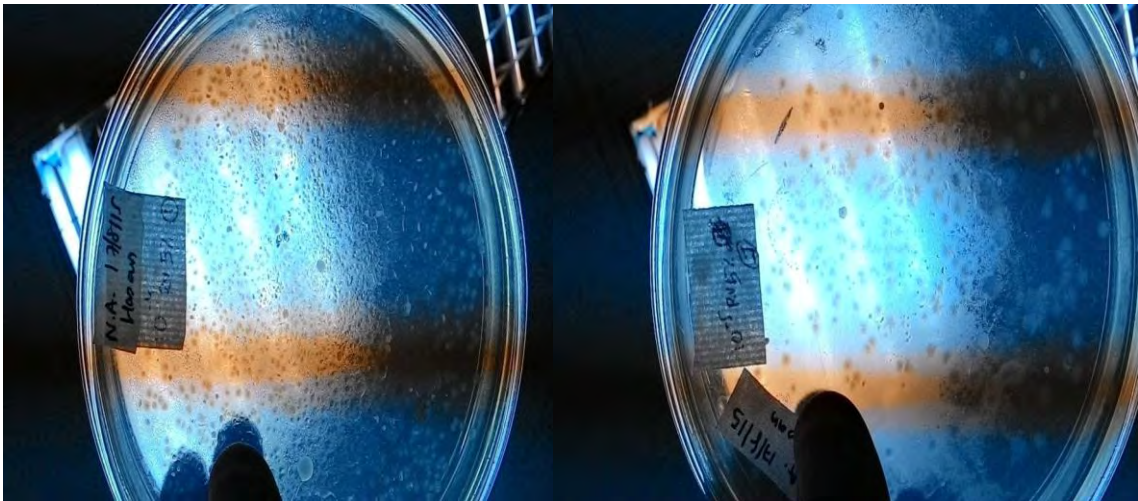


Fig 2.2.4e) colonies observed in  $10^{-4}$  reactive violet 5R, 5% for sample no. 1 and  $10^{-5}$  reactive violet 5, 5% for sample no. 1

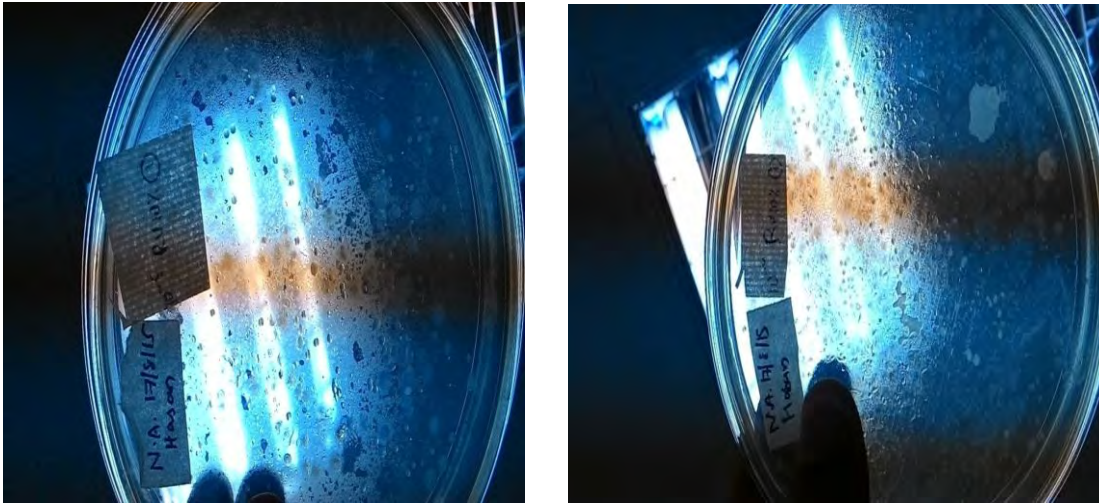


Fig 2.2.4f) colonies observed in  $10^{-5}$  reactive violet 5R, 10% for sample no.1 and  $10^{-4}$  reactive violet 5, 10% for sample 1

## 2.2.5 Biochemical identification

### 2.2.5.1 Starch hydrolysis

This biochemical test was carried out by preparing a starch agar in a petri dish. This was done by using 3 g/l of beef extract, 10g/l of soluble starch and 15g/l of agar which was autoclaved at 37 degrees Celsius for 24 hours. The next step was to inoculate the bacterial sample, this being done by forming a streak at a straight line. After that it was incubated for 24 hours at 37 degrees Celsius. The last step was to add iodine. If a clear zone was formed then the result would turn out positive. If not then it would be negative.

### 2.2.5.2 Triple sugar iron test

A triple-sugar iron agar (TSI) medium is a test which gives satisfactory reactions, that is, reactions which are more clear-cut for acid and gas, and more sensitive for  $H_2S$ .( A.A Hajna et al., 1945)

The triple sugar iron media was made in a volume of 140 ml, 7ml per test tube. The triple sugar iron media was added and it was kept slanted and placed in the autoclave for one and half hours at 15 psi for 121 degrees Celsius. Then the media was inoculated and incubated for 24 hours.

### **2.2.5.3 Gram staining**

A slide smear was prepared by transferring a drop of the suspended culture to be examined in a slide with an inoculation loop. A loopful of distilled water was added and then a minute amount of colony was transferred from the petri dish to glass slide, it was mixed over a small diameter. It was then left to air dry for some time with some heat fixation.

The initial step included adding crystal violet, it was then left for one minute. Then it was gently rinsed off and then grams iodine was added which was left to stand for 10 to 60 seconds. Then it was gently rinsed. Then ethyl alcohol was added and rinsed off within 5 seconds. The last step was to add safranin which was left to stand for 45 seconds. Then this too was rinsed off.

### **2.2.5.4 Simmon's citrate**

Simmons citrate agar tests the ability of organisms to utilize citrate as a carbon source. Simmons citrate agar contains sodium citrate as the sole source of carbon, ammonium dihydrogen phosphate as the sole source of nitrogen, other nutrients, and the pH indicator bromthymol blue.

Organisms which can utilize citrate as their sole carbon source use the enzyme citrase or citrate-permease to transport the citrate into the cell. These organisms also convert the ammonium dihydrogen phosphate to ammonia and ammonium hydroxide, which creates an alkaline environment in the medium. At pH 7.5 or above, bromthymol blue turns royal blue. At a neutral pH, bromthymol blue is green, as evidenced by the uninoculated media.

This test was carried out by adding simmon's citrate agar to screw cap tubes and then they were kept at 45 degrees slant position. The next step was to autoclave it at 15 psi and 121° C for one and half hours. Then they inoculated by stabbing a minute amount of colony taken from the petri dish and they were left to incubate at 37° C for 24 hours.



Results include that if the medium turns blue, the organism is citrate positive. If there is no color change, the organism is citrate negative. Some citrate negative organisms may grow weakly on the surface of the slant, but they will not produce a color change.

#### **2.2.5.5 Oxidase test**

The oxidase test is an important differential procedure in diagnostic bacteriology, especially useful for quickly confirming a tentative morphological identification of *Pseudomonas aeruginosa* or *Neisseria species*. This can be done as a filter paper spot test (A. L. Barry et al 1969) or by flooding colonies with one of several reagents, with or without alphanaphthol.( A. L. Barry et al.,1957)

This test was carried out by taking a filter paper soaked with a substrate tetramethyl-p-phenylenediamine dihydrochloride, the paper was moistened with distilled water. The colony to be tested was picked with a loop and smeared in the filter paper.

Positive results showed a change in colour within 10 to 30 seconds.

#### **2.2.5.6 Catalase test**

The importance of the catalase reactions, by which hydrogen peroxide is broken down to water and oxygen, is well known for its application to the differentiation of gram-positive organisms.(Welton Taylor et al., 1972).

The tests were carried out in glass slides. The bacterial samples were added into the glass slides with the help of a loop. The catalase enzyme was added to the glass slides and within a few seconds bubbles were formed showing the fact that hydrogen peroxide was degraded into water and oxygen.

#### **2.2.5.7 Nitrate reduction test**

The reduction of nitrate to nitrite was detected with dimethyl-a-naphthylamine (Wallace et al., 1927) and sulphanilic acid. The reaction was rapid with all the species tested; at 30 min. the results were consistent with the usual cultural method.

### **2.2.5.7.1 Nitrate broth media**

Nitrate broth consists of beef extract (3g/l), peptone (5g/l), potassium nitrate (5g/l). Nitrate broth was added to test tubes and Durham tubes were placed in an inverted manner. They were incubated for 24 to 48 hours. Sulfanilic acid and alpha naphthylamine each one drop was added after the incubation period. If no colour change was observed nitrite's absence was proven. Small amount of zinc was added to the nitrate reduction broth. At this point any colour change proves the test to be negative.

### **2.2.5.8 MRVP**

Bacterial culture are inoculated and incubated overnight in test tube at a temperature of 37 degrees Celsius. The day after, the MR and VP reagents are applied, the results produced either had to turn red or yellow, if yellow it proves the test results were negative, if red they were considered positive.

### **2.2.5.9 Indole test**

The production of indole from peptone depends on the presence of tryptophan in the medium. When the suspension from a culture grown on nutrient agar is incubated with tryptophan, indole is formed fairly rapidly and can be detected by any of the indole test reagents. Kovac's reagent was preferred to Bohme's as the results were easier to read and only one solution was needed. Kovac's reagent was made up in pure isoamyl alcohol, which Arnold & Weaver (1948) found gave better results than the other alcohols tested. In a dark bottle the solution remained stable on the bench for several months; the colour remained a golden yellow and no false colorations appeared in the test.

At first the bacterial colony was added to the tryptophan broth. It was incubated for 24 hours at 37 degrees Celsius. Kovac's reagent was added after that.

#### **2.2.5.10 Urease test**

Equal volumes of urea-buffer solution and suspension are mixed, sealed capillary tubes are incubated at 37°C and read for alkali production after 4 hours and 24 hours.

#### **2.2.5.11 Growth at 6.5% NaCl solution**

Nutrient agar was prepared, 6.5% solution of sodium chloride was added and after being autoclaved at standard conditions, it was inoculated and incubated using a loopful of colonies from the petri dish for 24 hours at 37 degrees Celsius.

#### **2.2.5.12 Acid production from Carbohydrates**

The constituents for carrying out this experiment are tryptase- 10g/l, NaCl-5g/l, Phenol red 0.018/l, sugar (Glucose, Fructose, Galactose, Lactose, Maltose, Mannitol, Sucrose, Treshalose) 5g/l

For 32 test tubes, 224 ml of the ingredients were to be measured out. Each test tube has a capacity of 7ml. Then they would be autoclaved at standard temperature and pressure for one and half hours. Lastly the samples were inoculated and incubated for 24 hours at 37 degrees Celsius. Color change would represent positive results.

#### **2.2.5.13 Decolorization rate**

The formula to measure the decolorization rate is  $[(\text{Initial reading} - \text{final reading})/\text{final reading}] \times 100\%$ . This formula is very important in determining the rate of decolorization that has taken place after an organism has been inoculated and incubated for a certain number of hours. This method helps to execute the precise and accurate level of an organism's efficiency to degrade or decolorize a dye.

