Chemical and Biological Oxidation of Naphthenic Acids - where Stoichiometry, Kinetics and Thermodynamics Meet

by

Reza Mahour

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

in

Environmental Engineering

Department of Civil and Environmental Engineering

University of Alberta

ABSTRACT

Open-pit mining of Alberta's oil sands deposits heavily depend on freshwater for the extraction of bitumen. It leaves 1.25 m^3 oil sands process affected water (OSPW) per barrel of produced oil. In spite of years of research on treatment of OSPW, currently, there are no approved economic and environmental friendly strategies for management of OSPW. Based on Alberta's zero discharge policy, OSPW must be kept on site in custom made tailings ponds that cover approximately 185 km^2 of area. Research results in various fields indicate that naphthenic acids (NAs) are the main group of complex organics in OSPW that require remediation. NAs have been shown to be the main contributor to toxicity in OSPW, as well NAs corrode the process infrastructure. Investigations on the role of indigenous microorganisms in tailings ponds address their positive effect in removal of NAs. However, a consistent concentration of NAs in aged oil sands tailings ponds indicates that NAs are not completely biodegradable. Generally, dealing with recalcitrant compounds, coupling of chemical and biological oxidation is a method of interest.

Sodium persulfate is an emerging oxidant used in OSPW treatment. Due to the challenging process of determining chemical composition, and measuring the exact concentration of NAs mixture in raw samples, for the first time in the field of NAs removal the concept of degree of reduction has been used for determining the stoichiometric amount of persulfate required for oxidation of NAs mixture. Estimated required concentrations based on proposed model show effective outcomes throughout the experiment. It also improved in optimization of the oxidation process by consuming approximately 5 times less oxidant reported in literature.

To reach the application of combining chemical oxidation by sodium persulfate and biodegradation, effect of stress of sodium persulfate on *Pseudomonas* sp. was studied. Quantitative physiology parameters determined for the bacteria to elucidate the effect of oxidative stress on their ability to consume NAs. Growth of the bacteria in the presence of sodium persulfate significantly is affected, which is illustrated by the maximum concentration of biomass. At 1000 mg/L of sodium persulfate, the maximum concentration of biomass decreased by ~25 % respect to non-stressed controls. At the dosage of 2000 mg/L, no growth was observed. However, quantitative physiological analyses showed no significant change in ability of *Pseudomonas* sp. for consumption of Merichem NAs (based on DOC). The biomass specific growth rate (0.1 h^{-1}), biomass specific substrate consumption rate (0.2 mgDOC/mgDCW/h) and yield of substrate to biomass (0.6 mgDCW/mgDOC) for each persulfate concentration series were not significantly different.

Thermodynamics is a very powerful tool in understanding the limits of a system and prediction of possibilities. Some model NAs within literature have indicated to be non-biodegradable, for example dicyclohexyl acetic acid. To answer the question why dicyclohexyl acetic acid is not biodegradable, thermodynamics study applied to understand the limits and predict the possibilities for biodegradation. The University of Minnesota Biocatalysis/Biodegradation database was used for prediction of possible catabolic pathways for biodegradation of dicyclohexyl acetic acid. Interestingly, thermodynamics suggest the possibility of biodegradation under aerobic conditions. Results from bio-thermodynamics analyses predict that dicyclohexyl acetic acid can be consumed by bacteria with the maximum yield of 0.6 g organic dry weight per g of dicyclohexyl acetic acid. A 13% relative error has previously been shown with the approach used in bio-thermodynamics studies. Therefore, thermodynamics can provide a foundation for

future for metabolic and genetic engineers to engineer microorganisms or microbial cultures with the objective of NAs biodegradation.

PREFACE

This thesis is an original work by Reza Mahour under supervision of Dr. Ania Ulrich.

The research completed in chapter 3 was planned, designed, conducted, analyzed, and compiled by myself. Chapter 4 of this work was planned and designed by Dr. Ania Ulrich and conducted, analyzed, and compiled by myself.

Chapter 5 of this thesis planned and designed by myself with collaboration of Prof. Sef Heijnen at Delft University of Technology and Prof. Urs von Stockar at École Polytechnique Fédérale de Lausanne.

To my mom's anxieties

To my dad's hands

To my sister's kindness

To my brother's jokes…

ACKNOWLEDGEMENTS

I always liked non-equilibrium thermodynamics since it tells you what is going on in the dynamic process! Because it gives you the idea of how to appreciate life! I mean it mathematically proves that you should enjoy the road not just by destination...sometimes a view on your road it is not as much as beautiful as you supposed it would be, but travel mates can turn the undesirable view into desirable ones. Dr. Ulrich, you were not only my supervisor but also you have been a great friend. You taught me that to be an active listener and you didn't just tell me that, you showed me by listening to me. You cared about my strengths and weaknesses, so now I understand what they are, and most of all you trusted me. Thank you Dr. Ulrich, great thanks!

The thermodynamics section of this thesis would have not been possible without invaluable comments of Prof. Dr. ir. Sef Heijnen (TU Delft, NL) and Prof. Urs von Stockar (EPFL, CH), I greatly appreciate your time and help for answering my countless questions. Dr. Vinay Prasad's comments about statistical analysis of experimental data is acknowledged.

I would like to acknowledge my supervisor Dr. Thomas Rexer at my research stay at Max Planck Institute of Dynamics of Complex Technical Systems. Thomas, thank you for all you have done for me during my stay in Germany. I will never forget your help and memories that we had in Magdeburg.

I also like to say thanks to my lab mates in Bioremediation Engineering group. Specifically, Dr. Dena Cologgi, Dr. Hamed Mahdavi and Sarah Miles. Thanks Dena for helping me to set-up my experiments. Thank you Hamed for your comments on working with naphthenic acids and teaching me statistical analysis. Hamed, you really are a great friend. Sarah, thank you for being a very good friend of mine. I will never forget your help to me for integrating into Canadian culture. And also thanks for your help in editing my thesis.

I am grateful to my funders, NSERC of Canada, Graduate Student Travel Award the Faculty of Graduate Studies and Research and Graduate Student Association at the University of Alberta.

I also like to say my thanks to my sister Nasim for being a great support. Khahari, you know that you are a huge part of my life, I always have and will be proud of having a sister and friend like you.

I'm also thankful to my friends in Edmonton who helped me in various aspects of life. My first friend in Canada, Vahid who always motivates me to follow my ideas. Thank you Ahura for being my Dai Asdola, you have always been like my elder brother! My awesome roommates Firouz and Ramin (I will never ever forget that how many times you guys asked me to make sure that I have enough funding for my trip to Germany). Thank you Parinaz for being my best friend and dance partner! I also thank to my friend Ehsan for the great time we had playing foosball and billiard. Thanks Behnam for our fruitful discussions in Tim Hortons in ETLC! Shiva, thanks for your help in editing. Thanks to Amir and Amirhossein for the good times we had. Special thanks to Masoud, Mohammadreza and Farzin (and Naser) for their beautiful minds and their nice comments about life! Masoud, you have been more than a friend to me. And great thanks to my very good friends for being good friends.

At the end, I want to hug my mom and dad to say I love you. One of the very best moments of life is when you realize that your parents are actually your best friends and I feel so blessed that I experienced this feeling so many times. Thank you for being my parent and my closest friends. Oh you read the whole acknowledgment! Thank you to you too!

April, 2016

Edmonton, Canada

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ACRONYMS

OSPW	Oil Sands Process-affected Water
NAs	Naphthenic Acids
AEOs	Acid Extractable Organics
COD	Chemical Oxygen Demand
DOC	Dissolved Organic Carbon
OD	Optical Density
DCW	Dry Cell Weight
DCM	Dichloromethane
FTIR	Fourier Transform Infrared Spectroscopy
NADH	Nicotinamide adenine dinucleotide
ETC	Electron Transport Chain

GLOSSARY

γ	Degree of reduction
ΔG	Gibbs free energy difference across a reaction
K_{eq}	Equilibrium constant
Q	Reaction quotient
$\boldsymbol{\Phi}$	Rate of dissipation of Gibbs free energy, dissipation function
μ_i	Chemical potential of unexchangeable substance i
μ_j	Chemical potential of freely exchangeable substance j
σ_s	Entropy production function
đ	Small change
$đ_e$	Small change due to exchange with the environment
$đ_i$	Small change due to internal process
$đ_e Q'$	Heat exchanged after correction for partial molar enthalpies of exchanged substabces
$đ_e Q''$	Pure heat exchanged after correction for partial molar enthalpy but including the entropy of exchanged particles
$đ_e W$	Work
$đ_e W'$	Work including enthalpy import through transport
$đ_e W''$	Work including transport work
$div(J_x)$	Divergence of J_x
E	Energy
J_α	Flux through branch α
J_{chem}	Fluc through chemical reaction
J_k	Flux through process k
J_q	Heat flow
V	Volume
P	Hydrostatic pressure
F	Elastic

S	Entropy
K_s	Substrate affinity
q_i	Biomass specific rate of compound i
Y_{ij}	Yield of compound i to compound j
μ	Biomass specific growth rate
$C_1H_{1.8}O_{0.5}N_{0.2}$	General formula of biomass

CHAPTER 1. INTRODUCTION AND RESEARCH OBJECTIVES

1.1 General Introduction

Oil sands consist of quartz sand grains, covered by a layer of water and clay and then surrounded by a slick of heavy oil called bitumen (Speight, 2007). Canada's oil sands deposits are the third largest oil sands deposits in the world, which contain 1.8 trillion barrels of oil reserves (Government of Alberta, 2016). 170 billion barrels are located in Alberta with 168 billion barrels of recoverable bitumen (with today's technologies). Alberta's oil sands are found in three deposits: Athabasca, Cold Lake and Peace River. These three deposits cover an area equal in size to the province of New Brunswick (Government of Alberta, 2016). One of the most common methods for oil sands extraction, is hot water extraction which was proposed by Dr. Karl A. Clark in 1920 (Speight, 2007). This method mixes oil sands with hot water to form a slurry. The slurry is transported to separation vessels where it is separated into three different layers (from bottom to top): sand settles to the bottom, middlings which is sand, and clay, water and a layer of bitumen froth at the top (Government of Alberta, 2016; Speight, 2007). The bitumen froth is transported to upgrading units for additional processing. The remaining layers of sand, clay and water from the separation vessel are pumped to large on site custom made tailings ponds for storage and re-use. Overall, this method produces 1.25 m^3 of oil sands process-affected water (OSPW) for each barrel of oil (Allen, 2008a). Since 1993 any release of these wastewaters into the environment is prohibited by the Alberta Environmental Protection and Enhancement Act, Section 23 (1993), commonly referred to as the zero discharge policy (Giesy et al., 2010; Government of Alberta, 2016). To satisfy the environmental regulations, promote settling of solids, and mineralization of organics, custom made tailings ponds are constructed on site to

collect OSPW (Government of Alberta, 2010). Currently, tailings ponds cover 77 square kilometers in Alberta (Government of Alberta, 2010). Utilization of OSPW in tailings ponds for water recycling reduces the dependency to fresh water by 80-85% (Allen, 2008a). However, recycling OSPW increases concentrations of the minerals, trace metals, residual bitumen, and organics in OSPW (Allen, 2008a). Therefore, finding an economic, environmentally friendly and efficient strategy for treatment of OSPW is one of the biggest challenges facing the oil sands industry (Allen, 2008b).

One consequence of on-site tailings ponds is seepage of OSPW from tailings ponds and their supporting dykes into the subsurface (Oiffer et al., 2009). There are several studies on the infiltration of OSPW into the subsurface, groundwater and its subsequent transport to surface water (Abolfazlzadehdoshanbehbazari et al., 2013; Holden et al., 2011; Holden et al., 2013). From these studies, the migration of salts, metals and naphthenic acids are a key concern.

Naphthenic Acids (NAs) are natural components of petroleum, which are complex mixtures of alkyl-substituted acyclic, monocyclic and polycyclic carboxylic acids with the general formula of $C_nH_{2n+z}O_2$ where n represents the number of carbons and z represents the number of rings (Holowenko, 2002). Concentration of NAs in crude oil varies depending on the source of crude oil. There are two potential sources that contribute to the presence of NAs in crude oil and oil sands: biological (diagenesis) and/or geological (catagenesis) (Tissot and Welte, 1978)., Catagenesis is a physical process that takes place at high temperature (between 50 and 150 ^{0}C) and pressure (Tissot and Welte, 1978). Elimination of carbonyl group (it is also found in NAs) is occurred at this step. Diagenesis is an anaerobic process where methane and kerogen are produced by microorganisms (Tissot and Welte, 1978). In general, when petroleum exposed to non-indigenous bacteria through i.e. earthquake resulted in biodegradation of petroleum (Riva,

1983). Carboxylic acids are by-products of biodegradation of crude oil. The presence of carboxylic acids is found in biodegradable crude oil deposits (Meredith et al., 2000; Nascimento et al., 1999) and laboratory research on biodegradation of crude oil (Roques et al., 1994; Watson et al., 2002). NAs in Alberta's oil sands are mainly by-products of bitumen biodegradation (Clemente and Fedorak, 2005; Tissot and Welte, 1978). NAs and AEOs need to be extracted from the aqueous phase to analyse. In literature there are three different type of NAs are mentioned. First, surrogate NAs which are mainly model NAs with exact molecular formula i.e. 2-Methyl-1-cyclohexanecarboxylic acid ($C_8H_{14}O_2$). Second, commercial NAs with suppliers like Merichem, Kodak which are mainly complex mixture of carboxylic acids. The third one is known as the OSPW associated NAs. To characterize NAs in OSPW an extraction step with a proper organic solvent i.e. dichloromethane needs to be conducted at acidic pHs (<2) (Brown and Ulrich, 2015). Analysis by high resolution analytical instruments show different a composition of OSPW sourced NAs in comparison of the classical NAs formula $C_nH_{2n+z}O_2$. For instance, only 20% of OSPW associated NAs are 2-oxygen NAs (Grewer et al., 2010; Headley and McMartin, 2004). To distinguish between the classic (general formula) and non-classic (OSPW associated) NAs, the term oil sands tailings water acid-extractable organics (OSTWAEO) or acid extractable organics (AEOs) should be used. One should realize that NAs are portion of AEOs or in other words AEOs are some organics plus NAs (Grewer et al., 2010).

$$\text{NAs } (C_nH_{2n+z}O_2) + \text{Organics} = \text{AEOs} \qquad (1.1)$$

Understanding which bitumen-derived compounds contribute to the acute toxicity of OSPW is complex (Klamerth et al., 2015; Morandi et al., 2015). To unravel the toxicity of organics in OSPW, Morandi et al. (2015) extracted the organics in OSPW at different pHs. Results from this work have shown that the non-acidic organic portion of OSPW (extracted at pH 11) are less-

toxic (Morandi et al., 2015). In contrast, results heavily support that the NAs are the main compounds that create acute toxicity in OSPW (MacKinnon and Boerger, 1986; Morandi et al., 2015; Zhang et al., 2011).

After many years of natural attenuation in tailings ponds, the remaining AEOs in aged OSPW are thought to be resistant to natural attenuation (Allen, 2008b). Application of chemical oxidants are very common in the treatment of non-biodegradable compounds (Oller et al., 2011). However, to completely remove the recalcitrant contaminants, utilizing chemical oxidation strategies is not always applicable due to the resistance of oxidation intermediates to complete oxidation (Muñoz et al., 2005; Oller et al., 2011). As such more oxidant is added to complete the oxidation process, which is both costly and time-consuming. One modification to this method is the use of microorganisms to bioremediate the oxidation intermediates. This process is attractive because of its low operation costs (Rittmann and McCarty, 2012; Tchobanoglous et al., 2003).

Various studies have shown that chemical oxidation as a pre-treatment step improves the biodegradability of wastewaters as it converts compounds from recalcitrant to biodegradable (Brown et al., 2013; Hwang et al., 2013; Martin et al., 2010). Based on this concept, coupling of chemical and biological oxidation is of interest in treatment of recalcitrant compounds (Oller et al., 2011). Due to the huge volume of tailings ponds, *in situ* treatment strategies for treatment of OSPW are more desirable specifically because of the lower costs. One of the challenges of *in situ* coupling of chemical oxidation and biological treatment is vulnerability of micro-organisms to chemical oxidants. Oxidants (proportional to their dosage and properties) can kill off or damage the cells because of their high redox potential and high reactivity (Guzel-Seydim et al., 2004; Veschetti et al., 2003). Many of these oxidants are used as disinfectants i.e. ozone for drinking water treatment (Camel and Bermond, 1998; Guzel-Seydim et al., 2004). However, our

lab completed a study demonstrating the survival of indigenous microbes after ozonation with the purpose of improving biodegradability of aged OSPW (Brown et al., 2013).

A number of issues are associated with working on an OSPW treatment. OSPW is a complex mixture of organics where the analysis of these organics is a significant challenge (Brown and Ulrich, 2015). To date, a cost effective and accurate analytical technique for organics whitin OSPW has not been developed (Brown and Ulrich, 2015). Analytical instruments with different degrees of accuracy and sensitivity give us different feedback about the real content of organics either acidic or nonacidic organics (Brown and Ulrich, 2015; Clemente and Fedorak, 2005; Headley et al., 2013).

To fully understand any reactions there are three main components to consider: stoichiometry, kinetics and thermodynamics. Determining these three fundamental components greatly help environmental engineers in treating wastewaters (as shown in Fig. 1.1).

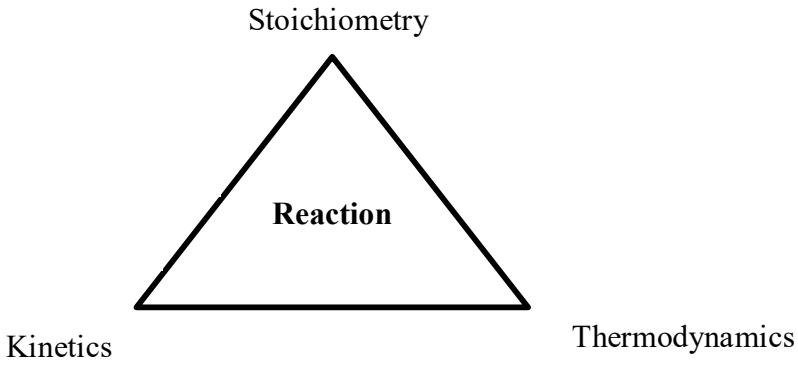


Figure 1-1 Connection of the three fundamental concepts of a reaction

Without understanding these connections it is difficult to optimize remediation processes. For instance, persulfate has been shown to be reactive toward NAs (Drzewicz et al., 2012; Sohrabi et al., 2013). Sohrabi et al. (2013) used 10 g/L of sodium persulfate and 5 g/L of potassium permanganate (separately) to degrade NAs in OSPW. However, there is no mention in their work why they chose 10 g/L and 5 g/L oxidant doses. It is understandable that determining oxidant

dose is difficult when target compounds (electron donors) are found within a complex mixture. However, appreciation of stoichiometry would provide practitioners more confidence when calculating oxidant doses for treatment. A knowledge gap exists where a simple and **applicable** approach for determining the appropriate oxidant dose for treating OSPW or NAs in contaminated wastewater is needed.

As mentioned above, coupling of chemical and biological oxidation is of interest due to the effective and economical nature of this treatment train. With respect to the remediation of tailings ponds water the approach we are interested in investigating includes both *in situ* treatment and combined chemical and biological oxidation. In this scenario, the addition of chemical oxidants will cause an increase in extracellular concentration of oxidants rather than their intracellular concentration. This phenomenon causes oxidative stress for the organisms (Sies, 1997). Previous research in our lab demonstrated the ability of indigenous microorganisms to survive *in situ* ozonation with a dose of 50 mg/L (Brown et al., 2013). This provides a solid foundation that this behaviour will be mimicked for other oxidants as well. However, a knowledge gap exists since there is no physiological information (kinetics + stoichiometry) about the oxidative stress effect on the growth of *Pseudomonas* sp. on NAs.

Thermodynamics inform us about the limitation and capacities that we are having. This is not completely wrong to say that there is no study about the thermodynamics behind of biodegradation of NAs. Recent advancements in systems biology provide researchers the capacity to design new pathways for biodegradation of recalcitrant compounds (Finley et al., 2009; Finley et al., 2010). And with the knowledge of metabolic and genetic engineering it might be a hope that engineered strains can help us in treatment of NAs contaminated wastewater

(Finley et al., 2009; Singh et al., 2008). The first step in this process involves studying the thermodynamics (Finley et al., 2009).

1.2 Purpose of Study

The overall objective of this dissertation is to understand the stoichiometric, kinetic and thermodynamic components of AEOs and NAs treatment in OSPW using chemical oxidation; chemical oxidation coupled with biodegradation, and biodegradation.

Chemical oxidation of NAs in OSPW: persulfate has been studied as a potential oxidant for treatment of NAs in OSPW. Usually the first *question* a practitioner asks when using chemical oxidation as a treatment strategy (after ensuring the oxidant works) is *how much oxidant do we need?* Since we are dealing with a complex mixture of organics, which do not have elucidated chemical formulas, it is impossible to stoichiometrically calculate the dosage of oxidant needed. In this section, for the first time a model has been proposed based on the available electron of the solution for determination of needed oxidant. We hypothesized that by understanding the capability of the target solution for chemical oxidation, it is possible to develop a model for prediction of needed oxidant. The proposed model has been validated to confirm theoretical background and compared with literature to verify its advantages.

As mentioned above, using bioremediation techniques is almost every environmental engineer's goal! But NAs are partially biodegradable. Coupling chemical and biological treatment could be an alternative. But a *question* arises here! *"What would happen to microorganisms once they subjected to an oxidant? Are they still able to degrade NAs?*" In this step, we focused on the main ability of microorganisms which is degradation of NAs after oxidant exposure. In this chapter, for the first time physiological properties of the cell is studied to elucidate the effect of oxidative stress on biodegradation. We hypothesized that survival strategies of the cell would

effect on growth due to oxidative stress. In this chapter, for the first time physiological properties of the cell is studied to elucidate the effect of oxidative stress on biodegradation.

Due to the author's knowledge, systems biology approaches hasn't been considered for biodegradation of naphthenic acids. However, it is shown that systems biology tools are promising tools in engineering of microorganisms for desired purposes. In this thesis for the first time we conducted thermodynamic analysis as the fundamental step in the world of systems biology on bioremediation of model NAs. The aim of this work was to build up a foundation for genetic and metabolic engineers to improve the power of microorganisms in removal of NAs and understanding the limits in the world of NAs biodegradation.

1.3 Organization of Thesis

This thesis contains six chapters. Chapter 2 consists of a review of relevant literature and theoretical background that one might need to understand this work. Chapter 3 is about the proposing of a model based on the electron content of the contaminant and validation of the model, and comparison the results with a published peer-reviewed paper. Chapter 4 addresses the effect of oxidative stress on the growth of *Pseudomonas* sp. on Merichem NAs. Chapter 5 provides a thermodynamic analysis for biodegradation of NAs. In Chapter 6 conclusions and recommendations for future work are discussed.

1.4 References

Abolfazlzadehdoshanbehbazari M, Birks SJ, Moncur MC, Ulrich AC. 2013. Fate and transport of oil sand process-affected water into the underlying clay till: a field study. *J. Contam. Hydrol.* **151**:83–92.

Allen EW. 2008a. Process water treatment in Canada's oil sands industry: I. Target pollutants and treatment objectives. *J. Environ. Eng. Sci.* **7**:123–138.

Allen EW. 2008b. Process water treatment in Canada's oil sands industry: II. A review of emerging technologies. *J. Environ. Eng. Sci.* **7**:499–524.

Brown LD, Pérez-Estrada L, Wang N, El-Din MG, Martin JW, Fedorak PM, Ulrich AC. 2013. Indigenous microbes survive in situ ozonation improving biodegradation of dissolved organic matter in aged oil sands process-affected waters. *Chemosphere* **93**:2748–55.

Brown LD, Ulrich AC. 2015. Oil sands naphthenic acids: a review of properties, measurement, and treatment. *Chemosphere* **127**:276–90.

Camel V, Bermond A. 1998. The use of ozone and associated oxidation processes in drinking water treatment. *Water Res.* **32**:3208–3222.

Clemente JS, Fedorak PM. 2005. A review of the occurrence, analyses, toxicity, and biodegradation of naphthenic acids. *Chemosphere* **60**:585–600.

Drzewicz P, Perez-Estrada L, Alpatova A, Martin JW, Gamal El-Din M. 2012. Impact of peroxydisulfate in the presence of zero valent iron on the oxidation of cyclohexanoic acid and naphthenic acids from oil sands process-affected water. *Environ. Sci. Technol.* **46**:8984–91.

Finley SD, Broadbelt LJ, Hatzimanikatis V. 2009. Thermodynamic analysis of biodegradation pathways. *Biotechnol. Bioeng.* **103**:532–41.

Finley SD, Broadbelt LJ, Hatzimanikatis V. 2010. In silico feasibility of novel biodegradation pathways for 1,2,4-trichlorobenzene. *BMC Syst. Biol.* **4**:7.

Giesy JP, Anderson JC, Wiseman SB. 2010. Alberta oil sands development. *Proc. Natl. Acad. Sci. U. S. A.* **107**:951–2.

Government of Alberta. 2010. Tailings.

Government of Alberta. 2016. Oil sands quarterly. Open Text Web Site Management.

Grewer DM, Young RF, Whittal RM, Fedorak PM. 2010. Naphthenic acids and other acid-extractables in water samples from Alberta: what is being measured? *Sci. Total Environ.*

408:5997–6010.

Guzel-Seydim ZB, Greene AK, Seydim AC. 2004. Use of ozone in the food industry. *LWT - Food Sci. Technol.* **37**:453–460.

Headley J V, Peru KM, Mohamed MH, Frank RA, Martin JW, Hazewinkel RRO, Humphries D, Gurprasad NP, Hewitt LM, Muir DCG, Lindeman D, Strub R, Young RF, Grewer DM, Whittal RM, Fedorak PM, Birkholz DA, Hindle R, Reisdorph R, Wang X, Kasperski KL, Hamilton C, Woudneh M, Wang G, Loescher B, Farwell A, Dixon DG, Ross M, Pereira ADS, King E, Barrow MP, Fahlman B, Bailey J, McMartin DW, Borchers CH, Ryan CH, Toor NS, Gillis HM, Zuin L, Bickerton G, Mcmaster M, Sverko E, Shang D, Wilson LD, Wrona FJ. 2013. Chemical fingerprinting of naphthenic acids and oil sands process waters-A review of analytical methods for environmental samples. *J. Environ. Sci. Health. A. Tox. Hazard. Subst. Environ. Eng.* **48**:1145–63.

Headley J V., McMartin DW. 2004. A review of the occurrence and fate of naphthenic acids in aquatic environments. *J. Environ. Sci. Heal. - Part A Toxic/Hazardous Subst. Environ. Eng.*

Holden AA, Haque SE, Mayer KU, Ulrich AC. 2013. Biogeochemical processes controlling the mobility of major ions and trace metals in aquitard sediments beneath an oil sand tailing pond: laboratory studies and reactive transport modeling. *J. Contam. Hydrol.* **151**:55–67.

Holden AA, Donahue RB, Ulrich AC. 2011. Geochemical interactions between process-affected water from oil sands tailings ponds and North Alberta surficial sediments. *J. Contam. Hydrol.* **119**:55–68.

Holowenko F. 2002. Characterization of naphthenic acids in oil sands wastewaters by gas chromatography-mass spectrometry. *Water Res.* **36**:2843–2855.

Hwang G, Dong T, Islam MS, Sheng Z, Pérez-Estrada LA, Liu Y, Gamal El-Din M. 2013. The impacts of ozonation on oil sands process-affected water biodegradability and biofilm formation characteristics in bioreactors. *Bioresour. Technol.* **130**:269–77.

Klamerth N, Moreira J, Li C, Singh A, McPhedran KN, Chelme-Ayala P, Belosevic M, Gamal El-Din M. 2015. Effect of ozonation on the naphthenic acids' speciation and toxicity of pH-dependent organic extracts of oil sands process-affected water. *Sci. Total Environ.* **506-507**:66–75.

MacKinnon MD, Boerger H. 1986. DESCRIPTION OF TWO TREATMENT METHODS FOR DETOXIFYING OIL SANDS TAILINGS POND WATER. *Water Pollut. Res. J. Canada*

21:496–512.

Martin JW, Barri T, Han X, Fedorak PM, El-Din MG, Perez L, Scott AC, Jiang JT. 2010. Ozonation of oil sands process-affected water accelerates microbial bioremediation. *Environ. Sci. Technol.* **44**:8350–6.

Meredith W, Kelland S-J, Jones D. 2000. Influence of biodegradation on crude oil acidity and carboxylic acid composition. *Org. Geochem.* **31**:1059–1073.

Morandi GD, Wiseman SB, Pereira A, Mankidy R, Gault IGM, Martin JW, Giesy JP. 2015. Effects-Directed Analysis of Dissolved Organic Compounds in Oil Sands Process-Affected Water. *Environ. Sci. Technol.* **49**:12395–404.

Muñoz I, Rieradevall J, Torrades F, Peral J, Domènech X. 2005. Environmental assessment of different solar driven advanced oxidation processes. *Sol. Energy* **79**:369–375.

Nascimento LR, Rebouças LMC, Koike L, de A.M Reis F, Soldan AL, Cerqueira JR, Marsaioli AJ. 1999. Acidic biomarkers from Albacora oils, Campos Basin, Brazil. *Org. Geochem.* **30**:1175–1191.

Oiffer AAL, Barker JF, Gervais FM, Mayer KU, Ptacek CJ, Rudolph DL. 2009. A detailed field-based evaluation of naphthenic acid mobility in groundwater. *J. Contam. Hydrol.* **108**:89–106.

Oller I, Malato S, Sánchez-Pérez JA. 2011. Combination of Advanced Oxidation Processes and biological treatments for wastewater decontamination--a review. *Sci. Total Environ.* **409**:4141–66.

Rittmann B, McCarty P. 2012. Environmental biotechnology: principles and applications.

Riva JP. 1983. World petroleum resources and reserves. Westview Press 355 p.

Roques DE, Overton EB, Henry CB. 1994. Using Gas Chromatography/Mass Spectroscopy Fingerprint Analyses to Document Process and Progress of Oil Degradation. *J. Environ. Qual.* **23**:851.

Sies H. 1997. Oxidative stress: oxidants and antioxidants. *Exp. Physiol.* **82**:291–295.

Singh S, Kang SH, Mulchandani A, Chen W. 2008. Bioremediation: environmental clean-up through pathway engineering. *Curr. Opin. Biotechnol.* **19**:437–44.

Sohrabi V, Ross MS, Martin JW, Barker JF. 2013. Potential for in situ chemical oxidation of acid extractable organics in oil sands process affected groundwater. *Chemosphere* **93**:2698–703.

Speight JG. Liquid Fuels from Oil Sand 197–221. http://www.drjamesspeight.qpg.com/.

Tchobanoglous G, Burton FL, Stensel HD, Eddy M&. 2003. Wastewater Engineering: Treatment and Reuse. McGraw-Hill Education 1819 p.

Tissot BP, Welte DH. 1978. Petroleum Formation and Occurrence. Berlin, Heidelberg: Springer Berlin Heidelberg.

Veschetti E, Cutilli D, Bonadonna L, Briancesco R, Martini C, Cecchini G, Anastasi P, Ottaviani M. 2003. Pilot-plant comparative study of peracetic acid and sodium hypochlorite wastewater disinfection. *Water Res.* **37**:78–94.

Watson J., Jones D., Swannell RP., van Duin AC. 2002. Formation of carboxylic acids during aerobic biodegradation of crude oil and evidence of microbial oxidation of hopanes. *Org. Geochem.* **33**:1153–1169.

Zhang X, Wiseman S, Yu H, Liu H, Giesy JP, Hecker M. 2011. Assessing the toxicity of naphthenic acids using a microbial genome wide live cell reporter array system. *Environ. Sci. Technol.* **45**:1984–91

CHAPTER 2. LITERATURE REVIEW

2.1 Introduction

Canada possesses the third largest oil sands deposit in the world with ~173 billion barrels of oil reserves. The common strategy for extraction of bitumen from oil sands is Clark hot water method. For this method of extraction, the volumetric ratio of used water and produced oil is more than seven (~7.8) (Government of Alberta, 2016). Therefore, a huge volume of process-affected water is produced in the extraction process. Due to zero discharge policy, all of the process-affected water should be stored in instructed tailings ponds (Government of Alberta, 2016). However, 80 to 85% water recycling in oil sands project is one of the positive points in water management (Allen, 2008a; Giesy et al., 2010; Government of Alberta, 2016). Long term storage of tailings ponds are associated with serious problems i.e. greenhouse gas emission (Government of Alberta, 2016; Holowenko et al., 2011; Iranmanesh et al., 2014), leakage to groundwater channels (Abolfazlzadehdoshanbehbazari et al., 2013; Holden et al., 2013; Ross et al., 2012), the toxicity of oil sands process-affected water (OSPW) to (micro-) organisms(Garcia-Garcia et al., 2011; Shu et al., 2014; Wang et al., 2013a). Accordingly, there is no doubt treatment of wastewater is tailings ponds is a must(Allen, 2008b). Efforts in the treatment of OSPW suggested that naphthenic acids are the target compounds that should be considered the main concerns in treatment processes (Grewer et al., 2010; Headley and McMartin, 2007). As followed in this chapter, naphthenic acids are introduced and conducted approaches and ideas for treatment of OSPW and naphthenic acids contaminated wastewater is discussed.

2.2 Naphthenic Acids

NAs are classified as a group of alkyl-substituted acyclic and cycloaliphatic carboxylic acids with the conventional formula of $C_nH_{2n+z}O_2$; where n shows the carbon number and Z indicates the hydrogen deficiency because of the ring formation (Clemente and Fedorak, 2005; Grewer et al., 2010). The Z is an even integer number which varies between 0 to -12. Saturated ring-containing compounds typically have five or six carbon atoms in their structure where each multiple of -2 represents another ring. In general, there are three main components in their structure: 1. cycloalkane rings, 2. an aliphatic chain, and 3. a carboxylic group. The carbon number (n) ranges from 5 to 33 and therefore the molecular weight ranges from 100 to 500 g/mol (Brown and Ulrich, 2015; Clemente and Fedorak, 2005; Headley and McMartin, 2004).

Detailed analyses of NAs in OSPW indicate some deviation in chemical structure and classic formula of $C_nH_{2n+z}O_2$. Results from elemental mass spectra are representing that <20% of the peaks related to classical NAs (2-oxygen) and oxy-NAs (3 to 5 atom oxygen). The deviation from the classic formula is not limited to differences in number of oxygen. Unsaturated bonds, aromatic compounds and presence of nitrogen and sulfur are also other deviations that occur from the classic formula (Grewer et al., 2010). It can be understood that the term of "NAs" does not completely cover the range of organic acids in OPSW matrix. Therefore, Grewer et al. (2010) suggested the term "oil sand tailing water acid extractable organics (OSTWAEO) or acid extractable organics (AEOs)" instead of NAs. The term "acid" refers to the procedure used when extracting and analysing water samples. Fig 1.2 shows the range of chemical structures found in AEOs.

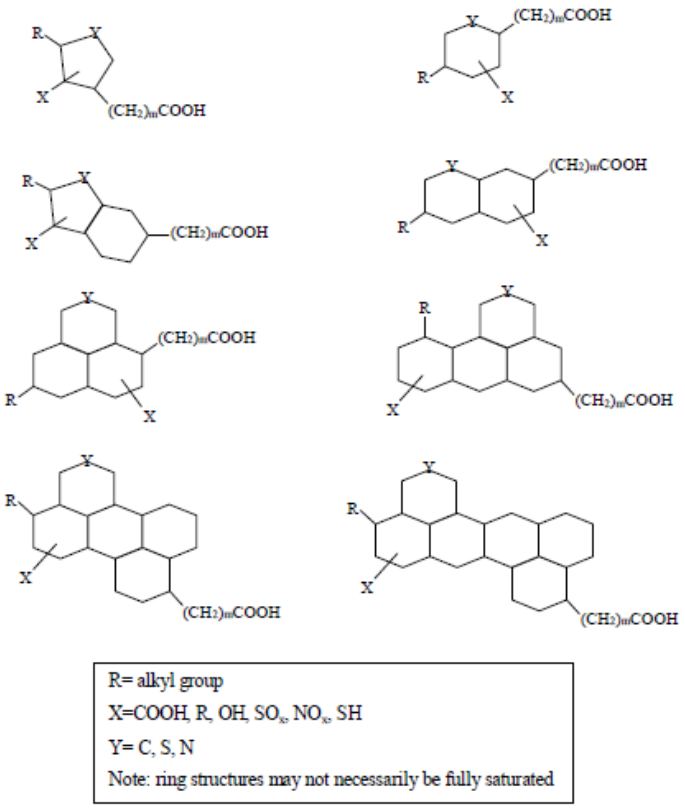


Figure 2-1 represents the structure of NAs fraction in acid extractable organics of OSPW. It can be seen that –COOH or carboxyl functional group is shared among any fraction. Other elements can be presented or not (reprinted with permission from Headley et al., (2007))

2.2.1 Properties of Naphthenic Acids

The chemical and structural composition of a mixture plays an important role in determining the chemical and physical properties of the mixture (Kannel and Gan, 2012). However, there are some general physical and chemical properties shared among the group of NAs compounds. Their vapor pressure is ~ 2.4×10^{-6} atm (Rogers, 2002). As the molecular weight of NAs increases their polarity and non-volatility also increase. The boiling point of NAs varies between 250°C and 350°C (J.A. Brient, P.J. Wessner, 1995). Their dissociation constant (pK_a) ranges from five to six (Kanicky et al., 2000). In aqueous solutions the solubility of NAs directly

depends on the pH of the solution (Quagraine et al., 2005). Organic solvents like dichloromethane and methanol completely dissolve NAs. In Table 1.2 some general properties of NAs are summarised.

Table 2-1 General physical and chemical properties of NAs adapted from Quinlan and Tam, (2015).

Parameter	General characteristics
Color	Pale yellow to dark amber
State	Viscous liquid
Molecular weight	140 - 450 g $mole^{-1}$
Density	0.97 – 0.99 gcm^{-3}
Water solubility	~ 50 mgL^{-1}
Dissociation constant	5 – 6
Boiling point	250 - 350°C
Acid number	150 – 310 mg KOH g^{-1}
Refractive index	~1.5

NAs demonstrate hydrophilic properties because of the carboxyl functional group in their structure. On the other hand, they have an alicyclic end which creates hydrophobic properties (Brown and Ulrich, 2015a). This structure has NAs behave as a surfactant (Headley and McMartin, 2004). These surfactant properties improve the bitumen extraction process at alkaline conditions by increasing the efficiency of bitumen recovery (Allen, 2008a). .

2.2.2 Application of Napphthenic Acids

Naphthenic acids, naphthenic acids esters, and their metal salts have various applications in industry. Some industrial usages include: wood preservative by inhibiting the growth of fungi, flame retardants in fabrics, and adhesion promoters in tire manufacturing (J.A. Brient, P.J. Wessner, 1995). To make commercial NAs, they are further refined at 200 to 370°C through caustic extraction followed by ethanol extraction to eliminate unsaponifiable material. The

purified extract will then be acidified to get NAs into their protonated form (J.A. Brient, P.J. Wessner, 1995).

2.2.3 Disadvantages of Naphthenic Acids

2.2.3.1 Corrosive to process infrastructures

NAs have been found to be corrosive to extraction facilities (Derungs, 1956). Crude oils with high concentration of NAs must be refined and processed in units with stainless steel infrastructure (Slavcheva et al., 1999). The mechanism of corrosion by NAs is not yet fully understood. However, it has been proposed that at high temperatures multimers in NAs dissociate into their monomer (Chakravarti et al., 2013). Monomers near a metallic surface have the tendency to form metal carboxylates. To understand the synthesis of metal carboxylates three different complexations have been proposed: 1st: bidentate bridging, 2nd: bidentate chelating, and 3rd: monodentate bridging (Chakravarti et al., 2013). The proposed complexes are shown in Figure 2.2.

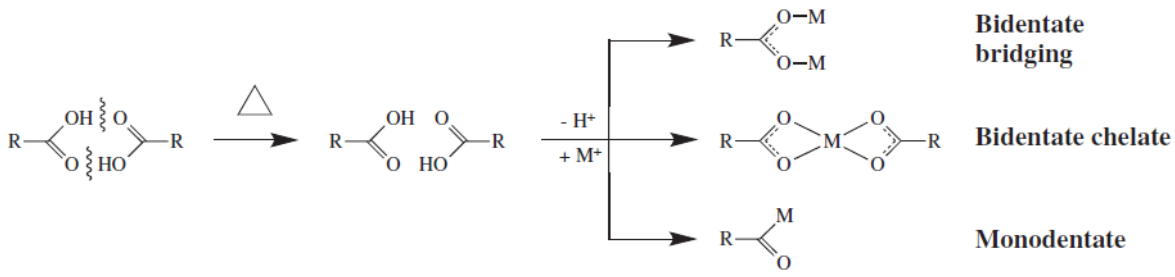


Figure 2-2 Proposed corrosion mechanisms by NAs at high temperature (reprinted with permission from Chakravarti et al., (2013)).

As discussed in introduction, since hot water extraction is used for extraction of oil sands, more than 3 times of water is consumed to extract oil in unit of volume. Accordingly, huge volume of

water needs to be managed to reduce the environmental and financial cost of the process. The 80 to 85% water recycling rate greatly reduces the dependency of oil sands operations on fresh water (Allen, 2008a). The disadvantage of the recycling process includes progressive concentration of contaminants in the process affected water in tailings ponds (Allen, 2008a).

2.2.3.2 AEOs Toxicity

Research has demonstrated that NAs cause acute and chronic toxicity to a broad range of organisms such as microorganisms, plants, birds, invertebrates and mammals (Headley and McMartin, 2004; Kindzierski et al., 2012; Miskimmin et al., 2010). However, measuring NAs concentration is insufficient for determining a relation between its concentration and toxicity level (Clemente and Fedorak, 2005; Kindzierski et al., 2012). It is interesting that Headley and McMartin (2004) discussed the contribution of surfactant behaviour of NAs to its toxicity. This phenomenon called narcosis is where cell death (cell damage) occurs because of the entrance of a hydrophobic compound into the lipid bilayer of cell membrane (Frank et al., 2009; Frank et al., 2010; Tollefsen et al., 2012).

There are several extensive reviews on OSPW and NAs toxicity. As previously described, OSPW has different portions of organics i.e. NAs and AEOs. For instance, AEOs represent the acidic organics in the OSPW matrix. If toxicity tests are completed using AEOs, this procedure neglects the other organics i.e. neutral organics. Recently, Morandi et al. (2015) conducted an interesting experiment to study the contribution of different organics to acute toxicity of OSPW (Morandi et al., 2015). The contribution of each portion was based on an extraction completed at different pHs. They used two different toxicity tests a 96 h fathead minnow embryo lethality test and a 15 min Microtox bioassay. Based on the results, the NAs (O_2^-) are among the most

acutely toxic (organic) compounds in OSPW. However, nonacidic species (O^+, O_2^+, SO^+) also cause acute toxicity (Morandi et al., 2015).

Since the AEOs and NAs are a complex group of compounds they are challenging to analyse. The following section discusses the analysis of NAs where different instruments can give us a different understanding of the system.

2.2.4 Quantification of Naphthenic Acids

Fourier Transform Infra-Red (FT-IR) has been utilized to analyse NAs and AEOs extensively for academic and industrial purposes (Brown and Ulrich, 2015a; Jiveraj, 1995). Its popularity is due to the fact that it is industries standard method for quantifying NAs. The instrument is user friendly and analysis can be completed in a short time. In this method, a sample is first acidified to a pH<2. To extract AEOs which contain NAs an extraction step is completed with an appropriate organic solvent such as dichloromethane (Brown and Ulrich, 2015). During analysis FTIR absorbance intensities of monomeric (single bond with a peak height of 1705 cm-1) and dimeric (double bond with the peak height of 1743 cm-1) form of carboxylic groups ae summed up to measure the concentration of NAs (Jiveraj, 1995). One should realize that FTIR has given us the concentration of total carboxylic acids in a sample since it measures only the carboxyl group.

Gas Chromatography is another technique used for AEOs measurements (Holowenko, 2002). Samples after extraction need to be derivatized to transform NAs in AEOs to methyl esters. The chromatogram results are shown as an unresolved hump (Brown and Ulrich, 2015). To analyse the data, the integration of the hump is compared with the area of an internal standard.

Mass spectroscopy is one of the most common tools for NAs analysis. In this method one can identify AEOs by plotting the relative response of each mass with respect to their n (number of carbon) and Z (hydrogen deficiency) (Headley et al., 2013). Fig 2.2 illustrates the profile of AEOs in OSPW. There are extensive reviews on the analysis of NAs with different resolution techniques (Brown and Ulrich, 2015; Headley et al., 2013).

2.2.5 Treatment of OSPW and NAs contaminated wastewater

Based on aforementioned reasons, there is no doubt we need to remove NAs from waste streams. To have a better understanding of available treatment technologies we will discuss both chemical and biological treatments.

2.2.5.1 Chemical Treatment

Chemical treatment refers to the application of specific chemicals which react with a pollutant and degrade them into environmentally safe end-products (Kabdasli and Arslan-Alaton, 2010). A common practice is to use chemical oxidants which are a group of compounds that have high redox potential (Siegrist et al., 2011). Oxidants accept electrons (from a pollutant in our case), convert to their reduced form and leave the pollutant in an oxidized form. Organic pollutants mainly exist in the reduced form of carbon where they have available electrons in their structure (Tchobanoglous et al., 2003). To degrade reduced compounds they need to be oxidized. This reaction happens by means of an oxidant. One question arises here! How can we know a given compound can be an oxidant?

The general format of writing a redox reaction has been shown below (Bard and Faulkner, 2000):

$$\text{Reduced compound} + \text{electron} \longrightarrow \text{Oxidized compound} \qquad (2.1)$$

$$[Re + e^- \longrightarrow Ox]$$

Each half reaction has its own redox potential or cell potential that can be calculated based on this formula (Bard and Faulkner, 2000):

$$E_{cell} = E^0_{cell} - \frac{RT}{nF} \ln Q \quad (2.2)$$

E^0_{cell}: is a standard cell potential at standard conditions (T= 25°C ; P= 1 atm; 1 molar concentration) [V volt]

n: number of electrons transferred

R: ideal gas law constant (8.314 J mol^{-1} K^{-1})

F: Faraday constant (96500 J mol^{-1} V^{-1})

Q: reaction quotient

The reaction represents the ratio of products and reactants. For instance for a given reaction:

$$aA + bB \leftrightarrow cC + dD \quad (2.3)$$

The reaction quotient is:

$$Q = \frac{C_C^C C_D^D}{C_A^a C_B^b} \quad (2.4)$$

Where equilibrium constant is:

$$K_{eq} = \frac{C_{C,eq}^C C_{D,eq}^D}{C_{A,eq}^a C_{B,eq}^b} \quad (2.5)$$

Based on thermodynamic laws we know that for a spontaneous reaction to occur the Gibbs free energy needs to be negative ($\Delta G < 0$). Gibbs free energy and redox potential are related as follows (Bard and Faulkner, 2000):

$$\Delta G = - n F E^0_{cell} \quad (2.6)$$

Based on this relationship when E^0_{cell} is positive, the Gibbs free energy is negative which means accepting electrons can occur spontaneously. The higher the redox potential the stronger the oxidant.

The three main things to consider when choosing a suitable oxidant for treatment purposes include (Siegrist et al., 2011):

1. Stability in the environment
2. Selective reactivity to target pollutant
3. Redox potential of oxidant (oxidative power)

There are four commonly used oxidants in wastewater treatment based on chemical oxidation (shown in Table 2-2).

Table 2-2 Common oxidants for chemical oxidation treatment goals (Siegrist et al., 2011).

Oxidant	**Chemical Formula**	**Redox Potential (V)**
Hydroxyl Radical	$HO^{-}\cdot$	2.7
Sulfate Radical	$SO_4^{2-}\cdot$	2.6
Ozone	O_3	2.2
Persulfate	$S_2O_8^{2-}$	2.1
Hydrogen Peroxide	H_2O_2	1.8
Permanganate	MnO_4^{-}	1.7

Generally, advanced oxidation processes (AOPs) are more applicable and efficient than oxidation processes (Oturan and Aaron, 2014). AOPs refer to an oxidation process with hydroxyl radicals (HO·) (Oturan and Aaron, 2014). AOPs are more efficient because hydroxyl radicals are very reactive to many organic pollutants and have high oxidizing power. There are different ways to produce hydroxyl radicals, for example by activation of H_2O_2 by means of transitional metal catalysts, UV light and ozone (Oturan and Aaron, 2014).

Due to the high degree of saturation in NAs they are a good candidate for treatment with hydroxyl radicals (Andreozzi, 1999; Quinlan and Tam, 2015). Oxidation with HO· typically occurs by hydrogen abstraction of the organics, in our case AEOs and NAs. The hydroxyl radicals react with the NAs converting them to highly reactive organic radicals (Quinlan and Tam, 2015). By progressing the oxidation reaction and propagation of the radical through the organic compounds, complete degradation can be achieved. Complete oxidation (mineralization) occurs when stable end products such as carbon dioxide, water and salts are produced.

Ozone (O_3) is another favorable oxidant for the degradation of NAs (Andreozzi, 1999). Two common ways to produce HO· with O_3 is through alkaline pH or catalytic decomposition (Oturan and Aaron, 2014). There is extensive research on ozonation of OSPW and elucidation of the structure-reactivity relationship for NAs with ozone or H_2O_2/UV (Klamerth et al., 2015; Pérez-Estrada et al., 2011; Scott et al., 2008; Wang et al., 2013b).

Other chemical approaches for treatment of NAs and OSPW have also been investigated. Mishra et. al. (2010) studied the treatment of commercial NAs and AEOs in OSPW with microwave in the presence of TiO_2. An energy efficient method based on solar UV/chlorine advanced oxidation process has also been reported (Shu et al., 2014). Using Gamma ray irradiation is one of the newest technologies for removal of NAs and other organic contaminants (Weisener et al., 2013; Jia et al., 2015). Gamma rays electronically excite water molecules, which result in the production of hydroxyl radicals and other molecules like hydrogen and hydrogen peroxide (Jia et al., 2015). It is interesting to mention that so far Gamma ray irradiation has been environmentally friendly, cost effective and high efficiency in removal of pollutants in lab scale research (Weisener et al., 2013; Jia et al., 2015).

2.2.5.2 Biological Treatment

Bioremediation is an economical and effective technique for the degradation of organic compounds. Bioremediation is based on the ability of microorganisms to consume organic compounds as their source of energy and carbon. Microorganisms can take up soluble organic components and leave carbon dioxide (in the case of complete mineralization), water, and new biomass as the end products of their metabolism. Bioremediation techniques can use mixed microbial cultures or pure cultures (Alexander, 1985; Alexander, 1999; Rittmann and McCarty, 2012; Tchobanoglous et al., 2003).

