CHEMICAL OXYGEN DEMAND REDUCTION OF PINEAPPLE INDUSTRY WASTEWATER BY LOCALLY ISOLATED MICROBES IN COLUMN SYSTEM

NOR SYAMIMI BINTI MUSA

UNIVERSITI TEKNOLOGI MALAYSIA

CHEMICAL OXYGEN DEMAND REDUCTION OF PINEAPPLE INDUSTRY WASTEWATER BY LOCALLY ISOLATED MICROBES IN COLUMN SYSTEM

NOR SYAMIMI BINTI MUSA

A thesis submitted in fulfilment of the
requirements for the award of the degree of
Master of Science (Chemistry)

Faculty of Science
Universiti Teknologi Malaysia

JULY 2012

This thesis is dedicated to:

Mum and dad… Zabedah Hamid and Musa Sulaiman.

Brother and Sister… Mohd. Iqbal and Izni Wahidah.

Friends.

Love you all.

ACKNOWLEDGEMENTS

In the name of Allah S.W.T, the Most Gracious, the Most Merciful,

I would like to express my deepest gratitude to my supervisor, Professor Dr. Wan Azlina Ahmad with all regards for giving me her advice, guidance, opinion, and understanding in accomplishing this research. I have gained a lot of knowledge and experience during doing this research.

I am indebted to the Ministry of Science, Technology and Innovation (MOSTI), Malaysia for the National Science Fellowship (NSF) Scholarship. Special thanks to lecturers and staff of Chemistry Department, Faculty of Science, UTM. I am also thankful to Mr. Jefri from Material Science Laboratory and Mr. Faizal from Ibnu Sina Institute, UTM for their help with FESEM and SEM analysis.

I also would like to acknowledge and thank to Biotechnology lab members, Dr. Zainul, K. Diana, K. Lini, A. Sopi, K. Fad, K. Ika, Sue, Jay, K. Nisa, K. Wan, K. Rozi, K. Shakila, Risha, Shaikh and Mr. Ali for their great assistance, encouragement and support throughout the research.

Last but not least, I wish to express my sincere appreciation to my beloved family for their understanding, inspiration and generous support for me to complete my research .Thank you so much.

ABSTRACT

Wastewater from pineapple canning industry contributes to high levels of Chemical Oxygen Demand (COD), colors and suspended solids when discharged into water environments. Currently, there are many methods that have been used to remove organic pollutants in industrial wastewater such as ozonation, chemical coagulation and electrochemical oxidation. However, these methods involve high operational costs and are energy extensive, besides producing large amounts of sludge. A biological approach may be a good alternative since the operational cost is usually lower and it is environmentally friendly compared to the physico-chemical methods. In this study, the effectiveness of locally isolated microbial agents in reducing COD level in pineapple industry wastewater was investigated. Two bacterial strains identified as *Kurthia gibsonii* and *Klebsiella pneumoniae* and a fungal strain (*Candida tropicalis*) were tested using continuous systems. Rubber wood husk, solid pineapple waste and sugarcane bagasse were utilized as support materials in single packed-bed columns. Parallel packed-bed columns containing sugarcane bagasse were used to enhance the performance of COD reduction. The COD reduction was monitored for five days and analyzed using a Hach DR/5000 spectrophotometer. Growth on microbial biofilms on sugarcane bagasse surface in both systems was analyzed using FESEM. In addition, the ability of an integrated biological system consisting of parallel packed-bed columns containing *Kurthia gibsonii* immobilized onto sugarcane bagasse to reduce COD level and Cr(VI) concentration in ChromeBac^{TM} effluent was also explored. The results obtained showed that at 50% (v/v) initial COD concentration, the presence of single microbial cultures resulted in reduction of COD by 93-95% whereas at 100% (v/v) initial COD concentration, reduction of 64-84% were observed. The mixed microbial culture resulted in 71% reduction in both cases while in the columns without bacteria, COD reduction of 49% and 37% were observed, respectively. Analysis by FESEM showed the presence of abundant EPS surrounding the cells in the bioreactor. The integrated biological system showed complete removal of Cr(VI) for both synthetic and real electroplating wastewater. The percentage of COD reduction in five batches was observed between 92-96% for synthetic Cr(VI) containing wastewater. The COD reductions for real electroplating wastewater were between 92-95%.

