Enhanced Degradation of a Model Naphthenic Acid Compound in Bioreactors

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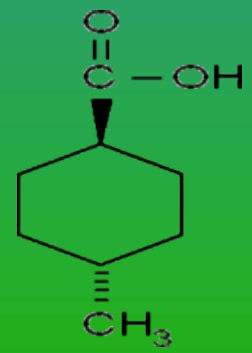
(Dr. John Headley)

October 25, 2007

Outline

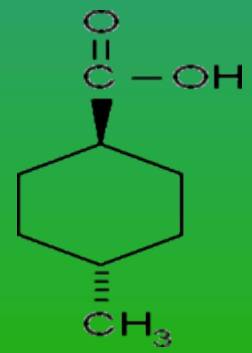
- Brief Overview of Oilsands
- Review of NAs and Background
- Objectives of this Study
- Materials and Methods
- Results
- Discussion and Direction



Background

- 1995 Goal: 1 million barrels/day by 2020
- Between 1995 and 2004, production rates increased to 1.1 million barrels/day
- Current rates projected to 2015:

  2.7 million barrels/day
- By 2030: 5 million barrels/day

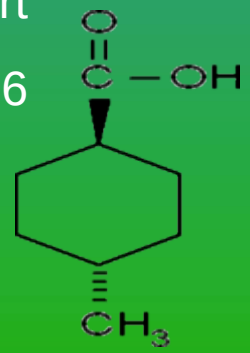


“Given these projections, the question of how to manage the associated environmental impacts becomes even more urgent.

The magnitude of the risks and opportunities is unprecedented in the history of Canadian energy production.”

- Pembina Report

August 23, 2006

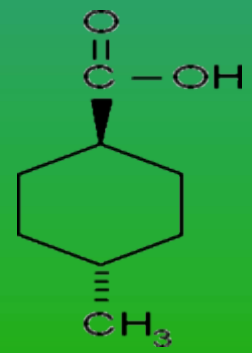


Clean-up approach still deemed a “mess” – Edmonton Journal

Photo by the Edmonton Journal, July 28, 2006

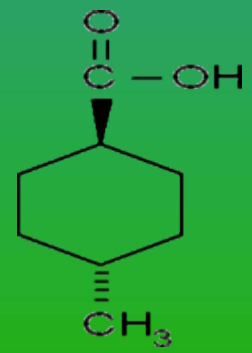
Background (cont'd)

- Naphthenic acids – natural organic compounds
- Corrosive to refinery processes
- Caustic soda extraction method for extraction of naphthenic acids



Background (cont'd)

- Naphthenic acids are toxic to aquatic algae and other micro organisms
- NAs are acutely toxic to aquatic organisms
- NAs have shown cellular respiratory inhibition in mammalian evaluations (Headley *et. al*, 2002)

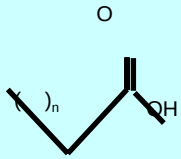


Naphthenic Acids: Review

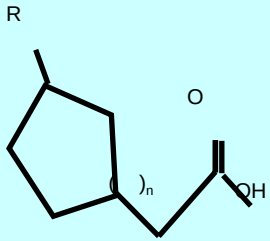
- Natural components found in most petroleum sources including the Athabasca oil sands
- Released from the oil sands during aqueous extraction at elevated pH
- General chemical formula: $C_nH_{2n+z}O_2$
- n indicates the carbon number
- *z* indicates hydrogen deficiency number

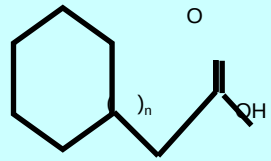
Structure of NAs

Z = 0

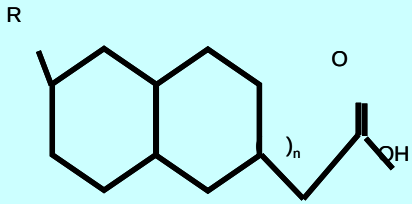


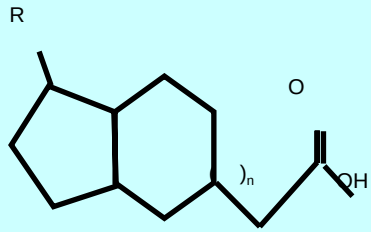
Z = -2

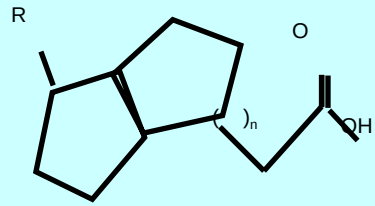




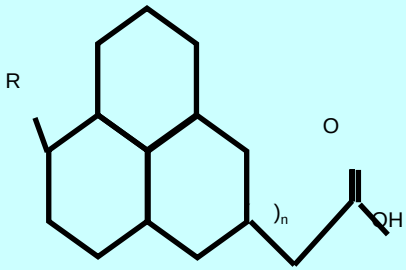
Z = -4

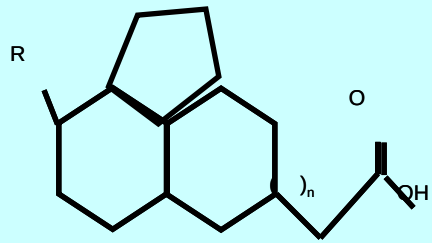


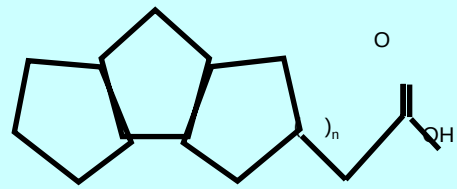




Z = -6







Naphthenic Acids (cont'd)

