DECOLOURIZATION OF TEXTILE DYES AND TEXTILE INDUSTRY EFFLUENT IN A FIXED BED BIOFILM REACTOR USING NATIVE MICROORGANISMS

Hewayalage Gimhani Madhushika

(158038H)

Degree of Doctor of Philosophy

Department of Chemical and Process Engineering

University of Moratuwa

Sri Lanka

May 2021
DECOLOURIZATION OF TEXTILE DYES AND TEXTILE INDUSTRY EFFLUENT IN A FIXED BED BIOFILM REACTOR USING NATIVE MICROORGANISMS

Hewayalage Gimhani Madhushika

(158038H)

Dissertation submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy

Department of Chemical and Process Engineering

University of Moratuwa
Sri Lanka

May 2021
DECLARATION OF THE CANDIDATE AND SUPERVISOR

I declare that this is my own work and this dissertation does not incorporate without acknowledgement any material previously submitted for a Degree or Diploma in any other University or institute of higher learning and to the best of my knowledge and belief it does not contain any material previously published or written by another person except where the acknowledgement is made in the text.

Also, I hereby grant to University of Moratuwa the non-exclusive right to reproduce and distribute my thesis/dissertation, in whole or in part in print, electronic or other medium. I retain the right to use this content in whole or part in future works (such as articles or books).

Signature: ..................................... Date: 02/05/2021........

H.G.Madhushika
Department of Chemical & Process Engineering
University of Moratuwa
Sri Lanka

The above candidate has carried out research for the PhD thesis/ dissertation under my supervision.

Signature of the supervisor: ........................................ Date: 02/05/2021....

Supervisor (PI): Dr. S.H.P.Gunawardena
Department of Chemical & Process Engineering
University of Moratuwa
Sri Lanka

Signature of the supervisor: ........................................ Date: 02/05/2021....

Supervisor: Dr. H.L.T.U. Ariyadasa
Department of Chemical & Process Engineering
University of Moratuwa
Sri Lanka
Abstract

Textile and apparel industry produces huge quantities of wastewater with unfixed dyes, which generate colour and toxicity in discharged water, creating environmental pollution. Physical and chemical effluent decolourization techniques are widely used at present to remove colour in effluents in textile industries, however, they have several drawbacks and therefore not productive. Compared to physical and chemical methods, biological treatments have gained much attention globally as environmental-friendly and cost-effective techniques to decolourize textile industry effluent. Hence, in this work, decolourization potential of textile dyes by microbial strains, which were isolated from local environment, and their applicability in industrial wastewater decolourization were investigated.

Five bacterial strains, with dye decolourizing potential were isolated from an effluent treatment facility of a local textile industry and identified using 16S rRNA gene sequencing analysis. Ability of these strains to decolourize selected textile dyes as individual strains and in a bacterial consortium was investigated using free bacterial cells cultured in 250 ml Erlenmeyer flasks containing 100 ml of decolourization media. Out of the isolated bacteria, Proteus mirabilis showed the highest capability to decolourize all dyes and was able to decolourize 50 ppm dye solutions of Yellow EXF, Red EXF, Blue EXF, Black WNN and Rhodamine under static conditions at 35 °C. Colour removal of 96, 94, 83, 95 and 30% respectively was observed after 72 h of treatment when decolourization media was inoculated with 2% (v/v) of bacterial culture. The developed bacterial consortium composed of Proteus mirabilis, Morganella morganii and Enterobacter cloacae, decolourized more than 90% of all four reactive dyes and 36% of Rhodamine dye after 72 h of incubation. Furthermore, the developed bacterial consortium was able to decolourize more than 83% of the synthetic dye mixture and 60% of the textile industry effluent, respectively after 46 h and 138 h of incubation at 35 °C temperature under static condition.

Effects of physico-chemical parameters (pH, temperature, concentration of dye, agitation and sources of carbon) for biological decolourization of dyes were studied in batch cultures with free cells. It was observed that dye decolourization was more effective under oxygen-limited, static conditions than shaking conditions and the maximum decolourization of dyes was observed at 40 °C and pH 7-8 in the media containing yeast extract as the carbon source.