NAs are good candidates as biomarkers for oil source maturation because of their recalcitrance. Used as a wood preservative (in the form of metal naphthenates) further indicates their resistance to biological degradation. Recalcitrance does not mean the compounds cannot biologically degrade at all. Sometimes these organic compounds are partially biodegradable. Biodegradation of NAs was reported for the first time by Herman et al. (1994) (Herman et al., 1994). There are a number of microorganisms that can grow on NAs, either model or mixture, in pure culture for example *Acinetobacter anitratum* (Rho and Evans, 1975) , *Alcaligenes faecalis* (Blakley, 1974; Whitby, 2010) and *Pseudomonas putida* (Blakley and Papish, 1982). There have been several studies documenting the biodegradation of commercial NAs and AEOs in OSPW mainly under aerobic conditions (Brown and Ulrich, 2015; Whitby, 2010). There is also an extensive review on biodegradation of NAs (Clothier and Gieg, 2016; Whitby, 2010; Yue et al., 2015). One point about biodegradation of NAs is the remaining presence of certain NAs in aged oil sands tailings ponds that are resisting biodegradation. This fact confirms that NAs are partially biodegradable.

Natural attenuation, the process where indigenous microorganisms biodegrade organic compounds *in situ* has been observed for NAs in tailings ponds. NAs with a high degree of

branching are shown to be more resistant to biodegradation by indigenous microorganisms. Similar to AOPs (or any other case of bioremediation of specific organic compounds) there are some relations between structure and biodegradation of NAs. For instance, aerobic batch biotransformation assay with NAs enriched mixed culture has shown that NAs with a quaternary carbon at the α- and β-position are non-biodegradable or have limited degree of biotransformation (Misiti et al., 2014a). In biodegradation of 4-methyl-cyclohexane acetic acid ($C_9H_{16}O_2$), *trans*- isomers have higher (~2 times) biodegradation rate compare to *cis*- isomers which shows the effect of alkyl side branching as one of the factors in biodegradability of NAs (Huang et al., 2012). Results has shown that biodegradability of NAs decreases as side chain increases (Johnson et al., 2011; Smith et al., 2008).

Approximately, the required time to achieve 50% biodegradation of commercial NAs strongly depends on the Z number, which change from 1 to 8 days. A comparison of biodegradation of commercial NAs and NAs in AEOs of OSPW can be seen in Fig 2-3.

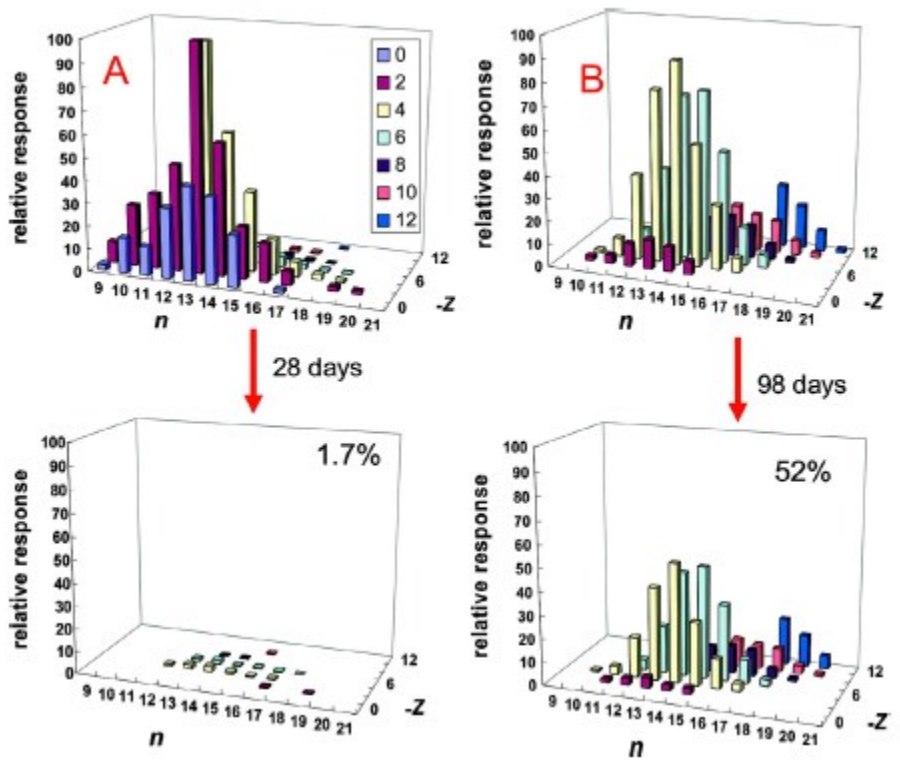


Figure 2-3. Difference between biodegradation of commercial NAs and NAs in AEOs from OSPW (reprinted with permission from Han et al (2008)).

Graph A shows the biodegradation of Merichem NAs where only 1.7% of NAs remains after 28 days of incubation. Graph B shows the biodegradation of NAs in AEOs in OSPW where 52% of the initial concentration remains after 98 days of incubation. The remaining fraction is mostly NAs with more rings and higher molecular weight. One of the reasons why commercial NAs are more biodegradable than AEOs in OSPW is that commercial NAs are from petroleum sources that have **not** undergone extensive biodegradation. NAs in OSPW (AEOs) have already undergone biodegradation in tailings ponds. From a structural point of view, NAs in OSPW have more alkyl chain branches and have more cyclic-compounds in their structure than commercial NAs. These two properties are unfavorable from a biodegradation perspective.

Biodegradation of model NAs has also been conducted under anoxic conditions (Clothier and Gieg, 2016; Dong and Nemati, 2016; Gunawan et al., 2014). Gunawan et al. (2014) studied the

biodegradation of trans-4-methyl-1-cyclohexane carboxylic under denitrifying conditions. Under denitrificating conditions, NAs act as the electron donor and carbon source and nitrate acts as the electron acceptor where it is reduced to N_2 gas.

2.2.5.3 Coupling of Chemical and Biological Remediation

To optimize the removal of NAs and AEOs in OSPW the coupling of chemical (fast yet expensive) and biological (slow yet affordable) treatment techniques is beneficial (Oller et al., 2011). This concept is under development for the oil sands industry for treatment of OSPW. Ozonation as a pre-treatment followed by aerobic biodegradation has shown an increase in biodegradability of NAs as a result of ozonation pre-treatment (Brown et al., 2013; Martin et al., 2010; Vaiopoulou et al., 2015). Based on Vaiopoulou et al.'s results, preferential degradation of polycylclic and high molecular weight NAs by ozonation lead to a removal efficiency up to 89%. Toxicity tests showed 15 times less toxic water as compared to untreated water (Vaiopoulou et al., 2015).

One important point for consideration when coupling AOPs and biological remediation for *in situ* remediation is the impact of the oxidant on the microorganisms. For *in situ* conditions a pond will act as a reactor for both chemical oxidation and biological degradation. Strong oxidants like ozone have been widely used as antimicrobial agents for disinfection purposes (Camel and Bermond, 1998; Guzel-Seydim et al., 2004; Tchobanoglous et al., 2003). So how will a mixed culture of microorganisms or a pure culture respond to the oxidation step in an *in situ* application? For elucidating this phenomena we need to understand why oxidation pre-treatment is harmful to microorganisms.

2.3 Oxidative Stress

Oxidative stress is defined by Sies et. al. (1997) as "the imbalance between oxidants and antioxidant in favor of the oxidants, potentially leading to damage." (Sies, 1997). Oxidants have a high redox potential and can also produce high energy radicals (Tchobanoglous et al., 2003). In biology, specifically in aerobic metabolism there are some intracellular oxidants. For example, reduction of oxygen to water accompanied by three reduction step. The addition of electron to oxygen produces superoxide (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($HO^{\cdot}$), respectively (Imlay, 2013).

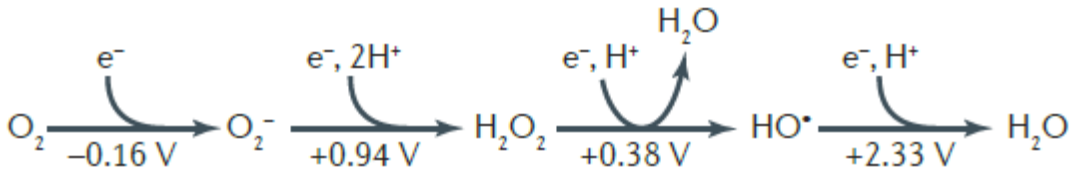


Figure 2-4. Reduction of oxygen to water; the redox potential of O_2; O_2^-; H_2O_2; $HO^{\cdot}$ are presented (in volt), respectively; potentially all compounds except oxygen are oxidant. (reprinted with permission from Imlay. (2013)); The oxidants in this figure are known as the intracellular oxidants. However, environmental (extracellular) oxidizing agents can cause injuries to cell.

Reduction takes place by one electron, two electron and three electron with the formation of superoxide anion radical, hydrogen peroxide and the hydroxyl radical, respectively (Pruchniak et al., 2015). Due to the high energy level of oxidants and their corresponding radicals, they have devastating effect for cell organelles that can be resulted in death of the cell (Lambert et al., 2001). On the other hand, antioxidants are the compounds that have high reductive power. It means that they are willing to donate electron. Obviously, oxidants and anti-oxidants, as its obvious from the names, have exact opposite function in nature. Therefore, antioxidants are very

useful in neutralizing the oxidants by reducing their redox potential through accepting electrons. Generally, an antioxidant is any compound, enzymatic or non-enzymatic, that can protect an oxidizable substrate from being oxidized by an oxidant (Halliwell & Gutteridge, 2007).

Oxidative stress also happens through *in vitro* circumstances (Chapman, 2003). One such example is the application of disinfectants like quaternary ammonium compounds, ozone, and hypochlorite for disinfection purposes (Tezel and Pavlostathis, 2015;Stewart et al., 2001). The disinfectant concentration is applied is in the order of hundreds to thousands times higher than their minimum inhibitory concentration (MIC) (Andersson and Hughes, 2014). MIC is the minimum concentration that prevents the observable growth of a given bacterial population (Lambert et al., 2001;Andrews, 2001). Exposure of microbial populations to antimicrobial agents below their MIC concentrations can have three different effects (Andersson and Hughes, 2014; Lorian, 1975):

I. Selection of strategy for resistance (enrichment of resistant bacteria in mixed cultures and selection of *de novo* methods)
II. Cause variation in genetic and phenotypic properties by enhancing the ability for adaptive evolution and modification in resistance strategy
III. Change in physiological activities, biofilm formation or in general gene expression

The last effect, variation in **physiological** properties or adaptive responses, is the main bridge between **environmental engineering goals and oxidative stress effect**. In chapter 4 the impact of oxidative stress due to persulfate on a *Pseudomonas* sp. will be determined.

2.4 Bioremediation Kinetics and Stoichiometry

When studying microbial growth kinetics (suspended cells) three different cultivation systems are typically used: batch, continuous and fed-batch (Rittmann and McCarty, 2012). In batch systems, the decrease in substrate concentration and increase in biomass is monitored over time (Kristiansen, 1999; Rittmann and McCarty, 2012; Tchobanoglous et al., 2003). In continuous systems the substrate and biomass concentration is constant over time (Kovarova-Kovar and Egli, 1998). In other words, substrate and biomass concentration is time-dependent in a batch system and time independent in a continuous system (Kovarova-Kovar and Egli, 1998). In continuous systems (chemostat), microorganism(s) cultivate under stable environmental condition i.e. dilution rate (Doran, 1995). Thus, in an ideal chemostat more precise, reproducible and statistically relevant data can be obtained as compared to a batch system (steady state vs. dynamic). However, data from a continuous system are not a good representation of growth conditions found in nature. Generally, in a real environment microbial growth is best represented somewhere between a closed system (batch) and open system (chemostat) (Kovarova-Kovar and Egli, 1998).

There are two main concepts related to microbial growth: (1) nutrient limitation, and (2) substrate limited growth. It should be noted that “limitation” refers to the stoichiometric aspects of growth (since “limitation” indicates the amount or concentration!). Nutrient limitation is when the nutrient amount becomes a decisive factor in biomass production. In other words, growth is a function of the nutrient concentration. Secondly, substrate limited growth refers to the rate of biosynthesis of new biomass governed by the specific substrate(s) concentration (Bailey, 1986; Clark and Blanch, 1995; Kovarova-Kovar and Egli, 1998; Kristiansen, 1999).

2.4.1 Kinetic and Stoichiometric Analysis of bioremediation: Quantitative Physiology

Growth of microorganism(s) on a substrate can be challenged by two fundamental questions: How fast (kinetic) and how much (stoichiometry)? In the case of biodegradation, there are four parameters that can elucidate the kinetics and stoichiometry of a given system (Kovárová et al., 1996; Nielsen et al., 2012; Rittmann and McCarty, 2012).

Two of them are **kinetic** parameters:

- μ^{max} : biomass maximum specific growth rate [time $^{-1}$]
- K_s : substrate affinity constant [mass/volume]

The other two are **stoichiometric** parameters:

- Y : yield (ratio of rates)
- m_s : threshold concentration of substrate [mass/volume]

To develop a better understanding of these parameters the following mathematical relationships will be described.

The Monod equation has been widely using to describe the kinetics of substrate depletion (biodegradation) and biomass production (Monod, 1949).

$$\mu = \mu^{max} \frac{C_s}{K_S + C_s} \tag{2.7}$$

$$r_s = \frac{dC_s}{dt} = \frac{1}{Y_{sx}} \mu^{max} \frac{C_s}{K_S + C_s} C_x \tag{2.8}$$

$$r_x = \frac{dC_x}{dt} = \mu^{max} \frac{C_s}{K_S + C_s} C_x \tag{2.9}$$

$$Y_{sx} = \frac{dC_x}{dC_s} = \frac{r_x}{r_s} \tag{2.10}$$

In the above equations, C_s substrate concentration and C_x biomass concentration are dependent variables and t time is an independent variable. μ is specific biomass growth rate which describes the rate of biosynthesis of new cells per amount of present biomass (Bailey, 1986; Monod, 1949; Nielsen et al., 2012; Rittmann and McCarty, 2012). Eq. 2.8 describes the rate of the substrate consumption. It expresses how fast substrate is consumed in a bioreactor by means of microorganisms. It can be seen that, r_s has a direct relation to concentration of biomass. However, in engineering working with independent parameters are more desirable. Because in design problems or in analysis of a system, less parameters need to be considered, which is good! In section 2.3.2 independent rates equation are described.

Eq. 2.10 expresses the yield of substrate to biomass. It shows the amount of substrate produced per unit of consumed substrate. Logically, this amount (yield) can be obtained by diving the rates. This parameter is one of the most important physiological parameter in design and analysis of a biosystem. Because this parameter tells us the amount of biomass is produced in a bioreactor and amount of biomass is very important in optimization purposes. Therefore, by knowing two of the following quantities we can calculate another one: substrate consumption rate, biomass production rate and yield. One should be realized that since yield is the ratio of two rates, obtained results is independent of time. Since μ describes the rate of biomass production we can correlate this expression to the doubling time $\mathbf{t_d}$ [time] of the cells.

$$t_d = \frac{\ln 2}{\mu} \tag{2.11}$$

K_s is the concentration of substrate where $\mu = 0.5\ \mu^{max}$. K_s can also be used as an affinity constant of the substrate toward the cell (Kovárová et al., 1996; Kristiansen, 1999). Based on definition, $1/K_s$ demonstrates the affinity of the **cells** to substrate.

A term **not** considered in the Monod model is m_s, which is related to maintenance energy - the amount of substrate cells need to survive at zero growth rate (Alexander, 1999; Kovarova-Kovar and Egli, 1998; Kristiansen, 1999). Maintenance is also known as the amount of substrate needed for endogenous metabolism and biosynthesis of new cells (Stephanopoulos et al., 1998). There are other models, such as the Herbert-Pirt model (Pirt, 1965), which consider the maintenance term when calculating the substrate uptake rate (Kristiansen, 1999; Nielsen et al., 2012; Stephanopoulos et al., 1998). A detailed explanation of maintenance can be found in the thermodynamic analysis section of this thesis (Section 5.4.1.1).

When the ratio between the consumed compounds and produced products are constant these ratios are called stoichiometric coefficient or yield. In general, yields are the ratio of rates (Kovarova-Kovar and Egli, 1998; Kristiansen, 1999; Rittmann and McCarty, 2012). One of the most important yields in biodegradation processes is the ratio of produced biomass per consumed substrate.

$$Y_{SX} = \frac{amount\ of\ biomass\ produced}{amount\ of\ susbtrate\ consumed} \tag{2.12}$$

In general:

$$Y_{ij} = \frac{amount\ of\ j}{amount\ of\ i} = \frac{rate_j}{rate_i} \tag{2.13}$$

2.4.2 Biomass Specific Rate: q-rate

In biological processes the substrate consumption rate is heavily dependent on the biomass concentration. As such, it is important to quantify the rates per unit amount of biomass. To overcome the dependency of the rates to the biomass concentration we can use the **biomass specific rate q_i** (Kristiansen, 1999; Smolke, 2009).

$$q_i = \frac{rate_i}{C_x} \tag{2.14}$$

These q_i rates are very informative since we can easily track and then characterize the activity of the cells (e.g. physiological parameters). q_i rates are influenced by two factors (Smolke, 2009):

- The cells: genotype effects such as genes, etc. [Genotype]
- The environment of the cells: temperature, concentration of nutrient, etc. [Environment]

These q rates nicely represent the physiological properties of the cells (Nielsen et al., 2012; Roels, 1983; Smolke, 2009; Stephanopoulos et al., 1998). It should be noted that q_x which is a biomass specific growth rate is equal to μ. q-rates are relevant and accurate for comparing of the performance of two different systems (Boender et al., 2009; Smolke, 2009) . Similar to the Monod model (hyperbolic model), q_s (substrate specific uptake rate) can be described by a hyperbolic function (Boender et al., 2009; Kristiansen, 1999; Van Loosdrecht and Henze, 1999).

$$q_s = q_s^{max} \frac{C_s}{K_s + C_s} \tag{2.15}$$

2.4.3 Biodegradation Thermodynamics

Generally, in engineering sciences one needs a combination of three concepts: kinetics (e.g. transport phenomena, growth kinetics), balances (e.g. mass and energy balance) and equilibria (e.g. force and phase equilibria) (von Stockar and van der Wielen, 1997). In environmental biotechnology, balances and kinetics models have been extensively developed while thermodynamic models have received little attention (Jaworska et al., 2002; Ramos et al., 2011; Singh et al., 2008). A rough comparison shows that biological processes are not optimized as much as chemical processes. This discrepancy can be attributed to a lack of fundamental information. For example, mathematical models for deriving forces and energy efficiency and

position of thermodynamic equilibrium (and non-equilibrium state) do not exist (Urs von Stockar, 2013).

2.4.3.1 Importance of Thermodynamics Analysis

When we complete a thermodynamic analysis, what can we find out and what can we do with the results? Here are some of the possibilities (Heijnen, 1994; Kristiansen, 1999; Urs von Stockar, 2013):

- Effect of temperature, pH, pressure, solutes and solvents on activity and selectivity
- Correction of deriving force models for bioprocess design
- Efficiency of cellular metabolism: optimal biomass and product yield
- Thermodynamic effects in cellular growth including heat generation
- Prediction of stoichiometric and kinetic parameters
- Investigation of opportunities for novel product formation with a given substrate
- Design of novel pathways for bioremediation purposes
- Improvement of scale up approaches in bioprocesses

As it can be seen results from thermodynamic analysis are very informative specifically its power in prediction of desired process parameters (Cueto-Rojas et al., 2015; Finley et al., 2010). These possibilities are the main inspiration for investigating NAs biodegradation from a thermodynamic perspective. More detailed discussions on this topic can be found in chapter five of this thesis.

2.4.3.2 Thermodynamics of Naphthenic Acids biodegradation

Why Naphthenic Acids biodegradation is interesting for thermodynamic study?

When we are talking about biodegradation there are two very basic different cases to think about: first is substrate (pollutant) and second one is microorganism. As we already knew NAs are partially biodegradable. It means that microorganism cannot take up all substrate as source of energy and carbon. The second topic that researchers can think is the ability of microorganisms to remove NAs with focusing on the cell. And of course, we do not want to apply any changes in substrate however, we are very interested in manipulation of the cells respect to our benefits.

Misiti et al. 2013 used thermodynamic analysis in their experiments for biodegradation of commercial NAs under different electron accepting condition (Misiti et al., 2013; Misiti et al., 2014b). However, the main points in thermodynamic analysis of bioremediation processes is prediction of stoichiometry and kinetic parameters (von Stockar and van der Wielen, 1997) which were not reported in their studies. Detailed related thermodynamics background is described in chapter 5 of this thesis.

2.4 References

Abolfazlzadehdoshanbehbazari M, Birks SJ, Moncur MC, Ulrich AC. 2013. Fate and transport of oil sand process-affected water into the underlying clay till: a field study. *J. Contam. Hydrol.* **151**:83–92.

Alexander M. 1985. Biodegradation of organic chemicals. *Environ. Sci. Technol.* **19**:106–111.

Alexander M. 1999. Biodegradation and Bioremediation. Gulf Professional Publishing 453 p.

Allen EW. 2008a. Process water treatment in Canada's oil sands industry: I. Target pollutants and treatment objectives. *J. Environ. Eng. Sci.* **7**:123–138.

Allen EW. 2008b. Process water treatment in Canada's oil sands industry: II. A review of emerging technologies. *J. Environ. Eng. Sci.* **7**:499–524.

Andersson DI, Hughes D. 2014. Microbiological effects of sublethal levels of antibiotics. *Nat. Rev. Microbiol.* **12**:465–78.

Andreozzi R. 1999. Advanced oxidation processes (AOP) for water purification and recovery. *Catal. Today* **53**:51–59.

Andrews JM. 2001. Determination of minimum inhibitory concentrations. *J. Antimicrob. Chemother.* **48**:5–16.

Bailey JE. 1986. Biochemical Engineering Fundamentals. McGraw-Hill 984 p.

Bard AJ, Faulkner LR. 2000. Electrochemical Methods: Fundamentals and Applications, 2nd Edition. John Wiley & Sons 856 p.

Blakley ER, Papish B. 1982. The metabolism of cyclohexanecarboxylic acid and 3-cyclohexenecarboxylic acid by *Pseudomonas putida*. *Can. J. Microbiol.* **28**:1324–9.

Blakley ER. 1974. The microbial degradation of cyclohexanecarboxylic acid: a pathway involving aromatization to form p hydroxybenzoic acid. *Can. J. Microbiol.*

Boender LGM, de Hulster EAF, van Maris AJA, Daran-Lapujade PAS, Pronk JT. 2009. Quantitative physiology of Saccharomyces cerevisiae at near-zero specific growth rates. *Appl. Environ. Microbiol.* **75**:5607–14.

Brown LD, Pérez-Estrada L, Wang N, El-Din MG, Martin JW, Fedorak PM, Ulrich AC. 2013. Indigenous microbes survive in situ ozonation improving biodegradation of dissolved organic matter in aged oil sands process-affected waters. *Chemosphere* **93**:2748–55.

Brown LD, Ulrich AC. 2015. Oil sands naphthenic acids: a review of properties, measurement,

and treatment. *Chemosphere* **127**:276–90.

Camel V, Bermond A. 1998. The use of ozone and associated oxidation processes in drinking water treatment. *Water Res.* **32**:3208–3222.

Chakravarti R, Patrick BN, Barney M, Kusinski G, Devine TM. 2013. Toward the Mechanism of Corrosion in Crude Oil: A Study Using Vibrational Spectroscopic Techniques at Elevated Temperatures. *Energy & Fuels* **27**:7905–7914.

Chapman JS. 2003. Disinfectant resistance mechanisms, cross-resistance, and co-resistance. *Int. Biodeterior. Biodegradation* **51**:271–276.

Christopher Grant Weisener JJHC. 2013. Gamma Radiation Apparatus for Remediation of Organic Compounds in Waste Slurries.

Clark DS, Blanch HW. 1995. Biochemical Engineering, Second Edition. CRC Press 702 p.

Clemente JS, Fedorak PM. 2005. A review of the occurrence, analyses, toxicity, and biodegradation of naphthenic acids. *Chemosphere* **60**:585–600.

Clothier LN, Gieg LM. 2016. Anaerobic biodegradation of surrogate naphthenic acids. *Water Res.* **90**:156–66.

Cueto-Rojas HF, van Maris AJA, Wahl SA, Heijnen JJ. 2015. Thermodynamics-based design of microbial cell factories for anaerobic product formation. *Trends Biotechnol.* **33**:534–46.

Derungs WA. 1956. Naphthenic Acid Corrosion —An Old Enemy Of the Petroleum Industry . *Corrosion* **12**:41–46.

Dong F, Nemati M. 2016. Anoxic biodegradation of a surrogate naphthenic acid coupled to reduction of nitrite. *Biochem. Eng. J.*

Doran PM. 1995. Bioprocess Engineering Principles. *Bioprocess Eng. Princ.* Elsevier 426-439 p.

Finley SD, Broadbelt LJ, Hatzimanikatis V. 2010. In silico feasibility of novel biodegradation pathways for 1,2,4-trichlorobenzene. *BMC Syst. Biol.* **4**:7.

Frank RA, Sanderson H, Kavanagh R, Burnison BK, Headley J V, Solomon KR. 2010. Use of a (quantitative) structure-activity relationship [(Q)SAR] model to predict the toxicity of naphthenic acids. *J. Toxicol. Environ. Health. A* **73**:319–29.

Frank RA, Fischer K, Kavanagh R, Burnison BK, Arsenault G, Headley J V., Peru KM, Kraak G Van Der, Solomon KR. 2009. Effect of Carboxylic Acid Content on the Acute Toxicity of Oil Sands Naphthenic Acids. *Environ. Sci. Technol.* **43**:266–271.

Garcia-Garcia E, Ge JQ, Oladiran A, Montgomery B, El-Din MG, Perez-Estrada LC, Stafford

JL, Martin JW, Belosevic M. 2011. Ozone treatment ameliorates oil sands process water toxicity to the mammalian immune system. *Water Res.* **45**:5849–57.

Giesy JP, Anderson JC, Wiseman SB. 2010. Alberta oil sands development. *Proc. Natl. Acad. Sci. U. S. A.* **107**:951–2.

Government of Alberta. 2016. Oil sands quarterly. Open Text Web Site Management.

Grewer DM, Young RF, Whittal RM, Fedorak PM. 2010. Naphthenic acids and other acid-extractables in water samples from Alberta: what is being measured? *Sci. Total Environ.* **408**:5997–6010.

Gunawan Y, Nemati M, Dalai A. 2014. Biodegradation of a surrogate naphthenic acid under denitrifying conditions. *Water Res.* **51**:11–24.

Guzel-Seydim ZB, Greene AK, Seydim AC. 2004. Use of ozone in the food industry. *LWT - Food Sci. Technol.* **37**:453–460.

Halliwell B, Gutteridge JMC. 1985. Free radicals in biology and medicine.

Han X, Scott AC, Fedorak PM, Bataineh M, Martin JW. 2008. Influence of Molecular Structure on the Biodegradability of Naphthenic Acids. Environ. Sci. Technol. 42:1290–1295.

Headley J V, Peru KM, Mohamed MH, Frank RA, Martin JW, Hazewinkel RRO, Humphries D, Gurprasad NP, Hewitt LM, Muir DCG, Lindeman D, Strub R, Young RF, Grewer DM, Whittal RM, Fedorak PM, Birkholz DA, Hindle R, Reisdorph R, Wang X, Kasperski KL, Hamilton C, Woudneh M, Wang G, Loescher B, Farwell A, Dixon DG, Ross M, Pereira ADS, King E, Barrow MP, Fahlman B, Bailey J, McMartin DW, Borchers CH, Ryan CH, Toor NS, Gillis HM, Zuin L, Bickerton G, Mcmaster M, Sverko E, Shang D, Wilson LD, Wrona FJ. 2013. Chemical fingerprinting of naphthenic acids and oil sands process waters-A review of analytical methods for environmental samples. *J. Environ. Sci. Health. A. Tox. Hazard. Subst. Environ. Eng.* **48**:1145–63.

Headley J V, Peru KM, Barrow MP, Derrick PJ. 2007. Characterization of naphthenic acids from athabasca oil sands using electrospray ionization: the significant influence of solvents. *Anal. Chem.* **79**:6222–9.

Headley J V., McMartin DW. 2004. A review of the occurrence and fate of naphthenic acids in aquatic environments. *J. Environ. Sci. Heal. - Part A Toxic/Hazardous Subst. Environ. Eng.*

Headley J V., McMartin DW. 2007. A Review of the Occurrence and Fate of Naphthenic Acids in Aquatic Environments. *J. Environ. Sci. Heal. Part A*.

Heijnen SJ. 1994. Thermodynamics of microbial growth and its implications for process design. *Trends Biotechnol.* **12**:483–492.

Herman DC, Fedorak PM, MacKinnon MD, Costerton JW. 1994. Biodegradation of naphthenic acids by microbial populations indigenous to oil sands tailings. *Can. J. Microbiol.* **40**:467–477.

Holden AA, Haque SE, Mayer KU, Ulrich AC. 2013. Biogeochemical processes controlling the mobility of major ions and trace metals in aquitard sediments beneath an oil sand tailing pond: laboratory studies and reactive transport modeling. *J. Contam. Hydrol.* **151**:55–67.

Holowenko F. 2002. Characterization of naphthenic acids in oil sands wastewaters by gas chromatography-mass spectrometry. *Water Res.* **36**:2843–2855.

Holowenko FM, MacKinnon MD, Fedorak PM. 2011. Methanogens and sulfate-reducing bacteria in oil sands fine tailings waste. *Can. J. Microbiol.*

Huang J, Nemati M, Hill G, Headley J. 2012. Batch and continuous biodegradation of three model naphthenic acids in a circulating packed-bed bioreactor. *J. Hazard. Mater.* **201-202**:132–40.

Imlay JA. 2013. The molecular mechanisms and physiological consequences of oxidative stress: lessons from a model bacterium. *Nat. Rev. Microbiol.* **11**:443–54.

Iranmanesh S, Harding T, Abedi J, Seyedeyn-Azad F, Layzell DB. 2014. Adsorption of naphthenic acids on high surface area activated carbons. *J. Environ. Sci. Health. A. Tox. Hazard. Subst. Environ. Eng.* **49**:913–22.

J.A. Brient, P.J. Wessner MND. 1995. Kirk-Othmer Encyclopedia of Chemical Technology. Hoboken, NJ, USA: John Wiley & Sons, Inc.

Jaworska J, Dimitrov S, Nikolova N, Mekenyan O. 2002. Probabilistic assessment of biodegradability based on metabolic pathways: catabol system. *SAR QSAR Environ. Res.* **13**:307–323.

Jia W, He Y, Ling Y, Hei D, Shan Q, Zhang Y, Li J. 2015. Radiation-induced degradation of cyclohexanebutyric acid in aqueous solutions by gamma ray irradiation. *Radiat. Phys. Chem.* **109**:17–22.

Jiveraj MN. 1995. Naphthenic acids extraction and quantitative analy; fourth ed. Syncrude Canada Ltd.; Research Department; Edmonton; AB.

Johnson RJ, Smith BE, Sutton PA, McGenity TJ, Rowland SJ, Whitby C. 2011. Microbial

biodegradation of aromatic alkanoic naphthenic acids is affected by the degree of alkyl side chain branching. *ISME J.* **5**:486–96.

Kabdasli I, Arslan-Alaton I. 2010. Chemical Oxidation Applications for Industrial Wastewaters. IWA Publishing 341 p.

Kanicky JR, Poniatowski AF, Mehta NR, Shah DO. 2000. Cooperativity among Molecules at Interfaces in Relation to Various Technological Processes: Effect of Chain Length on the p K a of Fatty Acid Salt Solutions †. *Langmuir* **16**:172–177.

Kannel PR, Gan TY. 2012. Naphthenic acids degradation and toxicity mitigation in tailings wastewater systems and aquatic environments: a review. *J. Environ. Sci. Health. A. Tox. Hazard. Subst. Environ. Eng.* **47**:1–21.

Kindzierski W, Jin J, Gamal El-Din M. 2012. Review of Health Effects of Naphthenic Acids: Data Gaps and Implications for Understanding Human Health Risk.

Klamerth N, Moreira J, Li C, Singh A, McPhedran KN, Chelme-Ayala P, Belosevic M, Gamal El-Din M. 2015. Effect of ozonation on the naphthenic acids' speciation and toxicity of pH-dependent organic extracts of oil sands process-affected water. *Sci. Total Environ.* **506-507**:66–75.

Kovárová K, Zehnder AJ, Egli T. 1996. Temperature-dependent growth kinetics of Escherichia coli ML 30 in glucose-limited continuous culture. *J. Bacteriol.* **178**:4530–9.

Kovarova-Kovar K, Egli T. 1998. Growth Kinetics of Suspended Microbial Cells: From Single-Substrate-Controlled Growth to Mixed-Substrate Kinetics. *Microbiol. Mol. Biol. Rev.* **62**:646–666.

Kristiansen CRB. 1999, eds. *Basic biotechnology*. Cambridge University Press

Lambert RJW, Skandamis PN, Coote PJ, Nychas G-JE. 2001. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *J. Appl. Microbiol.* **91**:453–462.

Lorian V. 1975. Some effects of subinhibitory concentrations of antibiotics on bacteria. *Bull. N. Y. Acad. Med.* **51**:1046–55.

Martin JW, Barri T, Han X, Fedorak PM, El-Din MG, Perez L, Scott AC, Jiang JT. 2010. Ozonation of oil sands process-affected water accelerates microbial bioremediation. *Environ. Sci. Technol.* **44**:8350–6.

Misiti TM, Tezel U, Pavlostathis SG. 2014a. Effect of alkyl side chain location and cyclicity on

the aerobic biotransformation of naphthenic acids. *Environ. Sci. Technol.* **48**:7909–17.

Misiti T, Tandukar M, Tezel U, Pavlostathis SG. 2013. Inhibition and biotransformation potential of naphthenic acids under different electron accepting conditions. *Water Res.* **47**:406–18.

Misiti T, Tandukar M, Tezel U, Pavlostathis SG. 2014b. Corrigendum to "Inhibition and biotransformation potential of naphthenic acids under different electron accepting conditions" [Water Res. 47 (1) (January 2013) 406–418]. *Water Res.* **48**:645.

Miskimmin, B., Fedorak P., Lauman R. & VK. 2010. Oil sands water toxicity: A critical review.

Monod J. 1949. The Growth of Bacterial Cultures. *Annu. Rev. Microbiol.* **3**:371–394.

Morandi GD, Wiseman SB, Pereira A, Mankidy R, Gault IGM, Martin JW, Giesy JP. 2015. Effects-Directed Analysis of Dissolved Organic Compounds in Oil Sands Process-Affected Water. *Environ. Sci. Technol.* **49**:12395–404.

Nielsen J, Villadsen J, Lidén G. 2012. Bioreaction Engineering Principles: Second Edition. Springer 528 p.

Oller I, Malato S, Sánchez-Pérez JA. 2011. Combination of Advanced Oxidation Processes and biological treatments for wastewater decontamination--a review. *Sci. Total Environ.* **409**:4141–66.

Oturan MA, Aaron J-J. 2014. Advanced Oxidation Processes in Water/Wastewater Treatment: Principles and Applications. A Review. *Crit. Rev. Environ. Sci. Technol.* **44**:2577–2641.

Pérez-Estrada LA, Han X, Drzewicz P, Gamal El-Din M, Fedorak PM, Martin JW. 2011. Structure-reactivity of naphthenic acids in the ozonation process. *Environ. Sci. Technol.* **45**:7431–7.

Pirt SJ. 1965. The Maintenance Energy of Bacteria in Growing Cultures. *Proc. R. Soc. B Biol. Sci.* **163**:224–231.

Pruchniak MP, Araźna M, Demkow U. 2015. Biochemistry of Oxidative Stress. *Adv. Exp. Med. Biol.*

Quagraine EK, Peterson HG, Headley J V. 2005. In Situ Bioremediation of Naphthenic Acids Contaminated Tailing Pond Waters in the Athabasca Oil Sands Region—Demonstrated Field Studies and Plausible Options: A Review. *J. Environ. Sci. Heal. Part A* **40**:685–722.

Quinlan PJ, Tam KC. 2015. Water treatment technologies for the remediation of naphthenic acids in oil sands process-affected water. *Chem. Eng. J.* **279**:696–714.

Ramos J-L, Marqués S, van Dillewijn P, Espinosa-Urgel M, Segura A, Duque E, Krell T, Ramos-González M-I, Bursakov S, Roca A, Solano J, Fernádez M, Niqui JL, Pizarro-Tobias P, Wittich R-M. 2011. Laboratory research aimed at closing the gaps in microbial bioremediation. *Trends Biotechnol.* **29**:641–7.

Rho EM, Evans WC. 1975. The aerobic metabolism of cyclohexanecarboxylic acid by Acinetobacter anitratum. *Biochem. J.* **148**:11–15.

Rittmann B, McCarty P. 2012. Environmental biotechnology: principles and applications.

Roels JA. 1983. Energetics and kinetics in biotechnology.

Rogers V V. 2002. Acute and Subchronic Mammalian Toxicity of Naphthenic Acids from Oil Sands Tailings. *Toxicol. Sci.* **66**:347–355.

Ross MS, Pereira A dos S, Fennell J, Davies M, Johnson J, Sliva L, Martin JW. 2012. Quantitative and qualitative analysis of naphthenic acids in natural waters surrounding the Canadian oil sands industry. *Environ. Sci. Technol.* **46**:12796–805.

Scott AC, Zubot W, MacKinnon MD, Smith DW, Fedorak PM. 2008. Ozonation of oil sands process water removes naphthenic acids and toxicity. *Chemosphere* **71**:156–60.

Shu Z, Li C, Belosevic M, Bolton JR, El-Din MG. 2014. Application of a solar UV/chlorine advanced oxidation process to oil sands process-affected water remediation. *Environ. Sci. Technol.* **48**:9692–701.

Siegrist R, Crimi M, Simpkin T. 2011. In situ chemical oxidation for groundwater remediation.

Sies H. 1997. Oxidative stress: oxidants and antioxidants. *Exp. Physiol.* **82**:291–295.

Singh S, Kang SH, Mulchandani A, Chen W. 2008. Bioremediation: environmental clean-up through pathway engineering. *Curr. Opin. Biotechnol.* **19**:437–44.

Slavcheva E, Shone B, Turnbull A. 1999. Review of naphthenic acid corrosion in oilrefining. *Br. Corros. J.* **34**:125–131.

Smith BE, Lewis CA, Belt ST, Whitby C, Rowland SJ. 2008. Effects of Alkyl Chain Branching on the Biotransformation of Naphthenic Acids. *Environ. Sci. Technol.* **42**:9323–9328.

Smolke C. 2009. The Metabolic Pathway Engineering Handbook: Fundamentals. CRC Press 678 p.

Stephanopoulos G, Aristidou AA, Nielsen J. 1998. Metabolic Engineering: Principles and Methodologies. Academic Press 725 p.

Stewart PS, Rayner J, Roe F, Rees WM. 2001. Biofilm penetration and disinfection efficacy of

alkaline hypochlorite and chlorosulfamates. *J. Appl. Microbiol.* **91**:525–532.

von Stockar U, van der Wielen LAM. 1997. Thermodynamics in biochemical engineering. *J. Biotechnol.* **59**:25–37.

Tchobanoglous G, Burton FL, Stensel HD, Eddy M&. 2003. Wastewater Engineering: Treatment and Reuse. McGraw-Hill Education 1819 p.

Tezel U, Pavlostathis SG. 2015. Quaternary ammonium disinfectants: microbial adaptation, degradation and ecology. *Curr. Opin. Biotechnol.* **33**:296–304.

Tollefsen KE, Petersen K, Rowland SJ. 2012. Toxicity of synthetic naphthenic acids and mixtures of these to fish liver cells. *Environ. Sci. Technol.* **46**:5143–50.

Urs von Stockar LAM van der W. 2013. Biothermodynamics: The Role of Thermodynamics in Biochemical Engineering. https://www.crcpress.com/Biothermodynamics-The-Role-of-Thermodynamics-in-Biochemical-Engineering/Stockar-van-der-Wielen/9781466582163.

Vaiopoulou E, Misiti TM, Pavlostathis SG. 2015. Removal and toxicity reduction of naphthenic acids by ozonation and combined ozonation-aerobic biodegradation. *Bioresour. Technol.* **179**:339–47.

VANLOOSDRECHT M, HENZE M. 1999. Maintenance, endogeneous respiration, lysis, decay and predation. *Water Sci. Technol.* **39**:107–117.

Wang N, Chelme-Ayala P, Perez-Estrada L, Garcia-Garcia E, Pun J, Martin JW, Belosevic M, Gamal El-Din M. 2013a. Impact of ozonation on naphthenic acids speciation and toxicity of oil sands process-affected water to Vibrio fischeri and mammalian immune system. *Environ. Sci. Technol.* **47**:6518–26.

Wang N, Chelme-Ayala P, Perez-Estrada L, Garcia-Garcia E, Pun J, Martin JW, Belosevic M, Gamal El-Din M. 2013b. Impact of ozonation on naphthenic acids speciation and toxicity of oil sands process-affected water to Vibrio fischeri and mammalian immune system. *Environ. Sci. Technol.* **47**:6518–26.

Whitby C. 2010. Microbial naphthenic Acid degradation. *Adv. Appl. Microbiol.* **70**:93–125.

Yue S, Ramsay BA, Ramsay JA. 2015. Biodegradation of naphthenic acid surrogates by axenic cultures. *Biodegradation* **26**:313–25.

CHAPTER 3. STOICHIOMETRIC DETERMINATION OF PERSULFATE DOSES FOR OXIDATION OF ACID EXTRACTABLE ORGANICS

3.1 Introduction

One of the largest industrial activities in the world is bitumen mining activities in the Alberta oil sands deposits (Leshuk et al., 2016). Due to the alkaline hot water process for extraction of surface-mined bitumen, large volumes of oil sands process affected water (OSPW) are produced. The extraction process produces 1.25 m^3 of tailings per barrel of produced oil and more than three liters of water will be consumed ($\frac{consumed\ water}{produced\ oil} > 3$) in the production of one liter of oil (Allen, 2008a; Government of Alberta, 2016). The zero discharge policy and lack of an established treatment strategy (for treatment of OSPW) results in the storage of OSPW in on-site tailings ponds (Government of Alberta, 2016). OSPW consists of different compounds i.e. suspended clays, dissolved organic compounds, trace heavy metals, and residual bitumen (Allen, 2008). Efforts in developing OSPW treatment methods have shown that naphthenic acids (NAs) are the most important compounds that need to be removed from OSPW (Allen, 2008; Brown and Ulrich, 2015; Clemente and Fedorak, 2005) due to their contribution in toxicity of OSPW (Garcia-Garcia et al., 2011; He et al., 2012; Marentette et al., 2015; Miskimmin, B., Fedorak P., Lauman R., 2010; Shu et al., 2014; Wang et al., 2013) and its corrsoivity to process instruments and vessels (Chakravarti et al., 2013; Derungs, 1956; Slavcheva et al., 1999).

NAs are naturally occurring compounds found in crude oil. They are a complicated mixture of carboxylic acids which are generally shown by the formula $C_nH_{2n+z}O_2$. In this formula ‘n’ represents carbon number, while ‘z’ represents the number of cycles in the NA structure

(Clemente and Fedorak, 2005). NAs concentration in OSPW may differ based on the source of crude oil, the age of the tailings ponds, and the process used in extraction (Allen, 2008a; Clemente and Fedorak, 2005; Headley and McMartin, 2004).

Naturally occurring NAs found in OSPW are just a portion of bigger group of organics called acid extractable organics (AEOs) (Grewer et al., 2010). The acid term comes from the process for extraction of this group of organics where a sample is acidified to pH<2 to have the acidic constituents of OSPW matrix in their protonated form (Brown and Ulrich, 2015). After the extraction with an organic solvent, the obtained complex mix of organic compounds are termed AEOs. Characterizations with high resolution techniques have determined that NAs are a fraction of these OSPW sourced AEOs (Grewer et al., 2010).

The use of chemical oxidants for treatment of industrial wastewatesr has always been of interest (Kabdasli and Arslan-Alaton, 2010; Oturan and Aaron, 2014). Some examples include the use of ozone for treatment of pharmaceutical wastewater and hydrogen peroxide for removal of micro pollutants in wastewater (Rice, 1996; Robinson et al., 2001). Oxidants are chemical compounds that have a high tendency to accept electrons. There are number of oxidants that can be used for remediation purposes, such as permanganate and persulfate (Kabdasli and Arslan-Alaton, 2010). The applicability of an oxidant depends on many factors such as: is the oxidant reactive toward the target compound? If yes, how much do we need to meet the pre-defined treatment requirements? Or how fast can the oxidant remove the pollutant?

Sodium persulfate with a redox potential of +2.01 V is one the newest oxidants used for remediation purposes (Liu et al., 2014; Tsitonaki et al., 2010). The persulfate ion reduces to sulfate by accepting two electrons based on following reaction (Tsitonaki et al., 2010):

$$S_2O_8^{2-} + 2e^- \rightarrow 2SO_4^{2-} \;;\; E^0 = +2.01\,V \quad (3.1)$$

However, the use of persulfate as an oxidant may have some unfavorable consequences, such as a decrease in pH of the contaminated aqueous phase to acidic levels (depends on the buffer capacity of the aquifer) (Huang et al., 2002; Liang et al., 2004) and a change in the redox regime of the treated area (Sutton et al., 2014). Production of sulfate as the reduced form of persulfate can stimulate the activity of sulfate reducing bacteria (SRB) since they use sulfate as their terminal electron acceptor (Muyzer and Stams, 2008). In SRB's metabolism, sulfate will be reduced to sulfide (S^{2-}) which can also create potential environmental concerns (Muyzer and Stams, 2008). For example, in tailings ponds an increase in hydrogen sulfide emissions, which is a toxic gas, has been observed (Holowenko et al., 2011; Ramos-Padrón et al., 2011). Therefore, the concentration of persulfate should be as low as possible to minimize secondary environmental issues.

The persulfate ion has shown to be reactive toward oxidation of NAs in OSPW (Drzewicz et al., 2012; Sohrabi et al., 2013). Sohrabi et al. (2013) used 10 g/L of unactivated sodium persulfate for removal of NAs in OSPW. To demonstrate the degradation of NAs over time, Sohrabi et al. (2010) measured the concentration of total carboxylic acids. Results demonstrate that almost complete removal of carboxylic acids was achieved after 111 days (Sohrabi et al., 2013). However, there was no discussion about the actual and/or needed amout of persulfate for treatment of NAs.

As previously mentioned, determining a stiochiometric amount of oxidant needed to react with the contaminant of concern is one of the main factors in designing and optimizing a remediation process. However, this process is challenging when dealing with NAs since they consist of a mixture containing thousands of complex organic compounds. Additionally, current analytical

techniques are semi-quantitative in nature due to the complexity of the mixture (Brown and Ulrich, 2015; Clemente et al., 2003; Headley et al., 2007; Holowenko, 2002). Previous lab-scale research has shown success in using oxidants in treating NAs in an OSPW matrix (Brown and Ulrich, 2015; Quinlan and Tam, 2015). However, there is no current strategy proposed for determining the practical dosage of oxidants that are needed for an industrial application.

Currently, there is a lack of a simple, cost effective and **practical** protocol for determination of oxidant doses needed for removal of NAs in OSPW or NAs contaminated wastewater. This knowledge gap provides the authors with an opportunity to develop a simple model to **approximately predict** the amount of needed persulfate (oxidant) for remediation purposes.

3.2 Materials and Methods

3.2.1 Chemicals and Material Preparation

Sodium persulfate (≥99%) was purchased from Sigma Aldrich. Methylene chloride (DCM) HPLC grade (99.9 % min_Scientific Fischer) was used as the organic solvent to extract AEOs from OSPW. Merichem NAs (Merichem Chemicals and Refinery Service, Houston, TX) was used as the calibration standard. All chemicals were certified A.C.S and all solutions were prepared in glass made containers.

Syncrude Mildred Lake settling basin (MLSB) tailings pond water was used as the source of OSPW. MLSB OSPW was filtered through a 0.22 μm (Milipore) nylon filter to eliminate microbial and solid state material in the liquid phase.

3.2.2 Kinetic Experiments

Experiments were performed using four different dosages of persulfate oxidant with respect to the predicted stoichiometric amount (D) – 0.25D, 0.5D, D, 2D. Sodium persulfate was dissolved in 500 mL OSPW contained in 1 L capped amber bottles. Magnetic stir bars were used to continuously mix the contents at room temperature.

3.2.3 Analytical Methods

Chemical oxygen demand (COD) of MLSB OSPW was measured with HACH COD digestion vials using a HACH thermoreactor and spectrophometer. To analyze the NAs in OSPW we first need to extract AEOs as per the following protocol:

Samples were immediately acidified to pH < 2 by addition of concentrated HCl. The purpose of acidification is to convert acidic contents to their protonated form. Then DCM was added to samples to transfer organic acids into the organic phase. The extraction step was repeated 5 times for extraction of AEOs from each sample and conducted in triplicate. One should note that by increasing the number of extraction steps better extraction efficiency can be obtained instead of using high volume of solvent.

Analysis of NAs in AEOs was performed by Fourier transform infrared (FTIR) spectroscopy over the time course of the reaction (Jiveraj, 1995). When using FTIR as an analytical technique one measures the concentration of total carboxylic acids. Standards were prepared gravimetrically using a known concentration of Merichem NAs in DCM. FTIR spectroscopy was performed using a PerkinElmer® Spectrum 100 instrument and PerkinElmer® Spectrum 10 software (Perkin Elmer, Waltham, MA). A FTIR cell with potassium bromide window, 3 mm-spaced (University of Alberta, Edmonton, AB) was used in the spectroscopy runs. Summation of

peak heights for monomers hydrogen-bond dimers, 1743 and 1705 cm^{-1} respectively were used in calculating the total NAs (AEOs) concentration. Each sample was extracted and ran in triplicate with 32 scans (Brown et al., 2013; Mahdavi et al., 2015).

3.3 Results and Discussion

3.3.1 Fundamentals of Proposed Model

This model is based on the concept of chemical oxygen demand (COD). COD is a concentration that determines the amount of oxygen needed to oxidize organic compounds in a mixture. In the proposed model, the assumption was all the COD originating from the concentration of NAs. The advantages of this assumption is contribution of other organic pollutants i.e. benzene, toluene, ethylbenzen and xylene or polycyclic aromatic compounds are also considered. As all of the organics contribute to COD of the solution, the estimated dosages is actually for treatment of all organics in the solution. This is applicable to a complex mixture of organic compounds just as is the case for NAs in OSPW. Since COD has units of $\frac{mass}{volume}$ that relate to $\frac{mass\ of\ O_2}{volume\ of\ liquid}$ one can measure the COD of a sample to get an idea of available electrons for oxidation (van Loosdrecht et al., 2016; Rittmann and McCarty, 2012).

Based on the half reaction of oxygen (Rittmann and McCarty, 2012):

$$O_2 + 4e^- + 4H^+ \rightarrow 2\,H_2O \;\; ; \;\; E^0 = \; +1.23\,V \qquad (3.2)$$

1 mole of O_2 accepts 4 moles of electrons (degree of reduction of oxygen = -4), which can be also expressed as 32 g of O_2 accepts 4 moles of electrons or as 32 gCOD accepts 4 moles of electrons. Or in its simplest form of 8 gCOD accepts 1 mole of electrons. For instance, for a wastewater with COD of 500 mg/L one can calculate the available electrons as follows on a per 1L basis:

$$500\ \frac{mgCOD}{l} \times 1\ l = 500\ mgCOD$$

$$500\ mgCOD \times \frac{1\ mmole\ e^-}{8\ mgCOD} = 62.5\ mmole\ e^-$$

Therefore, a solution with 500 mgCOD/L has 62.5 mmole e^- /L that can be oxidized. One important thing to note is that the total concentration of COD does not necessarily oxidize with oxygen since COD does not provide a detailed understanding on the structure, molecular weight, etc. of the electron acceptor.