#### **2.2.5.14 ABIS microbiology software**

ABIS microbiology software was used to determine the identification of the bacterial strains. This was done by individually inputting the biochemical test results obtained for each organism. This software also provides the users with a brief information about the biochemical tests to be carried out for the identification for each and every organism

CHAPTER THREE:  
RESULTS AND  
OBSERVATIONS

### 3. Results obtained

The results were obtained in the Microbiology and Biotechnology in the Mathematics and Natural science department.

#### 3.1 Biochemical test results

##### 3.1.1 Methyl red and Vogues-Proskauer test (MRVP)



Fig 3.1.1a) methyl red and vogues proskauer samples incubated for 24 hours at 37  
The above image states the condition of the samples before the barrit's agent was added.



Fig 3.1.1b) methyl red samples and vogues proskauer samples after adding the barrit reagent

After adding the barrit'sreagent the final results turned out to be negative. This also applied for the methyl red test done on the samples.

### 3.1.2 Catalase test results:



Fig3.1.2a) 1% (ii)D, 1% (ii) A, 10% (ii) C, all positive.

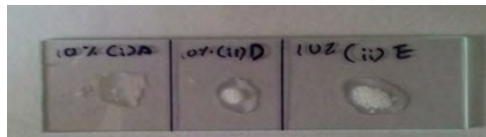


Fig3.1.2b) 10% (i) A, 10% (ii) D, 10% (ii) E, all positive

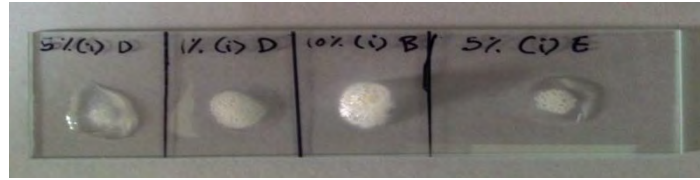


Fig 3.1.2c) 5% (i) D, 1% (i) D, 10% (i) B, 5% (i) E, all positive

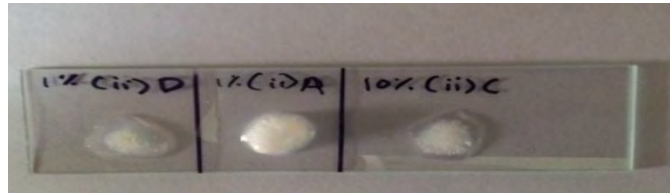


Fig 3.1.2d) 1% (ii) D, 1% (ii) A, 10% (ii) C, all positive

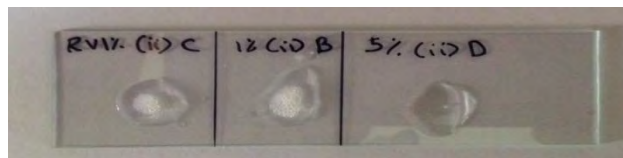


Fig 3.1.2e) 1% (ii) C, 1% (ii) B, 5% (ii) D, all positive except 5% (ii) D.



Fig 3.1.2f) 5% (i) B, 5% (ii) E, 1% (i) C, all positive except 1% (i) C

Most of the results turned out to be positive.



### 3.1.3 Oxidase test:

All the results turned out to be negative.



Fig 3.1.3a) slightly positive 5%(ii) E

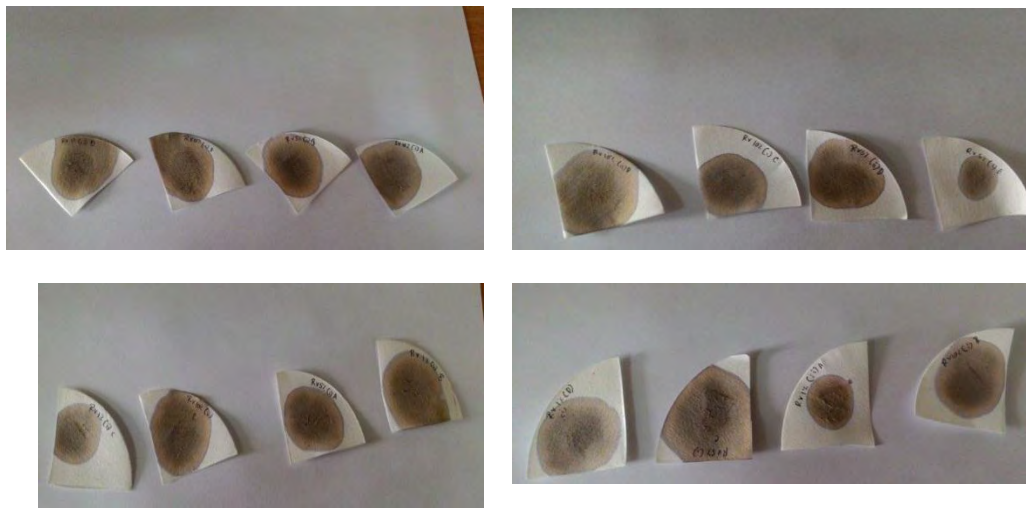


Fig 3.1.3b) all negative

### 3.1.4 Nitrate reduction test:



Fig 3.1.4a) positive test results

Only the samples 10% (ii) E, 10% (i) C, and 1% (i) C turned out to be positive.



Fig 3.1.4b) all negative

### 3.1.5 Simmon's citrate test:

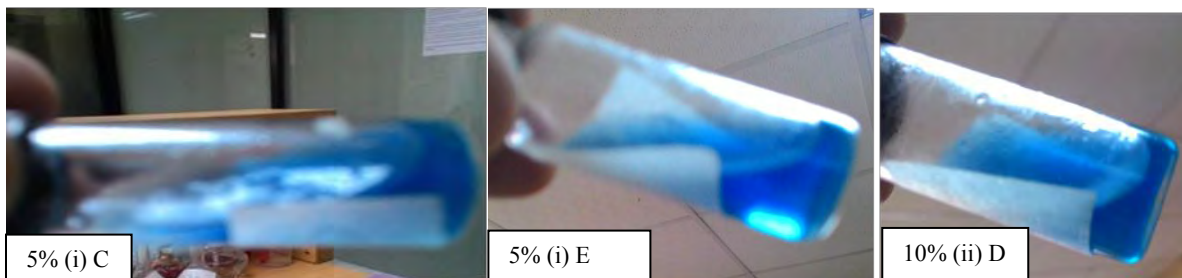


Fig 3.1.5 a) positive tested samples 5% (i) C, 5% (i) E, 10% (ii) D.

Only the samples 5%(i) C, 5% (i) E, 5%(i)A, 10%(ii) D proved to be positive. The rest were negative.

### 3.1.6 Triple sugar iron test:

Table 3.1.6.1 triple sugar iron test results

Samples	Slanted end	Rear end	Gas	H <sub>2</sub> S
Rv5% (i) D	Red	No change	negative	Negative
Rv10% (i)D	Yellow	No change	Negative	Negative
Rv10%(i) B	Yellow	No change	Negative	Positive
Rv10%(ii)C	Red	No change	Negative	Positive
Rv5%(ii)D	Red	No change	Negative	Negative
Rv5%(ii)E	Red	No change	Negative	Negative
Rv10%(ii)E	Yellow	No change	Negative	Negative
Rv1%(ii)A	No change	No change	Negative	Slightly positive
Rv1%(ii)B	Red	No change	Negative	Negative
Rv10%(i)A	Red	No change	Negative	Negative
Rv1%(ii)D	No change	No change	Negative	Negative
Rv5%(i)B	Red	No change	Negative	Negative
Rv1%(ii)C	Red	No change	Negative	Negative
Rv5%(i)E	Red	No change	Negative	Positive

Rv10%(i)D	Red	No change	Negative	Positive
Rv10%(ii)D	Red	No change	Negative	Positive
Rv1%(i)D	Red	No change	Negative	Negative
Rv5%(i)A	No change	Yellow	Negative	Positive
Rv5%(i)C	Red	No change	Negative	Negative
Rv1%(i)C	Yellow	No change	Negative	Negative

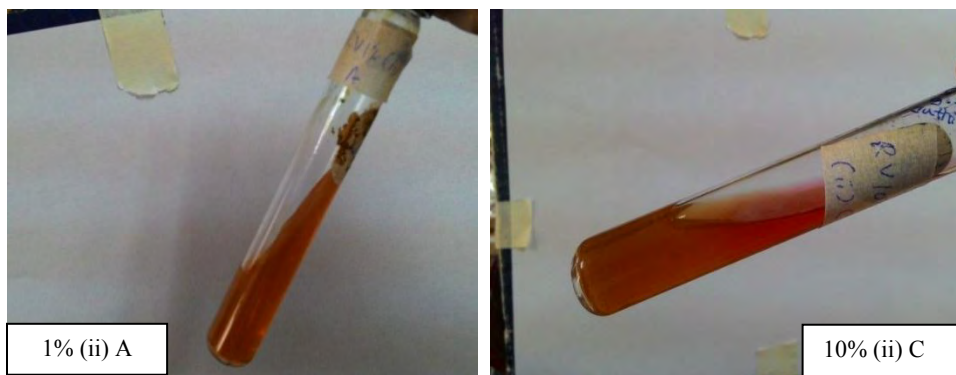


Fig 3.1.6a) all negative, Hydrogen sulfide slightly positive.

