CANADIAN LEADER IN
ENVIRONMENTAL EXPERTISE
& SPECIALIZED PRODUCTS

CHEMCO inc.

Enhanced Bio-Remediation of heavy petroleum hydrocarbons and PAHs through dedicated endogenous microorganisms bio-stimulation

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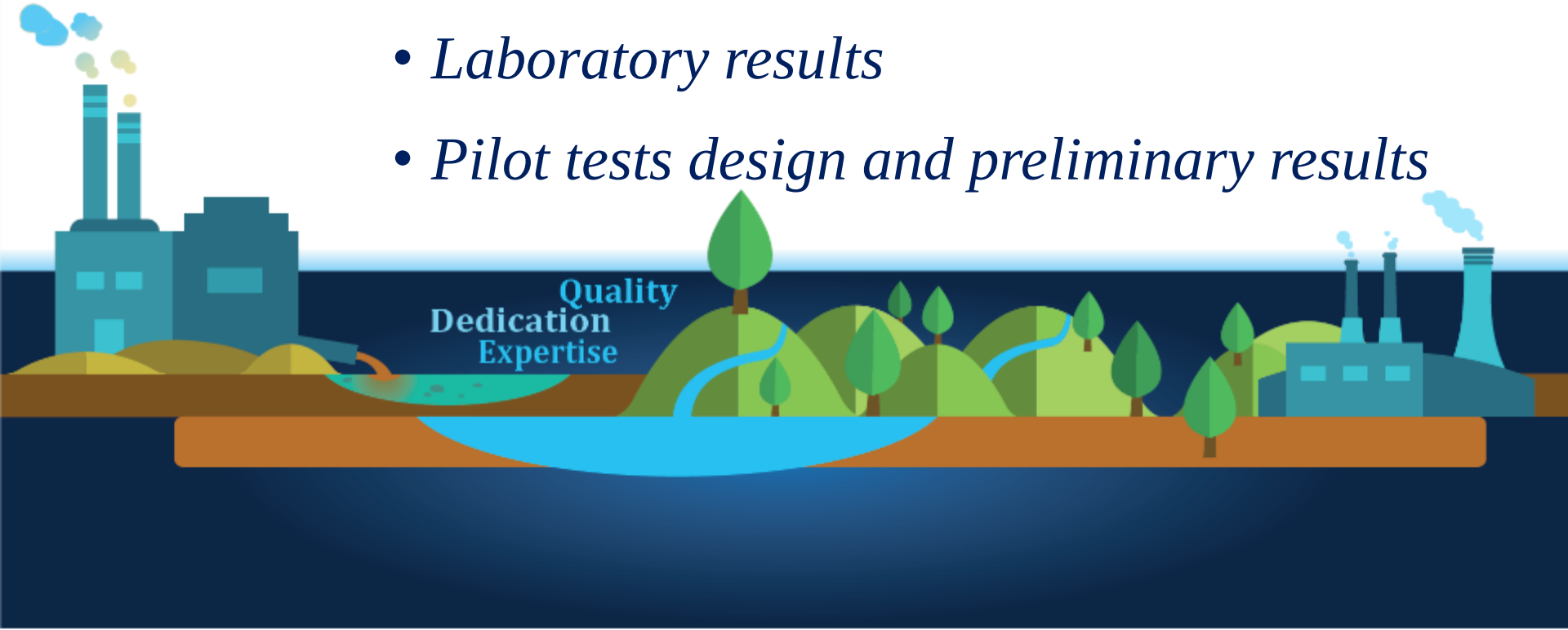
October 2017

Presentation Outline

- *About us*
- *Bio-Remediation – Principles*
- *ChemBio Formulation*
- *Laboratory results*
- *Pilot tests design and preliminary results*



About us

- Canadian Company founded in 1988
- **<u>Production</u> and warehouses throughout Canada**
  - Quebec
  - Ontario
  - Alberta
  - British Columbia
- **Sectors of activity:**

  - Industrial and Municipal Potable & Waste Water
  - Contaminated Soil and Groundwater
  - Air, Odours and Atmospheric Emissions (Activated Carbon, filtering medias)
  - Process Water & Thermal Exchange Fluids (Glycols)
  - Drilling Fluids (Oil and Gas & Diamond exploration)
  - Aircraft De-icing Fluids

Training & Education

Expert Technical Team

Field-Proven Technologies

Field Support

R&D and Treatability Laboratories

Technical and Design Support

Mixing and Handling Equipment

CANADIAN LEADER IN
ENVIRONMENTAL EXPERTISE
& SPECIALIZED PRODUCTS

- Chemical Oxidation
- Chemical Reduction
- Co solvent-Surfactant Washing
- Enhanced Bioremediation

Our product and services

CHEMCO inc.