ABSTRAK

Air sisa dari industri pengetinan nanas menyumbang kepada kehendak oksigen kimia (COD), warna dan pepejal terampai yang tinggi apabila dilepaskan ke persekitaran air. Pada masa sekarang, terdapat pelbagai kaedah yang telah digunakan untuk membuang bahan pencemar organik di dalam air sisa industri seperti pengozonan, penggumpalan kimia dan pengoksidaan elektrokimia. Walaubagaimanapun, kaedah ini memerlukan kos operasi yang tinggi dan tenaga yang intensif, selain menghasilkan sejumlah besar enapcemar. Pendekatan secara biologi merupakan alternatif yang baik memandangkan kos operasinya yang rendah dan lebih mesra alam berbanding dengan kaedah fizik-kimia. Dalam kajian ini, keberkesanan ejen mikroorganisma pencilan tulen dalam menurunkan paras COD dalam air sisa industri nanas telah dikaji. Dua jenis bakteria dikenal pasti sebagai *Kurthia gibsonii* dan *Klebsiella pneumoniae* dan kulat (*Candida tropicalis*) diuji menggunakan sistem berterusan. Sekam kayu getah, hampas nanas dan hampas tebu telah digunakan sebagai bahan sokongan di dalam turus terpadat tunggal. Turus terpadat selari yang mengandungi hampas tebu telah digunakan untuk meningkatkan prestasi penurunan COD. Penurunan COD telah dipantau selama lima hari dan dianalisa menggunakan Hach DR/5000 spektrofotometer. Pertumbuhan biofilem mikrob pada permukaan hampas tebu dalam kedua-dua sistem dianalisa menggunakan FESEM. Sebagai tambahan, keupayaan sistem biologi bersepadu terdiri dari turus terpadat selari yang mengandungi *Kurthia gibsonii* yang dipegunkan pada hampas tebu telah diuji untuk menurunkan paras COD dan kepekatan Cr(VI) dalam air sisa ChromeBac^{TM}. Keputusan yang diperolehi menunjukkan pada kepekatan awal COD 50% (v/v), kehadiran kultur mikrob tunggal menyebabkan penurunan COD sebanyak 93-95% manakala pada kepekatan awal COD 100% (v/v), penurunan COD sebanyak 64-84% telah diperhatikan. Kultur bakteria campuran menghasilkan penurunan sebanyak 71% dalam kedua-dua kes sementara di dalam turus tanpa bakteria, penurunan COD sebanyak 49% dan 37% telah diperhatikan. Analisis oleh FESEM menunjukkan kehadiran EPS yang banyak di sekeliling sel di dalam bioreaktor. Sistem biologi bersepadu menunjukkan penyingkiran lengkap Cr(VI) untuk kedua-dua sintetik dan air sisa sebenar penyaduran elektrik. Peratusan penurunan COD dalam lima kelompok telah diperhatikan antara 92-96% untuk air sisa sintetik yang mengandungi Cr(VI). Penurunan COD bagi air sisa sebenar penyaduran elektrik adalah antara 92-95%.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

$AgSO_4$	-	Silver Sulphate
APHA	-	American Public Health Association
BET	-	Brunauer-Emmet-Teller
BOD	-	Biochemical Oxygen Demand
BSA	-	Bovine Serum Albumin
CFU	-	Colony Forming Unit
C/N	-	Carbon per nitrogen ratio
COD	-	Chemical Oxygen Demand
Cr(VI)	-	Hexavalent Chromium
Cr(III)	-	Trivalent Chromium
^oC	-	Degree celcius
DPC	-	1,5-diphenylcarbazide
DW	-	Deionized water
EPS	-	Extracellular polymers
FAO	-	Food and Agriculture Organization
FESEM	-	Field Emission Scanning Electron Microscope
FTIR	-	Fourier Transform Infrared
g	-	Gram
g/L	-	Gram per liter
GDP	-	Gross Domestic Product
H_2SO_4	-	Sulphuric Acid
$HgSO_4$	-	Mercuric Sulphate
i.d.	-	Internal Diameter
IC	-	Inorganic Carbon
IBC	-	Indigenous Bacteria Colony
IUPAC	-	International Union of Pure and Applied Chemistry

$K_2Cr_2O_7$	-	Potassium Dichromate
kPa	-	kiloPascal
L	-	Liter
LB	-	Luria Broth
mg	-	Milligrams
mg/L	-	Milligram per liter
mL	-	Millilitres
mL/min	-	Millilitre per minute
NA	-	Nutrient Agar
NaOH	-	Sodium Hydroxide
NAP	-	National Agriculture Policy
NB	-	Nutrient Broth
NIRR	-	Near-Infrared Reflectance
NDIR	-	Nondispersive Infrared Detector
nm	-	Nanometer
o.d.	-	Outer Diameter
OD	-	Optical Density
rpm	-	Rotation per minute
RWH	-	Rubber Wood Husk
SEM	-	Scanning Electron Microscope
SS	-	Suspended Solids
SPW	-	Solid Pineapple Waste
SCB	-	Sugarcane Bagasse
TC	-	Total Carbon
TOC	-	Total Organic Carbon
TSS	-	Total Suspended Solid
UASB	-	Upflow Anaerobic Sludge Bed Bioreactor
v/v	-	Volume per volume

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background of Study

The trend towards strict environmental regulation and water quality improvement has increased public awareness on quality of the environment. Wastewater discharged from various industries such as pineapple industry contains hazardous and toxic chemicals, and contributes to high levels of Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD) and Suspended Solids (SS) (Somasiri *et al.*, 2008).

