CHARACTERIZATION OF DEHALOGENASE FOR THE DEGRADATION OF 3-CHLOROPROPIONIC ACID

WAFAA HASSAN MUSLEM

A thesis submitted in fulfilment of the
requirements for the award of the degree of
Doctor of Philosophy (Bioscience)

Faculty of Biosciences and Medical Engineering
Universiti Teknologi Malaysia

JULY 2017

To all my Lovely family

ACKNOWLEDGEMENT

In the name of Allah Subhanahu Wa Taala, the most Almighty and Merciful. All praise due to Him who granted me strength and perseverance throughout this research. Without His blessings, this research work would not have been completed.

I would like to express my sincere appreciation to my revered supervisors, Professor Fahrul Huyop and Dr. Roswanira Ab. Wahab for their valuable guidance, advice, motivation and constant encouragement during the course of this investigation. I have learned a great deal from them and it was a great pleasure and privilege to work under their direction. I am deeply grateful for what they have done for me during my study period including training me on molecular biology, biochemistry areas and how to write papers. Without them, I would not be able to complete my thesis and overcome the problems that occurred during this project.

In addition, I am grateful to all my fellow colleagues, especially, Dr. Mohamed Faraj Edbeib, Dr. Ahmed Lafi, Elham, Fasehah, Kalavani, Elizah and Fatimah for their encouragement, humor and friendship and I appreciate all the staff of Faculty of Biosciences and Medical engineering, Universiti Teknologi Malaysia.

Last but not least, special credits are given to my husband, Dr. Ahmad Bakheet and my great Father and lovely mother and all my family. The love and supports that they consistently offered were the most important elements in seeing this project to a timely and satisfying conclusion.

ABSTRACT

The use of herbicides that contain halogenated compounds, for example 3-chloropropionic acid (3-CP) poses significant environmental hazards as well as detrimental to human. The research detailed here investigated the isolation and identification of bacteria strains that could degrade 3-CP as its sole carbon source. Dehalogenase that can degrade 3-CP is rare in nature. In this study, two strains of dehalogenase producing bacteria capable of utilizing 3-CP were successfully isolated from abandoned agricultural land in Universiti Teknologi Malaysia. These bacteria were characterized by using 16S rRNA as well as biochemical analysis. Strain WH1 showed a 98 % sequence identity to *Burkholderia cepacia* with (accession number KU318403) whereas strain WH2 showed a 99% sequence identity to *Bacillus cereus* with (accession number KU721999). The results have shown that these bacteria were capable to grow in liquid minimal media supplied with 10 mM 3-CP as sole carbon source with doubling time of 43.62 h for WH1 and 14.75 h for WH2. Utilization of 3-CP was confirmed by detection of chloride ion released using halide ion assay technique for both strains which indicate their ability to degrade 3-CP. For further confirmation of 3-CP consumption, analysis by high performance liquid chromatography (HPLC) revealed that both *B. cepacia* WH1 and *B. cereus* WH2 effectively utilized ~100% of 10 mM 3-CP. This is the first report detailing both strains able to competently utilize 3-CP as their sole carbon source. Cell free extract of *B. cereus* strain WH2 was further characterized due to its faster growth on 3-CP compared to *B. cepacia* strain WH1. The intracellular dehalogenase from *B. cereus* WH2 was purified to homogeneity to afford a 2.5-fold (50 % yield) concentration with an estimated molecular mass of 37 kDa by SDS-PAGE analysis. Its highest enzyme activity was achieved at conditions of 30 ^{o}C and pH 7. While the activity of WH2 dehalogenase was substantially repressed by both Hg^{2+} and Ag^{2+}, the enzyme was not inhibited by DTT and EDTA. Pertinently, kinetics evaluation revealed a higher affinity of the WH2 dehalogenase towards 3-CP than 3-chlorobutyric acid (3-CB), affording K_m values of 0.32 mM (k_{cat} 3.97 s^{-1}) and 0.52 mM (k_{cat} 4.35 s^{-1}), respectively. The WH2 dehalogenase was ~1.6-fold catalytically more efficient (k_{cat}/K_m) in dehalogenating the three-carbon, 3-CP (12.4 mM^{-1} s^{-1}) over the four-carbon, 3-CB (8.27 mM^{-1} s^{-1}). From the data, it was identified that 3-CP degradation was not stimulated by co-factors, such as NAD^{+}, NADH, $NADP^{+}$, NADPH, FAD and CoA that did not affect the enzyme activity by demonstrating activities of <0.1 unite (g protein)$^{-1}$. The amplified dehalogenase gene fragment was designated "*deh-wh2*" and subsequent analysis showed it belongs to Group II dehalogenase. Eight conserved residues that line the active site were identified: Asp10, Thr14, Ser117, Lys150, Tyr156, Ser174, Asn176 and Asp179. These residues are consistent with the residues found in the active site of DhlB, DehIVa and L-DEX. The product of 3-CP degradation was 3-hydroxypropionic acid based on HPLC. In conclusion, this study confirmed the presence of new dehalogenase isolated from various bacteria that have potential to utilize 3-CP, especially from contaminated environment.

ABSTRAK

Penggunaan racun rumpai yang mengandungi sebatian, halogen seperti asid 3-kloropropionik (3-CP) boleh mendatangkan bahaya yang ketara kepada alam sekitar juga memudaratkan manusia sejagat. Penyelidikan yang dijalankan berkisar tentang pemencilan dan pengenalpastian strain bakteria yang boleh hidup menggunakan 3-CP sebagai sumber karbon tunggal. Dehalogenase yang boleh merendahkan 3-CP jarang ditemui secara semula-jadi. Dalam kajian ini, dua strain bakteria yang menghasilkan dehalogenase seumpama ini yang mampu menggunakan 3-CP telah berjaya dipencilkan dari tanah pertanian yang terbiar di Universiti Teknologi Malaysia. Bakteria ini dicirikan dengan menggunakan 16S rRNA serta analisis biokimia. Strain WH1 menunjukkan 98% identiti dengan penjujukan kepada strain *Burkholderia cepacia* dengan (nombor aksesi KU318403) sedangkan Strain WH2 menunjukkan 99% identiti dengan penjujukan kepada strain *Bacillus cereus* dengan (nombor aksesi KU721999). Keputusan ujikaji telah menunjukkan bahawa bakteria ini boleh tumbuh dalam kultur media-minima cecair yang dibekalkan dengan 10 mM 3-CP sebagai sumber karbon tunggal dengan selang masa dua kali ganda lebih 43.62 jam untuk WH1 dan 14.75 jam untuk WH2. Penggunaan 3-CP sebagai sumber karbon, telah ditentukan melalui kaedah pengesanan pengeluaran ion klorida oleh kedua-dua strain yang menunjukkan keupayaannya untuk menguraikan 3-CP. Bagi mengesahkan penggunaan 3-CP, analisis menggunakan kromatografi cecair berprestasi tinggi (HPLC) mendedahkan bahawa *B. cepacia* WH1 dan *B. cereus* WH2 menggunakan secara berkesan ~100% 10 mM 3CP. Ini merupakaan laporan yang pertama memperincikan kedua-dua strain yang mampu menggunakan 3-CP sebagai sumber karbon tunggal. Ekstrak dari sel *B. cereus* strain WH2 telah diuji selanjutnya kerana bakteria tersebut mempunyai kadar pertumbuhan yang jauh lebih tinggi daripada strain *B. cepacia* WH1. Dehalogenase yang diperoleh dari sel *B. cereus* WH2 telah ditulenkan, dan berupaya mencapai 2.5 kali ganda (50% hasil) kepekatan dengan anggaran jisim molekul 37 kDa melalui kaedah analisis SDS-PAGE. Aktiviti enzim yang tertinggi telah dicapai pada keadaan 30℃ dan pH 7. Manakala aktiviti dehalogenase WH2 telah mengurang dengan kehadiran kedua-dua Hg^{2+}dan Ag^{2+}, tetapi sebaliknya dengan kehadiran DTT dan EDTA. Bersangkutan dengan penilaian kinetik menunjukkan 3-CP merupakan substrat yang lebih baik daripada asid 3-klorobutirik (3-CB) terhadap enzim dehalogenase WH2, dengan nilai K_m masing-masing 0.32 mM (k_{cat} 3.97 s^{-1}) dan 0.52 mM (k_{cat} 4.35s^{-1}). Dehalogenase WH2 memangkin ~1.6 kali ganda lebih cekap (k_{cat}/K_m) terhadap bahan berhalogen yang mempunyai tiga karbon, 3-CP (12.4 mM^{-1} s^{-1}) daripada empat-karbon, 3-CB (8.27 mM^{-1} s^{-1}). Data telah membuktikan bahawa penguraian 3-CP tidak dirangsang oleh ko-faktor, seperti NAD^+, NADH, $NADP^+$, NADPH, FAD dan CoA yang tidak menjejaskan aktiviti enzim oleh aktiviti <0.1 unit (g protein)$^{-1}$. Serpihan gen dehalogenase diamplifikasikan yang telah diberi nama "*deh-wh2*" dan analisis berikutnya menunjukkan ia tergolong dalam kumpulan II dehalogenase. Lapan residu asid amino yang telah dikenal pasti melapisi tapak aktif terdiri daripada: Asp10, Thr14, Ser117, Lys150, Tyr156, Ser174, Asn176 dan Asp179. Penemuan residu asid amino ini selaras dengan residu yang ditemui pada tapak aktif DhlB, DehIVa dan L-DEX. Hasil penguraian 3-CP adalah asid 3-hidroxipropionik berdasarkan keputusan HPLC. Kesimpulannya, kajian ini mengesahkan kehadiran dehalogenase baharu yang telah dipencil dari pelbagai jenis bakteria yang berpotensi untuk menggunakan 3-CP, terutamanya dari persekitaran yang tercemar.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

