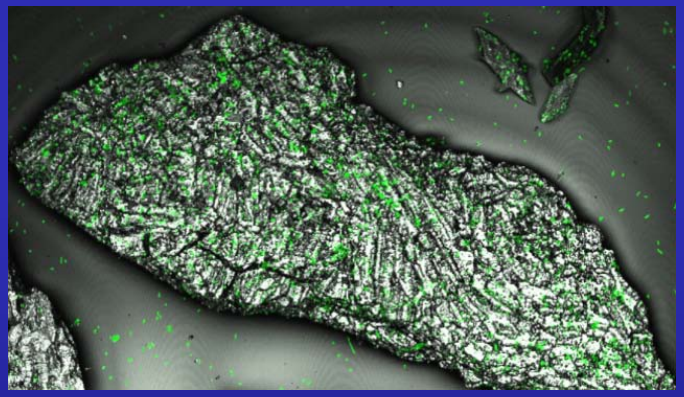
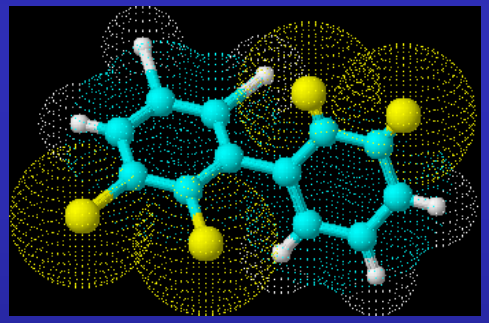
Bioremediation of polychlorinated biphenyls (PCBs) using biofilms



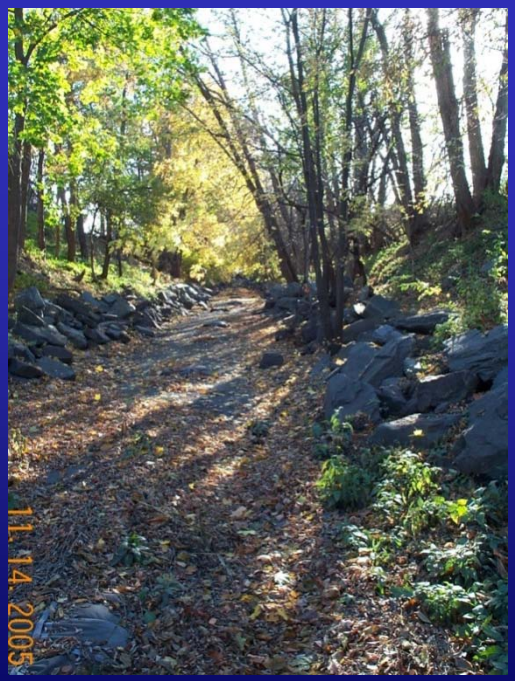


Birthe Veno Kjellerup, Ph.D.

University of Maryland at College Park
Department of Civil & Environmental Engineering

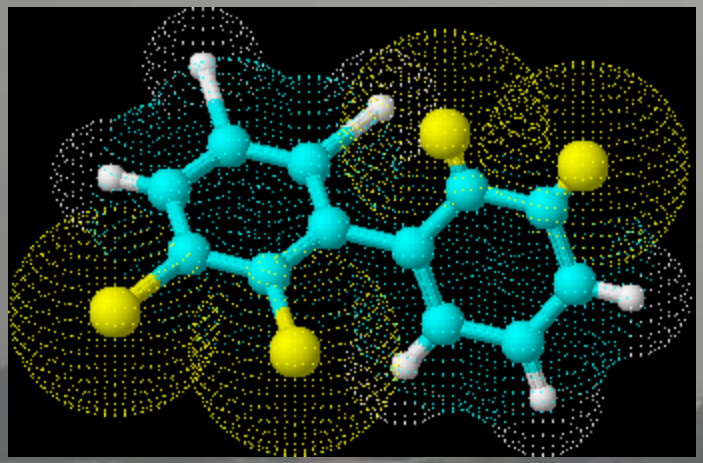
AGENDA

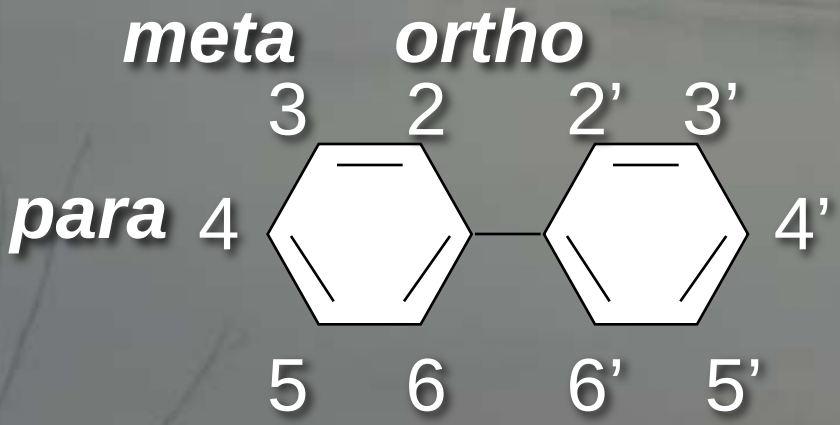
- Presence and concerns of PCBs?
- Biological fate of PCBs?
- Bioremediation using activated carbon
- Biofilms in bioremediation
- Aerobic-anaerobic biofilms in soil
- Future Research
- Questions



PCB contaminated soil

PCBs: Persistent organic pollutants





- 209 congeners
- Very Stable
- Bioaccumulate
- Toxicity concern
- Sediments/soils