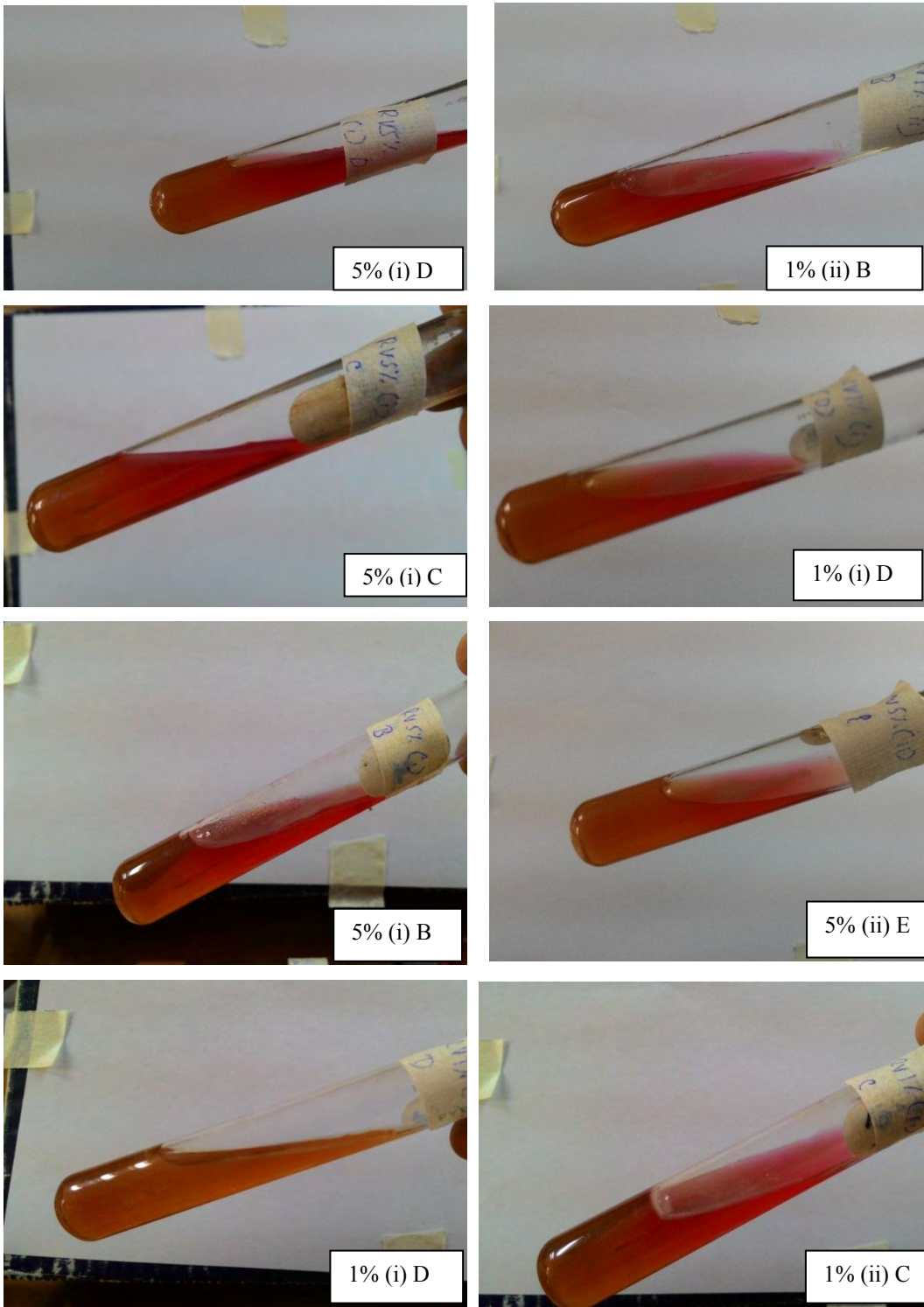


Fig 3.1.6b) all negative, slant red.

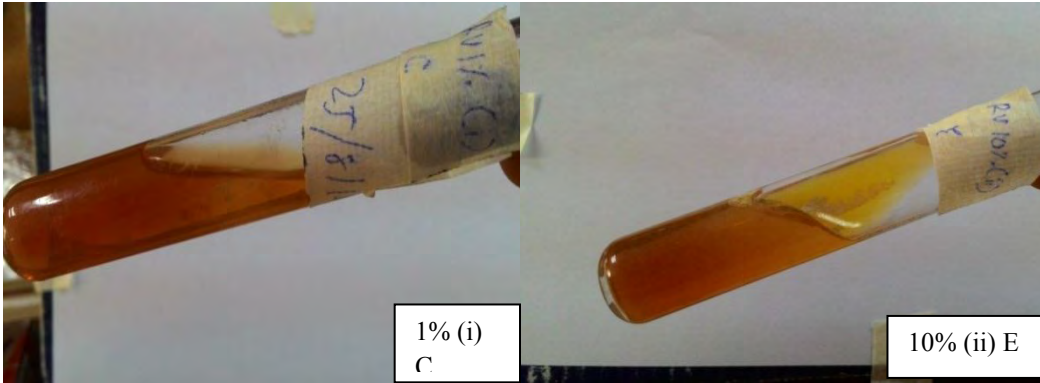


Fig 3.1.6c) slant yellow, all negative

### 3.1.7 Motility Indole urease test

Table 3.1.7.1 motility indole urease test results

Sample	Urease	Motility	Indole
10%(ii)E	Positive	Non-motile	Negative
5%(i)D	Negative	Non-motile	Negative
1%(ii)A	Negative	Non-motile	Negative
1%(ii)B	Negative	Motile	Negative
5%(ii)D	Negative	Non-motile	Negative
1%(i)C	Negative	Motile	Negative
10%(ii)D	Negative	Motile	Negative
1%(ii)C	Negative	Motile	Negative
10%(i)D	Negative	Motile	Negative
1%(ii)D	Negative	Motile	Negative
5%(i)A	Negative	Motile	Negative
10%(ii)C	Negative	Motile	Negative
5%(i)C	Negative	Motile	Negative
10%(i)C	Negative	Motile	Negative
10%(i)A	Negative	Motile	Negative



10%(i)B	Negative	Non-motile	Negative
1%(i)D	Negative	Motile	Negative
5%(ii)E	Negative	Motile	Negative
5%(i)E	Negative	Motile	Negative
5%(i)B	Negative	Motile	Negative

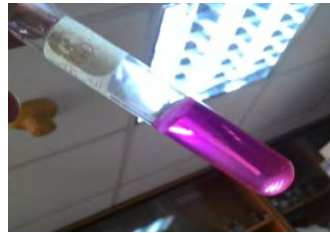


Fig 3.1.7a) 10% (ii) E, urease positive, indole negative, non, motile



Fig 3.1.7b) 5% (i) A, motile, rest negative

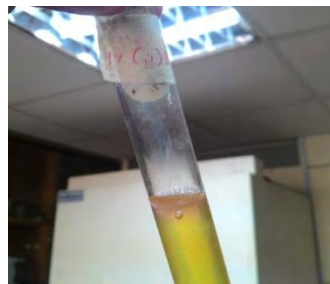


Fig 3.1.7c) 1% (ii) D, motile, rest negative

### 3.1.8 Starch hydrolysis:

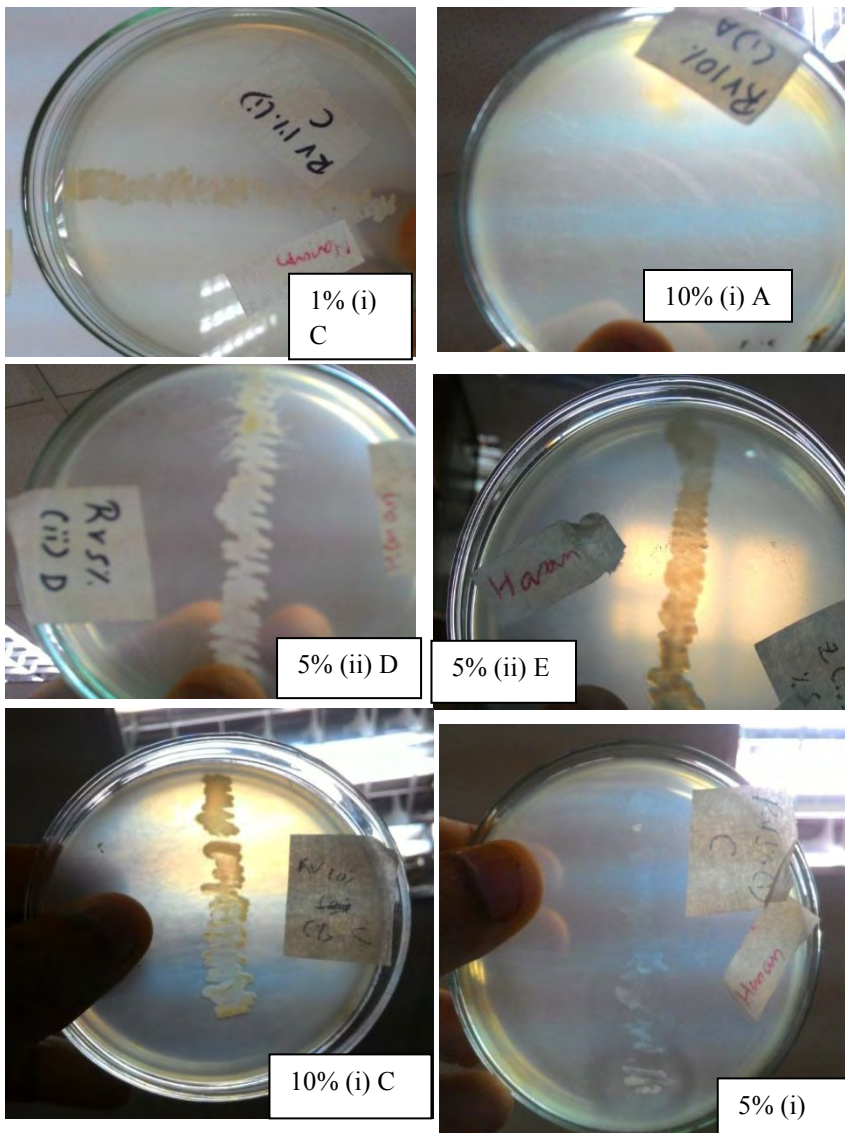


Fig 3.1.8a) all positive



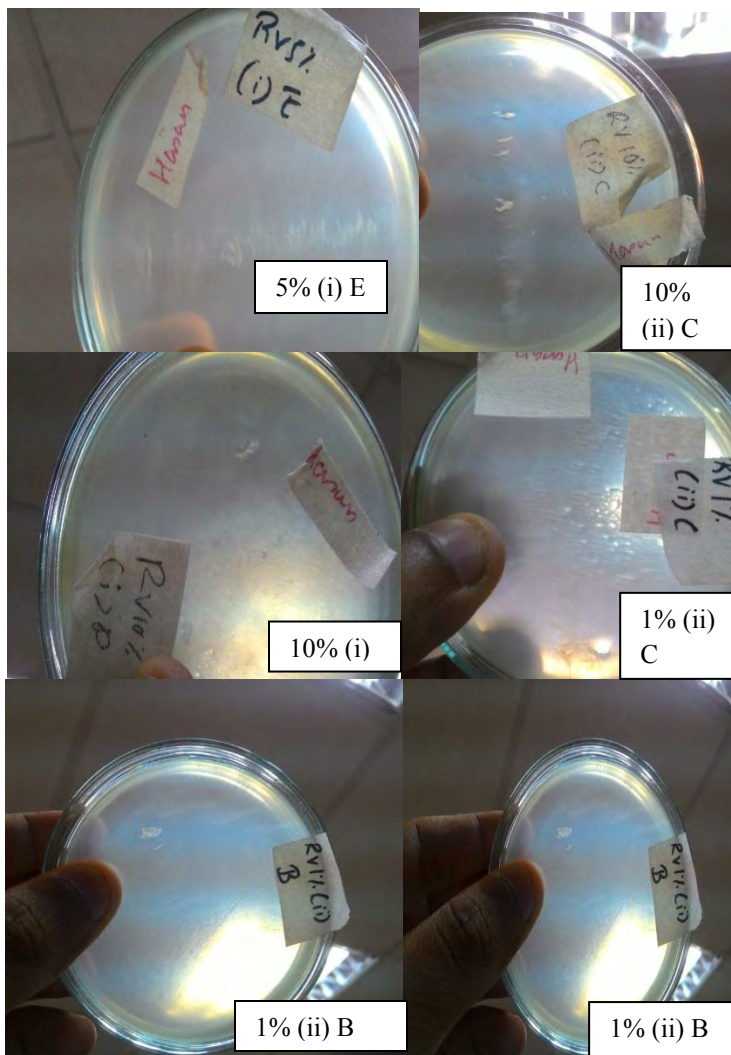


Fig 3.1.8b) all negative

Table 3.1.8.1: starch hydrolysis results

Sample	Result
Rv1%(i)D	Positive
Rv5%(i)A	Negative
Rv5%(i)C	Positive
Rv5%(i)E	Positive
Rv10%(i)B	Positive
Rv10%(ii)E	Positive