Dye decolourization was further investigated in a fixed bed biofilm reactor where the biofilm was composed with the developed bacterial consortium. Decolourization of the synthetic dye mixture was done with three different concentrations of yeast extract in the feed and more than 90% decolourization of the synthetic dye mixture was observed when the concentration was 2 and 1 g/l in batch operation of the reactor. However, even when the concentration was reduced to 0.25 g/l, 75% decolourization of synthetic dye mixture was achieved in both batch and continuous operation of the reactor. Results showed that dye decolourization was more effective with attached cells (bacterial consortium) in the reactor than with free cells (used in flasks). Stability of the dense microbial communities in biofilms and their ability to survive and degrade dyes at extreme conditions could be the reason for observed high colour removals in the decolourization studies conducted in the reactor. Structural changes occurred in dyes due to biological treatments were studied using ultraviolet-visible spectral and high-performance liquid chromatography analyses. Metabolites formed due to biological degradation were analyzed using gas chromatography-mass spectrophotometry and found to be non-toxic and benign.
A maximum of 45% colour removal was observed when the diluted textile effluent was treated in the fixed bed biofilm reactor operated in continuous mode whereas 70% colour removal was achieved in 48 h with undiluted textile wastewater treated in batch mode. This shows the ability of the developed bacterial consortium to endure in highly complex and toxic environment in the fixed bed biofilm reactor and the potential application in textile industry wastewater treatment.

Key words: biological, decolourization, dyes, fixed bed biofilm reactor, textile effluent
DEDICATION

Dedicated to my parents and husband for their unconditional love, endless support and encouragement.
ACKNOWLEDGEMENTS

I wish to express my most sincere gratitude to my supervisors, Dr. S.H.P. Gunawardena and Dr. H.L.T.U. Ariyadasa for their guidance, continuous support, supervision, assistance, motivation, and encouragement given throughout this period of study. I am also immensely thankful to the Senate Research Grant Committee for providing me with a valuable research grant (SRC/CAP/15/03) and all the academic staff at the Department of Chemical and Process Engineering, University of Moratuwa for their guidance.

I am thankful to Mrs. I.K Athukorala, Ms. P.D.M Rodrigo, Mrs. W.S.M De Silva, Mr. B. Karunathilaka, Mr. H. L. G. S. Peiris, Mr. M. P. A. J. Kumara and all the non-academic staff in the Department of Chemical and Process Engineering, University of Moratuwa for their support to succeed this research work. I would like to thank Mr. M.A.P.C. Gunawardana, Mr. M.T.M.R. Jayaweera from the Department of Material Science and Engineering and Mrs. S.M.N.D. Matino from the Department of Chemical and Process Engineering for their assistance in analytical studies.

Further, I would like to express my gratitude to Intertek Lanka (Pvt.) Ltd, Battaramulla for their support in sample analysis. My sincere thanks go to all my friends and staff members working in textile industry and Central Environmental Authority who have supported me to gather information for the industrial survey questionnaire.

I would also like to express my sincere gratitude to Rex Industries (Pvt) Ltd, Negombo, Sri Lanka for providing the plastic biofilm carrier materials required for this study. I am also thankful to the management of textile dyeing facilities for providing dyes and textile effluents required for this study. I am thankful to the progress review committee for their insightful comments and encouragement to carry out this work successfully.

I would like to thank the head and the staff of the Faculty of Graduate Studies, University of Moratuwa for facilitating this research degree. I would further express my most sincere gratitude to the Department of Civil Engineering, University of Moratuwa for allowing me to follow and complete subjects from their MSc. taught courses. I would like to thank my fellow lab mates and my friends for giving me hand whenever I needed. Finally, I must express my profound gratitude to my family, for having great faith and encouraging me to succeed in this study.
# TABLE OF CONTENTS