(v/v)	-	Volume percentage per 100 mL volume
(w/v)	-	Mass percentage per 100 mL volume
°C	-	Centigrade
β-HA	-	β-halo carboxylic acid
µL	-	Microliter
µm	-	Micrometre
µmol	-	Micromole
16S rRNA	-	ribosomal RNA gene
2,2,3-TB	-	2,2,3-trichlorobutyric acid
2,2,3-TCP	-	2,2,3-trichloropropionic acid
2,2-DCB	-	2,2-dichlorobutyric acid
2,2-DCP	-	2, 2-dichloropropionic acid
2-CB	-	2-chlorobutanoic acid
2-CP	-	2-chloropropionic acid
3,3-DCP	-	3,3-dichloropropanoic acid
3-CB	-	3-chlorobutanoic
3-CP	-	3-chloropropionic acid
3-HP	-	3-hydroxypropionic acid
Ag^{2+}	-	Silver ion
A_{nm}	-	Absorption spectroscopy at nm light source
APS	-	Ammonium persulfate

bar	-	Barometer
BLAST	-	Basic local alignment search tool
BLASTn	-	Basic local alignment search tool – nucleotide
bp	-	Base pairs
BS	-	Basal salts
BSA	-	Bovine serum albumin
Ca^{2+}	-	Calcium ions
CFE	-	Cell free extract
Co^{2+}	-	Cobalt ion
CoA	-	Coenzyme A
Cu^{2+}	-	Copper ion
DDT	-	Dichlorodiphenyltrichloroethane
deh	-	Dehalogenase
dH_2O	-	Distilled water
DNA	-	Deoxyribonucleic acid
EtBr	-	Ethidium bromide
FAD	-	Flavin adenine dinucleotide
g	-	Gram
h	-	Hour
Hg^{2+}	-	Mercury ion
HPLC	-	High performance liquid chromatography
HTUs	-	Hypothetical taxonomic units
kbp	-	Kilo base pair
KDa	-	kilodalton
LB	-	Luria Broth
LC-MS	-	Liquid chromatography–mass spectrometry

M	-	Molarity (molar)
MEGA	-	Molecular Evolutionary Genetics Analysis
mg	-	Milligram
Mg^{2+}	-	Magnesium ion
Min	-	Minute
mL	-	Milliliter
mM	-	Millimolar
mm	-	millimetre
MSA	-	multiple progression alignment
NaCl	-	Sodium chloride
NAD^{+}	-	Nicotinamide adenine dinucleotide
$NADP^{+}$	-	Nicotinamide adenine dinucleotide phosphate
NCBI	-	National center for biotechnology information
OD	-	Optical density
OTUs	-	Operational taxonomic units
PCBs	-	polychlorinated biphenyls
PCR	-	Polymerase chain reaction
Pfu		Plaque-forming unit
pKa	-	Acid dissociation constant
RNAse	-	Ribonuclease
rpm	-	Revolution Pper minute
rRNA	-	Ribosomal ribonucleic acid
RSM	-	response surface methodology
Rt	-	Retention time
s	-	Second

SDS- PAGE	-	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
TAE	-	Buffer solution containing a mixture of tris base, acetic acid and ethidium bromide
TEMED	-	Tetramethylethylenediamine
TS	-	Trace metal
W	-	Watt
WHO	-	World Health Organization
Zn^{2+}	-	Zinc ion

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background of Study

In recent time, rapid escalation of industrial and agricultural activities witnessed an excessive production of xenobiotic compounds and caused grave environmental concern. These man-made compounds are often described as non-naturally occurring chemicals (Rieger *et al*., 2002). Such chemicals at high concentrations are responsible for environmental pollution unless inhibited. The contamination of water and soil that is caused due to the presence of xenobiotic substances requires remediation. Lately, degradation of hazardous wastes including xenobiotic compounds using microbes became a prominent route (Yan *et al*., 2009).

It is needless to mention that some of these xenobiotic compounds are resistant to degradation. Studies revealed that specific microorganisms are not only capable of degrading such complexes but also remains resistant to such xenobiotics (Van Pée and Unversucht, 2003; Niknam *et al*., 2014). In this regard, halogenated organic compounds are considered as largest groups of xenobiotic compounds. They are known as prevalent pollutants. They are found in soil, air, water, and sediment. Halogenated organic compounds being extensively produced as industrial products are mostly used as herbicides, insecticides, fungicides, and solvents. Besides, they are considered as an important class of environmental pollutants. These halogenated compounds cause several human health problems due to its persistence, toxicity and hazardous metabolites (Fetzner, 1998; Fetzner and Lingens, 1994).

Since the beginning of the 20th century, physiologists and biochemists started realizing that halogenated compounds can affect our metabolic systems due to their toxicity (Slater *et al.*, 1995). Penfold (1913) first reported the microorganisms assisted degradation of halogenated compounds. Interestingly, these microorganisms owing to their short life cycle are capable of evolving new enzymes, pathways and regulatory mechanisms for the degradation of almost all xenobiotic compounds. Thus, the evolution of dehalogenase producing microorganisms exploiting some of these halogenated compounds is not only scientifically interesting but also have practical significance in terms of environmental pollution remediation via green chemical route (Penfold, 1913; Timmis and Pieper, 1999).

The compound, 3-chloropropionic acid (3-CP) belongs to the class of chlorinated monocarboxylic acid or β-chloro substituted haloalkanoic acids. It originates from the possible chemical inclusion in pesticides. This compound is a carcinogenic and genotoxic to the animals and humans (Alexander, 1981). Generally, they degrade into harmless substances *via* both non-biological and biological pathways in which the later are considered to be more economical, safer and environmentally amiable (Janssen *et al*., 2005). Primarily, biodegradation using microorganisms is a natural process that allows the removal of xenobiotic chemicals such as chloro-aliphatic compounds from the environment (Sinha *et al*., 2009; Abel *et al*., 2012; Edbeib *et al*., 2016).

Several studies are performed to isolate the bacterial species by enrichment and laboratory culture which used halogen-substituted organic acids as their carbon and energy resources (Janssen *et al.*, 2005). However, only a few organisms are found capable to degrade the β-halo carboxylic acid (β-HA) (Jing and Huyop, 2007; Mesri *et al*., 2009; Yusn and Huyop, 2009; Hamid *et al*., 2011; Muslem *et al*., 2015). The proposed reaction for dehalogenation of 3-CP by hydrolytic dehalogenation is similar to Group II dehalogenase enzyme. During nucleophilic substitution, the hydroxide ion of water attacks the partially positively charged carbon atom bonded to the electronegative chlorine atom. This causes the transfer of the electron pair from the electron-rich hydroxide to the partially positively charged carbon atom. Since the carbon atom can only accommodate four electron pairs (four covalent

bonds) in its valence state, this reaction subsequently results in the breaking of the carbon-chlorine bond to form the carbon-oxygen bond (van Pée and Unversucht, 2003). The chlorine atom is now liberated as a chloride ion carrying with it a pair of electrons.