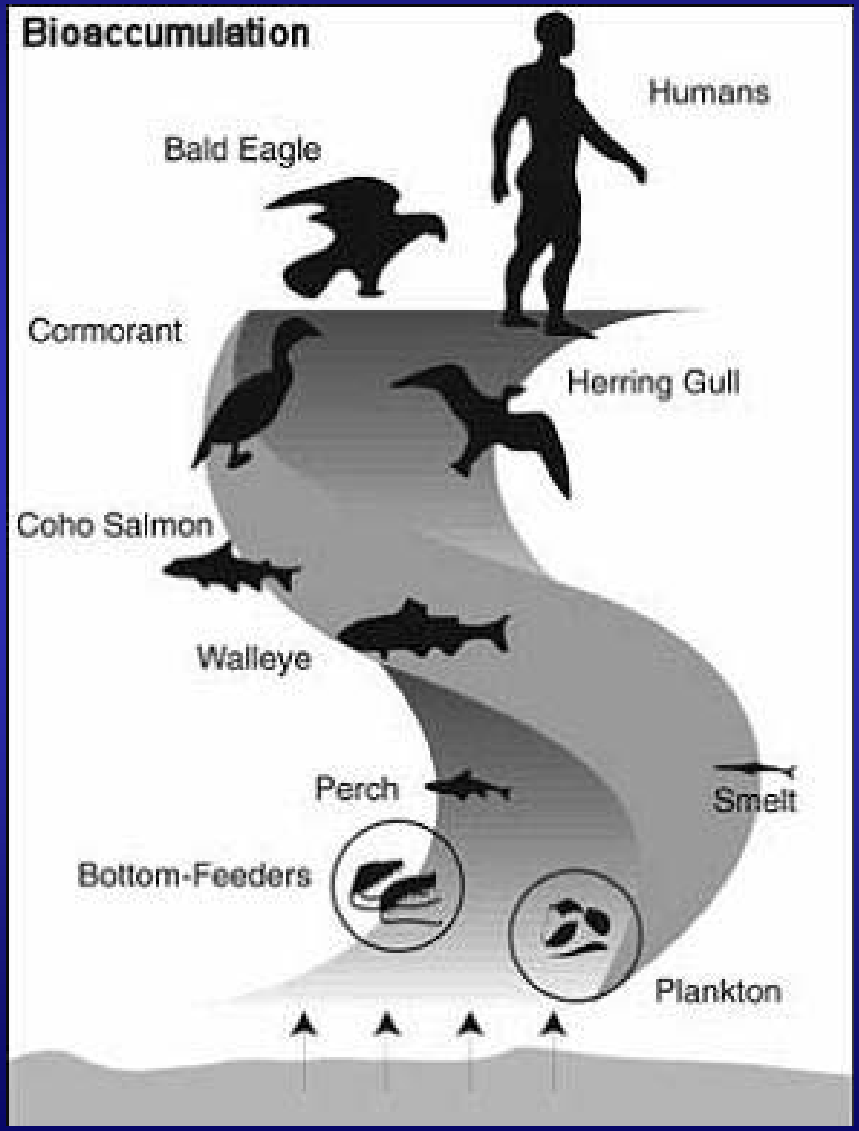
= global sinks

Microbial transformation of PCBs

An environmental legacy of PCBs

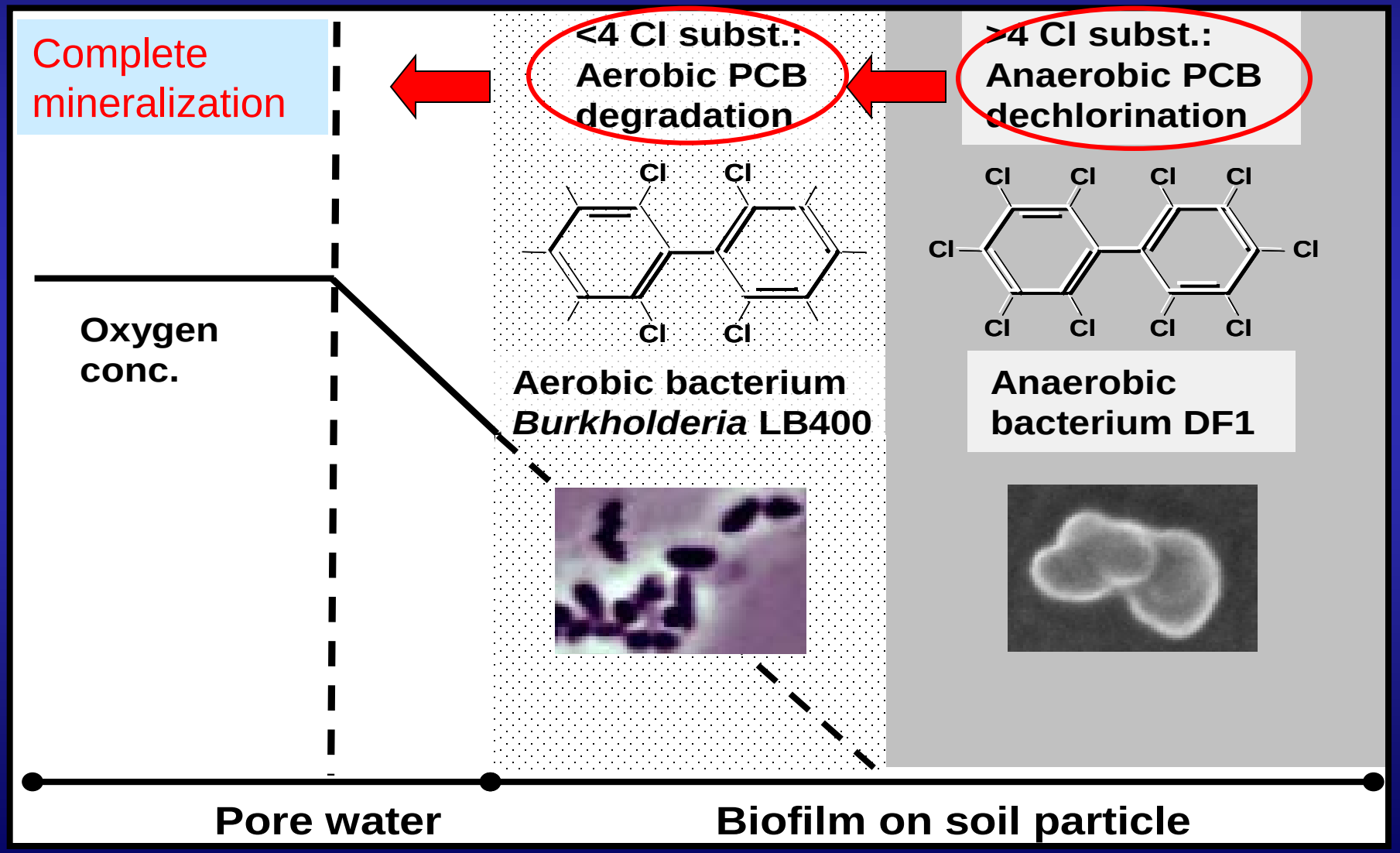
Estimated 0.6-1.2 billion kg worldwide

Why are PCBs of concern?

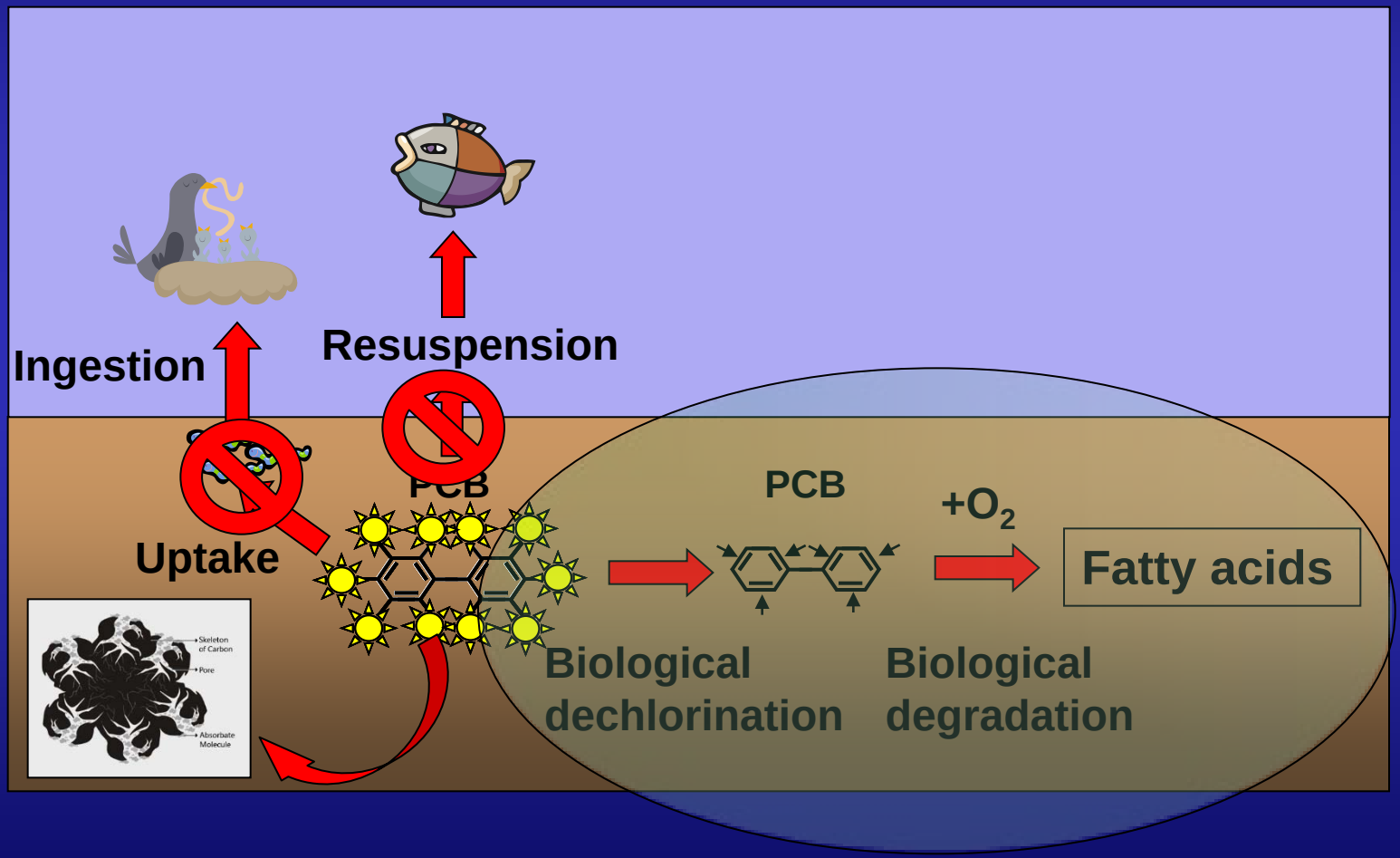


- Bioaccumulates and biomagnifies in the food chain
- Present in lipophilic tissue, blood and breast milk
- Toxicological effects: Cancer, problems with endocrine and reproductive organs as well as immunological issues
- Humans: Source - ingestion (sea food, meat, poultry etc.)