Effluent from the pineapple industry posses high COD levels due to the higher composition of sugars such as sucrose, glucose and fructose (Chareonsak *et al.*, 1980). The highest COD concentration in wastewater treatment systems are toxic to biological life and will affect aquatic communities such as aquatic plants and fishes (Somasiri *et al.*, 2008). According to the Environmental Quality (Industrial Effluents) Regulations 2009, the permissible concentration of COD in wastewater for discharge must be less than 80 mg/L for standard A and 200 mg/L for standard B.

Currently, there are many methods that have been used to treat organic pollutants in industrial wastewater such as ozonation, chemical coagulation, reverse osmosis, membrane filtration methods, photochemical oxidation and electrochemical oxidation. However, these methods require high operational costs and energy consumption, besides producing large amounts of sludge. Therefore, biological treatment may be a good alternative since it has lower operational cost.

1.2 Statement of Problem

This study is a continuation of the Cr(VI) Reduction System i.e. ChromeBac^{TM} which has been developed at the laboratory and pilot scale in UTM, Skudai, Johor, Malaysia. ChromeBac^{TM} is a novel, environmental-friendly system to treat Cr(VI)-bearing wastewater consisting of bioreactor packed with immobilized Cr(VI)-resistant-reducing bacteria. Cr(VI) bearing water, supplemented with minimal amount of pineapple industry wastewater as carbon source, was introduced into the bioreactor where it will be reduced to Cr(III) by bacterial action (Zakaria *et al.*, 2007). However, pineapple industry wastewater used in this system contributed to high concentrations of COD in the effluent before the post-treatment step. Hence, a system involving immobilization of bacteria onto agricultural waste as a support material in a column was employed to overcome the high COD problem.

1.3 Objective

The aim of this study is to reduce COD levels in the pineapple industry wastewater using locally isolated bacteria immobilized onto agricultural waste as support material in column system.

1.4 Scope of Study

In order to achieve the objective, the COD reducing bacteria will be isolated from pineapple industry wastewater and the selection of the most effective COD reducing bacteria will be carried out using batch system. The pineapple industry wastewater will be characterized in terms of COD, color, pH, protein, total carbohydrate, total nitrogen, total organic carbon, total suspended solids and turbidity. The reduction of COD using the most efficient COD reducing bacteria in batch and column system will be carried out. In column study, the agricultural waste such as solid pineapple waste, sugarcane bagasse and wood husk will be used as

support material and the performance of COD reduction using different agricultural wastes will be studied. COD reduction of pineapple industry wastewater using selected support material will be carried out in parallel packed-bed glass columns. The development of bacterial biofilm on surface of selected support material will be observed using Field Emission Scanning Electron Microscope (FESEM). Lastly, the system will be applied to reduce the COD level in ChromeBac^{TM} effluent.

1.5 Significance of Study

This research is important to ensure effluents that are discharged from the pineapple industry wastewater do not contain high levels of COD as it can be toxic to aquatic life. The abundant supply of agricultural waste can be used as a support material in column system to reduce COD level in pineapple industry wastewater.

REFERENCES

Abdullah and Mat, H. (2008). Characterisation of Solid and Liquid Pineapple Waste. *Reaktor*. 12(1), 48–52.

Ahmad, W. A., Zakaria, Z. A., Khasim, A. R., Alias, M. A. and Ismail, S. M. H. S. (2010). Pilot-Scale Removal of Chromium from Industrial Wastewater using the ChromeBac™ System. *Bioresource Technology*. 101(12), 4371–4378.

Ahmed, S. A. (2011). Batch and Fixed-Bed Column Techniques for Removal of Cu(II) and Fe(III) using Carbohydrate Natural Polymer Modified Complexing Agents. *Carbohydrate Polymers*. 83(4), 1470–1478.

Amit, B. and Mika, S. (2010). Utilization of Agro-Industrial and Municipal Waste Materials as Potential Adsorbents for Water Treatment—A Review. *Chemical Engineering Journal*. 157(2–3), 277–296.

Anisha, G. S. and Prema, P. (2008). Cell Immobilization Technique for the Enhanced Production of A-Galactosidase by *Streptomyces griseoloalbus*. *Bioresource Technology*. 99(9), 3325–3330.

Anwar, S. I. (2010). Determination of Moisture Content of Bagasse of Jaggery Unit using Microwave Oven. *Journal of Engineering Science and Technology*. 5(4), 472–478.

Azbar, N., Yonar, T. and Kestioglu, K. (2004). Comparison of Various Advanced Oxidation Processes and Chemical Treatment Methods for COD and Color Removal from a Polyester and Acetate Fiber Dyeing Effluent. *Chemosphere*. 55(1), 35–43.

Bahig, A. E., Aly E. A., Khaled A. A. and Amel K. A. (2008). Isolation, characterization and application of bacterial population from agricultural soil at Sohag Province, Egypt. *Malaysian Journal of Microbiology.* 4(2), 42–50.