Excellence & Science through proud Suppliers & Partners

ADVANCED OXIDATION TECHNOLOGY (AOT) *Since 2005*

PeroxyChem *Since 2005*

CARBONE CANADA INC *Since 2014*

CARUS® *Since 2014*

EthicalChem An Ethical Solutions, LLC Division *Since 2016*

EOS Remediation, LLC *Since 2016*

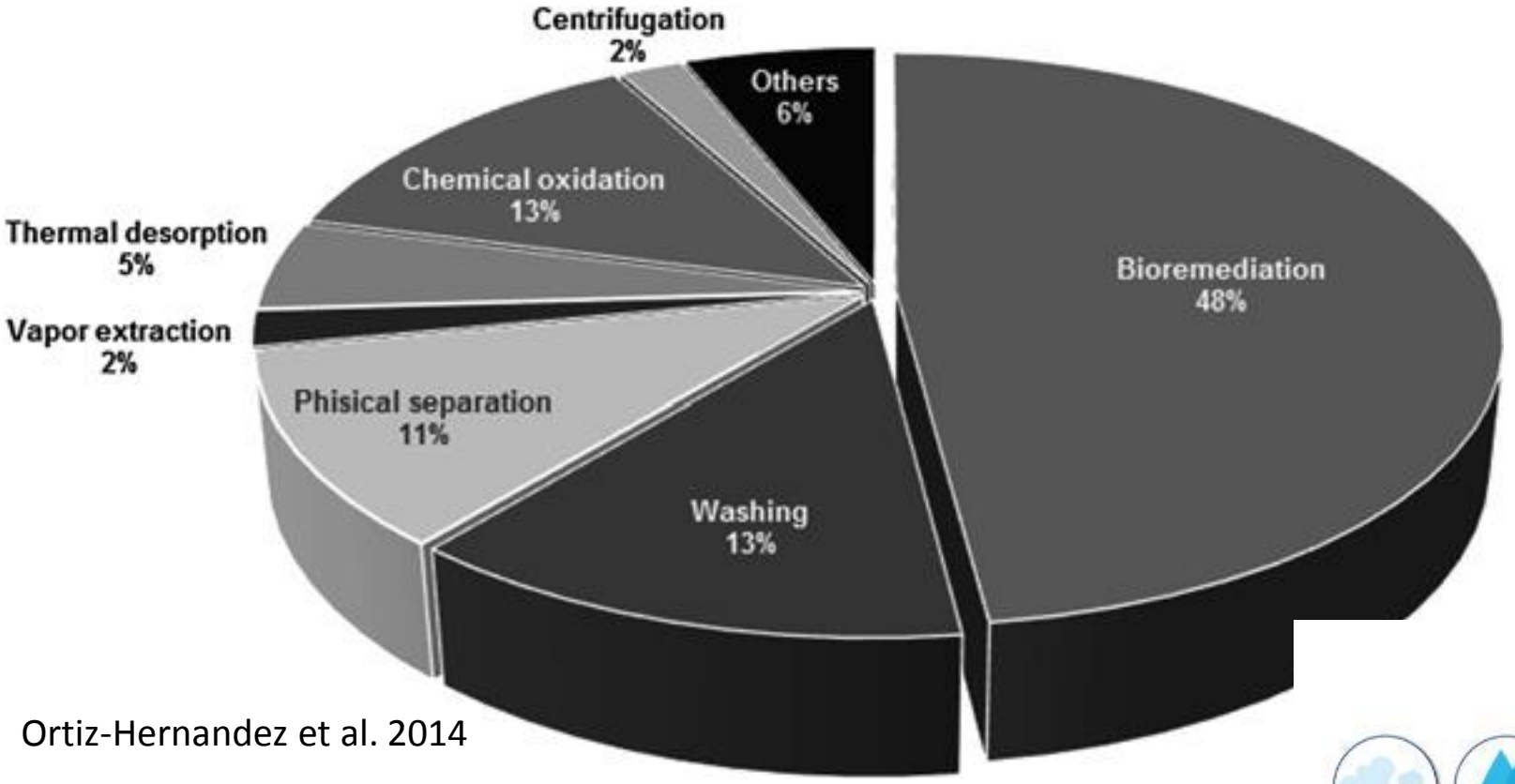
IVEY-SOL® Surfactant Technology www.iveyinternational.com *Since 2017*