Declaration of the candidate and supervisor .......................................................... i  
Abstract .................................................................................................................. ii  
Dedication ............................................................................................................... iv  
Acknowledgements ............................................................................................... v  
Table of contents ................................................................................................ vi  
List of figures .......................................................................................................... xi  
List of tables .......................................................................................................... xvii  
List of figures in appendices ................................................................................. xix  
List of abbreviations ............................................................................................ xx  
1 Introduction .......................................................................................................... 1  
1.1 Background to the research problem .............................................................. 1  
1.2 Problem statement ......................................................................................... 3  
1.3 Research objectives ...................................................................................... 4  
2 Literature Review ............................................................................................... 5  
2.1 Textile industry .............................................................................................. 5  
2.2 Textile dyes .................................................................................................... 6  
2.3 Environmental hazards and health issues caused by textile dyes ................. 8  
  2.3.1 Impact on aquatic environment ................................................................. 8  
  2.3.2 Impact on vegetation ................................................................................ 9  
  2.3.3 Impact on human health ........................................................................... 10  
2.4 Effluent decolourization techniques ............................................................. 11  
  2.4.1 Chemical and physical treatments ............................................................ 11  
  2.4.2 Biological treatments .............................................................................. 13  
2.5 Reactor studies ............................................................................................... 19  
  2.5.1 Decolourization of textile dyes in reactors .............................................. 19  
  2.5.2 Biofilm ................................................................................................... 23  
  2.5.3 Decolourization of textile dyes in fixed (packed) bed biofilm reactors . 24  
3 Materials and methods ...................................................................................... 26  
3.1 Industrial survey questionnaire .................................................................... 26  
3.2 Chemicals and reagents .............................................................................. 26
3.2.1 Dyes used.................................................................................................................. 26
3.2.2 Chemicals used............................................................................................................. 27
3.3 Dye stock solution preparation..................................................................................... 27
3.4 Luria–Bertani medium (LB) preparation ...................................................................... 28
3.5 Agar plate preparation.................................................................................................... 28
3.6 Decolourization media preparation .............................................................................. 29
3.7 Isolation of indigenous microbial strains that have potential in dye decolourization ................................................................. 29
  3.7.1 Effluent and sludge collection ................................................................................... 29
  3.7.2 Co-incubation of effluent samples in the nutrient medium ...................................... 29
  3.7.3 Isolation of bacterial species .................................................................................... 30
3.8 Nutrient broth screening for dye decolourizing isolates ............................................. 30
3.9 Preservation of bacterial strains .................................................................................... 30
3.10 Identification of isolated dye decolourizing strains ................................................... 30
  3.10.1 Gram staining ........................................................................................................ 30
  3.10.2 Capsule staining .................................................................................................... 31
  3.10.3 Endospore staining ............................................................................................... 31
  3.10.4 Catalase test ......................................................................................................... 31
  3.10.5 Polymerase chain reaction (PCR) ......................................................................... 32
  3.10.6 Agarose gel electrophoresis ................................................................................ 33
  3.10.7 Identification of microbial isolates ........................................................................ 35
3.11 Dye decolourization ................................................................................................... 35
3.12 Determination of cell growth ...................................................................................... 36
3.13 Determination of dye category ................................................................................... 37
3.14 Dye decolourization by bacterial consortium ............................................................ 37
3.15 Optimization of decolourization conditions .............................................................. 37
  3.15.1 Effect of agitation ................................................................................................. 38
  3.15.2 Effect of temperature ........................................................................................... 38
  3.15.3 Effect of pH .......................................................................................................... 38
  3.15.4 Effect of initial dye concentration ....................................................................... 38
  3.15.5 Effect of carbon source ....................................................................................... 39
3.16 Decolourization of Malachite green ......................................................................... 39