Rv10%(i)D	Positive
Rv10%(i)C	Positive
Rv1%(ii)B	Negative
Rv5%(ii)E	Positive
Rv5%(ii)d	Positive
Rv10%(ii)C	Negative
Rv1%(ii)D	Positive
Rv1%(ii)A	Positive
Rv1%(i)A	Positive
Rv10%(i)A	Negative
Rv5%(i)B	Negative

### 3.1.9 Gram staining results

Table 3.1.9.1: gram staining results

Sample	Results
10%(ii)B	Gram(-) cocci
1%(ii)C	Gram(+)cocci
5%(i)E	Gram(+)cocci
5%(i)B	Gram(+)cocci
1%(ii)A	Gram(+)rod
5%(i)D	Gram(+)rod
10%(i)C	Gram(+)rod
10%(i)A	Gram(+)rod
1%(ii)B	Gram(+)rod
5%(i)C	Gram(+)rod
10%(ii)C	Gram(+)rod
5%(i)A	Gram(+)rod
10%(i)D	Gram(+)rod
1%(i)D	Gram(+)rod

10%(ii)E	Gram(+)cocci
5%(ii)D	Gram(-)rod
1%(i)D	Gram(+)cocci
5%(ii)E	Gram(+)rod
1%(i)C	Gram(+)rod
10%(i)B	Gram(+)rod

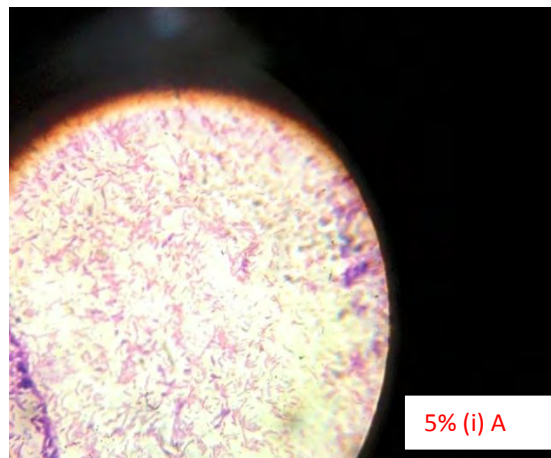


Fig 3.1.9a) gram positive, rod



Fig 3.1.9b) gram negative rod

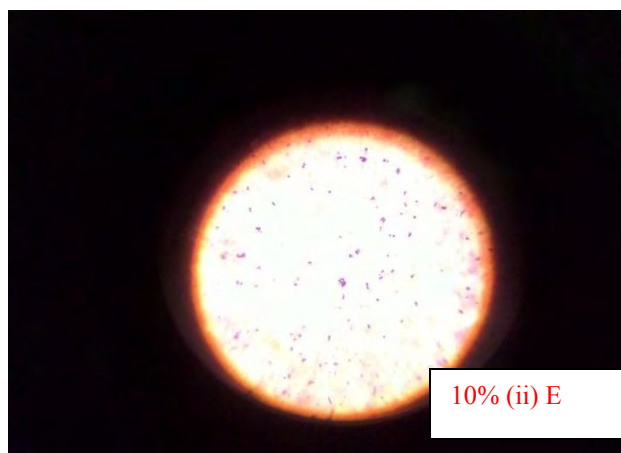


Fig 3.1.9c) gram positive cocci

### 3.2 Absorbance readings

After subsequent number of biochemical tests were done, it was determined that various samples previously tested showed the same results except for a number of organisms. An SM broth media was prepared for these organisms. The SM broth was distributed evenly into 8 conical flasks (each consisting of 200ml of the SM broth media). The next task was to add the dye reactive violet 5r in 1% concentration (v/v). The following samples were added to the solutions. After being incubated for a continuous 5 days the absorbance reading for these organisms were executed. These readings are listed below

Table 3.2.a organisms and their initial absorbance readings

Organisms	Absorbance at 410nm
Rv10%(i)D	0.361
Rv5% (i)A	0.470
Rv10%(ii)E	0.666
Rv5%(ii)D	0.409
Rv1%(i)C	0.334
Rv5%(i)C	0.324
Rv10%(i)A	0.366
Rv10%(i)C	0.312
Control	3.411

Thus a new set of samples were screened out and the ones to show the highest rate of absorbance were 10% (ii) E, 5% (i)A, 5%(ii)D and then a further 10 day observation of the change in the optical density was observed amongst the three. The results are provided below:

**Day 1**

Organism	Absorbance of control	Absorbance at 410 nm		
		1% reactive violet 5R	3% reactive violet 5R	5% reactive violet 5R
	3.411			
5% (i)A		0.168	0.202	0.293
5%(ii)D		0.204	0.189	0.206
10%(ii)E		0.240	0.260	0.270

**Day 2**

Organism	Absorbance at 410 nm		
	1% reactive violet 5R	3% reactive violet 5R	5% reactive violet 5R
5%(i)A	0.442	.252	.490
5%(ii)D	0.199	0.237	0.260
10%(ii)E	0.244	0.415	0.490

**Day 3**

Organism	Absorbance at 410 nm		
	1% reactive violet 5R	3% reactive violet 5R	5% reactive violet 5R
5%(i)A	0.148	0.279	0.224
5%(ii)D	0.425	0.358	1.764
10%(ii)E	0.923	1.669	2.337

**Day 4**

Organism	Absorbance at 410 nm		
	1% reactive violet 5R	3% reactive violet 5R	5% reactive violet 5R
5%(i)A	0.278	0.289	0.230
5%(ii)D	0.279	0.291	1.374
10%(ii)E	0.325	1.283	1.111

**Day 5**

Organism	Absorbance at 410 nm		
	1% reactive violet 5R	3% reactive violet 5R	5% reactive violet 5R
5%(i)A	0.186	0.314	0.301
5%(ii)D	0.490	0.461	2.087
10%(ii)E	1.099	2.671	2.043

Table 3.2.b five days of absorbance readings of the selected organisms at different concentrations

### 3.2.1 Further verification (acid production from carbohydrates)

10% (ii) E ended up having the highest rate of growth. Therefore the following experiments were carried out based on its morphological characteristics to determine its identity.

Table 3.2.1 results for the acid production from carbohydrates

Sugars	Positive	Negative
Fructose	Yes	
Galactose	Yes	
Glucose	Yes	
Lactose	Yes	
Maltose	Yes	
Mannitol		Yes
Sucrose	Yes	
Trehalose		Yes



Fig 3.2.1a) the results for the biochemical test

### 3.3 Decolorization

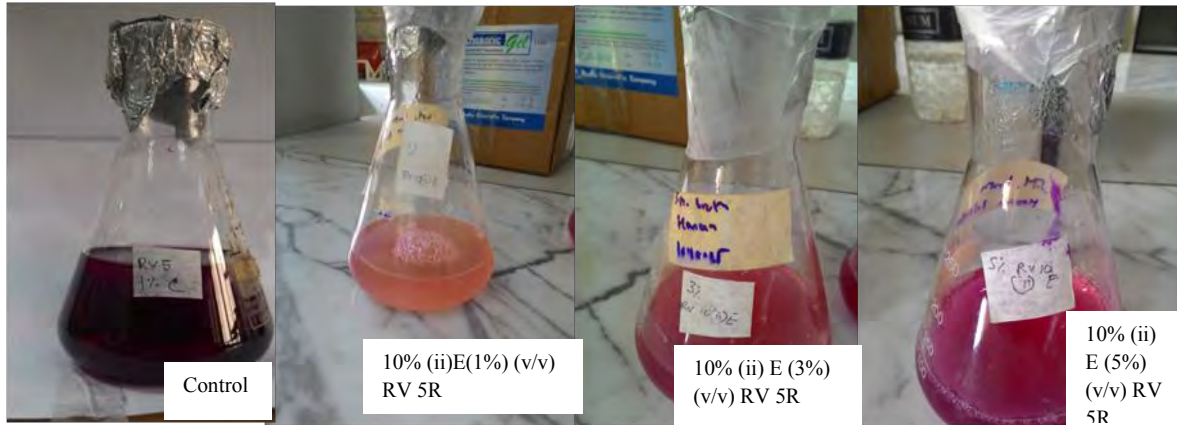


Fig 3.3a) decolorization of the dyes reactive violet 5R by the organism 10% (ii) E after 5 days

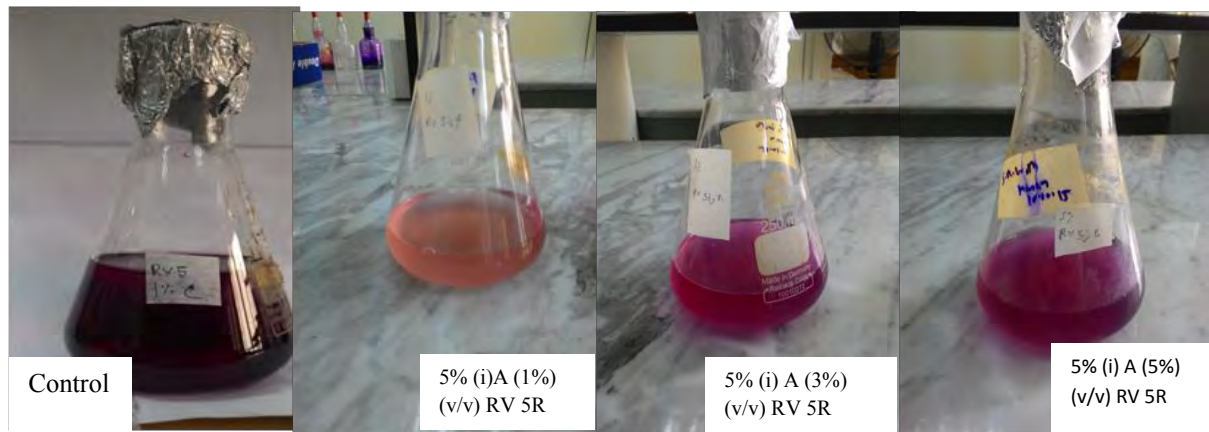


Fig 3.3b) decolorization of the reactive violet 5R by the organism 5% (i) A after 5 days



