

***Petroleum coke as a substratum for biofiltration of oil sands
process water: aerobic and anaerobic degradation
processes in the biofilters***

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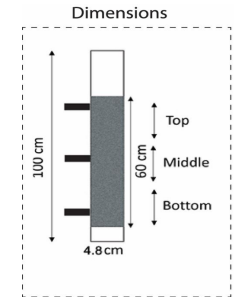
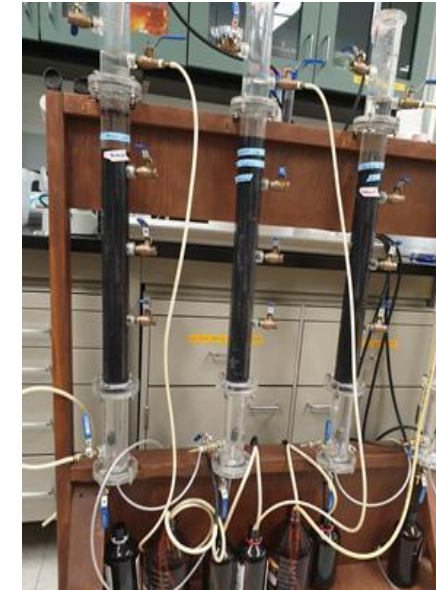
Background

- There is an increasing interest in developing **fix-bed biofilm reactors** (FBBR) for the remediation of oil sands processed water (OSPW).
- To this end, **choice of filter media** is crucial because it **selects specific microbial communities** to grow and colonize the bed followed by degradation of organics such as naphthenic acids (NAs).
- In this study, we investigated the potential of using **petroleum coke** (PC) as a filtering media because it is **readily available at the oil refining sites** and is a **by-product of bitumen upgrading process**.



Key Questions

- What microbial communities are selected by the PC during OSPW remediation in FBBR?
- What is the potential of FBBR towards NAs degradation?
- Whether bioaugmentation of NAs-degrading bacteria enhances biofiltration potential of FBBR?



Experimental setup of petroleum coke (PC) based fixed-bed biofilters used for OSPW remediation.



Experimental

Phase I

- PC characterization.
- FBBR were **operated continuously** to establish microbial communities on the filtering bed.
- Total and active microbial communities were analyzed (**DNA and RNA** sequencing).

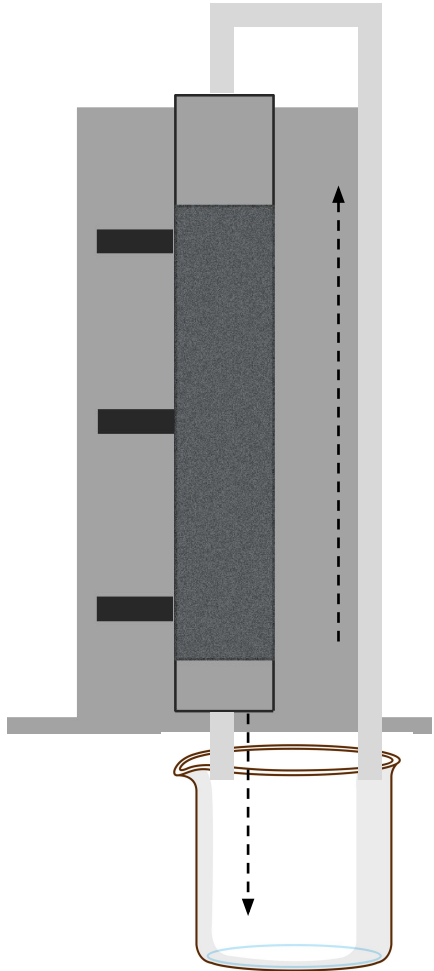
Phase II

- **Cultivable bacteria** were **isolated** from OSPW and oilsands tailings.
- The growth potential was tested on **15 model NAs** (straight chain, single ring, and multiple rings).
- **Consortium of 9 unique strains** was developed.

Phase III

- Consortium was applied on PC bed (**immobilization**).
- FBBR were operated continuously.
- Degradation of NAs and aromatics was investigated.
- Toxicity of treated water was studied.
- Persistence and colonization of immobilized bacteria was assessed.

Biofilters' Operation



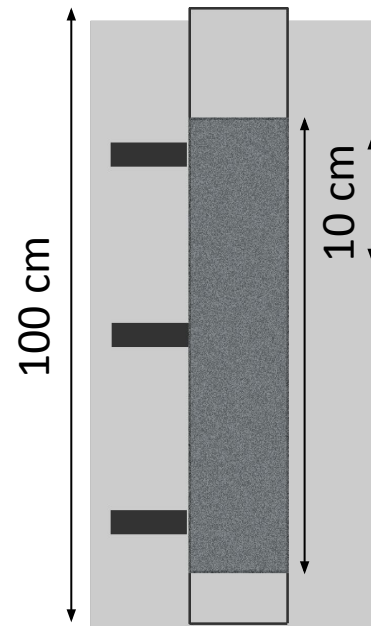
15 days
circulation



Control

Modified

Modified



Sample name	petroleum coke
BET Surface area (m ² /g)	0.903
Pore Size (nm)	<2, 2-4, 4-8



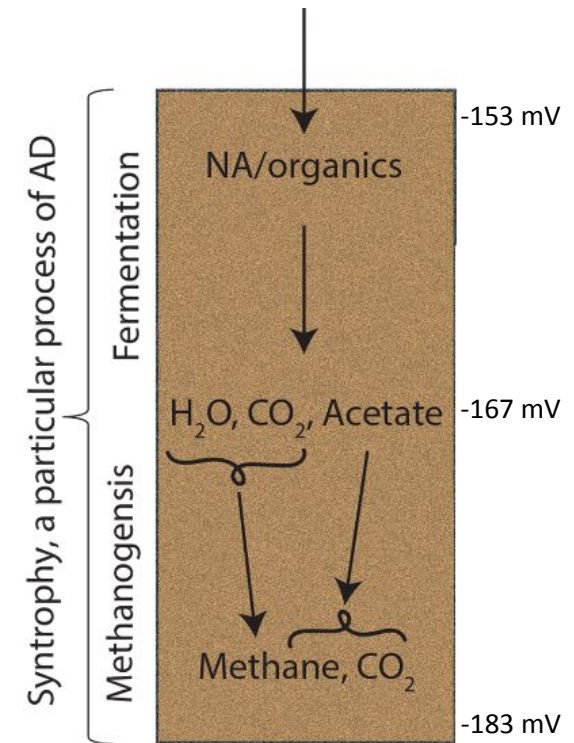
Results

Phase I

Active communities (RNA sequencing)

- Active bacterial community was **entirely anaerobic (99%)**.
- There were **no differences** in bacterial communities at different depths of FBBR.
- Deductions:** (1) Degradation of NAs/organics was subject to anaerobic digestion [a scheme similar to beta-oxidation], (2) PCR of methanogens (*mcrA gene*).