3.17 Decolourization of textile industry effluent by the bacterial consortium ..... 39
3.18 Yellow EXF degraded compounds analysis ........................................... 40
  3.18.1 HPLC analysis .................................................................................. 40
  3.18.2 Gas chromatography–mass spectroscopy (GCMS) analysis ............. 40
3.19 Decolourization of textile dyes and effluent in a reactor ..................... 41
  3.19.1 Reactor seeding material preparation ................................................. 41
  3.19.2 Reactor design and fabrication ......................................................... 44
  3.19.3 Biofilm formation on carrier materials in the fixed bed biofilm reactor
      (FBBR) .................................................................................................. 47
  3.19.4 Decolourization of synthetic dye mixture in batch FBBR ............... 48
  3.19.5 Decolourization of synthetic dye mixture in continuous FBBR ..... 48
  3.19.6 Decolourization of textile industry effluent in batch and continuous
      FBBR ................................................................................................... 49
3.20 Analysis of biofilm on carrier materials in FBBR .................................. 49
  3.20.1 Quantification of dry weight of biofilms ............................................ 49
  3.20.2 Scanning electron microscopy analysis .............................................. 50
  3.20.3 FTIR analysis of biofilm .................................................................... 50
3.21 Determination of the quality of water treated in FBBR ....................... 50
  3.21.1 COD analysis .................................................................................. 50
  3.21.2 Phytotoxicity studies ....................................................................... 51
  3.21.3 Analysis of the metabolites formed by biological degradation of dye
      mixture in FBBR ................................................................................ 52
4 Results and Discussion .............................................................................. 54
4.1 Industrial survey questionnaire ............................................................... 54
  4.1.1 Identification of the most widely used category of dyes in Sri Lankan
      textile industry ...................................................................................... 54
  4.1.2 Water consumption in textile dyeing and washing process ............... 55
  4.1.3 Textile effluent decolourizing methods used in Sri Lanka ............... 57
  4.1.4 Textile effluent characteristics .......................................................... 59
4.2 Isolation of indigenous microbial strains with dye decolourization potential
    .......................................................................................................... 65
  4.2.1 Isolation of microorganisms by spread and streak plate techniques .. 65
4.3 Nutrient broth screening for dye decolourizing isolates ......................... 66
4.4 Identification of dye decolourizing bacterial strains ........................................... 69
  4.4.1 Biochemical tests and morphological characteristics ..................................... 69
  4.4.2 16S rRNA gene sequencing analysis ............................................................. 71
4.5 Dye decolourization ........................................................................................... 73
  4.5.1 Decolourization of individual textile dyes by isolated bacteria ....................... 73
  4.5.2 UV-visible spectrophotometric analysis of dye decolourization ................. 78
4.6 Determination of dye category ......................................................................... 82
4.7 Dye decolourization by bacterial consortium ..................................................... 84
  4.7.1 Decolourization of individual dyes ................................................................. 84
  4.7.2 Decolourization of dye mixture .................................................................... 85
4.8 Parameter optimization ....................................................................................... 88
  4.8.1 Dye decolourization under shaking conditions .............................................. 88
  4.8.2 Effect of ph on dye decolourization ............................................................... 91
  4.8.3 Effect of temperature on dye decolourization .............................................. 94
  4.8.4 Effect of dye concentration .......................................................................... 96
  4.8.5 Effect of carbon sources on dye decolourization .......................................... 97
  4.8.6 Decolourization of Malachite green ............................................................. 101
4.9 Textile effluent decolourization by bacterial consortium .................................... 102
4.10 Yellow EXF degraded compounds analysis ......................................................... 106
  4.10.1 HPLC analysis ............................................................................................. 106
  4.10.2 GCMS analysis ........................................................................................... 108
4.11 Decolourization of textile dyes and effluent in FBBR ...................................... 112
  4.11.1 Reactor seeding material preparation ......................................................... 112
  4.11.2 Decolourization of synthetic dye mixture in batch FBBR ......................... 117
  4.11.3 Decolourization of synthetic dye mixture in continuous FBBR .............. 120
  4.11.4 Decolourization of textile effluent in batch and continuous FBBR modes .......... 122
4.12 Analysis of the biofilm formed on the carrier materials in FBBR ............ 126
  4.12.1 Biofilm formation on the carrier materials in the fixed bed biofilm reactor (FBBR) ........................................................................................................ 126
  4.12.2 FTIR analysis of biofilm ............................................................................ 128
4.13 Determination of quality of treated water in FBBR ...................................... 129
4.13.1 COD analysis ............................................................... 129
4.13.2 Phytotoxicity analysis .................................................. 130
4.13.3 Analysis of the metabolites formed by biological degradation of synthetic dye mixture in FBBR .......................................................... 133
5 Conclusions .................................................................................. 139
6 Recommendations and future work ............................................. 141
References ....................................................................................... 143
Appendix A ....................................................................................... 167
Appendix B ....................................................................................... 170
List of publications ............................................................................ 173
LIST OF FIGURES