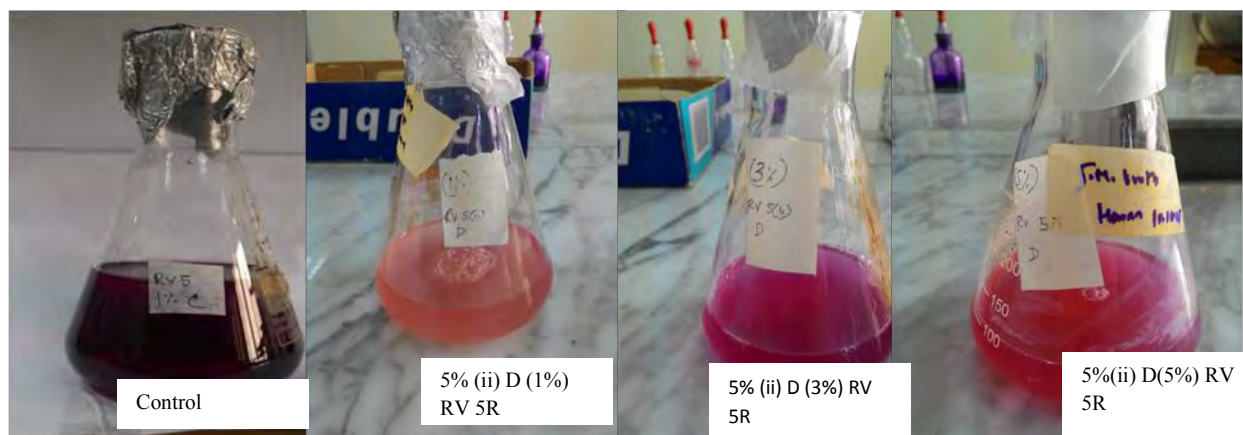


Fig 3.3c) decolorization of the reactive violet 5R by the organism 5% (ii) D after 5 days  
 The following formula was used to determine the rate of decolorization.

$$\text{Percentage(\%)} \text{ of decolorization} = \frac{[\text{Initial reading} - \text{final reading}]}{\text{final reading}} \times 100\%$$

Results of the degradation of the dyes as according to the absorbance readings of the organisms are listed below:

$$5\% \text{ (i)A } 1\% = \frac{(3.411 - 0.186)}{3.411} \times 100\% = \mathbf{94.55\%}$$

$$5\% \text{ (i) A } 3\% = \frac{(3.411 - 0.314)}{3.411} \times 100\% = \mathbf{90.79\%}$$

$$5\% \text{ (i) A } 5\% = \frac{(3.411 - 0.301)}{3.411} \times 100\% = \mathbf{91.17\%}$$

$$5\% \text{ (ii) D } 1\% = \frac{(3.411 - 0.490)}{3.411} \times 100\% = \mathbf{85.63\%}$$

$$5\% \text{ (ii) D } 3\% = \frac{(3.411 - 0.461)}{3.411} \times 100\% = \mathbf{86.48\%}$$

$$5\% \text{ (ii) D } 5\% = \frac{(3.411 - 2.087)}{3.411} \times 100\% = \mathbf{38.81\%}$$

$$10\% \text{ (ii) E } 1\% = \frac{(3.411 - 1.099)}{3.411} \times 100\% = \mathbf{67.78\%}$$

$$10\% \text{ (ii) E } 3\% = \frac{(3.411 - 2.671)}{3.411} \times 100\% = \mathbf{21.69\%}$$

$$10\% \text{ (ii) E } 5\% = \frac{(3.411 - 2.043)}{3.411} \times 100\% = \mathbf{40.10\%}$$

### 3.4 final identification results

After completing the biochemical tests, the results were analyzed in a software called ABIS microbiology, this software helps in analyzing the genus of the organism. The following results are demonstrated

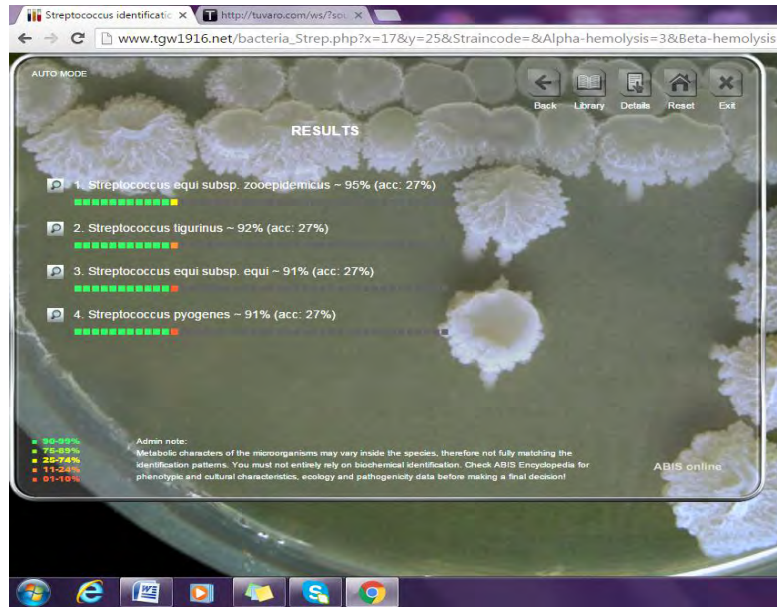


Fig 3.4a)10% (ii)E (*Streptococcus equi subsp. zooepidemicus*).

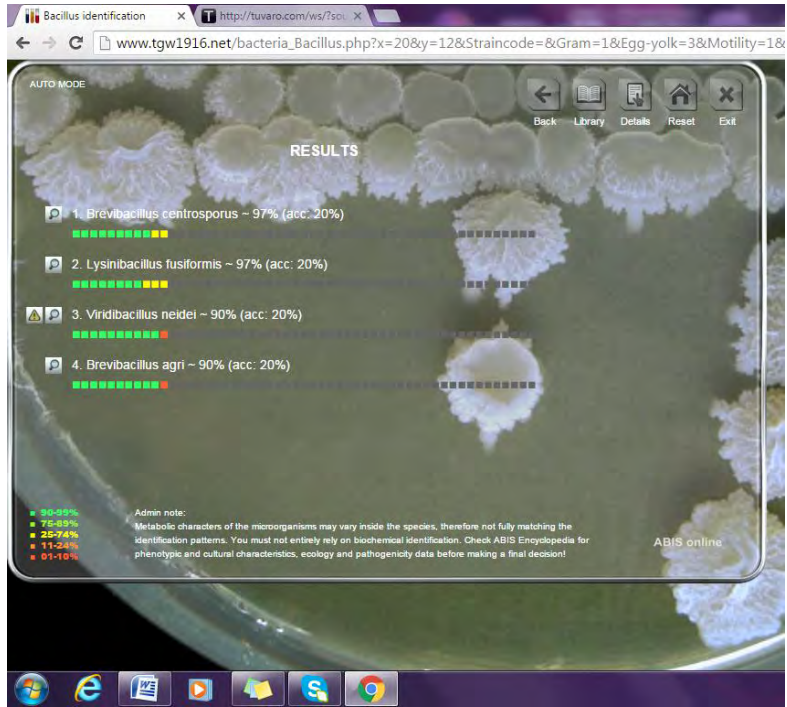


Fig 3.4b) 5% (i)A (*brevibacilluscentrosporus*)

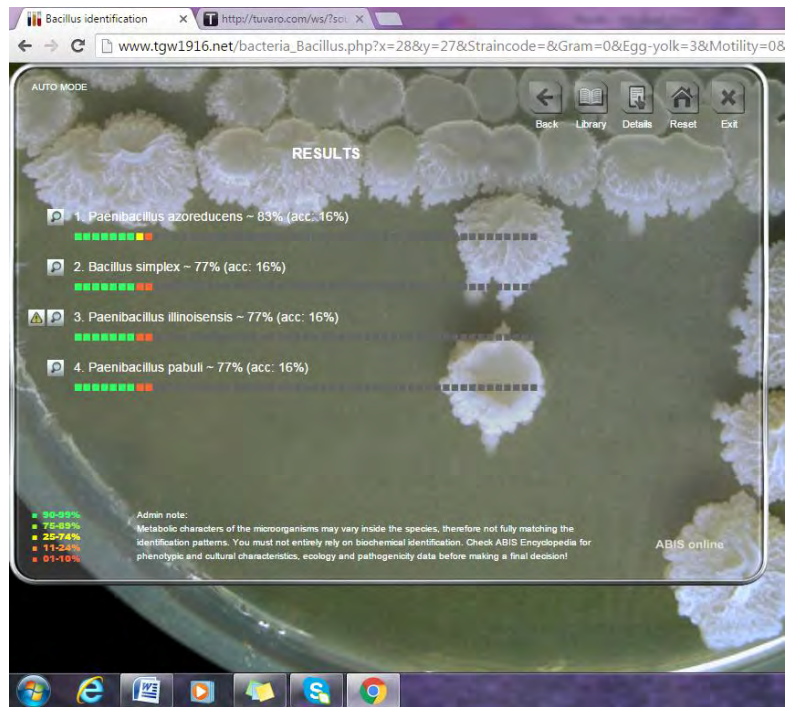


Fig 3.4 c) 5%(ii) D(*Paenibacillus azoreducens*)

These organisms degraded the dye to a partial amount

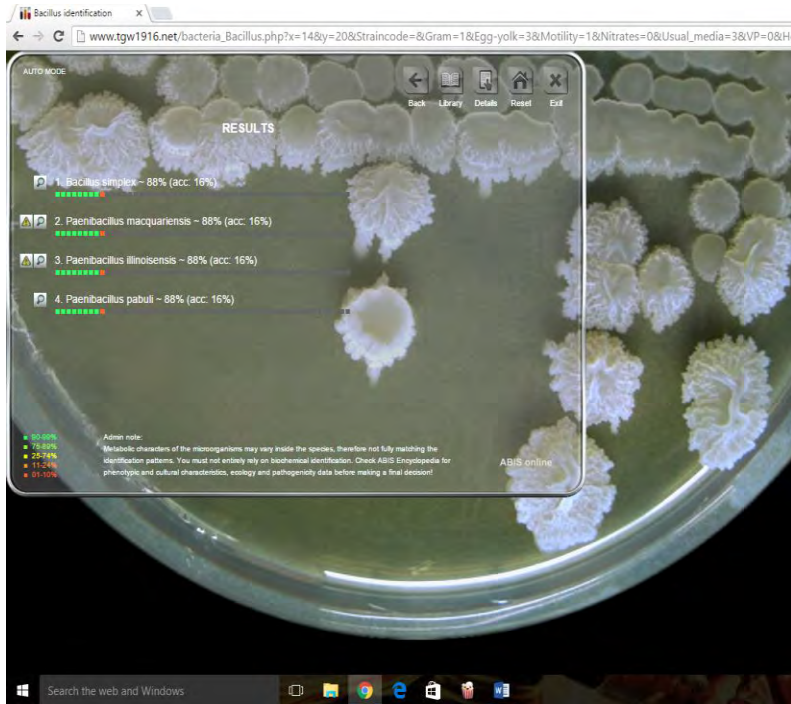


Fig 3.4 d) 10% (i) D (*Bacillus simplex*)