RioTinto QMP *Since 2017*

SiREM *Since 2017*

Bio-Remediation – Introduction

- Relevance



Ortiz-Hernandez et al. 2014

Bio-Remediation – Principles

- Bio-Augmentation: Addition of exogenous microorganisms.

  Issues:

  -Environmental concerns and legislations around the introduction of these organisms in an aquifer or soil matrix

  -Adaptability, competition

- Bio-Stimulation: Stimulation of endogenous microorganisms.

  Issues:

  -Targeting the specific organisms that are competent in the degradation of the contaminants

Bio-Remediation – Principles

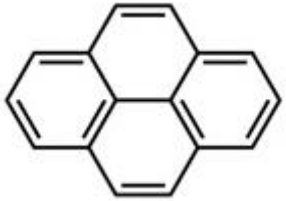
Optimizing Bio-degradation process:

Step 1-Feeding the beasts:

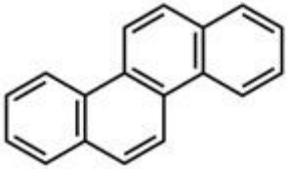
- Assuring the presence of
  temperature) & all esser
- In the soil, microorganis
  They need large amount
  energy source to functio
- Different substrates = di
  - Simple carbohydrate
    living things
  - Lipids: require lipase
  - Starch: require amyl
  - Cellulose: require ce
  - Lignin: require special oxidative enzymes (peroxidase, laccase)

Bio-Remediation – PAHs

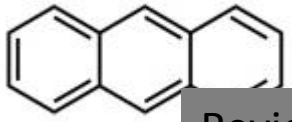
PAHs



Pyrene

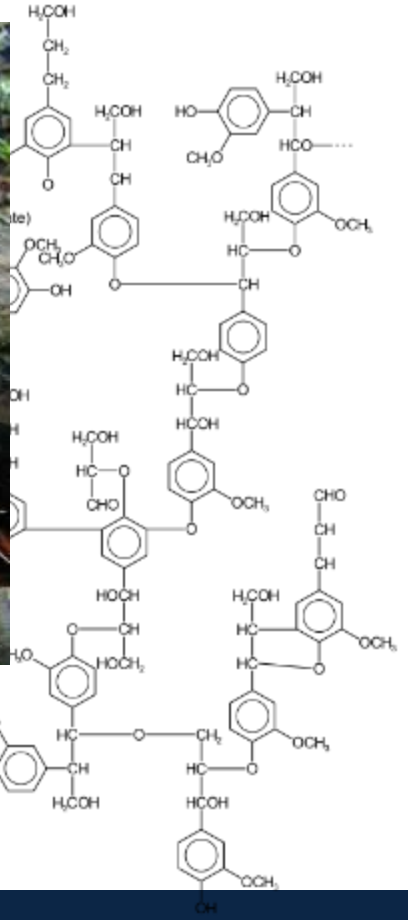


Chrysene



Anthrac

Lignin



Review: **Mushroom as a product and their role in mycoremediation,** S. Kulsheshtha et all, 2014.