Figure 2.1: Examples for azo dyes [29] (a) C.I. Solvent Yellow 14 (b) C.I. Disperse Red 13 and (c) C.I. Acid Black 1 ................................................................. 7
Figure 2.2: C.I. Disperse Red 60 dye with anthraquinone structure [29] ................ 7
Figure 2.3: Indigo dye with indigoid structure [29] ............................................ 7
Figure 2.4: Xanthene dye C.I. Acid Red 52 [29] .................................................. 8
Figure 2.5: Triarylmethane dye C.I. Acid Blue 93 [29] ......................................... 8
Figure 3.1: Structure of major dye in Black WNN ................................................. 27
Figure 3.2: Biofilm support material .................................................................. 42
Figure 3.3: Laboratory set-up for biofilm growth ................................................. 43
Figure 3.4: 3D drawing of the reactor ................................................................. 44
Figure 3.5: Male female connectors .................................................................. 46
Figure 3.6: Schematic diagram of the reactor ..................................................... 47
Figure 3.7: Conceptual diagram showing major experiments conducted in the study ........................................................................................................ 53
Figure 4.1: Temperature variation of textile industry effluent ......................... 60
Figure 4.2: pH variation of textile industry effluent being discharged into the inland surface waters ................................................................................. 61
Figure 4.3: pH variation of textile industry effluent being discharged into the common wastewater treatment plant ......................................................... 61
Figure 4.4: COD variation of textile industry effluent being discharged into the common wastewater treatment plant ......................................................... 62
Figure 4.5: COD variation of textile industry effluent being discharged into the inland surface waters ................................................................................. 62
Figure 4.6: BOD variation of textile industry effluent being discharged into the common wastewater treatment plant ......................................................... 63
Figure 4.7: BOD variation of textile industry effluent being discharged into the inland surface waters ................................................................................. 63
Figure 4.8: Colour variation of textile industry effluent (where, AT: after the treatments, BT: before the treatments) ......................................................... 64
Figure 4.9: Bacterial growth on dye-amended nutrient agar medium (a) Yellow EXF (b) Red EXF (c) Blue EXF (d) Black WNN and (e) Rhodamine ................................. 65
Figure 4.10: Agar plates streaked with some of the isolated bacteria.......................... 66
Figure 4.11: Yellow EXF dye-containing nutrient media inoculated with isolates 1, 2, 3, 4, 18, 19 and 20 after 4-days of incubation and control (first tube starting from left hand side) .......................................................................................................................... 66
Figure 4.12: Red EXF dye-containing nutrient media inoculated with isolates 5, 6, 7, 18 and 19 after 8-days of incubation and control (first tube starting from left hand side) .......................................................................................................................... 67
Figure 4.13: Blue EXF dye containing nutrient media inoculated with isolates 8, 9, 10, 11, 18, 19 and 20 after 10 days of incubation and control (first tube starting from left hand side) .......................................................................................................................... 67
Figure 4.14: Nova Black WNN dye-containing nutrient media inoculated with isolates 12, 13, 18, 19 and 20 after 10-days of incubation and control (first tube starting from left hand side) ................................................................. 68
Figure 4.15: Rhodamine dye-containing nutrient media inoculated with isolates 14, 15, 16, 17 and 20 after 10-days of incubation and control (first tube starting from right hand side) .......................................................................................................................... 69
Figure 4.16: Capsule-staining image of isolate 20 (x100)............................................. 70
Figure 4.17: Gram-staining response of isolate 19 (x100)........................................... 70
Figure 4.18: Endospore staining image of isolate 1 (x100) .......................................... 71
Figure 4.19: Gel image of the amplified DNA samples of the isolated bacteria ...... 71
Figure 4.20: Effect of bacterial isolates and incubation time on decolorization of Yellow EXF dye ................................................................................................. 73
Figure 4.21: Effect of bacterial isolates and incubation time on decolorization of Red EXF dye ................................................................................................................ 74
Figure 4.22: Effect of bacterial isolates and incubation time on decolorization of Blue EXF dye ................................................................................................................ 75
Figure 4.23: Effect of bacterial isolates and incubation time on decolorization of Nova Black WNN dye ................................................................................................. 75
Figure 4.24: Effect of incubation time on decolorization of Rhodamine dye by P. mirabilis .................................................................................................................. 76
Figure 4.25: Variation of UV-visible spectra of Yellow EXF dye solutions treated with P. mirabilis for different time intervals under static conditions ................................................. 79
Figure 4.26: Variation of UV-visible spectra of Red EXF dye solutions treated with P. mirabilis for different time intervals under static conditions ................................................. 80
Figure 4.27: Variation of UV-visible spectra of Blue EXF dye solutions treated with P. mirabilis for different time intervals under static conditions ................................................. 80
Figure 4.28: Variation of UV-visible spectra of Black WNN dye solutions treated with P. mirabilis for different time intervals under static conditions ................................................. 81
Figure 4.29: Variation of UV-visible spectra of Rhodamine dye solutions treated with P. mirabilis for different time intervals under static conditions ................................................. 81
Figure 4.30: UV-visible spectra of Yellow EXF dye solutions decolourized with P. mirabilis in glucose containing medium .................................................................................. 82
Figure 4.31: FTIR spectra of Yellow EXF, Red EXF, Blue EXF and Black WNN .. 83
Figure 4.32: Effect of bacterial consortium on dye decolourization over time. ........ 84
Figure 4.33: Effect of I₁ and the bacterial consortium on Rhodamine dye decolourization over time .................................................................................................................. 85
Figure 4.34: Time dependent cell growth and dye mixture decolourization by bacterial consortium ...................................................................................................................... 86
Figure 4.35: UV–visible spectra of the dye mixture decolourization with the bacterial consortium ...................................................................................................................... 86
Figure 4.36: Dye containing nutrient media in flasks before biological treatments .. 87
Figure 4.37: Dye containing nutrient media in flasks after 48 h biological treatments .................................................................................................................. 87
Figure 4.38: Bacterial cells and the supernatants of decolourized dye samples separated by centrifugation ............................................................................................................. 88
Figure 4.39: Effect of aeration on time-dependent cell growth and decolourization of Yellow EXF dye by P. mirabilis ............................................................................................................. 89
Figure 4.40: Effect of aeration on time-dependent decolourization of Red EXF, Blue EXF and Black WNN dyes by P. mirabilis ..................................................................................................... 90
Figure 4.41: Effect of aeration on time-dependent decolourization of synthetic dye mixture by P. mirabilis. ..................................................................................................................... 91
Figure 4.42: Effect of pH on time dependent decolourization of Yellow EXF dye by
P. mirabilis.............................................................................................................................................. 91