The Microbial Fate of PCBs

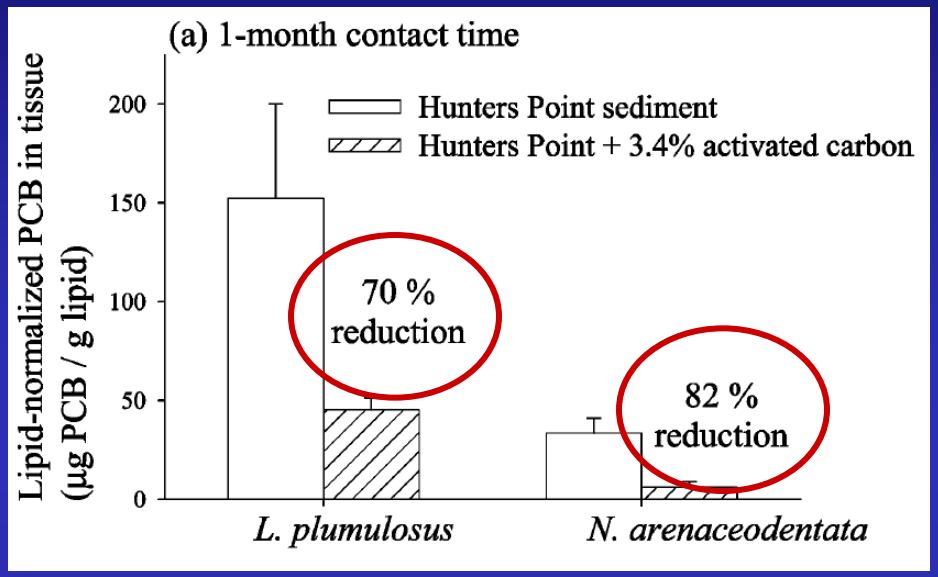


PCBs - Processes in sediment



> 50 years

Effect of Activated Carbon



L. ***plumulosus***

N. ***arenaceodentata***

Conclusion:

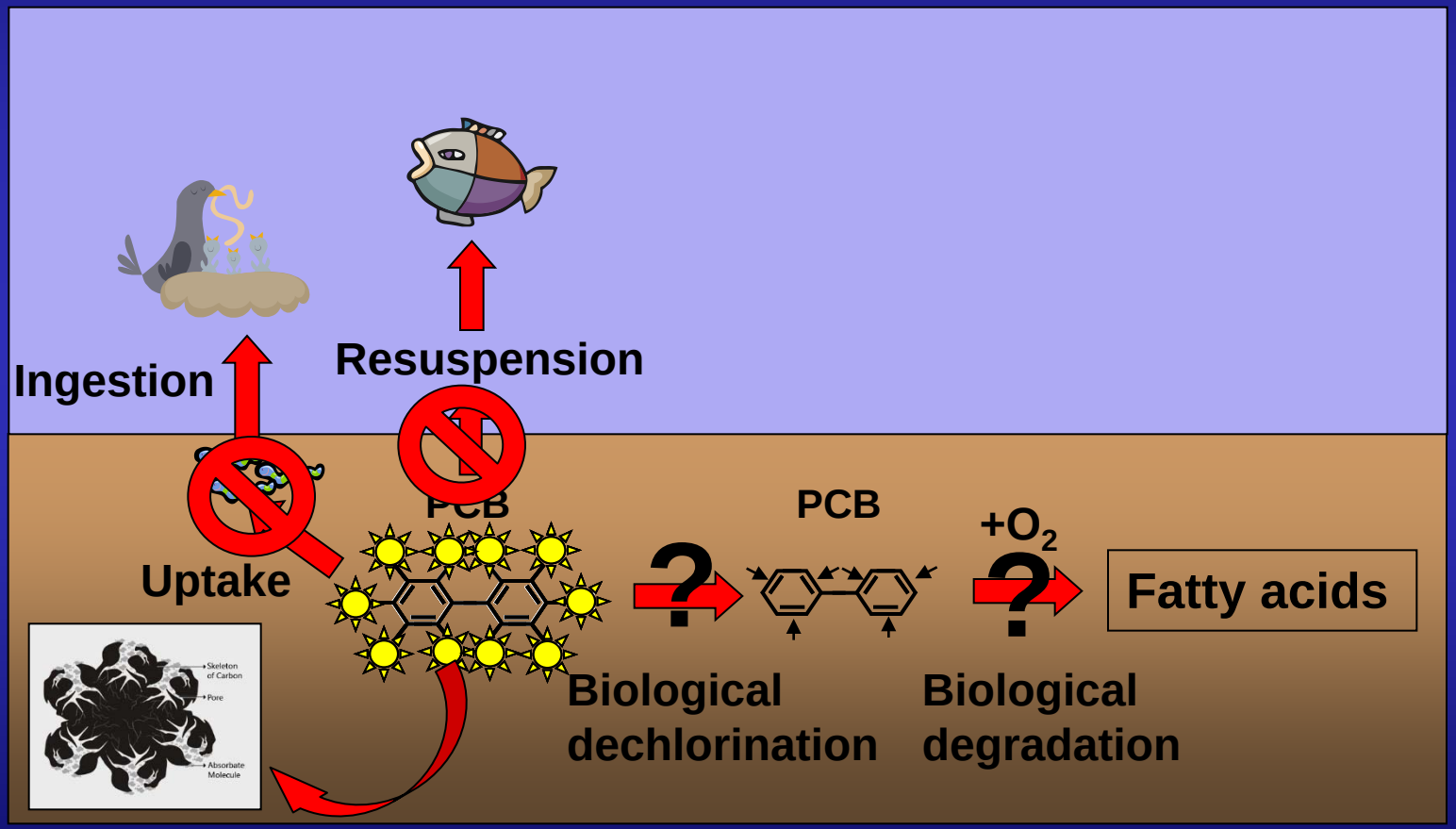
- Reduced uptake of PCB in tissue
- Reduced bioavailability for tested species

Questions:

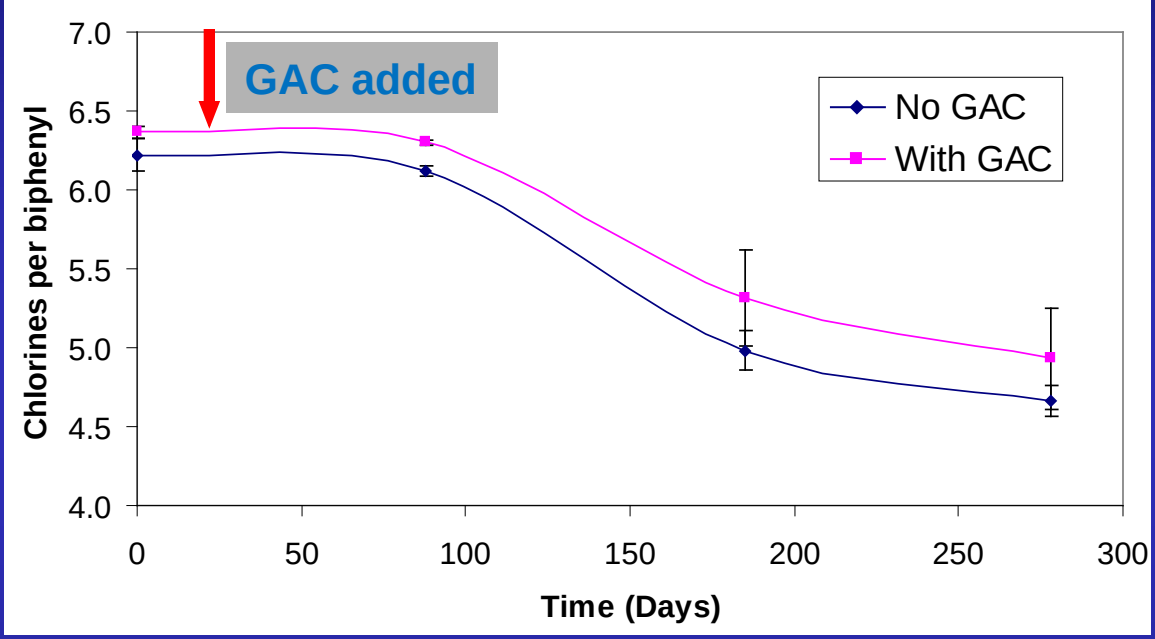
- Reduced bioavailability for bacteria?
- Effects on dechlorination rates and products?

Millward et al, Environ. Sci. Technol. 2005

PCBs - Processes in sediment



Are PCBs available for bacteria?