	Top	Middle	Lower
Ruminococcus; Firmicutes-	15.1	17	18
[Eubacterium]; Firmicutes-	11.5	11.5	11.1
Faecalibacterium; Firmicutes-	11	10.6	9.6
Dorea; Firmicutes-	6.6	7.4	7.4
Gemmiger; Firmicutes-	7.2	6.8	6.6
Collinsella; Actinobacteria-	4.9	5	5.1
Clostridium; Firmicutes-	4.3	4.7	4.5
Catenibacterium; Firmicutes-	4.5	4.2	4.5
_Ruminococcaceae_OTU_23; Firmicutes-	3.8	3.6	3.5
Erysipelotrichaceae_OTU_27; Firmicutes-	3	2.0	3.2
Streptococcus; Firmicutes-	2.4	2.3	2.4
Butyrivibrio; Firmicutes-	2.3	2.2	2.2
Roseburia; Firmicutes-	2.1	1.8	1.9



PC-based biofiltere operated under natural conditions

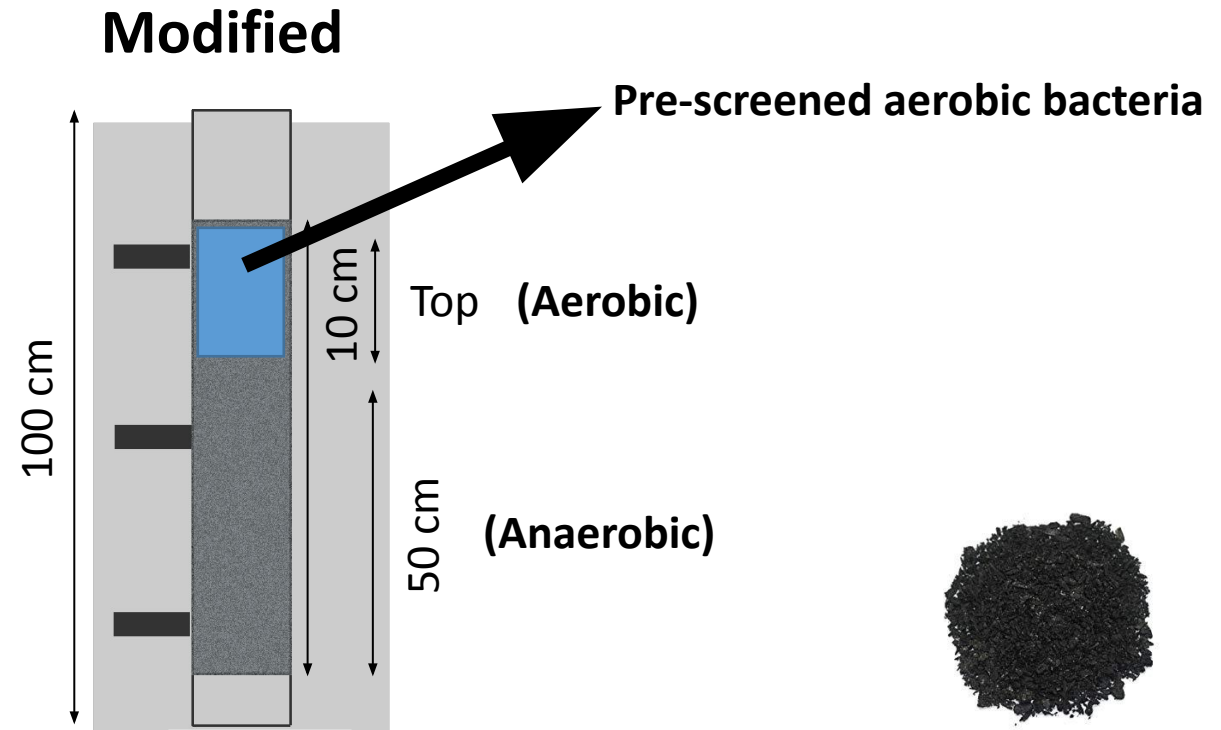
Biofilters' Operation

Phase II



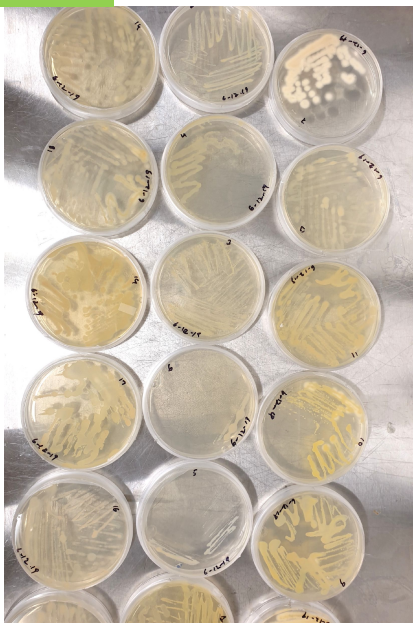
Control

Modified



Pre-screening of NAs degrading bacteria

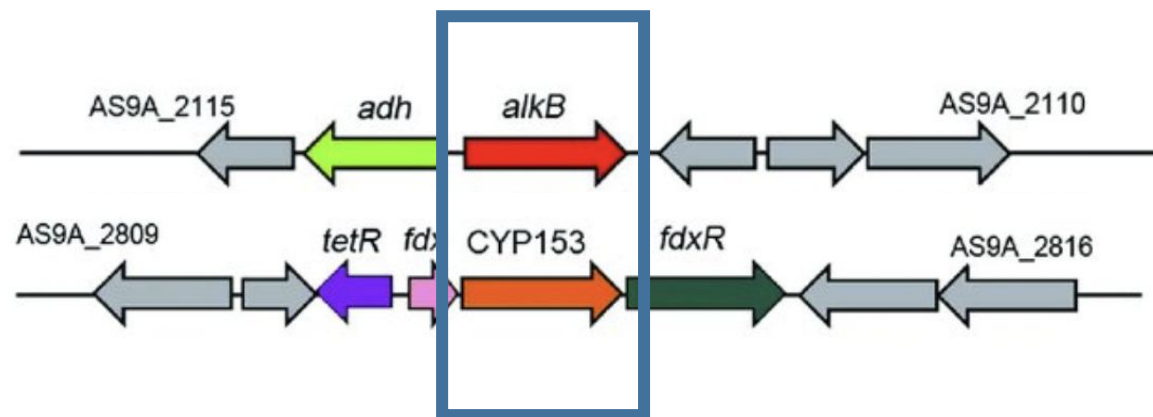
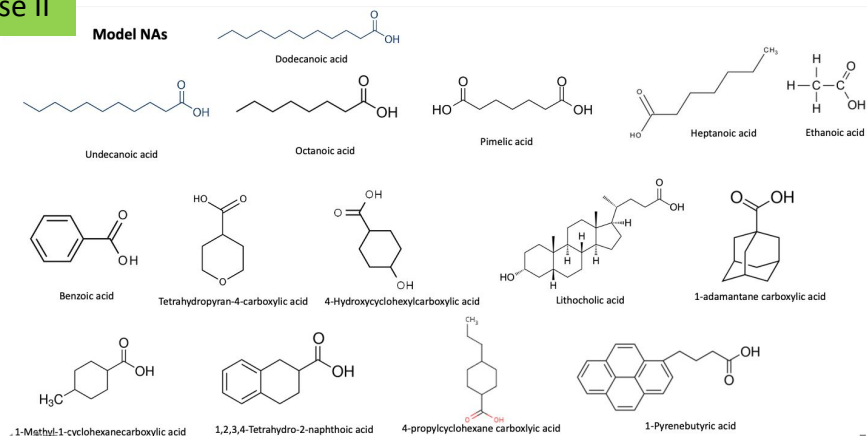
Phase II



