

**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

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requirements for the award of the degree of  
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*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.*

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## 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™ 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™. 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%.

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**LIST OF ABBREVIATIONS**

AgSO <sub>4</sub>	-	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
°C	-	Degree celcius
DPC	-	1,5-diphenylcarbazine
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 <sub>2</sub> SO <sub>4</sub>	-	Sulphuric Acid
HgSO <sub>4</sub>	-	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

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## 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 possesses 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™ which has been developed at the laboratory and pilot scale in UTM, Skudai, Johor, Malaysia. ChromeBac™ 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™ 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.

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