- Eventual biodegradation of petroleum leads to the formation of naphthenic acids
- Industrial uses: manufacture of tires and paints
- Used as lubricants, fuel additives and corrosion inhibitors
- Naphthates have been used as ground contact wood preservatives

Biryukova *et al*. 2007

Naphthenic Acids (cont'd)

- Oil Sand is 10 – 12% Bitumen (Clemente et. al. 2005)
- Average naphthenic acid concentration 200 mg/kg ore (Syncrude)
- Syncrude processes approx. 500 000 tonnes of ore/day
- 100 tonnes of naphthenic acid from ore each day!

What do we know?

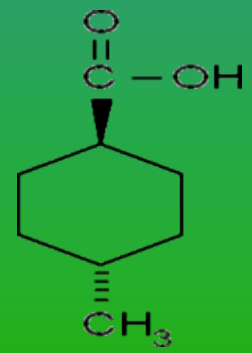
- We can achieve microbial growth in both commercial NAs and tailings pond water
- Microbial growth varies within the range of NA molecular structures
- There has been no separation and identification of individual NA compounds (Scott *et al*., 2005)

What do we know?

- Some success with biodegradation of standard NAs
- Biodegradation of tailings NAs is considerably slower (Scott *et al*., 2005)
- Low molecular mass NAs ($<C_{18}$) are more readily biodegraded than high molecular mass NAs (Scott *et al*., 2005)
- Toxicity is dominant in lower molecular weight NAs ($<C_{22}$) (MacKinnon, 2001)

What we need to do?

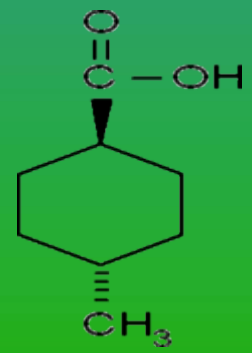
- Understand and quantify the growth kinetics of biodegradation for individual compounds as a basis for evaluating and engineering enhanced biodegradation systems



Objectives

- To develop a microbial consortium capable of biodegrading a NA model compound
- To experimentally determine the biokinetic parameters associated with biodegradation of the model compound
- To study the biodegradation of a model NA in bioreactors with freely suspended cells

Selection of a Model Compound

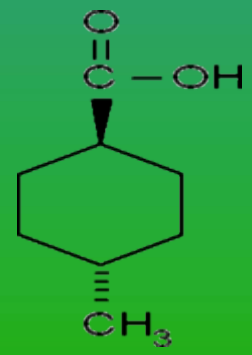


Trans 4-methyl-1-cyclohexane carboxylic acid

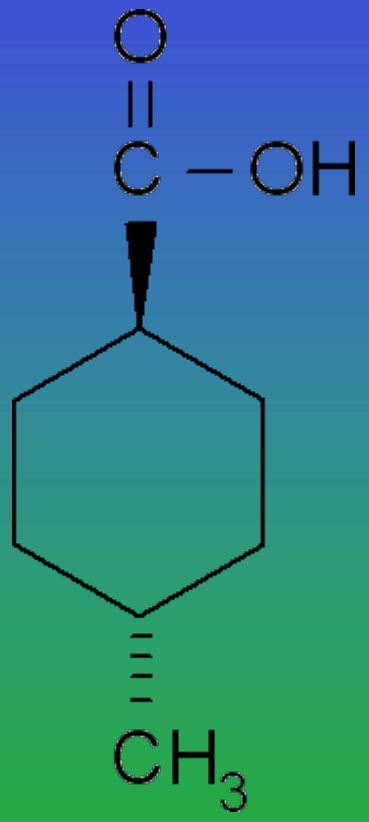
$CH_3C_6H_{10}CO_2H$

Molecular Weight
142.20

CAS Number
13064-83-0



Trans 4-methyl-1-cyclohexane carboxylic acid



$CH_3C_6H_{10}CO_2H$

Molecular Weight

142.20

CAS Number

13064-83-0

- Sigma-Aldrich

Why 4MCHCA trans?

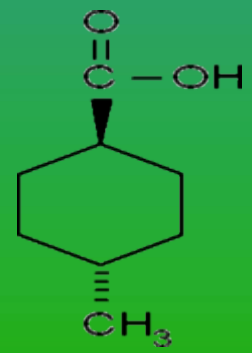
- Readily soluble similar to NAs in tailings water
- Trans isomers seem to be more consistently degradable (Tanapat, 2002)
- n=8 (C_8) outside the range of strong sorption to soils (Janfada, 2006)
- Within the range of NA compounds that are more toxic to fish ($<C_{22}$)
- Timely and direct quantitative analyses

Materials and Methods

- **A timely and reliable analytical method**
- A consortium capable of degrading the model compound
- A consistent means of measuring the microbial concentration
- Bioreactors
- Toxicity reduction evaluation