	NAs surrogates	Molecular formula	Nature
1	Ethanoic acid	CH ₃ COOH	Straight chain
2	Heptanoic acid	CH ₃ (CH ₂) ₅ COOH	Straight chain
3	Octanoic acid	CH ₃ (CH ₂) ₆ COOH	Straight chain
4	Undecanoic acid	CH ₃ (CH ₂) ₉ COOH	Straight chain
5	Dodecanoic acid	CH ₃ (CH ₂) ₁₀ COOH	Straight chain
6	Pimelic acid	HO ₂ C(CH ₂) ₅ COOH	Straight chain
7	Benzoic acid	C ₆ H ₅ COOH	Single ring
8	4-Hydroxycyclohexylcarboxylic acid	C ₇ H ₁₂ O ₃	Single ring
9	1-Methyl-1-cyclohexanecarboxylic acid	C ₈ H ₁₄ O ₂	Single ring
10	4-Propylcyclohexane carboxylic acid	C ₁₀ H ₁₈ O ₂	Single ring
11	Tetrahydropyran-4-carboxylic acid	C ₁₀ H ₁₈ O ₄	Single ring
12	1,2,3,4-Tetrahydro-2-naphthoic acid	C ₁₁ H ₁₂ O ₂	Two rings
13	1-Adamantane carboxylic acid	C ₁₁ H ₁₆ O ₂	Complex single ring
14	1-Pyrenebutyric acid	C ₂₀ H ₁₆ O ₂	Four rings
15	Lithocholic acid	C ₂₄ H ₄₀ O ₃	Complex multiple rings

Culturing NAs-degrading bacteria

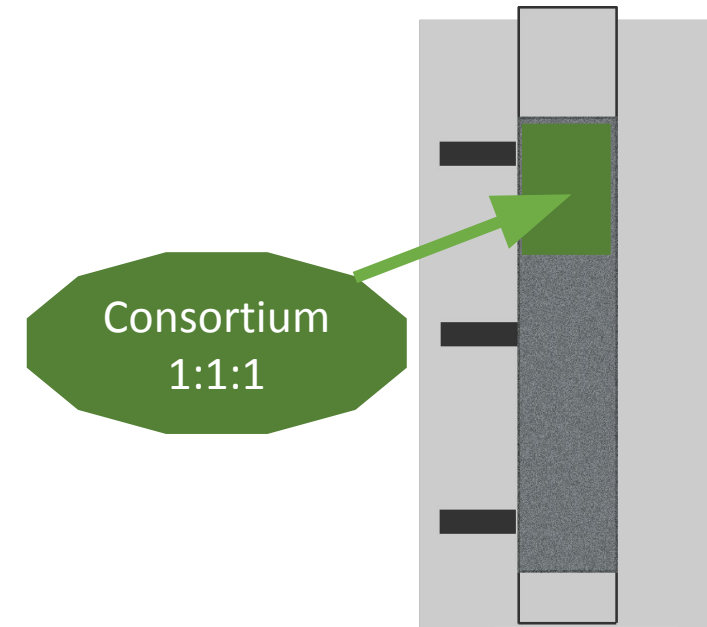
Phase II



Consortium development and application

Phase II

ID	Strain name	Source	Percent Identity	Accession Number	Catabolic genes
1	<i>Pseudomonas stutzeri</i>	Tailings	100%	MT729811	CYP153
2	<i>Bosea lathyri</i>	Tailings	99.26%	MT729812	CYP153 and alkB
3	<i>Sphingopyxis witflariensis</i>	Tailings	99.36%	MT729813	CYP153 and alkB
4	<i>Pseudomonas vancouverensis</i>	Tailings	94.23%	MT729814	CYP153
5	<i>Pseudomonas knackmussii</i>	Tailings	96.98%	MT729815	alkB
6	<i>Aquamicrobium aestuarii</i>	OSPW	98.11%	MT729816	CYP153
7	<i>Aquamicrobium terrae</i>	OSPW	99.34%	MT729817	Not detected
8	<i>Pseudomonas turukhanskensis</i>	Tailings	98.33%	MT729818	CYP153 and alkB
9	<i>Staphylococcus hominis</i>	OSPW	99.48%	MT729819	Not detected



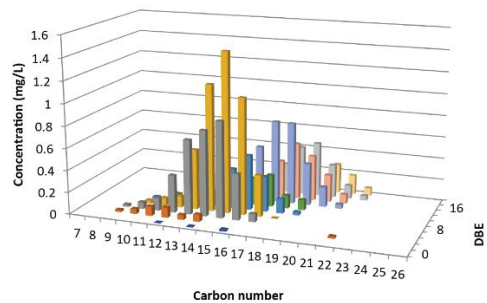
Immobilization efficiency : 23%

- Three bacteria were selected for consortium development.