Figure 4.43: Effect of pH on time dependent decolourization of Red EXF dye by P.
mirabilis. .............................................................................................................................................. 92
Figure 4.44: Effect of pH on time dependent decolourization of synthetic dye mixture
by bacterial consortium. .......................................................................................................................... 93
Figure 4.45: Effect of temperature on time dependent decolourization of Yellow EXF
dye by P. mirabilis. .............................................................................................................................. 94
Figure 4.46: Effect of temperature on time dependent decolourization of Red EXF dye
by P. mirabilis....................................................................................................................................... 95
Figure 4.47: Effect of temperature on time dependent decolourization of synthetic dye
mixture by bacterial consortium. .......................................................................................................... 95
Figure 4.48: Effect of dye concentration on decolourization of Yellow EXF by P.
mirabilis after 72 h of incubation ........................................................................................................ 97
Figure 4.49: Effect of carbon sources on Yellow EXF decolourization by P. mirabilis
.............................................................................................................................................................. 99
Figure 4.50: Effect of yeast extract concentration in decolourization of the synthetic
mixture of dyes and cell growth by bacterial consortium ............................................................ 100
Figure 4.51: Malachite green containing samples after 48 h incubation with bacterial
consortium in (a) 5 g/l (b) 5 g/l control and (c) 2 g/l yeast extract containing media
.............................................................................................................................................................. 101
Figure 4.52: UV-visible spectra for Malachite green decolourization by bacterial
consortium.............................................................................................................................................. 102
Figure 4.53: UV-visible spectra of company A effluent before and after biological
treatments ............................................................................................................................................... 103
Figure 4.54: UV-visible spectra of company Z effluent before and after biological
treatments ............................................................................................................................................... 104
Figure 4.55: UV-visible spectra of company D effluent before and after biological
treatments (study I) .............................................................................................................................. 105
Figure 4.56: UV-visible spectra of company D effluent before and after biological
treatments (study II) ............................................................................................................................ 105
Figure 4.57: Decolourization of textile effluents obtained from company (a) A (b) Z and (c) D (study I) (d) D (study II) ........................................................................................................... 106
Figure 4.58: HPLC chromatogram of Yellow EXF dye .................................................... 107
Figure 4.59: HPLC chromatogram of dye degraded compounds .................................. 107
Figure 4.60: Gas chromatogram of the dye degraded compounds formed after static treatment.................................................................................................................... 109
Figure 4.61: Gas chromatogram of the mineralized degraded compounds formed after both static and shaking treatments .................................................................................. 109
Figure 4.62: GC–mass spectra of degraded compounds of Yellow EXF by P. mirabilis for the peaks corresponding to RT value of (a) 4.825 (b) 11.521 (c) 12.208 and (d) 10.604................................................................................................................................. 111
Figure 4.63: Detection of biofilm formation by each microorganism by microtiter plate method (wells 1A to 1E: P. mirabilis, 2A to 2E: M. morganii, 3A to 3E: E. cloacae and 4A to 4E: control)................................................................................................................................. 114
Figure 4.64: SEM image of biofilm support material (x7500) (control) ....................... 115
Figure 4.65: SEM images of P. mirabilis biofilms at (a) 2500x and (b) 7500x magnifications ................................................................................................................................. 116
Figure 4.66: SEM images of M. morganii biofilms at (a) 2500x and (b) 7500x magnifications ................................................................................................................................. 116
Figure 4.67: SEM images of E. cloacae biofilms at (a) 2500x and (b) 7500x magnifications ................................................................................................................................. 116
Figure 4.68: Fixed bed biofilm reactor .............................................................................. 117
Figure 4.69: Effect of the concentration of yeast extract on decolourization of the synthetic dye mixture after 44h in batch FBBR ............................................................................. 118
Figure 4.70: Decolourization of synthetic dye mixture in FBBR in continuous mode ................................................................................................................................. 120
Figure 4.71: a) Influent (50 mg/l synthetic dye mixture containing medium) and b) treated water obtained during phase III operation of FBBR ................................................................. 121
Figure 4.72: Decolourization of 50% diluted textile effluent in continuous FBBR 122
Figure 4.73: a) Feed (Undiluted textile effluent containing medium) and b) 48 h treated water obtained from the batch FBBR ..................................................................................... 123
Figure 4.74: UV-visible spectra of undiluted textile effluent before and after treat in FBBR ................................................................. 123
Figure 4.75: Biofilms formed on the plastic carrier material after (a) one month and (b) two months of reactor operation................................................................. 126
Figure 4.76: SEM images of a) carrier material (x5000) and b) biofilms grown on the carrier material (x5000)................................................................. 126
Figure 4.77: a) biofilm attached on carrier particle (dried) and control b) biofilms attached on carriers (wet) and controls ................................................................. 127
Figure 4.78: SEM image showing the adherence of bacterial cells on to the surface of carrier material. Cells covered with EPS (□); EPS (o)........................................ 127
Figure 4.79: FTIR spectrum of the biofilms attached to the carrier.......................... 129
Figure 4.80: Germination of cowpea seeds watered with (a) control water (b) treated wastewater and (c) untreated wastewater................................................................. 131
Figure 4.81: Growth of cowpea seeds watered with (a) control water (b) treated wastewater and (c) untreated wastewater................................................................. 132
Figure 4.82: UV-visible spectra of reactor feed (2 g/l yeast extract containing media with 50 mg/l synthetic dye mixture) and treated water samples obtained from FBBR operated in batch and continuous mode ................................................................. 134
Figure 4.83: UV-visible spectra of reactor feed (1 g/l yeast extract containing media with 50 mg/l synthetic dye mixture) and treated water samples obtained from FBBR operated in batch and continuous mode ................................................................. 134
Figure 4.84: UV-visible spectra of reactor feed (0.25 g/l yeast extract containing media with 50 mg/l synthetic dye mixture) and treated water samples obtained from FBBR operated in batch and continuous mode ................................................................. 135
Figure 4.85: HPLC chromatogram of the reactor feed that containing 50 mg/l of synthetic dye mixture ................................................................. 136
Figure 4.86: HPLC chromatogram of the feed treated in FBBR in batch mode ..... 136
Figure 4.87: GC– mass spectra of compounds present in the biologically treated synthetic dye mixture (a) phenol, 4-methyl- (b) 1H-Indole, 3-methyl .......... 138
LIST OF TABLES