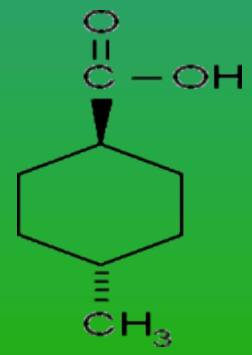
Materials and Methods

- A timely and reliable analytical method
- **A consortium capable of degrading the model compound**

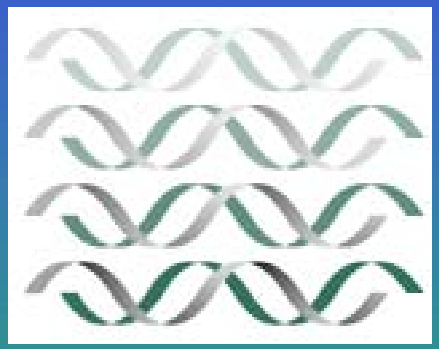


Development of a Microbial Consortium

- *Pseudomonas putida* (ATCC 17484)
- Culture developed using tailings pond water
- Culture developed using commercial NAs



Microbial Identification



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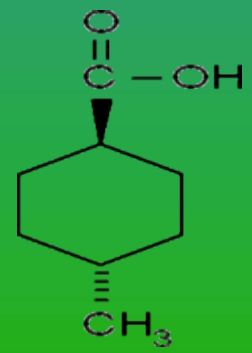
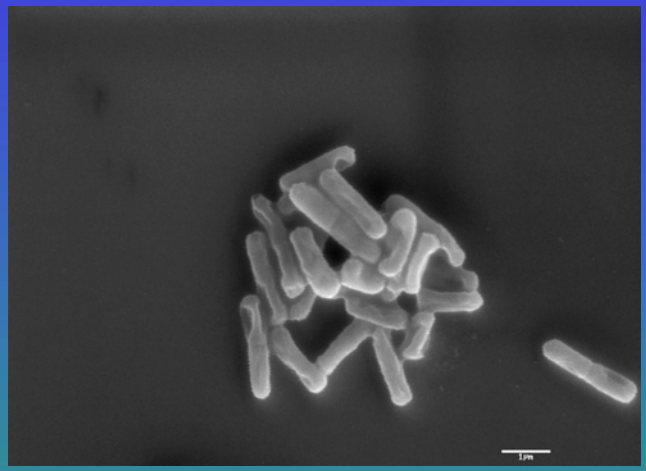
Variovorax paradoxus

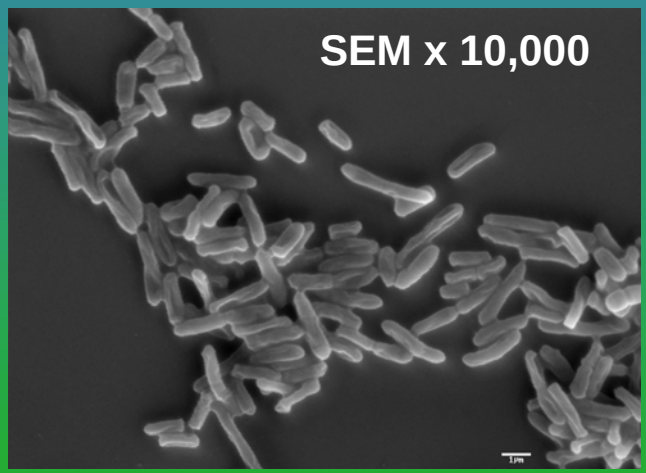
(Alcaligenes paradoxus)

Pseudomonas putida



Mixed Culture





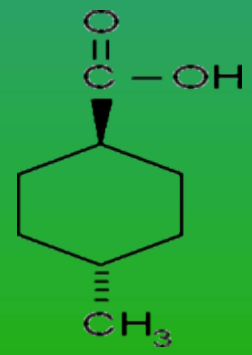
Psuedomonas

http://www-micro.msb.le.ac.uk

- Gram-negative
- Rod shaped
- Approximately 1 to 3 microns in length

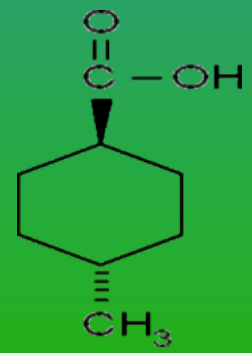
Materials and Methods

- A timely and reliable analytical method
- A consortium capable of degrading the model compound
- **A consistent means of measuring the microbial concentration**



Measurement of Biological Growth

- Spectrophotometer (optical density)
- Most probable number (MPN)
- Plate counts