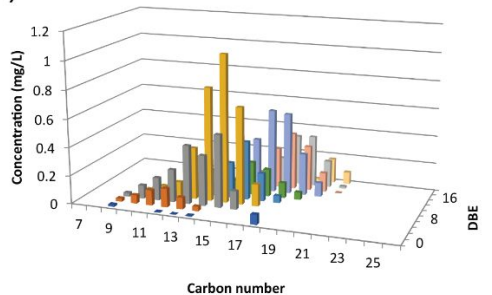
Naphthenic Acids

Phase III

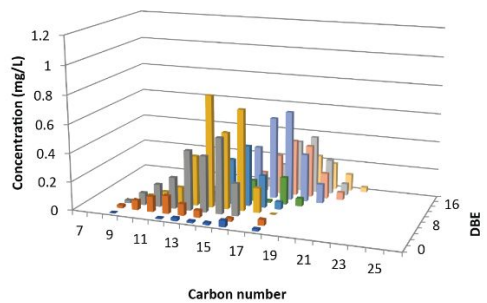
a). Raw OSPW (0-day) **16.1 mg/L**



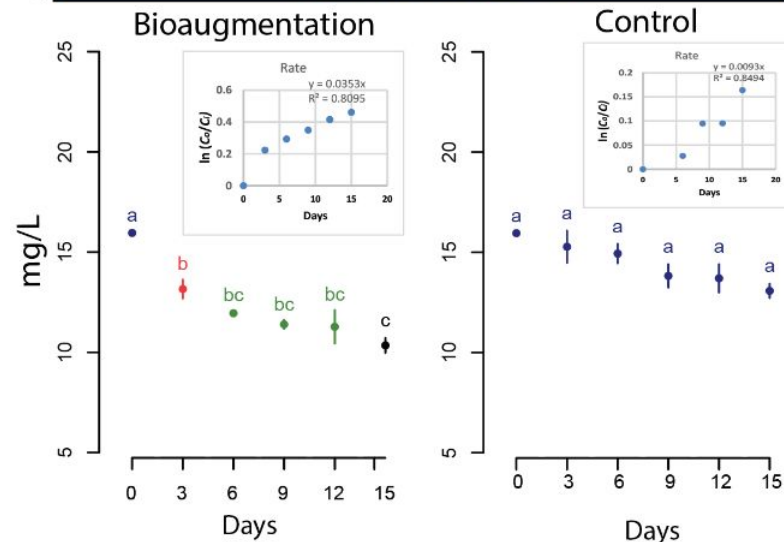
b). Control (15-days) **12.8 mg/L**



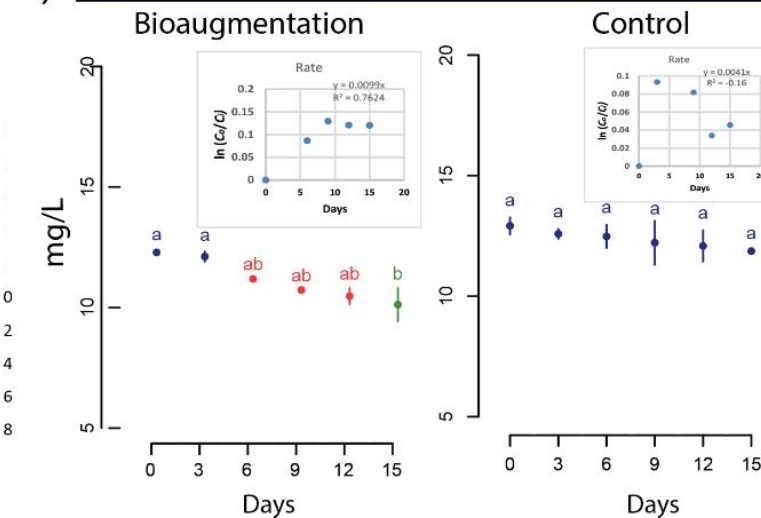
c). Bioaugmentation (15-day) **10.4 mg/L**



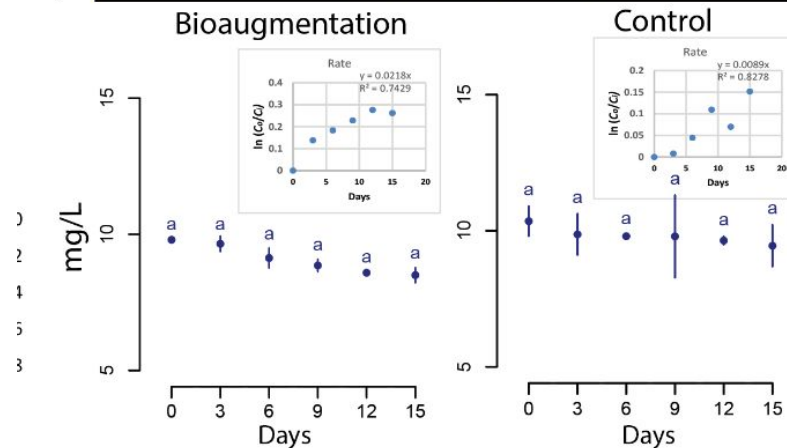
d). Classical NAs (O₂-NAs)



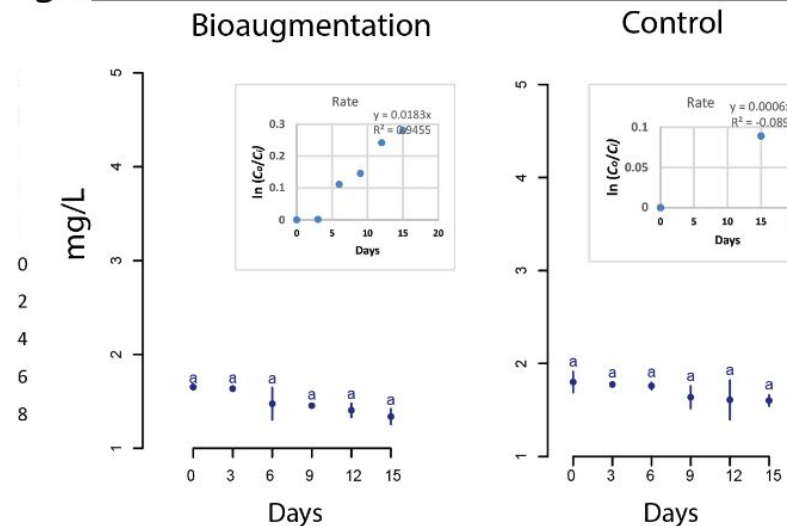
e). Oxidized NAs (O₃-NAs)



f). Oxidized NAs (O₄-NAs)



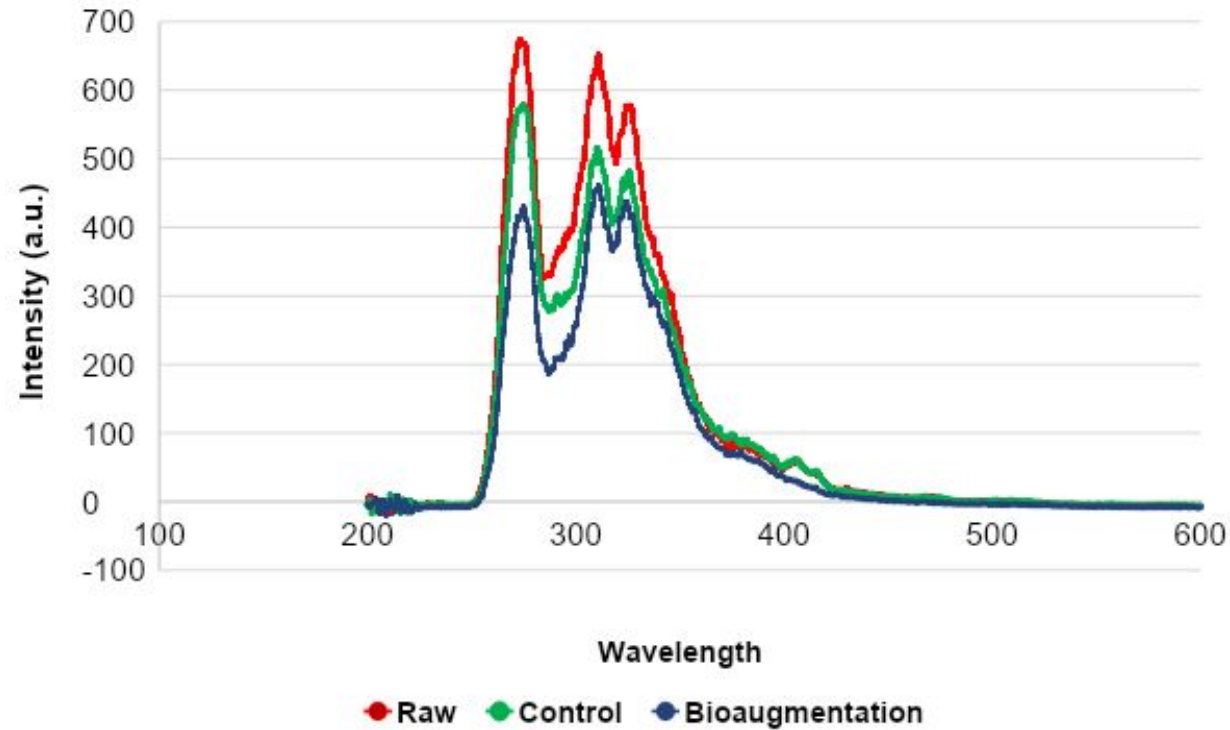
g). Oxidized NAs (O₅-NAs)