Our samples of MLSB OSPW were measured for COD and found to contain 125 mgCOD/L. Calculations for the conversion of COD to electrons is described below.

Electron donor:

$$125\ \frac{mgCOD}{l} \times \frac{1\ mmole\ e^-}{8\ mgCOD} = 15.6\ \frac{mmole\ e^-}{l}$$

Electron acceptor: Based on equation (3.4) 0.5 mole of persulfate accepts one mole of electrons.

Based on the electron donor and acceptor relationship below:

$$8\ g\ COD \rightarrow e^- \tag{3.3}$$

$$\frac{1}{2}S_2O_8^{2-} + e^- \rightarrow SO_4^{2-} \tag{3.4}$$

Theoretically we need 0.5 moles of persulfate ion to remove 8 gCOD.

Here, we want to connect the relation of COD to the available oxidant.To mathematically determine treatment dosages for a given oxidant with molecular weight, MW with the following reaction:

$$Ox + ne^- \rightarrow Red \tag{3.5}$$

$$theoretical\ dosage\ \ of\ needed\ oxidant\ D\left[\frac{g\ ox}{l}\right] = \frac{COD\ \left[\frac{g\ COD}{l}\right] \times MW\left[\frac{g\ ox}{mol\ ox}\right]}{8\left[\frac{g\ COD}{mol\ e^-}\right] \times n\left[\frac{mol\ e^-}{mol\ ox}\right]} \tag{3.6}$$

Specifically, for the sodium persulfate ion we can calculcate the dose (D) as follows:

$$D = \frac{COD \times 238}{8 \times 2} \tag{3.7}$$

3.3.2 Determination of Required Dosage of Oxidant

COD of MLSB OSPW was measured as 125 mg/L. Based on the proposed model, one can determine the stoichiometrically needed dosage of persulfate (D) to be 1.86 g/L for sodium salt of persulfate. Calculation of obtained result is described below:

$$D = \frac{125\ \left[\frac{mg\ COD}{l}\right] \times 238\ \left[\frac{mg\ Na_2S_2O_8}{mmol\ Na_2S_2O_8}\right]}{8\left[\frac{mg\ COD}{mmol\ e^-}\right] \times 2\left[\frac{mmol\ e^-}{mmol\ Na_2S_2O_8}\right]} = 1860\ \left[\frac{mg\ Na_2S_2O_8}{l}\right] = 1.86\left[\frac{g\ Na_2S_2O_8}{l}\right]$$

Theoretically, this amount of persulfate can oxidize all organic electron donors in OSPW. To test the ability of persulfate to oxidize the COD in OSPW. However, our target in this work is to oxidize NAs. In measurement of COD, all the organic pollutants are considered, and a fraction of measured organics are NAs. Therefore, we tested oxidant doses ranging from lower predicted dosage to higher. Dosages are 0.25, 0.5, 1 and 2 times of the theoretical calculated value (D), which correspond to actual concentrations of 0.47, 0.93, 1.86 and 3.72 g/L of sodium persulfate. The concentration of total carboxylic acids was monitored over a 28 day period as shown in Fig 3-1.

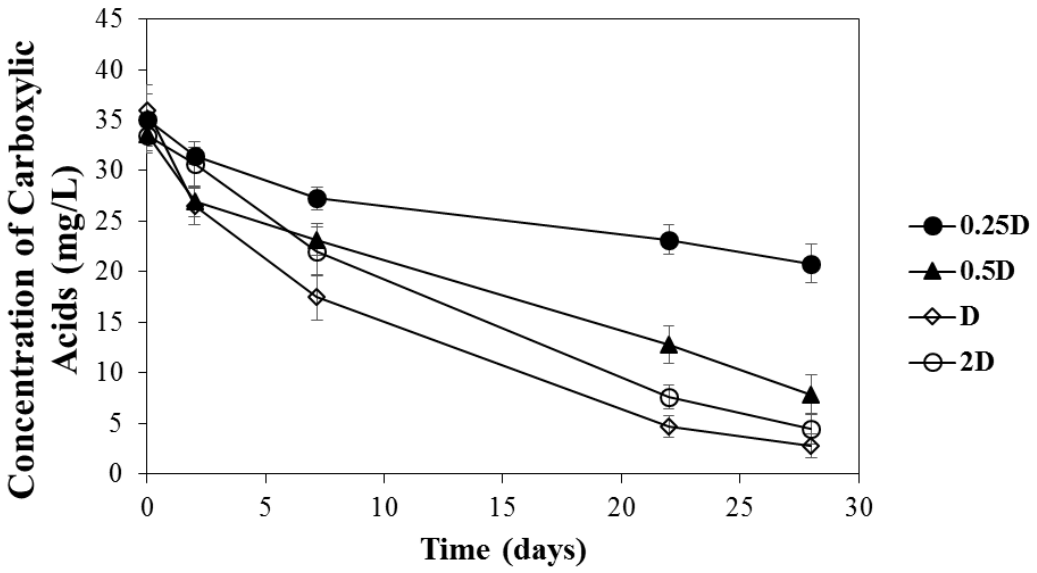


Figure 3-1 Degradation of NAs (carboxylic acids) vs. time based on FTIR measurement

Fig 3-1 shows the degradation of AEOs which presents by measuring the toal concentration of carboxylic acids. It can be seen that proposed strategy for determination of theoretical and/or actual needed persulfate for degradation of AEOs (total carboxylic acids) were effective.

The first order kinetic constants (k_{obs}) were determined by plotting the Ln (C_0/C) vs. time (Fig. 3.2). Results of the regression analysis for kinetic data are shown in table 1.3. In chemical oxidation processes or advanced oxidation processes partial or complete mineralization can be the goal of the process. It absolutely depends on the aim of the experimenter.

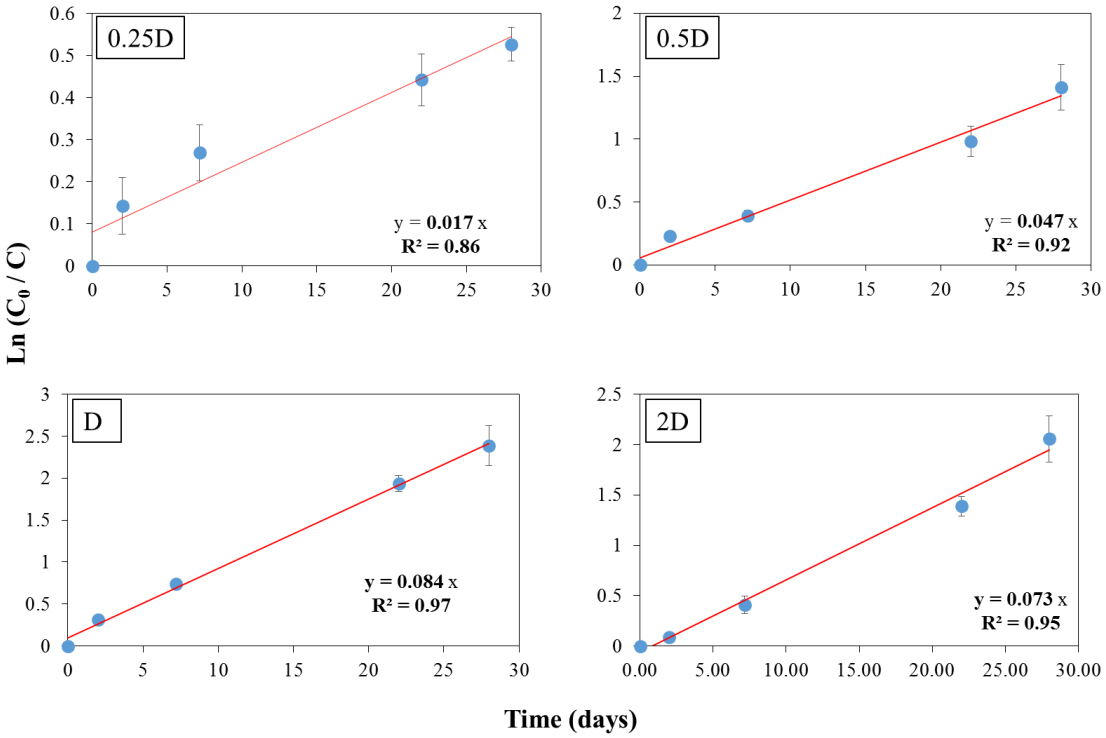


Figure 0-2. The linear regression analysis of degradation data to first order rate model; shows the samples

It is the same even at industrial level (practical scale). However, for each case of complete or partial degradation we need to apply a **proper** concentration of oxidant. With this proposed method, one can **approximately** determine the needed oxidant concentration for each case of partial or complete oxidation. One of the advantages of a COD based model is its power to determine an organic-based electron in solution. It also predicts the formation of by-products (the number of electron is constant, they just transfer between compunds). The reactivity of by-products and oxidant present may be of interest for future research.

Table 0-1. Kinetic constants of first order rate; mean multicomparison statistical analyses conducted to determine the significany of differences between treatments (See Appendix A.1); statistical analyses results show that all treatments are significantly different ($p < 0.05$) except treatments D and 2D ($p=0.359$) based on Tukey's test

Series	Concentration of sodium persulfate (mg/L)	First order rate constant (day^{-1})		R^2	Percentage of removal (%)
		Average	95% confidence interval		
0.25D	465	0.017	0.013-0.021	0.86	41
0.5D	930	0.047	0.039-0.055	0.92	76
D	1860	0.085	0.075-0.094	0.97	91
2D	3720	0.073	0.062-0.083	0.95	88

As discussed earlier in this work, the goal of this chapter is to propose a model to understand the concentration of oxidant required. Table 3-2 gives a comparison of our results with the only published work for degradation of carboxylic acids in AEOs with inactivated persulfate.

Persulfate is more active in high pHs (>10) and has lower activity at low pHs (<3). As persulfate dissolves in an aqueous solution it releases protons which decrease the pH. Therefore, there is an expected decrease in persulfate activity when used at high concentration (because it results in lowering the pH of the solution).

Table 0-2 Comparison of result of this work and relevant literature

	OSPW	*Initial concentration (FTIR) (mgL^{-1})*	*Oxidant Dosage (gL^{-1})*	*% of Removal*	*Time (day)*
This work	MLSB	~34	1.86	91%	28
Sohrabi et al. 2013	STP	~48	10	98%	111

Generally, optimization of remediation process is not possible without unraveling the stoichiometry behind of the reactions. Specifically, for the case of *in situ* application for remiditation of tailings ponds, the huge volume of OSPW creates the situation where optimization is needed for treatment. For example, there is 13.4×10^6 m^3 of OSPW contained withing the Mildred lake settling basin (Tailing Ponds, 2010). Table 3-3 shows the comparioson of cost estimation for treatment of OSPW in MLSB tailings ponds by proposed method and published data.

Table 0-3 Estimation of the cost of treatment process, and comparison of the cost based on different methods. Total volume of water in MLSB is reported by Syncrude and cost of the sodium persulfate considered as 2.7 $ per kg (Chen et al., 2015)

Method	*Required sodium persulfate (Tonne)*	*Cost of sodium persulfate (Million $)*	*Approximate time (month)*
Sohrabi et al. (2014)	1.34×10^5	361.8	~ 3.5
COD-based model	2.5×10^4	67.3	~ 1

Here we have shown the application of COD-based model for determination of the amount of oxidant other than persulfate.

3.3.3 Application of Proposed Model to other Oxidants

As with any model, the ability to account for multiple treatment processes is important, for example our COD model not only describes persulfate, it can also be applied to various other oxidation treatments.

One of the widely used oxidant is ozone; in the case of ozone:

$$O_3 + 2H^+ + 2e^- \rightarrow O_2 + H_2O \;\; ; \; E^0 = +2.07\, V \tag{3.8}$$

Based on half reaction of ozone, it accepts two electron. Based on the model, for example MLSB OSPW (COD is 125 mg/L):

$$D = \frac{COD \times MW}{8 \times n} = \frac{125 \times 48}{8 \times 2} = 375 \frac{mg}{L} of ozone\ is\ needed$$

For permanganate (Neutral pH):

$$MnO_4^{2-} + 3e^- + 2H_2O \rightarrow MnO_2 + 4\, OH^- \;\; ; \; E^0 = +2.09\, V \tag{3.9}$$

Degree of reduction of Permanganate (at neutral pH) is -3, thus:

$$D = \frac{COD \times MW}{8 \times n} = \frac{125 \times 119}{8 \times 3} = 619.43\ \frac{mg}{l}\ permanganate\ is\ needed$$

To theoretically verify the COD-based model, a stoichiometric analysis of degradation based on the half reaction (elemental balance) of a single model NA is used, as well as the COD of a solution.

For example, the model NA, trans-4-pentylcyclohexanecarboxylic acid ($C_{12}H_{22}O_2$) (Rittmann and McCarty, 2012):

Calculation of 1 mmole $C_{12}H_{22}O_2$:

$$C_{12}H_{22}O_2 + \frac{33}{2} O_2 \rightarrow 12\, CO_2 + 11\, H_2O \tag{3.10}$$

Hence, 1 mmole of $C_{12}H_{22}O_2$ has a COD of 528 mgCOD. On a 1 liter basis, based on the model (3.6), 1 mmole of $C_{12}H_{22}O_2$, the required sodium persulfate is:

$$D = \frac{528 \times 238}{8 \times 2} = 7854 \frac{mg}{L} of\ sodium\ persulfate$$

And now based on oxidant and target compound half reactions:

$$\frac{1}{66}\, C_{12}H_{22}O_2 + \frac{22}{66} H_2O \rightarrow \frac{12}{66}\, CO_2 + H^+ + e^- \quad (3.11)$$

$$\frac{1}{2} S_2O_8^{2-} + e^- \rightarrow SO_4^{2-} \quad (3.12)$$

$$C_{12}H_{22}O_2 + 33\, S_2O_8^{2-} + 22\, H_2O \rightarrow 12\, CO_2 + 66\, SO_4^{2-} + 66\, H^+ \quad (3.13)$$

It is established that reaction (13.3) is sum of reactions of (11.3) and (12.3). Accordingly, for 1 mmole of $C_{12}H_{22}O_2$, it needs 33 mmole of persulfate, which is equal to 7854 mg of $Na_2S_2O_8$ per liter.

Lack of a logical model for understanding the stoichiometry of NA degradation in wastewater is notated through extensive literature review. This work proposes a suitable model based on the available organic-based electrons to cover this knowledge gap. Since tailings ponds are contained huge volume of OSPW, coming up with an optimized strategy is a must. Optimization of a process can have different aspects. Here, establishing a cost effective and envrionemtally friendly process for treatment of OSPW is two of the main important points in optimization. In this chapter, a strategy based on the electron concent of OSPW is proposed to oxdize organic pollutants with different oxidant. Sodium persulfate was used as the tested oxidants to observe the results of the model in real world. The purpose of degradation experiment was mainly track the reactivity of sodium persulfate toward NAs. COD-based model can use as a rigorous tool in onset of optimization of chemical oxidation process for OSPW treatment. However, as mentioned before (Sections 3.1 and 2.2.5.1), in chemical oxidation process, genrally, complete removal of target comtaminats is not an ultimate goal. The removal percentages could be 100%, or partial degradation, it depends on the experimenter goal!

The other advantage of this model is its user friendliness. The only experiment that needs to be done in application of this model is COD test which is a cheap and simple test.

3.4 References

Allen EW. 2008a. Process water treatment in Canada's oil sands industry: I. Target pollutants and treatment objectives. *J. Environ. Eng. Sci.* **7**:123–138.

Allen EW. 2008b. Process water treatment in Canada's oil sands industry: II. A review of emerging technologies. *J. Environ. Eng. Sci.* **7**:499–524.

Brown LD, Pérez-Estrada L, Wang N, El-Din MG, Martin JW, Fedorak PM, Ulrich AC. 2013. Indigenous microbes survive in situ ozonation improving biodegradation of dissolved organic matter in aged oil sands process-affected waters. *Chemosphere* **93**:2748–55.

Brown LD, Ulrich AC. 2015. Oil sands naphthenic acids: a review of properties, measurement, and treatment. *Chemosphere* **127**:276–90.

Chakravarti R, Patrick BN, Barney M, Kusinski G, Devine TM. 2013. Toward the Mechanism of Corrosion in Crude Oil: A Study Using Vibrational Spectroscopic Techniques at Elevated Temperatures. *Energy & Fuels* **27**:7905–7914.

Chen K-F, Chang Y-C, Chiou W-T. 2015. Remediation of diesel-contaminated soil using in situ chemical oxidation (ISCO) and the effects of common oxidants on the indigenous microbial community: a comparison study. *J. Chem. Technol. Biotechnol.*:n/a–n/a.

Clemente JS, Fedorak PM. 2005. A review of the occurrence, analyses, toxicity, and biodegradation of naphthenic acids. *Chemosphere* **60**:585–600.

Clemente JS, Prasad NG., MacKinnon MD, Fedorak PM. 2003. A statistical comparison of naphthenic acids characterized by gas chromatography–mass spectrometry. *Chemosphere* **50**:1265–1274.

Derungs WA. 1956. Naphthenic Acid Corrosion —An Old Enemy Of the Petroleum Industry ★. *Corrosion* **12**:41–46.

Drzewicz P, Perez-Estrada L, Alpatova A, Martin JW, Gamal El-Din M. 2012. Impact of peroxydisulfate in the presence of zero valent iron on the oxidation of cyclohexanoic acid and naphthenic acids from oil sands process-affected water. *Environ. Sci. Technol.* **46**:8984–91.

Garcia-Garcia E, Ge JQ, Oladiran A, Montgomery B, El-Din MG, Perez-Estrada LC, Stafford

JL, Martin JW, Belosevic M. 2011. Ozone treatment ameliorates oil sands process water toxicity to the mammalian immune system. *Water Res.* **45**:5849–57.

Government of Alberta. 2016. Oil sands quarterly. Open Text Web Site Management.

Grewer DM, Young RF, Whittal RM, Fedorak PM. 2010. Naphthenic acids and other acid-extractables in water samples from Alberta: what is being measured? *Sci. Total Environ.* **408**:5997–6010.

He Y, Patterson S, Wang N, Hecker M, Martin JW, El-Din MG, Giesy JP, Wiseman SB. 2012. Toxicity of untreated and ozone-treated oil sands process-affected water (OSPW) to early life stages of the fathead minnow (Pimephales promelas). *Water Res.* **46**:6359–68.

Headley J V, Peru KM, Barrow MP, Derrick PJ. 2007. Characterization of naphthenic acids from Athabasca oil sands using electrospray ionization: the significant influence of solvents. *Anal. Chem.* **79**:6222–9.

Headley J V., McMartin DW. 2004. A review of the occurrence and fate of naphthenic acids in aquatic environments. *J. Environ. Sci. Heal. - Part A Toxic/Hazardous Subst. Environ. Eng.*

Holowenko F. 2002. Characterization of naphthenic acids in oil sands wastewaters by gas chromatography-mass spectrometry. *Water Res.* **36**:2843–2855.

Holowenko FM, MacKinnon MD, Fedorak PM. 2011. Methanogens and sulfate-reducing bacteria in oil sands fine tailings waste. *Can. J. Microbiol.*

Huang K-C, Couttenye RA, Hoag GE. 2002. Kinetics of heat-assisted persulfate oxidation of methyl tert-butyl ether (MTBE). *Chemosphere* **49**:413–420.

Jiveraj MN. 1995. Naphthenic acids extraction and quantitative analy; fourth ed. Syncrude Canada Ltd.; Research Department; Edmonton; AB.

Kabdasli I, Arslan-Alaton I. 2010. Chemical Oxidation Applications for Industrial Wastewaters. IWA Publishing 341 p.

Leshuk T, Wong T, Linley S, Peru KM, Headley J V, Gu F. 2016. Solar photocatalytic degradation of naphthenic acids in oil sands process-affected water. *Chemosphere* **144**:1854–61.

Liang C, Bruell CJ, Marley MC, Sperry KL. 2004. Persulfate oxidation for in situ remediation of

TCE. I. Activated by ferrous ion with and without a persulfate-thiosulfate redox couple. *Chemosphere* **55**:1213–23.

Liu H, Bruton TA, Doyle FM, Sedlak DL. 2014. In situ chemical oxidation of contaminated groundwater by persulfate: decomposition by Fe(III)- and Mn(IV)-containing oxides and aquifer materials. *Environ. Sci. Technol.* **48**:10330–6.

van Loosdrecht, M. C., Nielsen, P. H., Lopez-Vazquez, C. M., & Brdjanovic D. 2016. Experimental Methods in Wastewater Treatment | IWA Publishing. *Water Intell. Online*. http://www.iwapublishing.com/books/9781780404745/experimental-methods-wastewater-treatment.

Mahdavi H, Prasad V, Liu Y, Ulrich AC. 2015. In situ biodegradation of naphthenic acids in oil sands tailings pond water using indigenous algae-bacteria consortium. *Bioresour. Technol.* **187**:97–105.

Marentette JR, Frank RA, Bartlett AJ, Gillis PL, Hewitt LM, Peru KM, Headley J V, Brunswick P, Shang D, Parrott JL. 2015. Toxicity of naphthenic acid fraction components extracted from fresh and aged oil sands process-affected waters, and commercial naphthenic acid mixtures, to fathead minnow (Pimephales promelas) embryos. *Aquat. Toxicol.* **164**:108–17.

Miskimmin, B., Fedorak P., Lauman R. & VK. 2010. Oil sands water toxicity: A critical review.

Muyzer G, Stams AJM. 2008. The ecology and biotechnology of sulphate-reducing bacteria. *Nat. Rev. Microbiol.* **6**:441–54.

North SML and A. 2010. Home - Canada's Oil Sands. http://www.canadasoilsands.ca/topics/Tailings/Pages/default.aspx.

Oturan MA, Aaron J-J. 2014. Advanced Oxidation Processes in Water/Wastewater Treatment: Principles and Applications. A Review. *Crit. Rev. Environ. Sci. Technol.* **44**:2577–2641.

Quinlan PJ, Tam KC. 2015. Water treatment technologies for the remediation of naphthenic acids in oil sands process-affected water. *Chem. Eng. J.* **279**:696–714.

Ramos-Padrón E, Bordenave S, Lin S, Bhaskar IM, Dong X, Sensen CW, Fournier J, Voordouw G, Gieg LM. 2011. Carbon and sulfur cycling by microbial communities in a gypsum-treated oil sands tailings pond. *Environ. Sci. Technol.* **45**:439–46.

Rice RG. 1996. Applications of ozone for industrial wastewater treatment — A review. *Ozone Sci. Eng.* **18**:477–515.

Rittmann B, McCarty P. 2012. Environmental biotechnology: principles and applications.

Robinson T, McMullan G, Marchant R, Nigam P. 2001. Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative. *Bioresour. Technol.* **77**:247–255.

Shu Z, Li C, Belosevic M, Bolton JR, El-Din MG. 2014. Application of a solar UV/chlorine advanced oxidation process to oil sands process-affected water remediation. *Environ. Sci. Technol.* **48**:9692–701.

Slavcheva E, Shone B, Turnbull A. 1999. Review of naphthenic acid corrosion in oilrefining. *Br. Corros. J.* **34**:125–131.

Sohrabi V, Ross MS, Martin JW, Barker JF. 2013. Potential for in situ chemical oxidation of acid extractable organics in oil sands process affected groundwater. *Chemosphere* **93**:2698–703.

Sutton NB, Kalisz M, Krupanek J, Marek J, Grotenhuis T, Smidt H, de Weert J, Rijnaarts HHM, van Gaans P, Keijzer T. 2014. Geochemical and microbiological characteristics during in situ chemical oxidation and in situ bioremediation at a diesel contaminated site. *Environ. Sci. Technol.* **48**:2352–60.

Tsitonaki A, PETRI B, CRIMI M, MOSBÆK H, SIEGRIST RL, BJERG PL. 2010. In Situ Chemical Oxidation of Contaminated Soil and Groundwater Using Persulfate: A Review. *Crit. Rev. Environ. Sci. Technol.* **40**:55–91.

Wang N, Chelme-Ayala P, Perez-Estrada L, Garcia-Garcia E, Pun J, Martin JW, Belosevic M, Gamal El-Din M. 2013. Impact of ozonation on naphthenic acids speciation and toxicity of oil sands process-affected water to Vibrio fischeri and mammalian immune system. *Environ. Sci. Technol.* **47**:6518–26.

CHAPTER 4. OXIDATIVE STRESS INFLUENCE ON BIODEGRADATION: QUANTITATIVE PHYSIOLOGICAL STUDY

4.1 Introduction

Implementation of chemical oxidants is common in the treatment of recalcitrant compounds or partially biodegradable organics in wastewaters (Oturan and Aaron, 2014). As mentioned in chapter 2 (Section 2.2.5.1) oxidants are chemicals that have a high tendency to accept electrons. This tendency is demonstrated by their positive and high redox potential.

Peroxydisulfate or persulfate ($S_2O_8^{2-}$) with a redox potential of 2.1 V is used as a microbial disinfectant and a fundamental ion in detergents (Ghanizadeh et al., 2015; Oh et al., 2014; Veschetti et al., 2003; Wordofa, 2014). Recently, persulfate has received much attention as the newest oxidant in chemical oxidation for environmental engineering purposes specifically for *in situ* chemical oxidation (Liu et al., 2014; Siegrist et al., 2011; Sohrabi et al., 2013; Tsitonaki et al., 2010). There are a number of reasons that make sodium persulfate a good candidate (Liu et al., 2014; Tsitonaki et al., 2010; Waldemer et al., 2007):

- Low price point for the sodium salt
- High potential to generate free sulfate radicals under certain conditions
- Reactivity to various contaminants
- Production of non-toxic by products
- High water solubility and high persistency in the environment

There are limitations associated with chemical oxidation treatment of contaminated streams. These include: the high tendency of intermediates to be persistent to chemical oxidation, and the high cost of the process to reach complete mineralization (Muñoz et al., 2005). As such, interest in coupling chemical and biological treatment is increasing (Alaton et al., 2004; Esteves et al., 2016; Guieysse and Norvill, 2014; Marsolek and Rittmann, 2016; Oller et al., 2011). Many studies have confirmed that chemical oxidant treatment increases biodegradability of recalcitrant compounds (Anfruns et al., 2013; Brown et al., 2013; Kiliç et al., 2013; Martin et al., 2010; Merayo et al., 2013; Rizzo, 2011; Sanchis et al., 2013). Cassidy et al. (2009) investigated the effect of ozone and sodium persulfate on subsequent biodegradation of 2,4-dinitrotoluene. Removal efficiency of biological degradation of 2,4-dinitrotoluene was 66% by itself. Ozone pretreatment had efficiency up to 70% oxidation; however, it took 60 days for microorganism to rebound to uptake remaining pollutant. Sodium persulfate (~12 gL^{-1}) oxidized only 37% of the 2,4-dinitrotoluene and it there were no lag time for 2,4-dinitrotoluene degraders after sodium persulfate treatment. It means that sodium persulfate did not have any effect on 2,4-dinitrotoluene degraders (Cassidy et al., 2009). On the other hand, another study about oxidation of diesel contaminated site with sodium persulfate, potassium permanganate and hydrogen peroxide shows different results. Based on Chen et al. (2015) findings sodium persulfate has the most lethal effect on bacterial community (CFU g^{-1}) i.e. complete death at the dosage of 5% wt/wt (in 30 mL water and 30 g soil) (Chen et al., 2015).

Lee et al. (2000) found that 98% of Fenton-pretreated benz(a)thracene degradaed after 63 days of incubation; however, for the case of no pre-treatment only 12% of benz(a)thracene degraded over the same period of time (Lee and Hosomi, 2001). Another study about the persulfate oxidation for treatment of gasoline contaminated site has interestingly shown absolute decrease

in microbial population. Based on 16S rRNA analyses, population returned to their initial value after 3 months of firs injection of persulfate. However, increase in copy number of *alkB* gene imply on relative enrichment of alkane degrading microorganism (Sutton et al., 2014). Pre-treatment of pendimethaline contaminated soil by Fenton's reagent is investigated by Miller et al. (1996). In spite of overall decrease in population of heterotrophic microorganisms, increase in the concentration of *Pseudomonas* species was observed (Miller et al., 1996). It can be realized that chemical pre-treatment provides favorable condition for bioremediation.

Comparison of concentration of methane and sulfate in a mixture of oil sands process affected water and fluid fine tailings before and after gamma irradiation demonstrated in slight increases in microbial activity. Aerobic batch biodegradation of commercial NAs (TCI chemical) can have removal efficiency of 83%. However, pre-ozonated NAs can be degradaed by microorganisms up to 89% (Boudens et al., 2016).

In general, chemical oxidation as a pre-treatment enhances the bioavailibity of parent compounds (Kulik et al., 2006; Miller et al., 1996; Sutton et al., 2010) and produces biodegradable oxidized daughter compounds (Lee and Hosomi, 2001; Oller et al., 2011; Sutton et al., 2010).

Just as chemical oxidations improve biodegradability of recalcitrant compounds they also induce an oxidative stress response in microorganisms, which can lead to death. Brown et al. (2013) studied the survival of the indigenous microbial community during *in situ* ozonation of aged oil sands process-affected water. Results showed that a 50 mg/L ozone dose allowed survival of the indigenous microbial community and that subsequent biodegradation significantly improved in the treatments (Brown et al., 2013). It is important to note that few studies of this nature exist

thus indicating an important knowledge gap when coupling chemical and biological treatment for *in situ* treatment scenarios.

The basic physiology of a microorganism can be described by the following equation (Kristiansen, 1999; Lehninger et al., 2005):

$$\text{Phenotype} = \text{Genotype} + \text{Environment} \quad (4.1)$$

Phenotype expresses the observable properties of a cell either quantitative or qualitative. The phenotype of a cell originating from two sources: the genotype and the environment of the (micro-) organisms. The genotype addresses the information contained in DNA (The informational biopolymer DNA (deoxyribonucleic acid) contains all the cell's hereditary information) (Bailey, 1986; Lehninger et al., 2005). Cell physiology is categorized as one of the branches of phenotype of the cell. In cell physiology all the activities that are taken place in cell to keep the cell alive is studied. Therefore, parameters such as the rate of substrate consumption, amount of biomass produced per unit of consumed substrate, doubling time, respiration of the cell, metabolism are quantities that describe physiology of the cell. Some of mentioned parameters are interface of engineering and biology i.e. substrate consumption rate. Engineering parameters that imply on the rate (kinetics) and amount (stoichiometry) is applied to explain some of the quantitative aspects of cell physiology.

As it can be seen in the equation, physiological parameters cover the intrinsic properties of the cell. Based on equation 4.1, a change in a cell's phenotype may have a genetic and/or environmental origin. As such, if environmental conditions are held constant, differences in the physiology of the cell are due to changes in the genetic properties of the cell. Thus, studying physiological parameters are very informative for characterizing the behaviour of cells under

different conditions (Boender et al., 2009; Heyland et al., 2010; Jørgensen, 2011; Kristiansen, 1999; LaRowe and Amend, 2015; Nielsen et al., 2012; Rittmann and McCarty, 2012; Strous et al., 1999).

Bacteria have evolved sophisticated molecular mechanisms to activate antioxidant defense genes during oxidative stress (Harding et al., 2003; Pruchniak et al., 2015; Sies, 1997). Oxidative damage to proteins are the main physiological signal that activate antioxidant genes (Cabiscol et al., 2000; Farr and Kogoma, 1991; Storz et al., 1990). The transcriptional activator OxyR and SoxR are the main redox sensing proteins in bacteria which contain specific regulators for antioxidant genes (Cabiscol et al., 2000; Chung et al., 2015; Demple, 1991; Farr and Kogoma, 1991; Honn et al., 2016; Liu et al., 2016; Storz et al., 1990). The most common defense mechanism against oxidative stress is the production of reducing agents by antioxidant genes such as: NADHP, NADH, β-carotene, α-tocopherol (Cabiscol et al., 2000; Demple, 1991; Gambino and Cappitelli, 2016; Harding et al., 2003; Pruchniak et al., 2015). However, a defence mechanism against oxidative stress by means of persulfate salts is still unclear and there are no biomarkers currently identified (Mörtstedt et al., 2011). Current research suggests that persulfate directly attacks the proteins around the cell and biomolecules (Cruz et al., 2009; Mensing et al., 1998; Mörtstedt et al., 2011; Muñoz et al., 2004). Up to date, a significant knowledge gap still exists in understanding the impact of persulfate on microorganisms.

In this work, for the first time quantitative physiological parameters are tracked for a *Pseudomonas sp.* (isolated in our lab group) to elucidate the effect of oxidative stress by the persulfate ion. We hypothesized that oxidative stress would affect the ability of *Pseudomonas sp.* in depletion of Merichem NAs. To characterize the behaviour of *Pseudomonas* sp. before and after oxidative stress, the following quantitative physiological parameters will be determined:

biomass specific growth rate, biomass specific substrate consumption rate, yield of substrate to biomass. The biomass specific oxygen consumption rate, and yield of oxygen to biomass as the representation of the amount of electron acceptor consumed is also discussed (Boender et al., 2009; Heyland et al., 2010; Jørgensen, 2011; Kristiansen, 1999; LaRowe and Amend, 2015; Nielsen et al., 2012; Rittmann and McCarty, 2012; Strous et al., 1999).

4.2 Materials and Methods

4.2.1 Substrate, Oxidant and Microorganism Used in Experimental Set-up

Organic substrate. Naphthenic acids (NAs) were selected as the sole source of carbon and electrons (as discussed above, the selected substrate has to be partially biodegradable) Commercial NAs were purchased from Merichem Co. (Houston, Texas) and dissolved in 1 N filtered NaOH as the stock solution of 100 mg/mL for the carbon source.

Chemical oxidant. Sodium persulfate salt 99% was purchased from Sigma-Aldrich. The stock solution prepared at the concentration of 25 g/L. Assigned amount of sodium persulfate added to the stress test flasks.

Microorganisms. The *Pseudomonas* sp. in this study have been isolated from Suncor's South Tailings Pond in our lab. Bushnell-Haas minimal medium (Sigma-Aldrich) 3.27 g/L containing $MgSO_4$ 0.2 g, $CaCl_2$ 0.02 g, KH_2PO_4 1 g, K_2HPO_4 1 g, NH_4NO_3 1 g, $FeCl_3$ 0.05 g per litre of water and LB Broth Lennox (Fisher Scientific) containing casein peptone 10 g/L, yeast extract 5 g/L, NaCl 5 g/L was used as the bacterial growth media. LB broth used in oxidative stress experiments and HB broth used in biodegradation experiments.

4.2.2 Measurement of Cell Growth and DOC

To track bacterial growth in LB broth optical density at 600 nm was measured over time with NanoDrop 2000 UV-Vis spectrophotometer (Thermo Scientific). Dry cell weight (DCW) in BH broth was determined by filtering 25 ml of culture through previously weighed 0.22 µm filters (Millipore) and then dried at 40°C for 48 hours (Elmén et al., 1997). The DCW measuring techniques was desired in biodegradation studies for more industrial applicability, and dimensionally relevant (Elmén et al., 1997; Rittmann and McCarty, 2012) for prediction of respiratory parameters (oxygen consumption) (Colin Ratledge, 2006; Flickinger, 2009). To track the depletion of the Merichem NAs, dissolved organic carbon (DOC) measurements were completed with a TOC Analyser (Shimadzu TOC-L CPH, Japan). For DOC analysis, potassium hydrogen phathalate ≥ 99.95% (Sigma-Aldrich) used to make standard solution for calibration. And inorganic carbon was removed by adding 2.3% (v/v) 2 N HCl and airflow was adjusted to 50 ml/min for 3 min and organic carbon combusted at 680°C.

4.2.3 Oxidative Stress Test

Oxidative stress tests were carried out in batch cultivation using 1 L shaking flasks with 500 mL of LB broth. The same amount of the *Pseudomonas* sp. isolate (at OD of 1.2) was added into each flask. Four different concentrations of $Na_2S_2O_8$ were added to each flask: 0, 250, 500, 1000 and 2000 mg/L. Tested dosages were determined based on the obtained results in chapter 3. The dosages of persulfate selected based on the **estimated** amount of persulfate needed for NAs degradation (See section 3.3.2). Each treatment was conducted in triplicate at room temperature (21±3° C) and flasks were shake at 200 rpm. Once stationary phase was achieved in each treatment, a 500 µL aliquot was prepped and transferred to freshly set-up flasks to determine the

ability of the "oxidatively stressed" *Pseudomonas* sp. to degrade commercial NAs as outlined below.

4.2.4 Biodegradation Experiment

BH broth contained all compounds that are needed for growth except carbon and energy source. NAs added to the media up to the concentration of 50 mg/L (gravimetric amount). Each biodegradation experiment performed in a 1 litre shaking flasks with an effective volume of 500 mL as the volume of broth. Each treatment was conducted in triplicate at room temperature (21±3° C) and flasks were shake at 200 rpm. Table 4-1 summarizes the biodegradation experiment.

Table 4-1 Treatment conditions to test the biodegradative capacity of oxidatively stressed *Pseudomonas* sp. isolates.

Names	**Substrate**	**Electron Acceptor**	**Nutrient Medium**	**Stress test with $Na_2S_2O_8$ (mg/L)**
I – Series	Merichem NAs 50 mg/L	O_2/H_2O	BH	0
A – Series	Merichem NAs 50 mg/L	O_2/H_2O	BH	250
B – Series	Merichem NAs 50 mg/L	O_2/H_2O	BH	500
C – Series	Merichem NAs 50 mg/L	O_2/H_2O	BH	1000

A 500 µL aliquot at stationary phase was taken out from the stressed flasks and centrifuged at 5000 ×g for 10 min. The pellet was then re-suspended in 500 µL BH media and transferred to the flasks. All treatments were completed in triplicate under the same conditions with the only difference being the source of biomass which was exposed to different dosages of stress (0, 250, 500 and 1000 mg/L of $Na_2S_2O_8$).

4.3 Results and Discussion

4.3.1 Biodegradative Capacity of *Pseudomonas* sp.

Pseudomonas species have shown a strong ability to uptake a wide range of organic substrates as their sole carbon and energy source (Hack et al., 2015; Huang et al., 2016; Patel et al., 2012; Reardon et al., 2000; Silver et al., 1990; Zhang et al., 2016). *Pseudomonas* species have nonspecificity of induced enzymes and convergence of catabolic pathways, allowing them to utilize mixed substrates without any surplus genetic coding for enzyme production (Hutchinson and Robinson, 1988; Silver et al., 1990). Based on this capacity, *Pseudomonas* species, can be considered a potential candidate for degradation of NAs (Blakley and Papish, 1982; Demeter et al., 2015; Paslawski et al., 2009; Del Rio et al., 2006; Whitby, 2010).

4.3.2 Impact of Persulfate Oxidative Stress on *Pseudomonas* sp

To determine the impact of persulfate oxidative stress on the *Pseudomonas* sp. the growth curve of the isolate was monitored as it was exposed to 0- 2000 mg/L of persulfate in a LB broth media. As shown in figure 4.1 the maximum biomass concentration decreases from 1.260 AU (ideal growth) to 0 (2000 mg/L of sodium persulfate) with increasing the persulfate concentration.

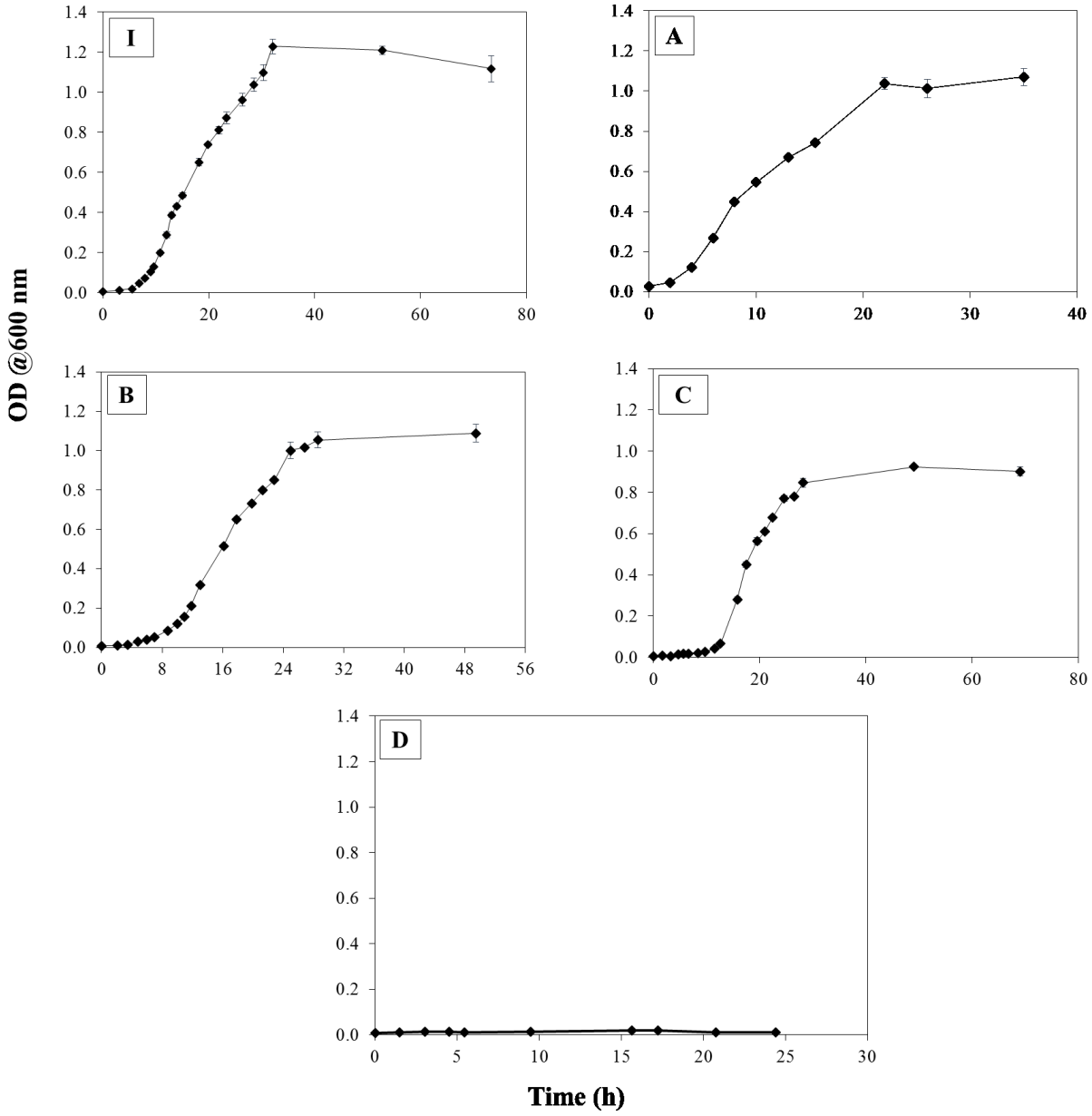


Figure 4-1Growth of *Pseudomonas sp*. in LB broth; Graph (I) represents growth under zero stress; Graph (A) illustrates growth under 250 mg/L of $Na_2S_2O_8$; Graph (B) illustrates growth under 500 mg/L of $Na_2S_2O_8$; Graph (C) illustrates growth under 1000 mg/L of $Na_2S_2O_8$; Graph (D) illustrates growth under 2000 mg/L of $Na_2S_2O_8$; Error bars are plus minus of standard error of triplicate

As expected, by increasing the persulfate concentration, maximum biomass concentration correspondingly decreased. A persulfate dose of 2000 mg/L prevented the *Pseudomonas* sp. from growing as shown by their OD that never reached higher than 0.02. Maximum biomass concentrations, as measured, are presented in table 4.2.

Table 4-2. Maximum biomass concentration (in absorbance unit (AU) @600 nm) under different persulfate oxidant concentrations. Data are shown ± standard error of triplicate

Growth Systems	$Na_2S_2O_8$ Dose (mg/L)	C_x^{max} (AU)
Ideal Growth	0	1.260 ± 0.006
A – Series	250	1.117 ± 0.017
B – Series	500	1.156 ± 0.021
C – Series	1000	0.934 ± 0.003
D – Series	2000	-

Statistical analysis conducted to compare the mean of maximum concentration of biomass and investigate their differences statistically. Bonferroni and Tukey's multiple comparison tests applied to compare each series to another (for results see Appendix 2). Statistical analysis present that the only series (based on the maximum concentration of biomass) that are not significantly different ($p>0.05$) are A and B. However, all series respect to the non-stress bacteria are significantly different ($p<0.05$). This observation shows the effect of oxidative stress on the bacteria. For the I, A and B series which are stressed by 0, 250 and 500 mg/L of sodium persulfate, no lag time were observed. While for C- series that stressed by 1000 mg/L of sodium persulfate, there were a lag time of ~10 hr. It shows the adaptability time of bacteria to persulfate stress.

Pseudomonas sp. are aerobic bacteria that are uses oxygen as the electron acceptor. To have an understanding of oxygen mass transfer in flasks as the bioreactor, mass transfer analysis perforemed, as follows.

4.3.3 Mass Transfer Analysis

One of the main factors that might affect the aerobic bioprocesses is limitation oxygen transfer. To ensure that oxygen was not a limiting factor in our system and keep the degree of the freedom of the system to one (the only degree of freedom in our system was concentration of substrate which was Merichem NAs). Transfer of oxygen takes place through two phases in our experiments. First, the transfer of oxygen from gas phase (from the bottleneck of the flask to the interface of liquid and gas phases). Second, transfer of oxygen in the liquid phase.

4.3.3.1 Oxygen Mass Transfer Coefficient in Gas Phase

To calculate the oxygen mass transfer coefficient in the gas phase (k_{Ga}) we need to know the turbulence factor (T_G) in the gas phase. There is an empirical formula for calculating the turbulence factor as a function of flask to liquid volume ratio and rate of shaking (Nikakhtari and Hill, 2006):

$$T_G = \frac{V}{L} \times N^{\alpha} \tag{4.1A}$$

For a flask without a closure, α is equal to 1.80. T_G represents the gas phase turbulence factor. By knowing the T_G; one can calculate the oxygen mass transfer coefficient in the gas phase as (Nikakhtari and Hill, 2006):

$$k_{G_a} = 0.176 \cdot \exp(0.0002 \times T_G) \tag{4.2}$$

In the above equations, k_{Ga} is the mass transfer coefficient in the liquid phase; V is the volume of the flask; L is the volume of liquid; T_G is the gas phase turbulence factor and N is the shaking rate (Nikakhtari and Hill, 2005). However, this equation is valid for T_G =11000 ± 400; for T_G more than 11000 an average value of 86.8 ± 15.7 h^{-1} is considered. Practically, for this range of k_{Ga} (~86.8 ± 15.7 h^{-1}) resistance against mass transfer of oxygen through the neck of the shake flask to the headspace is negligible (Gupta and Rao, 2003; Henzler and Schedel, 1991; Nikakhtari and Hill, 2006; Van Suijdam et al., 1978; Veglio et al., 1998). It can be seen that in table 4-3 the k_{Ga} for our experiments is high enough, therefore, the resistance against mass transfer of oxygen in gas phase is negligible. Table 4-3 shows the T_G value for this work.

Table 4-3. Oxygen mass transfer coefficient in the gas phase is calculated. V shows the volume of the flask, L represents the liquid volume of the flask; N is the mixing rate; α is constant and T_G is turbulence factor which is calculated based on Eq. 4.27.

	V (L)	*L (L)*	*N (rpm)*	*α*	*T_G*	*k_{Ga} (h^{-1})*
Beginning of the experiment	1	0.5	200	1.80	27726	86.8 ± 15.7
End of the experiment	1	0.25	200	1.80	55452	86.8 ± 15.7

4.3.3.2 Oxygen Mass Trasnfer Coefficient in Liquid Phase

Oxygen mass transfer coefficient in the liquid phase can be calculated based on the following equations (Nikakhtari and Hill, 2005):

$$k_{L_a} = 0.0182 \times (A \times T_L) \tag{4.3}$$

$$A = 142 \times (V - L)^{2/3} \tag{4.4}$$

$$T_L = \frac{V^{0.463}}{L} \times \frac{N}{60} \quad (4.5)$$

In the above equations k_{La} is the mass transfer coefficient in the liquid phase; A is liquid surface area in (cm^2); V is the volume of the flask; L is the volume of liquid; T_L is the liquid phase turbulence factor and N is the shaking rate (Nikakhtari and Hill, 2005). The calculated k_{La} for this work is shown in table 4-4

Table 4-4. Calculated related parameters for estimation of oxygen mass transfer coefficient in liquid phase;1 litre flasks used as a bioreactor with the effective liquid volume of 0.5 L (broth)

	V (L)	*L (L)*	*A (cm^2)*	*N (rpm)*	*T_L*	*k_{La} (h^{-1})*
Beginning of the batch	1	0.5	90	200	6.67	10.92
End of the batch	1	0.25	117	200	13.34	28.45

It can be seen that the mass transfer coefficient for the liquid phase is increasing with over the the experiment (due to increase in surface area of liquid phase because of sampling). (Comparing the predicted specific oxygen consumption rate and mass transfer coefficient for liquid phase conducted in Appendix 2)

4.3.4 Biodegradation of Merichem Naphthenic Acids Post Oxidative Stress

After completion of the stress test, *Pseudomonas* sp. from stationary phase were transferred to new flasks with fresh BH media amended with 50 mg/L of Merichem NAs to test their degradative abilities post oxidative stress. Sampling of growth and degradation started after inoculation of bacteria. Lag time was considered as the time when less than 2% of the substrate is depleted (Reardon et al., 2000). Consumption of Merichem NAs, which is shown in the form

of dissolved organic carbon shows the capacity for the *Pseudomonas* sp. to grow on Merichem NAs as the sole source of carbon and energy (as shown in Figure 4-2). Kinetic and stoichiometric (physiological) analysis was completed to compare the performance of bacteria under different cultivation conditions. It is interesting to mention that the "bottle effect" or surface to volume ratio has no effect on biodegradation and concentration of biomass in batch growth conditions (Hammes et al., 2010).

Calculation of stoichiometric and kinetic parameters can be quite challenging for partially biodegradable substrates. As can be seen Merichem NAs are also partially biodegradable by *Pseudomonas* sp. As mentioned in chapter 2, physiological parameters from batch data are not as reliable as chemostat cultivation (Kovarova-Kovar and Egli, 1998). For instance, there is no information about K_s (substrate affinity) of biodegradation of commercial NAs or AEOs to a given microorganism. Lack of such data affects parameter estimation. Ellis et al. (1996) conducted a sensitivity analysis to estimate the influence of each parameter of the Monod equation in parameter estimation processes and suggested that when $\frac{C_0}{K_s} < 0.1$, unique estimation of q^{max} and K_s is not possible (Ellis et al., 1996; Knightes and Peters, 2000).

Linearization of the Monod equation and then using linear regression analysis to estimate the parameter is another method that can be used (Cornish-Bowden, 2013; Krull and Peterat, 2016). This method is widely used in the estimation of parameters in biodegradation studies, fermentation and enzymes kinetic studies, however, it is infamous for being prone to error (Cornish-Bowden, 2013; Leatherbarrow, 1990). Because the Monod model (since it is a linear regression) assumes the errors are normally distributed and since linearization highly alters the

errors, results cannot be reliable (Cornish-Bowden, 2013; Knightes and Peters, 2000; Leatherbarrow, 1990).