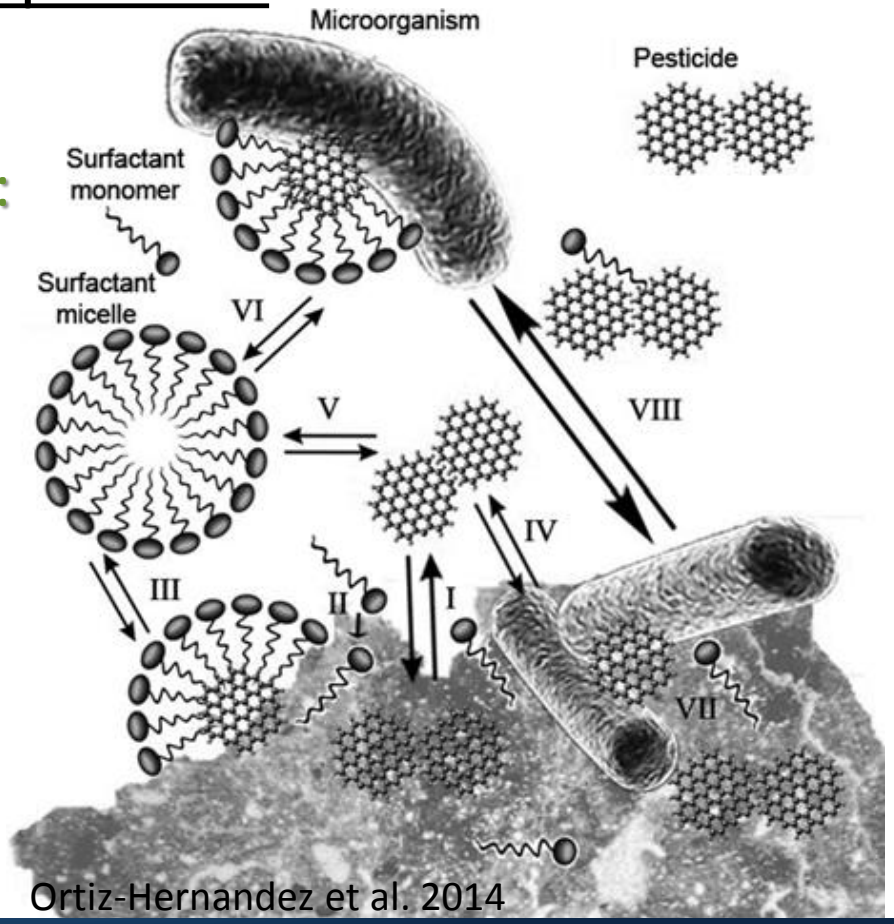
Bio-Remediation – Principles

Optimizing Bio-degradation process:

Feeding the beasts

Step 2-Enhancing bioavailability:

- Adding tension-active compounds (surfactant)
- Favoring the production of Bio-surfactant
- Increasing and maintaining high temperatures (35-55^{o} C)



Ortiz-Hernandez et al. 2014

Bio-Remediation – Principles

Optimizing Bio-degradation process:

Step 3-Controlling the pH & Modifying the ORP

Controlling the pH

-Extremes: 1-4 and 9-12 ➔ lower biological activity

-Slightly alkaline: 8-9 ➔ risk of NH_3 toxicity and discourage fungi

-Slightly acidic to neutral: 5-8 ➔ Promote bio-diversity

Modifying the ORP and the populations

Contaminant degradation mechanism can be positively increase by conducting a chemical shock before starting the aerobic bio-degradation.

Plenty of oxygen

-Increase the decontamination speed and efficiency

-Air movement allows for the elimination of gaseous and toxic by-products

Just enough water (will vary depending on soil type)

-Too little: Molecules stop to move and process stall

-Too much: Air movement is impaired, anaerobic conditions take place, process slow down.

ChemBio-formulation

ChemBio composition: Integration of all the bio-degradation principles

- Feeding the competent species: Specific energy sources and a large panel of nutriments
- Surfactant presence that further increase bio-surfactants production
- Initiate slightly acidic conditions
- Increasing the ORP and challenging the populations
- **Low cost**

Note: Addition of wood chips for improved soil structure and induce PAHs degradation (for fine grain geology and/or contaminant type)

ChemBio-formulation

Bio-stimulants drastically change the nature of the predominant microorganisms

Bacteria driven Remediation →

Fungi driven Remediation →

Enhanced Bio-Remediation - Tools, testing and tricks

Adjusting the formulation before you get to the field

Do competent microorganisms are naturally present in the soil matrix ?

Carbone source added: Heavy alcanes (C20-C50)

No carbone source added

Enhanced Bio-Remediation - Tools, testing and tricks

Adjusting the formulation: Laboratory testing

Duration: one week (growth pattern, pH Control, etc.)