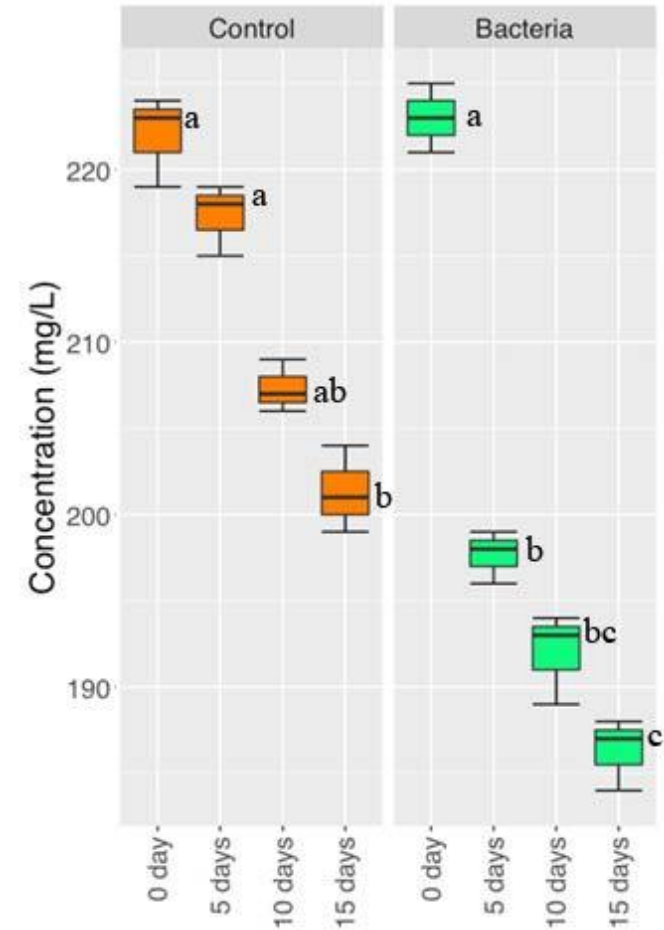
Fluorophores (aromatic compounds) and Chemical Oxygen Demand

Phase III

Synchronous fluorescence spectra

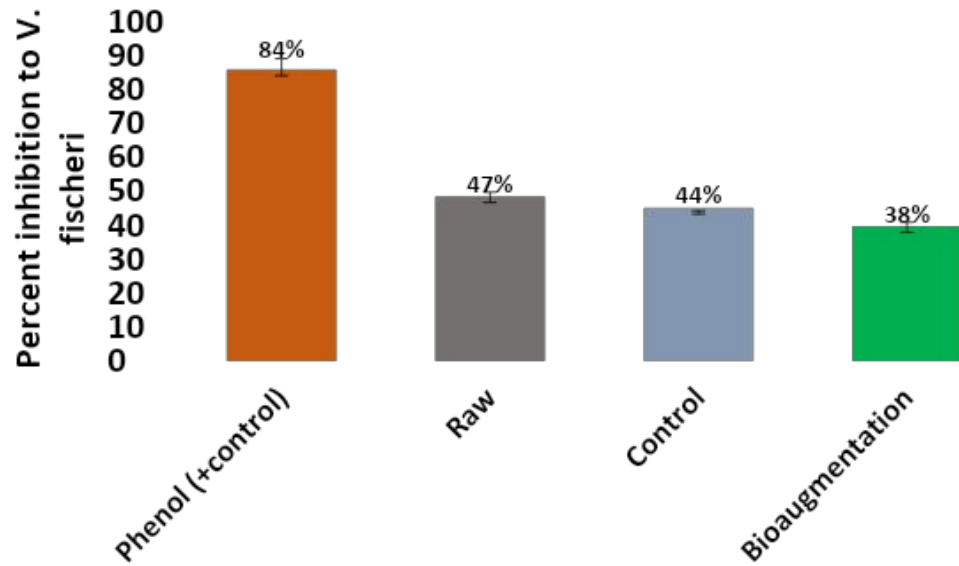


Chemical Oxygen Demand



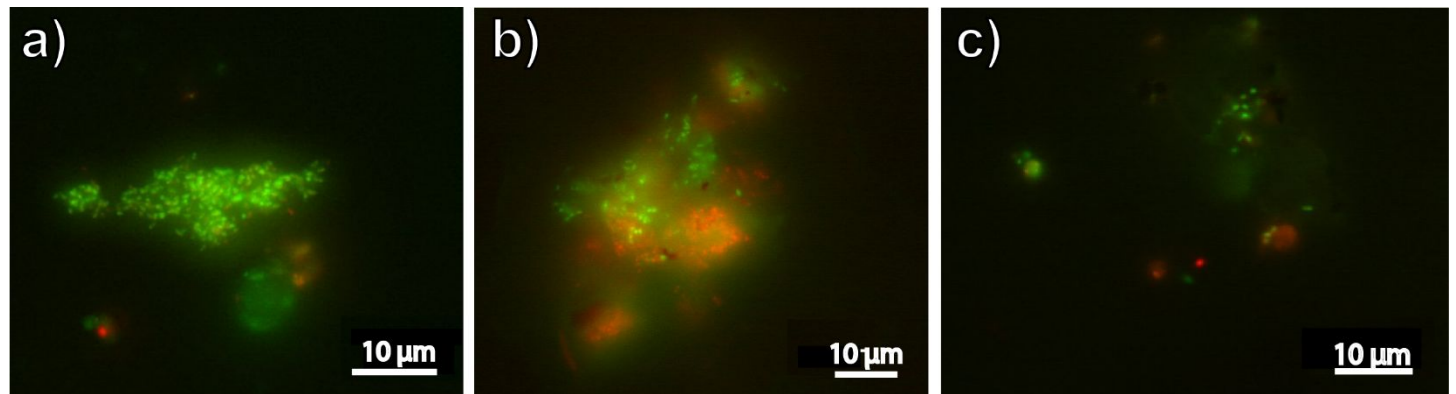
- Bacterial inoculation displayed higher removal of aromatic compounds, as well as COD reduction