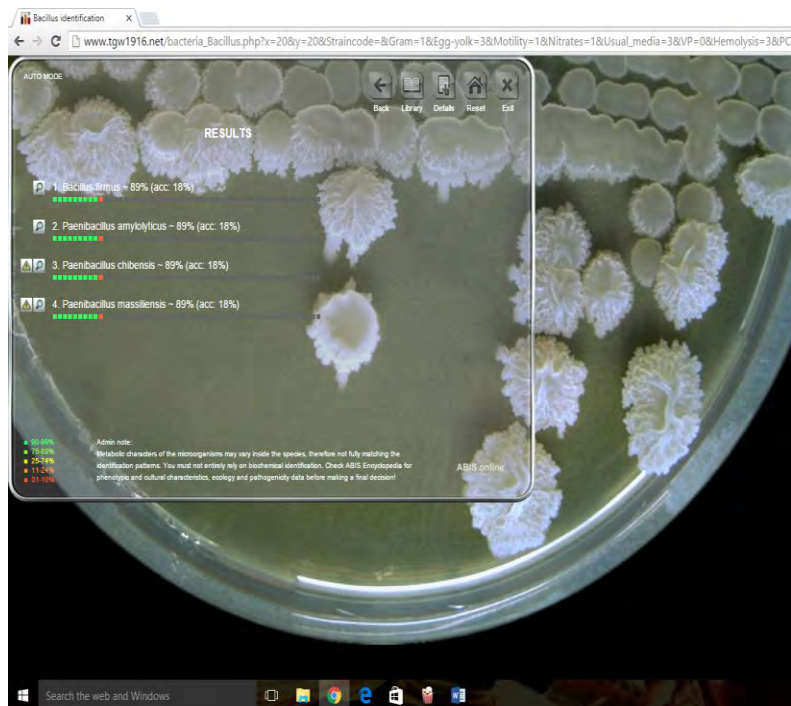


Fig 3.4 e) 1% (i) C *Bacillus firmus*



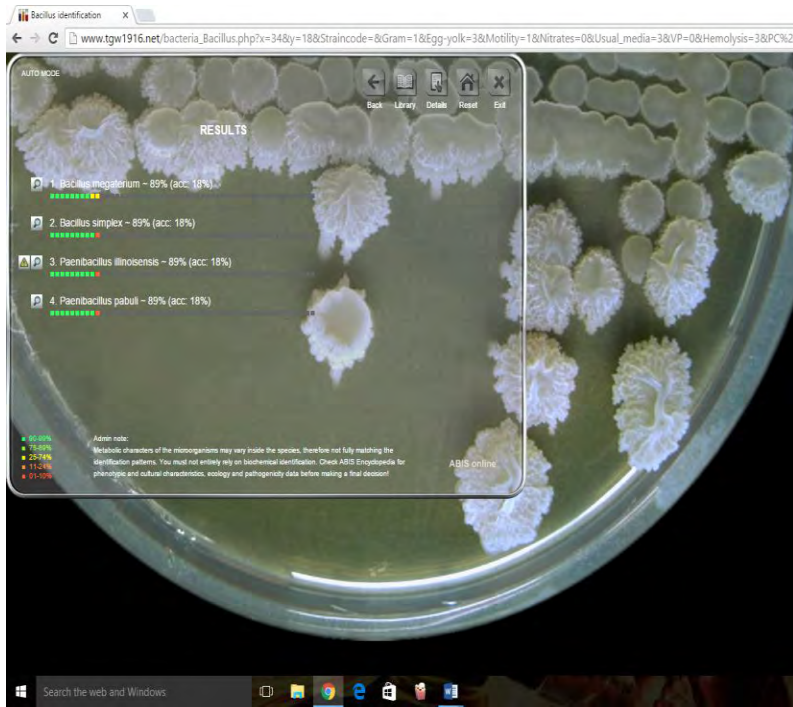


Fig 3.4 f) 5% (i) C (*Bacillus megaterium*)

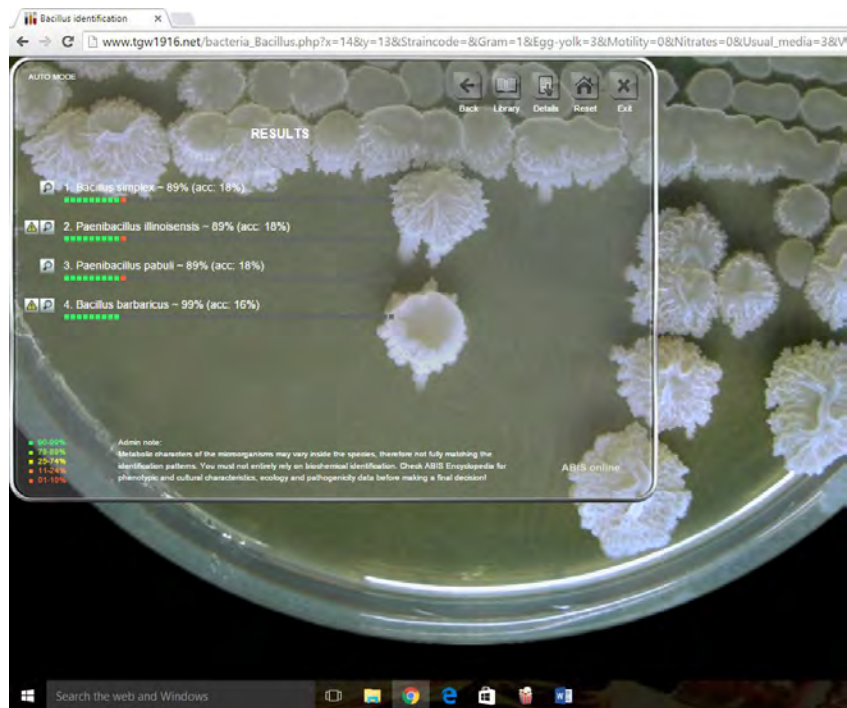


Fig 3.4 g) 10% (i) B (*Bacillus simplex*)

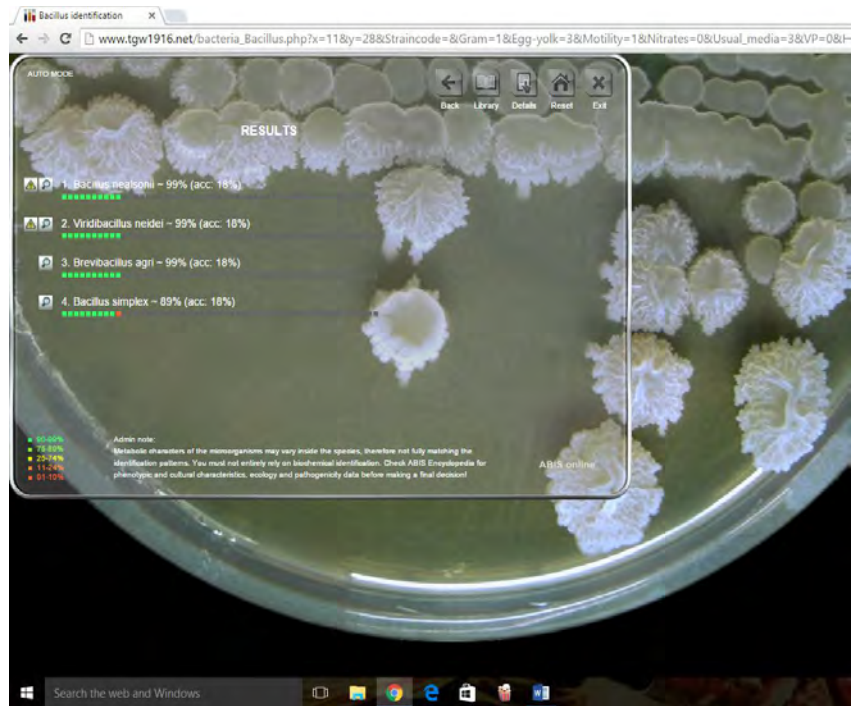


Fig 3.4 h) 10% (i) A (*Bacillus Nealsonii*)

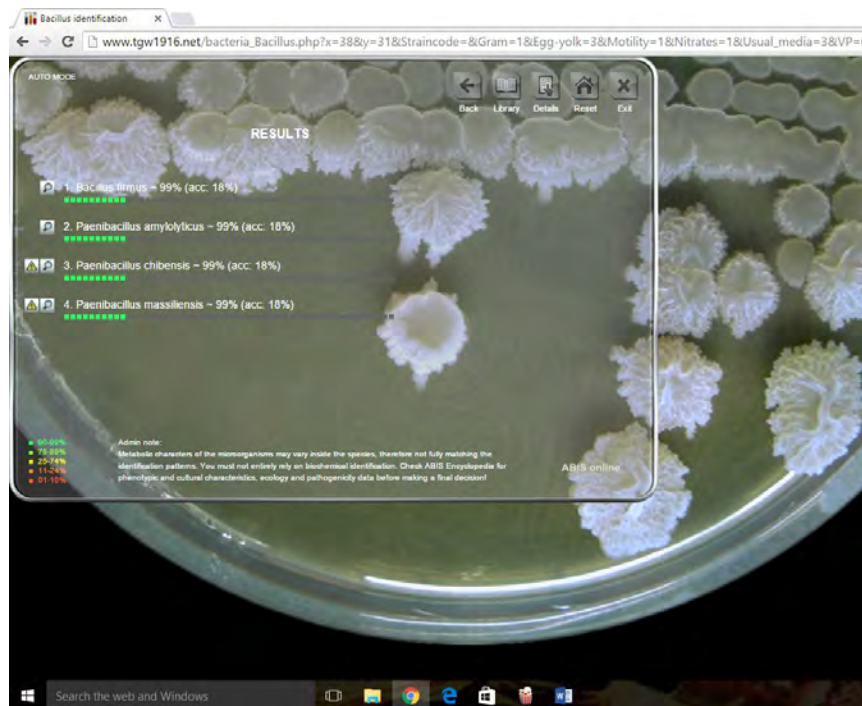


Fig 3.4 i) 10% (i) C (*Bacillus firmus*)

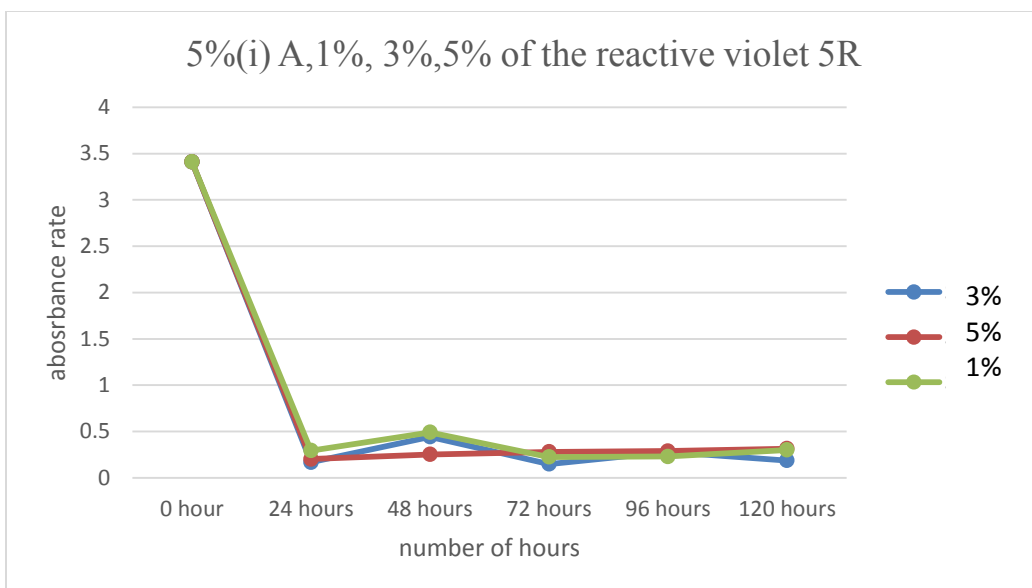


Fig 3.4.1a) decolorization rates of 1% (v/v), 3% (v/v), 5% (v/v) of the azo dye reactive violet 5R decolorized by organism (5% (i) A) (*brevibacillus centrosporus*)