To **overcome** all the difficulties associated with parameter estimation we can use a direct method to calculate **certain** kinetic and stoichiometric parameters. Direct method analysis is completed based on a mass balance and rate calculation (Smolke, 2009; Stephanopoulos et al., 1998; Villadsen et al., 2011). In this approach to be scientifically relevant, we consider the log-phase when analysing the data. We take into account data which are after the lag time. As it mentioned above, the lag time is a time when less than 2% of the substrate is depleted (Reardon et al., 2000). Each batch ends at the first time point when it reaches the highest concentration of biomass. And as it can be seen in figure 4-2, the highest concentration of biomass is the time when the batch reaches the lowest concentration of substrate.

4.3.5 Mathematical Modelling Based on Mass Balance

The mass balance in batch cultivation can be described as follows (Kristiansen, 1999; Rittmann and McCarty, 2012; Smolke, 2009):

$$\text{Accumulation of compound i} = \text{Rate i} \tag{4.6}$$

$$Rate\ i = \frac{d(V.C_i)}{dt} = \frac{dM_i}{dt} \tag{4.6A}$$

In the above equation, V represent the volume, C_i shows the concentration of compound I; t is time and M_i illustrates the total mass of the compound i in the system. By definition, for consumed compounds the rate is negative and for produced compounds is positive. One very important point about the calculation of physiological parameters is considering the volume changes through batch time. So by considering the changes in volume (Smolke, 2009):

$$Rate\ i = \frac{d(V.C_i)}{dt} = C_i \frac{dV}{dt} + V \frac{dC_i}{dt} \quad (4.7)$$

Throughout the experiment the changes in volume due to evaporation were negligible. So instead of using total mass we can continue using concentration in our rate calculations.

q-rates which are biomass specific rates are defined as follows (See section 2.4.2) (Smolke, 2009; Stephanopoulos et al., 1998):

$$q_i = \frac{r_i}{C_x} \quad (4.8)$$

In Eq. (4.8) q_i represents the biomass specific rate of compound i. For instance, r_s is the substrate consumption rate. Based on Eq. (2.8) the substrate consumption rate has a direct relation to concentration of biomass. It means that r_s will increase by increasing in concentration of biomass. Therefore, r_s is a dependent parameter which means one cannot use this parameter to describe the performance of the biomass in the system. For instance, $r_{s,1}$ = 30 mg L^{-1} h^{-1} and $r_{s,2}$ =20 mg L^{-1} h^{-1} for two different independent system. It is obvious that bioreactor one has higher rate i.e. in removal of substrate. However, we do not know about the performance of biomass in these two systems and we cannot analyse the ability of microorganisms in consumption of substrate unless we know the concentration of biomass. The concentration of biomass is as follows: $C_{x,1}$=10 mg L^{-1} and $C_{x,2}$=4 mg L^{-1}. Based on the Eq. (4.8); $q_{s,1}$= 3 mg C_s mg C_x^{-1} h^{-1} $q_{s,2}$= 5 mg C_s mg C_x^{-1} h^{-1} ; therefore, it can be seen that substrate consumption rate is faster per unit of biomass in bioreactor 2. It shows the microorganisms are better for our goal (i.e. remediation purposes). Accordingly, it is very important to compare appropriate parameters in evaluation of the performance of bioprocesses. By definition, q_x is equal to μ which is a specific growth rate. Now we can write up the equation for calculation of biomass growth rate

(Kristiansen, 1999; Smolke, 2009; Stephanopoulos et al., 1998). Mass balance for biomass (Rittmann and McCarty, 2012; Smolke, 2009):

$$\frac{dC_x}{dt} = q_x\, C_x = \mu\, C_x \tag{4.9}$$

Mass balance for substrate:

$$\frac{dC_s}{dt} = q_s\, C_x \tag{4.10}$$

By solving the mass balance differential equation for biomass:

$$\frac{dC_x}{dt} = \mu\, C_x \tag{4.11}$$

$$\frac{dC_x}{C_x} = \mu\, dt \tag{4.12}$$

$$\int_{C_{x_0}}^{C_{x_t}} \frac{dC_x}{C_x} = \int_0^t \mu\ dt \tag{4.13}$$

And by recalling $\frac{dy}{y} = d(lny)$:

$$\ln C_x = \mu\, t + C_{x_0} \tag{4.14}$$

$$\ln \frac{C_x}{C_{x_0}} = \mu\, t \tag{4.15}$$

$$C_x(t) = C_{x_0}\, e^{\mu\, t} \tag{4.16}$$

Equation 4.15 shows that the graph of ln C_x over time is a straight line with a slope of μ. So based on this graph we can calculate the biomass specific growth rate.

This equation shows that concentration of biomass changes exponentially in batch cultivation. To derive the equation for substrate consumption we need to solve the differential equation for substrate mass balance.

$$\frac{dC_s}{dt} = q_s\, C_x \tag{4.17}$$

$$\frac{dC_s}{dt} = q_s\, C_{x_0}\, e^{\mu\, t} \tag{4.18}$$

$$dC_s = (q_s\, \cdot C_{x_0})[(e^{\mu \cdot t})dt] \tag{4.19}$$

By recalling $\frac{de^{u(x)}}{dx} = e^{u(x)}\frac{du(x)}{dx}$:

$$dC_s = \left(\frac{(q_s \cdot\, C_{x_0})}{\mu}\right)[d(e^{\mu \cdot t})] \tag{4.20}$$

By integrating the above equation:

$$\int_{C_{s_0}}^{C_{s_t}} dC_s = \int_0^t \left(\frac{(q_s\, C_{x_0})}{\mu}\right)[d(e^{\mu \cdot t})] \tag{4.21}$$

$$C_s(t) - C_{s_0} = \left(\frac{(q_s\, C_{x_0})}{\mu}\right)((e^{\mu \cdot t}) - 1) \tag{4.22}$$

In equation 4.22 the only unknown parameter is q_s. We can determine μ from equation 4.15, therefore to calculate q_s:

$$C_s(t) - C_{s_0} = \left(\frac{q_s}{\mu}\right)(C_{x_0}\,(e^{\mu\, t}) - C_{x_0}) \tag{4.23}$$

Term of $C_{x_0}\,(e^{\mu \cdot t})$ is equal to $C_x(t)$ based on equation 4.16. Thus:

$$C_s(t) - C_{s_0} = \left(\frac{q_s}{\mu}\right)(C_x(t) - C_{x_0}) \tag{4.24}$$

$$\frac{C_x(t) - C_{x_0}}{C_s(t) - C_{s_0}} = \frac{\mu}{q_s} \tag{4.25}$$

This reltionship demonstrates that the plot of $C_s(t) - C_{s_0}$ and $C_x(t) - C_{x_0}$ is a straight line. The slope of this straight line is equal to $\frac{\mu}{q_s}$. This slope represents the ratio of these two rates which corresponds to the yield. One important yield, as a stoichiometric parameter, is the yield of substrate to the biomass. This yield demonstrates the biomass production per unit of consumed substrate. We can calculate this yield by dividing the rate of biomass growth and substrate consumption rate (Kovarova-Kovar and Egli, 1998; Rittmann and McCarty, 2012; Smolke, 2009; Stephanopoulos et al., 1998).

$$Y_{sx} = \frac{Rate\ C_x}{Rate\ C_s} = \frac{\mu\ C_x}{q_s\ C_x} = \frac{\mu}{q_s} \tag{4.26}$$

This yield can be calculated from the slope of the plot of ($C_x - C_{x0}$) and ($C_s - C_{s0}$). In Fig 4.3 Ln (C_x/C_{x0}) is plotted over time to calculate the specific growth rate. In Fig 4.4 ($C_x - C_{x0}$) is plotted over ($C_s - C_{s0}$) to calculate the yield of the substrate to biomass.

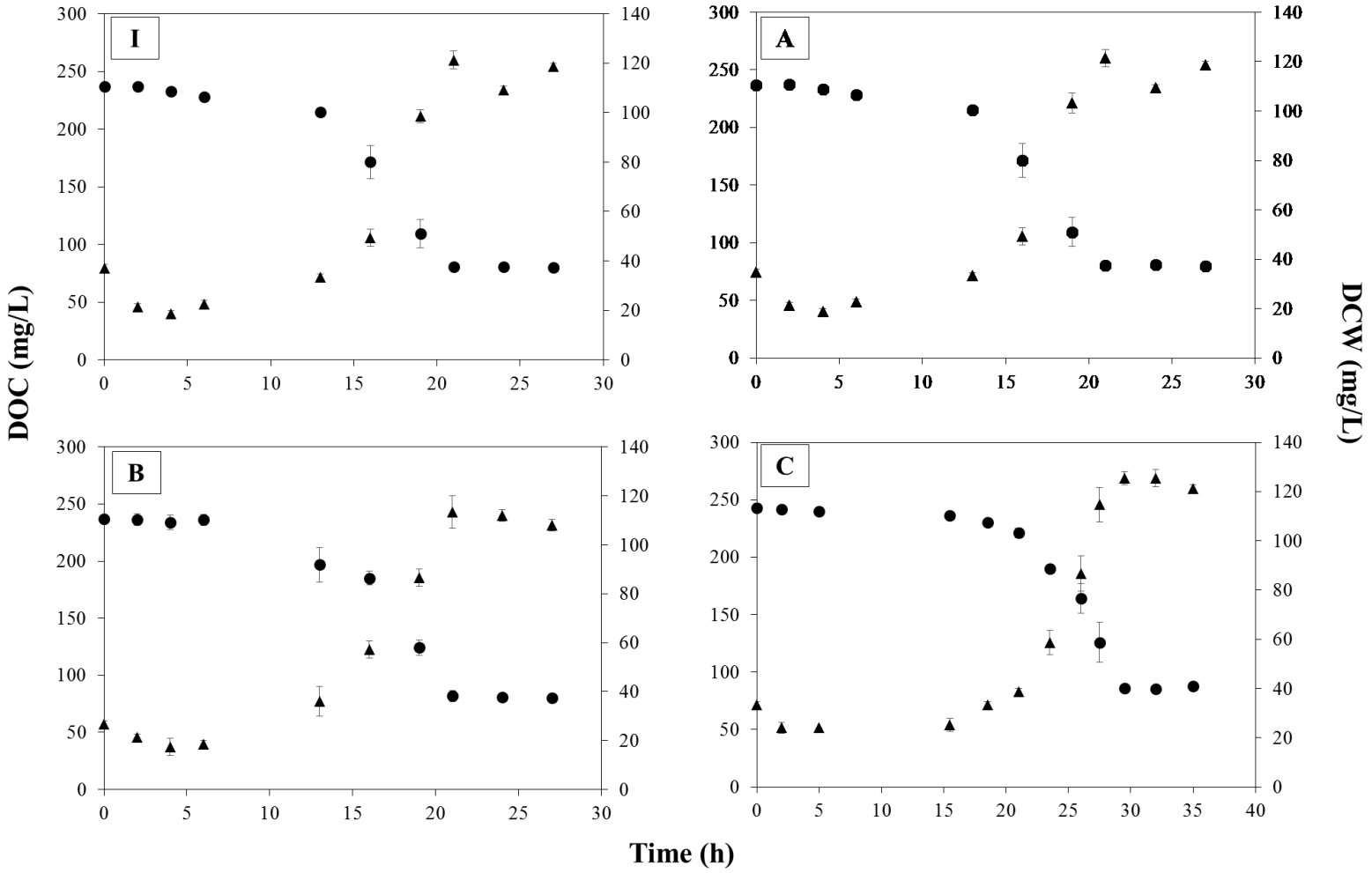


Figure 4-2. Biodegradation of Merichem NAs under aerobic condition by *Pseudomonas sp.* isolated from oil sands tailings pond. Series (I) represents the non-stressed bacteria; (A) shows the bacteria previously stressed with 250 mg/L sodium persulfate; (B) bacteria stressed with 500 mg/L and (C) with 1000 mg/L of sodium persulfate. (▲) represents the dry cell weight (DCW) and (●) illustrates the concentration of dissolved organic carbon (DOC); error bars are plus minus standard error

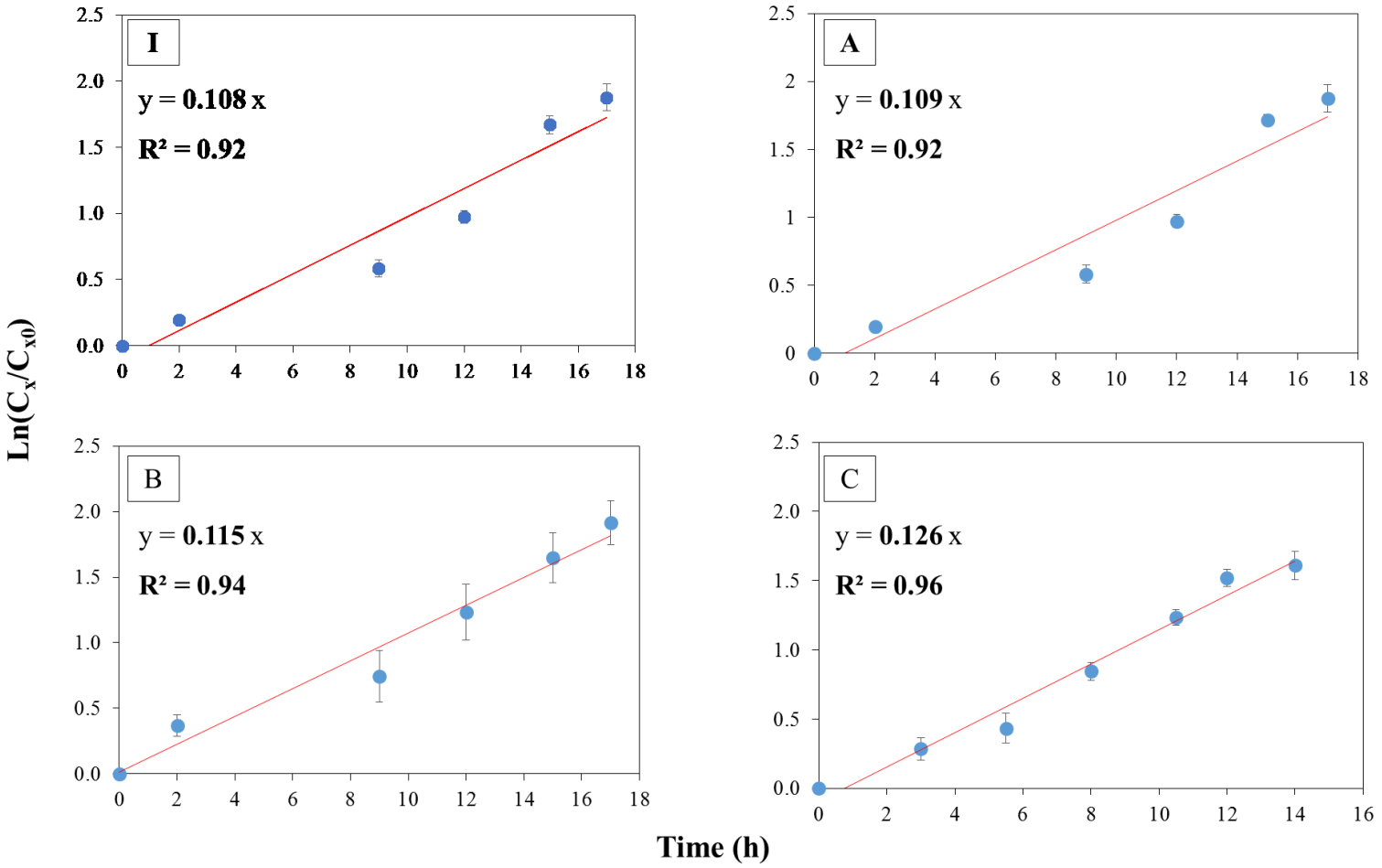


Figure 4-3. Linear regression of Ln (C_x/C_{x0}) vs. time. Based on Eq. 4.15, the slope shows the biomass specific growth rate. (I), (A),(B), (C) represent the different stress condition from 0, 250, 500,1000 mg/L of sodium persulfate. Error bars are plus minus of standard error of triplicate

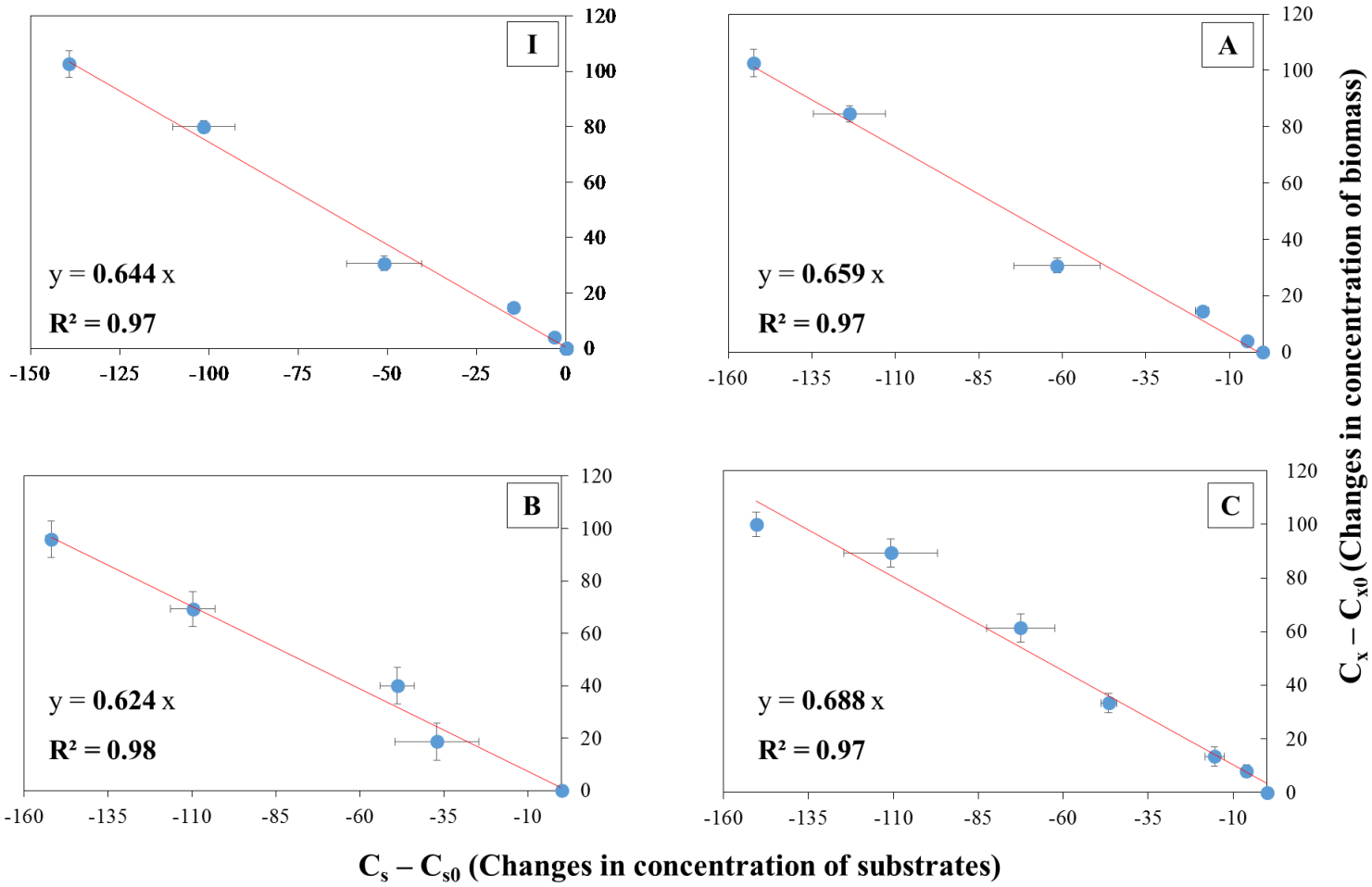


Figure 4-4. Yield of substrate to biomass or Y_{sx} can be obtained from the slope of the above graphs; (I), (A), (B) and (C) are differences only in terms of the source of biomass where stressed by 0, 250, 500 and 1000 mg/L of $Na_2S_2O_8$; error bars are plus minus of standard error of triplicate

4.3.5.1 Biomass specific growth rate

Results that are derived from Eq (4.15) are summerised in table 4-5. Biomass doubling time of the biomass is also shown to have better representation of growth rate.

Table 4-5. Biomass specific growth rate and doubling time of biomass

	μ biomass specific growth rate (h^{-1})		**t_D doubling time (h)**	
	Average	95% Confidence Interval	Average	95% Confidence Interval
I	0.108	0.091 - 0.124	6.42	7.62 - 5.59
A	0.109	0.092 - 0.126	6.36	7.53 - 5.50
B	0.115	0.099 - 0.131	6.03	7.00 - 5.29
C	0.126	0.114 - 0.138	5.50	6.08 - 5.02

Biomass specific growth rate represents the rate of biosynthesis of new cells. This rate is a function of different parametres i.e. temperature, amount of nutrient, concentration of substrate. This rate is representation of anabolism which is an intrinsic property of a cell. The changes in the rate of anabolism (biomass specific growth rate) in the batch process can have (in general) two degree of freedom, one for the environment and one for its intrinsic properties. Therefore, in the same environmental condition a change in the rate of anabolism has only one degree of freedom. This degree of freedom originates from the gene expression of the microorganisms, as genes control all of the cell's activity.

Biomass specific growth rates for each set of experiment determined as an independent parameter for comparison of the performance of bacteria. This parameter as introduced in chapter 2 (section 2.4.2), is a kinetic parameter. Results are showing no significant ($p>>0.05$) changes in kinetic of biomass specific growth rate. It means that changes in genetic expression of *Pseudomonas* sp. due to oxidative stress did not effect on the ability of the bacteria to grow on Merichem NAs. This observation enhances the chance of coupling chemical and biological

oxidation. However, there is a lag time in biodegrdation, after rebounding, the bacteria can **grow** as the same as non-stressed bacteria. For treatment purposes, the growth rate is one of the (many) factors are implementation of a process. Therefore, other related parameters are discussed, as follows.

4.3.5.2 Yield of Substrate to Biomass (Y_{sx})

In analyses of the amount of biomass that is produced per unit of consumed substrate based on Fig 4-4, following results are obtained. Generally, understanding of yield of substrate to biomass is among the most important parameters in design and application of a biological process (Rittmann and McCarty, 2012). In design of a bioprocess, the amount of produced biomass is very important. Lack of this information would result in i.e. high production rate of CO_2 (mainly because of catabolism), high rate of biomass production which increases the cost of sludge handling.

Table 4-6. Yield of substrate to biomass for non-stressed and previously stressed bacteria

	Y_{sx} *(mg DCW mgDOC $^{-1}$)*	
	Average	95% Confidence Interval
I	0.644	0.586 - 0.703
A	0.659	0.600 - 0.717
B	0.624	0.575 - 0.673
C	0.688	0.628 - 0.748

Here, by comparing the yields from each set of experiments, no changes were observed. This observation expresses that oxiadtively stressed bacteria use the same stoichiometric amount of substrate to produce biomass. Yield of substrate to biomass is the result of coupling of catabolism and anabolism. Surprisingly, the yield of substrate to biomass did not change

(significantly) for stressed and non-stressed bacteria. This observation would increase the possibility of coupling persulfate and biological oxidation.

4.3.5.3 Biomass Specific Substare Consumption Rate (q_s)

q-rates are introduced in Eq. (4.8). It has been described that q-rates are very informative in terms of explaining the activity of the cell. Since, it shows a given quantity per unit of biomass, for instance here, the rate of consumption of NAs (by tracking the concentration of dissolved organic carbon) is calculated per unit of biomass.

Table 4-7. Biomass specific substrate consumption rate for different oxidatively stressed bacteria

	q_s (mg DOC mg DCW^{-1} h^{-1})	
	Average	95% Confidence Interval
I	0.168	0.155 - 0.176
A	0.165	0.153 - 0.176
B	0.184	0.172 - 0.195
C	0.183	0.182 - 0.184

Biomass specific substrate consumption rate is also very relevant in design and application of a bioprocess. For instance, in environmental engineering purposes we are interested in higher biomass specific consumption rate, since it shows the rate of system. Similar to μ and yield, q_s is also did not change for oxidatively stressed bacteria.

By determining of three important parameters that governed stoichiometry and kinetics of NAs consumption and bacterial growth, possibility of using persulfate oxidation as a pretreatment step can be verified. Fom biodegradation graphs (Fig. 4-2), a lag time; specifically for C-series can be seen. Lag time in biodegradation is discussed below.

4.3.6 Lag Time of Biodegradation

The 2% biodegradation removal time considered as the lag time of system (Reardon et al., 2000). Lag time for I, A and B series calculated as 4 hours. 0, 250 and 500 mg/L of sodium persulfate stressed bacteria has the same amount of lag time. However, for the series of C the calculated lag time was 15.5 hours. The bacetria that stressed with highest concentration of sodium persulfate had the longest lag time. The lag time generally defines as the time of adaptability of microorganisms to current condition. After adaptation of bacteria to the current condition, the process parameters, as shown above, were not significantly different.

4.4 Conclusions

In this chapter, quantitave physiological study applied to elucidate the effect of oxidative stress on the ability of microorganisms in using Merichem NAs as the sole source of carbon and energy. The parameters that are used in quantitative physiological study were chosen base on their applicability in future desin of the process. The process parametres that are obtained from non-stressed microorganism are considered as the control, and therefore, parametres from stressed bacteria compared to the non-stressed ones. Results are showing no significant change in process parameters of biodegradation, except lag time. For instance, for the bacteria that previously stressed with 1000 mg/L of sodium persulfate, the lag time was more than 3 times higher respect to other series.

Other investigation on the effect of persulfate on microorganism have similar findings. However, changes in kinetic and stoichiometric parameters due to persulfate stress have not been reported. Palmroth et al. (2006) used counting techniques to demonstrate the effect of persulfate on indigenous population of soil. Based on their founding, after 2 days of exposure of soil to the persulfate with the dosage of 10 g/kg soil, the concentration of microorganism decreased from

$10^{7.5}$ to $10^{7.4}$ CFU/g soil (Palmroth et al. 2006). Tsitonaki et al. (2007) tested the range of 0.02 to 2 g $S_2O_8^{2-}$/kg soil on soil indigenous microorganisms. After 2 days as the exposure time, significant change in the concentration of indigenous microorganisms did not observe. While, the concentration of spiked *Pseudomonas putida* decreased from 2.96×10^6 live cells/g soil to 3.4×10^5 cells/g soil for heat activated persulfate (0.8 g/L) (Tsitonaki et al., 2008). Cassidy et al. (2009) compared the effect of three oxidants as follows ozone, Fenton and sodium persulfate. Results have shown that the least amount of effect occurred by sodium persulfate. Rebounding time (lag time of biodegradation) were as follows: 60 days for ozone, 20 days for Fenton treated matrix and 2 days for persulfate treated soil (Cassidy et al., 2009).

However, there is a lack of kinetic and stoichiometric parameters of microorganisms before and after oxidative stress, other findings are imply on the fact that persulfate does not significantly affect miroorganisms.

Findings of this work can be used in design of a coupled process of chemical and biological oxidation with persulfate as the oxidant. Tested dosages in chapter 3 of this work shows the reactivity of persulfate toward main pollutant in OSPW which is NAs. Stressed microorganisms with the ranages of persulfate that are reactive to NAs, do not significantly affect biodegradability of *Pseudomonas* sp. Therefore, combination of persulfate oxidation and biological oxidation is feasible. However, further experiments is required to apply the process at industrial scales.

4.5 References

Alaton IA, Dogruel S, Baykal E, Gerone G. 2004. Combined chemical and biological oxidation of penicillin formulation effluent. *J. Environ. Manage.* **73**:155–63.

Anfruns A, Gabarró J, Gonzalez-Olmos R, Puig S, Balaguer MD, Colprim J. 2013. Coupling anammox and advanced oxidation-based technologies for mature landfill leachate treatment. *J. Hazard. Mater.* **258-259**:27–34.

Bailey JE. 1986. Biochemical Engineering Fundamentals. McGraw-Hill 984 p.

Blakley ER, Papish B. 1982. The metabolism of cyclohexanecarboxylic acid and 3-cyclohexenecarboxylic acid by Pseudomonas putida. *Can. J. Microbiol.* **28**:1324–9.

Boender LGM, de Hulster EAF, van Maris AJA, Daran-Lapujade PAS, Pronk JT. 2009. Quantitative physiology of Saccharomyces cerevisiae at near-zero specific growth rates. *Appl. Environ. Microbiol.* **75**:5607–14.

Boudens R, Reid T, VanMensel D, Prakasan M R S, Ciborowski JJH, Weisener CG. 2016. Bio-physicochemical effects of gamma irradiation treatment for naphthenic acids in oil sands fluid fine tailings. *Sci. Total Environ.* **539**:114–24.

Brown LD, Pérez-Estrada L, Wang N, El-Din MG, Martin JW, Fedorak PM, Ulrich AC. 2013. Indigenous microbes survive in situ ozonation improving biodegradation of dissolved organic matter in aged oil sands process-affected waters. *Chemosphere* **93**:2748–55.

Cabiscol E, Tamarit J, Ros J. 2000. Oxidative stress in bacteria and protein damage by reactive oxygen species. *Int. Microbiol.*

Cassidy D, Northup A, Hampton D. 2009. The effect of three chemical oxidants on subsequent biodegradation of 2,4-dinitrotoluene (DNT) in batch slurry reactors. *J. Chem. Technol. Biotechnol.* **84**:820–826.

Chen K-F, Chang Y-C, Chiou W-T. 2015. Remediation of diesel-contaminated soil using in situ chemical oxidation (ISCO) and the effects of common oxidants on the indigenous microbial community: a comparison study. *J. Chem. Technol. Biotechnol.*:n/a–n/a.

Chung C-H, Fen S-Y, Yu S-C, Wong H-C. 2015. Influence of oxyR on Growth, Biofilm Formation, and Mobility of Vibrio parahaemolyticus. *Appl. Environ. Microbiol.* **82**:788–96.

Colin Ratledge BK. 2006. Basic Biotechnology. Cambridge University Press 666 p.

Cornish-Bowden A. 2013. Fundamentals of Enzyme Kinetics. John Wiley & Sons 510 p.

Cruz M-J, De Vooght V, Muñoz X, Hoet PHM, Morell F, Nemery B, Vanoirbeek JAJ. 2009. Assessment of the sensitization potential of persulfate salts used for bleaching hair. *Contact Dermatitis* **60**:85–90.

Demeter MA, Lemire JA, Yue G, Ceri H, Turner RJ. 2015. Culturing oil sands microbes as mixed species communities enhances ex situ model naphthenic acid degradation. *Front. Microbiol.* **6**:936.

Demple B. 1991. Regulation of bacterial oxidative stress genes. *Annu. Rev. Genet.* **25**:315–37.

Ellis TG, Barbeau DS, Smets BF, Grady CPL. 1996. Respirometric technique for determination of extant kinetic parameters describing biodegradation. *Water Environ. Res.* **68**:917–926.

Elmén J, Pan W, Leung SY, Magyarosy A, Keasling JD. 1997. Kinetics of toluene degradation by a nitrate-reducing bacterium isolated from a groundwater aquifer. *Biotechnol. Bioeng.* **55**:82–90.

Esteves BM, Rodrigues CSD, Boaventura RAR, Maldonado-Hódar FJ, Madeira LM. 2016. Coupling of acrylic dyeing wastewater treatment by heterogeneous Fenton oxidation in a continuous stirred tank reactor with biological degradation in a sequential batch reactor. *J. Environ. Manage.* **166**:193–203.

Farr SB, Kogoma T. 1991. Oxidative stress responses in Escherichia coli and Salmonella typhimurium. *Microbiol. Mol. Biol. Rev.* **55**:561–585.

Flickinger MC. 2009. Encyclopedia of Industrial Biotechnology. Hoboken, NJ, USA: John Wiley & Sons, Inc.

Gambino M, Cappitelli F. 2016. Mini-review: Biofilm responses to oxidative stress. *Biofouling* **32**:167–78.

Ghanizadeh G, Naseri Ara A, Esmaili D, Masoumbeigi H. 2015. Demonstration of the Enhanced Disinfection of E. coli Water Contamination by Associated Solar Irradiation with Potassium Persulfate. *Iran. J. Public Health* **44**:1376–86.

Guieysse B, Norvill ZN. 2014. Sequential chemical-biological processes for the treatment of industrial wastewaters: review of recent progresses and critical assessment. *J. Hazard. Mater.* **267**:142–52.

Gupta A, Rao G. 2003. A study of oxygen transfer in shake flasks using a non-invasive oxygen sensor. *Biotechnol. Bioeng.* **84**:351–8.

Hack N, Reinwand C, Abbt-Braun G, Horn H, Frimmel FH. 2015. Biodegradation of phenol, salicylic acid, benzenesulfonic acid, and iomeprol by Pseudomonas fluorescens in the capillary fringe. *J. Contam. Hydrol.* **183**:40–54.

Hammes F, Vital M, Egli T. 2010. Critical evaluation of the volumetric "bottle effect" on microbial batch growth. *Appl. Environ. Microbiol.* **76**:1278–81.

Harding HP, Zhang Y, Zeng H, Novoa I, Lu PD, Calfon M, Sadri N, Yun C, Popko B, Paules R, Stojdl DF, Bell JC, Hettmann T, Leiden JM, Ron D. 2003. An Integrated Stress Response Regulates Amino Acid Metabolism and Resistance to Oxidative Stress. *Mol. Cell* **11**:619–633.

Henzler H-J, Schedel M. 1991. Suitability of the shaking flask for oxygen supply to microbiological cultures. *Bioprocess Eng.* **7**:123–131.

Heyland J, Fu J, Blank LM, Schmid A. 2010. Quantitative physiology of Pichia pastoris during glucose-limited high-cell density fed-batch cultivation for recombinant protein production. *Biotechnol. Bioeng.* **107**:357–68.

Honn M, Lindgren H, Sjöstedt A. 2016. OxyR : an important regulator of the oxidative stress response of Francisella tularensis LVS.

Huang H, Wu K, Khan A, Jiang Y, Ling Z, Liu P, Chen Y, Tao X, Li X. 2016. A novel Pseudomonas gessardii strain LZ-E simultaneously degrades naphthalene and reduces hexavalent chromium. *Bioresour. Technol.* **207**:370–8.

Hutchinson D, Robinson C. 1988. Kinetics of the simultaneous batch degradation of p-cresol and phenol by Pseudomonas putida. *Appl. Microbiol. Biotechnol.* **29**:599–604.

Jørgensen BB. 2011. Deep subseafloor microbial cells on physiological standby. *Proc. Natl. Acad. Sci. U. S. A.* **108**:18193–4.

Kiliç MY, Yonar T, Kestioğlu K. 2013. Pilot-scale treatment of olive oil mill wastewater by physicochemical and advanced oxidation processes. *Environ. Technol.* **34**:1521–31.

Knightes CD, Peters CA. 2000. Statistical analysis of nonlinear parameter estimation for Monod biodegradation kinetics using bivariate data. *Biotechnol. Bioeng.* **69**:160–70.

Kovarova-Kovar K, Egli T. 1998. Growth Kinetics of Suspended Microbial Cells: From Single-Substrate-Controlled Growth to Mixed-Substrate Kinetics. *Microbiol. Mol. Biol. Rev.* **62**:646–666.

Kristiansen CRB. 1999, eds. *Basic biotechnology*. Cambridge University Press

Krull R, Peterat G. 2016. Analysis of reaction kinetics during chemostat cultivation of Saccharomyces cerevisiae using a multiphase microreactor. *Biochem. Eng. J.* **105**:220–229.

Kulik N, Goi A, Trapido M, Tuhkanen T. 2006. Degradation of polycyclic aromatic hydrocarbons by combined chemical pre-oxidation and bioremediation in creosote contaminated soil. *J. Environ. Manage.* **78**:382–91.

LaRowe DE, Amend JP. 2015. Catabolic rates, population sizes and doubling/replacement times of microorganisms in natural settings. *Am. J. Sci.* **315**:167–203.

Leatherbarrow RJ. 1990. Using linear and non-linear regression to fit biochemical data. *Trends Biochem. Sci.* **15**:455–458.

Lee B-D, Hosomi M. 2001. A hybrid Fenton oxidation–microbial treatment for soil highly contaminated with benz(a)anthracene. *Chemosphere* **43**:1127–1132.

Lehninger AL, Nelson DL, Cox MM. 2005. Lehninger Principles of Biochemistry. W. H. Freeman 1119 p.

Liu H, Bruton TA, Doyle FM, Sedlak DL. 2014. In situ chemical oxidation of contaminated groundwater by persulfate: decomposition by Fe(III)- and Mn(IV)-containing oxides and aquifer materials. *Environ. Sci. Technol.* **48**:10330–6.

Liu X, Sun M, Cheng Y, Yang R, Wen Y, Chen Z, Li J. 2016. OxyR Is a Key Regulator in Response to Oxidative Stress in Streptomyces avermitilis. *Microbiology*.

Marsolek MD, Rittmann BE. 2016. Effect of substrate characteristics on microbial community structure, function, resistance, and resilience; application to coupled photocatalytic-biological treatment. *Water Res.* **90**:1–8.

Martin JW, Barri T, Han X, Fedorak PM, El-Din MG, Perez L, Scott AC, Jiang JT. 2010. Ozonation of oil sands process-affected water accelerates microbial bioremediation. *Environ. Sci. Technol.* **44**:8350–6.

Mensing T, Marek W, Raulf-Heimsoth M, Baur X. 1998. Acute exposure to hair bleach causes airway hyperresponsiveness in a rabbit model. *Eur. Respir. J.* **12**:1371–4.

Merayo N, Hermosilla D, Blanco L, Cortijo L, Blanco A. 2013. Assessing the application of advanced oxidation processes, and their combination with biological treatment, to effluents from pulp and paper industry. *J. Hazard. Mater.* **262**:420–7.

Miller CM, Valentine RL, Roehl ME, Alvarez PJJ. 1996. Chemical and microbiological assessment of pendimethalin-contaminated soil after treatment with Fenton's reagent. *Water Res.* **30**:2579–2586.

Mörtstedt H, Jeppsson MC, Ferrari G, Jönsson BAG, Kåredal MH, Lindh CH. 2011. Strategy for identification and detection of multiple oxidative modifications within proteins applied on persulfate-oxidized hemoglobin and human serum albumin. *Rapid Commun. Mass Spectrom.* **25**:327–40.

Muñoz I, Rieradevall J, Torrades F, Peral J, Domènech X. 2005. Environmental assessment of different solar driven advanced oxidation processes. *Sol. Energy* **79**:369–375.

Muñoz X, Cruz MJ, Orriols R, Torres F, Espuga M, Morell F. 2004. Validation of specific inhalation challenge for the diagnosis of occupational asthma due to persulphate salts.

Occup. Environ. Med. **61**:861–6.

Nielsen J, Villadsen J, Lidén G. 2012. Bioreaction Engineering Principles: Second Edition. Springer 528 p.

Nikakhtari H, Hill G. 2005. Modelling Oxygen Transfer and Aerobic Growth in Shake Flasks and Well-Mixed Bioreactors. *Can. J. Chem.* ….

Nikakhtari H, Hill GA. 2006. Closure effects on oxygen transfer and aerobic growth in shake flasks. *Biotechnol. Bioeng.* **95**:15–21.

Oh J, Salcedo DE, Medriano CA, Kim S. 2014. Comparison of different disinfection processes in the effective removal of antibiotic-resistant bacteria and genes. *J. Environ. Sci. (China)* **26**:1238–42.

Oller I, Malato S, Sánchez-Pérez JA. 2011. Combination of Advanced Oxidation Processes and biological treatments for wastewater decontamination--a review. *Sci. Total Environ.* **409**:4141–66.

Oturan MA, Aaron J-J. 2014. Advanced Oxidation Processes in Water/Wastewater Treatment: Principles and Applications. A Review. *Crit. Rev. Environ. Sci. Technol.* **44**:2577–2641.

Palmroth MRT, Langwaldt JH, Aunola TA, Goi A, Münster U, Puhakka JA, Tuhkanen TA. 2006. Effect of modified Fenton's reaction on microbial activity and removal of PAHs in creosote oil contaminated soil. *Biodegradation* **17**:131–41.

Paslawski J, Nemati M, Hill G, Headley J. 2009. Biodegradation kinetics of trans -4-methyl-1-cyclohexane carboxylic acid in continuously stirred tank and immobilized cell bioreactors. *J. Chem. Technol. Biotechnol.* **84**:992–1000.

Patel V, Jain S, Madamwar D. 2012. Naphthalene degradation by bacterial consortium (DV-AL) developed from Alang-Sosiya ship breaking yard, Gujarat, India. *Bioresour. Technol.* **107**:122–30.

Pruchniak MP, Araźna M, Demkow U. 2015. Biochemistry of Oxidative Stress. *Adv. Exp. Med. Biol.*

Reardon K, Mosteller D, Rogers JB. 2000. Biodegradation kinetics of benzene, toluene, and phenol as single and mixed substrates for Pseudomonas putida F 1. *Biotechnol. ….*

Del Rio LF, Hadwin AKM, Pinto LJ, MacKinnon MD, Moore MM. 2006. Degradation of naphthenic acids by sediment micro-organisms. *J. Appl. Microbiol.* **101**:1049–61.

Rittmann B, McCarty P. 2012. Environmental biotechnology: principles and applications.

Rizzo L. 2011. Bioassays as a tool for evaluating advanced oxidation processes in water and wastewater treatment. *Water Res.* **45**:4311–40.

Sanchis S, Polo AM, Tobajas M, Rodriguez JJ, Mohedano AF. 2013. Degradation of chlorophenoxy herbicides by coupled Fenton and biological oxidation. *Chemosphere* **93**:115–22.

Siegrist R, Crimi M, Simpkin T. 2011. In situ chemical oxidation for groundwater remediation.

Sies H. 1997. Oxidative stress: oxidants and antioxidants. *Exp. Physiol.* **82**:291–295.

Silver S, Chakrabarty AM (Illinois U. CI (USA). D of M and I, Iglewski B (Rochester U. N (USA). D of M and I, Kaplan S (eds. . (Texas U. HT (USA). D of M and I. 1990. Pseudomonas: Biotransformations, pathogenesis, and evolving biotechnology.

Smolke C. 2009. The Metabolic Pathway Engineering Handbook: Fundamentals. CRC Press 678 p.

Sohrabi V, Ross MS, Martin JW, Barker JF. 2013. Potential for in situ chemical oxidation of acid extractable organics in oil sands process affected groundwater. *Chemosphere* **93**:2698–703.

Stephanopoulos G, Aristidou AA, Nielsen J. 1998. Metabolic Engineering: Principles and Methodologies. Academic Press 725 p.

Storz G, Tartaglia LA, Farr SB, Ames BN. 1990. Bacterial defenses against oxidative stress. *Trends Genet.* **6**:363–368.

Strous M, Kuenen JG, Jetten MSM. 1999. Key Physiology of Anaerobic Ammonium Oxidation.

Appl. Envir. Microbiol. **65**:3248–3250.

Van Suijdam JC, Kossen NWF, Joha AC. 1978. Model for oxygen transfer in a shake flask. *Biotechnol. Bioeng.* **20**:1695–1709.

Sutton NB, Kalisz M, Krupanek J, Marek J, Grotenhuis T, Smidt H, de Weert J, Rijnaarts HHM, van Gaans P, Keijzer T. 2014. Geochemical and microbiological characteristics during in situ chemical oxidation and in situ bioremediation at a diesel contaminated site. *Environ. Sci. Technol.* **48**:2352–60.

Sutton NB, Grotenhuis JTC, Langenhoff AAM, Rijnaarts HHM. 2010. Efforts to improve coupled in situ chemical oxidation with bioremediation: a review of optimization strategies. *J. Soils Sediments* **11**:129–140.

Tsitonaki A, Petri B, Crimi M, Mosbak H, Siegrist RL, Bjerg PL. 2010. In Situ Chemical Oxidation of Contaminated Soil and Groundwater Using Persulfate: A Review. *Crit. Rev. Environ. Sci. Technol.* **40**:55–91.

Tsitonaki A, Smets BF, Bjerg PL. 2008. Effects of heat-activated persulfate oxidation on soil microorganisms. *Water Res.* **42**:1013–22.

Veglio F, Beolchini F, Ubaldini S. 1998. Empirical models for oxygen mass transfer: a comparison between shake flask and lab-scale fermentor and application to manganiferous ore bioleaching. *Process Biochem.* **33**:367–376.

Veschetti E, Cutilli D, Bonadonna L, Briancesco R, Martini C, Cecchini G, Anastasi P, Ottaviani M. 2003. Pilot-plant comparative study of peracetic acid and sodium hypochlorite wastewater disinfection. *Water Res.* **37**:78–94.

Villadsen J, Nielsen J, Lidén G. 2011. Bioreaction Engineering Principles. Boston, MA: Springer US.

Waldemer RH, Tratnyek PG, Johnson RL, Nurmi JT. 2007. Oxidation of Chlorinated Ethenes by Heat-Activated Persulfate: Kinetics and Products. *Environ. Sci. Technol.* **41**:1010–1015.

Whitby C. 2010. Microbial naphthenic Acid degradation. *Adv. Appl. Microbiol.* **70**:93–125.

Wordofa DN. 2014. Application of Iron Activated Persulfate for Disinfection in Water Treatment.

Zhang R, Xu X, Chen W, Huang Q. 2016. Genetically engineered Pseudomonas putida X3 strain and its potential ability to bioremediate soil microcosms contaminated with methyl parathion and cadmium. *Appl. Microbiol. Biotechnol.* **100**:1987–97.

CHAPTER 5. THERMODYNAMICS OF NAPHTHENIC ACIDS BIODEGRADATION UNDER AEROBIC CONDITION

5.1 Introduction

Environmental pollution is considered one of the main consequences of industrial progress. The widespread utilization of man-made chemicals, which are not naturally occurring in nature, result in the accumulation of anthropogenic chemicals in the environment. Some of the xenobiotic compounds are polycyclic aromatic hydrocarbons (PAHs), pesticides and personal care products. An increase in recalcitrant anthropogenic chemicals leads to contamination of air, soil and water (Gavrilescu et al., 2015; Petrie et al., 2014). Physico-chemical strategies for remediation of contaminated sites, such as incineration and land filling are not cost effective and also have adverse effects on the environment (Dua et al., 2002; Paul et al., 2005). As such, alternative cleanup methods need to be effective while minimally hazardous and economically feasible (Oller et al., 2014).

One promising strategy for removal of contamination is bioremediation. Bioremediation is based on the ability of microorganisms to utilize these foreign (mainly organic) substances for their growth and maintenance. Microorganisms or consortia of microorganisms are able to use different pathways under different physiological conditions for degradation of xenobiotic compounds. There are three basic principles that need to be considered when selecting an optimum bioremediation strategy. First is the availability of the pollutant to microorganisms, second is the opportunity for optimization of biological activity and the third is the amenability

of the contaminant to biotransformation to less toxic by-products (Alexander, 1999; Boopathy, 2000).

One effective tool for evaluating biodegradation potential (both from blackbox and metabolic model) is thermodynamics analysis (Chauhan et al., 2015; Dolfing et al., 2008; Dolfing et al., 2009; Erickson et al., 1995; Finley et al., 2009a; Finley et al., 2009b; Finley et al., 2010; Meckenstock et al., 2015).

Biodegradation of NAs has been reported in the literature under various physiological conditions with either mixed or pure cultures (Dong and Nemati, 2016; Huang et al., 2012; Paslawski et al., 2009). Studying mixed or pure cultures each has its own advantages and disadvantages, but we are mainly interested in pure culture biodegradation. Studies with pure cultures provide an easier model for validating theoretical data with experimental data and for validating novel pathways. Because one of the first point in improvement of a strain is manipulation of its metabolic pathway. To manipulate the pathway, specific enzymes (depends on experimenter goal) need to be increase, decrease or eliminate. To do so, genome of the microorganism need to be known. Therefore, with a pure culture, a system under study will not have high degree of freedom in terms of changes in metabolic pathways and responses (due to manipulation).

The goal of this chapter is to provide theoretical stoichiometry and kinetic parameters of growth of a given microorganism with general composition of $C_1H_{1.8}O_{0.5}N_{0.2}$ on a model naphthenic acid. Based on the author's knowledge, there is a gap in theoretical study with respect to thermodynamics of NAs biodegradation. This fundamental study would be the first step needed for improvement of NAs biodegradation and for metabolic and genetic engineers in optimizing bioremediation of NAs.

5.2 Cellular Growth Concepts

5.2.1 Cellular Metabolism

Generally, all chemical processes conducted by a cell are called metabolism. Metabolism is divided into two general categories: catabolism and anabolism. Catabolism refers to the reactions involved in yielding energy for a cell to survive and grow. The processes of growth or biosynthesis of new cells are covered by anabolism (Lehninger et al., 2005; Russell and Cook, 1995).

5.2.1.1 Catabolism

In chemoorganotroph microorganisms catabolism depends on the oxidation of electron donor and reduction of electron acceptor. The electron donor is considered the substrate or "food" of the microorganisms. Organic compounds, which have carbon in a reduced state, are the most common electron donor (and carbon source) for microorganisms. However, electron acceptors are in an oxidized state i.e. oxygen, nitrite, Fe (III) (Lehninger et al., 2005).

The purpose of catabolism is to produce energy for anabolism. So it is important to quantify this amount of energy (Russell and Cook, 1995). Using half reactions for the electron donor and acceptor and their associate Gibbs free energy one can write a complete reaction and the associated Gibbs free energy associated with the reaction. (Rittmann and McCarty, 2012). The produced energy in catabolism is diverse in nature from 30 to 3000 kJ per mole of electron donor and greatly depends on the electron acceptor (Kristiansen, 1999). For example, catabolism of glucose under aerobic conditions produces 2843.1 kJ $mole^{-1}$, while under anaerobic (glucose to ethanol) conditions produces 225.4 kJ $mole^{-1}$ (Alberty, 1998; Rittmann and McCarty, 2012). In Fig. 5.1 the dependency of redox couple to redox potential is illustrated (Reddy and DeLaune,

2008). For example, a given redox couple can be active only in the proper redox potential zone i.e. CO_2/CH_4 cannot be a redox couple at redox potential of +350 mV. In table 5.1 several catabolic reactions with donor and acceptor couples showing the wide range of produced energy (Alberty, 1998; Smolke, 2009).