MicroTox® and Microscopy

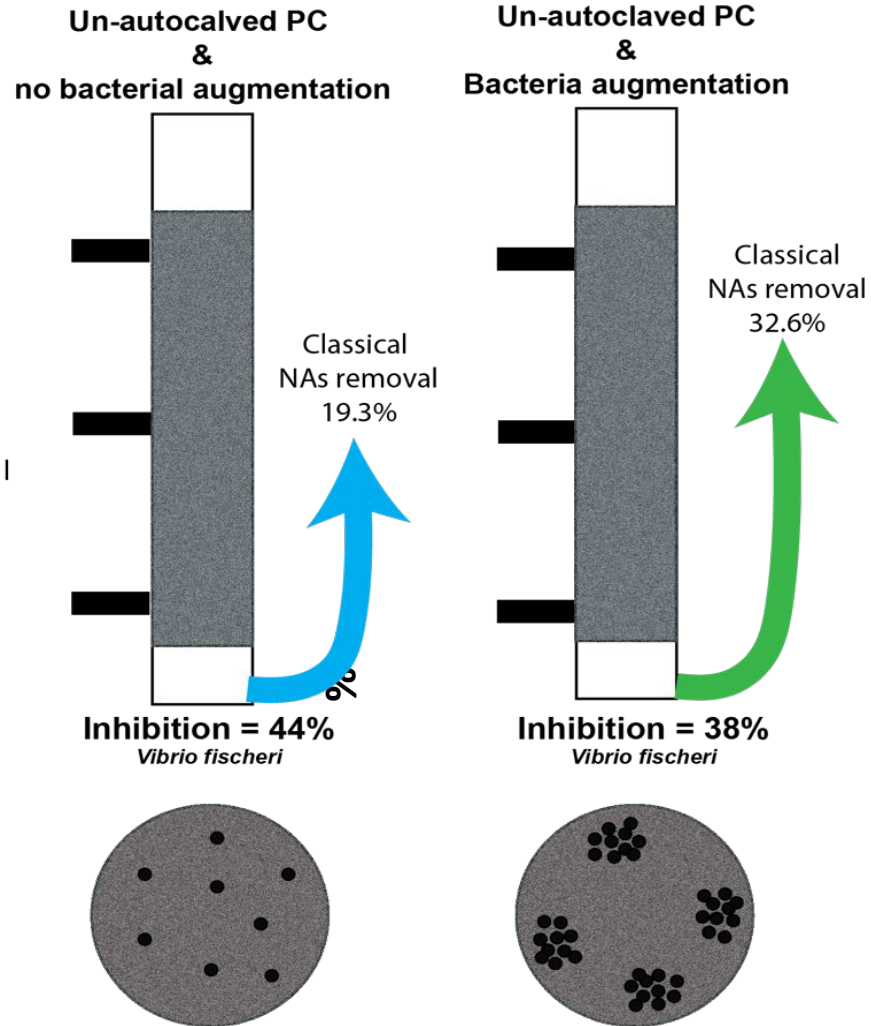


- Highest reduction in toxicity was observed when OSPW was treated in the presence of bacterial augmentation.

- Bacterial inoculation displayed colonization in the form of clusters.



Performance Evaluation

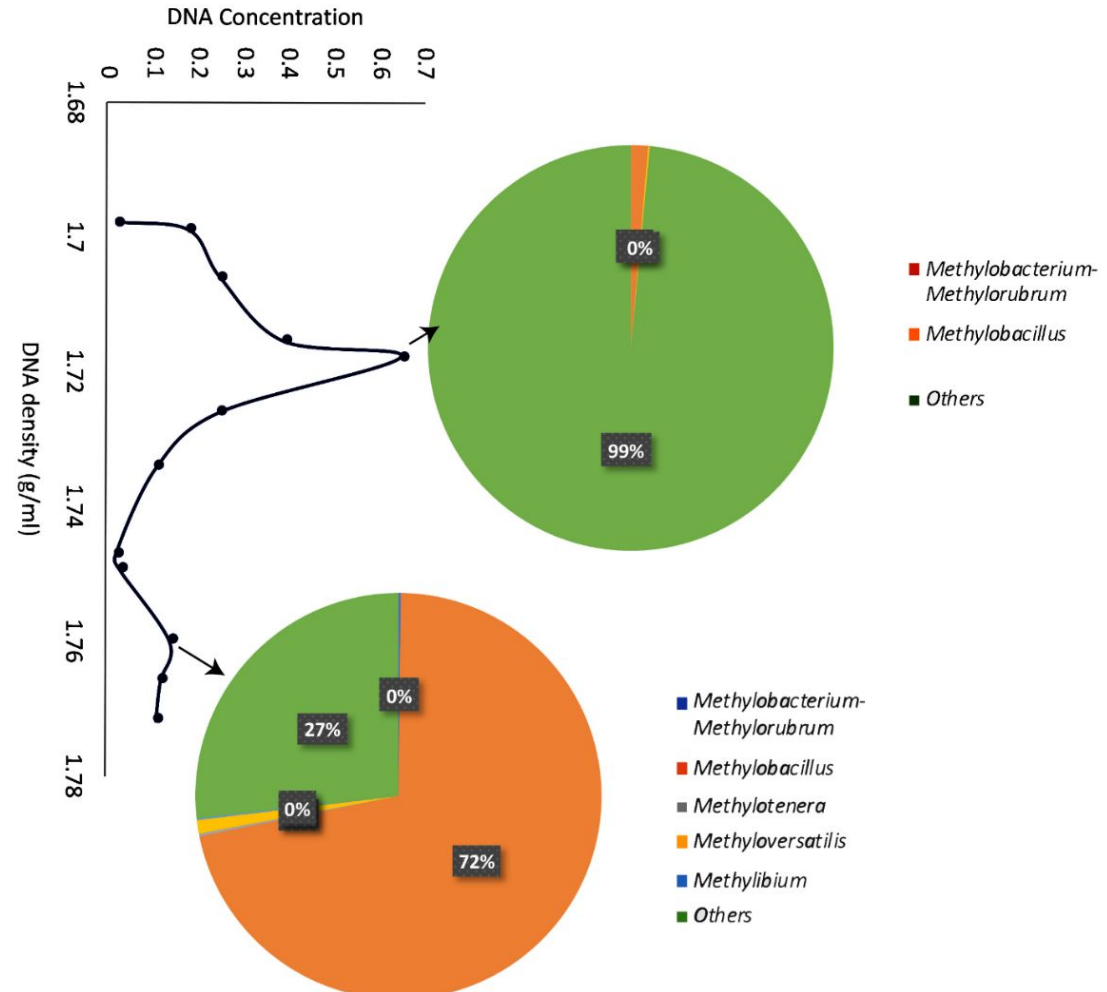
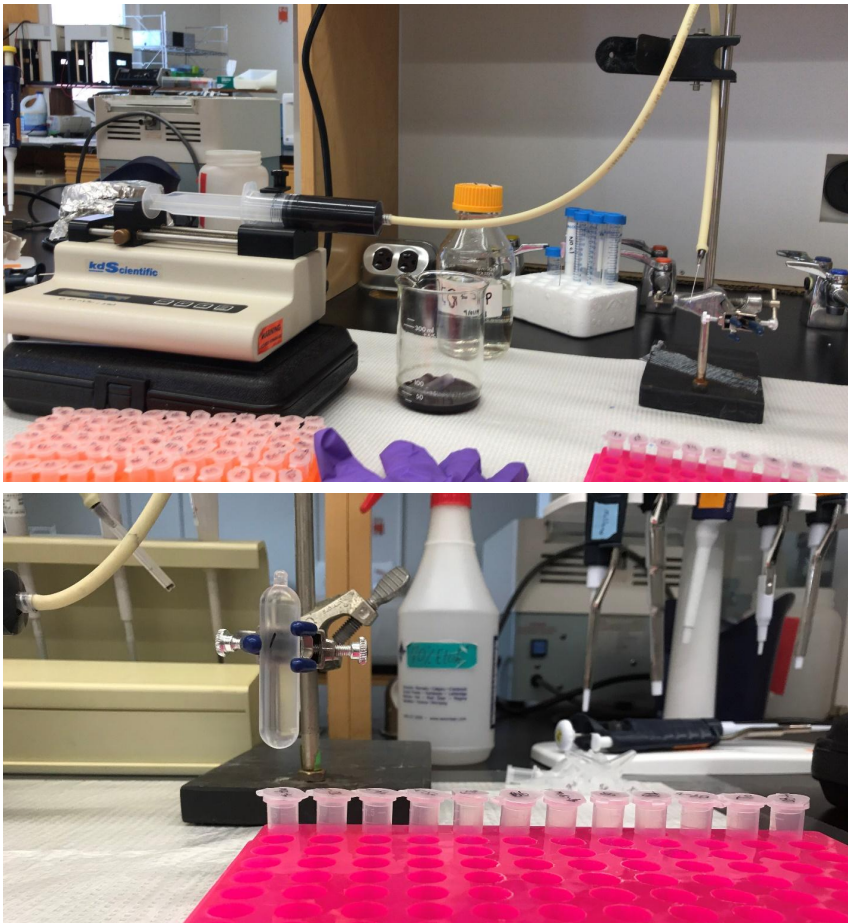