Baig, S. and Liechti, P. A. (2001). Ozone Treatment for Biorefractory COD Removal. *Water Science and Technology.* 43(2), 197–204.

Bansode, R.R., Losso, J.N., Marshall, W.E., Rao, R.M. and Portier, R.J. (2004). Pecan Shell-Based Granular Activated Carbon for Treatment of Chemical Oxygen Demand (COD) in Municipal Wastewater. *Bioresource Technology.* 94(2), 129–135.

Benefield, L. D. and Randall, C. W. (1980). Biological Process Design for Wastewater Treatment. Englewood Cliffs, N.J., Prentice-Hall, Inc. 73–74.

Black, J. G. (2002). *Microbiology: Principles and Expl*oration. USA: John Wiley and Sons, Inc.

Blonskajaa, V., Kamenevb, I. and Zubc, S. (2006). Possibilities of Using Ozone for the Treatment of Wastewater from the Yeast Industry. *Proc. Estonian Acad. Sci. Chem.*. 55(1), 29–39.

Brandao, P. C., Souza, T. C., Ferreira, C. A. Hori, C. E. and Romanielo, L. L. (2010) Removal of Petroleum Hydrocarbons from Aqueous Solution using Sugarcane Bagasse as Adsorbent. *Journal of Hazardous Materials*. 175(1–3), 1106–1112.

Breed, R. S. and Murray, E. G. (1984). *Bergey's Manual of Determinative Bacteriology [M]*. USA: The Williams and Wilkins Company.

Britz, T. J., Trnovec, W. and Fourie, P. C. (2000). Influence of Retention Time and Influent Ph on the Performance of an Upflow Anaerobic Sludge Bioreactor Treating Cannery Waste Waters. *International Journal of Food Science & Technology*. 35(3), 267-274.

Carvalho, L. M. J., Castro, I. M. and Silva, C. A. B. (2008). A Study of Retention of Sugars in the Process of Clarification of Pineapple Juice (*Ananas comosus*, L. Merril) by Micro- and Ultra-Filtration. *Journal of Food Engineering.* 87(4), 447–454.

Castilla, C. M., Toledo, I. B., Garcia, M. A. F. and Utrilla, J. R. (2003). Influence of Support Surface Properties on Activity of Bacteria Immobilised on Activated Carbons for Water Denitrification. *Carbon*. 41(9), 1743–1749.

Chareonsak, C., Charoensiri, K. and Vananuvat, P. (1980). Protein Production by *Candida utilis* from Pineapple Wastewater. *Journal of the National Research Council.* 12(1), 1–24.

Chen, C. Y., Kao, C. M. and Chen, S. C. (2008). Application of *Klebsiella oxytoca* Immobilized Cells on the Treatment of Cyanide Wastewater. *Chemosphere*. 71(1), 133–139.

Chowdhury, S., Chakraborty, S. and Saha, P. (2011). Biosorption of Basic Green 4 from Aqueous Solution by *Ananas comosus* (Pineapple) Leaf Powder. *Colloids and Surfaces B: Biointerfaces*. 84(2), 520–527.

Chughtai, M. I. D. (1991). Biological Treatment of Liquid Effluents. In Martin, A. M. *Biological Degradation of Wastes* (pp. 323–338). New York, USA: Elsevier Science Publishing CO., Inc.

Coates, J. (2000). Interpretation of Infrared Spectra, a Practical Approach. In Meyers, R.A. (Ed.). *Encyclopaedia of Analytical Chemistry* (pp. 10815–10837). Chichester: John Wiley and Sons Ltd.

Department of Agriculture. (2003). *Crop Statistics of Malaysia 2001*. Ministry of Agriculture and Agro-Based Industry. Malaysia.

Devi, R., Singh, V. and Kumar, A. (2008). COD and BOD Reduction from Coffee Processing Wastewater using Avocado Peel Carbon. *Bioresource Technology*. 99(6), 1853–1860.

Domini, C. E., Vidal, L. and Canals, A. (2009). Trivalent Manganese as an Environmentally Friendly Oxidizing Reagent for Microwave- and Ultrasound-Assisted Chemical Oxygen Demand Determination. *Ultrasonics Sonochemistry*. 16(5), 686–691.

DR5000 Spectrophotometer Procedures Manual (2005) *DOC082.98.00670*. (2nd ed.) Germany: Hach Company.

Dudek, R. W. (2007). *High Yield Kidney*. (pp. 171–173). Philadelphia: Lippincott Williams & Wilkins.

Eaton, A. D., Clesceri, L. S., Rice, E. W. and Greenberg, A. E. (2005). *Standard Methods for the Examination of Water and Wastewater*. (21st ed.). United State of America: American Public Health Association.

Enviromental Quality Act and Regulations Handbook. (2009). Laws of Malaysia: Details on Eniromental Quality Act 1974 and Regulations Amendments up to June 2009: Act 127 with Inde. (pp. 34–35). Kuala Lumpur: MDC Publishers.