It is known that long term exposure of microorganism to halogenated compounds found in soil and water triggers certain microorganisms to produce specialized enzymes that enable the utilization of such compounds as their energy and carbon source. Generally, the aforementioned bacterial species possess certain common characteristics in utilizing halogenated organic compounds as sole carbon and energy source. However, some fundamental characteristics must be satisfied for the bacteria to be able to utilize halogenated organic compounds for their growth. The bacteria must either possess or synthesize dehalogenase in response to the halogenated compound which, in turn confer the bacteria special abilities to remove the halogenated substituent(s) from the compound. Furthermore, the product of the dehalogenation must be non-toxic and easily convertible to an intermediate in the central metabolic pathway of the bacteria. Next, the halogenated compound must enter the cell either *via* passive or active transport mechanism to reach the active site of dehalogenase. Finally and most importantly, the halogenated compound must be non-toxic to the bacteria at normal intracellular concentrations. These halogenated organic compounds (herbicides) are widespread in agriculture sectors including 2-chloropropionic acid (2-CP); 2, 2-dichloropropionic acid (2,2-DCP) and 3-chloropropionic acid (3-CP). Despite some studies, following intensive literature search, the available information on 3-CP degradation is still lacking. Chan *et al*. (2010) had acknowledged that many microbial genomes carrying enzyme families contain dehalogenases that are useful for xenobiotic compounds remediation.

It is pertinent to highlight here the degradation of 3-CP, a representative of the β-substituted chloroalkanoic acids has been quite problematic as compared to that of the α-chloro-substituted haloalkanoic acids such as 2, 2-DCP. This is because the α-chloroalkanoic acid-degrading microorganisms cannot utilize/degrade the β-substituted chloroalkanoic acids as the former secrete α-specific dehalogenases that only cleave α-chloro-substituted haloalkanoic acids. Furthermore, only a few isolated

microorganisms have been described to be able to degrade the β-halocarboxylic acids (β-HA) (Mesri *et al*., 2009; Yusn and Huyop, 2009). The present work proposes the isolation of bacteria from soil taken from an abandoned agricultural land and characterizes them to determine their potency in degrading 3-CP. Evaluation of the dehalogenating capacity of such bacteria towards 3-CP was the main aim of this study. The knowledge gathered from the abovementioned evaluation can be beneficial for the application of *in situ* bioremediation of contaminated soil and waterways, especially those containing pollutants comprising of chlorinated xenobiotics. Degradation of 3-CP was the prime focus of this study because the compound represents a group of commonplace bioactive ingredient (β-substituted chloroalkanoic acids) prevalent in formulations of herbicides used extensively in the agricultural sector to manage weed growth in plantations (Jing and Huyop, 2007). Furthermore, 3-CP is a more complex structure than the α-chloro-substituted haloalkanoic acids i.e. 2, 2-DCP, as 3-CP is resistant to enzymatic attack as well as being a representative of a well-known group of pollutants in the environment. Hence, 3-CP has been chosen as a model pollutant associated with pesticides in this study.

1.2 Problem Statement

The expansion of agricultural area, crop production, and subsequent use of pesticides and herbicides result in the liberation of high concentrations of the xenobiotic compounds that pollutes the environment. The major environmental pollutants namely the xenobiotic halogenated compounds i.e. β-substituted chloroalkanoic acids are weakly degradable chemicals. Presently, the tendency of such environmentally hazardous compounds towards bioaccumulation is harmful to human health and hygiene (Birnbaum and Fenton, 2003; Hayes *et al*., 2006; Qing Li *et al*., 2006) as well as their intractability remains a global concern. Consequently, dedicated efforts are needed to develop safe, economic and effective strategies to eliminate such toxic substances from the environment.

It is known that certain microbes are effective in degrading β-substituted chloroalkanoic acids; hence the exploitation of such beneficial microbes for the safer removal of such compounds from the environment is of great interest. Herein, the study suggests a greener biotechnological approach which involves the isolation, identification, characterization and evaluation of bio-degradation efficacy of β-producing bacteria. The study believes the natural ability of the bacteria to utilize such toxic compounds for growth while rendering these compounds safer to the environment and makes them perfect bioremediation agents for neutralizing the toxicity of β-substituted chloroalkanoic acids.

The study hypothesized the bioprospecting for dehalogenase-producing bacteria from environments previously exposed to continuous spraying of herbicides (i.e. agricultural land in Universiti Teknologi Malaysia (UTM) would yield bacterial isolates competent for *in-situ* bioremediation of 3-CP (i.e. β-substituted chloroalkanoic acid) as its sole source of carbon and energy for growth. It was expected the environmental stress from growing in an environment high in concentrations of β -substituted chloroalkanoic acid would trigger certain bacteria to develop exceptional catalytic machinery that allows them to utilize the abovementioned toxic compound.

It is pertinent to indicate here, isolation studies focusing on the biodegradation effectiveness of certain bacterial isolates to degrade β-chlorosubstituted haloalkanoic acids such as a 3-CP (Mesri *et al*., 2009; Muslem *et al*., 2015) remain lacking. Similarly, the precise mechanism for the β-chlorosubstituted degradation by such unique bacterial isolates is poorly understood. Even the product of the degradation of 3-CP has never been accumulated as proof of their degradation pathway. Moreover, there is yet any detailed report on the physicochemical characteristics and the full gene sequence of these β-specific dehalogenases.

1.3 Objectives of the Study

Based on the abovementioned problem statement the following objectives are set:

1. To isolate and identify the dehalogenase-producing bacteria effective in degrading 3-CP as the sole source of carbon and energy.
2. To characterize the biochemical properties and evaluate 3-CP dehalogenation efficiency of the bacterial isolates.
3. To purify and study the physicochemical characteristics of the β-haloalkanoic acid dehalogenase.
4. To amplify and analyse the full gene sequence of the β-haloalkanoic acid dehalogenase.

1.4 Scope of the Study

The soil sample expected to harbor β-specific haloalkanoic acid degrading bacteria was collected from soil samples in an abandoned agricultural land in UTM. The study used enrichment techniques that utilized only 3-CP as the sole carbon source. Hence, only bacteria effective in utilizing the 3-CP-enriched broth as the sole source of carbon would be isolated from the soil samples and used in the subsequent evaluation of the study. The bacterial isolates were initially subjected to halo ion assay for confirming their ability to utilize 3-CP for their growth by using HPLC. Subsequently, the morphological and molecular identification on the isolates using 16S rRNA was used to determine the gene sequence of 16S rRNA gene of bacterial DNA. Results are further used to make a comparison with genomic database for determining microorganism's identity. Phylogeny tree was constructed to identify the evolutionary relationship distance of the microorganism to the nearest known microorganisms.

The identified bacterial isolates were assessed for their biochemical properties using Biolog^{TM} Gen III. Next, their efficacy for degrading 3-CP using a time course growth profile versus the production of the degraded product i.e. 3-hydroxypropionic

acid (3-HP) using HPLC. The degradation efficiency of the 3-CP for the selected bacterial isolates was assessed over an unstipulated period until the degradation of 3-CP was almost completed. After this, only the bacterium showing the fastest degradation of 3-CP was selected for the subsequent study.

The next scope was to obtain the pure form of the β-specific haloalkanoic acid dehalogenase for the physicochemical assessments on the enzyme. The purification was carried using ammonium sulphate precipitation and subsequently dialyzed to obtain the pure form of the dehalogenase. The purified dehalogenase was characterized for parameters: enzyme substrate assays, pH, temperature, co-factors, substrate specify and kinetic parameter.

The study then determines the full gene sequence of the β-haloalkanoic acid dehalogenase using primers from the Group II dehalogenase. Lastly, the study carried out qualitative and quantitative assay to monitor the liberation of 3-CP degraded product i.e. 3-HP monitored by HPLC catalyzed by the purified β-specific haloalkanoic acid dehalogenase.

1.5 Significance of the Study

This study hopes to provide new knowledge on the safe remediation of environmentally hazardous xenobiotic halogenated compounds such as β-haloalkanoic acids through the use of an exceptional dehalogenase producing bacteria. Furthermore, the properties of the β-haloalkanoic acid dehalogenase isolated in this study will be better understood while the product of the 3-CP degradation can be confirmed. For that particular reason, it will be interesting to study this β-specific novel dehalogenase enzyme which can degrade 3-CP, in order to recognize their catalytic properties and the biodegradation adopted by their catalytic machinery.

