

## **WRC PROJECT K/1367**

# **BIO-REMEDIATION AND BIO-UTILIZATION OF PULPING AND BLEACHING WASTE WATERS**

### **TABLE OF CONTENTS FOR FINAL REPORT TO THE WRC**

<b>EXECUTIVE SUMMARY</b>	<b>ii</b>
<b>ACKNOWLEDGEMENTS</b>	<b>vii</b>
<b>LIST OF SYMBOLS AND ABBREVIATIONS</b>	<b>ix</b>
<b>GLOSSARY OF SPECIALIZED TERMS</b>	<b>x</b>
<b>TABLE OF CONTENTS</b>	<b>xi</b>
<b>CHAPTER 1. INTRODUCTION</b>	<b>1</b>
1.1. Introduction	1
1.2. Environmental considerations	2
1.3. Biotechnology in the pulp and paper industry	2
<b>CHAPTER 2. LITERATURE SURVEY</b>	<b>5</b>
2.1. Introduction	5
2.2. Chemical composition of waste water	7
2.2.1. High molecular mass compounds	7
2.2.1. Low molecular mass compounds	8
2.3. Biobleaching-based waste water bioremediation	8
2.3.1. Introduction	8
2.3.2. Bleaching with polyoxometalates	9
2.3.2.1. Structure and reaction mechanisms	9
2.3.2.2. Delignification of wood and pulp	11
2.3.2.3. Bleaching of pulp	11
2.3.3. Bleaching with enzymes	13
2.3.3.1. Biobleaching of kraft pulps	14
2.3.3.2. Biobleaching of sulfite pulps	15

2.3.3.3. Scale-up of biobleaching	16
2.4. Bio-utilization of pulp and paper waste waters	17
2.5. Conclusions	20
<b>CHAPTER 3. WASTE WATER BIO-REMEDIATION</b>	<b>21</b>
3.1. Polyoxometalate-based waste water bioremediation	21
3.1.1. Abstract	21
3.1.2. Introduction	21
3.1.3. Materials and methods	22
3.1.3.1. Pulps	22
3.1.3.2. Synthesis of polyoxometalates	22
3.1.3.3. Delignification of pulps with polyoxometalates	22
3.1.3.4. Chemical bleaching of pulps	22
3.1.3.5. Determination of pulp properties	23
3.1.4. Results and discussion	23
3.1.4.1. Bleaching of hardwood soda-aq pulp with polyoxometalates	23
3.1.4.2. Bleaching of bagasse soda pulp with polyoxometalates	24
3.1.4.3. Recycling and recovery efficiency of polyoxometalates	24
3.1.5. Conclusions	25
3.2. Xylanase-based waste water bioremediation	27
3.2.1. Abstract	27
3.2.2. Introduction	28
3.2.3. Materials and methods	29
3.2.3.1. Enzyme assay	29
3.2.3.2. Pulps	30
3.2.3.3. Enzyme treatment of pulp	30
3.2.3.4. Chemical bleaching of pulp	30
3.2.3.5. Analyses	30
3.2.3.6. Colour determinations	31
3.2.3.7. Analysis of chemical oxygen demand	31
3.2.3.8. Chloride determinations	31