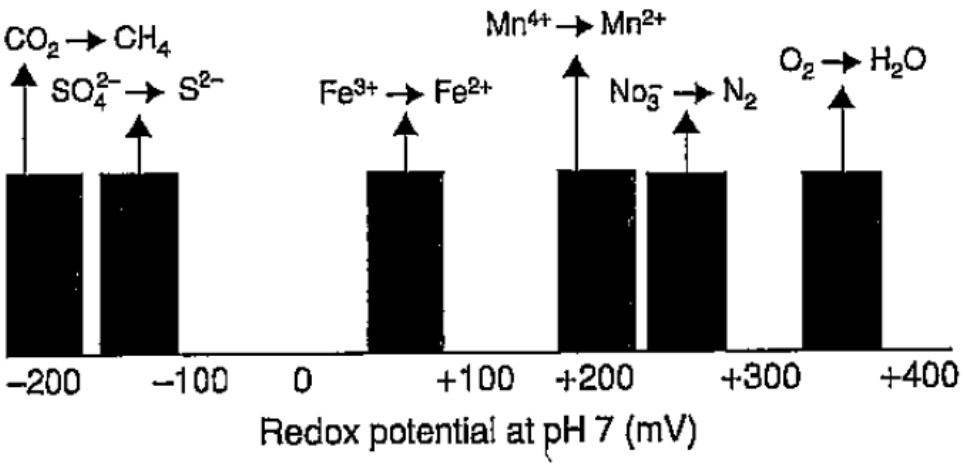


Figure 5-1 Critical redox potentials for the transformation of redox couples (reprinted with permission from Reddy and DeLaune, (2008))

Table 5-1 Catabolic reactions of some organic compounds under various electron accepting condition; redox couple of NO_3^{-1}/N_2 and CO_2 / CH_4 are representing denitrification and methanogen condition, respectively (Smolke, 2009)

Catabolic reaction for 1 mole electron donor	Donor	Acceptor	$\Delta_{cat}G^{0'}$ (kJ/mol)
$\boldsymbol{C_6H_{12}O_6 + 6O_2 \rightarrow 6HCO_3^- + 6H^+}$	$C_6H_{12}O_6 / HCO_3^-$	O_2/H_2O	-2843.1
$\boldsymbol{C_2H_6O + 3O_2 \rightarrow 2HCO_3^- + 2H^+ + H_2O}$	C_2H_6O / HCO_3^-	O_2/H_2O	-1308.9
$\boldsymbol{C_2H_3O_2^- + 2O_2 \rightarrow 2HCO_3^- + H^+}$	$C_2H_3O_2^- / HCO_3^-$	O_2/H_2O	-844.16
$\boldsymbol{CH_4O + 1.2\,NO_3^- + 0.2\,H^+ \rightarrow 0.6\,N_2 + 1.6\,H_2O + HCO_3^-}$	CH_4O / HCO_3^-	NO_3^- / N_2	-649.36
$\boldsymbol{C_6H_{12}O_6 + 2H_2O \rightarrow 2HCO_3^- + 2H^+ + 2C_2H_6O}$	$C_6H_{12}O_6 / HCO_3^-$	$C_6H_{12}O_6 / HCO_3^-$	-225.4
$\boldsymbol{H_2 + 0.25\,HCO_3^- + 0.25\,H^+ \rightarrow 0.25\,CH_4 + 0.75\,H_2O}$	H_2 / H^+	CO_2/CH_4	-33.90
$\boldsymbol{Fe^{2+} + 0.25\,O_2 + H^+ \rightarrow Fe^{3+} + 0.5\,H_2O}$	Fe^{2+}/Fe^{3+}	O_2/H_2O	-33.78
$\boldsymbol{C_2H_3O_2^- + H_2O \rightarrow HCO_3^- + CH_4}$	$C_2H_3O_2^- / HCO_3^-$	CO_2/CH_4	-31.0

5.2.1.2 Anabolism

Anabolism is the part of metabolism involved in the biosynthesis of new cells (Lehninger et al., 2005). Simple chemical compounds i.e. organics, ammonia are converted into complex building blocks and macromolecules like proteins, lipids, carbohydrates and nucleic acid. Therefore, macromolecules are built with smaller molecules. This process needs energy! So anabolism, in terms of energy, is quite the opposite of catabolism (Stephanopoulos et al., 1998). Anabolism is subdivided into two categories: heterotrophy and autotrophy. The source of the carbon in heterotrophy is organic and the source of carbon in autotrophy is inorganic carbon such as CO_2 (Rittmann and McCarty, 2012). The energy needed for new biomass synthesis is much less in heterotrophs than autotrophs (Kristiansen, 1999).

Microbial biomass has relatively similar composition with 40-70% protein, 1-2% DNA, 5-15% RNA, 2-10% lipid and 3-10% carbohydrate (Smolke, 2009). This similarity allowed for the development of a common 1 carbon formula to show the organic portion of biomass = $C_1H_{1.8}O_{0.5}N_{0.2}$ (Kristiansen, 1999; Rittmann and McCarty, 2012; Stephanopoulos et al., 1998; Tchobanoglous et al., 2003). One can also consider other elements in biomass such as P, S^{2-}, K^{+}, Mg^{2+} and other inorganic compounds, but here we neglected these minor elements since their contribution in biomass content is <1-2% (Rittmann and McCarty, 2012; Stephanopoulos et al., 1998). In heterotrophic growth (our case), the substrate is an organic compound and is the sole source of carbon and energy at the same time (Lehninger et al., 2005). The biosynthesis reaction of biomass for heterotrophic growth is (Kristiansen, 1999):

$$\frac{1}{Y_{SX}}\ electron\ donor/C\ source + \ b\ N - source \rightarrow dH_2O + eCO_2 + C_1H_{1.8}O_{0.5}N_{0.2}$$

In above reaction Y_{SX} is the yield of substrate to biomass, b, c, d e, f, g, h are the stoichiometric coefficients of each compound. Also, source refers to the compounds that act as the source of carbon i.e. glucose and nitrogen source i.e. ammonia. $C_1H_{1.8}O_{0.5}N_{0.2}$ represents the composition of biomass. A schematic overview of metabolism is shown in Fig 5.2.

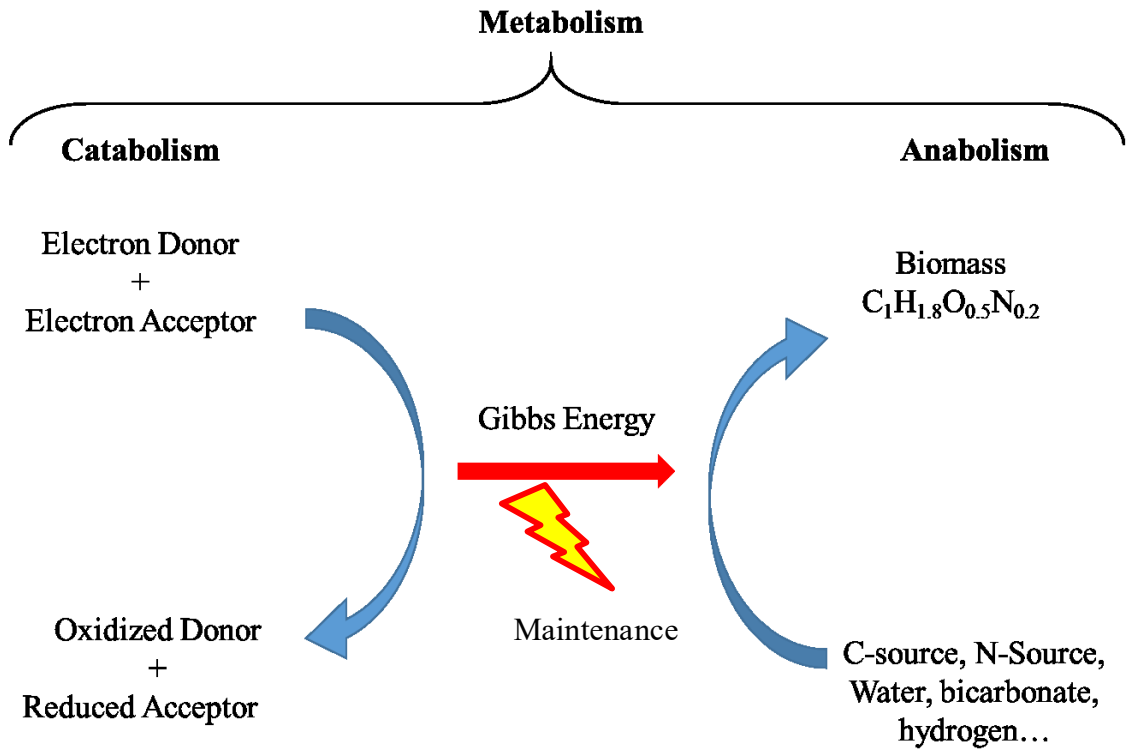


Figure 5-2 General representation of microbial metabolism. During catabolism, energy will be produced for maintenance and growth. In anabolism the carbon source, nitrogen source and other necessary components are used in anabolic reactions for biosynthesis of new cells.

5.2.1.3 Maintenance

In all microorganisms, damage and decay also occur i.e. protein denaturation and leakage of molecules from membranes. These processes are commonly referred to as endogenous metabolism: the amount of substrate that is needed at zero growth rate (Stephanopoulos et al., 1998; Van Loosdrecht and Henze, 1999). Interestingly, it has been found that the amount of

energy needed for maintenance is similar for a broad range of microorganisms (Smolke, 2009). These processes need to be compensated. And logically some energy must be spent! This amount of energy (generally) has a direct relationship with temperature (approximately doubles with every 8^{o}C increase in temperature) (Tijhuis et al., 1993). It is interesting to mention that in the Monod model, maintenance of the cell is not considered (Kovarova-Kovar and Egli, 1998).

5.3 Thermodynamics Background

5.3.1 Equilibrium Thermodynamics

Thermodynamics is divided into two different categories: equilibrium and non-equilibrium (Demirel, 2014; Groot and Mazur, 1962; Smith et al., 2001). Cell growth and its metabolism take place far or near equilibrium (Zotin, 1990). Thus, equilibrium thermodynamics fails to provide insights into biotransformation of compounds and growth of a cell (Demirel, 2014; S. Roy Caplan, 1983; Stephanopoulos et al., 1998; Westerhoff and Dam, 1987). Living systems are open systems, constantly exchanging mass and energy with the environment (Roels, 1983).

The first law of thermodynamics expresses the total amount of energy in a process is constant (Smith et al., 2001).

$$E = constant \tag{5.1}$$

The second law of thermodynamics states that spontaneity of a process is determined from the overall change in Gibbs free energy. Gibbs free energy (G) or free energy was introduced by Willard Gibbs in 1878. The concept of free energy is the maximum amount of work (non-displacement) that can be done by a system at constant pressure and temperature (Smith et al., 2001). When $\Delta G < 0$ the reaction is spontaneous or exogenic and the system can conduct work! On the other hand, when $\Delta G > 0$ the reaction (or process) is endergonic and free energy is

needed for the reaction to proceed. And at equilibrium, ΔG = 0 (Smith et al., 2001; Wylen et al., 1994). For a given reaction, ΔG can be calculated (Stephanopoulos et al., 1998):

$$aA + bB \rightarrow cC + dD \tag{5.2}$$

$$\Delta G = \Delta G^{0\prime} + RT \ln\left(\frac{C_c^c C_D^d}{C_A^a C_B^b}\right) \tag{5.3}$$

Where:

$$\Delta G^{0\prime} = RT \ln\left(K_{eq}\right) \tag{5.4}$$

And K_{eq} is:

$$K_{eq} = \frac{C_{c,eq}^c C_{D,eq}^d}{C_{A,eq}^a C_{B,eq}^b} = e^{-\frac{\Delta G^{0\prime}}{RT}} \tag{5.5}$$

As we mentioned above, cellular systems are open systems, which perform far from equilibrium stage. Generally, to study non-equilibrium systems, first we investigate the system at equilibrium, then we apply some changes to deviate the system from equilibrium (Demirel, 2014; Prigogine, 1968; Westerhoff and Dam, 1987).

5.3.2 *Non*-Equilibrium Thermodynamics

The mathematical foundation for non-equilibrium thermodynamics is described as follows (Westerhoff and Dam, 1987). For context, "system" refers to a set of defined boundaries creating separation of the system from the rest of the world. A system can interact with the outside world through its boundaries (Smith et al., 2001; Wylen et al., 1994). These interactions can be through (Westerhoff and Dam, 1987):

- exchange of heat to environment ($đ_e Q$)
- pressure work carried out on the environment ($đ W = -p \cdot đ_e V$)
- chemical work through exchange of certain chemical (j) ($\sum_j \mu_j \cdot đ_e N_j$)
- mechanical work ($F \cdot đ_e L$)

Here, “p” represents the pressure; "μ_j" shows the chemical potential of chemical j and “F” demonstrates the elastic force. N_j, V and L are number of molecules of chemical j, volume and length of the contractile element of the system. The notation "đ" means “a small change in” and the subscript “e” refers to the interaction with environment (out of the system’s boundaries) (Westerhoff and Dam, 1987).

$$đ_e E = đ_e Q + đ W + \sum_j \mu_j \cdot đ_e N_j - F \cdot đ_e L \tag{5.6}$$

$$đ_e E = đ_e Q - p \cdot đ_e V + \sum_j \mu_j \cdot đ_e N_j - F \cdot đ_e L \tag{5.7}$$

The energy balance equation can be divided into “**work**” and “**heat**” components. The interaction of energy with environment in terms of “work” can be described by the following equation:

$$đ_e W'' = -p \cdot đ_e V + \sum_{i=1}^{m} \mu_i \cdot đ_e m_i + \sum_{j=1}^{n} \mu_j \cdot đ_e n_j - F \cdot đ_e L \tag{5.8}$$

And the interaction of energy with environment in terms of “heat” can be described by the following equation, where $\overline{S_j}$ is the partial molar entropy of substance j:

$$đ_e Q'' = đ_e Q' + \sum_{j=1}^{n} T \cdot \overline{S_j} \cdot đ_e n_j - F \cdot đ_e L \tag{5.9}$$

In above equations, $đ_e Q''$means the pure heat exchanged (after correction for partial moral enthalpy) and including the entropy of exchanged particles ($đ_e Q'$ shows the heat exchanged after correction for partial molar enthalpies of exchanged substances). The $đ_e W''$demonstrates the work including transport work (Westerhoff and Dam, 1987).

Work can be converted to heat (i.e. friction) and vice versa. To mathematically show this balance:

$$đ_e E \overset{\text{def}}{=} đ_e Q'' + đ_e W'' \tag{5.10}$$

Based on conservation of energy, we can write:

$$đE = đ_e E + đ_i E \tag{5.11}$$

And due to the first law of thermodynamics, for a closed system (Smith et al., 2001; Wylen et al., 1994):

$$đ_i E = 0 \tag{5.12}$$

Eq. 5.12 indicates the total amount of energy in a closed system is constant.

As we already know, $đ_e Q''$corresponds to heat exchanged (with environment) in the process (through reversible processes). By dividing $đ_e Q''$by absolute temperature T, “entropy” is obtained:

$$đ_e S = \frac{đ_e Q''}{T} \tag{5.13}$$

Entropy is a state function. When a property is a state function it means, the quantity of the property does not depend on the pathway and length of the process, it only depends on the initial and final state of the process.

The second law of thermodynamics implies that for a spontaneous reaction:

$$đ_e S \geq 0 \tag{5.14}$$

Where the > sign is valid for systems that are not in equilibrium and the = sign is valid for the systems that are in equilibrium (Westerhoff and Dam, 1987).

Similar to equation 5.11:

$$đS = đ_e S + đ_i S \tag{5.15}$$

$$đS - đ_i S = đ_e S \tag{5.16}$$

$$đS - đ_i S = đ_e S = +\frac{đE}{T} + \frac{p}{T} \cdot đ_e V - \sum_{i=1}^{m} \frac{\mu_i}{T} \cdot đ_e m_i - \sum_{j=1}^{n} \frac{\mu_j}{T} \cdot đ_e n_j + \frac{F}{T} \cdot đ_e L \tag{5.17}$$

This equation can be re-written as:

$$\mathrm{d}E = T \cdot \mathrm{d}S - T \cdot đ_i S - p \cdot đ_e V + \sum_{i=1}^{m} \mu_i \cdot đ_e m_i + \sum_{j=1}^{n} \mu_j \cdot đ_e n_j - F \cdot đ_e \tag{5.18}$$

Based on the Gibbs free energy (G) definition (Smith et al., 2001):

$$\mathrm{d}G \stackrel{\text{def}}{=} \mathrm{d}(E + p \cdot V - T \cdot S) = đ_i G + đ_e G \tag{5.19}$$

Where:

$$-đ_i G \stackrel{\text{def}}{=} +T \cdot đ_i S \tag{5.20}$$

And

$$đ_e G \stackrel{\text{def}}{=} -S \cdot \mathrm{d}T + V \cdot \mathrm{d}P + \sum_{i=1}^{m} \mu_i \cdot đ_e m_i + \sum_{j=1}^{n} \mu_j \cdot đ_e n_j - F \cdot đ_e L \tag{5.21}$$

For a spontaneous reaction we have:

$$(\mathrm{d}G)_{T,P,m_i,n_j,L} = \text{đ}_i G \leq 0 \tag{5.22}$$

The latter equation refers to second law of thermodynamics. The purpose of these calculation is to provide an equation as a fundamental relationship in non-equilibrium thermodynamics.

5.3.3 Free Energy Dissipation in Spontaneous Process

To analyse a non-equilibrium situation in a real world, an example is provided. Let us assume a chemical reaction:

$$\boldsymbol{A \leftrightarrow B} \tag{5.23}$$

This reaction proceeds by an amount of dξ:

$$\text{đ}_i n_A = -\mathrm{d}\xi = -\text{đ}_i n_B \tag{5.24}$$

However, this reaction looks reversible, we cannot use equilibrium thermodynamic approaches to treat this reaction before it reaches equilibrium. For example, at time zero we have only compound A. And this reaction for example needs 20 min to reach equilibrium! In this 20 minutes the reaction is not reversible (so we cannot calculate $\text{đ}_i G$). To simulate this reaction with a reversible reaction we are can supply energy from the outside world (outside the system boundary). To achieve this goal, which is having a reversible reaction, we add substance B to the system and take the compound A out.

Therefore:

$$\text{đ}_e n'_B = \text{đ}_i n_B \tag{5.25}$$

And

$$đ_e n'_A = đ_i n_A \tag{5.26}$$

By recalling equation 5.11, we may write:

$$\mathrm{d}G = \ đ_i G + \ đ_e G \tag{5.27}$$

Since we are dealing with a reversible reaction:

$$đ_i G = 0 \tag{5.28}$$

By using equation:

$$\mathrm{d}G' = \ đ_e G = \ \mu_A \cdot đ_e n'_A + \ \mu_B \cdot đ_e n'_B \tag{5.29}$$

Since G is a function of state and due to equation 5.21:

$$\mathrm{d}G' = \ đ_i G = \ \mu_A \cdot đ_i n_A + \ \mu_B \cdot đ_i n_B \tag{5.30}$$

$$-(\ \mu_A - \mu_B) \cdot d\xi = -\Delta\mu_{AB} \cdot d\xi = \ - T \cdot \ đ_i S \tag{5.31}$$

The reaction is proceeded to forward, from A to B and not *vice versa* when $\mu_A > \mu_B$. By considering the role of time in the reaction:

$$\boldsymbol{\Phi} \stackrel{\text{def}}{=} -\frac{đ_i G}{\mathrm{dt}} = T \cdot \frac{đ_i S}{\mathrm{dt}} = \Delta G_{AB} \cdot \frac{d\xi}{\mathrm{dt}} \tag{5.32}$$

Where $\boldsymbol{\Phi}$ is the "dissipation function" and ΔG_{AB} is the thermodynamic affinity of reaction 5.23 (Prigogine, 1968; Welch, 1985; Westerhoff and Dam, 1987).

5.3.4. Conservation and flows laws in non-equilibrium thermodynamics

An increase or decrease in the amount of a property in a system under study, can be because of influx ($đ_e$) or production ($đ_i$). To describe the flux (J) of a give substance A through a fixed surface area O and a fixed volume, we may write (Westerhoff, 1983):

$$\frac{đ_e n_A}{dt} = -\oiint \langle \overrightarrow{J_A} \cdot \vec{O} \rangle \tag{5.33}$$

To mathematically show the production of an arbitrary compound A, in the volume of V, we can write:

$$\frac{đ_i n_A}{dt} = \iiint \sigma_A \cdot dV \tag{5.34}$$

σ_A is the source term of compound A. Combination of flux and source term result in a balanced equation for compound A:

$$\frac{dn_A}{dt} = \frac{đ_e n_A}{dt} + \frac{đ_i n_A}{dt} = -\oiint \langle \overrightarrow{J_A} \cdot d\vec{O} \rangle + \iiint \sigma_A \cdot dV \tag{5.35}$$

When concentration of A (C_A) is available, mass of "A" can be calculated:

$$\frac{dn_A}{dt} = \iiint \frac{dC_A}{dt} \cdot dV \tag{5.36}$$

The mathematical Gauss theorem states that:

$$\oiint \langle \overrightarrow{J_A} \cdot d\vec{O} \rangle = -\iiint div(\overrightarrow{J_A}) \cdot dV \tag{5.37}$$

Where:

$$div(\overrightarrow{J_A}) = \frac{\partial J_{A,x}}{\partial x} + \frac{\partial J_{A,y}}{\partial y} + \frac{\partial J_{A,z}}{\partial z} = \langle \vec{\nabla} \cdot \overrightarrow{J_A} \rangle \tag{5.38}$$

Now the total mass balance for compound A will be:

$$\iiint \left[\frac{dC_A}{dt} + \langle \vec{\nabla} \cdot \overrightarrow{J_A} \rangle - \sigma_A \right] \cdot dV = 0 \tag{5.39}$$

This equation should be valid for an arbitrary volume element. The integration will be droped:

$$\frac{dC_A}{dt} = \sigma_A - \langle \vec{\nabla} \cdot \overrightarrow{J_A} \rangle \tag{5.40}$$

5.3.4 Entropy Production in a Continuous System

Based on equilibrium thermodynamics, a spontaneous process, $\sigma_S \geq 0$ (Smith et al., 2001; Westerhoff and Dam, 1987). To have a better understanding for non-equilibrium conditions, we write equation 5.40 in terms of entropy and then compare it to the equilibrium form of the Gibbs equation (Westerhoff and Dam, 1987):

$$\frac{dC_S}{dt} = \sigma_S - \langle \vec{\nabla} \cdot \overrightarrow{J_S} \rangle \tag{5.41}$$

At equilibrium đ$_i S$=0 and by neglecting the elastic work term; and make it time dependent and divide it by volume (eq. 5.17):

$$\frac{dC_S}{dt} = \frac{1}{T} \cdot \frac{đC_E}{dt} - \sum_j \frac{\mu_j}{T} \frac{dC_j}{dt} \tag{5.42}$$

Doing the same for energy:

$$\frac{dC_S}{dt} = \frac{\sigma_E}{T} - \frac{\langle \vec{\nabla} \cdot \overrightarrow{J_E} \rangle}{T} - \sum_j \frac{\mu_j}{T} \sigma_j + \sum_j \frac{\mu_j}{T} \cdot \langle \vec{\nabla} \cdot \overrightarrow{J_J} \rangle \tag{5.43}$$

Due to the first law of thermodynamics the source term for the energy is zero (eq 5.12). The source term for chemical compound j is related to the rates of the chemical reaction in the system.

The combination of equations of source term of compound j and equation 5.42, and 5.43 we can write:

$$\frac{dC_S}{dt} = \overrightarrow{J_E} \cdot \overrightarrow{grad}\left(\frac{1}{T}\right) - \sum_j \overrightarrow{J_J} \cdot \overrightarrow{grad}\left(\frac{\mu_j}{T}\right) + \sum_r \frac{(J_{chem})_r}{T} \cdot \Delta G_r$$

$$+ div\left(\frac{-\overrightarrow{J_E}}{T} + \sum_j \left(\frac{\overrightarrow{J_J} \cdot \mu_j}{T}\right)\right) \tag{5.44}$$

Above calculation is due to mathematical property, for the vector $\vec{V}$ and the scalar λ:

$$div(\lambda \cdot \vec{V}) = \lambda \cdot div(\vec{V}) + \langle \overrightarrow{\text{grad}}(\lambda) \cdot \vec{V} \rangle \tag{5.45}$$

The rewritten version (Eq. 5.44) of the Gibbs equation (Eq. 5.17) is very similar to the theoretical entropy balance equation (Eq. 5.41). Thus, equation 5.44 has both entropy production and entropy flow terms:

$$\sigma_S = \overrightarrow{J_E} \cdot \overrightarrow{grad}\left(\frac{1}{T}\right) + \sum_j \overrightarrow{J_J} \cdot \overrightarrow{grad}\left(\frac{-\mu_j}{T}\right) + \sum_r \frac{(J_{chem})_r}{T} \cdot \Delta G_r \tag{5.46}$$

And

$$\overrightarrow{J_S} = \frac{\overrightarrow{J_E}}{T} - \sum_j \overrightarrow{J_J} \cdot \left(\frac{\mu_j}{T}\right) \tag{5.47}$$

Now, one can rewrite the entropy production equation by using $\overrightarrow{J_S} = \left(\frac{\overrightarrow{J_q'}}{T}\right)$:

$$\mathbf{\Phi} = T \cdot \sigma_S = \overrightarrow{J_S} \cdot \overrightarrow{grad}\,(-T) + \sum_j \overrightarrow{J_J} \cdot \overrightarrow{grad}(-\mu_j) + \sum_r (J_{chem})_r \cdot \Delta G_r \tag{5.48}$$

This equation is equal to dissipation of Gibbs free energy per unit of volume. Equation 5.48 is widely known as the "dissipation function".

Note:

It is very important to realize that the entropy production and dissipation functions consist of the sum of products of **flows** and **forces**. For example, by flows, it means flux in space or reaction

rate and by forces it means i.e. temperature differences. Equation 5.48 shows which flows belong to which forces.

Explanation of equation 5.48:

$\overrightarrow{J_S} \cdot \overrightarrow{grad}\,(-T)$: Free energy dissipation in the form of heat flow; the driving force of this energy is differences in temperature.

$\sum_j \overrightarrow{J_j} \cdot \overrightarrow{grad}(-\mu_j)$: Free energy dissipation in the form of diffusion; the driving force of this energy is differences in (electro) chemical potential.

$\sum_r (J_{chem})_r \cdot \Delta G_r$: Free energy dissipation in the form of chemical reaction; the driving force of this term is the free-energy differences of reaction (affinity of the reaction).

Equation 5.46 represents the production of entropy and Eq. 5.48 describes the dissipation of free energy at every point in a system.

5.3.5 Thermodynamic Coupling

The second law of thermodynamics states that total entropy production cannot be negative (Demirel, 2014; Smith et al., 2001; Westerhoff and Dam, 1987). In other words, the summation of all terms in equation 5.46 must be more than zero, but it does **not** mean all terms must exceed zero (Westerhoff and Dam, 1987). For example, in some processes we can have both entropy production and consumption. This phenomenon, when some processes drive other non-spontaneous systems, is called thermodynamic coupling (Groot and Mazur, 1962; Onsager, 1931; Prigogine, 1968; Westerhoff and Dam, 1987).

As is mentioned above, entropy production or dissipation of free energy consists of the sum of the products of flows and their affinity. Thus, for a system with n couples of flow (J_k) and force (X_k), one can write the entropy production function (Westerhoff and Dam, 1987):

$$\sigma_S = \sum_{k=1}^{n} J_k \cdot X_k \tag{5.49}$$

It seems that each flux is driven by its affinity. However, thermodynamic coupling suggests a flux may also be effected by any other forces in the system. Based on thermodynamic coupling concepts, it is possible for flux $J_1$to be influenced by both X_1 force and additional factors such as, α_1. To express this idea, we develop a Taylor expansion of flux J_j around a certain reference point (Westerhoff and Dam, 1987):

$$\begin{aligned} J_j = {} & J_j^{\varphi} + \left(\frac{\partial J_j}{\partial X_j}\right) \cdot \left(X_j - X_j^{\varphi}\right) + \frac{1}{2} \cdot \left(\frac{\partial^2 J_j}{\partial^2 X_j}\right) \cdot \left(X_j - X_j^{\varphi}\right)^2 \\ & + \sum_{\substack{k=1 \\ k \neq j}}^{n} \left(\frac{\partial J_j}{\partial X_k}\right)^{\varphi} \cdot \left(X_k - X_k^{\varphi}\right) + \sum_{l} \left(\frac{\partial J_j}{\partial \alpha_l}\right) \cdot \left(\alpha_l - \alpha_l^{\varphi}\right) \\ & + \frac{1}{2} \cdot \sum_{\substack{k=1 \\ k \neq j}}^{n} \sum_{\substack{m=1 \\ m \neq j}}^{m} \left(\frac{\partial^2 J_j}{\partial X_k \cdot \partial X_m}\right)^{\varphi} \cdot \left(X_k - X_k^{\varphi}\right) \cdot \left(X_m - X_m^{\varphi}\right) \\ & + \frac{1}{2} \cdot \sum_{l} \sum_{l'} \left(\frac{\partial^2 J_j}{\partial \alpha_l \cdot \partial \alpha_{l'}}\right)^{\varphi} \cdot \left(\alpha_l - \alpha_l^{\varphi}\right) \cdot \left(\alpha_{l'} - \alpha_{l'}^{\varphi}\right) \\ & + \frac{1}{2} \cdot \sum_{l} \sum_{k} \left(\frac{\partial^2 J_j}{\partial X_k \cdot \partial \alpha_l}\right)^{\varphi} \cdot \left(X_k - X_k^{\varphi}\right) \cdot \left(\alpha_l - \alpha_l^{\varphi}\right) + \vartheta^{\varphi}(3) \end{aligned} \tag{5.50}$$

In the above equation subscript φ refers to the reference state and the properties α are properties that can have some influence on the flux. $\vartheta^{\varphi}(3)$ represents the third and higher order terms in the Taylor expansion. The mathematical description of thermodynamic coupling can be

simplified if we consider the reference state as the equilibrium state. In this case, if we work around the equilibrium point, we can simply neglect the second and higher orders in Taylor expansion for force X and properties.

Hence,

$$J_j = J_j^{\varphi} + \left(\frac{\partial J_j}{\partial X_j}\right) \cdot \left(X_j - X_j^{\varphi}\right) + \sum_{\substack{k=1 \\ k \neq j}}^{n} \left(\frac{\partial J_j}{\partial X_k}\right)^{\varphi} \cdot \left(X_k - X_k^{\varphi}\right) \tag{5.51}$$

$$J_j = L_{jj} \cdot X_j + \sum_{\substack{k=1 \\ k \neq j}}^{k} L_{jk} \cdot X_k \tag{5.52}$$

The phenomenological coefficient "L_{jk}" defined as (Demirel, 2014; Onsager, 1931; Prigogine, 1968):

$$L_{jk} \stackrel{\text{def}}{=} \left(\frac{\partial J_j}{\partial X_k}\right)_{X_j, eq} \tag{5.53}$$

For the entropy production relation we have:

$$\sigma_S = \sum_{j=1}^{n} L_{jj} \cdot X_j^2 + \sum_{\substack{k=1 \\ k \neq j}}^{k} L_{jk} \cdot X_j \cdot X_k \tag{5.54}$$

As a note, at equilibrium all forces are equal to zero.

Onsager discovered a general principle that might be called “the fourth law of thermodynamics” (Onsager, 1931). This principle is an empirical law and also can be derived through statistical mechanical principles. Here we consider it as an empirical law, which says:

$$L_{jk} = L_{kj} \tag{5.55}$$

This law implies that, around equilibrium, the first order dependence of any flow J_j on any force X_k is identical to the dependence of the flow J_k on the force X_j. Now for a system with three force-flow couples we can write (Onsager, 1931):

$$J_1 = L_{11} \cdot X_1 + L_{12} \cdot X_2 + L_{13} \cdot X_3 \tag{5.56}$$

$$J_2 = L_{21} \cdot X_1 + L_{22} \cdot X_2 + L_{23} \cdot X_3 \tag{5.57}$$

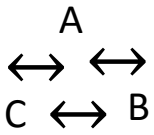
$$J_3 = L_{31} \cdot X_1 + L_{32} \cdot X_2 + L_{33} \cdot X_3 \tag{5.58}$$

And we will have $\left(\frac{3 \cdot 2}{2}\right) = 3$ reciprocal relations:

$$L_{21} = L_{12}\,;\; L_{23} = L_{32}\,;\; L_{13} = L_{31} \tag{5.59}$$

5.3.6 Chemical Reatcion as One of the Examples of Force-Flow Relation and Reciprocal Relation

Onsager's reciprocal relation can be shown for a process with a chemical reaction. Assume the following set of reactions:



(5.60)

Based on the formula for Gibbs free energy we can describe the above reactions:

$$\Delta G = RT\left(\ln\left(\frac{C_A}{C_{A,eq}}\right) + \ln\left(\frac{C_B}{C_{B,eq}}\right) + \ln\left(\frac{C_C}{C_{C,eq}}\right)\right) \tag{5.61}$$

To realize the distance of the system from equilibrium we shall introduce the notation:

$$x_A = C_A - C_{A,eq} \tag{5.62}$$

$$x_B = C_B - C_{B,eq} \tag{5.63}$$

$$x_C = C_C - C_{C,eq} \tag{5.64}$$

Based on the Taylor's expansion we can approximate the thermodynamics affinity of each compound towards the reactions.

$$\Delta G_A = RT \ln \frac{C_A}{C_{A,eq}} \sim \frac{RT}{C_{A,eq}} \left(C_A - C_{A,eq}\right) \tag{5.65}$$

$$\Delta G_B = RT \ln \frac{C_B}{C_{B,eq}} \sim \frac{RT}{C_{B,eq}} \left(C_B - C_{B,eq}\right) \tag{5.66}$$

$$\Delta G_C = RT \ln \frac{C_C}{C_{C,eq}} \sim \frac{RT}{C_{C,eq}} \left(C_C - C_{C,eq}\right) \tag{5.67}$$

Mass balance of the system is written below. Since we are investigating on a batch process, mass balance of each compound in the system results in the rate of consumption and/or production of corresponding compound.

$$\frac{dC_A}{dt} = -(k_{BA} + k_{CA}) \cdot C_A + k_{AB} \cdot C_B + k_{AC} \cdot C_C \tag{5.68}$$

$$\frac{dC_B}{dt} = -(k_{AB} + k_{CB}) \cdot C_B + k_{BA} \cdot C_A + k_{BC} \cdot C_C \tag{5.69}$$

$$\frac{dC_C}{dt} = -(k_{AC} + k_{BC}) \cdot C_C + k_{CB} \cdot C_B + k_{CA} \cdot C_A \tag{5.70}$$

To analyse the above equations (or system in general) at non-equilibrium conditions, we need to consider a concentration other than equilibrium concentration. Therefore, we write:

$$\frac{dx_A}{dt} = -(k_{BA} + k_{CA}) \cdot x_A + k_{AB} \cdot x_B + k_{AC} \cdot x_C \tag{5.71}$$

Due to equation (5.65):

$$\left(C_A - C_{A,eq}\right) = x_A = \frac{C_{A,eq}}{RT} \cdot \Delta G_A \tag{5.72}$$

So for equation (5.71) we have:

$$\frac{dx_A}{dt} = -(k_{BA} + k_{CA}) \cdot \frac{C_{A,eq}}{RT} \cdot \Delta G_A + k_{AB} \cdot \frac{C_{B,eq}}{RT} \cdot \Delta G_B + k_{AC} \cdot \frac{C_{C,eq}}{RT} \cdot \Delta G_C \tag{5.73}$$

$$\frac{dx_B}{dt} = -(k_{AB} + k_{CB}) \cdot \frac{C_{B,eq}}{RT} \cdot \Delta G_B + k_{BA} \cdot \frac{C_{A,eq}}{RT} \cdot \Delta G_A + k_{BC} \cdot \frac{C_{C,eq}}{RT} \cdot \Delta G_C \tag{5.74}$$

$$\frac{dx_C}{dt} = -(k_{AC} + k_{BC}) \cdot \frac{C_{C,eq}}{RT} \cdot \Delta G_C + k_{CB} \cdot \frac{C_{B,eq}}{RT} \cdot \Delta G_B + k_{CA} \cdot \frac{C_{A,eq}}{RT} \cdot \Delta G_A \tag{5.75}$$

By comparison equations (5.73) to equation (5.76), the analogy between equation terms can be seen. Comparison is as follows:

$$J_A = L_{11} \cdot X_1 + L_{12} \cdot X_2 + L_{13} \cdot X_3 \tag{5.76}$$

For compound A, we have:

$$L_{11} = -(k_{BA} + k_{CA}) \cdot \frac{C_{A,eq}}{RT} \; ; X_1 = \Delta G_A \tag{5.77}$$

$$L_{12} = k_{AB} \cdot \frac{C_{B,eq}}{RT}; \; X_2 = \Delta G_B \tag{5.78}$$

$$L_{13} = k_{AC} \cdot \frac{C_{C,eq}}{RT}; \; X_3 = \Delta G_C \tag{5.79}$$

And one can show the reciprocal relations in this process. For example, for $L_{13} = L_{31}$we can compare these two relationships:

$$L_{13} = k_{AC} \cdot \frac{C_{C,eq}}{RT} \tag{5.80}$$

$$L_{31} = k_{CA} \cdot \frac{C_{A,eq}}{RT} \tag{5.81}$$

$$k_{AC} \cdot \frac{C_{C,eq}}{RT} = k_{CA} \cdot \frac{C_{A,eq}}{RT} \tag{5.82}$$

$$k_{AC} \cdot C_{C,eq} = k_{CA} \cdot C_{A,eq} \tag{5.83}$$

Similarly we have:

$$k_{CB} \cdot C_{B,eq} = k_{BC} \cdot C_{C,eq} \tag{5.84}$$

$$k_{BA} \cdot C_{A,eq} = k_{AB} \cdot C_{B,eq} \tag{5.85}$$

The equations (5.83) and (5.84) and (5.85) are explaining the equilibrium stage of reactions. Equilibrium thermodynamics satisfies the relationships in equations (5.83), (5.84) and (5.85).

5.3.7 Phenomenological non-Equilibrium Thermodynamics and Microbial Growth

Phenomenological non-equilibrium thermodynamics considers any system in the form of black box that converts an input flow to an output flow. Fig. 5.3 represents the simulation of microbial growth based on phenomenological non-equilibrium thermodynamics. "J_c" describes the flow of energy into the system through catabolism (energy producing step). And "- J_a" demonstrates the output of energy which here is the rate of biomass production through anabolism (energy consuming step) (Westerhoff and Dam, 1987).

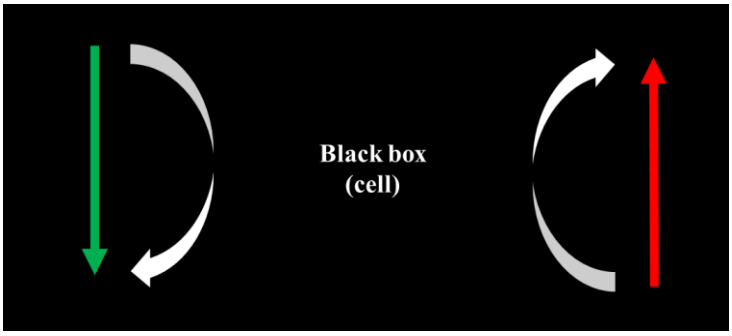


Figure 5-3 Illustrates the macroscopic interaction of energetic terms in microbial growth. In J_c energy will be produced and in J_a energy will be consumed through irreversible processes to produce new cells.

By implementing the Onsager reciprocal relation, one can describe the flow and forces for the microbial growth system (Westerhoff and Dam, 1987):

$$J_c = L_{cc} \cdot (\Delta G_c - \Delta G_c^{\#}) + L_{ca} \cdot (\Delta G_a - \Delta G_a^{\#}) \tag{5.86}$$

$$J_a = L_{ac} \cdot (\Delta G_c - \Delta G_c^{\#}) + L_{aa} \cdot (\Delta G_a - \Delta G_a^{\#}) \tag{5.87}$$

In the above equations, ΔG_c and ΔG_a are free energy differences for catabolism and anabolism respectively and $\Delta G_c^{\#}$ and $\Delta G_a^{\#}$ are independent constants. If we write equations 5.86 and 5.87 at the equilibrium point, $\Delta G_c^{\#} = \Delta G_a^{\#} = 0$:

$$J_c = L_{cc} \cdot \Delta G_c + L_{ca} \cdot \Delta G_a \tag{5.88}$$

$$J_a = L_{ac} \cdot \Delta G_c + L_{aa} \cdot \Delta G_a \tag{5.89}$$

Equation (5.88) represents the flux of catabolism and equation (5.89) shows the flux of anabolic reactions. To describe the total substrate flux we can sum these two equations:

$$J_s = J_c + (-J_a) \tag{5.90}$$

$$J_s = \Delta G_c \, (L_{cc} - L_{ac}) + \Delta G_a \, (L_{ca} - L_{aa}) \tag{5.91}$$

ΔG_c can be written based on equation (5.88). By replacing ΔG_c from equation (5.88) and put it into equation (5.91), following equation is obtained:

$$J_s = \left(\left(\frac{L_{ca} - L_{cc}}{L_{ac}} \right) \cdot (-J_a) \right) - \left(\Delta G_a \cdot \left((L_{cc} - L_{ac}) \cdot \left(\frac{L_{aa}}{L_{ac}} \right) + (L_{ca} - L_{aa}) \right) \right) \tag{5.92}$$

We can also represent the above linear equation in this format (Westerhoff and Dam, 1987):

$$J_s = \alpha \cdot (-J_a) + \beta \tag{5.93}$$

This equation is useful since it demonstrates a linear relationship between substrate consumption rate and biomass production rate (Demirel, 2014; S. Roy Caplan, 1983; Stephanopoulos et al., 1998; Westerhoff and Dam, 1987). This thermodynamic driven equation is similar to the Herbert-Pirt relationship (Pirt, 1965), which describes the substrate consumption rate "q_s" as a function of biomass specific growth rate "μ" in a linear equation (Pirt, 1965; Stephanopoulos et al., 1998):

$$\boldsymbol{q_s = \frac{\mu}{Y_{sx}^{max}} + m_s} \tag{5.94}$$

This equation will be used as the main model in the further thermodynamics analysis. In the Herbert-Pirt equation, "$\frac{1}{Y_{sx}^{max}}$" is maximum yield of substrate to biomass and term of "m_s" shows the maintenance or endogenous metabolism. Since the Herbert-Pirt equation is the model that matches best with the thermodynamics background of our process, we use this model to predict the stoichiometry and kinetic parameters of biodegradation.

5.4 Thermodynamics of Microbial Growth

5.4.1 Prediction of Stoichiometric and Kinetics parametres from Thermodynamics Perspective

Based on the Herbert-Pirt equation there is a linear relationship between substrate uptake rate, growth rate and maintenance of the cell (Pirt, 1965). By quantifying the parameters in the Herbert-Pirt equation we can describe the biodegradation process. With the help of thermodynamics we are going to predict the parameters of Herbert-Pirt equation to model the biodegradation process (Beeftink et al., 1990; Cueto-Rojas et al., 2015; Smolke, 2009; von Stockar et al., 2006; Tijhuis et al., 1993).

5.4.1.1 Prediction of Substrate Needed for Maintenance

Even at zero growth rate, cells need to spend energy to support their life through maintenance processes (Stephanopoulos et al., 1998). Maintenance energy was found to be independent of substrate (carbon source) and nitrogen source (Heijnen et al., 1992; Tijhuis et al., 1993). However, as mentioned in section (5.2.1.3) temperature is the most significant factor in determining maintenance energy (Beeftink et al., 1990; Tijhuis et al., 1993). This amount of energy m_G is expressed in kJ of Gibbs free energy per 1 C-mole of biomass ($C_1H_{1.8}O_{0.9}N_{0.2}$) per **hour** (Beeftink et al., 1990; Tijhuis et al., 1993). The difference in maintenance energy under aerobic and anaerobic conditions are not significant (Heijnen, 2010; Tijhuis et al., 1993). Fig. 5.4 shows the correlation between m_G and temperature under aerobic and anaerobic conditions.

5.4.1.1.1 Required Substrate for Maintenance

We know that the source of energy for maintenance is the catabolic reaction. ΔG_{cat} has units of kJ per mole substrate and m_G has units of kJ per C-moleC_x per hour.

Hence:

$$m_S = \frac{m_G}{\Delta G_{cat}} \left[\frac{\frac{kJ}{C-moleX.h}}{\frac{kJ}{mole\ S}} \right] \tag{5.95}$$

$$m_S = \frac{m_G}{\Delta G_{cat}} \left[\frac{mole\ S}{C-moleX.h} \right] \tag{5.96}$$

Where m_s shows the amount of needed substrate for maintenance. Based on this formula (Eq. 5.97), one can calculate the needed substrate for cell maintenance (Colin Ratledge, 2006; Heijnen, 2010; Smolke, 2009).

5.4.1.1.2 Required Energy for Maintenance

To calculate the maintenance energy (m_G) based on the above correlations (Colin Ratledge, 2006; Heijnen, 2010; Smolke, 2009; Tijhuis et al., 1993), following equation is proposed:

$$m_G = 4.5\ exp\left[-\frac{69000}{R}\left(\frac{1}{T}-\frac{1}{298}\right)\right]\left[\frac{kJ}{C-mole C_x.h}\right] \quad (5.97)$$

In this equation:

R: is universal gas constant 8.314 J mole^{-1} K^{-1}

T: is absolute temperature

m_G: is maintenance Gibbs free energy kJ h^{-1} C-moleC_x^{-1}

This equation tells us that at standard condition (T=25 ^{o}C), in one hour, one C-mole of the cell needs 4.5 kJ energy **at least** to survive. However, theses correlations have the confidence interval of 41% for aerobic growth and 47% for anaerobic growth. The accuracy of proposed equation is not high, however, up to date, it has been the only equation that proposed and used widely in biotechnology and environmental biotechnology purposes.

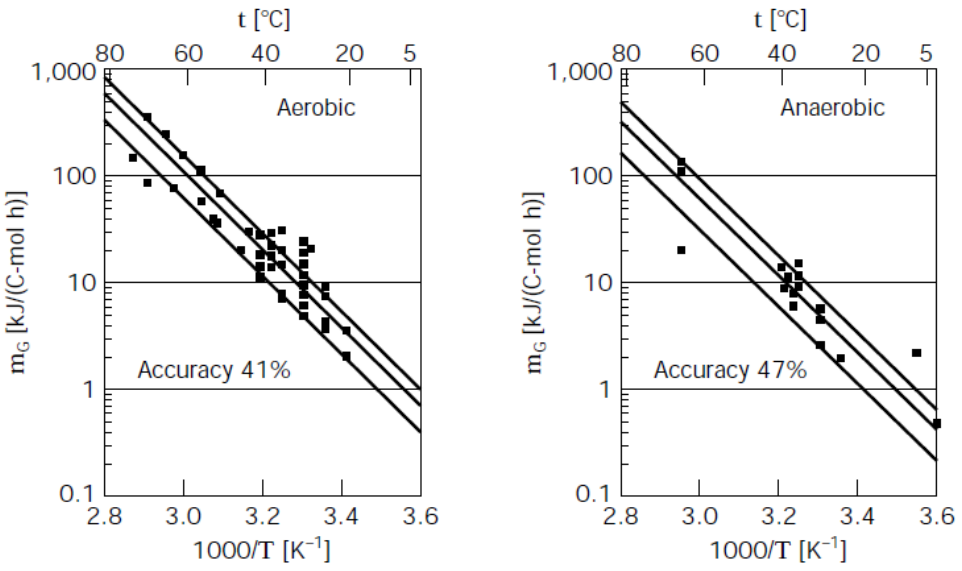


Figure 5-4 There is 41% accuracy (aerobic growth) for prediction of maintenance energy based on Eq. 5.97 and 47% accuracy for anaerobic growth (reprinted with permission from Tijhuis et al., (1993)).

The amount of maintenance energy comes from the catabolism of electron donor. Now based on the type of the electron donor and acceptor we can calculate the required amount of substrate needed to supply maintenance energy (Colin Ratledge, 2006; Heijnen, 2010).

5.4.1.2 Prediction of Gibbs Energy Needed for Growth

"$\frac{1}{Y_{SX}^{max}}$" is a stoichiometric parameter that shows us the amount of consumed substrate for biosynthesis of 1 C-mole biomass. This stoichiometric parameter is equal to the amount of energy needed for biosynthesis of biomass $\left(\frac{1}{Y_{GX}^{max}}\right)$ (Heijnen, 2010; Roels, 1983; Urs von Stockar, 2013). This amount of energy represents the amount of biochemical work required to produce biomass from an available C-source through irreversible **carbon-carbon** coupling and **oxidation/reduction** reactions. Therefore, dissipation energy greatly depends on two main properties of a carbon source (Heijnen and Van Dijken, 1992):

- Degree of reduction: biomass has the degree of reduction of 4.2 (see Appendix 3). Therefore, dissipation energy or $\frac{1}{Y_{GX}^{max}}$ is minimal for $\gamma_D \approx 3.5$ to 4.5 (see Fig. 5.5.) and it increases for more reduced or oxidized compounds. Because for more reduced compounds $\gamma_D > 4.2$ or more oxidized compounds $\gamma_D < 4.2$, microorganism needs to perform additional work which leads to higher Gibbs energy dissipation.
- Carbon chain length: biomass consists of a polymer that contains monomers of 4 to 6 carbon atoms. So when C-source has less carbon than 4 to 6 C-atoms, microorganisms have to spend more energy to achieve $C - C$ coupling. Therefore, $\frac{1}{Y_{GX}^{max}}$ increases for carbon sources with less carbon atoms.

The effect of carbon number and degree of reduction on dissipation energy is shown in Fig. 5.5. An example of a heterotrophic bacteria using glucose ($C_6H_{12}O_6$) as a substrate for growth will be used to illustrate how Fig. 5-5 can be interpreted. Glucose has six carbons with a degree of reduction of 4, which correlates to the lowest Gibbs energy of dissipation on Fig 5.5. Under these conditions, the microorganism needs to complete the least amount of biological work to produce new cells from glucose (~ 236 kJ Gibbs energy per C-mol biomass). In contrast, when methane (CH_4) is used as a substrate with 1 carbon atom and degree of reduction of 8, Gibbs free energy dissipation for biosynthesis of 1 C-mol biomass is among the highest amounts (~1087 kJ Gibbs energy for production of one C-mol biomass).

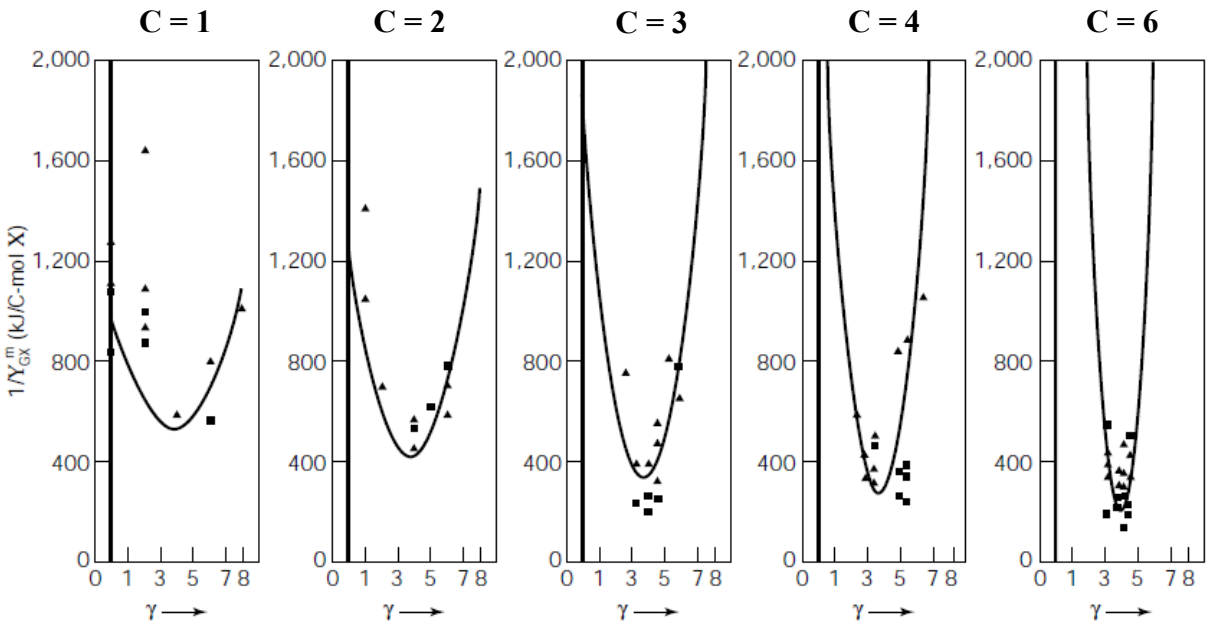


Figure 5-5. Gibbs free energy of dissipation based on number of carbon atoms and degree of reduction of carbon source (reprinted with permission from Heijnen, (1992)).