Eva, M.T. and Springaely, D. (2003). The Role of Mobile Genetic Elements in Bacterial Adaptation to Xenobiotic Organic Compounds. *Current Opinion in Biotechnology*. 14(3), 262–269.

Everett, D. H. (1972). Manual of Symbols and Terminology for Physicochemical Quantities and Units, Appendix II: Definitions, Terminology and Symbols in Colloid and Surface Chemistry. *Pure and Applied Chemistry*. 31(4), 577–638.

Farinella, N.V., Matos, G.D. and Arruda, M.A.Z. (2007). Grape Bagasse as a Potential Biosorbent of Metals in Effluent Treatments. *Bioresource Technology*. 98(10), 1940–1946.

Food and Agriculture Organization (FAO) of the United Nations databases.

Gerardi, M. H. (2006). *Wastewater Bacteria*. (1st ed.). Hoboken, New Jersey: John Wiley & Sons, Inc.

Gonçalves, A. R., Esposito, E. and Benar, P. (1998). Evaluation of *Panus tigrinus* in the Delignification of Sugarcane Bagasse by FTIR-PCA and Pulp Properties. *Journal of Biotechnology*. 66(2–3), 177–185.

Gray, N. F. (2004). *Biology of Wastewater Treatment*. (2nd ed.). Covent Garden, London: Imperial College Press.

Greenberg, A. E., Trussell, R. R. and Clesceri, L. S. (1985). *Standard Methods for the Examination of Water and Wastewater*. (16th ed.). Washington D. C.: American Public Health Association.

Hamilton, M., Heersink, J., Buckingham-meyer, K. and Goeres, P. L. (2003). *The Biofilm Laboratory: Step-by-Step Protocols for Experimental Design, Analysis, and Data Interpretation*. Bozeman: Montana University.

Harley, J.P. and Prescott, L.M. (1990). *Lab Exercise in Microbiology*. USA: Wm. C. Brow Publisher.

Hui, Y. H. and Khachatourians, G. G. (1995). *Food Biotechnology: microorganisms*. (pp 315–320). Canada: Wiley-VCH Inc.

Imandi, S. B., Bandaru, V. V. R., Somalanka, S. R., Bandaru, S. R. and Garapati, H. R. (2008). Application of Statistical Experimental Designs for the Optimization of Medium Constituents for the Production of Citric Acid from Pineapple Waste. *Bioresource Technology*. 99(10), 4445–4450.

Iwai, S. and Kitao, T. (1994). *Wastewater Treatment with Microbial Films*. (pp 24–25). Pensylvania, USA: Technomic Publishing Company, Inc.

Kapdan, I. K. and Kapdan, S. (2005). Application of Anaerobic-Aerobic Sequential Treatment System to Real Textile Wastewater for Color and COD removal. *Enzyme and Microbial Technology.* 36(2), 273–279.

Kapdan, I. K. and Oztekin, R. (2006). The Effect of Hydraulic Residence Time and Initial COD Concentration on Color and COD Removal Performance of the Anaerobic–Aerobic SBR System. *Journal of Hazardous Materials*. 136(3), 896-901.

Kartal, B., Koleva, M., Arsov, R., Star, W., Jetten, M. S. M. and Strous, M. (2006). Adaptation of a Freshwater Anammox Population to High Salinity Wastewater. *Journal of Biotechnology.* 126(4), 546–553.

Khalil, H. P. A., Ismail, H., Rozman, H. D. and Ahmad, M. N. (2001). The Effect of Acetylation on Interfacial Shear Strength between Plant Fiber and Various Matrices. *European Polymer Journal*. 37(5), 1037–1045.

Kilic, N. K., Nielsen, J. L., Yüce, M. and Dönmez, G. (2007) Characterization of a Simple Bacterial Consortium for Effective Treatment of Wastewaters with Reactive Dyes and Cr(VI). *Chemosphere*. 67(4), 826–831.

Kim, S., Park, C., Kim, T.H., Lee, J.W. and Kim, S.W. (2003). COD Reduction and Decolourization of Textile Effluent using Combined Process. J. *Biosci. Bioeng*. 95(1), 102–105.

Kobayashi, H., Oethinger, M. M., Tuohy, M. J., Procop, G. W. and Bauer, T. W. (2009). Improved Detection of Biofilm-formative Bacteria by Vortexing and Sonication: A Pilot Study. *Clinical Orthopaedics and Related Research*. 467(5), 1360–1364.

Konovalova, V. V., Dmytrenko, G. M., Nigmatullin, R. R., Bryk, M. T. and Gvozdyak, P. I. (2003). Chromium(VI) Reduction in a Membrane Bioreactor with Immobilized *Pseudomonas* Cells. *Enzyme and Microbial Technology*. 33(7), 899–907.

Kosińska, K., and MiśKiewicz, T. (2009). Performance of an Anaerobic Bioreactor with Biomass Recycling, Continuously Removing COD and Sulphate from Industrial Wastes. *Bioresource Technology*. 100(1), 86–90.