Biodegradation process	Circulation	OSPW	Influent NA concentration (mg/L)	Effluent NA concentration (mg/L)	NA removal efficiency (%)	References
Petroleum coke biofilter	15 days	Raw	15.93	12.85	19.3%	This study
Petroleum coke biofilter + bacteria	15 days	Raw	16.10	10.87	32.5%	This study
Sand biofilter	23 days	Raw	13.1	10.2	22.1%	Zhang et al., 2018
MBBR	214 days	Raw	19.8	12.9	34.8%	Shi et al., 2015
IFAS	335 days	Raw	25.1	14.3	43.0%	Huang et al., 2015

Microbial Community Structures (Bioaugmentation)

DNA-Stable Isotope Probing (^{13}C and ^{12}C labeling of NA surrogates)

Oleic acid, palmitic acid, myristic acid, benzoic acid, phenylalanine, 2-keto-4-methylpantonic acid

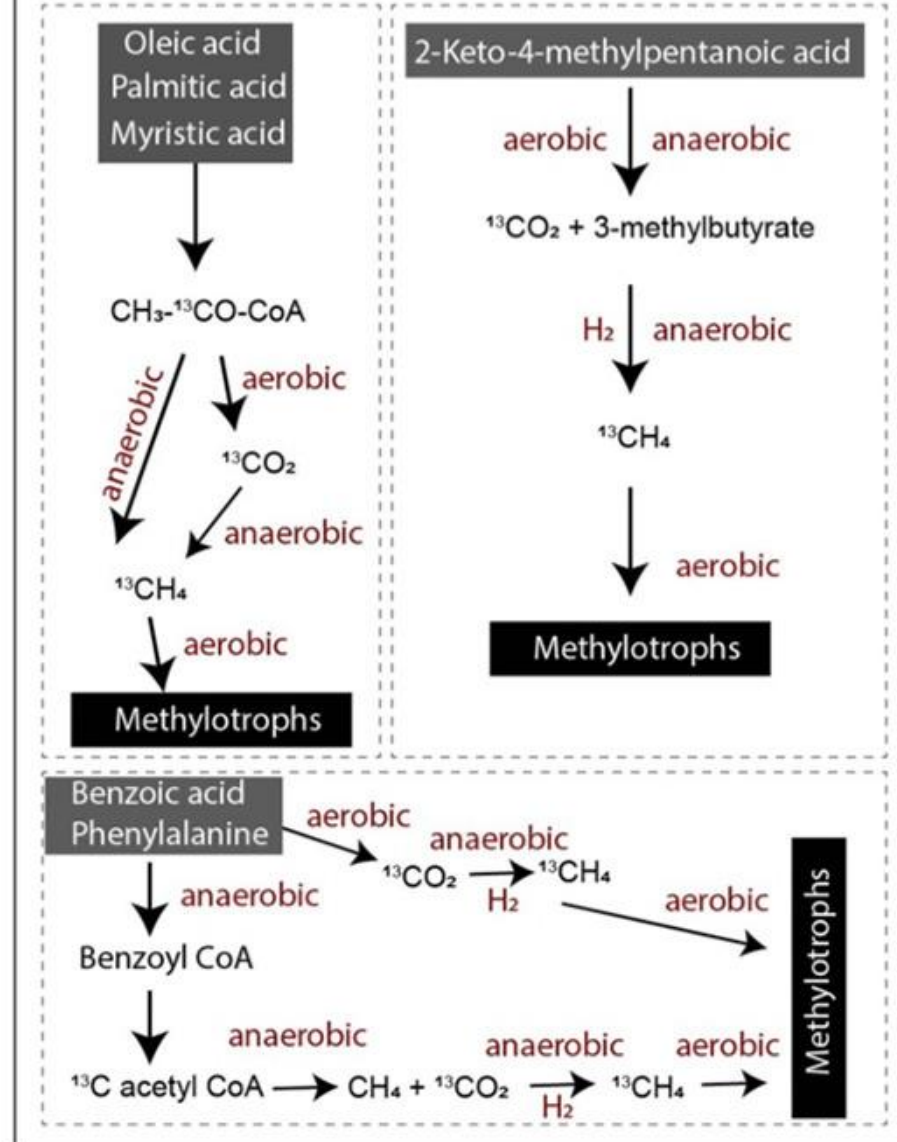


Microbial Community Structures (Bioaugmentation)

	13C-NAs	13C-NAs	
Methylotrophy	<i>Proteobacteria; Methylobacillus</i>	68.1	69
	Unclassified; Unclassified	0.3	0.2
	<i>Proteobacteria; Unclassified</i>	0.9	1.4
Inoculated	<i>Proteobacteria; Pseudomonas</i>	6.4	5.2
NAs/HC degradation	<i>Proteobacteria; Pseudoxanthomonas</i>	2.7	1.3
	<i>Bacteroidota; Sediminibacterium</i>	0.6	2.2
	<i>Proteobacteria; Methyloversatilis</i>	0.6	1.3
	<i>Proteobacteria; Rhodobacter</i>	1.3	1.5
	<i>Verrucomicrobiota; Neochlamydia</i>	3.5	1.1
	<i>Cyanobacteria; Obscuribacteraceae</i>	1.5	2.1
	<i>Proteobacteria; Hyphomicrobium</i>	1.2	1.1
	<i>Bacteroidota; Subsaxibacter</i>	2.2	1.5
	<i>Bacteroidota; OLB12</i>	0	0
	<i>Bacteroidota; Lacibacter</i>	0.5	1
	<i>Bacteroidota; Unclassified</i>	0.2	0.4
	<i>Proteobacteria; Legionella</i>	0.2	0.4
	<i>Gemmatimonadota; Gemmatimonas</i>	0.3	0.5
	<i>Acidobacteriota; Bryobacter</i>	0.7	0.4
	<i>Actinobacteriota; Cutibacterium</i>	0.3	0.4
	<i>Bacteroidota; Terrimonas</i>	0.5	0.2