Average chlorine content

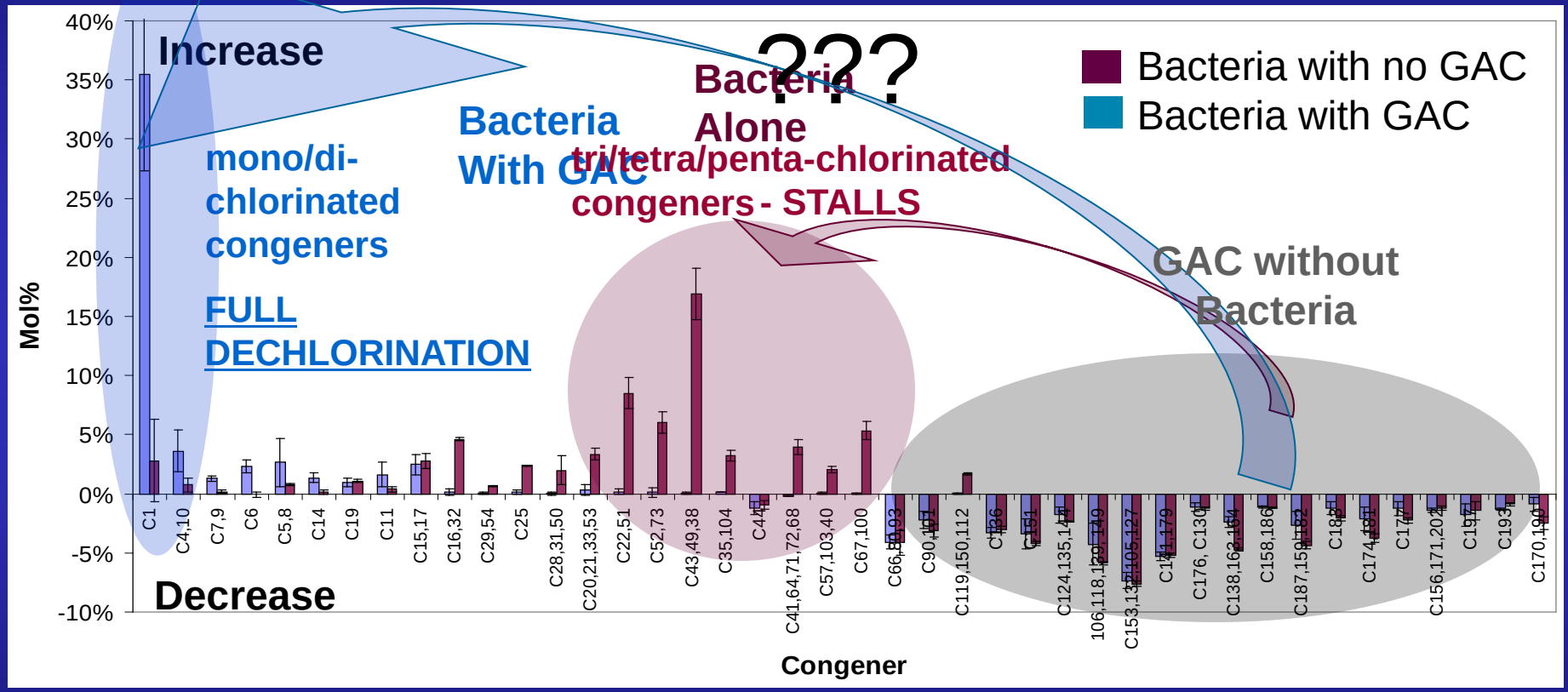
Conclusion:

- Dechlorination of Aroclor 1260 in sediment with GAC

⇒ No effect of GAC based on average chlorine content

Kjellerup et al, Water Res (2014), Apr 1;52C:1-10

Are PCBs available for bacteria?



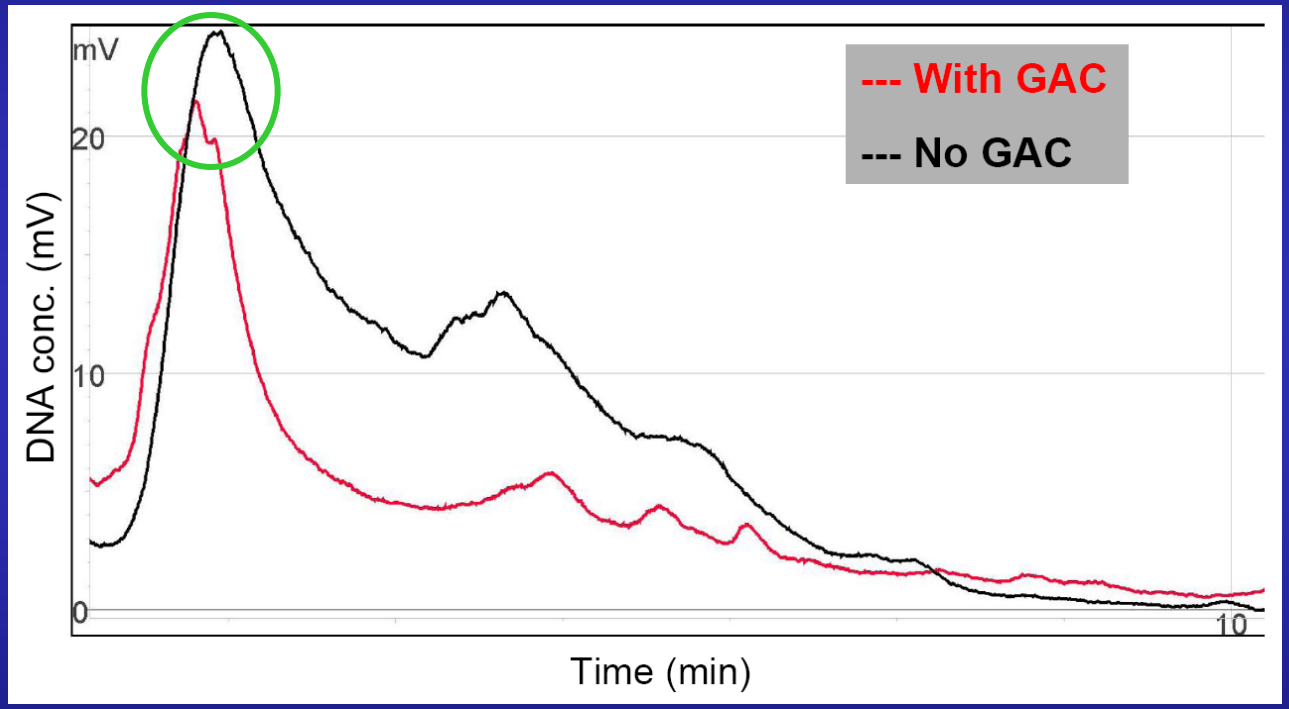
Conclusion:

- With GAC, full dechlorination possible
- Aerobic microbes can now degrade biphenyl rings

Kjellerup et al, Water Res. 2014

Different bacterial populations?

Screening of bacterial diversity using DHPLC and primers targeting dechlorinating bacteria (16S rRNA)

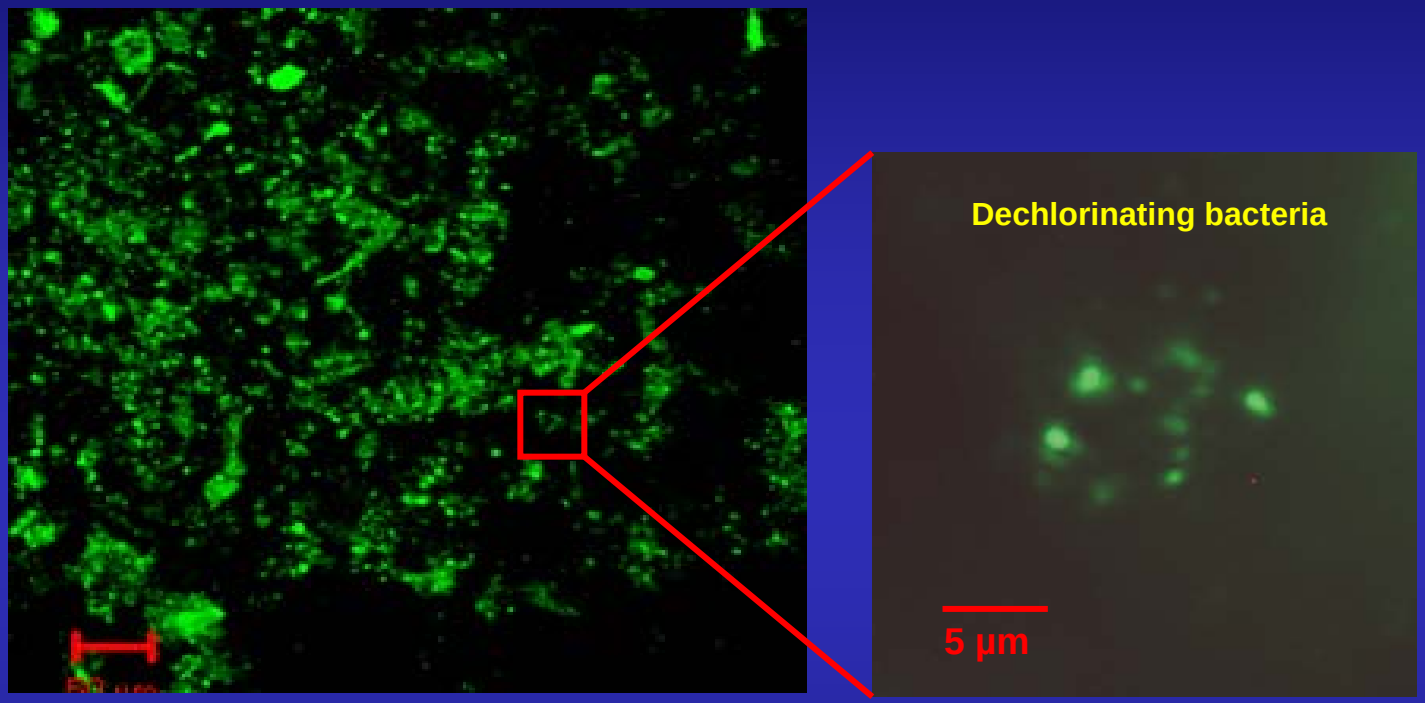


Conclusion:

- Dominant dechlorinating phylo-types are the same → Not responsible for difference in dechlorination

Kjellerup et al, Water Res (2014), Apr 1;52C:1-10

Biofilm on GAC in sediment

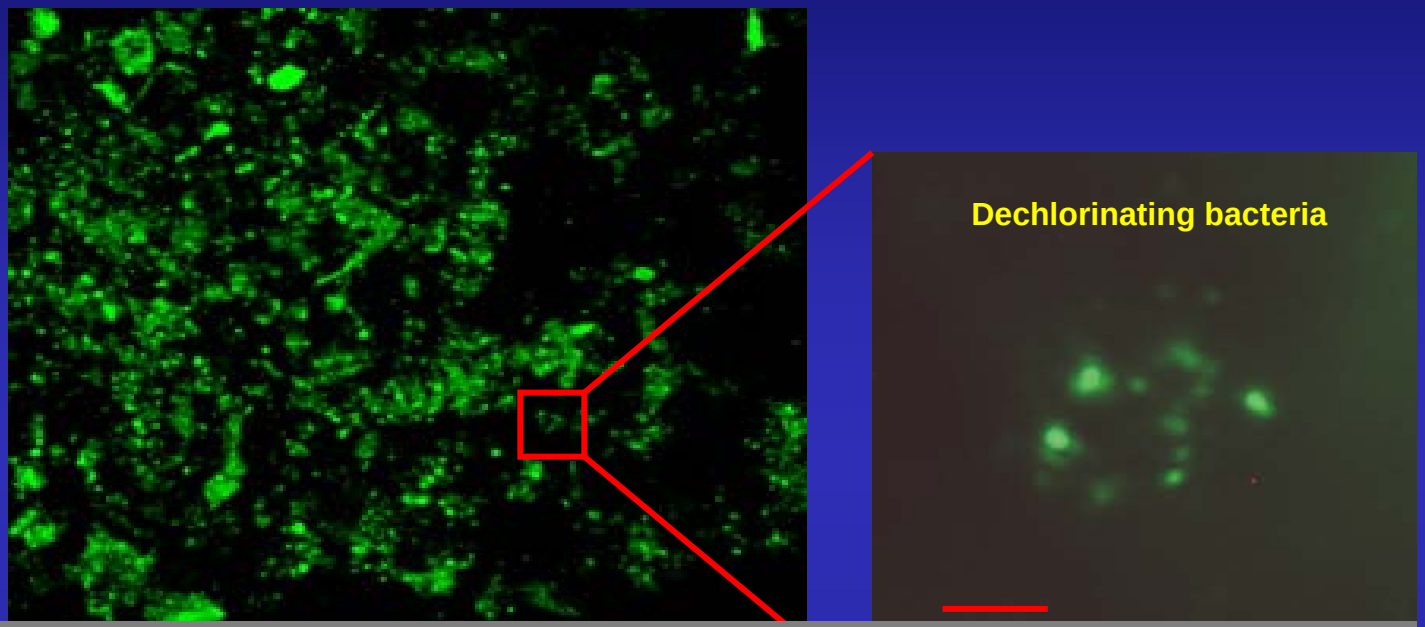


Conclusion:

- Biofilms are present in sediment
- Natural mode of growth

- Can we utilize this observation?

Biofilm on GAC in sediment



Dual approach:

1. Adsorption of PCBs on activated carbon
2. Biofilm instead of liquid inoculum for bioaugmentation?

Objective:

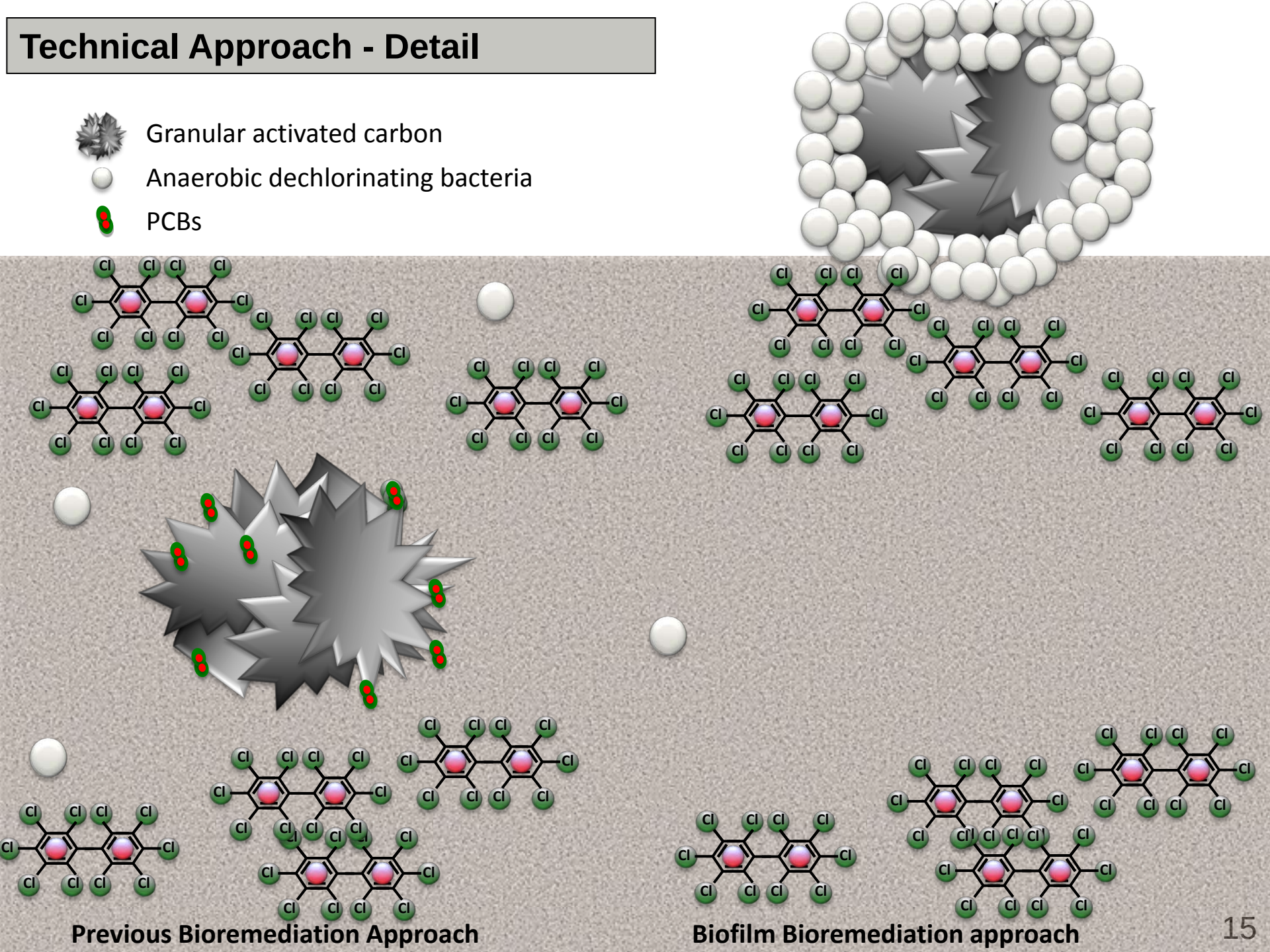
Apply **biofilm** communities to PCB contaminated sediment as a **delivery system** to enhance dechlorination