Table 2.1: Classification of dyes according to usage [28] .................................................. 6
Table 2.2: Chemical and physical dye decolourization methods widely used in industry ................................................................................................................................. 12
Table 2.3: Decolourization of textile dyes using bacteria and algae ........................................ 16
Table 2.4: Decolourization of textile dyes using fungi and yeast ........................................... 18
Table 2.5: Dye decolourization studies conducted using different biological reactors ............................................................................................................................. 21
Table 2.6: Dye decolourization studies conducted in packed bed biofilm reactors with natural support materials .................................................................................................................. 25
Table 3.1: Composition of LB medium [107] ........................................................................ 28
Table 3.2: Composition of agar medium .................................................................................. 28
Table 3.3: Composition of decolourization medium [13] ....................................................... 29
Table 3.4: Reagents used for PCR master mix preparation ..................................................... 32
Table 3.5: PCR cycling conditions ......................................................................................... 33
Table 3.6: Dimensions and details of the reactor components .............................................. 45
Table 3.7: Different phases of synthetic dye mixture decolourization in continuous FBBR ............................................................................................................................. 48
Table 4.1: Consumption of dyes in textile processing companies in Sri Lanka (from industrial survey) ........................................................................................................................... 54
Table 4.2: Water consumption and wastewater generation in textile processing companies in Sri Lanka ....................................................................................................................... 56
Table 4.3: Distribution of textile-related industries in Sri Lanka according to EPL listed classification .......................................................................................................................... 57
Table 4.4: Effluent treatment techniques used in textile dyeing facilities in Sri Lanka ............................................................................................................................... 58
Table 4.5: Tolerance limits for the textile industry effluents discharges (for the selected quality parameters) in Sri Lanka ........................................................................................................... 60
Table 4.6: Bacteria isolated from each dye-amended medium with code numbers ... 65
Table 4.7: Bacterial isolates capable of decolourizing each dye as per the nutrient broth screening .......................................................................................................................... 69
Table 4.8: Description of the identified bacterial isolates