Kruger, N.J. (2002). The Bradford Method for Protein Quantitation. In: *The Protein Protocols Handbook* (2nd ed.). Totowa, New Jersey: Humana Press Inc.

Lappin-Scott, H. M. and Costerton, J. W. (1995). Microbial Biofilms. Cambridge, United Kingdom: Cambrige University Press.

Larone, D. H. (1995). *Medically Important Fungi - A Guide to Identification*. (3rd ed.). Washington D.C.: ASM Press.

Leenen. E. J. T. M., Santos, V. A. P. D., Grolle, K. C. F., Tramper, J. and Wijffels, R. (1996). Characteristics of and Selection Criteria for Support Materials for Cell Immobilization in Wastewater Treatment. *Water Research*. 30(12), 2985–2996.

Lewandowski, Z. and Beyenal, H. (2007). *Fundamentals of Biofilm Research*. Boca Raton, Florida, USA: CRC Press, Taylor & Francis Group.

Liu, J., Björnsson, L. and Mattiasson, B. (2000). Immobilised Activated Sludge Based Biosensor for Biochemical Oxygen Demand Measurement. *Biosensors & Bioelectronics*. 14(12), 883–893.

Lo´pez-Pasquali, C.E. and Herrera, H. (1997). Pyrolysis of Lignin and IR Analysis of Residues. *Thermochimica. Acta*. 293(1–2), 39–46.

Lucas, M. S. and Peres, J. A. (2009). Removal of COD from Olive Mill Wastewater by Fenton's Reagent: Kinetic Study. *Journal of Hazardous Materials.* 168(2–3), 1253–1259.

Madaeni, S. S. and Mansourpanah, Y. (2006). Screening Membranes for COD Removal from Dilute Wastewater. *Desalination*. 197(1–3), 23–32.

Madigan, M.T., Martinko, J.M. and Parker, J. (2000). *Brock Biology of Microorganism*. New Jersey: Prentice Hall International, Inc.

Malaysian Standard (2003). Animal Feeding Stuffs Determination of Moisture and Other Volatile Matter Content. *MS ISO 6496:2003*. Malaysia: SIRIM Berhad.

Mecozzi, M. (2005). Estimation of Total Amount in Environmental Samples by the Phenol-Sulphuric Acid Method Assisted by Multivariate Calibration. *J. Chemometrics & Intelligent Laboratory System.* 79(1–2), 84–90.

Ministry of Agriculture and Agro-Based Industry. (2010). *Agriculture Statistic.* Ministry of Agriculture and Agro-Based Industry. Malaysia.

Miqueletoa, A. P., Dolosica, C. C., Pozzia, E., Forestia, E. and Zaiat, M. (2010). Influence of Carbon Sources and C/N Ratio on EPS Production in Anaerobic Sequencing Batch Biofilm Reactors for Wastewater Treatment. *Bioresource Technology*. 101(4), 1324–1330.

Mishra, B. K., Arora, A. and Lata. (2004). Optimization of a Biological Process for Treating Potato Chips Industry Wastewater using a Mixed Culture of *Aspergillus foetidus* and *Aspergillus niger*. *Bioresource Technology*. 94(1), 9–12.

Nagadomi, H., Takahasi, T., Sasaki, K. and Yang, H.C. (2000). Simultaneous Removal of Chemical Oxygen Demand and Nitrate in Aerobic Treatment of Sewage Wastewater using an Immobilized Photosynthetic Bacterium of Porous Ceramic Plates. *World J. Microbiology & Biotechnology.* 16(1), 57–62.

Nielsen, L. E., Kadavy, D. R., Rajagopal, S., Drijber, R. and Nickerson, K. W. (2005). Survey of Extreme Solvent Tolerance in Gram-Positive Cocci: Membrane Fatty Acid Changes in *Staphylococcus haemolyticus* Grown in Toluene. *Applied and Environmental Microbiology*. 71(9), 5171–5176.

Nigam, J. N. (1999). Continuous Ethanol Production from Pineapple Cannery Waste. *Journal of Biotechnology*. 72(3), 197–202.

Nordin, N. (2009). Two-stage Copper(II) and Nickel(II) Removal using Rubber Wood Shavings and Strontium Alginate Immobilized Bacteria. M.Sc. Thesis. Universiti Teknologi Malaysia.

Ofomaja, A. E., Naidoo, E. B. and Modise, S. J. (2009). Removal of Copper(II) from Aqueous Solution by Pine and Base Modified Pine Cone Powder as Biosorbent. *Journal of Hazardous Materials*. 168(2–3), 909–917.

Parande, A. K., Sivashanmugam, A., Beulah, H. and Palaniswamy, N. (2009). Performance Evaluation of Low Cost Adsorbents in Reduction of COD in Sugar Industrial Effluent. *Journal of Hazardous Materials.* 168(2–3), 300–305.