Technical Approach - Detail

Granular activated carbon

Anaerobic dechlorinating bacteria

PCBs



Previous Bioremediation Approach

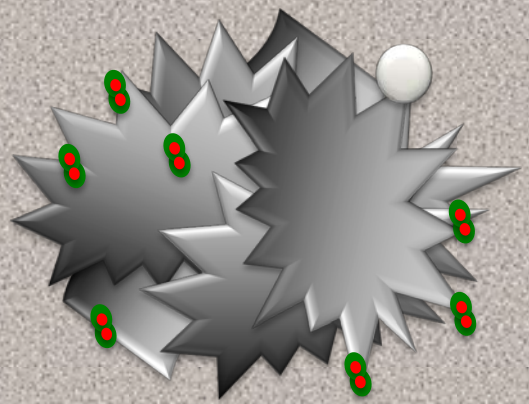
Biofilm Bioremediation approach

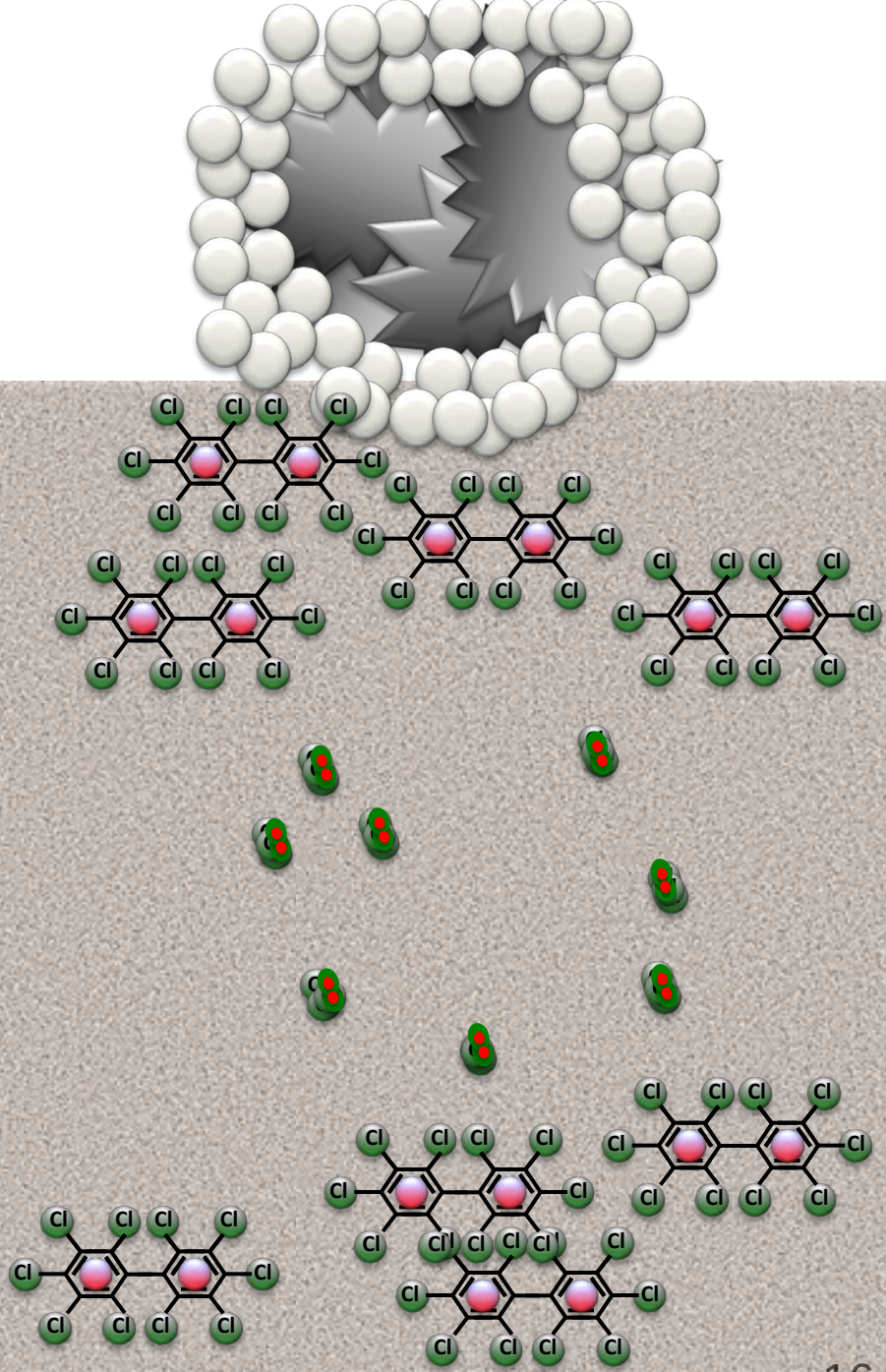
Technical Approach - Detail

Granular activated carbon

Anaerobic dechlorinating bacteria

PCBs

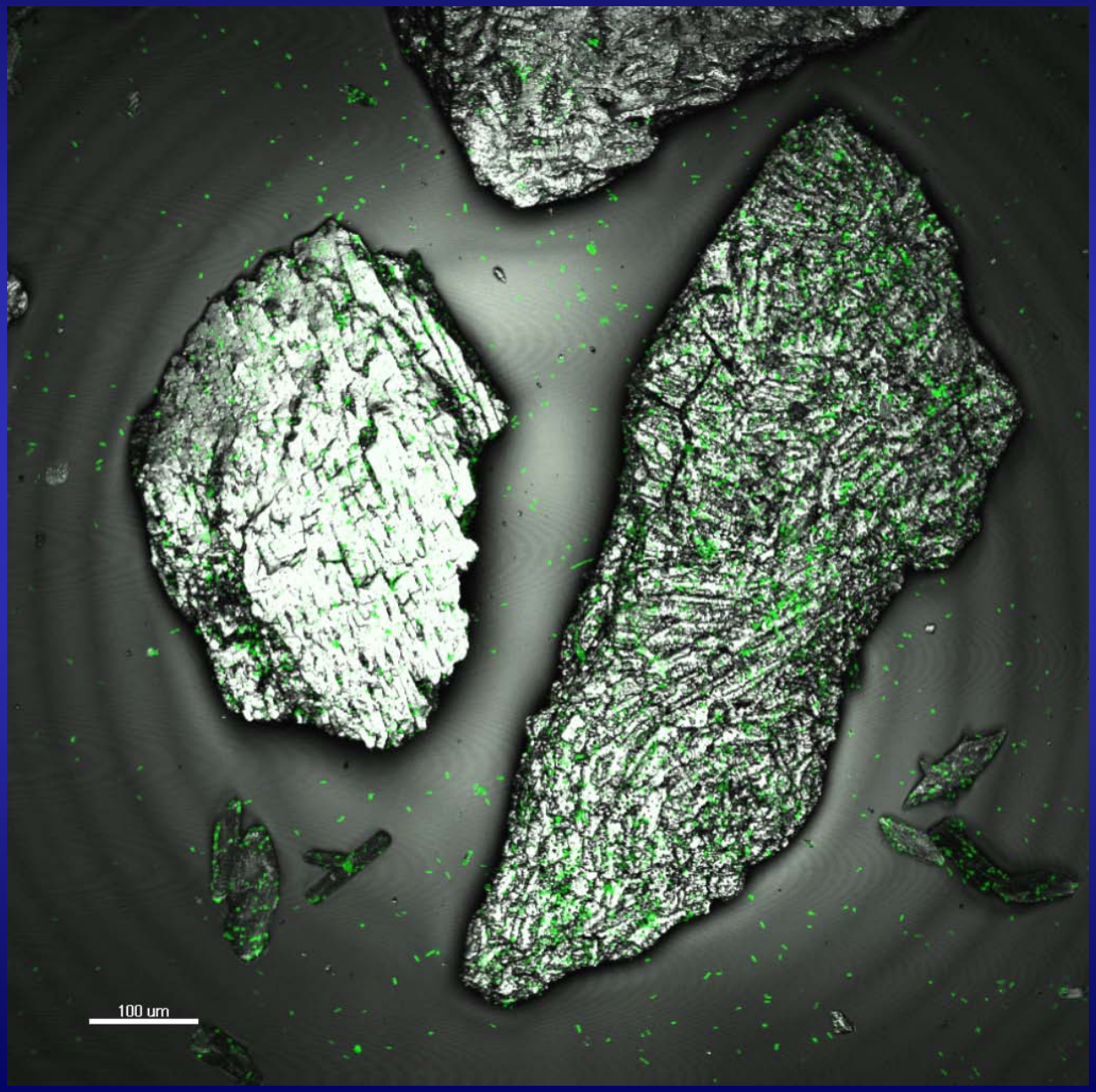