1.6 Operational Framework of the Research

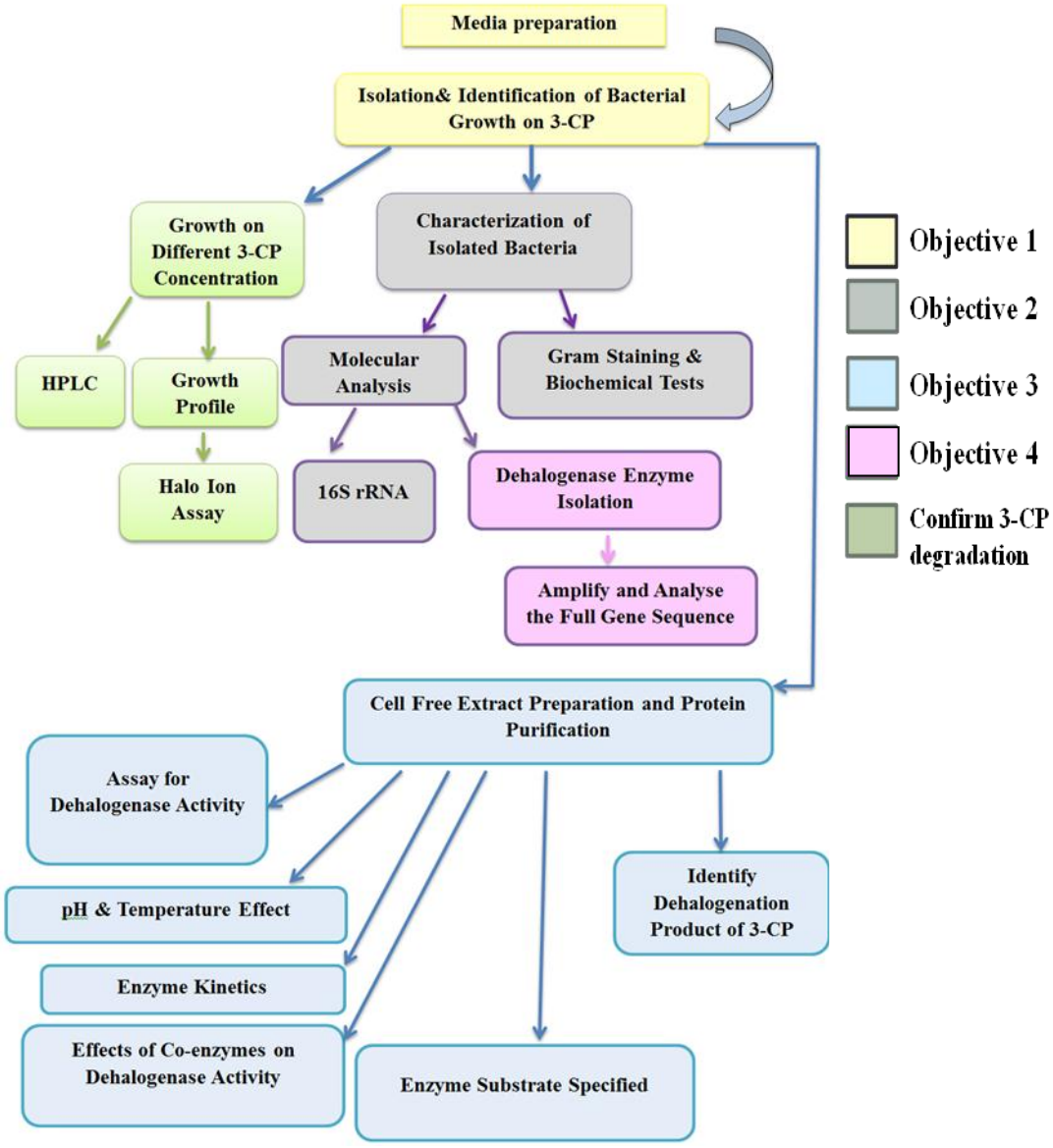

Figure 1.1: Operational framework of the research methodology.

REFERENCES

Abel, E., Ibrahim, N. and Huyop, F. (2012). Identification of *Serratia marcescens* SE1 and Determination of its Herbicide 2, 2-Dichloropropionate (2, 2-DCP) Degradation Potential. *Malaysian Journal of Microbiology*. 8, 259-265.

Isah, A. A., Mahat, N. A., Jamalis, J., Attan, N., Zakaria, I. I., Huyop, F. and Wahab, R. A. (2017). Synthesis of Geranyl Propionate in a Solvent-Free Medium Using *Rhizomucor miehei* Lipase Covalently Immobilized on Chitosan–Graphene Oxide Beads. *Preparative Biochemistry and Biotechnology*. 47(2), 199-210.

Aislabie, J. M., Richards, N. K. and Boul, H. L. (1997). Microbial Degradation of DDT and its Residues—a Review. *New Zealand Journal of Agricultural Research*. 40(2), 269-282.

Alexander, M. (1981). Biodegradation of Chemicals of Cnvironmental Concern. *Science*. 211(4478), 132-138.

Allison, N., Skinner, A. J., and Cooper, R. A. (1983). The Dehalogenases of a 2, 2-Dichloropropionate-Degrading Bacterium. *Microbiology*. 129(5), 1283-1293.

Allpress, J. D., and Gowland, P. C. (1998). Dehalogenases: Environmental Defence Mechanism and Model of Enzyme Evolution. *Biochemical Education*. 26(4), 267-276.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W. and Lipman, D. J. (1990). Basic Local Alignment Search Tool. *Journal of Molecular Biology*. 215(3), 403-410.

Aráoz, B. and Viale, A. A. (2004). Microbial Dehalogenation of Polychlorinated Biphenyls in Aerobic Conditions. *Revista Argentina de Microbiologia*. 36(1), 47-51.

Baggi, G., Bernasconi, S., and Zangrossi, M. (2005). 3-Chloro-, 2,3-and 3,5-Dichlorobenzoate Co-metabolism in a 2-Chlorobenzoate-Degrading Consortium: Role of 3,5-Dichlorobenzoate as Antagonist of 2-Chlorobenzoate Degradation. *Biodegradation*. 16(3), 275-282.

Bergmann, J.G. and Sanik, Jr. J. (1957). Determination of Trace Amounts of Chlorine in Naphtha. *Analytical Chemistry*. 29(2), 241-243.

Berry, E. K., Allison, N., Skinner, A. J., and Cooper, R. A. (1979). Degradation of the Selective Herbicide 2, 2-Dichloropropionate (Dalapon) By a Soil Bacterium. *Microbiology*. 110(1), 39-45.

Birnbaum, L. S. and Fenton, S. E. (2003). Cancer and Developmental Exposure to Endocrine Disruptors. *Environmental Health Perspectives*. 111(4), 389-394.

Biswanger H. (2014). Enzyme Assays. Perspect. Sci. 1, 41-55.

Besseling, E., Wegner, A., Foekema, E. M., Van Den Heuvel-Greve, M. J. and Koelmans, A. A. (2012). Effects of Microplastic on Fitness and PCB Bioaccumulation by the Lugworm *Arenicola marina* (L.). *Environmental Science and Technology*. 47(1), 593-600.

Bollag, J. M. and Alexander, M. (1971). Bacterial Dehalogenation of Chlorinated Aliphatic Acids. *Soil Biology and Biochemistry*. 3(2), 91-96.

Borodina, I., Kildegaard, K.R., Jensen, N.B., Blicher, T.H., Maury, J., Sherstyk, S., Schneider, K., Lamosa, P., Herrgård, M.J., Rosenstand, I. and Öberg, F., (2015). Establishing a Synthetic Pathway for High-Level Production of 3-Hydroxypropionic Acid in Saccharomyces Cerevisiae via β-Alanine. *Metabolic Engineering*. *27*, 57-64.

Bradford, M. M. (1976). A Rapid And Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Analytical Biochemistry*. 72(1-2), 248-254.

Bruce, E. R. and Perry, L. M. (2001). Environmental Biotechnology: Principles and Applications. *New York: McGrawHill*, 400.

Carepo, M. S., Azevedo, J., Porto, J. I., Bentes-Souza, A., Batista, J. d. S., Silva, A. and Schneider, M. P. (2004). Identification of *Chromobacterium violaceum*

Genes with Potential Biotechnological Application in Environmental Detoxification. *Genetics and Molecular Research*. 3(1), 181-194.

Cairns, S. S., Cornish, A., and Cooper, R. A. (1996). Cloning, Sequencing and Expression in Escherichia Coli of Two *Rhizobium* sp. Genes Encoding Haloalkanoate Dehalogenases of Opposite Stereospecificity. *European Journal of Biochemistry*. 235(3), 744-749.

Chan, W. Y., Wong, M., Guthrie, J., Savchenko, A. V., Yakunin, A. F., Pai, E. F. and Edwards, E. A. (2010). Sequence- and Activity- Based Screening of Microbial Genomes for Novel Dehalogenases. *Microbial Biotechnology*. 3(1), 107-120.

Chiarini, L., Bevivino, A., Dalmastri, C., Tabacchioni, S., and Visca, P. (2006). *Burkholderia cepacia* Complex Species: Health Hazards and Biotechnological Potential. *Trends in Microbiology*. 14(6), 277-286.

Cirello-Egamino, J., and Brindle, I. D. (1995). Determination of Chloride Ions by Reaction with Mercury Thiocyanate in the Absence of Iron (III) Using a UV-Photometric, Flow Injection Method. *Analyst*. 120(1), 183-186.