Based on this relationship between Gibbs free energy for heterotrophic growth systems on the degree of reduction of substrate and number of carbon (up to 6 carbon compounds) Heijnen et al. (1991) proposed the following equation (Heijnen, 1991; Heijnen, 2010; Smolke, 2009; Tijhuis et al., 1993):

$$\frac{1}{Y_{GX}^{max}} = 200 + 18(6 - C)^{1.8} + exp[|3.8 - \gamma|^{0.32} \times (3.6 + 0.4C)] \left[\frac{kJ}{C - moleC_x}\right] \quad (5.98)$$

In this equation:

C: is carbon number

γ: is the degree of reduction of substrate **per C-atom of substrate**

$\frac{1}{Y_{GX}^{max}}$ is dissipation energy – as the amount of Gibbs free energy that needs to be dissipated for production of 1 C-mole biomass. Therefore, equation 5.98 determines the energy needed to produce 1 C-mole biomass from an available substrate under heterotrophic conditions.

5.4.1.3 Prediction of Biomass Maximum Specific Growth Rate (μ^{max})

Kinetic parameters have various values for different microbial growth systems. For example, specific growth rates vary enormously between 0.005 to 2 h^{-1} depending on the substrate and electron acceptor (Smolke, 2009). These differences in specific growth rate can be explained thermodynamically as follows: Cells have **limits** in the rate of production of catabolic energy (Colin Ratledge, 2006; Flickinger, 2009; Smolke, 2009). The transportation of electrons from electron donor to electron acceptor occurs via electron transport chain. These chains are made of protein that are located in the cell's membrane (Lehninger et al., 2005). The rate of electron transport is limited by the amount of electron transport chain proteins in the cell membrane and the available space for electron transport (also known as electron transport capacity). A correlation has been proposed to quantify the maximum rate of electron transport capacity (ETC) (Colin Ratledge, 2006; Heijnen, 2010; Urs von Stockar, 2013):

$$(q_G^{max})ETC = 3\ exp\left[-\frac{69000}{R}\left(\frac{1}{T}-\frac{1}{298}\right)\right]\left[\frac{\frac{mole\ electron}{h}}{C-mole\ C_x}\right] \quad (5.99)$$

Where T is temperature; R is the ideal gas law constant. Based on equation 5.99, when T=298 K, the maximum rate of electron transfer is 3 electron-mole per hour per C-mole of biomass. The amount of Gibbs free energy per mole of electron that is catabolized in catabolism (Heijnen, 2010; Smolke, 2009; Urs von Stockar, 2013):

$$energy\ produced\ per\ catabolized\ electron = \frac{\Delta G\ of\ catabolism}{n}\left[\frac{kJ}{mole\ electron}\right] \quad (5.100)$$

Where "n" is the number of catabolized electrons. Finally, the product of these two expressions (5.99 and 5.100) determines the maximum production rate of Gibbs energy per catabolized electrons (Heijnen, 2010; Smolke, 2009):

$$q_G^{max} = 3 \cdot \left(\frac{\Delta G_{cat}}{n}\right) exp\ \left[-\frac{69000}{R}\left(\frac{1}{T} - \frac{1}{298}\right)\right]\left[\frac{kJ}{C-mole\ C_x.h}\right] \quad (5.101)$$

Where q_G^{max} is equal to the term of maximum biomass specific substrate consumption rate in terms of energy. It represents the maximum amount of energy produced **by** 1 C-mole of biomass. Based on the Herbert-Pirt equation with energetic terms:

$$q_G^{max} = \frac{1}{Y_{GX}^{max}} \cdot \mu^{max} + m_G \quad (5.102)$$

Now we have all the necessary relationships to predict all the parameters in equation 5.102 except μ^{max}, which can now be determined as follows:

$$\mu^{max} = \left(\frac{\left(3 \cdot \frac{\Delta G_{cat}}{n}\right) - m_G}{\frac{1}{Y_{GX}^{max}}}\right) exp\ \left[-\frac{69000}{R}\left(\frac{1}{T} - \frac{1}{298}\right)\right]\left[\frac{1}{h}\right] \quad (5.103)$$

5.5 Case Study: Biodegradation of Model Naphthenic Acids

As mentioned previously, biodegradation takes place when microorganisms use available organics (pollutant) for their growth and maintenance (Alexander, 1999). It is possible to quantify the growth of a microorganism on known organic compounds – such as model NAs. To provide a framework for this process, we will determine the catabolism, anabolism and maintenance of a cell when using a model NA - dicyclohexylacetic acid ($C_{14}\ H_{24}\ O_2$) as its organic substrate under aerobic conditions. This specific compound was chosen as is it is known

to be recalcitrant (non-biodegradable) under aerobic conditions (Misiti et al., 2014). This study can then be expanded to other model NA compounds to help us optimize the NAs biodegradation process.

5.5.1 Catabolic Pathways

Catabolic reactions produce energy for a cell. The majority of the proposed equations for predicting kinetic and stoichiometric parameters based on thermodynamic analysis can apply for organic compounds with up to 6 carbons. This is due to the assumption that a 6 carbon organic compound can be used directly by a microorganism as a carbon source (Colin Ratledge, 2006; Stephanopoulos et al., 1998; Urs von Stockar, 2013). As such, there is a lack of bioenergetics studies for compounds with more than 6 carbons (personal communication, Prof. Urs von Stockar). Instead, an understanding of catabolism for larger organic compounds, such as dicyclohexylacetic acid, can be determined using biodegradation databases.

The University of Minnesota Biocatalysis/Biodegradation database uses biochemistry rules to predict catabolic pathways under aerobic conditions (Ellis et al., 2006). Biotransformation rules are based on known biochemical reactions of functional groups. By using the chemical structure of a compound as an input, functional groups are identified, and through rule matching, (rules are based on known biochemical reactions by respected enzymes) biodegradation starts. The produced compounds based on reactions can have various different functional groups. Subsequently, further reactions will be different (as it mentioned before, different functional groups use different rules) and result in multiple different catabolic pathways. Therefore, the amount of different pathways is unique for each compound. The theoretical degradation process continues until an intermediate metabolite is produced. It means that, as soon as a known

metabolite is produced the reaction is stopped. This reasonable since known metabolites (by known means it is in cellular metabolism like C1 to C6 sugars) are readily biodegradable by microorganisms (Ellis et al., 2006; Hou et al., 2003; Hou et al., 2004a; Wackett and Ellis, 1999). The end product of the catabolic pathway, is considered as the C-source for the anabolic reactions.

5.5.2 *in silico* Biodegradation of Dicyclohexyl acetic acid

Dicyclohexylacetic acid is shown to be non-biodegradable based on Misiti et al., (2014) investigation. However, Pathway analysis demonstrates that dicyclohexylacetic acid can be mineralized by catabolizing to acetate.

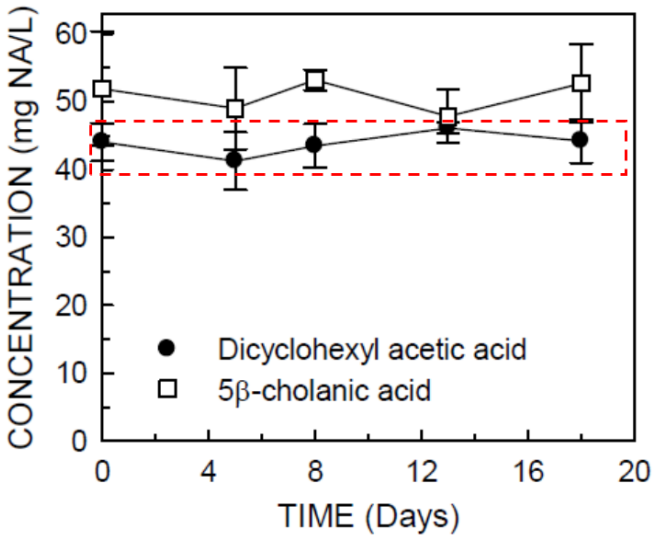


Figure 5-6 Batch biodegradation of dicyclohexyl acetic and 5β-cholanic acid in separate bioreactors (reprinted with permission from Misiti et al., (2014))

Fig. 5-6 shows that the concentration of dicyclohexyl acetic acid did not change over the time of 20 days under aerobic condition. No change in concentration of dicyclohexyl acetic acid expresses its non-biodegradability. The experimental conditions above show that dicyclohexyl acetic acid is not degradabale, however, thermodynamics implies that aerobic biodegradation is possible.

5.5.2.1 Catabolism of Dicyclyhexyl acetic acid

Dicyclohexyl acetic acid introduced into the database by sketching the chemical structure of the compound. As it mentioned, a compound can have different catabolic pathways. Selecting a

pathway can be based on different reasons. One might choose a pathway based on less thermodynamic bottlenecks, based on the end product, or based on the length of the pathway. For dicyclohexyl acetic acid, we picked up a pathway based on the end product. The importance of the pathway is its role in anabolism. For example, dicyclohexyl acetic acid specifically the end product of the catabolic pathway is used as the carbon source for anabolism. In our analysis, a pathway with the end product of acetic acid has selected to study. The degradation pathway is illustrated in Fig 5-7 Biodegradation starts by monooxygenase enzyme that acts on two hydrogen-donors (NADH – nicotinamide adenine dinucletide) and one oxygen (reaction 5.104). The second reaction takes place by dehydrogenase enzyme; two electrons are released by using NAD^+ as the electron acceptor (5.105). The third reaction (5.106) is similar to reaction (5.104) as it takes place by a monooxygenase enzyme. The reaction (5.107) is a hydrolases reaction. The reaction (5.108) generally known as the β-oxidation reaction that results in production of acetate as the by-product. Next reaction (5.109) is alcohol dehydrogenase that uses NAD^+ as the electron acceptor. It acts on CH-OH group as the electron donor. Reaction (5.110) takes place by oxidoreductase that oxidizes aldehydes to their corresponding acid, by using NAD^+ as the electron acceptor. The next reaction is (5.111) which is as the same as reaction (5.104). Reaction (5.112) is dehydrogenase that acts on secondary alcohol through production of NADH. The Reaction (5.113) is oxidoreductase that uses NAD^+ as electron acceptor. The reaction (5.114) is similar to reaction (5.104). The nest reaction is a decarboxylase reaction that produces carbon dioxide (5.115). The next reaction (5.116) takes place by dehydrogenase. The reaction (5.117) conducts by oxidoreductase enzymes that act on two hydrogen donors, and oxygen. The next reaction is a hydrolysis reaction (5.118). The reaction (5.119) is similar to reaction (5.113). The reaction (5.120) performs by dehydrogenases enzymes, the same happens for reaction (5.121). Reaction (5.122) is similar to reaction (5.104). The reaction

(5.123) conducts by dehydrogenase by using NAD^+ as electron acceptor. The reaction (5.124) is oxidoreductase that acts on single molecular oxygen. Reaction (5.125) and reaction (5.104) is the same again. Reaction (5.126) occurs by dehydrogenases. The next reaction (5.127) and reaction (5.125) are the same. The reactions (5.128) and (5.129) are decarboxylases reaction that produces CO_2. And the last reaction (5.130) produces two acetic acid by using NADH as the source of hydrogen.

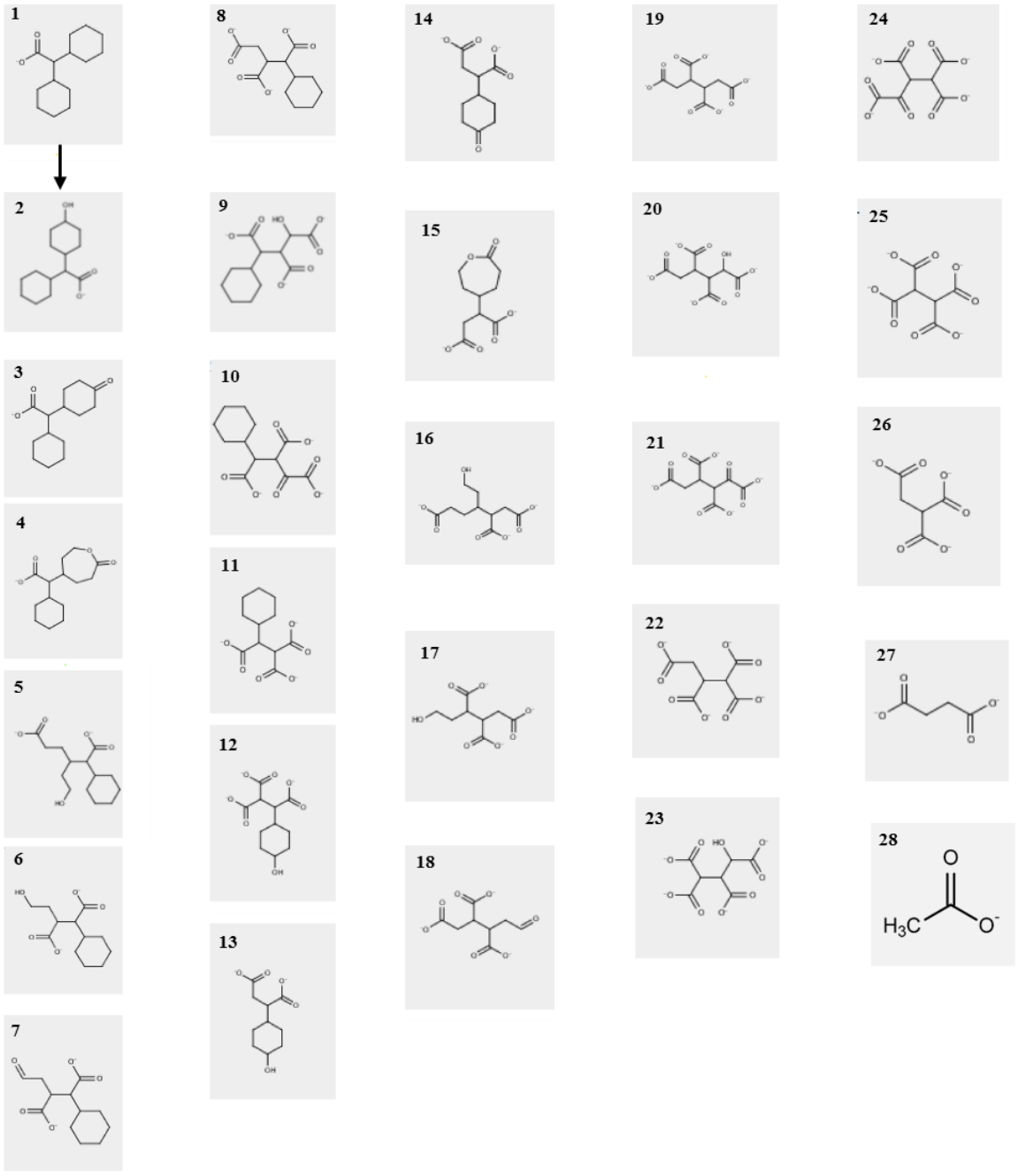


Figure 5-7. Catabolic pathway of dicyclohexyl acetic acid biodegradation under aerobic condition; numbers are shown in the order of biodegradation

$$C_{14}H_{23}O_2^- + O_2 + NADH \rightarrow C_{14}H_{22}O_3^- \quad (5.104)$$

$$C_{14}H_{22}O_3^- \rightarrow C_{14}H_{21}O_3^- + NADH \quad (5.105)$$

$$C_{14}H_{21}O_3^- + O_2 + NADH \rightarrow C_{14}H_{21}O_4^- \quad (5.106)$$

$$C_{14}H_{21}O_4^- + H_2O \rightarrow C_{14}H_{22}O_5^{2-} \quad (5.107)$$

$$C_{14}H_{22}O_5^{2-} \rightarrow C_{12}H_{18}O_5^{2-} + C_2H_3O_2^- + 2NADH \quad (5.108)$$

$$C_{12}H_{18}O_5^{2-} \rightarrow C_{12}H_{16}O_5^{2-} + NADH \quad (5.109)$$

$$C_{12}H_{16}O_5^{2-} \rightarrow C_{12}H_{15}O_6^{3-} + NADH \quad (5.110)$$

$$C_{12}H_{15}O_6^{3-} \rightarrow C_{12}H_{15}O_7^{3-} + NADH \quad (5.111)$$

$$C_{12}H_{15}O_7^{3-} \rightarrow C_{12}H_{13}O_7^{3-} + NADH \quad (5.112)$$

$$C_{12}H_{13}O_7^{3-} \rightarrow C_{11}H_{13}O_6^{3-} + CO_2 + NADH \quad (5.113)$$

$$C_{11}H_{13}O_6^{3-} + O_2 + NADH \rightarrow C_{11}H_{13}O_7^{3-} \quad (5.114)$$

$$C_{11}H_{13}O_7^{3-} \rightarrow C_{10}H_{14}O_5^{2-} + CO_2 \quad (5.115)$$

$$C_{10}H_{14}O_5^{2-} \rightarrow C_{10}H_{12}O_5^{2-} + NADH \quad (5.116)$$

$$C_{10}H_{12}O_5^{2-} + O_2 + NADH \rightarrow C_{10}H_{12}O_6^{2-} \quad (5.117)$$

$$C_{10}H_{12}O_6^{2-} + H_2O \rightarrow C_{10}H_{13}O_7^{3-} \quad (5.118)$$

$$C_{10}H_{13}O_7^{3-} \rightarrow C_8H_9O_7^{3-} + C_2H_3O_2^- + 2NADH \quad (5.119)$$

$$C_8H_9O_7^{3-} \rightarrow C_8H_7O_7^{3-} + NADH \quad (5.120)$$

$$C_8H_7O_7^{3-} \rightarrow C_8H_6O_8^{4-} + NADH \quad (5.121)$$

$$C_8H_6O_8^{4-} + O_2 + NADH \rightarrow C_8H_6O_9^{4-} \quad (5.122)$$

$$C_8H_6O_9^{4-} \rightarrow C_8H_4O_9^{4-} + NADH \quad (5.123)$$

$$C_8H_4O_9^{4-} + 0.5O_2 \rightarrow C_7H_4O_8^{4-} + CO_2 \quad (5.124)$$

$$C_7H_4O_8^{4-} + O_2 + NADH \rightarrow C_7H_4O_9^{4-} \quad (5.125)$$

$$C_7H_4O_9^{4-} \rightarrow C_7H_2O_9^{4-} + NADH \quad (5.126)$$

$$C_7H_2O_9^{4-} + 0.5O_2 \rightarrow C_6H_2O_8^{4-} + CO_2 \quad (5.127)$$

$$C_6H_2O_8^{4-} \rightarrow C_5H_3O_6^{3-} + CO_2 \quad (5.128)$$

$$C_5H_3O_6^{3-} \rightarrow C_4H_4O_4^{2-} + CO_2 \quad (5.129)$$

$$C_4H_4O_4^{2-} + NADH \rightarrow 2C_2H_3O_2^{-} \quad (5.130)$$

Above reactions are lumped into the following reaction:

$$-C_{14}H_{24}O_2 - 7O_2 - 4\,H_2O + 6\,CO_2 + 4\,C_2H_4O_2 + 8\,NADH \quad (5.131)$$

$$C_{14}H_{24}O_2 + 7O_2 + 4\,H_2O \rightarrow 6\,CO_2 + 4\,C_2H_4O_2 + 8\,NADH \quad (5.131A)$$

The reactions (5.131) and (5.131A) are the same. In this format (5.131) the minus signs indicate consumption and positive signs indicate production. When setting up a reaction in this form it helps us in elemental balance analysis, which is the most common way of representing a reaction in the metabolic engineering field. This overall reaction demonstrates that acetic acid is the end product of catabolism and it is used by microorganisms as the carbon source.

5.5.2.2 Anabolism

By knowing the source of carbon is acetic acid (and nitrogen as ammonium) we can write the overall reaction in anabolism as follows:

$$a\,C_2H_4O_2 + b\,NH_3 + c\,H_2O + d\,CO_2 + \,CH_{1.8}O_{0.5}N_{0.2} \quad (5.132)$$

By setting up an elemental balance analysis we can calculate the stoichiometric coefficients as follows:

$$\begin{pmatrix} 2 & 0 & 0 & 1 & 1 \\ 4 & 3 & 2 & 0 & 1.8 \\ 2 & 0 & 1 & 2 & 0.5 \\ 0 & 1 & 0 & 0 & 0.2 \\ 8 & 0 & 0 & 0 & 4.2 \end{pmatrix} \cdot \begin{pmatrix} a \\ b \\ c \\ d \\ 1 \end{pmatrix} = 0 \tag{5.133}$$

By multiplying the above matrix we have:

$$C - balance: 2a + d + 1 = 0 \tag{5.134}$$

$$H - balance: 4a + 3b + 2C + 1.8 = 0 \tag{5.135}$$

$$O - balance: 2a + c + 2d + 0.5 = 0 \tag{5.136}$$

$$N - balance: b + 0.2 = 0 \tag{5.137}$$

$$\gamma - balance: 8\,a + 4.2 = 0 \tag{5.138}$$

Solving the above equation results in:

$$\begin{pmatrix} a \\ b \\ c \\ d \\ 1 \end{pmatrix} = \begin{pmatrix} -0.525 \\ -0.2 \\ 0.45 \\ 0.05 \\ 1 \end{pmatrix} \tag{5.139}$$

Now we have the anabolic reaction based on the production of 1 C-mol biomass using acetic acid as a carbon source.

By recalling Eq (5.98):

$$\frac{1}{Y_{GX}^{max}} = 200 + 18(6 - C)^{1.8} + exp[|3.8 - \gamma|^{0.32} \times (3.6 + 0.4C)] \left[\frac{kJ}{C - mole C_x}\right]$$

Based on this equation we can calculate the Gibbs energy of dissipation for growth of 1 C-mole biomass on acetate. Acetate has 2 carbon with degree of reduction of 4 mole electron per C-mole atom.

$$\frac{1}{Y_{GX}^{max}} = 200 + 18(6 - 2)^{1.8} + exp[|3.8 - 4|^{0.32} \times (3.6 + 0.4 \times 2)] \left[\frac{kJ}{C - mole C_x}\right]$$

$$\frac{1}{Y_{GX}^{max}} = 432.12 \left[\frac{kJ}{C - mole C_x}\right]$$

Thus,

$$-0.525\ C_2H_4O_2 - 432.12\ (kJ\ Gibbs\ energy) - 0.2\ NH_3 + 0.45\ H_2O + 0.05\ CO_2 + CH_{1.8}O_{0.5}N_{0.2} \quad (5.140)$$

Based on Eq. (5.131) growth takes place on four moles of acetic acid. Hence:

$$-4\ C_2H_4O_2 - 3292.75(kJ\ Gibbs\ energy) - 1.524\ NH_3 + 3.428\ H_2O + 0.38\ CO_2 + 7.62\ CH_{1.8}O_{0.5}N_{0.2} \quad (5.141)$$

It can be determined that for growth on 4 moles of acetic acid, 3292.75 kJ Gibbs energy is needed. One possibility for the cells to gain this energy comes from the oxidation of produced NADH:

$$-8\ NADH - 4O_2 + 8\ H_2O + 8\ NAD^+ + 1840\ (kJ\ Gibbs\ energy) \quad (5.142)$$

From this reaction there is a gain of 1840 kJ, therefore 1452.75 kJ Gibbs energy to support anabolic reaction.

$$3292.75 - 1840 = 1452.75\ kJ\ Gibbs\ energy \quad (5.143)$$

The remaining amount of energy must come from additional catabolism of substrate.

$$-C_{14}H_{24}O_2 - 7O_2\ (mono-\ and\ di-oxygenase) \\ -12\ O_2(electron\ trasnport\ chain) \\ +14\ CO_2 + 12\ H_2O + (5520\ kJ\ Gibbs\ energy) \quad (5.144)$$

To obtain the ratio of energy needed from equation 5.149 we used the following:

$$\left(\frac{1452.75}{5520}\right) = 0.263 \quad (5.145)$$

Which then translates to the reaction for extra catabolism as follows:

$$-0.263\ C_{14}H_{24}O_2 - 1.841\ O_2(mono-ox) - 3.156\ O_2(ETC) + 3.682\ CO_2 + 3.156\ H_2O + 1452.75\ kJ\ Gibbs\ energy \quad (5.146)$$

To get the overall growth (biodegradation) reaction we need to sum up reactions (5.131), (5.141),(5.142), and (5.146):

$$-1.263\ C_{14}H_{24}O_2 - 15.997\ O_2 - 1.524\ NH_3 + 7.62\ CH_{1.8}O_{0.5}N_{0.2} + 10.584\ H_2O + 10.062\ CO_2 \quad (5.147)$$

5.8.4. Calculation of yield of substrate to biomass

To calculate the yield of substrate to biomass using equation 5.147 we have:

$$Y_{sx} = \frac{7.62}{1.263 \times 14} = 0.431\ \frac{C-mol\ biomass}{C-mol\ substrate} = 0.663\ \frac{g\ organic\ dry\ weight}{g\ substrate} \quad (5.148)$$

The accuracy in the prediction of yield of substrate to biomass by the proposed approach, has a relative error of 13%.

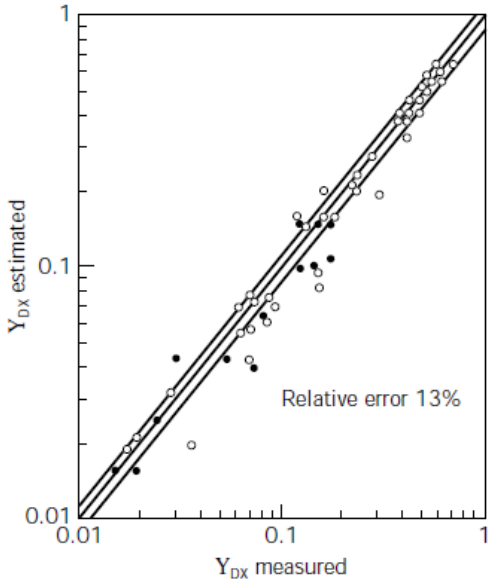


Figure 5-8 Comparison of measured and predicted yield of substrate to biomass; (●) shows the fermentative growth system and (○) shows the aerobic growth system; taken from (reprinted with permission from Heijnen et. al, (1992))

5.5.2.1.1 Calculation of μ^{max}

In production of energy through electron transport chain ~ 460 kJ Gibbs energy is produced per mole of electron. This number comes from following reaction:

$$O_2 + NADH \rightarrow 2NAD^+ + 2H_2O \;;\; \Delta G = -460\ kJ/mol$$

Therefore, 115 kJ energy is produced per mole of catabolized electron (1 mole of oxygen contains 4 mole of electron). With this the maximum specific growth rate of biomass can be calculated.

By recalling following equation:

$$q_G^{max} = 3 \cdot \left(\frac{\Delta G_{cat}}{n}\right) exp\ \left[-\frac{69000}{R}\left(\frac{1}{T} - \frac{1}{298}\right)\right]\left[\frac{kJ}{C - mole\ C_x . h}\right]$$

At T=298 K:

$$q_G^{max} = 3 \times 115 = 345\left[\frac{kJ}{C - mole\ C_x . h}\right]$$

Thus,

$$\mu^{max} = \left(\frac{q_G^{max} - m_G}{\frac{1}{Y_{GX}^{max}}}\right) = \left(\frac{345 - 4.5}{432}\right) = 0.788\ h^{-1}$$

5.5.2.3 Needed Substrate for Maintenance

By using equation (5.97), the energy required for maintenance can be calculated.

$$m_G = 4.5\ exp\left[-\frac{69000}{R}\left(\frac{1}{T}-\frac{1}{298}\right)\right]\left[\frac{kJ}{C-mole C_x.h}\right]$$

At T=298 K we have:

$$m_G = 4.5\left[\frac{kJ}{C-mole C_x.h}\right]$$

Hence, due to equation (5.96)

$$m_s = \frac{4.5}{5520} = 8.15\times10^{-4}\ \frac{mol\ C_s}{C-mol\ C_x\cdot h}$$

Maintenance substrate for other compounds:

$$m_{O_2} = \frac{4.5\times(7+12)}{5520} = 0.0154\ \frac{mol\ O_2}{C-mol\ C_x\cdot h}$$

$$m_{CO_2} = \frac{4.5\times14}{5520} = 0.0114\ \frac{mol\ CO_2}{C-mol\ C_x\cdot h}$$

$$m_{H_2O} = \frac{4.5\times12}{5890} = 0.0098\ \frac{mol\ H_2O}{C-mol\ C_x\cdot h}$$

Above calculations demonstrate the substrate needed (oxygen and substrate) and produced (carbon dioxide and water) in maintenance. As maintenance is defined as the needed substrate at zero growth rate and at zero growth rate, no biomass is produced. Therefore, a nitrogen source is not needed here.

Since the yields and maintenance substrate have been determined, a Herbert-Pirt equation can be written for each compound.

$$q_s = \left(\frac{1.263}{7.62}\right)\mu + 8.15\times10^{-4}\ \left[\frac{mol\ C_s}{C-mol\ C_x\cdot h}\right]$$

$$q_{O_2} = \left(\frac{15.997}{7.62}\right)\mu + 0.0154 \ \left[\frac{mol\ O_2}{C-mol\ C_x \cdot h}\right]$$

$$q_{CO_2} = \left(\frac{10.062}{7.62}\right)\mu + 0.0114 \ \left[\frac{mol\ CO_2}{C-mol\ C_x \cdot h}\right]$$

$$q_{H_2O} = \left(\frac{10.587}{7.62}\right)\mu + 0.0098 \ \left[\frac{mol\ H_2O}{C-mol\ C_x \cdot h}\right]$$

$$q_{NH_3} = \left(\frac{1.524}{7.62}\right)\mu \ \left[\frac{mol\ NH_3}{C-mol\ C_x \cdot h}\right]$$

Based on these results an estimated stoichiometric coefficient of each compound in biodegradation of dicyclohexyl acetic acid at different growth rate can be determined. Followed, simulation of biodegradation of dicyclohexyl acetic acid based on derived Herbert-Pirt equation is discussed.

5.5.3 Simulation of the stoichiometric and kinetics parameters

Once kinetic and stoichiometric parameters of biodegradation of given compound is available, A simulation of process can be done. Here, graphs of changes in stoichiometric parameters of the process at different growth rates are presented.

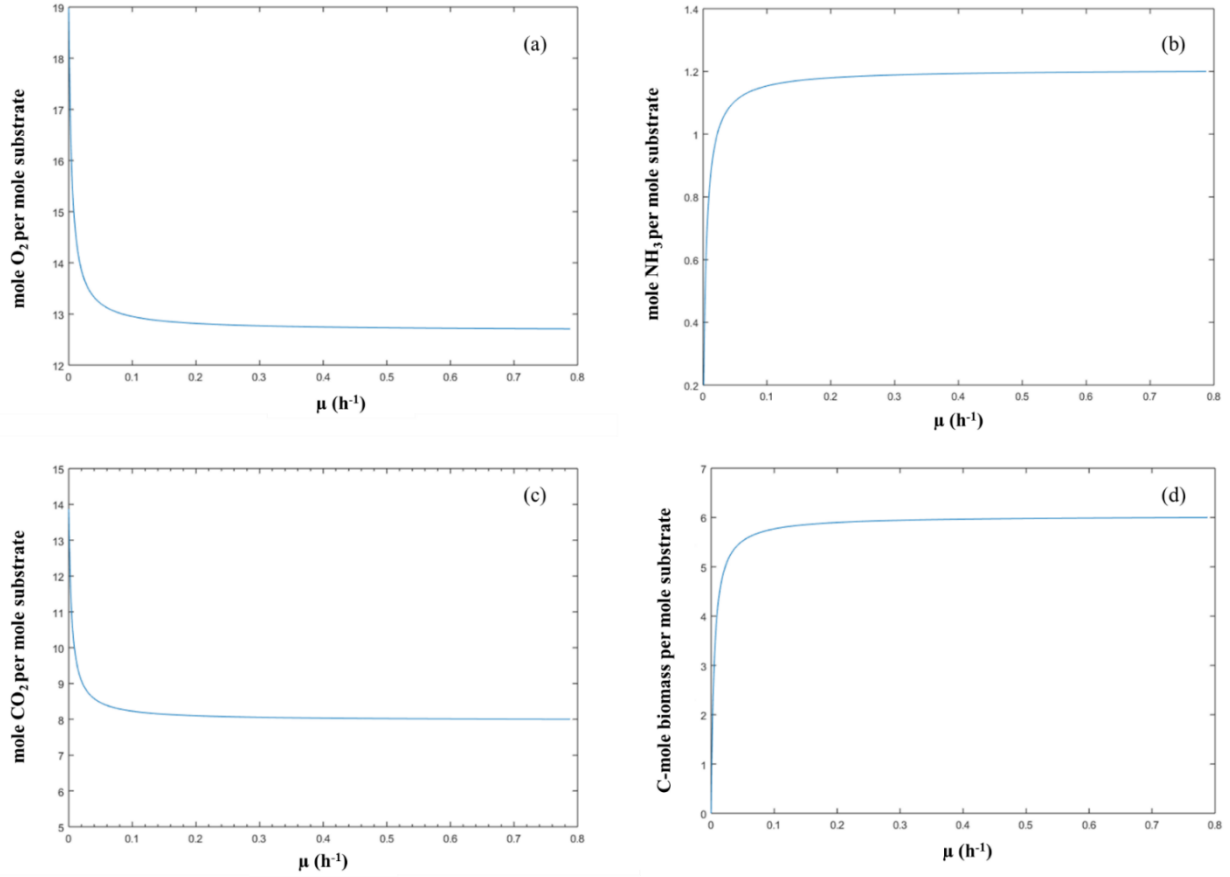


Figure 5-9. Yield of each compound respect to the specific growth rate (a), (b), (c) and (d) are yield substrateto oxygen, ammonium, carbon dioxoid and biomass, respectively

In graph 5-9(a), y-axis shows the mole of oxygen consumed per unit of biomass specific consumption rate. This axis represents the amount of required oxygen per unit of consumed substrate. The x-axis represents the biomass specific growth rate. For instance, environmental engineering would want a fast and efficient treatment. Therefore, with the faster a q_s (rate of consumption of substrate per unit of biomass), the more efficient systems produced. And graph (a) in Fig. 5.9 illustrates that when μ is more than 0.2 h^{-1}, 13 moles of oxygen are needed for consumption of one mole of substrate; however, at specific growth rate ~0.02, this ratio is 15. At the first glance, the difference between 15 mole and 13 moles of oxygen is not very big. However, it should be realized that this small difference can be very significant in long runs and huge volumes. The same explanation can be done on other parameters i.e. CO_2 and NH_3. Accordingly, relation which are provided by thermodynamics can help us in prediction and optimization of the process. Following section discusses the *in silico* biodegradation of dicyclohexyl acetic acid and comparison of results with experimental data.

5.5.3.1 Estimation of Substrate Affinity

In general there is no thermodynamic approach for prediction of substrate affinity. However, one of the logical approaches for estimation of substrate affinity for low soluble compounds is using their solubility (Colin Ratledge, 2006; Smolke, 2009). It means that solubility can be directly considered as the substrate affinity. Therefore, solubility of dicyclohlohexyl acetic acid estimated as 0.724 mM at 25 degree of C and pH=7 (Hou et al., 2004b; Shoghi et al., 2013).

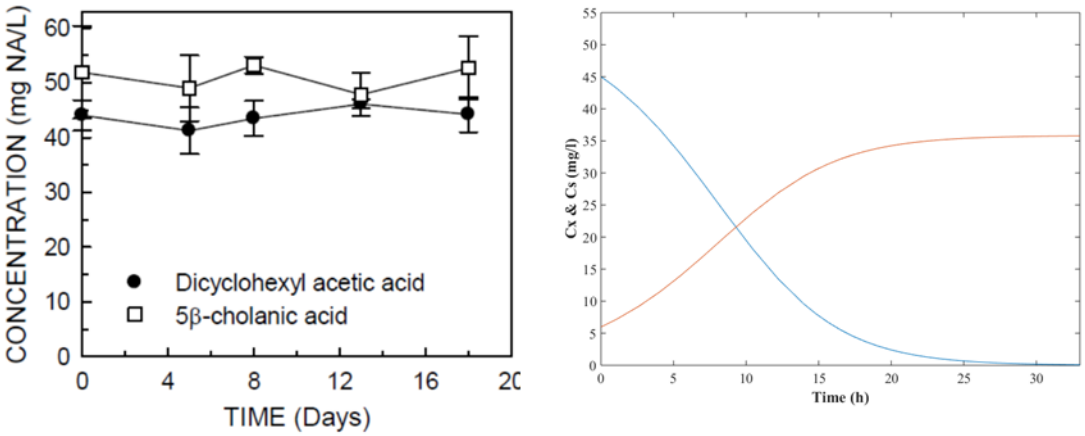


Figure 5-10. Comparison of experimental and *in silico* biodegradation of dicyclohlohexyl acetic acid with the same environmental condition. (Graph on the right reprinted with permission from Misiti et. al, (2014))

Results from this simulation indicate complete mineralization of dicyclohlohexyl acetic acid is possible. Efforts for biodegradation of dicyclohlohexyl acetic acid with wild type microorganisms have failed to succeed, however, thermodynamic analysis or feasibility studies have shown engineered pathways may result in complete removal of this recalcitrant compound.

However, one should be noted that thermodynamics study does not ensure biodegradation. For application of proposed pathway more detailed analysis i.e. metabolic flux analysis (MFA) based on similar experimental data or thermodynamic MFA should be performed to figure out about bottlenecks of a pathway under study. It means that for practical purposes a pathway should biologically and thermodynamically debottleneck.

5.6 Conclusion

In the world of NAs treatment, there has been a successful advancement in molecular, chemical, and kinetic analysis for either model NAs, commercially produced or OSPW associated NAs. As well, kinetic and stoichiometric parameters for bioremediation of NAs under different physiological condition has been determined. Partial biodegradability of commercial, tailings associated and various model NAs have been proved.

Computational tools to predict novel biodegradation pathways of target compounds allow us to investigate the power of microorganisms to consume pollutants as their source of carbon and energy (Darvas, Gómez et al., 2007; Greene et al., 1999; Henry et al., 2006; Jaworska et al., 2002; Klopman et al., 1994; Rodrigo et al., 2008). Therefore, these computational tools i.e. UM-BBD gives us the opportunity to explore the possible metabolic pathways for biodegradation of model NAs.

Results from *in silico* investigation of NAs biodegradation illustrate that by implementing genetic and metabolic engineering tools, it is possible to design a cell factory to degrade NAs. For bioremediation purposes, design of catabolic pathways is the main interest (Finley et al., 2009a; Finley et al., 2009b; Hatzimanikatis et al., 2005). Through a thermodynamic favorable catabolic pathway with non-toxic (end-) products, complete mineralization of a pollutant is achievable (Alexander, 1999; Finley et al., 2009b; de la Peña Mattozzi et al., 2006; Walker and Keasling, 2002). However, thermodynamic favourability can be manipulated through genetic manipulation (i.e. expression of specific gene for production of higher protein respect wild type) (Henry et al., 2006; Henry et al., 2007; Smolke, 2009).

In general, due to author's knowledge, systems biology approaches have not been conducted for the case of NAs biodegradation. Successful investigation of systems biology aspects of NAs bioremediation may result in development of more capable microbial cultures for consumption of NAs. Moreover, establishment of microbial consortium is another advantages of systems and synthetic biology field. Synergies among microbes can be seen as an asset in improving the mineralization of organic pollutants. These interactions may result in detoxification of some metabolites (Gilbert et al., 2003; Mukherji and van Oudenaarden, 2009).

5.7 References

Alberty RA. 1998. Calculation of standard transformed formation properties of biochemical reactants and standard apparent reduction potentials of half reactions. *Arch. Biochem. Biophys.* **358**:25–39.

Alexander M. 1985. Biodegradation of organic chemicals. *Environ. Sci. Technol.* **19**:106–111.

Alexander M. 1999. Biodegradation and Bioremediation. Gulf Professional Publishing 453 p.

Beeftink HH, Heijden RTJM, Heijnen JJ. 1990. Maintenance requirements: energy supply from simultaneous endogenous respiration and substrate consumption. *FEMS Microbiol. Lett.* **73**:203–209.

Boopathy R. 2000. Factors limiting bioremediation technologies. *Bioresour. Technol.* **74**:63–67.

Chauhan G, Stein M, Seidel-Morgenstern A, Pant KK, Nigam KDP. 2015. The thermodynamics and biodegradability of chelating agents upon metal extraction. *Chem. Eng. Sci.* **137**:768–785.

Colin Ratledge BK. 2006. Basic Biotechnology. Cambridge University Press 666 p.

Cueto-Rojas HF, van Maris AJA, Wahl SA, Heijnen JJ. 2015. Thermodynamics-based design of microbial cell factories for anaerobic product formation. *Trends Biotechnol.* **33**:534–46.

Darvas F. MetabolExpert, an expert system for predicting metabolism of substances. *QSAR Environ. Toxicol.*

Demirel Y. 2014a. Nonequilibrium Thermodynamics. *Nonequilibrium Thermodyn.* Elsevier 1-74 p.

Demirel Y. 2014b. Nonequilibrium Thermodynamics. *Nonequilibrium Thermodyn.* Elsevier 752-758 p.

Dolfing J, Larter SR, Head IM. 2008. Thermodynamic constraints on methanogenic crude oil biodegradation. *ISME J.* **2**:442–52.

Dolfing J, Xu A, Gray ND, Larter SR, Head IM. 2009. The thermodynamic landscape of

methanogenic PAH degradation. *Microb. Biotechnol.* **2**:566–74.

Dong F, Nemati M. 2016. Anoxic biodegradation of a surrogate naphthenic acid coupled to reduction of nitrite. *Biochem. Eng. J.*

Dua M, Singh A, Sethunathan N, Johri AK. 2002. Biotechnology and bioremediation: successes and limitations. *Appl. Microbiol. Biotechnol.* **59**:143–52.

Ellis LBM, Roe D, Wackett LP. 2006. The University of Minnesota Biocatalysis/Biodegradation Database: the first decade. *Nucleic acids Res.* **34**.

Erickson LE, Davis LC, Narayanan M. 1995. Bioenergetics and bioremediation of contaminated soil. *Thermochim. Acta* **250**:353–358.

Feist AM, Henry CS, Reed JL, Krummenacker M, Joyce AR, Karp PD, Broadbelt LJ, Hatzimanikatis V, Palsson BØ. 2007. A genome-scale metabolic reconstruction for Escherichia coli K-12 MG1655 that accounts for 1260 ORFs and thermodynamic information. *Mol. Syst. Biol.* **3**:121.

Finley SD, Broadbelt LJ, Hatzimanikatis V. 2009a. Thermodynamic analysis of biodegradation pathways. *Biotechnol. Bioeng.* **103**:532–41.

Finley SD, Broadbelt LJ, Hatzimanikatis V. 2009b. Computational framework for predictive biodegradation. *Biotechnol. Bioeng.* **104**:1086–97.

Finley SD, Broadbelt LJ, Hatzimanikatis V. 2010. In silico feasibility of novel biodegradation pathways for 1,2,4-trichlorobenzene. *BMC Syst. Biol.* **4**:7.

Flickinger MC. 2009. Encyclopedia of Industrial Biotechnology. Hoboken, NJ, USA: John Wiley & Sons, Inc.

Gavrilescu M, Demnerová K, Aamand J, Agathos S, Fava F. 2015. Emerging pollutants in the environment: present and future challenges in biomonitoring, ecological risks and bioremediation. *N. Biotechnol.* **32**:147–56.

Gilbert ES, Walker AW, Keasling JD. 2003. A constructed microbial consortium for biodegradation of the organophosphorus insecticide parathion. *Appl. Microbiol. Biotechnol.*

61:77–81.

Gómez MJ, Pazos F, Guijarro FJ, de Lorenzo V, Valencia A. 2007. The environmental fate of organic pollutants through the global microbial metabolism. *Mol. Syst. Biol.* **3**:114.

Greene N, Judson PN, Langowski JJ, Marchant CA. 1999. Knowledge-based expert systems for toxicity and metabolism prediction: DEREK, StAR and METEOR. *SAR QSAR Environ. Res.* **10**:299–314.

Groot SR De, Mazur P. 1962. Non-Equilibrium Thermodynamics. Courier Corporation 528 p.

Hatzimanikatis V, Li C, Ionita JA, Henry CS, Jankowski MD, Broadbelt LJ. 2005. Exploring the diversity of complex metabolic networks. *Bioinformatics* **21**:1603–9.

Heijnen JJ. 2010. Impact of thermodynamic principles in systems biology. *Adv. Biochem. Eng. Biotechnol.* **121**:139–62.

Heijnen JJ. 1991. A new thermodynamically based correlation of chemotrophic biomass yields. *Antonie Van Leeuwenhoek* **60**:235–256.

Heijnen JJ, Van Dijken JP. 1992. In search of thermodynamic description of biomass yields for the chemotrophic growth of microorganisms. *Biotechnol. Bioeng.*

Henry CS, Broadbelt LJ, Hatzimanikatis V. 2007. Thermodynamics-based metabolic flux analysis. *Biophys. J.* **92**:1792–805.

Henry CS, Jankowski MD, Broadbelt LJ, Hatzimanikatis V. 2006. Genome-scale thermodynamic analysis of Escherichia coli metabolism. *Biophys. J.* **90**:1453–61.

Hoijnen JJ, van Loosdrecht MC, Tijhuis L. 1992. A black box mathematical model to calculate auto- and heterotrophic biomass yields based on Gibbs energy dissipation. *Biotechnol. Bioeng.* **40**:1139–54.

Hou BK, Ellis LBM, Wackett LP. 2004a. Encoding microbial metabolic logic: predicting biodegradation. *J. Ind. Microbiol. Biotechnol.* **31**:261–72.

Hou BK, Wackett LP, Ellis LBM. 2003. Microbial pathway prediction: a functional group

approach. *J. Chem. Inf. Comput. Sci.* **43**:1051–7.

Hou TJ, Xia K, Zhang W, Xu XJ. 2004b. ADME evaluation in drug discovery. 4. Prediction of aqueous solubility based on atom contribution approach. *J. Chem. Inf. Comput. Sci.* **44**:266–75.

Huang J, Nemati M, Hill G, Headley J. 2012. Batch and continuous biodegradation of three model naphthenic acids in a circulating packed-bed bioreactor. *J. Hazard. Mater.* **201-202**:132–40.

Jankowski MD, Henry CS, Broadbelt LJ, Hatzimanikatis V. 2008. Group contribution method for thermodynamic analysis of complex metabolic networks. *Biophys. J.* **95**:1487–99.

Jaworska J, Dimitrov S, Nikolova N, Mekenyan O. 2002. Probabilistic assessment of biodegradability based on metabolic pathways: catabol system. *SAR QSAR Environ. Res.* **13**:307–323.

Klopman G, Dimayuga M, Talafous J. 1994. META. 1. A program for the evaluation of metabolic transformation of chemicals. *J. Chem. Inf. Comput. Sci.* **34**:1320–1325.

Kovar K, Chaloupka V, Egli T. 2002. A Threshold Substrate Concentration Is Required to Initiate the Degradation of 3-Phenylpropionic Acid in Escherichia coli. *Acta Biotechnol.* **22**:285–298.

Kovarova-Kovar K, Egli T. 1998. Growth Kinetics of Suspended Microbial Cells: From Single-Substrate-Controlled Growth to Mixed-Substrate Kinetics. *Microbiol. Mol. Biol. Rev.* **62**:646–666.

Kristiansen CRB. 1999, eds. *Basic biotechnology*. Cambridge University Press

de la Peña Mattozzi M, Tehara SK, Hong T, Keasling JD. 2006. Mineralization of paraoxon and its use as a sole C and P source by a rationally designed catabolic pathway in Pseudomonas putida. *Appl. Environ. Microbiol.* **72**:6699–706.

Lehninger AL, Nelson DL, Cox MM. 2005. Lehninger Principles of Biochemistry. W. H. Freeman 1119 p.

Mavrovouniotis ML. 1990. Group contributions for estimating standard gibbs energies of formation of biochemical compounds in aqueous solution. *Biotechnol. Bioeng.* **36**:1070–82.

Mavrovouniotis ML. 1991. Estimation of standard Gibbs energy changes of biotransformations. *J. Biol. Chem.* **266**:14440–14445.

Meckenstock RU, Elsner M, Griebler C, Lueders T, Stumpp C, Aamand J, Agathos SN, Albrechtsen H-J, Bastiaens L, Bjerg PL, Boon N, Dejonghe W, Huang WE, Schmidt SI, Smolders E, Sørensen SR, Springael D, van Breukelen BM. 2015. Biodegradation: Updating the concepts of control for microbial cleanup in contaminated aquifers. *Environ. Sci. Technol.* **49**:7073–81.

Misiti T, Tezel U, Pavlostathis S. 2014. Effect of alkyl side chain location and cyclicity on the aerobic biotransformation of naphthenic acids. *Environ. Sci. ...*.

Mukherji S, van Oudenaarden A. 2009. Synthetic biology: understanding biological design from synthetic circuits. *Nat. Rev. Genet.* **10**:859–71.

Oller I, Polo-López I, Miralles-Cuevas S, Fernández-Ibáñez P, Malato S. 2014. Advanced Technologies for Emerging Contaminants Removal in Urban Wastewater:1–25.

Onsager L. 1931. Reciprocal Relations in Irreversible Processes. I. *Phys. Rev.* **37**:405–426.

Paslawski JC, Headley J V, Hill GA, Nemati M. 2009. Biodegradation kinetics of trans-4-methyl-1-cyclohexane carboxylic acid. *Biodegradation* **20**:125–33.

Paul D, Pandey G, Pandey J, Jain RK. 2005. Accessing microbial diversity for bioremediation and environmental restoration. *Trends Biotechnol.* **23**:135–42.

Petrie B, Barden R, Kasprzyk-Hordern B. 2014. A review on emerging contaminants in wastewaters and the environment: Current knowledge, understudied areas and recommendations for future monitoring. *Water Res.* **72**:3–27.

Pirt SJ. 1965. The Maintenance Energy of Bacteria in Growing Cultures. *Proc. R. Soc. B Biol. Sci.* **163**:224–231.

Prigogine I. 1968. Introduction to thermodynamics of irreversible processes. Interscience

Publishers 147 p.

Reddy KR, DeLaune RD. 2008. Biogeochemistry of Wetlands: Science and Applications. CRC Press 800 p.

Rittmann B, McCarty P. 2012. Environmental biotechnology: principles and applications.

Rodrigo G, Carrera J, Prather KJ, Jaramillo A. 2008. DESHARKY: automatic design of metabolic pathways for optimal cell growth. *Bioinformatics* **24**:2554–6.

Roels JA. 1983. Energetics and kinetics in biotechnology.

Russell JB, Cook GM. 1995. Energetics of bacterial growth: balance of anabolic and catabolic reactions. *Microbiol. Rev.* **59**:48–62.