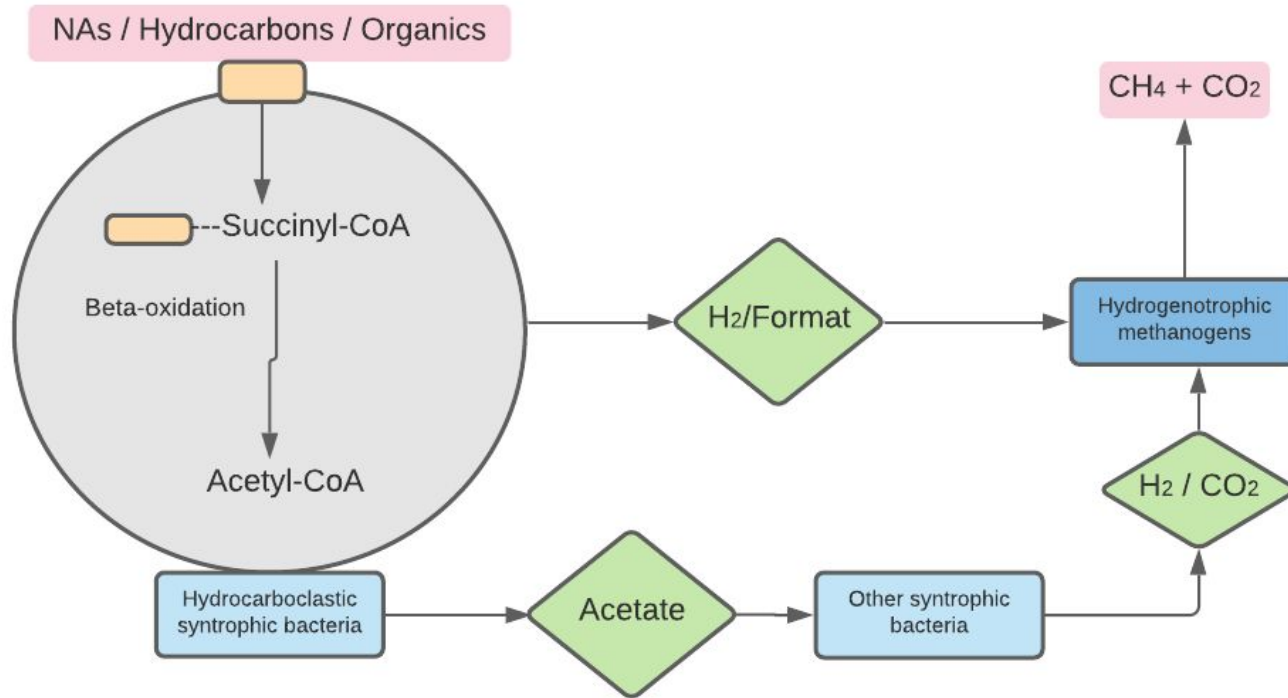
	12C-NAs	12C-NAs	
	<i>Cyanobacteria; Obscuribacteraceae</i>	24.5	27.4
	<i>Proteobacteria; Rhodobacter</i>	13.9	12.7
Inoculated	<i>Proteobacteria; Sphingomonas</i>	6	7.1
	<i>Proteobacteria; Sphingopyxis</i>	4.4	6.5
	<i>Proteobacteria; Hyphomicrobium</i>	4	4.4
	k__d_Bacteria_OTU_8; k__d_Bacteria_OTU_8	14.9	2.6
	<i>Planctomycetota; Gemmata</i>	1.1	6.4
	<i>Proteobacteria; Blastomonas</i>	3.2	3.9
	<i>Acidobacteriota; Bryobacter</i>	2.4	2.2
	<i>Gemmatimonadota; Gemmatimonas</i>	2.1	2.5
	<i>Proteobacteria; Methylobacterium-Methylorubrum</i>	1.2	1.6
	<i>Spirochaetota; Leptospira</i>	1.3	1.1
	<i>Proteobacteria; Reyranella</i>	2.2	1.7
	<i>Actinobacteriota; f__Microbacteriaceae_OTU_16</i>	2.5	3.1
	k__d_Bacteria_OTU_13; k__d_Bacteria_OTU_13	0	0
Inoculated	<i>Proteobacteria; Bosea</i>	1.3	1.5
	<i>Proteobacteria; f__Sphingomonadaceae_OTU_25</i>	0.4	2
	<i>Proteobacteria; Caulobacter</i>	1.2	0.8
	<i>Actinobacteriota; f__Microbacteriaceae_OTU_24</i>	0	0
	<i>Proteobacteria; f__Rhizobiaceae_OTU_27</i>	0.5	1.3

DNA-Stable Isotope Probing

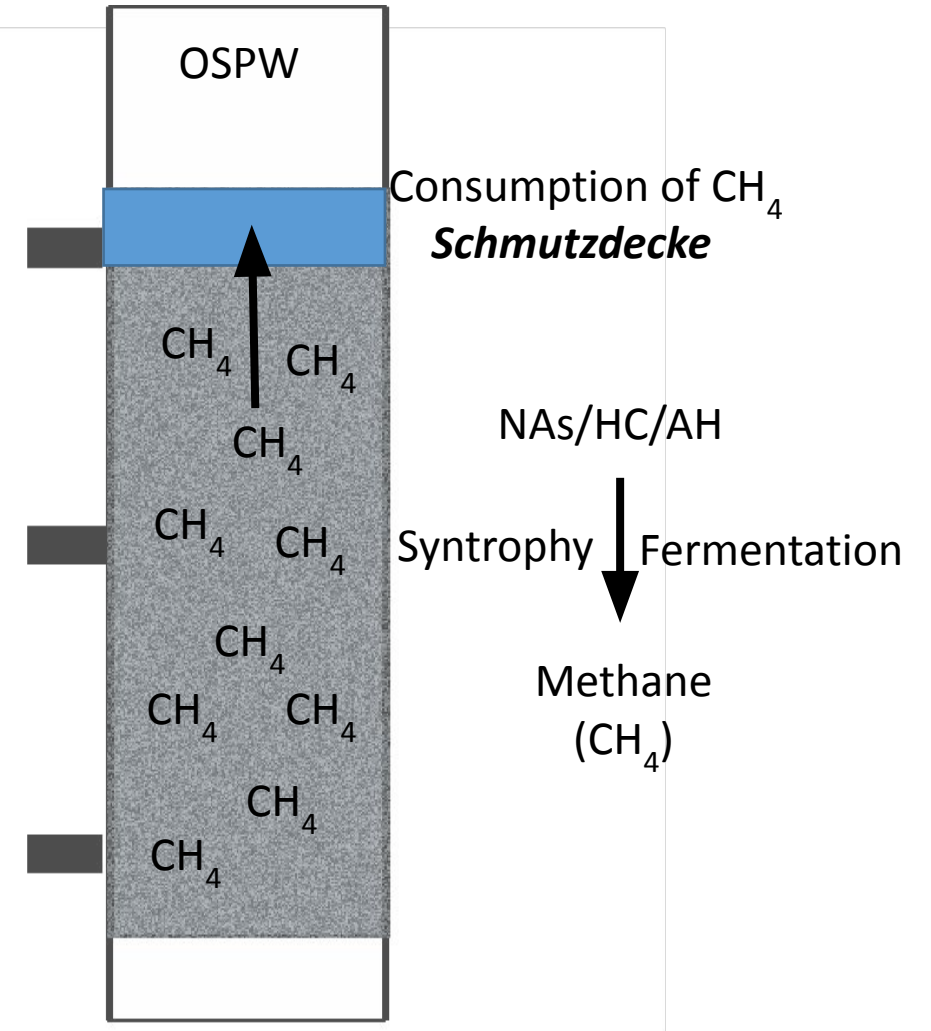


Summary Mechanisms

Control



Modified



Concluding Remarks

- **Active community** in PC comprises anaerobes, which allow remediation via anaerobic digestion.
- OSPW remediation under **natural conditions** are coupled with **methanogenesis** in a **syntrophic** mechanism.
- Removal of **classical NAs**, **O₃-NAs**, and **fluorophore** (aromatic) compounds was evident in the presence of **bacterial augmentation**.
- DNA-SIP revealed a high degree of **methylophony** in the *Schumutzdecke*.
- **Oxygen** was primarily **consumed** by **methylophony** rather than used for **degradation of NAs**.
- **PC + bioaugmentation** for aerated **OSPW** is beneficial and **release of CH₄** can be minimized



Teck



Thank You!



**Time For
QUESTIONS**