Materials and Methods

- A timely and reliable analytical method
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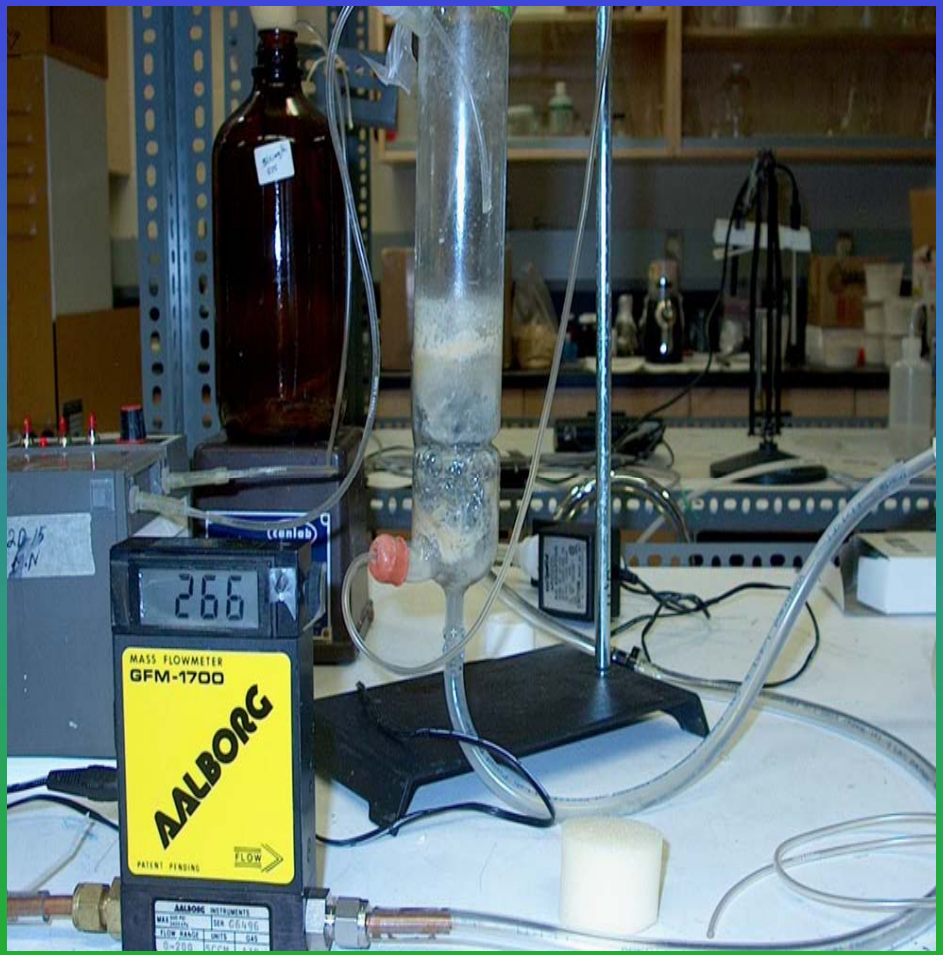
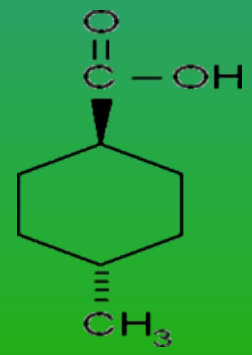
- **Bioreactors (batch; continuous; immobilized cell)**