Enhanced Bio-Remediation – Laboratory results

Mixte-L01 Silty sand

	T0	T28	M1 40-1-1	M1 40-1-1	M1 40-1-1 BP	M1 40-1-1 BP	Moyenne	Reduction %	Reduction mg/Kg
F2 (10-16 C)	240	20	0	0	0	0	0	-100	240
F3 (16-34 C)	1935	1605	980	840	790	820	820	-58	1115
F4 (34-50 C)	6170	4650	2810	2330	2300	2370	2370	-62	3800
F4g (>50 C)	15150	13850	6600	5400	6700	6440	6440	-57	8710
Total	23495	17245	10390	8570	9790	9630	9630	-59	13865
PAH	212,5	240,5	242	181	122	136	129	-39	84

THP-25 Heavy clay

	T0	T28	T1 40-1-1	T1 40-1-1	T1 40-1-1 BP	T1 40-1-1 BP	Moyenne	Reduction %	Reduction mg/Kg
F2 (10-16 C)	10	15	50	40	30	40	40	300	-30
F3 (16-34 C)	2315	1530	210	180	290	210	222,5	-90	2093
F4 (34-50 C)	660	720	20	30	100	50	50	-92	610
Total	2985	2265	280	250	420	300	312,5	-90	2673

Data in mg/Kg T0: Initial concentration BP: Wood chips

Enhanced Bio-Remediation – Pilot tests

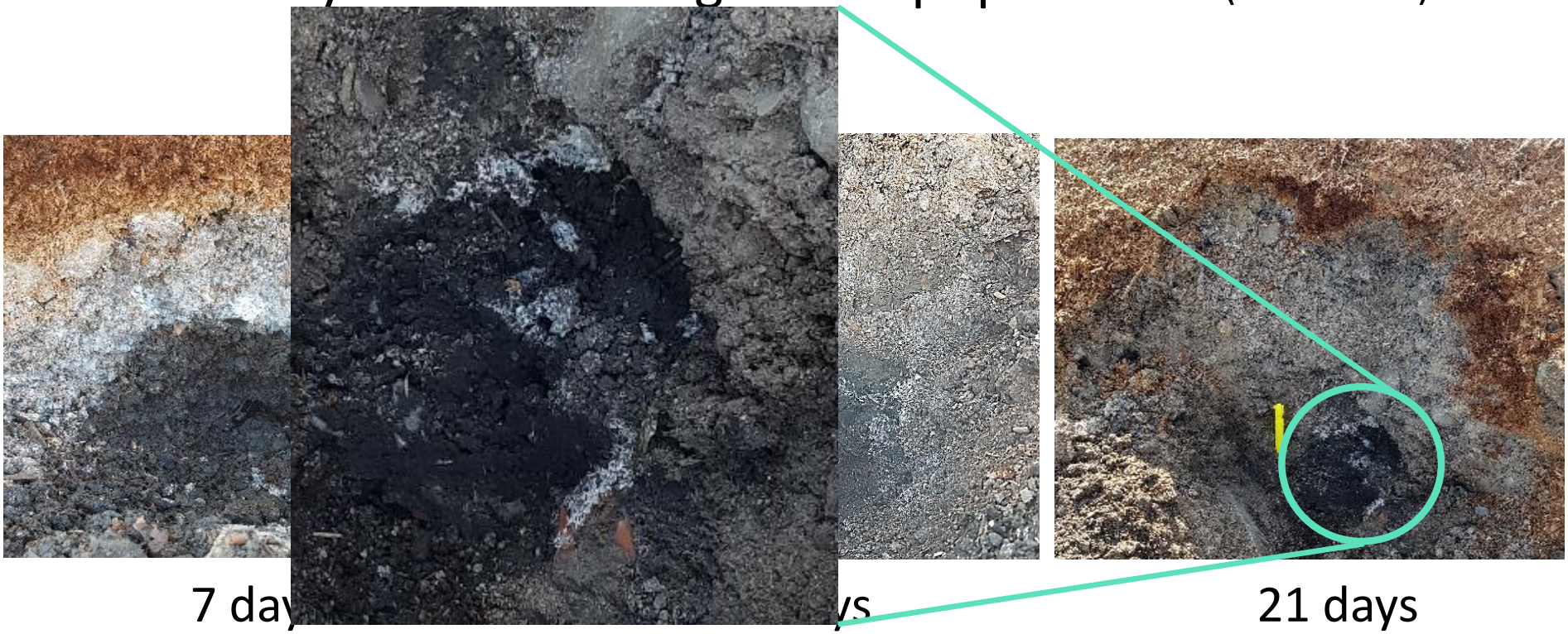
Getting to the field: Preliminary design