Rahman, M. S. and Islam, M. R. (2009). Effects of pH on Isotherms Modeling for Cu(II) Ions Adsorption using Maple Wood Sawdust. *Chemical Engineering Journal*. 149(1–3), 273–280.

Rani, D. S. and Nand, K. (2004). Ensilage of Pineapple Processing Waste for Methane Generation. *Waste Management*. 24(5), 523–528.

Rapoport, A., Borovikova, D., Kokina, A., Patmalnieks, A., Polyak, N., Pavlovska, I., Mezinskis, G. and Dekhtyar, Y. (2011). Immobilisation of Yeast Cells on the Surface of Hydroxyapatite Ceramics. *Process Biochemistry*. 46(3), 665–670.

Ravella, S. R., Quiñones, T. S., Retter, A., Heiermann, M., Amon, T. and Hobbs, P. J. (2010). Extracellular Polysaccharide (EPS) Production by a Novel Strain of Yeast-Like Fungus *Aureobasidium pullulans. Carbohydrate Polymers*. 82(3), 728–732.

Riemann, H. P. and Cliver, D. O. (2006). *Foodborne Infections and Intoxications*. (3rd ed.). California, USA: Elsevier Academic Press.

Rosma, A. and Ooi, K. I. (2006). Production of *Candida utilis* Biomass and Intracellular Protein Content: Effect of Agitation Speed and Aeration Rate. *Malaysian Journal of Microbiology.* 2(2), 15–18.

Sairi, M.B., 2005. The Deacidification of Pineapple Juice using Electrodialysis with Monopolar Ion Exchange Membranes. Msc Thesis. Universiti Teknologi Malaysia.

Santos, D. T., Sarrouh, B. F., Rivaldia, J. D., Converti, A. and Silva, S. S. (2008). Use of Sugarcane Bagasse as Biomaterial for Cell Immobilization for Xylitol Production. *Journal of Food Engineering*. 86(4), 542–548.

Sarrouh, B. F., Silva, S. S., Santos, D. T. and Converti, A. (2007). Technical/Economical Evaluation of Sugarcane Bagasse Hydrolysis for Bioethanol Production. Chemical *Engineering & Technology*. 30(2), 270–275.

Sene, L., Converti, A., Felipe, M. G. and Zilli, M. (2002). Sugarcane Bagasse as Alternative Packing Material for Biofiltration of Benzene Polluted Gaseous Streams: A Preliminary Study. *Bioresource Technology*.83(2), 153–157.

Sheng, G. P., Yu, H. Q. and Li, X. Y. (2010). Extracellular Polymeric Substances (EPS) of Microbial Aggregates in Biological Wastewater Treatment Systems: A Review. *Biotechnology Advances*. 28(6), 882–894.

Singh, R., Kumar, A., Kirrolia, A., Kumar, R., Yadav, N., Bishnoi, N. R. and Lohchab, R. K. (2011). Removal of Sulphate, COD and Cr(VI) in Simulated and Real Wastewater by Sulphate Reducing Bacteria Enrichment in Small Bioreactor and FTIR Study. *Bioresource Technology.* 102(2), 677–682.

Soliev, A. B., Hosokawa, K. and Enomoto, K. (2011). Bioactive Pigments from Marine Bacteria: Applications and Physiological Roles. *Evidence-Based Complementary and Alternative Medicine*. 2011(670349), 1–17.

Somasiri, W., Li, X., Ruan, W. and Jian, C. (2008). Evaluation of the Efficacy of Upflow Anaerobic Sludge Blanket Reactor in Removal of Colour and Reduction of COD in Real Textile Wastewater. *Bioresource Teechnology*. 99(9), 3692–3699.

Sousa, A. C., Lucio, M. M. L. M., Neto, O. F. B., Marcone, G. P. S. , Pereira, A. F. C. , Dantas, E. O., Fragoso, W. D., Araujo, M. C. U. and Galvão, R. K. H. (2007). A Method for Determination of COD in a Domestic Wastewater Treatment Plant by using Near-Infrared Reflectance Spectrometry of Seston. *Analytica Chimica Acta*. 588(2), 231–236.

Souza, S. M. A. d. A. G. U., Bonilla, K. A. S. and Souza, A. A. U. (2010). Removal of COD and Color from Hydrolyzed Textile Azo Dye by Combined Ozonation and Biological Treatment. *Journal of Hazardous Materials*. 179(1–3), 35–42.

Sperling, M. (2007). *Biological Wastewater Treatment, Wastewater Characteristics, Treatment and Disposal*. (pp. 80–82). London, UK: IWA Publishing.

Stackebrandt, E., Keddie, R. M. and Jones, D. (2006). The Genus *Kurthia*. *Prokaryotes*. 4, 519-529.

Sud, D., Mahajan, G. and Kaur, M. P. (2008). Agricultural Waste Material as Potential Adsorbent for Sequestering Heavy Metal Ions from Aqueous Solutions – A Review. *Bioresource Technology*. 99(14), 6017–6027.