Cleland, W. W. (1964). Dithiothreitol, a New Protective Reagent for SH Groups. *Biochemistry*. 3(4), 480-482.

Coenye, T. and Vandamme, P. (2003). Diversity and Significance of *Burkholderia Species* Occupying Diverse Ecological Niches. *Environmental Microbiology*. 5(9), 719-729.

Commandeur, L.C. and Parsons, J. R. (1990). Degradation of Halogenated Aromatic Compounds. *Biodegradation*. 1(2-3),207-220.

Copley, S. D. (1998). Microbial Dehalogenases: Enzymes Recruited to Convert Xenobiotic Substrates. *Current Opinion in Chemical Biology*. 2(5), 613-617.

Corpet, F. 1988. Multiple Sequence Alignment with Hierarchical Clustering. *Nucleic Acids Research*. 16(22), 10881–10890.

Dallaire, F., Dewailly, É., Muckle, G., Vézina, C., Jacobson, S. W., Jacobson, J. L., and Ayotte, P. (2004). Acute Infections and Environmental Exposure to Organochlorines in Inuit Infants from Nunavik. *Environmental Health Perspectives*. 112(14), 1359-1364.

Damalas, C. A. and Eleftherohorinos, I. G. (2011). Pesticide Exposure, Safety Issues, and Risk Assessment Indicators. *International Journal of Environmental Research and Public Health*. 8(5), 1402-1419.

Della Pina, C., Falletta, E. and Rossi, M. (2011). A Green Approach to Chemical Building Blocks: The Case of 3-Hydroxypropanoic Acid. *Green Chemistry*. 13(7), 1624-1632.

Drancourt, M., Berger, P., and Raoult, D. (2004). Systematic 16S Rrna Gene Sequencing of Atypical Clinical Isolates Identified 27 New Bacterial Species Associated With Humans. *Journal of Clinical Microbiology*. 42(5), 2197-2202.

Drienovska, I., Chovancova, E., Koudelakova, T., Damborsky, J. and Chaloupkova, R. (2012). Biochemical Characterization of a Novel Haloalkane Dehalogenase from a Cold-Adapted Bacterium. *Applied and Environmental Microbiology*. 78(14), 4995-4998.

Drivon, G. and Ruppin, C. (1998). *Process for the Preparation of 3-Chloropropionic Acid*. U.S. Patent No. 5,731,469.

Duarte, C. M., Holmer, M., Olsen, Y., Soto, D., Marba, N., Guiu, J., Black, K. and Karakassis, I. (2009). Will the Oceans Help Feed Humanity?. *Bioscience*. 59(11), 967-976.

Edbeib, M. F., Wahab, R. A. and Huyop, F. (2016). Characterization of an α-Haloalkanoic Acid–Degrading Pseudomonas Aeruginosa MX1 Isolated from Contaminated Seawater. *Bioremediation Journal*. 20(2), 89-97.

Farrington, J. W. and Takada, H. (2014). Persistent Organic Pollutants (POPs), Polycyclic Aromatic Hydrocarbons (PAHs), and Plastics: Examples of the Status, Trend, and Cycling of Organic Chemicals of Environmental Concern in the Ocean. *Oceanography*. 27(1), 196-213.

Fetzner, S. (1998). Bacterial Dehalogenation. *Applied Microbiology and Biotechnology*. 50(6), 633-657.

Fetzner, S., and Lingens, F. (1994). Bacterial Dehalogenases: Biochemistry, Genetics, and Biotechnological Applications. *Microbiological Reviews*. 58(4), 641-685.

Fox, G.E., Wisotzkey, J.D., and Jurtshuk, P. J.R. (1992). How Close Is Close: 16S rRNA Sequence Identity May Not Be Sufficient To Guarantee Species Identity. *International Journal of Systematic Bacteriology*. 42(1), 166-170.

Fulton, C. K. and Cooper, R. A. (2005). Catabolism of Sulfamate by *Mycobacterium* sp. CF1. *Environmental Microbiology*. 7(3), 378-381.

Chiba, Y., Yoshida, T., Ito, N., Nishimura, H., Imada, C., Yasuda, H. and Sako, Y. (2009). Isolation of a Bacterium Possessing a Haloacid Dehalogenase from a Marine Sediment Core. *Microbes and Environments*. 24(3), 276-279.

Chua, E. M., Shimeta, J., Nugegoda, D., Morrison, P. D. and Clarke, B. O. (2014). Assimilation of Polybrominated Diphenyl Ethers from Microplastics by the Marine Amphipod, *Allorchestes Compressa*. *Environmental Science and Technology*. 48(14), 8127-8134.

Gladen, B. C., and Rogan, W. J. (1995). DDE and Shortened Duration of Lactation in A Northern Mexican Town. *American Journal of Public Health*. 85(4), 504-508.

Goldman, P., Milne, G. and Keister, D. B. (1968). Carbon-Halogen Bond Cleavage. *Journal of Biological Chemistry*. 243(2), 428.

Gribble, G.W. (1994). The Natural Production of Chlorinated Compounds. *Environmental Science and Technology*. 28(7), 310A-319A.

Gutteridge, A. (2005). *Understanding the Relationship between Enzyme Structure and Catalysis*. Doctor Philosophy, University of Cambridge, UK.

Guo, H., Yao, J., Cai, M., Qian, Y., Guo, Y., Richnow, H. H., Blake, R. E., Doni, S. and Ceccanti, B. (2012). Effects of Petroleum Contamination on Soil Microbial Numbers, Metabolic Activity and Urease Activity. *Chemosphere*. 87(11), 1273-1280.

Hamid, A. A. A., Wong, E. L., Joyce-Tan, K. H., Shamsir, M. S., Hamid, T. H. T. A., and Huyop, F. (2013). Molecular Modelling and Functional Studies of the Non-Stereospecific A-Haloalkanoic Acid Dehalogenase (Dehe) from *Rhizobium* sp. RC1 and its Association with 3-Chloropropionic Acid (B-Chlorinated Aliphatic Acid). *Biotechnology and Biotechnological Equipment*. 27(2), 3725-3736.

Hamid, A. A. A., Hamid, T. H. T. A., Wahab, R. A., Omar, M. S. S., and Huyop, F. (2015). An S188V Mutation Alters Substrate Specificity of Non-Stereospecific α-Haloalkanoic Acid Dehalogenase E (Dehe). *PloS one*. 10(3), e0121687.

Hamid, T. A., Hamid, A. A. A., Zulkifly, A. H., Hamdan, S., Ariffin, S. H. Z., and Huyop, F. (2011). Purification and Properties of a New Dehalogenase Enzyme from *Pseudomonas* sp. B6P Grow In 3-Chloropropionate (3-CP). *African Journal of Biotechnology*. 10(4), 610-614.

Häggblom, M. M. and Bossert, I. D. (2003). *Microbial Processes and Environmental Applications*. Kluwer Academic Publishers, Dordrecht.

Hahada, T., and Hirabayashi, T. (1968). Utilization of Alcohols by *Hansenula miso*. *Agricultural and Biological Chemistry*. 32(9), 1175-1180.

Hardman, D. J. (1991). Biotransformation of Halogenated Compounds. *Critical Reviews in **B**iotechnology*. 11(1), 1-40.

Hareland, W. A., Crawford, R. L., Chapman, P. J., and Dagley, S. T. A. N. L. E. Y. (1975). Metabolic Function and Properties of 4-Hydroxyphenylacetic Acid 1-Hydroxylase from *Pseudomonas Acidovorans*. *Journal of Bacteriology*. 121(1), 272-285.

Hayes, T. B., Case, P., Chui, S., Chung, D., Haeffele, C., Haston, K., Lee, M., Mai, V. P., Marjuoa, Y., Parker, J. and Tsui, M. (2006). Pesticide Mixtures, Endocrine Disruption, and Amphibian Declines: are we Underestimating the Impact?. *Environmental Health Perspectives*. 114 (Suppl 1), 40-50.

Hill, K. E., Marchesi, J. R. and Weightman, A. J. (1999). Investigation of Two Evolutionarily Unrelated Halocarboxylic Acid Dehalogenase Gene Families. *Journal of Bacteriology*. 181(8), 2535-2547.

Hirsch, P., and Alexander, M. (1960). Microbial Decomposition of Halogenated Propionic and Acetic Acids. Canadian Journal of Microbiology. 6(3), 241-249.

Hodge, T., Jamie, M. and Cope, T. V. (2000). A Myosin Family Tree. *Journal of Cell Science*. 113(19), 3353-3354.

Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T., Williams, S.T. (1994). *In: Bergy's Manual of Determinative Bacteriology*, Ninth Ed. Williams and Wilkins Pub., MD, USA.

Hughes, S. (1988). *Microbial Growth on 3-Chloropropionic Acid*. Doctor Philosophy, University of Wales, Cardiff. U.K.

Huyop, F., Sabaruddin, N., Abdullah, A. A., Mesri, S., and Cooper, R. A. (2003). Bioinfomatic Studies of Different L-2-Haloacid and D-2-Haloacid Dehalogenases from *Rhizobium* sp. In *International Conference on Advancement in Science and Technology* (pp. 5-7).

Huyop, F. Z., and Cooper, R. A. (2012). A Potential Use of Dehalogenase D (DehD) from *Rhizobium* sp. for Industrial Process. *Jurnal Teknologi*. 38(1), 69-75.

Huyop, F., and Nemati, M. (2010). Properties of Dehalogenase from *Rhizobium* sp. RC1. *African Journal of Microbiology Research*. 4(25), 2836-2847.

Huyop, F., Rashid, N. A., Wahab, R. A., and Cooper, R. A. (2008a). Purification and Properties of Rhizobial DehL Expressed in Escherichia coli. *African Journal of Biotechnology*. 7(12), 1944-1949.

Huyop, F., Jing, N. H., and Cooper, R. A. (2008b). Overexpression, Purification and Analysis of Dehalogenase D of *Rhizobium* sp. *Canadian Journal of Pure and Applied Sciences*. 389 -392.

Huyop, F., Yusn, T. Y., Ismail, M., Wahab, R. A., and Cooper, R. A. (2004). Overexpression and Characterisation of Non-Stereospecific Haloacid Dehalogenase E (Dehe) of *Rhizobium* Sp. *Asia Pacific Journal of Molecular Biology and Biotechnology*. 12(1-2), 15-20.

Illanes, A. (Ed.). (2008). *Enzyme Biocatalysis: Principles and Applications*. Springer Science and Business Media.

Janssen, D. B., Dinkla, I. J., Poelarends, G. J. and Terpstra, P. (2005). Bacterial Degradation of Xenobiotic Compounds: Evolution and Distribution of Novel Enzyme Activities. *Environmental Microbiology*. 7(12), 1868-1882.

Janssen, D. B., Pries, F. and Van der Ploeg, J. R. (1994). Genetics and Biochemistry of Dehalogenating Enzymes. *Annual Reviews in Microbiology*. 48(1), 163-191.

Janssen, D. B., Pries, F., Van der Ploeg, J., Kazemier, B., Terpstra, P. and Witholt, B. (1989). Cloning of 1, 2-Dichloroethane Degradation Genes of *Xanthobacter Autotrophicus* GJ10 and Expression and Sequencing of the *dhla* Gene. *Journal of Bacteriology*. 171(12), 6791-6799.

Jensen, H. L. (1957). Decomposition of Chloro-Substituted Aliphatic Acids by Soil Bacteria. *Canadian Journal of Microbiology*, 3(2), 151-164.

Jiang, X., Meng, X., and Xian, M. (2009). Biosynthetic Pathways for 3-Hydroxypropionic Acid Production. *Applied Microbiology and Biotechnology*. 82(6), 995-1003.

Jing, N. H. and Huyop, F. (2007). Dehalogenation of Chlorinated Aliphatic Acid by *Rhodococcus* sp. *Asia Pacific Journal of Molecular Biology and Biotechnology*. 15, 147-151.

Jing, N. H., Taha, A. M., Wahab, R. A. and Huyop, F. (2008a). Dehalogenase from *Methylobacterium* sp. HJ1 Induced by the Herbicide 2, 2-Dichloropropionate (Dalapon). *African Journal of Microbiology Research*. 2(2), 32-36.

Jing, N. H., Wahab, R. A., Taha, A. M., Rashid, N. A. A. and Huyop, F. (2008b). A Further Characterization of 3-Chloropropionic Acid Dehalogenase from *Rhodococcus* sp. HJ1. *Research Journal of Microbiology*. 3(6), 482-488.

Jing N. H., Wahab R. A., Taha A. M., Rashid N. A. A. and Huyop F. (2010). Cloning and DNA Sequence Analysis of the Haloalkanoic Permease Uptake Gene from *Rhizobium* sp. RC1. *Biotechnology*. 9(3), 319-325.

Kawasaki, H., Toyama, T., Maeda, T., Nishino, H., and Tonomura, K. (1994). Cloning and Sequence Analysis of a Plasmid-Encoded 2-Haloacid Dehalogenase Gene from *Pseudomonas Putida* No. 109. *Bioscience, Biotechnology, and Biochemistry*. 58(1), 160-163.

Kearney, P. C., and Kellogg, S. T. (1985). Microbial Adaptation to Pesticides. *Pure and Applied Chemistry*. 57(2), 389-403.

Kohler-Staub, D. O. R. I. S., and Kohler, H. P. (1989). Microbial Degradation of Beta-Chlorinated Four-Carbon Aliphatic Acids. *Journal of Bacteriology*. 171(3), 1428-1434.

Kolbert, C. P., and Persing, D. H. (1999). Ribosomal DNA Sequencing as a Tool for Identification of Bacterial Pathogens. *Current Opinion in Microbiology*. 2(3), 299-305.

Kranz, R., Hafer, K. W., and Richards, E. (2006). *Identifying Unknown Bacteria Using Biochemical and Molecular Methods*. Washington:Washington University in Saint Louis.

Kumar, V., Ashok, S. and Park, S. (2013). Recent Advances in Biological Production of 3-Hydroxypropionic Acid. *Biotechnology Advances*. 31, 945-61.

Kupiec, T. (2004). Quality-Control Analytical Methods: High-Performance Liquid Chromatography. *International Journal of Pharmaceutical Compounding*. 8, 223-227.

Kwak, S., Park, Y. C. and Seo, J. H. (2013). Biosynthesis of 3-Hydroxypropionic Acid from Glycerol in Recombinant *Escherichia coli* Expressing *Lactobacillus brevis dhaB* and *dhaR* Gene Clusters and *E. coli* K-12 aldH. *Bioresource Technology*. 135, 432-9.

Laemmli, U.K. (1970). Cleavage of Structural Proteins during Assembly of Head of Bacteriophage T4. *Nature*. 227, 680-685.

Leigh, J., Skinner, A. and Cooper, R. (1988). Partial Purification, Stereospecificity and Stoichiometry of Three Dehalogenases from a *Rhizobium* species. *FEMS Microbiology Letters*, 49(3), 353-356.

Lewis, C., Pook, C. and Galloway, T. (2008). Reproductive Toxicity of the Water Accommodated Fraction (WAF) of Crude Oil in the Polychaetes *Arenicola Marina* (L.) and *Nereis virens* (Sars). *Aquatic Toxicology*. 90(1), 73-81.

Lin, C., Yang, L., Xu, G., and Wu, J. (2011). Biodegradation and Metabolic Pathway of β-Chlorinated Aliphatic Acid in *Bacillus* sp. CGMCC No. 4196. *Applied Microbiology and Biotechnology*. 90(2), 689-696.

Magee, L. A. and Colmer, A. R. (1959). Decomposition of 2, 2-Dichloropropionic Acid by Soil Bacteria. *Canadian Journal of Microbiology*. 5(3), 255-260.

Mahenthiralingam, E., Urban, T. A., ad Goldberg, J. B. (2005). The Multifarious, Multireplicon *Burkholderia cepacia* Complex. *Nature Reviews Microbiology*. 3(2), 144-156.

Manan, F.M.A., Rahman, I.N.A., Marzuki, N.H.C., Mahat, N.A., Huyop, F. and Wahab, R.A. (2016). Statistical Modelling of Eugenol Benzoate Synthesis Using *Rhizomucor miehei* Lipase Reinforced Nanobioconjugates. *Process Biochemistry*. 519(2),249-262.

Maniatis, T., Fritsch, E. F. and Sambrook, J. (1982). *Molecular Cloning: a Laboratory Manual* (545). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Marzuki, N. H. C., Mahat, N. A., Huyop, F., Buang, N. A., and Wahab, R. A. (2015). *Candida rugosa* Lipase Immobilized onto Acid-Functionalized Multi-Walled Carbon Nanotubes for Sustainable Production of Methyl Oleate. *Applied Biochemistry and Biotechnology*. 177(4), 967-984.

McAuliffe, J. C., Aehle, W., Whited, G. M. and Ward, D. E. (2007). Industrial Enzymes and Biocatalysis. In Kent, J. A. (Ed.) *Kent and Riegel's Handbook of Industrial Chemistry and Biotechnology* (pp. 1375-1420). Boston, MA: Springer US.