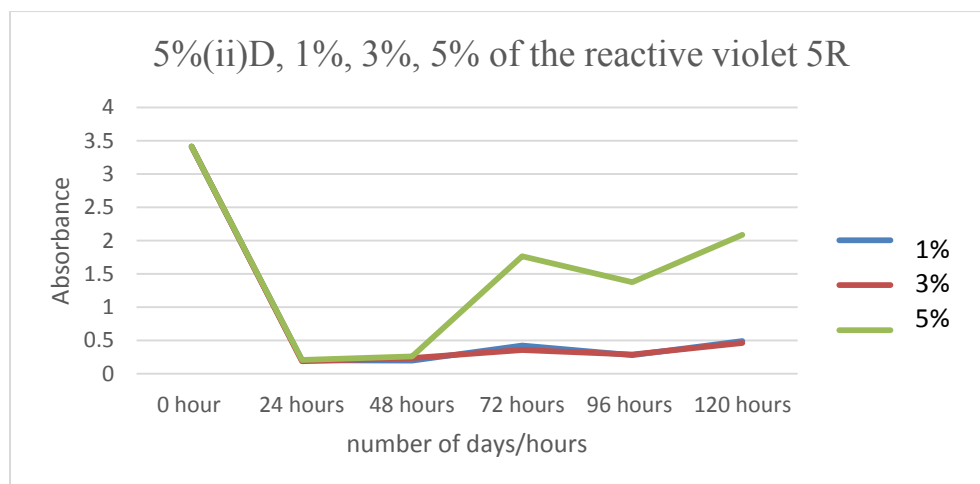


Fig 3.4.1 b) decolorization rates of 1% (v/v), 3% (v/v), 5% (v/v) of the azo dye reactive violet 5R decolorized by the organism (5%(ii)D) (*Paenibacillus azoreducens*)

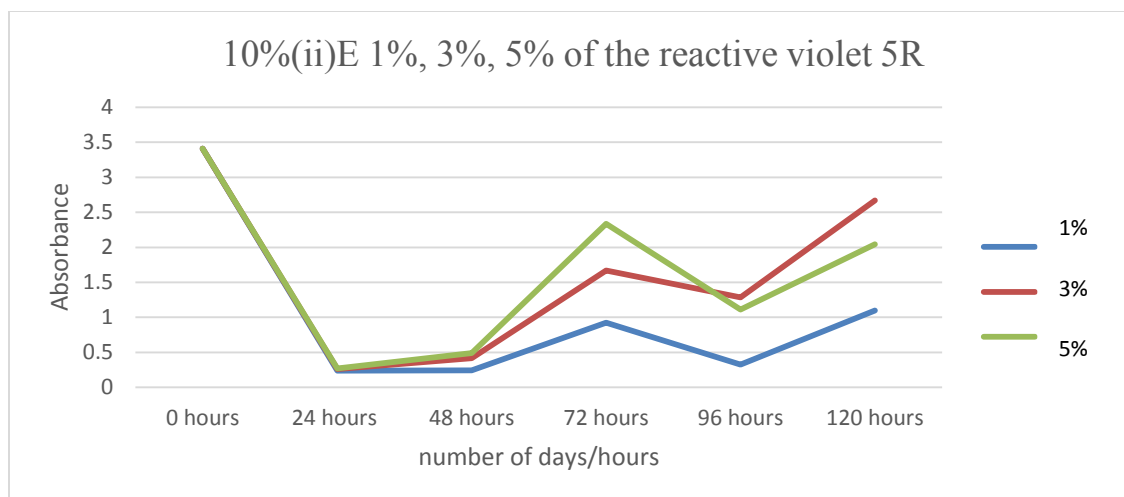


Fig 3.4.1c) decolorization rates of 1% (v/v), 3% (v/v), 5% (v/v) of the azo dye reactive violet 5R decolorized by the organism *Streptococcus equi subsp. zooepidemicus*

The highest decolorization rate was observed for *brevibacillus centrosporus* which are respectively 94.55%, 90.79%, and 91.17% for 1% (v/v), 3% (v/v), and 5% (v/v) concentrations of azo dye reactive violet 5R. The second highest decolorization rate was observed in *Paenibacillus azoreducens*, which are respectively 85.63%, 86.48%, and 38.81% for the 1% (v/v), 3% (v/v) and 5% (v/v) concentrations of azo dye reactive violet 5R. *Streptococcus equi subsp. zooepidemicus* projected a decolorization rate of 67.78%, 21.69%, and 40.10% for the concentrations of 1% (v/v), 3% (v/v), and 5%(v/v) of the reactive violet 5R



# CHAPTER FOUR: DISCUSSION

## 4. Discussion

Different micro-organisms react differently to the same dye. The decolorization performance of Reactive Violet 5 by the *Paracoccus* sp. GSM2 was studied by increasing initial dye concentration (100–800 mg/L). Then it was observed that the percentage of decolorization was decreased slowly with increasing dye concentration. (Bheemaraddi et al., 2014) Biodegradation of commercially available textile dyes namely Reactive Orange – M2R, Reactive Blue – MR, Reactive Yellow – M4G and Reactive Black-B were studied against five bacterial isolates which have been isolated from the dye effluent sample by pour plate method and percentage decolorization. In a study conducted, three different bacterial strains were isolated from the textile dye effluent. Based on preliminary tests, plating on selective media and biochemical tests, they were identified as *Bacillus* sp. *Escherichia coli* and *Pseudomonas fluorescens*. (Olukanni et al., 2005)

There were also experiments concluding to the analysis which showed that the *Staphylococcus aureus*, *Bacterioides fragilis*, *Bacillus subtilis*, *Bacillus cereus*, *Clostridium perfringens*, *Escherichia coli* and *Peptostreptococcus* sp. can reduce and stabilize textile effluents containing predominantly Indigo Blue. (Olukanni et al., 2005)

On the other hand in a study conducted (*Streptococcus equi* subsp. *zooepidemicus*, *Brevibacillus centrosporus*, *Paenibacillus azoreducens*) clearly showed a change in absorbance. *Brevibacillus centrosporus* showed the highest decolorization rates of 94.55%, 90.79%, and 91.17% when inoculated and incubated for 5 consecutive days in SM broth media containing dye reactive violet 5R at the respective concentrations of 1% (v/v), 3% (v/v), and 5% (v/v). In the same way *Paenibacillus azoreducens* projected a decolorization rate of 85.63%, 86.48% and 38.81% when inoculated and incubated for 5 consecutive days in SM broth containing the reactive violet 5R at the concentrations of 1% (v/v), 3% (v/v), and 5% (v/v) respectively. On the contrary *Streptococcus equi* subsp. *zooepidemicus* showed the highest growth rate for which the absorbance rate was much higher than compared to the other two bacterial strains but this led to a significant decrease in the decolorization rate which was 67.78%, 21.69% and 40.10% when

inoculated and incubated in SM broth containing reactive violet 5R at the respective concentrations of 1%(v/v), 3%(v/v) and 5%(v/v). *Bacillus simplex*, *Bacillus firmus*, *Bacillus megaterium* and *Bacillus nealsonii*, these microbial strains have also partially decolorized the azo dye reactive violet 5R. It is also scientifically proven that mixed bacterial culture can give a better degradation rate than the individual strain. (Chen et al., 2003)

There is a difference between each and every organisms' decolorization rates and growth rates when it comes to being inoculated and incubated in a specific type of azo dye. This difference arises due to the physiological and morphological characteristics of the microbes. The chemical properties of the dye is also a significant factor and the origin of the microbe also plays a major role in this case.

# CHAPTER FIVE: CONCLUSION

## 5 Conclusion

The azo dye reactive violet 5R was successfully degraded by the following microbes *Streptococcus equi subsp. zooepidemicus*, *Brevibacillus centrosporus*, *Paenibacillus azoreducens* but each organism has done it in an exclusively different rate. *Streptococcus equi subsp. zooepidemicus* showed decolorization rates of 67.78%, 21.69% and 40.10% after being inoculated and incubated in SM broth containing azo dye reactive violet 5R at respective concentrations of 1%(v/v), 3%(v/v) and 5%(v/v). This organism also showed the highest growth rate in terms of absorbance readings. *Brevibacillus centrosporus* showed decolorization rates of 94.55%, 90.79%, and 91.17% after being inoculated and incubated for 5 consecutive days in SM broth media containing dye reactive violet 5R at the respective concentrations of 1% (v/v), 3% (v/v), and 5% (v/v). , *Paenibacillus azoreducens* projected a decolorization rate of 85.63%, 86.48% and 38.81% when inoculated and incubated for 5 consecutive days in SM broth containing the reactive violet 5R at the concentrations of 1% (v/v), 3% (v/v), and 5% (v/v) respectively. These bacterial strains were all kept at an aerobic state. It is scientifically proven that bacterial strains showed good growth when cultured in aerobic or agitation culture. (Chen et al., 2003). The variance of rate also varied due to the physical characteristics at which the dye was kept and also at which the organisms were preserved. Their morphological characteristics also contributes immensely to their dye degrading qualities. It was seen that the optical density reading had varied remarkably from one day to another. This change in the optical density shows that the azo dye reactive violet 5R was decolorized within the course of time.

The future prospective of undertaking this particular study is to create awareness. There are solutions to these kind of remedies which are available within the elements of nature. This experiment itself proved to be cost efficient and also less time consuming. It also proved to be environmentally stable and economically efficient. The only problem lies within the isolation of the proper strain of microbes from its native environment. This problem can be countered if the genes responsible for the decolorization of these dyes are isolated and extracted from the following hosts. These preferable genes can be inserted into a host with high multiplying capacities. This can be brought to commercial which can become a revolutionizing weapon for the decolorization of the synthetic dyes polluting the environment. Textile sludge can be controlled and their carcinogenic effects can be brought to a minimum. In general,

bioremediation will become a strong aspect for controlling the water pollution. The eco system can be controlled and thus to conclude, there will be a strong and healthy balance between the human beings and the elements of nature making the world a better place to dwell in.

# CHAPTER SIX: REFERENCES

### References from Journals:

A. A. Hajna Bureau of Bacteriology, Maryland State Department of Health, Baltimore, Maryland  
Received for publication March 2, 1945

Abadulla,E., Tzanov, T., Costa, S., Robra, H.H., Cavaco- Paulo, A., and Gubitz, G.M. 2000.  
Decolorization and detoxification of textile dyes with a laccase from *Trametes hirsute*, *Applied Environmental Microbiology*, 66: 3357-3362

Ajibola, V.O., S.J. Oniye and C.E. Odeh, 2005. Biodegradation of Indigo containing textile effluent using some strains of bacteria. *Journal of Applied Sciences*, 5(5): 853-855.