Table 4.9: Biochemical test results of isolated bacterial strains

Table 4.10: Comparison of Rhodamine dye decolourization results of P. mirabilis with effluent discharge limits

Table 4.11: Percentage decolourization of textile effluents at different wavelengths

Table 4.12: Percentage decolourization of effluent obtained from company D by bacterial consortium after 48 h of incubation

Table 4.13: Optical density values measured from ELISA reader at 595 nm wavelength

Table 4.14: Maximum spectral absorption coefficient of reactor feed (influent), treated water samples (effluents) and the permissible discharge limits

Table 4.15: COD of the water samples collected from FBBR

Table 4.16: Effect of effluent on cowpea seed germination

Table 4.17: Results of the plant growth assay
LIST OF FIGURES IN APPENDICES

Appendix B Figure 1: Sectional view of the reactor lid ........................................170
Appendix B Figure 2: Sectional view of the reactor base..................................170
Appendix B Figure 3: Sectional view of the reactor column...............................171
Appendix B Figure 4: Bonding of the reactor column to the reactor base..........172
Appendix B Figure 5: Fixing of the reactor base to the reactor holding stand......172
Appendix B Figure 6: Fixing of the reactor lid to the reactor column.................173
<table>
<thead>
<tr>
<th>AEBR</th>
<th>Anaerobic expanded bed reactor</th>
</tr>
</thead>
<tbody>
<tr>
<td>AQDS</td>
<td>Anthraquinone-2, 6-disulfonate</td>
</tr>
<tr>
<td>AQS</td>
<td>Anthraquinone-2-sulfonate</td>
</tr>
<tr>
<td>BLAST</td>
<td>Basic local alignment search tool</td>
</tr>
<tr>
<td>BOD</td>
<td>Biochemical oxygen demand</td>
</tr>
<tr>
<td>CEA</td>
<td>Central environmental authority</td>
</tr>
<tr>
<td>COD</td>
<td>Chemical oxygen demand</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>dNTP</td>
<td>Deoxynucleoside triphosphate</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>EPL</td>
<td>Environmental protection license</td>
</tr>
<tr>
<td>EPS</td>
<td>Extracellular polymeric substances</td>
</tr>
<tr>
<td>EtBr</td>
<td>Ethidium bromide</td>
</tr>
<tr>
<td>FAD</td>
<td>Flavin adenine dinucleotide</td>
</tr>
<tr>
<td>FADH</td>
<td>Reduced form of flavin adenine dinucleotide</td>
</tr>
<tr>
<td>FBBR</td>
<td>Fixed bed biofilm reactor</td>
</tr>
<tr>
<td>FBR</td>
<td>Fluidized bed reactor</td>
</tr>
<tr>
<td>FMN</td>
<td>Flavin adenine mononucleotide</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier-transform infrared spectroscopy</td>
</tr>
<tr>
<td>GCMS</td>
<td>Gas chromatography–mass spectroscopy</td>
</tr>
<tr>
<td>GOTS</td>
<td>Global organic textile standards</td>
</tr>
<tr>
<td>HDPS</td>
<td>High density polystyrene</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>HRT</td>
<td>Hydraulic retention time</td>
</tr>
<tr>
<td>IV</td>
<td>Intravenous tubing</td>
</tr>
<tr>
<td>LB</td>
<td>Luria–Bertani</td>
</tr>
<tr>
<td>MBBR</td>
<td>Moving bed biofilm reactor</td>
</tr>
<tr>
<td>MRSL</td>
<td>Manufacturing restricted substances list</td>
</tr>
<tr>
<td>MSDS</td>
<td>Material safety data sheets</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>MTBE</td>
<td>Methyl tertiary butyl ether</td>
</tr>
<tr>
<td>MTP</td>
<td>Microtiter plate</td>
</tr>
<tr>
<td>NADH</td>
<td>Reduced form of nicotinamide adenine dinucleotide</td>
</tr>
<tr>
<td>NADPH</td>
<td>Reduced form of nicotinamide adenine dinucleotide phosphate</td>
</tr>
<tr>
<td>NCBI</td>
<td>National center for biotechnology information</td>
</tr>
<tr>
<td>NIST</td>
<td>National institute of standards and technology</td>
</tr>
<tr>
<td>OD</td>
<td>Optical density</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PE</td>
<td>Polyethylene</td>
</tr>
<tr>
<td>PMMA</td>
<td>Poly (methyl methacrylate)</td>
</tr>
<tr>
<td>PP</td>
<td>Polypropylene</td>
</tr>
<tr>
<td>PTFE</td>
<td>Polytetrafluoroethylene</td>
</tr>
<tr>
<td>PU</td>
<td>Polyurethane</td>
</tr>
<tr>
<td>PVC</td>
<td>Polyvinyl chloride</td>
</tr>
<tr>
<td>RBC</td>
<td>Rotating biological contactors</td>
</tr>
<tr>
<td>rRNA</td>
<td>Ribosomal ribonucleic acid</td>
</tr>
<tr>
<td>SAMBR</td>
<td>Submerged anaerobic membrane bioreactor</td>
</tr>
<tr>
<td>SBR</td>
<td>Sequencing batch reactor</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscope</td>
</tr>
<tr>
<td>TAE</td>
<td>Tris-acetate-EDTA</td>
</tr>
<tr>
<td>UASB</td>
<td>Up-flow anaerobic sludge blanket</td>
</tr>
<tr>
<td>US EPA</td>
<td>United States environmental protection agency</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>ZDHC</td>
<td>Zero discharge of hazardous chemicals</td>
</tr>
</tbody>
</table>