McPherson, R. A., and Pincus, M. R. (2016). *Henry's Clinical Diagnosis and Management by Laboratory Methods*. Elsevier Health Sciences.

Meng, C., He, Q., Huang, J.-W., Cao, Q., Yan, X., Li, S.-P. and Jiang, J.-D. (2015). Degradation of Chlorothalonil through a Hydrolytic Dehalogenase Secreted from *Bacillus subtilis* WB800. *International Biodeterioration and Biodegradation*. 104, 97-104.

Mesri, S., Wahab, R. A. and Huyop, F. Z. (2009). Degradation of 3- Chloropropionic Acid (3-CP) by *Pseudomonas* Sp. B6p Isolated from a Rice Paddy Field. *Annals of Microbiology*. 59 (3) 446.

Miller, J. H. (1972). *Experiments in molecular genetics*. Cold Spring Harbor Laboratory. New York.

Muslem, W. H., Huyop, F., Zakaria, I. I., and Wahab, R. A. Isolation and Characterization of a Biodegrading 3-Chloropropionic Acid *Burkholderia cepacia* WH1 isolated from abandoned agricultural land. *Asia-Pacific Journal of Molecular Biology and Biotechnology*. 23 (2), 268-279

Nardi-Dei, V., Kurihara, T., Park, C., Esaki, N. and Soda, K. (1997). Bacterial DL-2-Haloacid Dehalogenase from *Pseudomonas* sp. Strain 113: Gene Cloning and Structural Comparison with D-and L-2-Haloacid Dehalogenases. *Journal of Bacteriology*. 179(13), 4232-4238.

Nardi-Dei, V., Kurihara, T., Park, C., Miyagi, M., Tsunasawa, S., Soda, K. and Esaki, N. (1999). dl-2-Haloacid Dehalogenase from *Pseudomonas* sp. 113 Is a New Class of Dehalogenase Catalyzing Hydrolytic Dehalogenation Not Involving Enzyme-Substrate Ester Intermediate. *Journal of Biological Chemistry*. 274(30), 20977-20981.

Niknam, M. R., Huyop, F. and Wahab, R. A. (2014). Identification and Characterization of *Raoutella ornithilolytica* and Determination of its Herbicide 2, 2-Dichloropropionate (2, 2-DCP) Degradation Potential. *Malaysian Journal of Microbiology*. 10(4), 249-254.

Novak, H. R., Sayer, C., Isupov, M. N., Gotz, D., Spragg, A. M. and Littlechild, J. A. (2014). Biochemical and Structural Characterisation of a Haloalkane Dehalogenase from a Marine *Rhodobacteraceae*. *FEBS letters*. 588(9), 1616-1622.

Papachristodoulou, D., Snape, A., Elliott, W. H., and Elliott, D. C. (2014). *Biochemistry and Molecular Biology*. Oxford University Press.

Parvizpour, S., Abdul Hamid, T. H. T. and Huyop, F. (2013). Molecular Identification and Biodegradation of 3-Chloropropionic Acid (3-CP) by Filamentous Fungi-*Mucor* and *Trichoderma* Species Isolated from UTM Agricultural Land. *Malaysian Journal of Microbiology*. 9(1), 120-124.

Patel, J. B. (2001). 16S rRNA Gene Sequencing for Bacterial Pathogen Identification in the Clinical Laboratory. *Molecular Diagnosis*. 6(4), 313-321.

Penfold, W. J. (1913). The Inhibitory Selective Action on Bacteria of Bodies Related to Monochloracetic Acid (A Contribution to the Theory of Cell Intoxication.). *Journal of Hygiene*. 13(01), 35-48.

Pimentel, D. and Levitan, L. (1986). Pesticides: Amounts Applied and Amounts Reaching Pests. *Bioscience*. 36 (2), 86-91.

Qing Li, Q., Loganath, A., Seng Chong, Y., Tan, J. and Philip Obbard, J. (2006). Persistent Organic Pollutants and Adverse Health Effects in Humans. *Journal of Toxicology and Environmental Health, Part A*. 69(21), 1987-2005.

Ridder, I. S., Rozeboom, H. J., Kalk, K. H., Janssen, D. B. and Dijkstra, V. W. (1997). Three-Dimensional Structure of L-2-Haloacid Dehalogenase from *Xanthobacter autotrophicus* GJ10 Complexed with the Substrate-Analogue Formate. *The Journal of Biological Chemistry*. 272(52), 33015–33022.

Rieger, P. G., Meier, H. M., Gerle, M., Uwe Vogt, Groth, T. and Knackmuss, H. J. (2002). Xenobiotics in The Environment: Present and Future Strategies to Obviate the Problem of Biological Persistence. *Journal of Biotechnology*. 94(1), 101–123.

Ritter, L., Solomon, K. R., Forget, J., Stemeroff, M., and O'leary, C. (1995). A Review of Selected Persistent Organic Pollutants. *International Programme on Chemical Safety (IPCS). PCS/95.39. Geneva: World Health Organization*. 65, 66.

Rogan, W. J., Gladen, B. C., McKinney, J. D., Carreras, N., Hardy, P., Thullen, J., and Tully, M. (1987). Polychlorinated Biphenyls (PCBs) and Dichlorodiphenyl Dichloroethene (DDE) in Human Milk: Effects on Growth, Morbidity, and Duration of Lactation. *American Journal of Public Health*. 77(10), 1294-1297.

Rye, C. A., Isupov, M. N., Lebedev, A. A. and Littlechild, J. A. (2009). Biochemical and Structural Studies of a L-haloacid Dehalogenase from the Thermophilic Archaeon *Sulfolobus tokodaii*. *Extremophiles*. 13(1), 179-190.

Rylander, L., Rignell-Hydbom, A., and Hagmar, L. (2005). A Cross-Sectional Study of the Association between Persistent Organochlorine Pollutants and Diabetes. *Environmental Health*. 4(1), 28.

Saitou, N. and Nei, M. (1987). The Neighbor-Joining Method: A New Method for Reconstructing Phylogenetic Trees. *Molecular Biology and Evolution*. 4(4), 406-425.

Schmidberger, J. W., Wilce, J. A., Tsang, J. S. and Wilce, M. C. (2007). Crystal Structures of the Substrate Free-Enzyme, and Reaction Intermediate of the

HAD Superfamily Member, Haloacid Dehalogenase DehIVa from Burkholderia Cepacia MBA4. *Journal of Molecular Biology*. 368(3), 706-717.

Schneider, B., Müller, R., Frank, R. and Lingens, F.(1991). Complete Nucleotide Sequences and Comparison of the Structural Genes of Two 2-Haloalkanoic cid Dehalogenases from *Pseudomonas* sp. Strain CBS3 . *Journal of Bacrteriology*. 173(4), 1530-1535.

Schwarz, M., Köpcke, B., Weber, R. W., Sterner, O. and Anke, H. (2004). 3-Hydroxypropionic Acid as a Nematicidal Principle in Endophytic Fungi. *Phytochemistry*. 65(15), 2239-2245.

Schwarze, R., Brokamp, A. and Schmidt, F. R. J. (1997). Isolation and Characterization of Dehalogenases from 2, 2-Dichloropropionate-Degrading Soil Bacteria. *Current Microbiology*. 34(2), 103-109.

Sebastianes, F. L., Cabedo, N., El Aouad, N., Valente, A. M., Lacava, P. T., Azevedo, J. L., Pizzirani-Kleiner, A. A. and Cortes, D. (2012). 3-Hydroxypropionic Acid as an Antibacterial Agent from Endophytic Fungi Diaporthe phaseolorum. *Current Microbiology*. 65(5), 622-32.

Segel, I. H. (1975). *Enzyme kinetics*. Wiley, New York.

Selvamani S., Wahab R.A. and Huyop, F.Z. (2015). A Novel Putative Non-Ligninolytic Dehalogenase Activity for 3-Chloropropionic Acid (3CP) Utilization by *Trichoderma asperellum* Strain SD1. *Malaysian Journal of Microbiology*. 11(3), 265-272.

Senior, E., Bull, A. T. and Slater, J. H. (1976). Enzyme Evolution in a Microbial Community Growing on the Herbicide Dalapon. *Nature*, 263(5577), 476-479.

Sinha, S., Chatopadhyay, P., Pan, L., Chatterjee, S., Chanda, P., Bandyopadhaya, D., Das, K. and Sen, S. (2009). Microbial Transformation of Xenobiotics for Environmental Bioremediation. *African Journal of Biotechnology*. (22),6016-6027.

Singh, B. K., and Walker, A. (2006). Microbial Degradation of Organophosphorus Compounds. *FEMS Microbiology Reviews*. 30(3), 428-471.