- Passive aeration: applicable in remote areas
- Wood chips covering: Isolation, O_2 and NH_3 exchange, watering, monitoring…
- 1.5m height: reduce compaction

Enhanced Bio-Remediation – Pilot tests

Preliminary results

- Two layers of microorganisms populations (Mixte-L01)



7 day ys 21 days

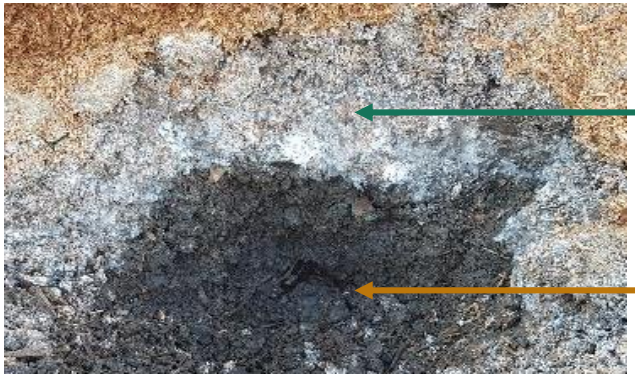
Enhanced Bio-Remediation – Pilot tests

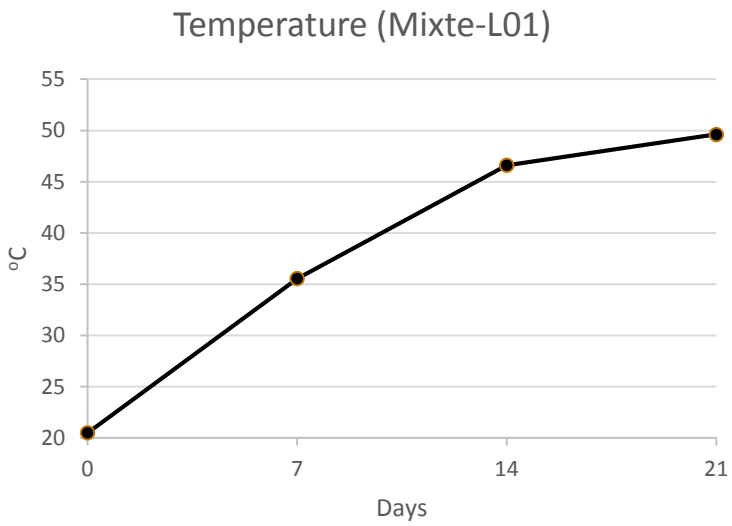
Preliminary results

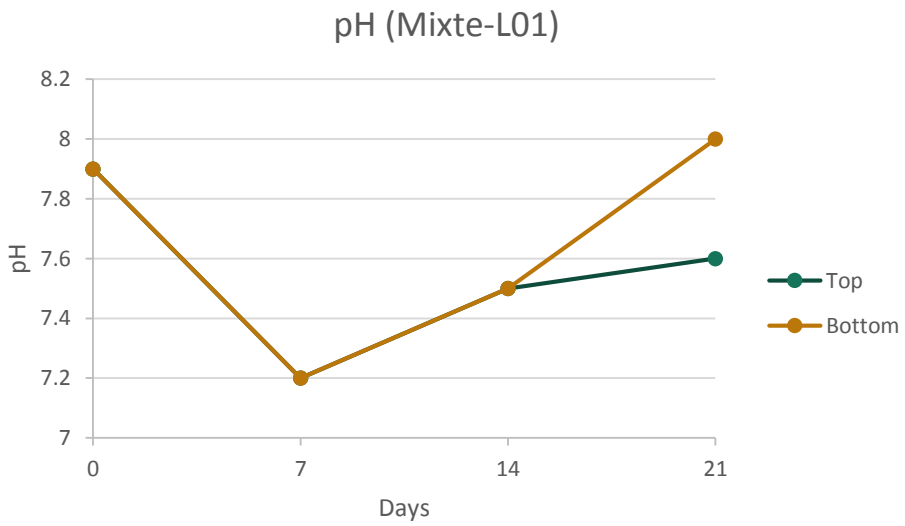
- Heat

Enhance Bio-Remediation – Pilot tests

Mixte-L01

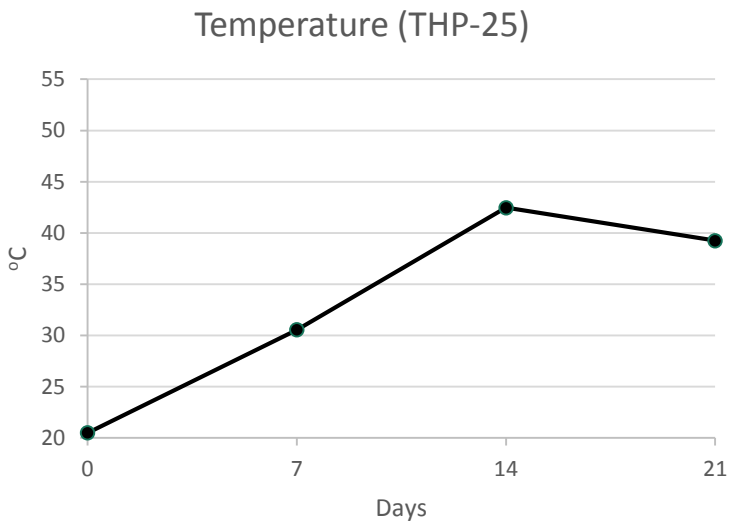