Continuous Stirred Tank Reactor

Immobilized Cell System



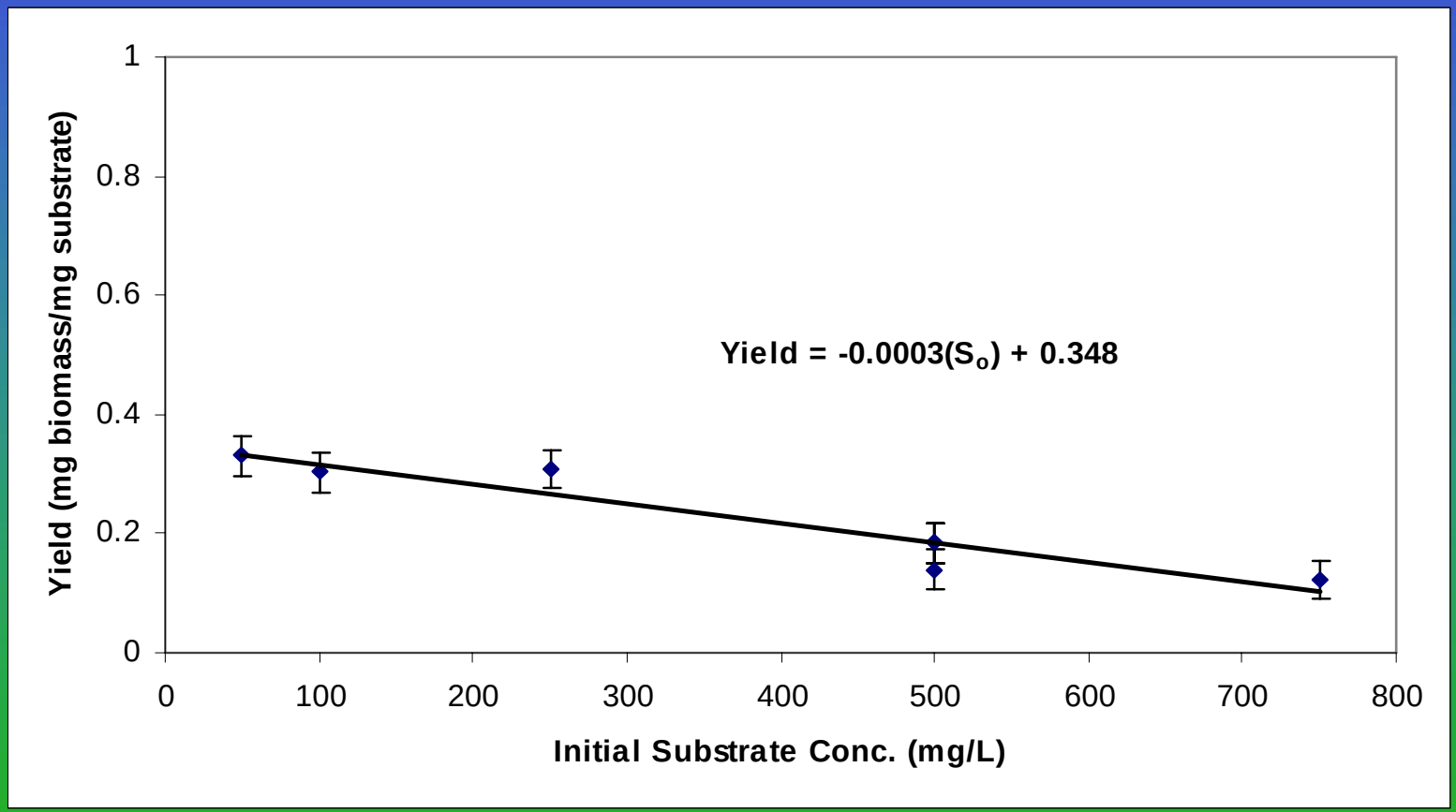
Results



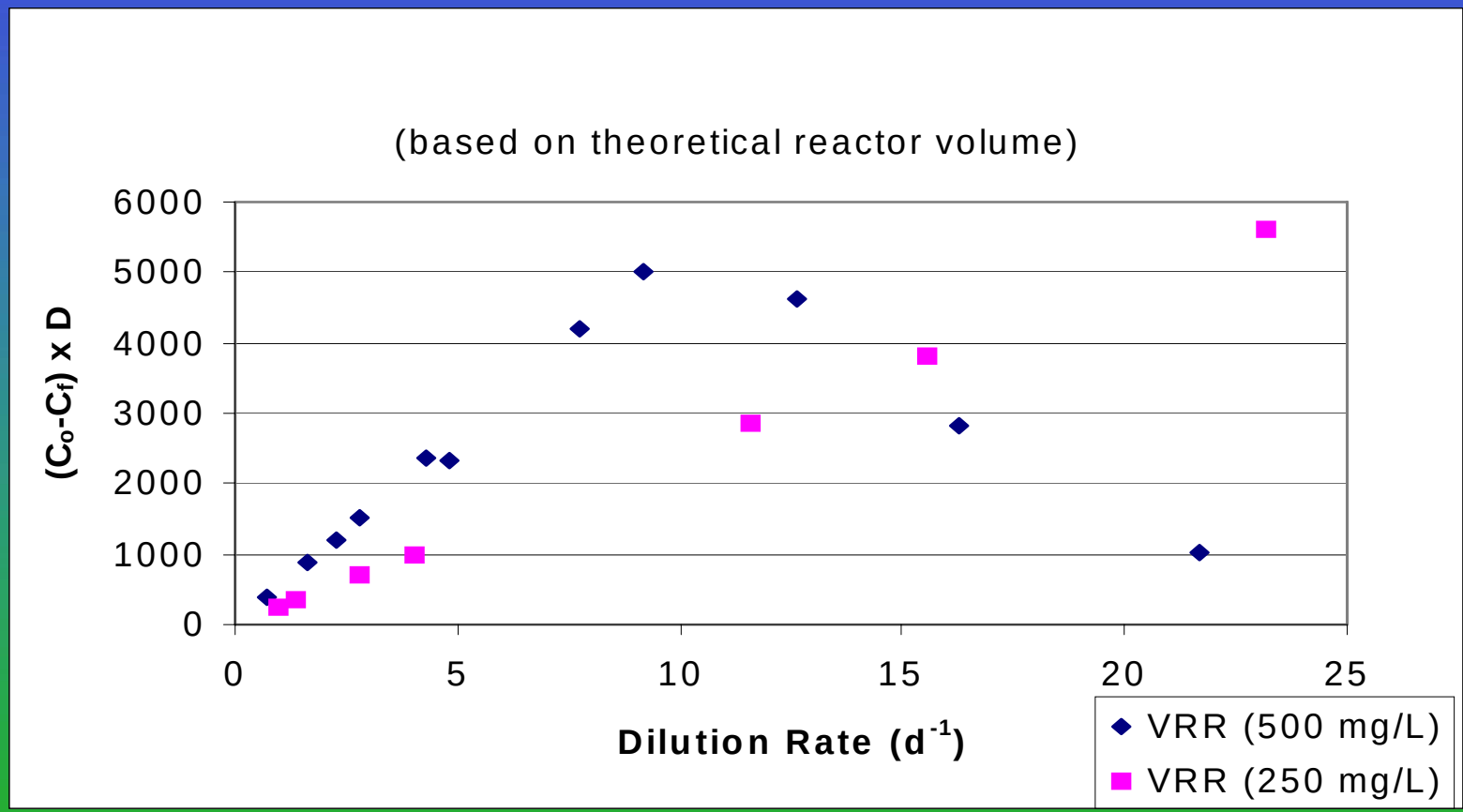
Quantifying Growth and Biodegradation Kinetics