Slater, J. H., Bull, A. T. and Hardman, D. J. (1995). Microbial Dehalogenation. *Biodegradation*. 6(3), 181-189.

Slater, J. H., Bull, A. T., and Hardman, D. J. (1996). Microbial Dehalogenation of Halogenated Alkanoic Acids, Alcohols and Alkanes. *Advances in Microbial Physiology*. 38, 133-176.

Snellman, E. A., and Colwell, R. R. (2004). Acinetobacter Lipases: Molecular Biology, Biochemical Properties and Biotechnological Potential. *Journal of Industrial Microbiology and Biotechnology*. 31(9), 391-400.

Soccol, C. R., Vandenberghe, L. P., Woiciechowski, A. L., Thomaz-Soccol, V., Correia, C. T., and Pandey, A. (2003). Bioremediation: An Important Alternative for Soil and Industrial Wastes Clean-Up. *Indian Journal of Experimental Biology*. 41, 1030- 1045.

Swanson, P. E. (1999). Dehalogenases Applied to Industrial-Scale Biocatalysis. *Current Opinion in Biotechnology*. 10(4), 365-369.

Strait, D. S. and Grine, F. E. (2004). Inferring Hominoid and Early Hominid Phylogeny Using Craniodental Characters: The Role of Fossil Taxa. *Journal of Human Evolution*. 47(6), 399-452.

Stringfellow, J.M., Cairns, S.S., Cornish, A. and Cooper, R.A. (1997). Haloalkanoate Dehalogenase II (DehE) of a *Rhizobium* sp. Molecular Analysis of the Gene and Formation of Carbon Monoxide from Trihaloacetate by the Enzyme. *European Journal of Biochemistry*. 250 (3), 789-93.

Sufian, N. H. M., Naim, M. A., Hamid, T. H. T. A., Huyop, F. and Hamid, A. A. A. (2015). Isolation and Identification of 3-Chloropropionic Acid Degrading Bacterium from Marine Sponge. *Jurnal Teknologi (Sciences and Engineering)*. 77(25), 71-75.

Suplatov, D., Panin, N., Kirilin, E., Shcherbakova, T., Kudryavtsev, P., and Švedas, V. (2014). Computational Design of a pH Stable Enzyme: Understanding Molecular Mechanism of Penicillin Acylase's Adaptation to Alkaline Conditions. *PloS One*. 9(6), e100643.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution*. 30(12), 2725-2729.

Thomas, A. W., Slater, J. H. and Weightman, A.J.(1992). The Dehalogenase Gene *dehI* from *Pseudomonas putida* PP3 Is Carried on an Unusual Mobile Genetic Element Designated DEH. *Journal of Bacreiology*. 174(6), 1932-1940.

Thomas, W. H., Seibert, D. and Koeller, P. (2012). Controlled Ecosystems: their Use in the Study of the Effects of Petroleum Hydrocarbons on Plankton. *Physiolgcl Resp of Marine Biota to Pol Z*. 323.

Towner, K. J. and Cockayne,A. (1993). Analysis of Nucleic Acid Profiles. In: Molecular Methods for Microbial Identification and Typingpp. 29-63. Towner K. J. and A. Cockayne (eds.) Chapman and Hall, London, UK.

Timmis, K. N. and Pieper, D. H. (1999). Bacteria Designed for Bioremediation. *Trends in Biotechnology*. 17(5), 200-204.

Tsang, J. S. H. and Sam, L. (1999). Cloning and Characterization of a Cryptic Haloacid Dehalogenase from *Burkholderia cepacia* MBA4. *Journal of Bacteriology*. 181(19), 6003-6009.

Umland, J. B., and Bellama, J. M. (1999). *General chemistry*. Brooks/Cole Publishing Company.

Van Pée, K.H. and Unversucht, S. (2003). Biological Dehalogenation and Halogenation Reactions. *Chemosphere*. 52(2),299 – 312.

Ventura,M. and Zink, R. (2002). Rapid Identification Differentiation and Proposed New Taxonomic Classification of *Bifidobacterium lactis*. *Applied and Environmental Microbiology*. 68 (12), 6429- 6434.

Vial, L., Chapalain, A., Groleau, M. C., and Déziel, E. (2011). The Various Lifestyles of the *Burkholderia cepacia* Complex Species: A Tribute to Adaptation. *Environmental Microbiology*. 13(1), 1-12.

Wade, L. G. Jr. (2009). *Organic Chemistry*. 7th ed. New Jersey: Prentice Hall.

Wahab, R. A., Basri, M., Rahman, R. N. Z. R. A., Salleh, A. B., Rahman, M. B. A., Chaibakhsh, N., and Leow, T. C. (2014). Enzymatic Production of a Solvent-Free Menthyl Butyrate via Response Surface Methodology Catalyzed by a Novel Thermostable Lipase from *Geobacillus zalihae*. *Biotechnology and Biotechnological Equipment*. 28(6), 1065-1072.

Wahab, R.A., Basri, M., Rahman, R.N.Z., Salleh, A.B., Rahman, M.B.A., Chaibakhsh, N. and Leow, T.C. (2016). Facile Modulation of Enantioselectivity of Thermophilic *Geobacillus zalihae* lipase by Regulating Hydrophobicity of its Q114 Oxyanion. *Enzyme and Microbial Technology*. 93, 174-181.

Weightman, A. J., Topping, A. W., Hill, K. E., Lee,L. L., Sakai, K., Slater, J. H. and Thomas, A. W. (2002). Transposition of DEH, a Broad-Host-Range Transposon Flanked by ISPpu12, in *Pseudomonas putida* is Associated with Genomic Rearrangements and Dehalogenase Gene Silencing. *Journal of Bacteroilogy*. 184 (23), 6581–6591.

Weightman, A. J., Weightman, A. L. and Slater, J. H. (1982). Stereospecificity of 2-Monochloropropionate Dehalogenation by the Two Dehalogenases of *Pseudomonas putida* PP3: Evidence for Two Different Dehalogenation Mechanisms. *Journal of General Microbiology*. 128(8), 1755.

World Health Organization (2010). Persistent Organic Pollutants: Impact on Child Health. WHO Library Cataloguing-in-Publication Data, ISBN 978 92 4 150110 1.

Wong, W. Y. and Huyop, F. (2012). Molecular Identification and Characterization of Dalapon-2, 2-Dichloropropionate (2, 2DCP)-Degrading Bacteria from a Rubber Estate Agricultural Area. *African Journal of Microbiology Research*. 6(7), 1520-1526.

Woese, C. R. and Fox, G. E. (1977). Phylogenetic Structure of the Prokaryotic Domain: the Primary Kingdoms. Proceedings of the National Academy of Sciences. 74(11), 5088-5090.

Woo, P. C. Y., Ng, K. H. I., Lau, S. K. P.,Yip, K.T., Fung, K.T., Leung, K. W.,Tam, D. M. W., Que, T.L. and Yuen, K.Y. (2003). Usefulness of the MicroSeq 500 16S ribosomal DNA-Based Identification System for Identification of

Clinically Significant Bacterial Isolates with Ambiguous Biochemical Profiles. *Journal Clinical Microbiology*. 41(5),1996-2001.

Yan, S., Subramanian, S. B., Tyagi, R. D., Surampalli, R. Y. and Zhang, T. C. (2009). Emerging Contaminants of Environmental Concern: Source, Transport, Fate, and Treatment. *Practice Periodical of Hazardous, Toxic, and Radioactive Waste Management*. 14(1), 2-20.

Young, R. G., and Gossett, J. M. (1997). Effect of Environmental Parameters and Concentration on Dechlorination of Chloroethenes. In *Fourth International In Situ and On-Site Bioremediation Symposium, New Orleans, LA.*

Yusn, T. Y., Huyop, F. (2009). Degradation of 3-Chloropropionic Acid by *E.coli* JM109 Expressing Dehalogenase (*deh*) Gene Used as Selection Marker. *Biotechnology*. 8(3), 385-388.

Zhang, J., Xin, Y., Cao, X., Xue, S. and Zhang, W. (2014). Purification and Characterization of 2-Haloacid Dehalogenase from Marine Bacterium *Paracoccus* sp. DEH99, isolated from Marine Sponge *Hymeniacidon perlevis*. *Journal of Ocean University of China*. 13(1), 91-96.

Zhu, J. G., Ji, X. J., Huang, H., Du, J., Li, S. and Ding, Y. Y. (2009). Production of 3-Hydroxypropionic Acid by Recombinant *Klebsiella Pneumoniae* Based on Aeration and ORP Controlled Strategy. *Korean Journal of Chemical Engineering*. 26(6), 1679-1685.