S. Roy Caplan AE. 1983. Bioenergetics and Linear Nonequilibrium Thermodynamics. *Harvard Univ. Press*. http://www.hup.harvard.edu/catalog.php?isbn=9780674732063.

Shoghi E, Fuguet E, Bosch E, Ràfols C. 2013. Solubility-pH profiles of some acidic, basic and amphoteric drugs. *Eur. J. Pharm. Sci.* **48**:291–300.

Smith JM, Ness HC Van, Abbott MM. 2001. Introduction to Chemical Engineering Thermodynamics. McGraw-Hill 789 p.

Smolke C. 2009. The Metabolic Pathway Engineering Handbook: Fundamentals. CRC Press 678 p.

Stephanopoulos G, Aristidou AA, Nielsen J. 1998. Metabolic Engineering: Principles and Methodologies. Academic Press 725 p.

von Stockar U, Maskow T, Liu J, Marison IW, Patiño R. 2006. Thermodynamics of microbial growth and metabolism: an analysis of the current situation. *J. Biotechnol.* **121**:517–33.

Tchobanoglous G, Burton FL, Stensel HD, Eddy M&. 2003. Wastewater Engineering: Treatment and Reuse. McGraw-Hill Education 1819 p.

Tijhuis L, Van Loosdrecht MCM, Heijnen JJ. 1993. A thermodynamically based correlation for maintenance Gibbs energy requirements in aerobic and anaerobic chemotrophic growth.

Biotechnol. Bioeng. **42**:509–519.

Urs von Stockar LAM van der W. 2013. Biothermodynamics: The Role of Thermodynamics in Biochemical Engineering. https://www.crcpress.com/Biothermodynamics-The-Role-of-Thermodynamics-in-Biochemical-Engineering/Stockar-van-der-Wielen/9781466582163.

Van Loosdrecht M, Henze M. 1999. Maintenance, endogeneous respiration, lysis, decay and predation. *Water Sci. Technol.* **39**:107–117.

Wackett LP, Ellis LB. 1999. Predicting biodegradation. *Environ. Microbiol.* **1**:119–24.

Walker AW, Keasling JD. 2002. Metabolic engineering of Pseudomonas putida for the utilization of parathion as a carbon and energy source. *Biotechnol. Bioeng.* **78**:715–21.

Welch GR. 1985. Some problems in the usage of gibbs free energy in biochemistry. *J. Theor. Biol.* **114**:433–446.

Westerhoff H V., Dam K van. 1987. Thermodynamics and control of biological free-energy transduction. Elsevier 568 p.

Westerhoff HV. 1983. Mosaic Non-equilibrium Thermodynamics and (the Control Of) Biological Free- Energy Transduction. Drukkerij Gerja 368 p.

Wylen GJ Van, Sonntag RE, Borgnakke C. 1994. Fundamentals of Classical Thermodynamics, Volume 1. Wiley 852 p.

Zotin AI. 1990. Thermodynamic Bases of Biological Processes: Physiological Reactions and Adaptations. Walter de Gruyter 293 p.

CHAPTER 6. CONCLUSIONS AND RECOMMENDATIONS

6.1 Thesis Overview

Devastating effects of oil sands process affected water (OSPW) and tailings ponds on the environment were the main driving forces of this research. There is no doubt OSPW in tailings ponds needs to be remediated. Efforts are still striving to achieve an efficient, and practical strategy to remediate OSPW. To accomplish this goal, many research teams are focusing on varying methods to determine a proven practical treatment strategy.

6.2 Summary of Findings

In 2013, inactivated sodium persulfate and potassium permanganate used for the first time for removal of naphthenic acids (NAs) in OSPW. Results showed the reactivity of persulfate and permanganate toward NAs. A challenge the obtained results from chemical oxidation experiments has is a lack of stoichiometric information in the process. Stoichiometric parameters are among the main parameters that have critical role in design, application and optimization of the oxidation process. In chapter 3 of this thesis, a stoichiometrically-based design was performed to estimate the required amount of persulfate for treatment purposes. The main factor in the proposed design was the amount of available electrons in the wastewater. The amount of available electrons was determined by a chemical oxygen demand measurement. Then, for the determination of required oxidant, the degree of reduction of oxidant was considered to calculate the theoretical oxidant dosage. Results showed the model successfully demonstrated outcomes which resulted in degradation of NAs. Summaries of findings described below:

- Complete understanding of detailed chemistry behind NAs oxidation is not necessarily needed for its application.

- More than 90% degradation in the NAs concentration achieved in 28 days with less than 20% of needed persulfate from the previously published data.
- Case study for treatment of MLSB OSPW demonstrates we can save 110,000 tonnes of persulfate, which equates to a 290 million dollars saving.
- Due to huge volume of OSPW in tailings ponds, when oxidation with a chemical oxidant is of interest, results from lab scale experiments need to be gone through optimzation processes.
- Understanding the stoichiometry of oxidation reactions can significantly improve the remediation process based on chemical oxidation.

After successful degradation of NAs by persulfate, the idea of coupling chemical and biological oxidation was considered as the next step. In chapter 4 of this work the effect of persulfate on ability of microorganisms in removal of NAs is discussed. Results are summarized below:

- Stress test affected the maximum concentration of biomass. By increasing the concentration of oxidant, the maximum concentration of *Pseudomonas* sp. is decreased. At the dosage of 2000 mg/L, no growth was observed.
- In spite the high oxidative stress, the bacteria could still consume Merichem NAs as the sole source of carbon and energy.
- Quantitative physiology applied to compare the effect of oxidative stress on biodegradation. Biomass, specific growth rate, yield of substrate to biomass, and biomass specific substrate consumption rate, were not significantly different among different series.
- The bacteria that are stressed with 1000 mg/L of sodium persulfate had a 15.5 hr lag time, while other series had almost the same lag time.

- Chemical oxidation with sodium persulfate can couple with biological oxidation.

In chapter 6 of this work, thermodynamics of NAs biodegradation was investigated for the first time as fundamental step in this field. Power of thermodynamics in prediction of unknown

processes has been proved before. For instance, in 1976 Broda published a paper entitle of "two lithotrophs are missing in nature" (Broda, 1976)[1]. He predicted based on thermodynamics calculation that ammonia can be anaerobically oxidized to nitrogen gas by using nitrite as the electron acceptor by microorganisms. Years later, in 1999, Strous and coworkers published a paper in nature entitle "missing lithotrophs identified as new planctomycetes" (Strous et al., 1999)[2]. This was one of the examples among so many other findings based on a thermodynamic understanding. Some important points of thermodynamics founding are:

- Thermodynamics feasibility study should be conducted as the first step in attempt to biodegrade recalcitrant compounds.
- Experimental results suggest a non-biodegradability of dicyclohexyl acetic acid, however, thermodynamics predict the complete mineralization of this compound under aerobic condition
- Catabolic pathways are the most important process in biodegradability of model NAs.
- Improvement of a strain of a wild type microorganism for degradation of a model NAs would result in enhancement of biodegradation of other model NAs. This is because almost all of classical NAs have same Gibbs energy of formation (~ 107 kJ per electron mole).
- Predicted stoichiometric and kinetics parameters i.e. yield of substrate to biomass, amount if needed nitrogen source, amount of needed oxygen, based on thermodynamics can be used in design and optimization of biodegradation processes.

1.Broda E. 1976. Two kinds of lithotrophs missing in nature. *Z. Allg. Mikrobiol.* **17**:491–493.

2.Strous M, Fuerst JA, Kramer EH, Logemann S, Muyzer G, van de Pas-Schoonen KT, Webb R, Kuenen JG, Jetten MS. 1999. Missing lithotroph identified as new planctomycete. *Nature* **400**:446–9.

6.3 Significance of the Research

In the chapters of this thesis, have tried to address the removal of NAs with different perspectives. For chemical oxidation (chapter 3), a simple model based on the available electrons in OSPW has been proposed to determine the needed amount of oxidant. Results have shown the model's results predict effective concentrations. This model has attempted to estimate the desired parameter (amount of required oxidant) with less available information. The model would be a good tool at industrial level. In chapter 4, design parameters that are required in design of a bioprocess is determined. Findings represent feasible coupling of chemical and biological oxidation by using sodium persulfate as the oxidant. Theoretical analyses based on the thermodynamics behind biodegradation of NAs can build up a new road with the expectation of biodegradation of previously believed non-biodegradable NAs.

6.4 Recommendations for Future Work

1. Simultaneous application of persulfate and biological oxidation

It is interesting to see the simultaneous chemical oxidation (by sodium persulfate) and biological oxidation of NAs. Chemical oxidation and biological oxidation have different rate in degradation of NAs. Differenet parameters can affect each rate. Therefore, rate-based modelling can be used with the aim of optimization of the process.

2. Thermodynamics metabolic flux analysis (TMFA)

Application of systems biology tools like TMFA can greatly impact the world of NAs biodegradation. These tools allow one to investigate the design of engineered microorganisms with more detailed perspective. By TMFA optimized flux of interacellular (and also extracelluar) metabolites can be determined. Results can help further improvmnets of microorganisms through gene manipulation for production of higher/lower determined enzymes.

3. Estimation of the threshold concentration of naphthenic acids for microorganisms

One of the gaps in the world of NAs biodegradation is lack of the knowledge of threshold concentration. This gap might arise a question that the remaining concentration of NAs in aged oil sands tailings ponds is below the threshold concentration for microorganisms, therefore, they cannot consume it. An experimental approach is proposed here to estimate the threshold concentration of NAs.

For this purpose, experiments to calculate steady state biodegradation rate at different concentration of NAs (highest possible to lowest possible) need to be conducted. Then, the biodegradation rate should be plotted versus its corresponding concentration. By extrapolating the hyperbolic curve to zero rate, where the curve meets x-axis, we can determine the threshold concentration. However, this threshold does not tell us the biological threshold. This threshold might have chemical and physical origin, for instance, it can be mass transfer limitation (diffusion) or dissolution limitation. To overcome this issue, the second part should be performed.

At this step, the same experiments need to be conducted but with highest rate of mixing. Higher mixing rate would eliminate transport phenomena limitations and dissolution limitation. Again, as the same as first part, biodegradation rate is plotted against concentration of substrate. Where, the hyperbolic curve, meets the x-axis which means that at the extrapolated concentration the biodegradation rate is zero. This point is a good approximate of biological threshold. However, determination of a threshold concentration for a specific substrate is very tedious. The proposed idea, however, can be used as a good first approximate.

4. Determination of true non-biodegradable portion of acid extractable organics

It is interesting to perform the biodegradation of OSPW associated NAs in pilot scale (~10 L). The purpose of this experiment is to extract the portion of non-biodegradable portion of organics. After extraction of organics, it should be dissolve in appropriate growth media i.e. Bushnell Hass broth.

In the second experiment lower volume of broth is needed since we want to have the concentration of organics to be higher than the remaining level in (i.e. ~ > 3 times). Tracking biodegradation in the second experiment may give important results. If degradation happens, then we cannot call the remaining portion as the non-biodegradable fraction of organics. It degradation does not happen, the approach can be used for enrichment of mixed culture of microorganisms. The selective strategy through powerful techniques like sequencing batch reactors can be conducted on the recalcitrant fraction of organics to select appropriate culture of microorganisms.

5. Recovery of elemental sulfur from oil sands process affected water

Sulfate is one of the main ions in OSPW and has a concentration of more than 500 mg/L. Sulfate reducing bacteria use sulfate as their electron acceptor and organic carbon as the source of electron donor and carbon source. For example, by considering acetate as the carbon source:

$$CH_3OOH + SO_4^{2-} \rightarrow HS^- + 2HC_3^- + H^+$$

Sulfate reducing bacteria, provide energy for anabolism by reducing sulfate to sulfide ion. Sulfide ion can be oxidized to elemental sulfur in a partial oxidation process by means of sulfur oxidizing bacteria.

$$HS^- + 0.5\ O_2 \rightarrow S^0 \downarrow\ + OH^-$$

The produced sulfur can be used as soil fertiliser or production of sulfuric acids.

6. Biological CO_2 sequestration

GHG emission from oil sands tailings ponds was 62 Mt (1 Mt= 1 million tonne) in 2013 and it was 55 Mt in 2011. Here, a strategy is proposed for biological CO_2 sequestration. Weathering of alkaline silicate minerals is one of the main processes that take place in the natural environment to balance the concentration CO_2 in the atmosphere.

$$2CO_2 + H_2O + CaSiO_3 \rightarrow\ Ca^{2+} + 2\ HCO_3^- + 2SiO_2$$

The above reaction is a slow process in the natural environment. This phenomenon, can be applied in oil sands tailings ponds to avoid emission of carbon dioxide into the atmosphere. These two following reactions are the key reactions for CO_2 sequestration:

$$(Ca/Mg)^{2+}_{(aq)} + CO_2 + 2OH^- \rightarrow (Ca/Mg)CO_{3\,(s)} + H_2O_{(aq)}$$

$$CaSiO_3 + CO_2 \rightarrow CaCO_{3(s)} + SiO_2$$

Providing alkalinity and divalent cations is considered among the main challenges for CO_2 sequestration. Biological activity in tailings ponds can supply alkalinity, proton and needed water for the process. Concentration of divalent cations is relatively low in tailings ponds, for instance in MLSB concentration of calcium and magnesium is 17 and 8 mg/L, respectively. However, alkalinity is relatively high. Mentioned reaction can explain the low concentration of divalent cations in tailings ponds. Therefore, it is interesting to see the effect of addition of calcium and magnesium in the emission rate of carbon dioxide.

7. Estimation of steady state concentration of sulfate radical and hydroxyl radical

Using activated sodium persulfate is very of interest due to it high reactivity and high oxidative power. Production of sulfate radical with persulfate is mainly associated with production of hydroxyl radicals. Generally, half-life of radicals is in the order of nano-seconds. Therefore, they mostly produce by a steady state rate. Measuring of the concentration of radicals in the reaction vessel needs instruments like electron paramagnetic resonance (EPR) or electron spin resonance (ESR). Mentioned instruments can directly measure the radical generation and concentration. However, having high knowledge of chemistry is needed to work with EPR and ESR machines. Here, the simple method is proposed to estimate the steady state concentration of hydroxyl and sulfate radicals. Two organic compounds with known second-order rate constant respect to hydroxyl and sulfate radical should be chosen (can be found in literature). Experimenter can use different strategies for production of radicals. Experiment with each compound needs to be done separately. For each degradation experiment we have observed degradation rate constant for assumed compounds of α and β:

$$k_{obs,\alpha} = k_{1,\alpha} \cdot [HO\cdot]_{ss} + k_{2,\alpha} \cdot [SO_4^-\cdot]_{ss}$$

$$k_{obs,\beta} = k_{1,\beta} \cdot [HO\cdot]_{ss} + k_{2,\beta} \cdot [SO_4^-\cdot]_{ss}$$

It can be seen that we have two equations with two unknown parameters which are steady state (ss) concentration of radicals. By solving two above equations, one can have an understanding about the concentrations of sulfate and hydroxyl radicals in condition of under study.

BIBLIOGRAPHY

Abolfazlzadehdoshanbehbazari M, Birks SJ, Moncur MC, Ulrich AC. 2013. Fate and transport of oil sand process-affected water into the underlying clay till: a field study. *J. Contam. Hydrol.* **151**:83–92.

Alaton IA, Dogruel S, Baykal E, Gerone G. 2004. Combined chemical and biological oxidation of penicillin formulation effluent. *J. Environ. Manage.* **73**:155–63.

Alberty RA. 1998. Calculation of standard transformed formation properties of biochemical reactants and standard apparent reduction potentials of half reactions. *Arch. Biochem. Biophys.* **358**:25–39.

Alexander M. 1985. Biodegradation of organic chemicals. *Environ. Sci. Technol.* **19**:106–111.

Alexander M. 1999. Biodegradation and Bioremediation. Gulf Professional Publishing 453 p.

Allen EW. 2008a. Process water treatment in Canada's oil sands industry: I. Target pollutants and treatment objectives. *J. Environ. Eng. Sci.* **7**:123–138.

Allen EW. 2008b. Process water treatment in Canada's oil sands industry: II. A review of emerging technologies. *J. Environ. Eng. Sci.* **7**:499–524.

Andersson DI, Hughes D. 2014. Microbiological effects of sublethal levels of antibiotics. *Nat. Rev. Microbiol.* **12**:465–78.

Andreozzi R. 1999. Advanced oxidation processes (AOP) for water purification and recovery. *Catal. Today* **53**:51–59.

Andrews JM. 2001. Determination of minimum inhibitory concentrations. *J. Antimicrob. Chemother.* **48**:5–16.

Anfruns A, Gabarró J, Gonzalez-Olmos R, Puig S, Balaguer MD, Colprim J. 2013. Coupling anammox and advanced oxidation-based technologies for mature landfill leachate treatment. *J. Hazard. Mater.* **258-259**:27–34.

Bailey JE. 1986. Biochemical Engineering Fundamentals. McGraw-Hill 984 p.

Bard AJ, Faulkner LR. 2000. Electrochemical Methods: Fundamentals and Applications, 2nd Edition. John Wiley & Sons 856 p.

Beeftink HH, Heijden RTJM, Heijnen JJ. 1990. Maintenance requirements: energy supply from simultaneous endogenous respiration and substrate consumption. *FEMS Microbiol. Lett.* **73**:203–209.

Blakley ER, Papish B. 1982. The metabolism of cyclohexanecarboxylic acid and 3-cyclohexenecarboxylic acid by *Pseudomonas putida*. *Can. J. Microbiol.* **28**:1324–9.

Blakley ER. 1974. The microbial degradation of cyclohexanecarboxylic acid: a pathway involving aromatization to form p hydroxybenzoic acid. *Can. J. Microbiol.*

Boender LGM, de Hulster EAF, van Maris AJA, Daran-Lapujade PAS, Pronk JT. 2009. Quantitative physiology of Saccharomyces cerevisiae at near-zero specific growth rates. *Appl. Environ. Microbiol.* **75**:5607–14.

Boopathy R. 2000. Factors limiting bioremediation technologies. *Bioresour. Technol.* **74**:63–67.

Boudens R, Reid T, VanMensel D, Prakasan M R S, Ciborowski JJH, Weisener CG. 2016. Bio-physicochemical effects of gamma irradiation treatment for naphthenic acids in oil sands fluid fine tailings. *Sci. Total Environ.* **539**:114–24.

Broda E. 1976. Two kinds of lithotrophs missing in nature. *Z. Allg. Mikrobiol.* **17**:491–493.

Brown LD, Pérez-Estrada L, Wang N, El-Din MG, Martin JW, Fedorak PM, Ulrich AC. 2013. Indigenous microbes survive in situ ozonation improving biodegradation of dissolved organic matter in aged oil sands process-affected waters. *Chemosphere* **93**:2748–55.

Brown LD, Ulrich AC. 2015. Oil sands naphthenic acids: a review of properties, measurement, and treatment. *Chemosphere* **127**:276–90.

Cabiscol E, Tamarit J, Ros J. 2000. Oxidative stress in bacteria and protein damage by reactive oxygen species. *Int. Microbiol.*

Camel V, Bermond A. 1998. The use of ozone and associated oxidation processes in drinking water treatment. *Water Res.* **32**:3208–3222.

Cassidy D, Northup A, Hampton D. 2009. The effect of three chemical oxidants on subsequent biodegradation of 2,4-dinitrotoluene (DNT) in batch slurry reactors. *J. Chem. Technol. Biotechnol.* **84**:820–826.

Chakravarti R, Patrick BN, Barney M, Kusinski G, Devine TM. 2013. Toward the Mechanism of

Corrosion in Crude Oil: A Study Using Vibrational Spectroscopic Techniques at Elevated Temperatures. *Energy & Fuels* **27**:7905–7914.

Chapman JS. 2003. Disinfectant resistance mechanisms, cross-resistance, and co-resistance. *Int. Biodeterior. Biodegradation* **51**:271–276.

Chauhan G, Stein M, Seidel-Morgenstern A, Pant KK, Nigam KDP. 2015. The thermodynamics and biodegradability of chelating agents upon metal extraction. *Chem. Eng. Sci.* **137**:768–785.

Chen K-F, Chang Y-C, Chiou W-T. 2015. Remediation of diesel-contaminated soil using in situ chemical oxidation (ISCO) and the effects of common oxidants on the indigenous microbial community: a comparison study. *J. Chem. Technol. Biotechnol.*:n/a–n/a.

Christopher Grant Weisener JJHC. 2013. Gamma Radiation Apparatus for Remediation of Organic Compounds in Waste Slurries.

Chung C-H, Fen S-Y, Yu S-C, Wong H-C. 2015. Influence of oxyR on Growth, Biofilm Formation, and Mobility of Vibrio parahaemolyticus. *Appl. Environ. Microbiol.* **82**:788–96.

Clark DS, Blanch HW. 1995. Biochemical Engineering, Second Edition. CRC Press 702 p.

Clemente JS, Fedorak PM. 2005. A review of the occurrence, analyses, toxicity, and biodegradation of naphthenic acids. *Chemosphere* **60**:585–600.

Clemente JS, Prasad NG., MacKinnon MD, Fedorak PM. 2003. A statistical comparison of naphthenic acids characterized by gas chromatography–mass spectrometry. *Chemosphere* **50**:1265–1274.

Clothier LN, Gieg LM. 2016. Anaerobic biodegradation of surrogate naphthenic acids. *Water Res.* **90**:156–66.

Colin Ratledge BK. 2006. Basic Biotechnology. Cambridge University Press 666 p.

Cornish-Bowden A. 2013. Fundamentals of Enzyme Kinetics. John Wiley & Sons 510 p.

Cruz M-J, De Vooght V, Muñoz X, Hoet PHM, Morell F, Nemery B, Vanoirbeek JAJ. 2009. Assessment of the sensitization potential of persulfate salts used for bleaching hair. *Contact Dermatitis* **60**:85–90.

Cueto-Rojas HF, van Maris AJA, Wahl SA, Heijnen JJ. 2015. Thermodynamics-based design of

microbial cell factories for anaerobic product formation. *Trends Biotechnol.* **33**:534–46.

Darvas F. MetabolExpert, an expert system for predicting metabolism of substances. *QSAR Environ. Toxicol.*

de la Peña Mattozzi M, Tehara SK, Hong T, Keasling JD. 2006. Mineralization of paraoxon and its use as a sole C and P source by a rationally designed catabolic pathway in Pseudomonas putida. *Appl. Environ. Microbiol.* **72**:6699–706.

Del Rio LF, Hadwin AKM, Pinto LJ, MacKinnon MD, Moore MM. 2006. Degradation of naphthenic acids by sediment micro-organisms. *J. Appl. Microbiol.* **101**:1049–61.

Demeter MA, Lemire JA, Yue G, Ceri H, Turner RJ. 2015. Culturing oil sands microbes as mixed species communities enhances ex situ model naphthenic acid degradation. *Front. Microbiol.* **6**:936.

Demirel Y. 2014b. Nonequilibrium Thermodynamics. *Nonequilibrium Thermodyn.* Elsevier 752-758 p.

Demple B. 1991. Regulation of bacterial oxidative stress genes. *Annu. Rev. Genet.* **25**:315–37.

Derungs WA. 1956. Naphthenic Acid Corrosion —An Old Enemy Of the Petroleum Industry ★. *Corrosion* **12**:41–46.

Dolfing J, Larter SR, Head IM. 2008. Thermodynamic constraints on methanogenic crude oil biodegradation. *ISME J.* **2**:442–52.

Dolfing J, Xu A, Gray ND, Larter SR, Head IM. 2009. The thermodynamic landscape of methanogenic PAH degradation. *Microb. Biotechnol.* **2**:566–74.

Dong F, Nemati M. 2016. Anoxic biodegradation of a surrogate naphthenic acid coupled to reduction of nitrite. *Biochem. Eng. J.*

Doran PM. 1995. Bioprocess Engineering Principles. *Bioprocess Eng. Princ.* Elsevier 426-439 p.

Drzewicz P, Perez-Estrada L, Alpatova A, Martin JW, Gamal El-Din M. 2012. Impact of peroxydisulfate in the presence of zero valent iron on the oxidation of cyclohexanoic acid and naphthenic acids from oil sands process-affected water. *Environ. Sci. Technol.* **46**:8984–91.

Dua M, Singh A, Sethunathan N, Johri AK. 2002. Biotechnology and bioremediation: successes and limitations. *Appl. Microbiol. Biotechnol.* **59**:143–52.

Ellis LBM, Roe D, Wackett LP. 2006. The University of Minnesota Biocatalysis/Biodegradation Database: the first decade. *Nucleic acids Res.* **34**.

Ellis TG, Barbeau DS, Smets BF, Grady CPL. 1996. Respirometric technique for determination of extant kinetic parameters describing biodegradation. *Water Environ. Res.* **68**:917–926.

Elmén J, Pan W, Leung SY, Magyarosy A, Keasling JD. 1997. Kinetics of toluene degradation by a nitrate-reducing bacterium isolated from a groundwater aquifer. *Biotechnol. Bioeng.* **55**:82–90.

Erickson LE, Davis LC, Narayanan M. 1995. Bioenergetics and bioremediation of contaminated soil. *Thermochim. Acta* **250**:353–358.

Esteves BM, Rodrigues CSD, Boaventura RAR, Maldonado-Hódar FJ, Madeira LM. 2016. Coupling of acrylic dyeing wastewater treatment by heterogeneous Fenton oxidation in a continuous stirred tank reactor with biological degradation in a sequential batch reactor. *J. Environ. Manage.* **166**:193–203.

Farr SB, Kogoma T. 1991. Oxidative stress responses in Escherichia coli and Salmonella typhimurium. *Microbiol. Mol. Biol. Rev.* **55**:561–585.

Feist AM, Henry CS, Reed JL, Krummenacker M, Joyce AR, Karp PD, Broadbelt LJ, Hatzimanikatis V, Palsson BØ. 2007. A genome-scale metabolic reconstruction for Escherichia coli K-12 MG1655 that accounts for 1260 ORFs and thermodynamic information. *Mol. Syst. Biol.* **3**:121.

Finley SD, Broadbelt LJ, Hatzimanikatis V. 2009. Thermodynamic analysis of biodegradation pathways. *Biotechnol. Bioeng.* **103**:532–41.

Finley SD, Broadbelt LJ, Hatzimanikatis V. 2009a. Computational framework for predictive biodegradation. *Biotechnol. Bioeng.* **104**:1086–97.

Finley SD, Broadbelt LJ, Hatzimanikatis V. 2010. In silico feasibility of novel biodegradation pathways for 1,2,4-trichlorobenzene. *BMC Syst. Biol.* **4**:7.

Flickinger MC. 2009. Encyclopedia of Industrial Biotechnology. Hoboken, NJ, USA: John Wiley & Sons, Inc.

Frank RA, Fischer K, Kavanagh R, Burnison BK, Arsenault G, Headley J V., Peru KM, Kraak G Van Der, Solomon KR. 2009. Effect of Carboxylic Acid Content on the Acute Toxicity of Oil Sands Naphthenic Acids. *Environ. Sci. Technol.* **43**:266–271.

Frank RA, Sanderson H, Kavanagh R, Burnison BK, Headley J V, Solomon KR. 2010. Use of a (quantitative) structure-activity relationship [(Q)SAR] model to predict the toxicity of naphthenic acids. *J. Toxicol. Environ. Health. A* **73**:319–29.

Gambino M, Cappitelli F. 2016. Mini-review: Biofilm responses to oxidative stress. *Biofouling* **32**:167–78.

Garcia-Garcia E, Ge JQ, Oladiran A, Montgomery B, El-Din MG, Perez-Estrada LC, Stafford JL, Martin JW, Belosevic M. 2011. Ozone treatment ameliorates oil sands process water toxicity to the mammalian immune system. *Water Res.* **45**:5849–57.

Gavrilescu M, Demnerová K, Aamand J, Agathos S, Fava F. 2015. Emerging pollutants in the environment: present and future challenges in biomonitoring, ecological risks and bioremediation. *N. Biotechnol.* **32**:147–56.

Ghanizadeh G, Naseri Ara A, Esmaili D, Masoumbeigi H. 2015. Demonstration of the Enhanced Disinfection of E. coli Water Contamination by Associated Solar Irradiation with Potassium Persulfate. *Iran. J. Public Health* **44**:1376–86.

Giesy JP, Anderson JC, Wiseman SB. 2010. Alberta oil sands development. *Proc. Natl. Acad. Sci. U. S. A.* **107**:951–2.

Gilbert ES, Walker AW, Keasling JD. 2003. A constructed microbial consortium for biodegradation of the organophosphorus insecticide parathion. *Appl. Microbiol. Biotechnol.* **61**:77–81.

Gómez MJ, Pazos F, Guijarro FJ, de Lorenzo V, Valencia A. 2007. The environmental fate of organic pollutants through the global microbial metabolism. *Mol. Syst. Biol.* **3**:114.

Government of Alberta. 2010. Tailings.

Government of Alberta. 2016. Oil sands quarterly. Open Text Web Site Management.

Greene N, Judson PN, Langowski JJ, Marchant CA. 1999. Knowledge-based expert systems for toxicity and metabolism prediction: DEREK, StAR and METEOR. *SAR QSAR Environ. Res.* **10**:299–314.

Grewer DM, Young RF, Whittal RM, Fedorak PM. 2010. Naphthenic acids and other acid-extractables in water samples from Alberta: what is being measured? *Sci. Total Environ.* **408**:5997–6010.

Groot SR De, Mazur P. 1962. Non-Equilibrium Thermodynamics. Courier Corporation 528 p.

Guieysse B, Norvill ZN. 2014. Sequential chemical-biological processes for the treatment of industrial wastewaters: review of recent progresses and critical assessment. *J. Hazard. Mater.* **267**:142–52.

Gunawan Y, Nemati M, Dalai A. 2014. Biodegradation of a surrogate naphthenic acid under denitrifying conditions. *Water Res.* **51**:11–24.

Gupta A, Rao G. 2003. A study of oxygen transfer in shake flasks using a non-invasive oxygen sensor. *Biotechnol. Bioeng.* **84**:351–8.

Guzel-Seydim ZB, Greene AK, Seydim AC. 2004. Use of ozone in the food industry. *LWT - Food Sci. Technol.* **37**:453–460.

Hack N, Reinwand C, Abbt-Braun G, Horn H, Frimmel FH. 2015. Biodegradation of phenol, salicylic acid, benzenesulfonic acid, and iomeprol by Pseudomonas fluorescens in the capillary fringe. *J. Contam. Hydrol.* **183**:40–54.

Halliwell B, Gutteridge JMC. 1985. Free radicals in biology and medicine.

Hammes F, Vital M, Egli T. 2010. Critical evaluation of the volumetric "bottle effect" on microbial batch growth. *Appl. Environ. Microbiol.* **76**:1278–81.

Han X, Scott AC, Fedorak PM, Bataineh M, Martin JW. 2008. Influence of Molecular Structure on the Biodegradability of Naphthenic Acids. Environ. Sci. Technol. 42:1290–1295.

Harding HP, Zhang Y, Zeng H, Novoa I, Lu PD, Calfon M, Sadri N, Yun C, Popko B, Paules R, Stojdl DF, Bell JC, Hettmann T, Leiden JM, Ron D. 2003. An Integrated Stress Response Regulates Amino Acid Metabolism and Resistance to Oxidative Stress. *Mol. Cell* **11**:619–633.

Hatzimanikatis V, Li C, Ionita JA, Henry CS, Jankowski MD, Broadbelt LJ. 2005. Exploring the diversity of complex metabolic networks. *Bioinformatics* **21**:1603–9.

He Y, Patterson S, Wang N, Hecker M, Martin JW, El-Din MG, Giesy JP, Wiseman SB. 2012. Toxicity of untreated and ozone-treated oil sands process-affected water (OSPW) to early life stages of the fathead minnow (Pimephales promelas). *Water Res.* **46**:6359–68.

Headley J V, Peru KM, Barrow MP, Derrick PJ. 2007. Characterization of naphthenic acids from athabasca oil sands using electrospray ionization: the significant influence of solvents. *Anal. Chem.* **79**:6222–9.

Headley J V, Peru KM, Mohamed MH, Frank RA, Martin JW, Hazewinkel RRO, Humphries D, Gurprasad NP, Hewitt LM, Muir DCG, Lindeman D, Strub R, Young RF, Grewer DM, Whittal RM, Fedorak PM, Birkholz DA, Hindle R, Reisdorph R, Wang X, Kasperski KL, Hamilton C, Woudneh M, Wang G, Loescher B, Farwell A, Dixon DG, Ross M, Pereira ADS, King E, Barrow MP, Fahlman B, Bailey J, McMartin DW, Borchers CH, Ryan CH, Toor NS, Gillis HM, Zuin L, Bickerton G, Mcmaster M, Sverko E, Shang D, Wilson LD, Wrona FJ. 2013. Chemical fingerprinting of naphthenic acids and oil sands process waters-A review of analytical methods for environmental samples. *J. Environ. Sci. Health. A. Tox. Hazard. Subst. Environ. Eng.* **48**:1145–63.

Headley J V, Peru KM, Mohamed MH, Frank RA, Martin JW, Hazewinkel RRO, Humphries D, Gurprasad NP, Hewitt LM, Muir DCG, Lindeman D, Strub R, Young RF, Grewer DM, Whittal RM, Fedorak PM, Birkholz DA, Hindle R, Reisdorph R, Wang X, Kasperski KL, Hamilton C, Woudneh M, Wang G, Loescher B, Farwell A, Dixon DG, Ross M, Pereira ADS, King E, Barrow MP, Fahlman B, Bailey J, McMartin DW, Borchers CH, Ryan CH, Toor NS, Gillis HM, Zuin L, Bickerton G, Mcmaster M, Sverko E, Shang D, Wilson LD, Wrona FJ. 2013. Chemical fingerprinting of naphthenic acids and oil sands process waters-A review of analytical methods for environmental samples. *J. Environ. Sci. Health. A. Tox. Hazard. Subst. Environ. Eng.* **48**:1145–63.

Headley J V., McMartin DW. 2004. A review of the occurrence and fate of naphthenic acids in aquatic environments. *J. Environ. Sci. Heal. - Part A Toxic/Hazardous Subst. Environ. Eng.*

Headley J V., McMartin DW. 2004. A review of the occurrence and fate of naphthenic acids in aquatic environments. *J. Environ. Sci. Heal. - Part A Toxic/Hazardous Subst. Environ. Eng.*

Headley J V., McMartin DW. 2004. A review of the occurrence and fate of naphthenic acids in aquatic environments. *J. Environ. Sci. Heal. - Part A Toxic/Hazardous Subst. Environ. Eng.*

Headley J V., McMartin DW. 2007. A Review of the Occurrence and Fate of Naphthenic Acids in Aquatic Environments. *J. Environ. Sci. Heal. Part A*.

Heijnen JJ, Van Dijken JP. 1992. In search of thermodynamic description of biomass yields for the chemotrophic growth of microorganisms. *Biotechnol. Bioeng.*

Heijnen JJ. 1991. A new thermodynamically based correlation of chemotrophic biomass yields. *Antonie Van Leeuwenhoek* **60**:235–256.

Heijnen JJ. 2010. Impact of thermodynamic principles in systems biology. *Adv. Biochem. Eng. Biotechnol.* **121**:139–62.

Heijnen SJ. 1994. Thermodynamics of microbial growth and its implications for process design. *Trends Biotechnol.* **12**:483–492.

Henry CS, Broadbelt LJ, Hatzimanikatis V. 2007. Thermodynamics-based metabolic flux analysis. *Biophys. J.* **92**:1792–805.

Henry CS, Jankowski MD, Broadbelt LJ, Hatzimanikatis V. 2006. Genome-scale thermodynamic analysis of Escherichia coli metabolism. *Biophys. J.* **90**:1453–61.

Henzler H-J, Schedel M. 1991. Suitability of the shaking flask for oxygen supply to microbiological cultures. *Bioprocess Eng.* **7**:123–131.

Herman DC, Fedorak PM, MacKinnon MD, Costerton JW. 1994. Biodegradation of naphthenic acids by microbial populations indigenous to oil sands tailings. *Can. J. Microbiol.* **40**:467–477.

Heyland J, Fu J, Blank LM, Schmid A. 2010. Quantitative physiology of Pichia pastoris during glucose-limited high-cell density fed-batch cultivation for recombinant protein production. *Biotechnol. Bioeng.* **107**:357–68.

Hoijnen JJ, van Loosdrecht MC, Tijhuis L. 1992. A black box mathematical model to calculate auto- and heterotrophic biomass yields based on Gibbs energy dissipation. *Biotechnol. Bioeng.* **40**:1139–54.

Holden AA, Donahue RB, Ulrich AC. 2011. Geochemical interactions between process-affected water from oil sands tailings ponds and North Alberta surficial sediments. *J. Contam. Hydrol.* **119**:55–68.

Holden AA, Haque SE, Mayer KU, Ulrich AC. 2013. Biogeochemical processes controlling the mobility of major ions and trace metals in aquitard sediments beneath an oil sand tailing pond: laboratory studies and reactive transport modeling. *J. Contam. Hydrol.* **151**:55–67.

Holowenko F. 2002. Characterization of naphthenic acids in oil sands wastewaters by gas chromatography-mass spectrometry. *Water Res.* **36**:2843–2855.

Holowenko FM, MacKinnon MD, Fedorak PM. 2011. Methanogens and sulfate-reducing bacteria in oil sands fine tailings waste. *Can. J. Microbiol.*

Honn M, Lindgren H, Sjöstedt A. 2016. OxyR : an important regulator of the oxidative stress response of Francisella tularensis LVS.

Hou BK, Ellis LBM, Wackett LP. 2004a. Encoding microbial metabolic logic: predicting biodegradation. *J. Ind. Microbiol. Biotechnol.* **31**:261–72.

Hou BK, Wackett LP, Ellis LBM. 2003. Microbial pathway prediction: a functional group approach. *J. Chem. Inf. Comput. Sci.* **43**:1051–7.

Hou TJ, Xia K, Zhang W, Xu XJ. 2004b. ADME evaluation in drug discovery. 4. Prediction of aqueous solubility based on atom contribution approach. *J. Chem. Inf. Comput. Sci.* **44**:266–75.

Huang H, Wu K, Khan A, Jiang Y, Ling Z, Liu P, Chen Y, Tao X, Li X. 2016. A novel Pseudomonas gessardii strain LZ-E simultaneously degrades naphthalene and reduces hexavalent chromium. *Bioresour. Technol.* **207**:370–8.

Huang J, Nemati M, Hill G, Headley J. 2012. Batch and continuous biodegradation of three model naphthenic acids in a circulating packed-bed bioreactor. *J. Hazard. Mater.* **201-202**:132–40.

Huang K-C, Couttenye RA, Hoag GE. 2002. Kinetics of heat-assisted persulfate oxidation of methyl tert-butyl ether (MTBE). *Chemosphere* **49**:413–420.

Hutchinson D, Robinson C. 1988. Kinetics of the simultaneous batch degradation of p-cresol and

phenol by Pseudomonas putida. *Appl. Microbiol. Biotechnol.* **29**:599–604.

Hwang G, Dong T, Islam MS, Sheng Z, Pérez-Estrada LA, Liu Y, Gamal El-Din M. 2013. The impacts of ozonation on oil sands process-affected water biodegradability and biofilm formation characteristics in bioreactors. *Bioresour. Technol.* **130**:269–77.

Imlay JA. 2013. The molecular mechanisms and physiological consequences of oxidative stress: lessons from a model bacterium. *Nat. Rev. Microbiol.* **11**:443–54.

Iranmanesh S, Harding T, Abedi J, Seyedeyn-Azad F, Layzell DB. 2014. Adsorption of naphthenic acids on high surface area activated carbons. *J. Environ. Sci. Health. A. Tox. Hazard. Subst. Environ. Eng.* **49**:913–22.

J.A. Brient, P.J. Wessner MND. 1995. Kirk-Othmer Encyclopedia of Chemical Technology. Hoboken, NJ, USA: John Wiley & Sons, Inc.

Jankowski MD, Henry CS, Broadbelt LJ, Hatzimanikatis V. 2008. Group contribution method for thermodynamic analysis of complex metabolic networks. *Biophys. J.* **95**:1487–99.

Jaworska J, Dimitrov S, Nikolova N, Mekenyan O. 2002. Probabilistic assessment of biodegradability based on metabolic pathways: catabol system. *SAR QSAR Environ. Res.* **13**:307–323.

Jia W, He Y, Ling Y, Hei D, Shan Q, Zhang Y, Li J. 2015. Radiation-induced degradation of cyclohexanebutyric acid in aqueous solutions by gamma ray irradiation. *Radiat. Phys. Chem.* **109**:17–22.

Jiveraj MN. 1995. Naphthenic acids extraction and quantitative analy; fourth ed. Syncrude Canada Ltd.; Research Department; Edmonton; AB.

Johnson RJ, Smith BE, Sutton PA, McGenity TJ, Rowland SJ, Whitby C. 2011. Microbial biodegradation of aromatic alkanoic naphthenic acids is affected by the degree of alkyl side chain branching. *ISME J.* **5**:486–96.

Jørgensen BB. 2011. Deep subseafloor microbial cells on physiological standby. *Proc. Natl. Acad. Sci. U. S. A.* **108**:18193–4.

Kabdasli I, Arslan-Alaton I. 2010. Chemical Oxidation Applications for Industrial Wastewaters. IWA Publishing 341 p.

Kanicky JR, Poniatowski AF, Mehta NR, Shah DO. 2000. Cooperativity among Molecules at

Interfaces in Relation to Various Technological Processes: Effect of Chain Length on the p K a of Fatty Acid Salt Solutions †. *Langmuir* **16**:172–177.

Kannel PR, Gan TY. 2012. Naphthenic acids degradation and toxicity mitigation in tailings wastewater systems and aquatic environments: a review. *J. Environ. Sci. Health. A. Tox. Hazard. Subst. Environ. Eng.* **47**:1–21.

Kiliç MY, Yonar T, Kestioğlu K. 2013. Pilot-scale treatment of olive oil mill wastewater by physicochemical and advanced oxidation processes. *Environ. Technol.* **34**:1521–31.

Kindzierski W, Jin J, Gamal El-Din M. 2012. Review of Health Effects of Naphthenic Acids: Data Gaps and Implications for Understanding Human Health Risk.

Klamerth N, Moreira J, Li C, Singh A, McPhedran KN, Chelme-Ayala P, Belosevic M, Gamal El-Din M. 2015. Effect of ozonation on the naphthenic acids' speciation and toxicity of pH-dependent organic extracts of oil sands process-affected water. *Sci. Total Environ.* **506-507**:66–75.

Klamerth N, Moreira J, Li C, Singh A, McPhedran KN, Chelme-Ayala P, Belosevic M, Gamal El-Din M. 2015. Effect of ozonation on the naphthenic acids' speciation and toxicity of pH-dependent organic extracts of oil sands process-affected water. *Sci. Total Environ.* **506-507**:66–75.

Klopman G, Dimayuga M, Talafous J. 1994. META. 1. A program for the evaluation of metabolic transformation of chemicals. *J. Chem. Inf. Comput. Sci.* **34**:1320–1325.

Knightes CD, Peters CA. 2000. Statistical analysis of nonlinear parameter estimation for Monod biodegradation kinetics using bivariate data. *Biotechnol. Bioeng.* **69**:160–70.

Kovar K, Chaloupka V, Egli T. 2002. A Threshold Substrate Concentration Is Required to Initiate the Degradation of 3-Phenylpropionic Acid in Escherichia coli. *Acta Biotechnol.* **22**:285–298.

Kovárová K, Zehnder AJ, Egli T. 1996. Temperature-dependent growth kinetics of Escherichia coli ML 30 in glucose-limited continuous culture. *J. Bacteriol.* **178**:4530–9.

Kovarova-Kovar K, Egli T. 1998. Growth Kinetics of Suspended Microbial Cells: From Single-Substrate-Controlled Growth to Mixed-Substrate Kinetics. *Microbiol. Mol. Biol. Rev.* **62**:646–666.

Kristiansen CRB. 1999, eds. *Basic biotechnology*. Cambridge University Press

Krull R, Peterat G. 2016. Analysis of reaction kinetics during chemostat cultivation of Saccharomyces cerevisiae using a multiphase microreactor. *Biochem. Eng. J.* **105**:220–229.

Kulik N, Goi A, Trapido M, Tuhkanen T. 2006. Degradation of polycyclic aromatic hydrocarbons by combined chemical pre-oxidation and bioremediation in creosote contaminated soil. *J. Environ. Manage.* **78**:382–91.

Lambert RJW, Skandamis PN, Coote PJ, Nychas G-JE. 2001. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *J. Appl. Microbiol.* **91**:453–462.

LaRowe DE, Amend JP. 2015. Catabolic rates, population sizes and doubling/replacement times of microorganisms in natural settings. *Am. J. Sci.* **315**:167–203.

Leatherbarrow RJ. 1990. Using linear and non-linear regression to fit biochemical data. *Trends Biochem. Sci.* **15**:455–458.

Lee B-D, Hosomi M. 2001. A hybrid Fenton oxidation–microbial treatment for soil highly contaminated with benz(a)anthracene. *Chemosphere* **43**:1127–1132.

Lehninger AL, Nelson DL, Cox MM. 2005. Lehninger Principles of Biochemistry. W. H. Freeman 1119 p.

Leshuk T, Wong T, Linley S, Peru KM, Headley J V, Gu F. 2016. Solar photocatalytic degradation of naphthenic acids in oil sands process-affected water. *Chemosphere* **144**:1854–61.

Liang C, Bruell CJ, Marley MC, Sperry KL. 2004. Persulfate oxidation for in situ remediation of TCE. I. Activated by ferrous ion with and without a persulfate-thiosulfate redox couple. *Chemosphere* **55**:1213–23.

Liu H, Bruton TA, Doyle FM, Sedlak DL. 2014. In situ chemical oxidation of contaminated groundwater by persulfate: decomposition by Fe(III)- and Mn(IV)-containing oxides and aquifer materials. *Environ. Sci. Technol.* **48**:10330–6.

Liu X, Sun M, Cheng Y, Yang R, Wen Y, Chen Z, Li J. 2016. OxyR Is a Key Regulator in

Response to Oxidative Stress in Streptomyces avermitilis. *Microbiology*.

Lorian V. 1975. Some effects of subinhibitory concentrations of antibiotics on bacteria. *Bull. N. Y. Acad. Med.* **51**:1046–55.

MacKinnon MD, Boerger H. 1986. DESCRIPTION OF TWO TREATMENT METHODS FOR DETOXIFYING OIL SANDS TAILINGS POND WATER. *Water Pollut. Res. J. Canada* **21**:496–512.

Mahdavi H, Prasad V, Liu Y, Ulrich AC. 2015. In situ biodegradation of naphthenic acids in oil sands tailings pond water using indigenous algae-bacteria consortium. *Bioresour. Technol.* **187**:97–105.

Marentette JR, Frank RA, Bartlett AJ, Gillis PL, Hewitt LM, Peru KM, Headley J V, Brunswick P, Shang D, Parrott JL. 2015. Toxicity of naphthenic acid fraction components extracted from fresh and aged oil sands process-affected waters, and commercial naphthenic acid mixtures, to fathead minnow (Pimephales promelas) embryos. *Aquat. Toxicol.* **164**:108–17.

Marsolek MD, Rittmann BE. 2016. Effect of substrate characteristics on microbial community structure, function, resistance, and resilience; application to coupled photocatalytic-biological treatment. *Water Res.* **90**:1–8.

Martin JW, Barri T, Han X, Fedorak PM, El-Din MG, Perez L, Scott AC, Jiang JT. 2010. Ozonation of oil sands process-affected water accelerates microbial bioremediation. *Environ. Sci. Technol.* **44**:8350–6.

Mavrovouniotis ML. 1990. Group contributions for estimating standard gibbs energies of formation of biochemical compounds in aqueous solution. *Biotechnol. Bioeng.* **36**:1070–82.

Mavrovouniotis ML. 1991. Estimation of standard Gibbs energy changes of biotransformations. *J. Biol. Chem.* **266**:14440–14445.

Meckenstock RU, Elsner M, Griebler C, Lueders T, Stumpp C, Aamand J, Agathos SN, Albrechtsen H-J, Bastiaens L, Bjerg PL, Boon N, Dejonghe W, Huang WE, Schmidt SI, Smolders E, Sørensen SR, Springael D, van Breukelen BM. 2015. Biodegradation: Updating the concepts of control for microbial cleanup in contaminated aquifers. *Environ. Sci. Technol.* **49**:7073–81.

Mensing T, Marek W, Raulf-Heimsoth M, Baur X. 1998. Acute exposure to hair bleach causes airway hyperresponsiveness in a rabbit model. *Eur. Respir. J.* **12**:1371–4.

Merayo N, Hermosilla D, Blanco L, Cortijo L, Blanco A. 2013. Assessing the application of advanced oxidation processes, and their combination with biological treatment, to effluents from pulp and paper industry. *J. Hazard. Mater.* **262**:420–7.

Meredith W, Kelland S-J, Jones D. 2000. Influence of biodegradation on crude oil acidity and carboxylic acid composition. *Org. Geochem.* **31**:1059–1073.

Miller CM, Valentine RL, Roehl ME, Alvarez PJJ. 1996. Chemical and microbiological assessment of pendimethalin-contaminated soil after treatment with Fenton's reagent. *Water Res.* **30**:2579–2586.

Misiti T, Tandukar M, Tezel U, Pavlostathis SG. 2013. Inhibition and biotransformation potential of naphthenic acids under different electron accepting conditions. *Water Res.* **47**:406–18.

Misiti T, Tandukar M, Tezel U, Pavlostathis SG. 2014b. Corrigendum to "Inhibition and biotransformation potential of naphthenic acids under different electron accepting conditions" [Water Res. 47 (1) (January 2013) 406–418]. *Water Res.* **48**:645.

Misiti T, Tezel U, Pavlostathis S. 2014. Effect of alkyl side chain location and cyclicity on the aerobic biotransformation of naphthenic acids. *Environ. Sci.* ….

Miskimmin, B., Fedorak P., Lauman R. & VK. 2010. Oil sands water toxicity: A critical review.

Monod J. 1949. The Growth of Bacterial Cultures. *Annu. Rev. Microbiol.* **3**:371–394.

Morandi GD, Wiseman SB, Pereira A, Mankidy R, Gault IGM, Martin JW, Giesy JP. 2015. Effects-Directed Analysis of Dissolved Organic Compounds in Oil Sands Process-Affected Water. *Environ. Sci. Technol.* **49**:12395–404.

Mörtstedt H, Jeppsson MC, Ferrari G, Jönsson BAG, Kåredal MH, Lindh CH. 2011. Strategy for identification and detection of multiple oxidative modifications within proteins applied on persulfate-oxidized hemoglobin and human serum albumin. *Rapid Commun. Mass Spectrom.* **25**:327–40.

Mukherji S, van Oudenaarden A. 2009. Synthetic biology: understanding biological design from synthetic circuits. *Nat. Rev. Genet.* **10**:859–71.

Muñoz I, Rieradevall J, Torrades F, Peral J, Domènech X. 2005. Environmental assessment of different solar driven advanced oxidation processes. *Sol. Energy* **79**:369–375.

Muñoz X, Cruz MJ, Orriols R, Torres F, Espuga M, Morell F. 2004. Validation of specific inhalation challenge for the diagnosis of occupational asthma due to persulphate salts. *Occup. Environ. Med.* **61**:861–6.

Muyzer G, Stams AJM. 2008. The ecology and biotechnology of sulphate-reducing bacteria. *Nat. Rev. Microbiol.* **6**:441–54.