Previous Bioremediation Approach

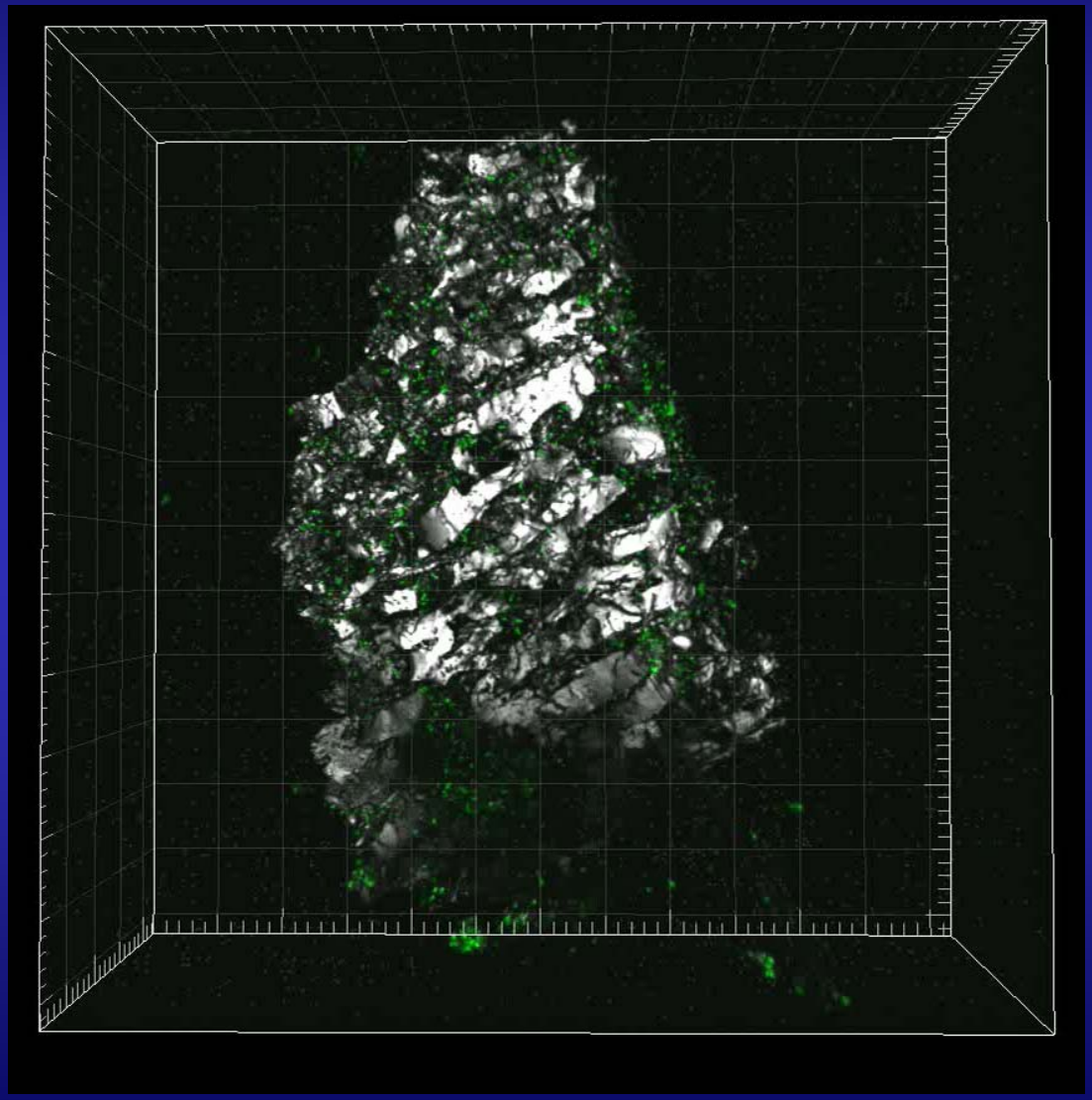
Biofilm Bioremediation approach

Direct SYBR Green staining - CLSM



Betsey Pitts,
MSU/CBE, 2012

Anaerobic Biofilm Formation



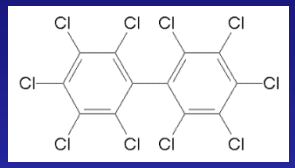
SYBR Green staining & CLSM

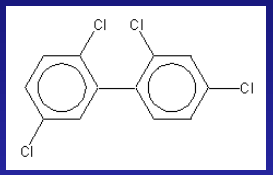
Edwards et al, 2016 (In prep)

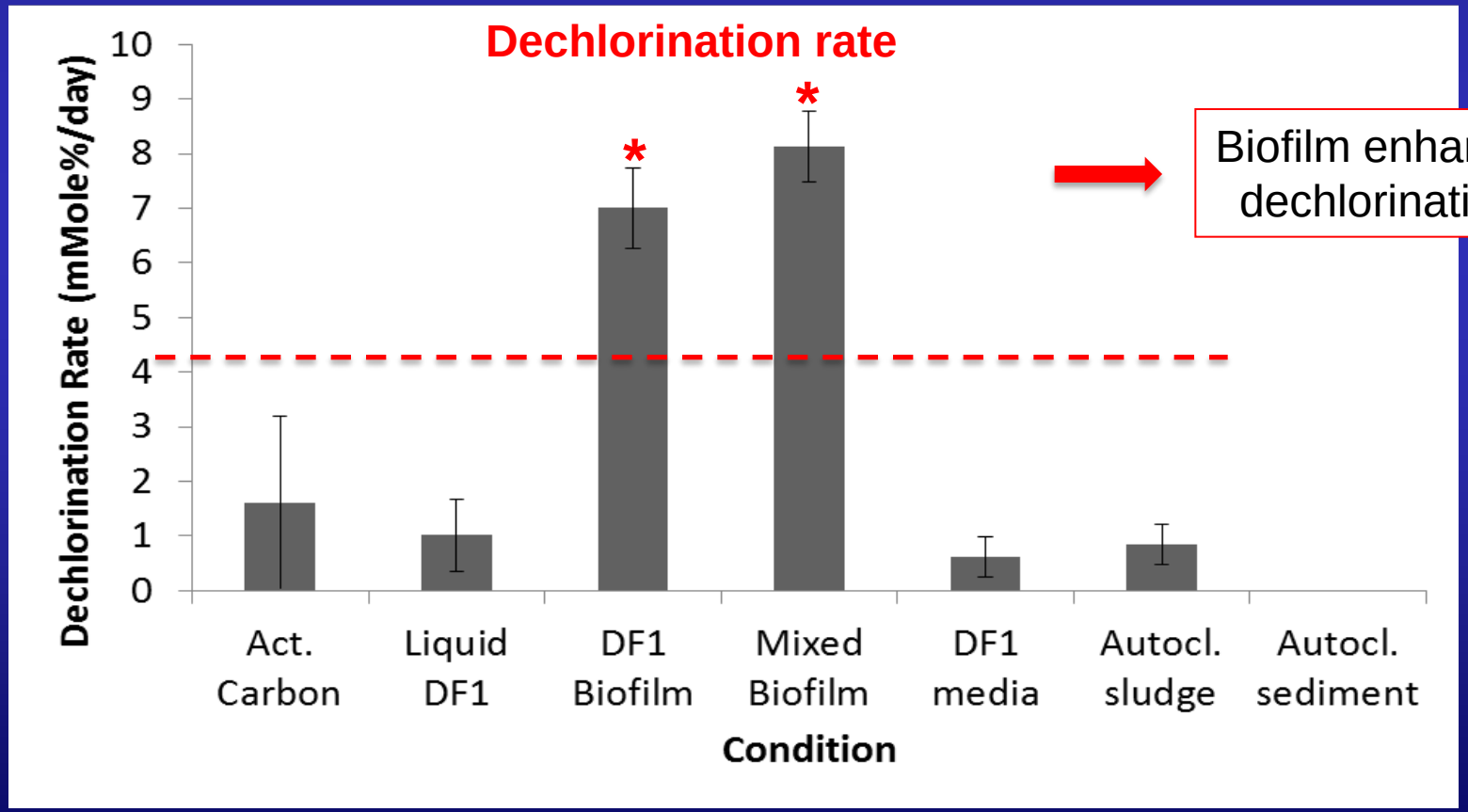
Effect on dechlorination?