A.L. Barry and K.L.Feeney.,1967.Eddy, B. P., 1961. The Voges-Proskauer reaction and its significance: a review. *J. Appl. Bacteriol.* 24:27-41.

A. L. Barry and K. L. Bernsohn Departments of Microbiology and Medicine, University of California, Irvine, College of Medicine, Irvine, California 92650, and Los Angeles County General Hospital (Unit 11), Los Angeles, California 90033 Received for publication 18 March 1969

Asad S, Amoozegar MA, Pourbabae AA, Sarbolouki MN, Dastgheib SMM. Decolorization of textile azo dyes by newly isolated halophilic and halotolerant bacteria. *Bioresource Technology*. 2007;98(11):2082–2088.

Chang J-S, Chou C, Lin Y-C, Lin P-J, Ho J-Y, Lee Hu T. Kinetic characteristics of bacterial azo-dye decolorization by *Pseudomonas luteola*. *Water Research*. 2001;35(12):2841–2850.



Cheriaa J, Khaireddine M, Rouabhia M, Bakhrouf A. Removal of triphenylmethane dyes by bacterial consortium. *The Scientific World Journal*. 2012;2012:9 pages.512454

Duran.N.,Rosa.M.A.,D Annibale.A., Gianfreda.L.,2002. Applications of laccasas and tyrosinases Phenoloxidases immobilized on different supports: a review. *Enzyme Microbial Technology*,31: 907-931.

Ewing, W. H., and J. G. Johnson. 1960. The differentiation of *Aeromonas* and C27 cultures from Enterobacteriaceae. *Int. Bull. Bacteriol. Nomencl. Taxon*. 10:223-230.

Gaby, W. L., and C. Hadley. 1957. Practical laboratory test for the identification of *Pseudomonas aeruginosa*. *J. Bacteriol*. 74:356-358.

Glenn.J.,Akileswaran.L.,Gold.M.H.,1986. MnII oxidation is the principal function of the extracellular Mn-peroxidase from *Phanerochete chrysosporium*, *Archieves of Biochemistry and Biophysics*.251:688-696

Gupta VK, Jain R, Nayak A, Agarwal S, Shrivastava M. Removal of the hazardous dye-Tartrazine by photodegradation on titanium dioxide surface. *Materials Science and Engineering C*. 2011;31(5):1062–1067.

H. J. Conn and R. S. Breed From the New York Agricultural Experiment Station, Geneva, New York Received for publication, January 29, 1919

Karimi L, Zohoori S, Yazdanshenas ME. Photocatalytic degradation of azo dyes in aqueous solutions under UV irradiation using nano-strontium titanate as the nanophotocatalyst. *Soc: J Saudi Chem*; 2011.

Kaushik P, Malik A. Fungal dye decolourization: recent advances and future potential. *Environment International*. 2009;35(1):127–141.

Kovacs, N. 1956. Identification of *Pseudomonas pyocyanea* by the oxidase reaction. *Nature* 178:703.

Leclerc, H., and H. Beerens. 1962. Une technique simple de mise en evidence de l'oxydase chez les bacteries. *Ann. Inst. Pasteur Lille* 13:187-192.

MacPherson, M. J. 1953. Isolation and identification of amylolytic streptococci from the rumen of the sheep. *J. Pathol. Bacteriol.* 66:95-102.

Mahvi AH, Ghanbarian M, Nasser S, Khairi A. Mineralization and discoloration of textile wastewater by TiO<sub>2</sub> nanoparticles. *Desalination*. 2009;239:309–316. doi: 10.1016/j.desal.2008.04.002.

Manivannan, M., D. Reetha and P. Ganesh, 2011. Decolourization and degradation of Direct azo dyes and biodegradation of textile dye effluent by using bacteria isolated from textile dye effluent. *Journal of Ecobiotechnology*, 2(7): 7-11.

Mallikarjun C. Bheemaraddi, Santosh Patil, Channappa T. Shivannavar, and Subhashchandra M. Gaddad *ScientificWorldJournal*. 2014; 2014: 410704.

Manuel De La Rosa Fraile, Dolores Vega Aleman, and Clotilde Fernandez Gutierrez Servicio de Bacteriologia, Ciudad Sanitaria Virgen de las Nieves, Granada, Spain

McMullan, G., Meehan, C., Conneely, A., Kirby, N., Robinson, T., Nigam, P., Banat, I.M., Marchant, R., and Smyth, W.F. 2001. Microbial decolorization and degradation of textile dyes. *Appl Microbial Biotechnol.* 56:1-2, 81-87;

Moosvi S, Kher X, Madamwar D. Isolation, characterization and decolorization of textile dyes by a mixed bacterial consortium JW-2. *Dyes and Pigments.* 2007;74(3):723–729.

Myslak ZW, Bolt HM. Occupational exposure to azo dyes and risk of bladder cancer. *ZblArbeitsmed.*1988;38:310–321.

Olukanni, O.D., A.A. Osuntoki and G.D. Gbenle, 2005. Textile effluent biodegradation potentials of textile effluent-adopted and non-adopted bacteria. *Applied Environmental Microbiology*, pp: 837-844.

O'Neill C, Hawkes FR, Hawkes DL, Lourenco ND, Pinheiro HM, Delee W. Color in textile effluents sources, measurement, discharge consents and simulation: a review. *Journal of Chemical Technology and Biotechnology.* 1999;74(11):1009–1018.

Palma.C.,Moreira.M.T.,Mielgo.I.,Feijoo.G.,Lema.J.M.,1999. Use of a fungal bioreactor as a pretreatment or post-treatment step for continuous decolorisation of dyes.

Rafii, F., Smith, D.B., Benson, W.R., and Cerniglia, C.E. 1992. Immunological homology among azoreductases from *Clostridium* and *Eubacterium* strains isolated from human intestinal microflora, *J Basic Microbiol.* 32, 99.

Rafii.F., Hall.D.J., Cerniglia.E.C., 1997. Mutagenicity azo dyes used in foods, drugs and cosmetic before and after reduction by *Clostridium* Species from the human intestinal tract. *Food Chem. Toxic.* 35:897-901.

Robinson, T.; McMullan, G.; Marchant, R. Nigam, P. 2001. Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative. *Bioresource technology*, 77, 247-255, ISSN 0960-8524

Rodgers, K. B. 1963. Oxidase reaction. *Lancet* 2:686.

Russ.R., Rau.J., and Stolz.A., 2000 The Function of cytoplasmic flavin reductases in the reduction of azo dyes by bacteria. *Appl.Environ.Microbiol.*, 66: 1429-1434.

Saranraj, P., V. Sumathi and D. Reetha, 2010. Decolourization and degradation of Direct azo dyes and Biodegradation of textile dye effluent by using bacteria isolated from textile dye effluent. *Journal of Ecobiotechnology*, 2(7): 7-11.

Saratale RG, Saratale GD, Kalyani DC, Chang JS, Govindwar SP. Enhanced decolorization and biodegradation of textile azo dye Scarlet R by using developed microbial consortium-GR. *Bioresource Technology*. 2009;100(9):2493–2500.

Skerman, V. B. D. 1969. Starch hydrolysis detection method, p. 808. In V. B. D. Skerman (ed.), *Abstracts of microbiological methods*. Wiley-Interscience, New York.

Steel, K. J. 1961. The oxidase reaction as a taxonomic tool. *J. Gen. Microbiol.* 25:297-306.

Stolz, A. 2001 Basic and applied aspects in the microbial degradation of azo dyes. *Appl. Microbiol. Biotechnol.* 56:pp.69-80.

Suassuna, I., U. R. Suassuna, and W. H. Ewing, 1961. The Methyl Red and Voges-Proskauer reactions of Enterobacteriaceae. *Public Health Lab.* 19:67-75.

Vandevivere PC, Bianchi R, Verstraete W. Treatment and reuse of wastewater from the textile wet-processing Industry: review of emerging technologies. *Journal of Chemical Technology and Biotechnology*.1998;72:289–302.

Wang H, Zheng X-W, Su J-Q, Tian Y, Xiong X-J, Zheng T-L. Biological decolorization of the reactive dyes Reactive Black 5 by a novel isolated bacterial strain *Enterobacter* sp. EC3. *Journal of Hazardous Materials*. 2009;171(1–3):654–659.

Welton I. Taylor and David Achanzar Grant Hospital of Chicago, Chicago, Illinois 60614

Received for publication 10 May 1972

Yoo.E.S.,Libra.J., and Adrain.L.,2001. Mechanism of decolorization of azo dyes in anaerobic mixed culture. *Journal of Environ.Eng.*1279:844-849.

Zollinger H. *Color Chemistry—Synthesis, Properties and Application of Organic Dyes and Pigments*. New York, NY, USA: VCH Publishers; 1987

Zollinger, H., 1987. *Color Chemistry-Synthesis, Properties and Application of Organic Dyes and Pigment*. VCH Publishers, New York, pp. 92 -/102.

**References from websites:**

[www.britannica.com](http://www.britannica.com)

[microbeworld.org](http://microbeworld.org)

[learn.genetics.utah.edu](http://learn.genetics.utah.edu)

## **Appendices:**

### **Apparatus:**

1. Aluminum foil papers
2. Beaker
3. Bunsen burner
4. Conical flask
5. Durham tubes
6. Filter paper
7. Falcon tubes
8. Filter papers
9. Glass pipette
10. Glass slides
11. Masking tape
12. Measuring cylinder
13. Micropipette
14. Para film
15. Petri dish
16. Spatula
17. Spirit lamp
18. Test tubes
19. Vials

### **Chemicals:**

1. Agar powder
2. Beef extract
3. Cimmon's citrate
4. Distilled water
5. Di potassium hydrogen phosphate

6. Ethanol
7. Fructose
8. Galactose
9. Glucose
10. Lactose
11. Magnesium sulfate
12. Maltose
13. Mannitol
14. MIU agar
15. Nutrient agar
16. Peptone
17. Phenol
18. Potassium di hydrogen phosphate
19. Potassium nitrate
20. Savlon
21. Sodium chloride
22. Starch agar
23. Treshalose
24. Trypticase
25. Urea
26. Zinc powder

**Machineries:**

1. Autoclave machine
2. Centrifuge
3. Incubator
4. Laminar air flow
5. Microscope
6. pH meter
7. Refrigerator



8. Shaker
9. Spectrophotometer
10. Vortex machine

Soil sample is the primary source of the bacterial cultures which were isolated and identified with the aid of biochemical tests. The dye degraded was reactive violet 5r.

Various types of reagents were used while carrying out the biochemical tests to identify the bacterial cultures.

For catalase test:

1. Hydrogen peroxide(  $H_2O_2$ )

For Gram staining

1. Crystal violet
2. Grams iodine
3. 100% ethanol
4. Safranin

For starch test:

1. Indole

For vogues proskauer:

1. Baritt's reagent A= 5% naphthol in absolute ethanol
2. Baritt's reagent B= 40% KOH in deionized water.

For oxidase test:

1. 1% kovacs oxidase reagent.