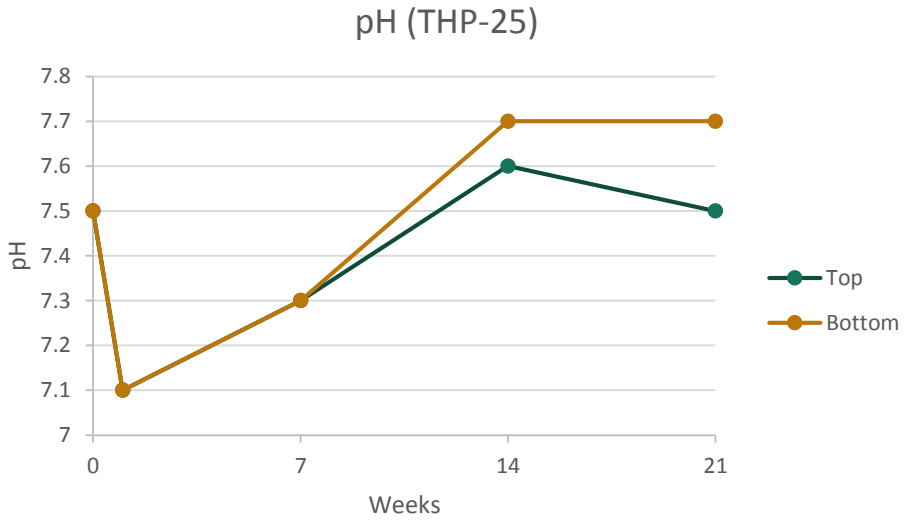




Enhanced Bio-Remediation – Pilot tests

THP-25





Enhanced Bio-Remediation – Pilot tests

Mixte-L01 – Silty Sand

Total petroleum hydrocarbon (mg/Kg)

Before treatment

C>10-C16 (F2)	246
C>16-C34 (F3)	1698
C>34-C50 (F4)	4381
C>C50 (F4g)	11142
Total	**17467**

Days after treatment

7	**14**	**21**
335	185	180
1730	1525	1760
1048	1155	947
6570	4205	0
9683	**7070**	**2887**
N/A	456	373
N/A	2240	2645
N/A	1020	1008
N/A	12100	0
N/A	**15816**	**4026**

Enhanced Bio-Remediation – Pilot tests

Mixte-L01 – Silty Sand

Total PAHs (mg/Kg)