- Microbial growth
- Specific growth rate
- Substrate utilization
- Removal rate of model NA
- Yield

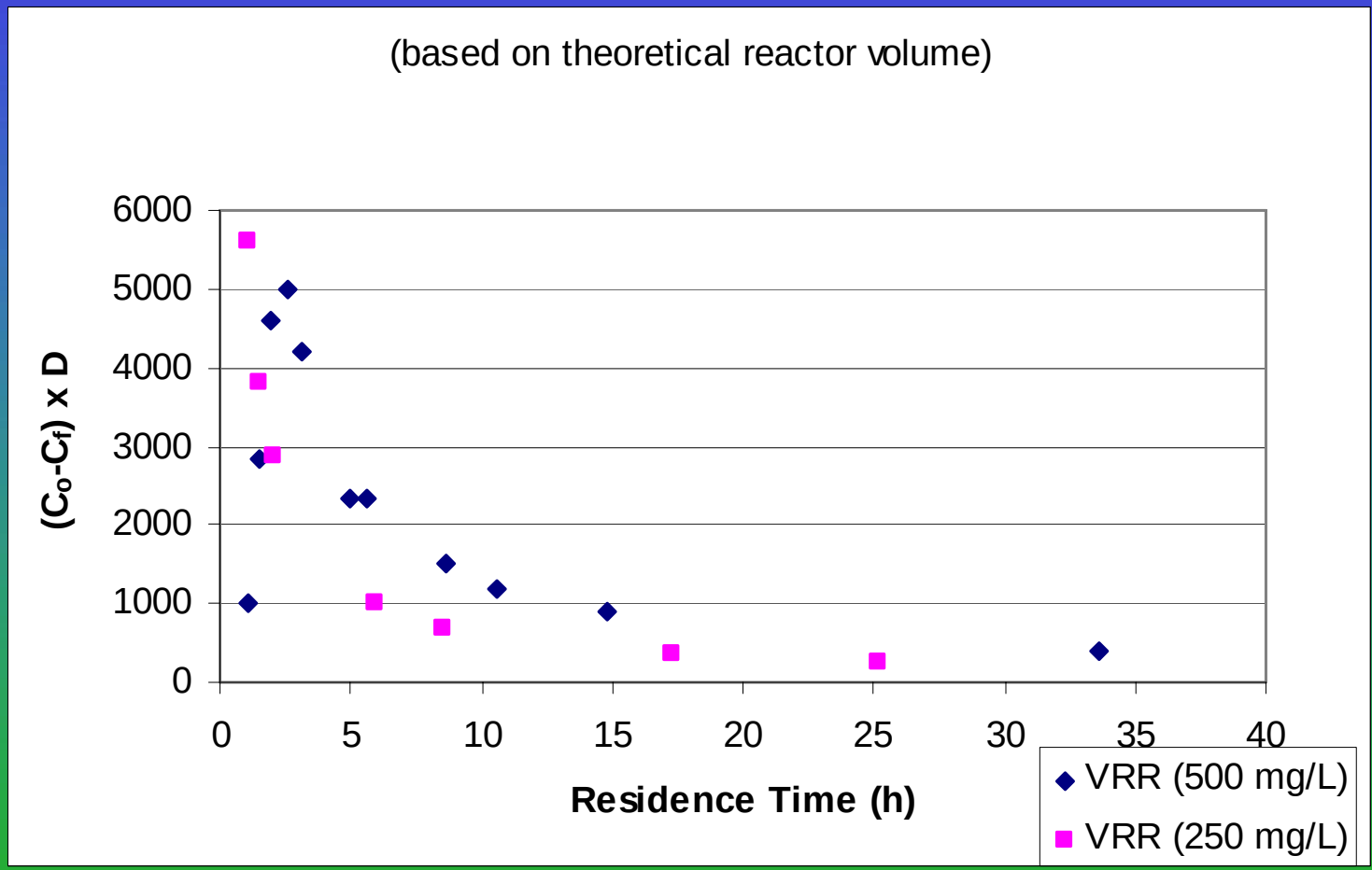
Yield v. Initial Substrate Concentration



Volumetric Reaction Rate Immobilized Cell System



Immobilized Cell Reactor (Residence Times)



Summary

- Yield for microbial degradation was 0.3 mg biomass/mg substrate
- The growth of this consortium on the model NA compound is up to 5 times slower than that of other environmental contaminants
- The maximum degradation rate occurred in the immobilized cell system

Discussion

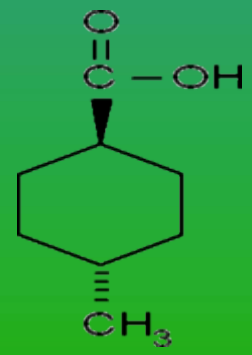
- The observed degradation rates increased significantly from batch systems to a continuously stirred tank reactor and an immobilized cell system
- The most efficient residence time decreased from 40 to 2.4 hours between the CSTR and the immobilized cell system (17 times more efficient)

Future Direction

- Continued evaluation of biokinetics in an immobilized cell system (biofilms in an immobilized cell system)
- Evaluation of the substrate capacity of the immobilized cell system
- Evaluation of the biokinetics of an immobilized cell system and continuous reactor in varying environmental conditions