3.2.3.9. Bacterial growth inhibition test	31
3.2.3.10. Solvent extraction	32
3.2.3.11. Thin layer chromatography procedures	32
3.2.3.12. Determination of absorbable organic halides	33
3.2.4. Results and discussion	33
3.2.4.1. Effect of xylanase on reduction of chemical consumption during bleaching	33
3.2.4.2. Effect of xylanase bleaching on waste water properties	36
3.2.5. Conclusions	41
<b>CHAPTER 4. WASTE WATER BIO-UTILIZATION</b>	<b>43</b>
4.1. Waste water utilization for xylanase production	43
4.1.1. Abstract	43
4.1.2. Introduction	44
4.1.3. Materials and methods	45
4.1.3.1. Characterization of spent sulfite liquor	45
4.1.3.2. Evaluation of fungal biomass using concentrated spent sulfite liquor as carbon feedstock	45
4.1.3.2.1. Fungal strains and cultivation conditions	45
4.1.3.2.2. Effect of pH on suspended solids in the concentrated spent sulfite liquor	46
4.1.3.2.3. Determination of fungal biomass cultivated on concentrated spent sulfite liquor-based medium	46
4.1.3.2.4. Analytical methods	47
4.1.3.3. Xylanase production with concentrated spent sulfite liquor as carbon substrate in shake flask cultures	47
4.1.3.3.1. Fungal strains	47
4.1.3.3.2. Media and cultivation conditions	47
4.1.3.3.3. Analytical methods	48
4.1.3.4. Pretreatment of spent sulfite liquor for xylanase production	49
4.1.3.4.1. Pretreatment of spent sulfite liquor	49
4.1.3.4.2. Microorganisms	49
4.1.3.4.3. Xylanase production	49

4.1.3.4.4. Analytical procedures	50
4.1.3.5. Xylanase production in batch cultures: Effect of culture pH	50
4.1.3.5.1. Cultivation conditions	50
4.1.3.5.2. Cultivation medium	51
4.1.3.5.3. Analyses	51
4.1.3.6. Xylanase production in batch cultures: Effect of agitation rate	52
4.1.3.6.1. Cultivation conditions	52
4.1.3.6.2. Cultivation medium	52
4.1.3.6.3. Analyses	52
4.1.3.7. Xylanase production in fed-batch cultures: Effect of xylose concentration	52
4.1.3.7.1. Cultivation conditions	52
4.1.3.7.2. Cultivation medium	53
4.1.3.7.3. Analyses	53
4.1.3.8. Partial characterization of xylanases	53
4.1.3.8.1. Media and cultivation conditions	53
4.1.3.8.2. Effect of pH and temperature on activity of xylanases	54
4.1.3.8.3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis	54
4.1.3.8.4. Zymogram analysis	55
4.1.3.9. Evaluation of xylanases in bleaching of pulp	55
4.1.4. Results and discussion	55
4.1.4.1. Characterization of spent sulfite liquor	55
4.1.4.2. Evaluation of fungal biomass	56
4.1.4.2.1. Biomass production with xylose as carbon substrate	56
4.1.4.2.2. Effect of pH on suspended solids in the concentrated spent sulfite liquor	57
4.1.4.2.3. Effect of pH adjustment on biomass concentration	58
4.1.4.2.4. Effect of acetate buffer on biomass concentration	60
4.1.4.2.5. Determination of total organic carbon	61
4.1.4.3. Xylanase production in shake flask cultures	62
4.1.4.4. Effect of pretreatment of spent sulfite liquor	65
4.1.4.4.1. True colour of waste waters	65
4.1.4.4.2. Ultrafiltration	66

4.1.4.4.3. Overliming and heat treatment	67
4.1.4.5. Xylanase production in batch cultures: Effect of culture pH	69
4.1.4.5.1. Xylanase production with xylan as carbon substrate	70
4.1.4.5.2. Xylanase production with concentrated spent sulfite liquor as carbon substrate	70
4.1.4.6. Xylanase production in batch cultures: Effect of agitation rate	73
4.1.4.7. Xylanase production in fed-batch cultures	76
4.1.4.8. Xylanase characterization	77
4.1.4.8.1. Physico-chemical properties	77
4.1.4.8.2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and zymograms	82
4.1.4.9. Application of xylanases in bleaching of pulp	83
4.1.5. Conclusions	84
<b>CHAPTER 5. GENERAL CONCLUSIONS AND RECOMMENDATIONS</b>	<b>86</b>
5.1. Conclusions	86
5.2. Recommendations	86
<b>REFERENCES</b>	<b>88</b>
<b>ANNEXURE PRODUCTS EMANATING FROM THE PROJECT</b>	<b>97</b>