Nascimento LR, Rebouças LMC, Koike L, de A.M Reis F, Soldan AL, Cerqueira JR, Marsaioli AJ. 1999. Acidic biomarkers from Albacora oils, Campos Basin, Brazil. *Org. Geochem.* **30**:1175–1191.

Nielsen J, Villadsen J, Lidén G. 2012. Bioreaction Engineering Principles: Second Edition. Springer 528 p.

Nikakhtari H, Hill G. 2005. Modelling Oxygen Transfer and Aerobic Growth in Shake Flasks and Well-Mixed Bioreactors. *Can. J. Chem.* ….

Nikakhtari H, Hill GA. 2006. Closure effects on oxygen transfer and aerobic growth in shake flasks. *Biotechnol. Bioeng.* **95**:15–21.

North SML and A. 2010. Home - Canada's Oil Sands. http://www.canadasoilsands.ca/topics/Tailings/Pages/default.aspx.

Oh J, Salcedo DE, Medriano CA, Kim S. 2014. Comparison of different disinfection processes in the effective removal of antibiotic-resistant bacteria and genes. *J. Environ. Sci. (China)* **26**:1238–42.

Oiffer AAL, Barker JF, Gervais FM, Mayer KU, Ptacek CJ, Rudolph DL. 2009. A detailed field-based evaluation of naphthenic acid mobility in groundwater. *J. Contam. Hydrol.* **108**:89–106.

Oller I, Malato S, Sánchez-Pérez JA. 2011. Combination of Advanced Oxidation Processes and biological treatments for wastewater decontamination--a review. *Sci. Total Environ.* **409**:4141–66.

Oller I, Polo-López I, Miralles-Cuevas S, Fernández-Ibáñez P, Malato S. 2014. Advanced Technologies for Emerging Contaminants Removal in Urban Wastewater:1–25.

Onsager L. 1931. Reciprocal Relations in Irreversible Processes. I. *Phys. Rev.* **37**:405–426.

Oturan MA, Aaron J-J. 2014. Advanced Oxidation Processes in Water/Wastewater Treatment: Principles and Applications. A Review. *Crit. Rev. Environ. Sci. Technol.* **44**:2577–2641.

Palmroth MRT, Langwaldt JH, Aunola TA, Goi A, Münster U, Puhakka JA, Tuhkanen TA. 2006. Effect of modified Fenton's reaction on microbial activity and removal of PAHs in creosote oil contaminated soil. *Biodegradation* **17**:131–41.

Paslawski JC, Headley J V, Hill GA, Nemati M. 2009. Biodegradation kinetics of trans-4-methyl-1-cyclohexane carboxylic acid. *Biodegradation* **20**:125–33.

Patel V, Jain S, Madamwar D. 2012. Naphthalene degradation by bacterial consortium (DV-AL) developed from Alang-Sosiya ship breaking yard, Gujarat, India. *Bioresour. Technol.* **107**:122–30.

Paul D, Pandey G, Pandey J, Jain RK. 2005. Accessing microbial diversity for bioremediation and environmental restoration. *Trends Biotechnol.* **23**:135–42.

Pérez-Estrada LA, Han X, Drzewicz P, Gamal El-Din M, Fedorak PM, Martin JW. 2011. Structure-reactivity of naphthenic acids in the ozonation process. *Environ. Sci. Technol.* **45**:7431–7.

Petrie B, Barden R, Kasprzyk-Hordern B. 2014. A review on emerging contaminants in wastewaters and the environment: Current knowledge, understudied areas and recommendations for future monitoring. *Water Res.* **72**:3–27.

Pirt SJ. 1965. The Maintenance Energy of Bacteria in Growing Cultures. *Proc. R. Soc. B Biol. Sci.* **163**:224–231.

Prigogine I. 1968. Introduction to thermodynamics of irreversible processes. Interscience Publishers 147 p.

Pruchniak MP, Araźna M, Demkow U. 2015. Biochemistry of Oxidative Stress. *Adv. Exp. Med. Biol.*

Quagraine EK, Peterson HG, Headley J V. 2005. In Situ Bioremediation of Naphthenic Acids Contaminated Tailing Pond Waters in the Athabasca Oil Sands Region—Demonstrated Field Studies and Plausible Options: A Review. *J. Environ. Sci. Heal. Part A* **40**:685–722.

Quinlan PJ, Tam KC. 2015. Water treatment technologies for the remediation of naphthenic acids in oil sands process-affected water. *Chem. Eng. J.* **279**:696–714.

Ramos J-L, Marqués S, van Dillewijn P, Espinosa-Urgel M, Segura A, Duque E, Krell T, Ramos-González M-I, Bursakov S, Roca A, Solano J, Fernádez M, Niqui JL, Pizarro-Tobias P, Wittich R-M. 2011. Laboratory research aimed at closing the gaps in microbial bioremediation. *Trends Biotechnol.* **29**:641–7.

Ramos-Padrón E, Bordenave S, Lin S, Bhaskar IM, Dong X, Sensen CW, Fournier J, Voordouw G, Gieg LM. 2011. Carbon and sulfur cycling by microbial communities in a gypsum-treated oil sands tailings pond. *Environ. Sci. Technol.* **45**:439–46.

Reardon K, Mosteller D, Rogers JB. 2000. Biodegradation kinetics of benzene, toluene, and phenol as single and mixed substrates for Pseudomonas putida F 1. *Biotechnol. ...*.

Reddy KR, DeLaune RD. 2008. Biogeochemistry of Wetlands: Science and Applications. CRC Press 800 p.

Rho EM, Evans WC. 1975. The aerobic metabolism of cyclohexanecarboxylic acid by Acinetobacter anitratum. *Biochem. J.* **148**:11–15.

Rice RG. 1996. Applications of ozone for industrial wastewater treatment — A review. *Ozone Sci. Eng.* **18**:477–515.

Rittmann B, McCarty P. 2012. Environmental biotechnology: principles and applications.

Riva JP. 1983. World petroleum resources and reserves. Westview Press 355 p.

Rizzo L. 2011. Bioassays as a tool for evaluating advanced oxidation processes in water and wastewater treatment. *Water Res.* **45**:4311–40.

Robinson T, McMullan G, Marchant R, Nigam P. 2001. Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative. *Bioresour. Technol.* **77**:247–255.

Rodrigo G, Carrera J, Prather KJ, Jaramillo A. 2008. DESHARKY: automatic design of metabolic pathways for optimal cell growth. *Bioinformatics* **24**:2554–6.

Roels JA. 1983. Energetics and kinetics in biotechnology.

Rogers V V. 2002. Acute and Subchronic Mammalian Toxicity of Naphthenic Acids from Oil

Sands Tailings. *Toxicol. Sci.* **66**:347–355.

Roques DE, Overton EB, Henry CB. 1994. Using Gas Chromatography/Mass Spectroscopy Fingerprint Analyses to Document Process and Progress of Oil Degradation. *J. Environ. Qual.* **23**:851.

Ross MS, Pereira A dos S, Fennell J, Davies M, Johnson J, Sliva L, Martin JW. 2012. Quantitative and qualitative analysis of naphthenic acids in natural waters surrounding the Canadian oil sands industry. *Environ. Sci. Technol.* **46**:12796–805.

Russell JB, Cook GM. 1995. Energetics of bacterial growth: balance of anabolic and catabolic reactions. *Microbiol. Rev.* **59**:48–62.

S. Roy Caplan AE. 1983. Bioenergetics and Linear Nonequilibrium Thermodynamics. *Harvard Univ. Press*. http://www.hup.harvard.edu/catalog.php?isbn=9780674732063.

Sanchis S, Polo AM, Tobajas M, Rodriguez JJ, Mohedano AF. 2013. Degradation of chlorophenoxy herbicides by coupled Fenton and biological oxidation. *Chemosphere* **93**:115–22.

Scott AC, Zubot W, MacKinnon MD, Smith DW, Fedorak PM. 2008. Ozonation of oil sands process water removes naphthenic acids and toxicity. *Chemosphere* **71**:156–60.

Shoghi E, Fuguet E, Bosch E, Ràfols C. 2013. Solubility-pH profiles of some acidic, basic and amphoteric drugs. *Eur. J. Pharm. Sci.* **48**:291–300.

Shu Z, Li C, Belosevic M, Bolton JR, El-Din MG. 2014. Application of a solar UV/chlorine advanced oxidation process to oil sands process-affected water remediation. *Environ. Sci. Technol.* **48**:9692–701.

Siegrist R, Crimi M, Simpkin T. 2011. In situ chemical oxidation for groundwater remediation.

Sies H. 1997. Oxidative stress: oxidants and antioxidants. *Exp. Physiol.* **82**:291–295.

Silver S, Chakrabarty AM (Illinois U. CI (USA). D of M and I, Iglewski B (Rochester U. N (USA). D of M and I, Kaplan S (eds. . (Texas U. HT (USA). D of M and I. 1990. Pseudomonas: Biotransformations, pathogenesis, and evolving biotechnology.

Singh S, Kang SH, Mulchandani A, Chen W. 2008. Bioremediation: environmental clean-up through pathway engineering. *Curr. Opin. Biotechnol.* **19**:437–44.

Slavcheva E, Shone B, Turnbull A. 1999. Review of naphthenic acid corrosion in oilrefining. *Br. Corros. J.* **34**:125–131.

Smith BE, Lewis CA, Belt ST, Whitby C, Rowland SJ. 2008. Effects of Alkyl Chain Branching on the Biotransformation of Naphthenic Acids. *Environ. Sci. Technol.* **42**:9323–9328.

Smith JM, Ness HC Van, Abbott MM. 2001. Introduction to Chemical Engineering Thermodynamics. McGraw-Hill 789 p.

Smolke C. 2009. The Metabolic Pathway Engineering Handbook: Fundamentals. CRC Press 678 p.

Sohrabi V, Ross MS, Martin JW, Barker JF. 2013. Potential for in situ chemical oxidation of acid extractable organics in oil sands process affected groundwater. *Chemosphere* **93**:2698–703.

Sohrabi V, Ross MS, Martin JW, Barker JF. 2013. Potential for in situ chemical oxidation of acid extractable organics in oil sands process affected groundwater. *Chemosphere* **93**:2698–703.

Speight JG. Liquid Fuels from Oil Sand 197–221. http://www.drjamesspeight.qpg.com/.

Stephanopoulos G, Aristidou AA, Nielsen J. 1998. Metabolic Engineering: Principles and Methodologies. Academic Press 725 p.

Stewart PS, Rayner J, Roe F, Rees WM. 2001. Biofilm penetration and disinfection efficacy of alkaline hypochlorite and chlorosulfamates. *J. Appl. Microbiol.* **91**:525–532.

Storz G, Tartaglia LA, Farr SB, Ames BN. 1990. Bacterial defenses against oxidative stress. *Trends Genet.* **6**:363–368.

Strous M, Fuerst JA, Kramer EH, Logemann S, Muyzer G, van de Pas-Schoonen KT, Webb R, Kuenen JG, Jetten MS. 1999. Missing lithotroph identified as new planctomycete. *Nature* **400**:446–9.

Strous M, Kuenen JG, Jetten MSM. 1999. Key Physiology of Anaerobic Ammonium Oxidation. *Appl. Envir. Microbiol.* **65**:3248–3250.

Sutton NB, Grotenhuis JTC, Langenhoff AAM, Rijnaarts HHM. 2010. Efforts to improve coupled in situ chemical oxidation with bioremediation: a review of optimization strategies.

J. Soils Sediments **11**:129–140.

Sutton NB, Kalisz M, Krupanek J, Marek J, Grotenhuis T, Smidt H, de Weert J, Rijnaarts HHM, van Gaans P, Keijzer T. 2014. Geochemical and microbiological characteristics during in situ chemical oxidation and in situ bioremediation at a diesel contaminated site. *Environ. Sci. Technol.* **48**:2352–60.

Tchobanoglous G, Burton FL, Stensel HD, Eddy M&. 2003. Wastewater Engineering: Treatment and Reuse. McGraw-Hill Education 1819 p.

Tezel U, Pavlostathis SG. 2015. Quaternary ammonium disinfectants: microbial adaptation, degradation and ecology. *Curr. Opin. Biotechnol.* **33**:296–304.

Tijhuis L, Van Loosdrecht MCM, Heijnen JJ. 1993. A thermodynamically based correlation for maintenance Gibbs energy requirements in aerobic and anaerobic chemotrophic growth. *Biotechnol. Bioeng.* **42**:509–519.

Tissot BP, Welte DH. 1978. Petroleum Formation and Occurrence. Berlin, Heidelberg: Springer Berlin Heidelberg.

Tollefsen KE, Petersen K, Rowland SJ. 2012. Toxicity of synthetic naphthenic acids and mixtures of these to fish liver cells. *Environ. Sci. Technol.* **46**:5143–50.

Tsitonaki A, Petri B, Crimi M, Mosbak H, Siegrist RL, Bjerg PL. 2010. In Situ Chemical Oxidation of Contaminated Soil and Groundwater Using Persulfate: A Review. *Crit. Rev. Environ. Sci. Technol.* **40**:55–91.

Tsitonaki A, Smets BF, Bjerg PL. 2008. Effects of heat-activated persulfate oxidation on soil microorganisms. *Water Res.* **42**:1013–22.

Urs von Stockar LAM van der W. 2013. Biothermodynamics: The Role of Thermodynamics in Biochemical Engineering.

Vaiopoulou E, Misiti TM, Pavlostathis SG. 2015. Removal and toxicity reduction of naphthenic acids by ozonation and combined ozonation-aerobic biodegradation. *Bioresour. Technol.* **179**:339–47.

Van Loosdrecht M, Henze M. 1999. Maintenance, endogeneous respiration, lysis, decay and predation. *Water Sci. Technol.* **39**:107–117.

van Loosdrecht, M. C., Nielsen, P. H., Lopez-Vazquez, C. M., & Brdjanovic D. 2016. Experimental Methods in Wastewater Treatment | IWA Publishing. *Water Intell. Online*.

Van Suijdam JC, Kossen NWF, Joha AC. 1978. Model for oxygen transfer in a shake flask. *Biotechnol. Bioeng.* **20**:1695–1709.

Veglio F, Beolchini F, Ubaldini S. 1998. Empirical models for oxygen mass transfer: a comparison between shake flask and lab-scale fermentor and application to manganiferous ore bioleaching. *Process Biochem.* **33**:367–376.

Veschetti E, Cutilli D, Bonadonna L, Briancesco R, Martini C, Cecchini G, Anastasi P, Ottaviani M. 2003. Pilot-plant comparative study of peracetic acid and sodium hypochlorite wastewater disinfection. *Water Res.* **37**:78–94.

Villadsen J, Nielsen J, Lidén G. 2011. Bioreaction Engineering Principles. Boston, MA: Springer US.

von Stockar U, Maskow T, Liu J, Marison IW, Patiño R. 2006. Thermodynamics of microbial growth and metabolism: an analysis of the current situation. *J. Biotechnol.* **121**:517–33.

von Stockar U, van der Wielen LAM. 1997. Thermodynamics in biochemical engineering. *J. Biotechnol.* **59**:25–37.

Wackett LP, Ellis LB. 1999. Predicting biodegradation. *Environ. Microbiol.* **1**:119–24.

Waldemer RH, Tratnyek PG, Johnson RL, Nurmi JT. 2007. Oxidation of Chlorinated Ethenes by Heat-Activated Persulfate: Kinetics and Products. *Environ. Sci. Technol.* **41**:1010–1015.

Walker AW, Keasling JD. 2002. Metabolic engineering of Pseudomonas putida for the utilization of parathion as a carbon and energy source. *Biotechnol. Bioeng.* **78**:715–21.

Wang N, Chelme-Ayala P, Perez-Estrada L, Garcia-Garcia E, Pun J, Martin JW, Belosevic M, Gamal El-Din M. 2013. Impact of ozonation on naphthenic acids speciation and toxicity of oil sands process-affected water to Vibrio fischeri and mammalian immune system. *Environ. Sci. Technol.* **47**:6518–26.

Watson J., Jones D., Swannell RP., van Duin AC. 2002. Formation of carboxylic acids during aerobic biodegradation of crude oil and evidence of microbial oxidation of hopanes. *Org.*

Geochem. **33**:1153–1169.

Welch GR. 1985. Some problems in the usage of gibbs free energy in biochemistry. *J. Theor. Biol.* **114**:433–446.

Westerhoff H V., Dam K van. 1987. Thermodynamics and control of biological free-energy transduction. Elsevier 568 p.

Westerhoff HV. 1983. Mosaic Non-equilibrium Thermodynamics and (the Control Of) Biological Free- Energy Transduction. Drukkerij Gerja 368 p.

Whitby C. 2010. Microbial naphthenic Acid degradation. *Adv. Appl. Microbiol.* **70**:93–125.

Wordofa DN. 2014. Application of Iron Activated Persulfate for Disinfection in Water Treatment.

Wylen GJ Van, Sonntag RE, Borgnakke C. 1994. Fundamentals of Classical Thermodynamics, Volume 1. Wiley 852 p.

Yue S, Ramsay BA, Ramsay JA. 2015. Biodegradation of naphthenic acid surrogates by axenic cultures. *Biodegradation* **26**:313–25.

Zhang R, Xu X, Chen W, Huang Q. 2016. Genetically engineered Pseudomonas putida X3 strain and its potential ability to bioremediate soil microcosms contaminated with methyl parathion and cadmium. *Appl. Microbiol. Biotechnol.* **100**:1987–97.

Zhang X, Wiseman S, Yu H, Liu H, Giesy JP, Hecker M. 2011. Assessing the toxicity of naphthenic acids using a microbial genome wide live cell reporter array system. *Environ. Sci. Technol.* **45**:1984–91.

Zotin AI. 1990. Thermodynamic Bases of Biological Processes: Physiological Reactions and Adaptations. Walter de Gruyter 293 p.

APPENDIX 1: SUPPLEMENTARY DATA FOR CHAPTER 3

Statistical analysis of *pseudo*-first order rate constant

Table A-0-1Statistical results of multiple comparison of 0.25, 0.5, 1 and 2 times of D; *p-value* is shown as "Sig"

Multiple Comparisons

Dependent Variable:

(I) Sample Type			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	0.25D	0.5D	-.030000*	.006839	.010	-.05190	-.00810
		D	-.067667*	.006839	.000	-.08957	-.04576
		2D	-.055667*	.006839	.000	-.07757	-.03376
	0.5D	0.25D	.030000*	.006839	.010	.00810	.05190
		D	-.037667*	.006839	.003	-.05957	-.01576
		2D	-.025667*	.006839	.023	-.04757	-.00376
	D	0.25D	.067667*	.006839	.000	.04576	.08957
		0.5D	.037667*	.006839	.003	.01576	.05957
		2D	.012000	.006839	.359	-.00990	.03390
	2D	0.25D	.055667*	.006839	.000	.03376	.07757
		0.5D	.025667*	.006839	.023	.00376	.04757
		D	-.012000	.006839	.359	-.03390	.00990
Bonferroni	0.25D	0.5D	-.030000*	.006839	.014	-.05379	-.00621
		D	-.067667*	.006839	.000	-.09146	-.04387
		2D	-.055667*	.006839	.000	-.07946	-.03187
	0.5D	0.25D	.030000*	.006839	.014	.00621	.05379
		D	-.037667*	.006839	.003	-.06146	-.01387
		2D	-.025667*	.006839	.034	-.04946	-.00187
	D	0.25D	.067667*	.006839	.000	.04387	.09146
		0.5D	.037667*	.006839	.003	.01387	.06146
		2D	.012000	.006839	.705	-.01179	.03579
	2D	0.25D	.055667*	.006839	.000	.03187	.07946
		0.5D	.025667*	.006839	.034	.00187	.04946
		D	-.012000	.006839	.705	-.03579	.01179

***. The mean difference is significant at the 0.05 level.**

Concept of degree of reduction (γ)

Degree of reduction or γ represents the available electron in a compound, therefore it can be calculated when the elemental composition is known (Kristiansen, 1999). This concept is based upon the redox half reaction of a compound when it converts to a set of reference compounds and electrons. Reference compounds are CO_2, H_2O, H^+, Fe^{3+}, SO_4^{2-}, PO_4^{3-} , N_2. After setting up the redox half reaction, the produced electrons are equal to γ. It should be noted that γ of reference compounds are equal to zero. The degree of reduction of some common elements are shown in table below.

Table A1-2. Degree of reduction of common elements

γ of Elements	
H	+1
O	-2
C	+4
+ Charge	-1
- Charge	+1
S	+6
P	+5
Fe	+3
N	-3 for NH_4^+
	0 for N_2
	+5 for NO_3^-

Since biological reactions are set of redox reactions; γ can be used as a stoichiometric quantity. And also we can do γ balance because electrons are conserved (Kristiansen, 1999; Smolke, 2009). To summarise the concept of degree of reduction following notes need to be understood:

- Electrons are conserved; therefore, we can do electron balance.
- For convenience, and based on the concept of degree of reduction we consider some compounds as the reference compounds. Reference compounds have the degree of

reduction of zero. This fact makes the electron balance easier by eliminating the coefficient of reference compounds. And interestingly, reference compounds are the compounds that are very common in biochemical reactions i.e. H_2O, CO_2

Example for calculation of degree of reduction:

First example: dicyclohexyl acetic acid $C_{14}H_{24}O_2$:

$$\gamma_{C_{14}H_{24}O_2} = 14 \times (+4) + 24 \times (+1) + 2 \times (-2) = 76 \left[\frac{e^- mol}{mol\ C_{14}H_{24}O_2}\right]$$

It shows that the compound $C_{14}H_{24}O_2$ has 76 electron that can be oxidized (because its positive)!

APPENDIX 2: SUPPLEMENTARY DATA FOR CHAPTER 4

Statistical Analyses of Chapter 4 Results

Analyses conducted with SPSS® (Ver 21;SPSS) software.

Table A2-1. Statistical analysis of maximum concentration of biomass

Multiple Comparisons

Statistica Analysis for maximum concentration of biomass

(I) Sample Type			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	I	A	.143000*	.019597	.000	.08024	.20576
		B	.103333*	.019597	.003	.04058	.16609
		C	.325333*	.019597	.000	.26258	.38809
	A	I	-.143000*	.019597	.000	-.20576	-.08024
		B	-.039667	.019597	.256	-.10242	.02309
		C	.182333*	.019597	.000	.11958	.24509
	B	I	-.103333*	.019597	.003	-.16609	-.04058
		A	.039667	.019597	.256	-.02309	.10242
		C	.222000*	.019597	.000	.15924	.28476
	C	I	-.325333*	.019597	.000	-.38809	-.26258
		A	-.182333*	.019597	.000	-.24509	-.11958
		B	-.222000*	.019597	.000	-.28476	-.15924
Bonferroni	I	A	.143000*	.019597	.001	.07482	.21118
		B	.103333*	.019597	.005	.03516	.17151
		C	.325333*	.019597	.000	.25716	.39351
	A	I	-.143000*	.019597	.001	-.21118	-.07482
		B	-.039667	.019597	.465	-.10784	.02851
		C	.182333*	.019597	.000	.11416	.25051
	B	I	-.103333*	.019597	.005	-.17151	-.03516
		A	.039667	.019597	.465	-.02851	.10784
		C	.222000*	.019597	.000	.15382	.29018
	C	I	-.325333*	.019597	.000	-.39351	-.25716
		A	-.182333*	.019597	.000	-.25051	-.11416
		B	-.222000*	.019597	.000	-.29018	-.15382

*. The mean difference is significant at the 0.05 level.

Table A2-2. Statistical analysis of maximum concentration of biomass

Multiple Comparisons

Statistical analysis of biomass specific growth rate

(I) Sample Type			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	I	A	-.00110	.01248	1.000	-.0411	.0389
		B	-.00703	.01248	.940	-.0470	.0329
		C	-.01827	.01248	.499	-.0582	.0217
	A	I	.00110	.01248	1.000	-.0389	.0411
		B	-.00593	.01248	.962	-.0459	.0340
		C	-.01717	.01248	.546	-.0571	.0228
	B	I	.00703	.01248	.940	-.0329	.0470
		A	.00593	.01248	.962	-.0340	.0459
		C	-.01123	.01248	.805	-.0512	.0287
	C	I	.01827	.01248	.499	-.0217	.0582
		A	.01717	.01248	.546	-.0228	.0571
		B	.01123	.01248	.805	-.0287	.0512
Bonferroni	I	A	-.00110	.01248	1.000	-.0445	.0423
		B	-.00703	.01248	1.000	-.0504	.0364
		C	-.01827	.01248	1.000	-.0617	.0251
	A	I	.00110	.01248	1.000	-.0423	.0445
		B	-.00593	.01248	1.000	-.0493	.0375
		C	-.01717	.01248	1.000	-.0606	.0262
	B	I	.00703	.01248	1.000	-.0364	.0504
		A	.00593	.01248	1.000	-.0375	.0493
		C	-.01123	.01248	1.000	-.0546	.0322
	C	I	.01827	.01248	1.000	-.0251	.0617
		A	.01717	.01248	1.000	-.0262	.0606
		B	.01123	.01248	1.000	-.0322	.0546

Table A2-3. Statistical analysis of yield of substrate to biomass

Multiple Comparisons

Statistical analysis of yield of substrate to biomass

(I) Sample Type			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	I	A	-.014333	.046270	.989	-.16251	.13384
		B	.020333	.046270	.970	-.12784	.16851
		C	-.043667	.046270	.783	-.19184	.10451
	A	I	.014333	.046270	.989	-.13384	.16251
		B	.034667	.046270	.875	-.11351	.18284
		C	-.029333	.046270	.918	-.17751	.11884
	B	I	-.020333	.046270	.970	-.16851	.12784
		A	-.034667	.046270	.875	-.18284	.11351
		C	-.064000	.046270	.542	-.21217	.08417
	C	I	.043667	.046270	.783	-.10451	.19184
		A	.029333	.046270	.918	-.11884	.17751
		B	.064000	.046270	.542	-.08417	.21217
Bonferroni	I	A	-.014333	.046270	1.000	-.17530	.14664
		B	.020333	.046270	1.000	-.14064	.18130
		C	-.043667	.046270	1.000	-.20464	.11730
	A	I	.014333	.046270	1.000	-.14664	.17530
		B	.034667	.046270	1.000	-.12630	.19564
		C	-.029333	.046270	1.000	-.19030	.13164
	B	I	-.020333	.046270	1.000	-.18130	.14064
		A	-.034667	.046270	1.000	-.19564	.12630
		C	-.064000	.046270	1.000	-.22497	.09697
	C	I	.043667	.046270	1.000	-.11730	.20464
		A	.029333	.046270	1.000	-.13164	.19030
		B	.064000	.046270	1.000	-.09697	.22497

Table A2-4. Statistical analysis of biomass specific substrate consumption rate

Multiple Comparisons

Statistical analysis of biomass specific substrate consumption rate

(I) Sample Type			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	I	A	.001667	.007937	.996	-.02375	.02708
		B	-.017333	.007937	.207	-.04275	.00808
		C	-.016667	.007937	.232	-.04208	.00875
	A	I	-.001667	.007937	.996	-.02708	.02375
		B	-.019000	.007937	.156	-.04442	.00642
		C	-.018333	.007937	.175	-.04375	.00708
	B	I	.017333	.007937	.207	-.00808	.04275
		A	.019000	.007937	.156	-.00642	.04442
		C	.000667	.007937	1.000	-.02475	.02608
	C	I	.016667	.007937	.232	-.00875	.04208
		A	.018333	.007937	.175	-.00708	.04375
		B	-.000667	.007937	1.000	-.02608	.02475
Bonferroni	I	A	.001667	.007937	1.000	-.02595	.02928
		B	-.017333	.007937	.363	-.04495	.01028
		C	-.016667	.007937	.414	-.04428	.01095
	A	I	-.001667	.007937	1.000	-.02928	.02595
		B	-.019000	.007937	.262	-.04661	.00861
		C	-.018333	.007937	.298	-.04595	.00928
	B	I	.017333	.007937	.363	-.01028	.04495
		A	.019000	.007937	.262	-.00861	.04661
		C	.000667	.007937	1.000	-.02695	.02828
	C	I	.016667	.007937	.414	-.01095	.04428
		A	.018333	.007937	.298	-.00928	.04595
		B	-.000667	.007937	1.000	-.02828	.02695

THEORETICAL APPROACH TO CALCULATE OXYGEN CONSUMPTION RATE

Following approach can be used to approximate the rate of oxygen consumption in the system. First of all we need to know the degree of reduction of substrate per C-atom. Since here determination of exact amount of degree of reduction was not possible because of lack of accurate formula for the substrate. To debottleneck this issue, an experimental approach based on concept of COD and TOC has been used to calculate the degree of reduction of substrate.

After determination of degree of reduction of substrate, yield of electron acceptor can be obtained through setting up a redox balance. It should be noted that for convenience in writing the redox balance, degree of reduction of reaction constituents is considered as zero except electron donor, electron acceptor and biomass. Further explanation can be found in chapter 5 of this thesis.

Order of materials is as follows:

1. Determination of degree of reduction of substrate per C-atom
    a. Calculation of COD based on degree of reduction
    b. Calculation of TOC
    c. Correlation for degree of reduction and TOC and COD
2. Setting up redox balance among electron donor, electron acceptor and biomass
    a. Redox balance
    b. Yield of electron acceptor to biomass (Y_{AX})
    c. Conversion of yield to biomass specific oxygen consumption rate

Calculation of degree of reduction of substrate

As it mentioned earlier, Merichem NAs used as the substrate. Since its exact formula is not available, we are not able to calculate the degree of reduction. However, since measurement of total organic carbon and chemical oxygen demand is applicable we are able to measure degree of reduction through its concept (Kristiansen, 1999; Nielsen et al., 2012; Villadsen et al., 2011).

Calculation of COD based on degree of reduction

It has been explained in Chapter 4 that each mole of electron represents 8 g COD. By recalling the formula of degree of reduction (Nielsen et al., 2012):

$$\gamma_D = \frac{available\ electrons}{number\ of\ carbon\ atoms\ (n)} \tag{A2.1}$$

For example for ethanol (C_2H_6O) we have:

Available electrons: $2 \cdot (+4) + 6 \cdot (+1) + 1 \cdot (-2) = 12$

Thus:

$$\gamma_D = \frac{12}{2} = 6\ mole\ electron\ per\ mole\ donor$$

Based on the concept of COD we have (Kristiansen, 1999; Rittmann and McCarty, 2012):

$$COD = n \cdot \gamma_D \cdot 8 \tag{A2.2}$$

Hence for 1 mole of ethanol we have:

$$COD\ of\ 1\ mole\ ethanol = 12\ mole\ electron \cdot \frac{8\ gCOD}{1\ mole\ electron} = 96\ g\ COD$$

Calculation of TOC

Total organic carbon for ethanol can be calculated based in its concept. One should be realized that 1 mole of carbon atom is equal to 12 g.

$$TOC\ of\ 1\ mole\ ethanol = 2\ mole\ C - atom\ \cdot \frac{12\ g\ TOC}{1\ mole\ C - atom} = 24\ g\ TOC$$

4A.1.c. Correlation for degree of reduction and TOC and COD

If we consider n as the number of carbon atoms in a compound, we can set up the following equations.

$$COD = n \cdot \gamma_D \cdot 8 \quad \text{(A2.2)}$$

$$TOC = n \cdot 12 \quad \text{(A2.3)}$$

Now we can connect these two above equations by eliminating "n":

$$\frac{TOC}{12} = \frac{COD}{\gamma_D \cdot 8} \quad \text{(A2.4)}$$

Now we can calculate the degree of reduction:

$$\gamma_D = 1.5 \cdot \left(\frac{COD}{TOC}\right) \quad \text{(A2.5)}$$

This equation means that one can calculate the degree of reduction of substrate by measuring total organic carbon and chemical oxygen demand.

Redox balance

Setting up a balance equation for degree of reduction (electron) over the bioreactor is possible with knowing of γ_D. This balance is described below (Colin Ratledge, 2006; Kristiansen, 1999):

$$-\frac{\gamma_D}{Y_{DX}}-\frac{\gamma_A}{Y_{AX}}+\gamma_X=0 \tag{A2.6}$$

In the above equation, $\gamma_D$and $\gamma_A$and $\gamma_X$demonstrates the degree of reduction of electron donor (substrate), electron acceptor and biomass, respectively. Also Y_{DX} $(=Y_{SX})$ and Y_{AX} shows the yield of substrate to biomass and electron acceptor to biomass, respectively. This balance is also known as the redox balance equation.

COD and TOC of the 50 mg/L Merichem NAs (in BH media) measured as 717 and 250 mg/L, respectively. Now we can calculate the degree of reduction of the substrate (Merichem NAs):

$$\gamma_{D\_NAs}=1.5\cdot\left(\frac{COD}{TOC}\right)=1.5\cdot\left(\frac{717}{242}\right)=4.45\ mole\ electron\ per\ C-mole\ substrate$$

The general proposed formula for biomass is $CH_{1.8}O_{0.5}N_{0.2}$. Once ammonia used as the nitrogen source, the degree of reduction of biomass will be (Smolke, 2009; Stephanopoulos et al., 1998; Urs von Stockar, 2013):

$$\gamma_X=\ 1\times4+1\times1.8+0.5\ \times(-2)+0.2\times(-3)=4.2$$

Calculation of yield of electron acceptor to biomass

Based on the calculated parameter, we can easily calculate the yield of electron acceptor (oxygen) to biomass for each case of biodegradation. For example for C-series:

$$\frac{1}{Y_{AX}} = \frac{\gamma_D}{(-\gamma_A)\cdot Y_{SX}} - \frac{\gamma_X}{(-\gamma_A)} \qquad \text{(A2.6)}$$

It is important to mention that the dimension of yield in above equation is $\left(\frac{C-mole\ C_x}{C-mole\ D}\right)$.

So to convert the yield of C-series we have:

Here, to have more accuracy, DCW divided by 0.95; since DCW contains organic and inorganic components of cell.

$$DCW = 24.6 \times \frac{1}{0.95} = 25.9\ \frac{gDCW}{C-mol\ C_x}$$

$$Y_{SX} = 0.7\ \left[\frac{mg\ DCW}{mg\ DOC}\right] \times \left[\frac{1\ C-mole\ C_x}{25.9\ g\ DCW}\right] \times \left[\frac{12\ g\ DOC}{1\ C-mole\ S\ (or\ D)}\right]$$

$$Y_{SX} = 0.32\left[\frac{C-mole\ C_x}{C-mole\ S}\right]$$

Now based on the equation A2.6, we have:

$$\frac{1}{Y_{AX}} = \frac{4.45}{(4)\cdot 0.32} - \frac{4.2}{(4)}$$

$$Y_{AX} = 0.41\ \left[\frac{C-mole\ C_x}{mole\ O_2}\right] = 0.33\left[\frac{mgDCW}{mg\ O_2}\right]$$

In above calculation, 24.6 g/C-mole C_x is the molecular weight of biomass.

Conversion of yield to biomass specific oxygen consumption rate

Based on the definition of yield we can calculate the oxygen specific consumption rate:

$$Y_{ij} = \frac{r_j}{r_i} \tag{2A.7}$$

$$Y_{AX} = \frac{r_X}{r_A} = \frac{\mu \cdot C_X}{q_{O_2} \cdot C_X} = \frac{\mu}{q_{O_2}} \tag{2A.8}$$

Therefore,

$$q_{O_2} = \frac{\mu}{Y_{AX}} = \frac{0.126}{0.33} = 0.38 \left[\frac{mg\ O_2}{mgDCW \cdot h}\right]$$

Based on above calculations, yield of oxygen to biomass, and biomass specific oxygen consumption rate are estimated.

Table A2-1. Yield of oxygen to biomass

	Yield of oxygen to biomass (mgDCW mgO_2^{-1})		
	Average	**95% Confidence Interval**	
I	0.302	0.266	0.342
A	0.312	0.274	0.352
B	0.289	0.259	0.321
C	0.332	0.292	0.375

Table A2-2. Biomass specific substrate consumption rate

	q_{O2} (mgDCW mgO_2^{-1} h^{-1})		
	Average	**95% Confidence Interval**	
I	0.357	0.343	0.362
A	0.349	0.336	0.358
B	0.398	0.382	0.408
C	0.380	0.391	0.368

Dimension of biomass specific substrate consumption rate is per time. On the other hand, oxygen mass transfer coefficient in liquid phase is also has the dimension of per time. By comparing the

oxygen mass transfer coefficient in liquid phase which changes from 11 to 28.5 h^{-1} to biomass specific oxygen consumption rate; it can be seen that oxygen was not a limiting factor through the experiment.

APPENDIX 3: SUPPLEMENTARY DATA FOR CHAPTER 5

Estimation of Gibbs Free Energy of Formation

Thermodynamic analysis based on entirely experimental data is not possible due to limited number of experimental data that are currently available (Jankowski et al., 2008). For instance, if one wants to use experimentally measured $\Delta_r G^0$ to analyse reactions in metabolism, only 8.1% reactions in *Eschericia coli* metabolism are available (Feist et al., 2007). To overcome this challenge, Mavrovouniotis proposed a group contribution method for estimating Gibbs free energy of formation and reaction in biochemical systems (Mavrovouniotis, 1990; Mavrovouniotis, 1991). Based on this method, it is possible to estimate $\Delta_r G'^0$ and $\Delta_f G'^0$ for most biochemical compounds.

Determination of Substructure Groups

In the group contribution method, a given compound with **known molecular structure** is decomposed to a set of smaller molecular structures that can be estimated. After the smaller group is identified, a linear model can estimate the $\Delta_f G'^0$ of the entire compound. The mathematical description of this process is described below (Jankowski et al., 2008):

$$\Delta_f G'^0_{est} = \sum_{i=1}^{N_{gr}} n_i \, \Delta_{gr} G'^0_i \qquad \text{(A3.1)}$$

In this equation:

$\Delta_f G'^0_{est}$ is the estimated $\Delta_f G'^0$

$\Delta_{gr} G'^0_i$ is the contribution of group *i* to $\Delta_f G'^0_{est}$.

And n_i is the number of instances of group *i* in the structure of compound.

Now similarly, $\Delta_r G'^{0}$ of a reaction can be estimated by summing the contribution of each structural group destroyed or created during the course of the reaction:

$$\Delta_r G'^{0}_{est} = \sum_{i=1}^{m} v_i \left(\sum_{j=1}^{N_{gr}} n_j \, \Delta_{gr} G'^{0}_{j} \right) \tag{A3.2}$$

In this equation, v_i is the stoichiometric coefficient of species *i* in the reaction and m is the number of species involved in the reaction (Jankowski et al., 2008). In table 5.3 the pre-defined groups based on known $\Delta_{gr} G'^{0}$ is presented (Jankowski et al., 2008).

Table A3-0-1. Structural groups used in group contribution method for estimation of Gibbs free energy of formation

Molecular substructures with oxygen	$\Delta_{gr}G'^{0}$ **(kcal/mol)**
-OH	-41.50
-O-	-23.20
-O- (participating in ring)	-36.60
>C=O	-28.40
>C=O (participating in ring)	-30.10
-CH=O	-30.40
$-COO^{-1}$	-83.10
-O-CO-	-75.3
-O-CO- (participating in ring)	-71.0
Molecular structures with saturated carbon	
$-CH_3$	-3.65
> CH_2	1.62
> CH_2 (participating in one ring)	3.18
> CH -	5.08
> CH - (participating in one ring)	4.84
> CH - (participating in two fused rings)	2.60
> C <	7.12
> C < (participating in one ring)	7.17
> C < (participating in two fused rings)	-3.89
Molecular structures with unsaturated carbon	
= CH -	12.8
= CH - (participating in non-aromatic ring)	8.46
> C =	15.7
= CH_2	6.87
= CH - (participating in one aromatic ring)	4.93
> C = (one single bond and one double bond participating in an aromatic ring)	6.95
> C = (two single bonds participating in one nonaromatic ring)	11.7
> C = (participating in two fused nonaromatic rings)	16.7
> C = (participating in two fused rings: one aromatic and one nonaromatic)	6.77
> C = (double bond and one single bond participating in a ring)	32.1
> C = (participating in two fused aromatic rings)	-0.0245
≡ CH	60.7
≡ CH -	41.6

Gibbs free energy of formation of a model naphthenic acid

As an example, the estimation of $\Delta_f G_{est}^{\prime 0}\ for$ 4-butylcyclohexanecarboxylic acid is described below using the group contribution method. The molecular structure of 4 butylcyclohexanecarboxylic acid, $C_{11}H_{20}O_2$ is illustrated in Fig. 5.6.

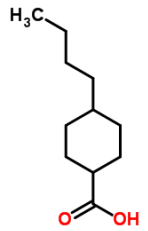


Figure A3-0-1. Molecular structure of 4-butylcyclohexanecarboxylic acid.

Table A3-0-2. Participated groups in calculation Gibbs energy of formation of $C_{11}H_{20}O_2$

Group	$\Delta_{gr}G^{\prime 0}$ (kcal/mol)	Number of instances
-OH	-41.50	
-O-	-23.20	
-O- (in ring)	-36.60	
>C=O	-28.40	
>C=O (in ring)	-30.10	
-CH=O	-30.40	
-COO-	-83.10	1
-CH3	-3.65	1
>CH2	1.62	3
>CH2 (in ring)	3.18	4
>CH-	5.08	
>CH- (in ring)	4.84	2
>C<	7.12	
>C< (in ring)	7.17	
>CH2 (in 2 fused ring)	2.6	

$$\Delta_f G_{est}^{\prime 0} = \sum_{i=1}^{N_{gr}} n_i \, \Delta_{gr} G_i^{\prime 0} =$$

$$(-83.1 \times 1) + (-3.65 \times 1) + (3 \times 1.62) + (4 \times 3.18) + (2 \times 4.84) = -59.49 \left[\frac{kcal}{mol}\right]$$

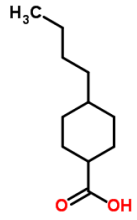
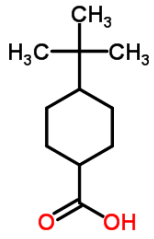
$$\Delta_f G^{\circ}_{est} = -59.49 \left[\frac{kcal}{mol}\right] = -248.90 \left[\frac{kJ}{mol}\right]$$

Thermodynamic Analysis of Different Catabolic Pathways

To see the differences in catabolic pathways, cumulative changes in Gibbs free energy in degradation of two model NAs are graphed.

Two model NAs are selected to show the thermodynamic favourability of different pathways.

Table A3-0-3Two model NAs for determination of thermodynamic favourability of catabolic pathwyas

Model NAs	Formula	ΔG^0_f (kcal/mole)	Molecular structure
4-Butylcyclohexanecarboxylic (I)	$C_{11}H_{20}O_2$	-59.49	
4-tert-Butylcyclohexanecarboxylic Acid (II)	$C_{11}H_{20}O_2$	-64.53	

Followed, degradation pathways of two above compounds (I) and (II) are shown. Selection of pathways was arbitrary. Following graphs represent that the cumulative Gibbs free energy in the pathway and also the length of the catabolic pathway. Understanding of thermodynamics pathway and determination of bottlenecks, and capacity for improvement of a pathway i.e. debottleneck of a thermodynamic bottleneck, elimination of a by-product by deletion of respected gene. For instance, for the 4-butylcyclohexanecarboxylic which is shown as compound

(I): the pathway 1 is shorter than pathway 2, in spite of the fact that they can have same end product (which result in the same Gibb free energy changes), pathway 2 is more thermodynamically favourable.

.

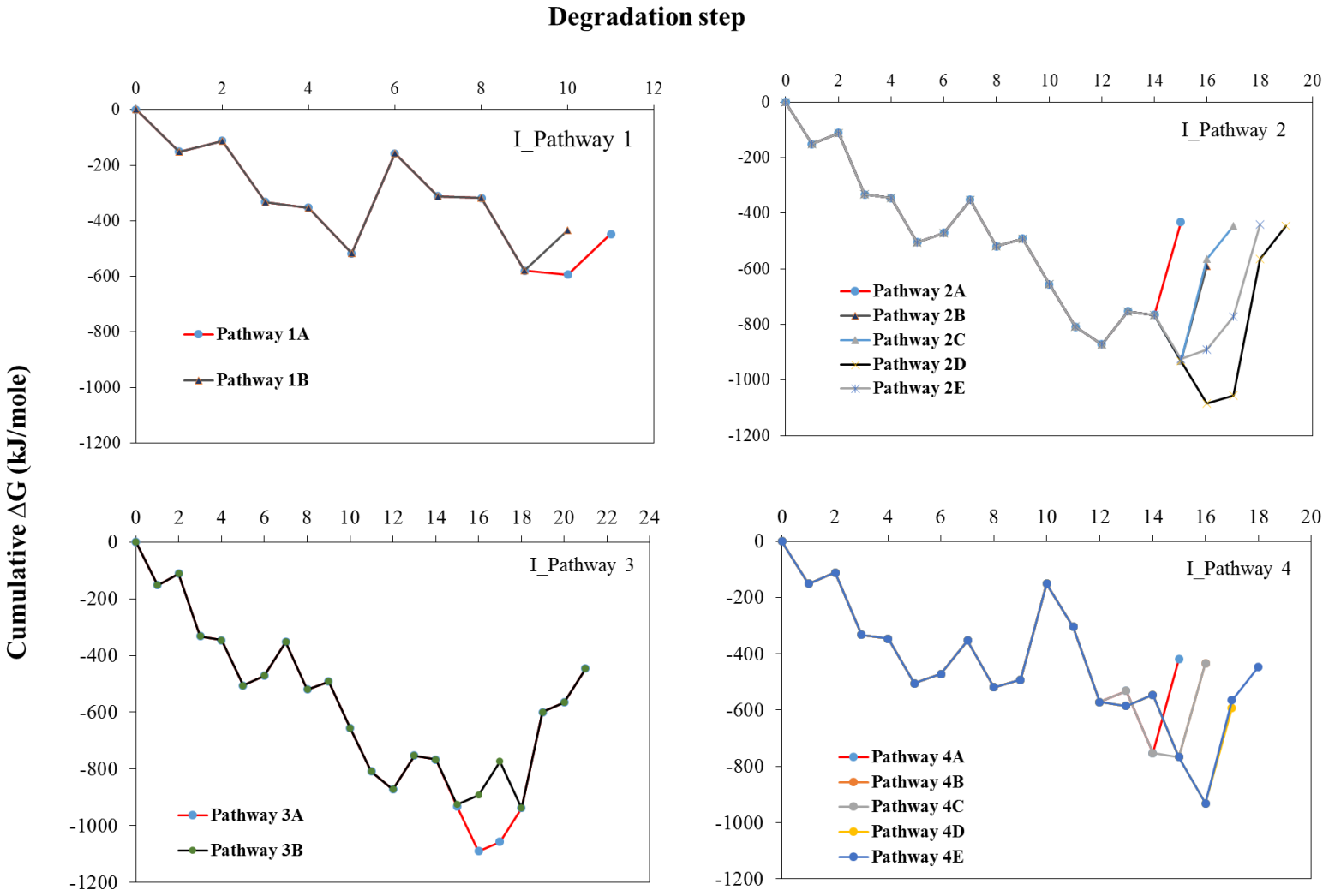


Figure A3 -0-2. Different catabolic pathways for biodegradation of 4-Butylcyclohexanecarboxylic

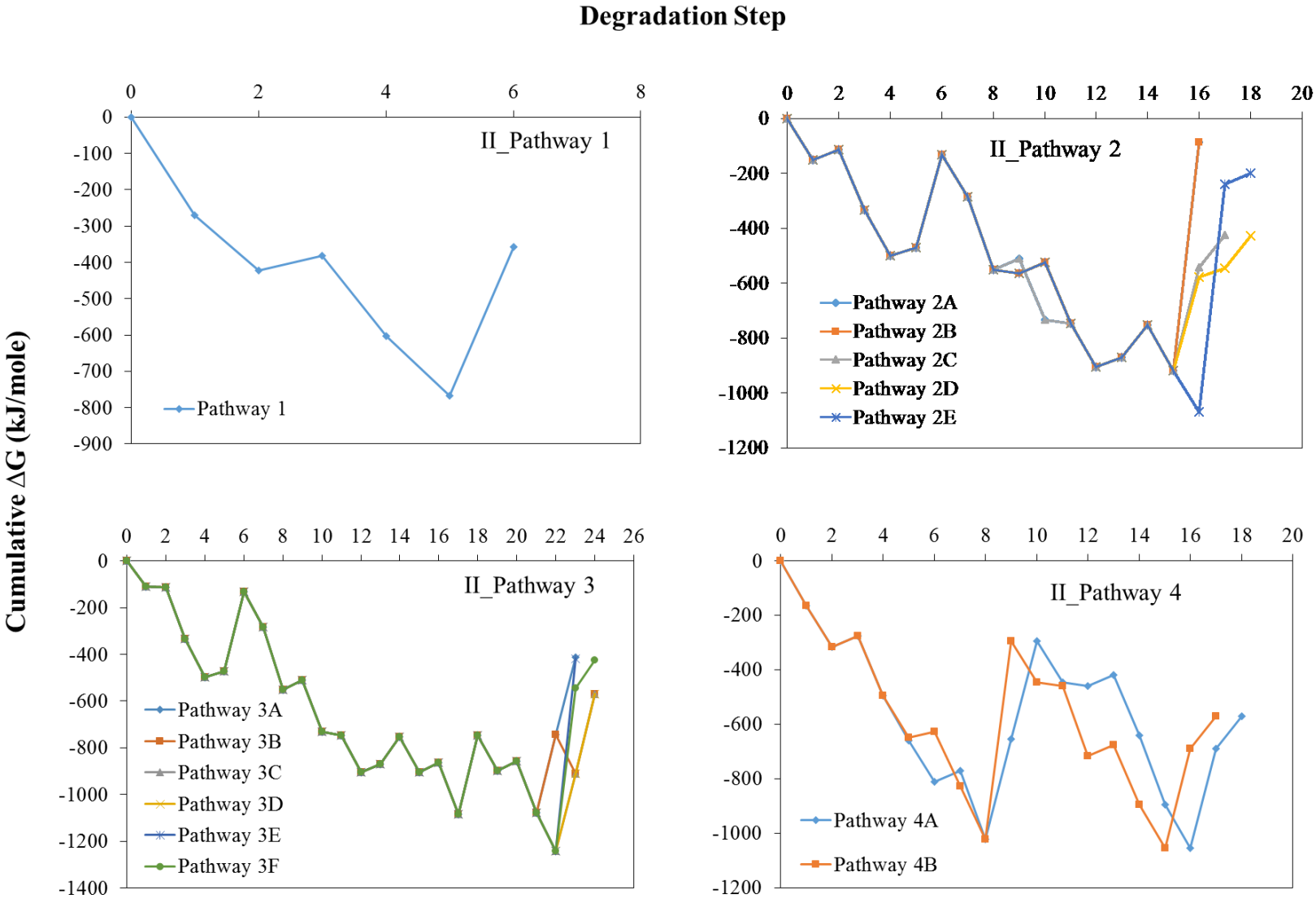


Figure A3-0-3. Different catabolic pathways for 4-tert-Butylcyclohexanecarboxylic acid

Another interesting result can be obtained by comparison of pathway 1 and 2 for compound II. Due to cumulative Gibbs free energy, it can be seen that pathway 2 of compound II is more favourable, however the last step is a huge thermodynamic bottleneck. It might be a possibility that a wild type microorganism(s) cannot overcome this issue. Therefore, pathway analyses create opportunities for improvement of biodegradation by engineered microorganisms.