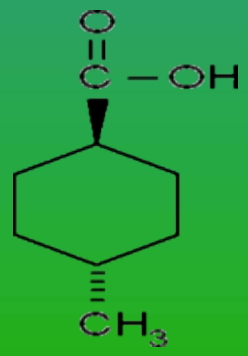
Acknowledgements

- University of Saskatchewan
- National Water Research Institute
- Natural Science and Engineering Research Council of Canada
- Canadian Council of Professional Engineers



Acknowledgements

- Dr. Hill and Dr. Nemati, Chemical Engineering, U of S
- Dr. Headley, NWRI

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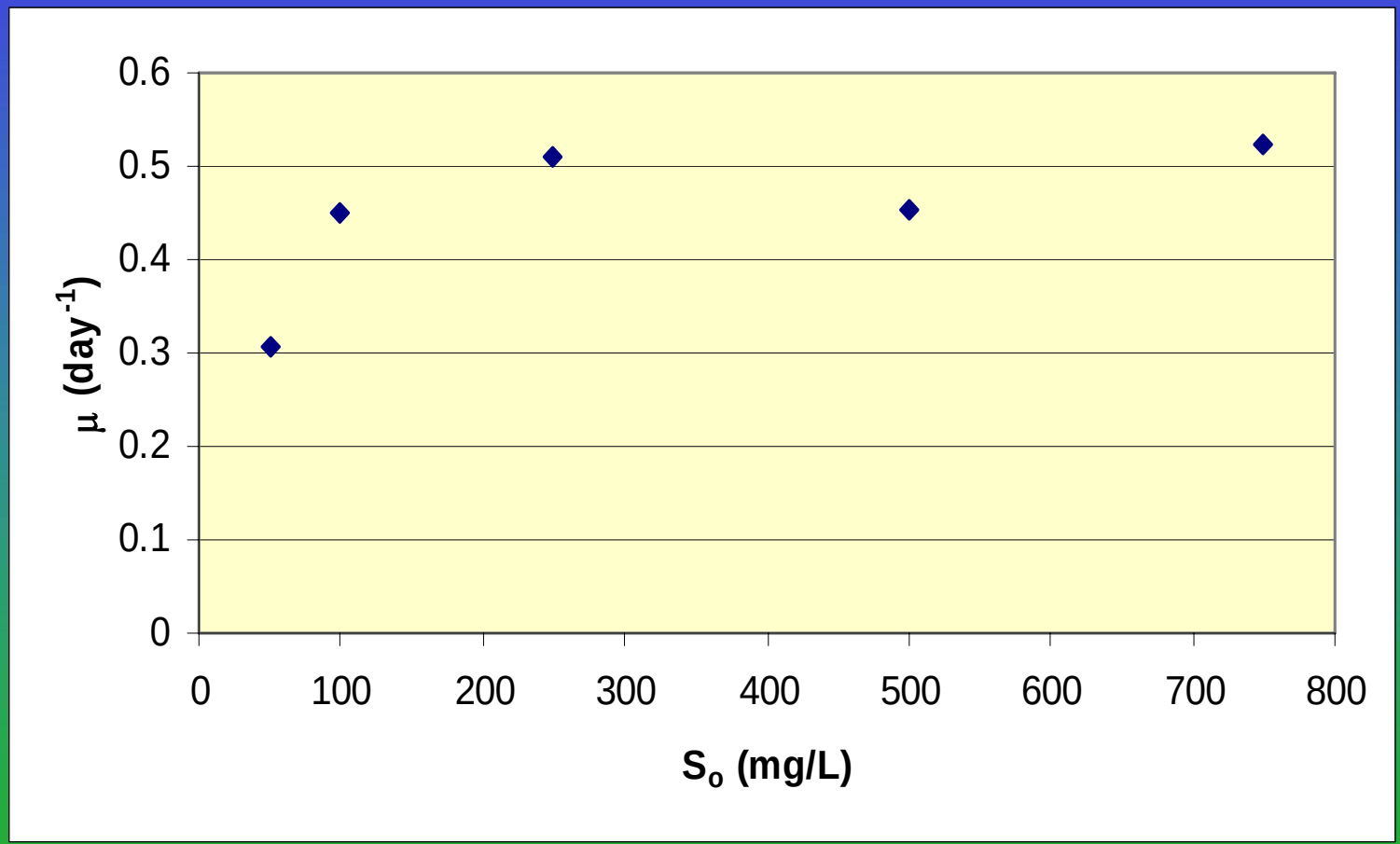
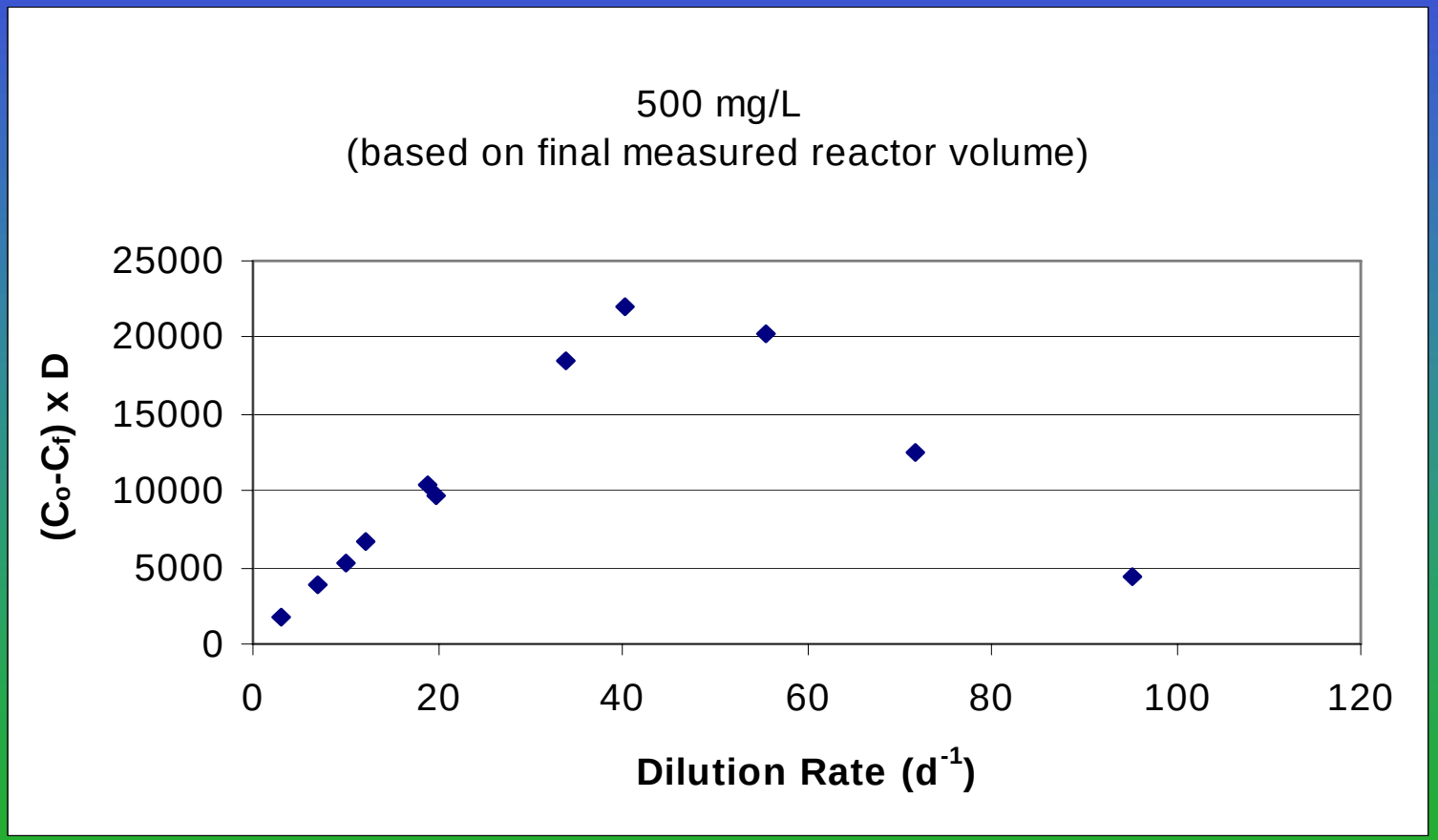
Comments and Questions?