Sun, X. F., Xu, F., Sun, R. C., Geng, Z. C., Fowler, P. and Baird, M. S. (2005). Characteristics of Degraded Hemicellulosic Polymers obtained from Steam Exploded Wheat Straw. *Carbohydrate Polymers*. 60(1), 15–26.

Tampion, J. and Tampion, M. D. (1987). *Immobilized Cells: Principles and Applications.* United Kingdom: Cambridge University Press.

Tansel, B. (2008). *New Technologies for Water and Wastewater Treatment: A Survey of Recent Patents*. (pp. 1874–4788). USA: Bentham Science Publishers Ltd.

Tayagi, R. D. and Venbu, K. (1990). *Wastewater Treatment by Immobilized Cells*. Boca Raton, Florida: CRC Press, Inc.

Teresa, Z. P., Gunther, G. and Fernando, H. (2007). Chemical Oxygen Demand Reduction in Coffee Wastewater through Chemical Flocculation and Advanced Oxidation Processes. *Journal of Environmental Sciences.* 19(3), 300–305.

Tortora, G. J., Funke, B. R. and Case, C. L. (2007). *Microbiology an Introduction*. (pp 68–71). United State of America: Pearson Education, Inc.

Trejo-Hernandez, M. R., Ortiz, A., Okoh, A. I., Morales, D. and Quintero, R. (2007). Biodegradation of Heavy Crude Oil Maya using Spent Compost and Sugarcane Bagasse Wastes. *Chemosphere*. 68(5), 848–855.

Tziotzios, G., Michailakis, S. and Vayenas, D.V. (2007). Aerobic Biological Treatment of Olive Mill Wastewater by Olive Pulp Bacteria. *International Biodeterioration & Biodegradation.* 60(4), 209–214.

Vero, A.F., Melo, T.M., Torres, A.P.R., Ferreira, N.R., Jr., G.L.S., Borges, C.P. and Santiago, V.M.J. (2008). The Effects of Long-Term Feeding of High Organic Loading in a Submerged Membrane Bioreactor Treating Oil Refinery Wastewater. *Journal of Membrane Science.* 319(1–2), 223–230.

Vijayaraghavan, K. and Yun, Y.S. (2008). Bacterial Biosorbents and Biosorption. *Biotechnology Advances.* 26(3), 266–291.

Volesky, B. (1990). Removal and Recovery of Heavy Metals by Biosorption. In: B. Volesky (Ed.). *Biosorption of Heavy Metals.* Florida, USA: Boca Raton Press.

Vyrides, I. and Stuckey, D.C. (2009). A Modified Method for the Determination of Chemical Oxygen Demand (COD) for Samples with High Salinity and Low Organics. *Bioresource Technology*. 100(2), 979–982.

Walker, G.M. and Weatherley, L.R. (2001). COD Removal from Textile Industry Effluent: Pilot Plant Studies. *Chemical Engineering Journal*. 84(2), 123–131.

Wang, W., Han, H., Yuan, M., Li, H., Fang, F. and Wang, K. (2011). Treatment of Coal Gasification Wastewater by a Two-Continuous UASB System with Step-Feed for COD and Phenol Removal. *Bioresource Technology*. 102(9), 5454–5460.

Wijffels, R. H. (2001). *Immobilized Cells – Laboratory Manuals*. Heidelberg: Sprinnger.

Wuertz, S., Bioshop, P. and Wilderer, P. (2003). *Biofilms in Wastewater Treatment*. United States of America: IWA publishing.

Zakaria, Z. A. (2006). Development of bacteria Based Remediation System for the Removal of Chromium (VI) from Electroplating Industrial Effluent. PhD thesis. Universiti Teknologi Malaysia.

Zakaria, Z. A., Zakaria, Z., Surif, S. and Ahmad, W. A. (2007). Biological Detoxification of Cr(VI) using Wood-husk Immobilized *Acinetobacter haemolyticus. Journal of Hazardous Materials*. 148(1–2), 164–171.

Zhang, J., Zhou, B., Zheng, Q., Li, J., Bai, J., Liu, Y. and Cai, W. (2009). Photoelectrocatalytic COD Determination Method using Highly Ordered TiO_2 Nanotube Array. *Water Research*. 43(7), 1986–1992.

Zhao, X., Wang, Y., Ye, Z., Alistair, G. L. and Jinren Ni, B. (2006). Oil Field Wastewater Treatment in Biological Aerated Filter by Immobilized Misroorganisms. *Process Biochemistry*. 41(7). 1475–1483.

Zhu, W., Chai, L., Ma, Z., Wang, Y., Xiao, H. and Zhao, K. (2008). Anaerobic Reduction of Hexavalent Chromium by Bacterial Cells of *Achromobacter* sp. Strain Ch1. *Microbial Research*. 163(6), 616–623.

Zularisam, A. W., Ismail, A. F. and Salim, R. (2006). Behaviours of Natural Organic Matter in Membrane Filtration for Surface Water Treatment – A Review. *Desalination*. 194(1–3), 211–231.