Before traitement

Total	**186**

	Days after traitement		
	7	14	21
Top	160	153	**120**
Bottom	N/A	195	163

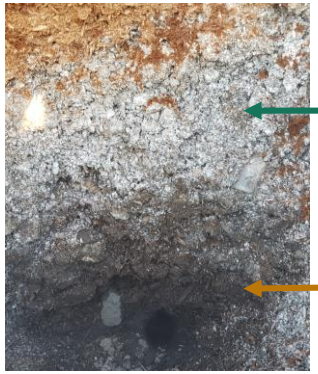
Enhanced Bio-Remediation – Pilot tests

THP-25 – Heavy Clay

Total petroleum hydrocarbon (mg/Kg)

Before traitement

C>10-C16 (F2)	750
C>16-C34 (F3)	10950
C>34-C50 (F4)	5740
C>C50 (F4g)	24250
Total	**41690**



Top

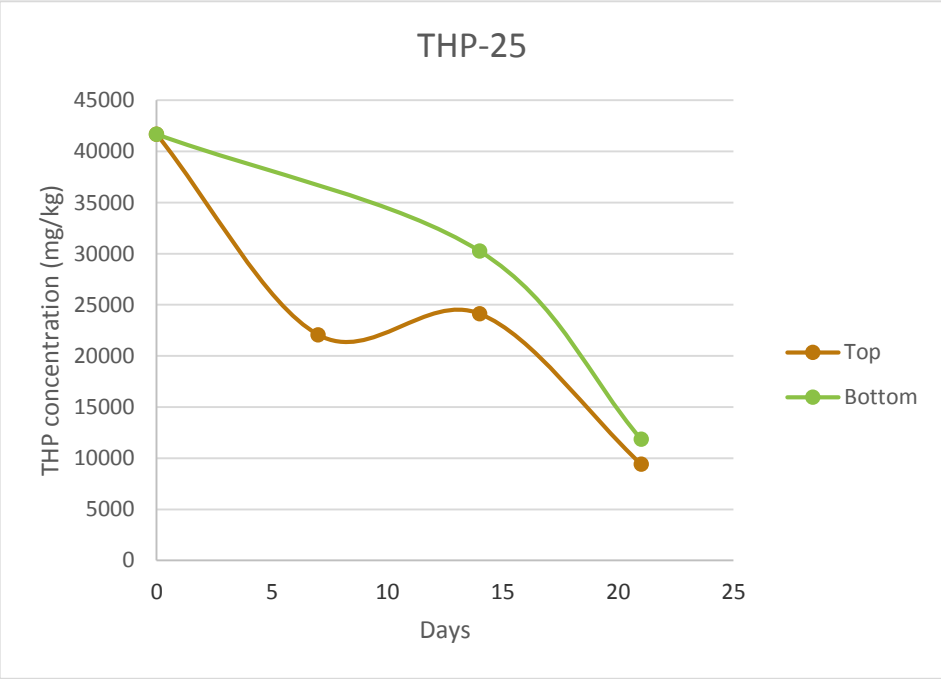
Bottom

Days after traitement

7	14	21
328	442	215
5250	6075	6650
1960	2420	2555
14550	15200	0
22088	**24137**	**9420**
N/A	500	506
N/A	7040	8450
N/A	2625	2910
N/A	20100	0
N/A	**30265**	**11866**

Enhanced Bio-Remediation – Pilot tests

Total petroleum hydrocarbon (mg/Kg)



Enhanced Bio-Remediation

Conclusions:

- A promising cost effective process of particular interest for difficult contaminants
- A strategy based on the possibility to sequentially harness different populations of indigenous microorganisms
- A formulation that encompasses many ingredients that cope with the presented principles of Bio-Remediation
- Pilot tests underway for further understand large scale potential of the research

Enhanced Bio-Remediation

Acknowledgements

Morelos University

CHEMCO inc.

Thank you for your attention !

Questions ?!?

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Chemco-inc.com

Enhanced Bio-Remediation - Tools, testing and tricks

Adjusting the formulation before you get to the field

- Does competent microorganisms are naturally present in the soil?
- What is the pH and the buffering capacity of the soil?
- Calibrating the C/N ratio around 30 to 50, including the contaminants
- To which extent the structure of the soil need to be improved?
- Is PAHs present in the soil?

Enhanced Bio-Remediation – Pilot tests

Evolution of microorganisms populations

- Two waves of biological attacks
- Possible explanation:

  Heat ➔ Water reduction ➔ Increase O_2

Enhanced Bio-Remediation – Pilot tests

- Fungal diversity