Specific Growth Rate v. S_o



Volumetric Reaction Rate Immobilized Cell System

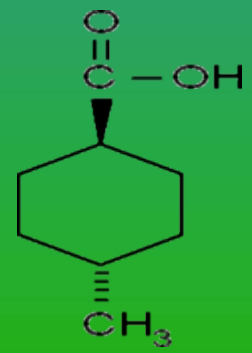


Materials and Methods

- A timely and reliable analytical method
- A consortium capable of degrading the model compound
- A consistent means of measuring the microbial concentration
- **Bioreactors (batch; continuous; immobilized cell)**
- **Toxicity reduction evaluation**

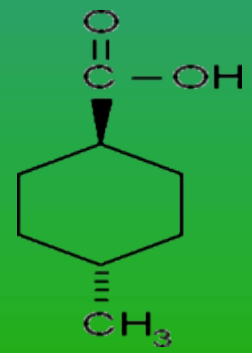
Toxicity Reduction Evaluation

- Acceptable toxicity bioassays
  - EUB Guide 50: Microtox^{R} EC50; *Daphnia* magna (LC50; EC50)
  - *Artemia* salina toxicity test



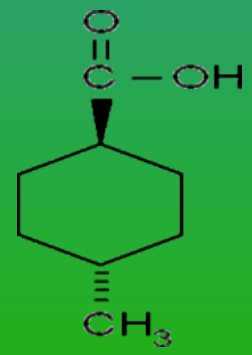
Toxicity Reduction Evaluation Results

- *Daphnia* magna (LC50)
- Microtox^{R} (EC50)
- *Artemia* salina toxicity reduction test



Daphnia magna 48 hour Acute Toxicity Test (LC50)

Treated Effluent	**Stock Solution** (500 mg/L)
79.5%	35.4%



Microtox^{R} Toxicity Test (EC50)

Treated Effluent	**Stock Solution** (500 mg/L)
>82%	14%