Sediment mesocosms from Grasse River, NY

- **Two types of biofilm inoculum**





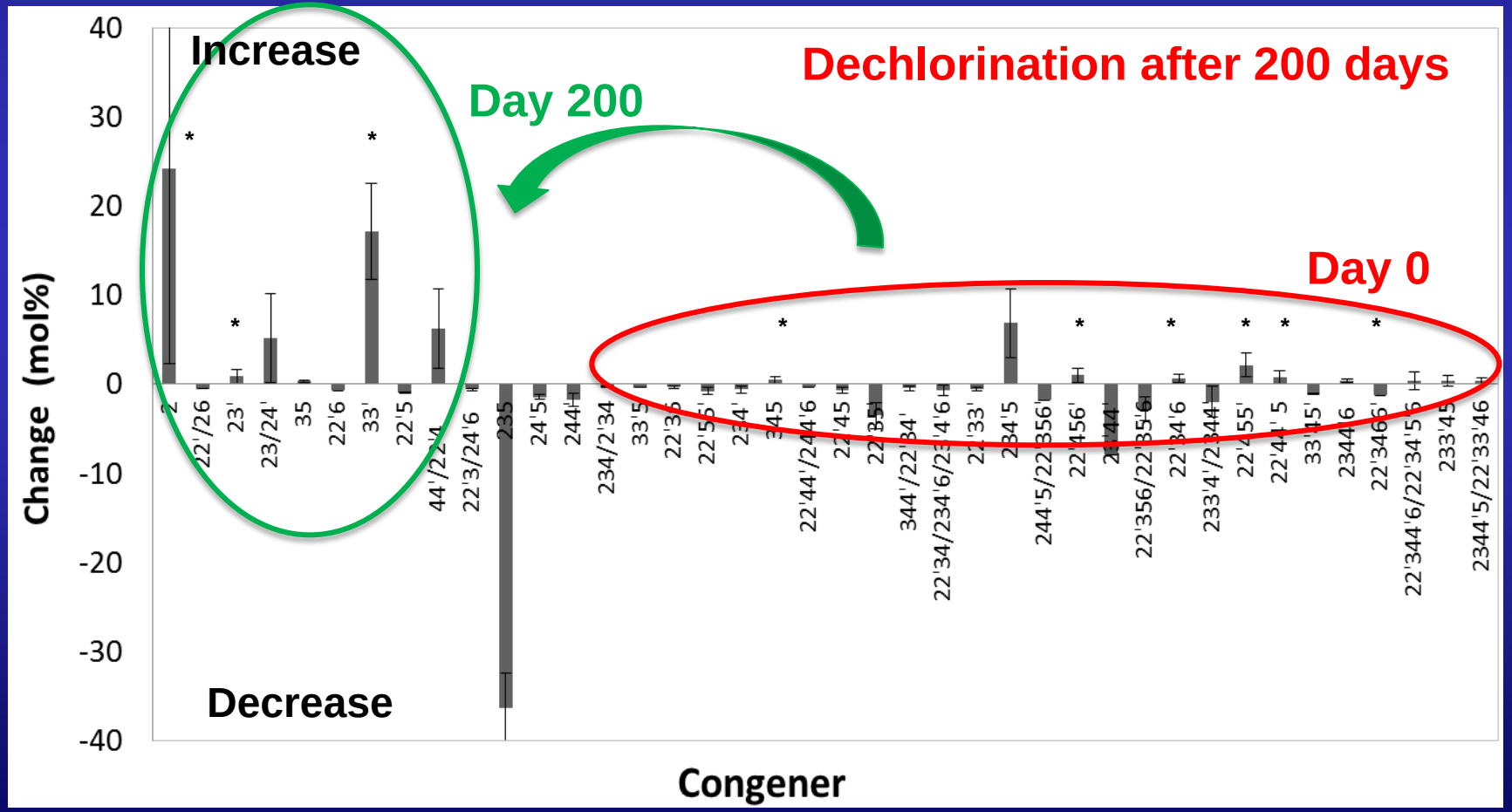


* Statistical significance <30% - EPA Standard

Edwards et al, 2016 (In prep)

Effect on dechlorination?

Mono and di-chlorinated congeners - significantly more in the presence of biofilm compared to GAC and liquid inoculum



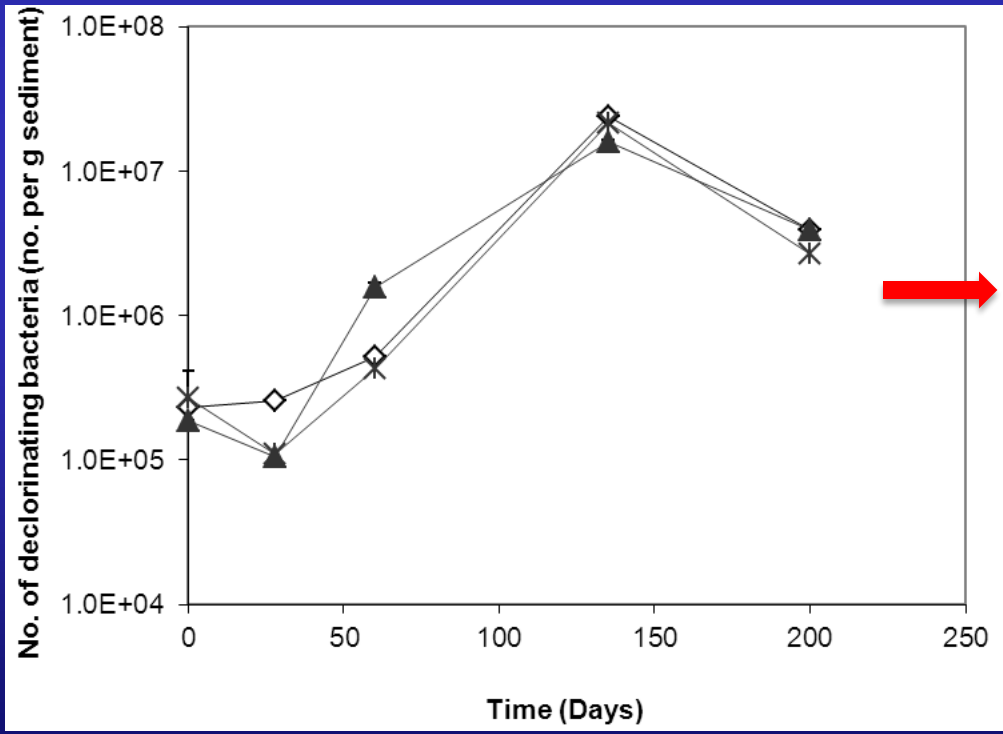
* Statistical significance <30% - EPA Standard

Edwards et al, 2016 (In prep)

Can the numbers explain?

Can the numbers of bacteria explain the difference in activity?

- Anaerobic DF1 biofilm



The numbers of dechlorinating bacteria are similar over time
⇒ Not responsible for the difference in activity

Reason:
⇒ Diversity?
⇒ Mode of growth?

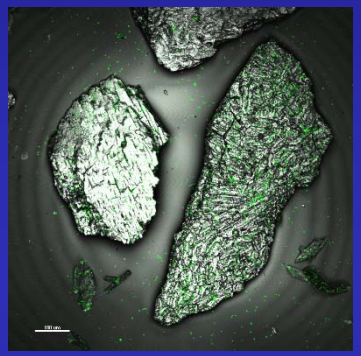
Edwards et al, 2016 (In prep)

Effect of Biofilms?

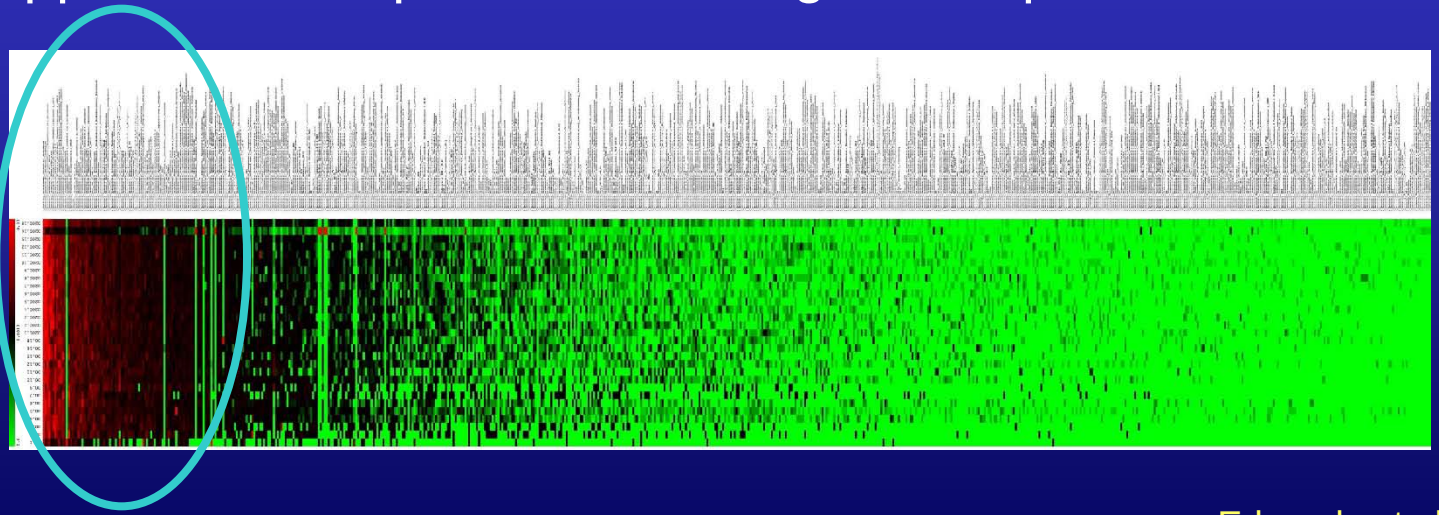
Looking at the microbial populations in the sediment

↓

What is the effect of Biofilm Augmentation?



- Approach: Multiplex 16S rRNA gene seq. - Illumina MiSeq



Edwards et al, 2016 (In prep)

Influence of Biofilms?

Taxon	Fold increase	Example organism/family	Function
Acidobacteria	2-35	*Holophagaceae*	Anaerobic cellulose degrader
Actinobacteria	3	*Micromonosporaceae*	Sediment bacterium
Alphaproteobacteria	2-10	*Rhodospirillaceae*	Metal (Se, Zn) reduction in soil
Betaproteobacteria	16	*Zoogloea*	Remediation of pharmaceutical and personal care products
Deltaproteobacteria	2-5	*Desulfobulbaceae*	Toluene-Degrading
Gammaproteobacteria	4	*Methylomonas*	Anaerobic methane oxidation
Armatimonadetes	3-5	-----	Anaerobic ammonium oxidation
Bacteroidetes	2-3	*Niabella*	Soil/Rhizosphere bacterium
Chloroflexi	2-8	*Anaerolineae*	Anaerobic degradation of oil-related compounds
Cyanobacteria	2-4	*Nostocaceae*	Nitrogen transformation
Elusimicrobia	3-4	*Elusimicrobiales*	Degradation of aromatic compounds
Firmicutes	4	*Ruminococcaceae*	Anaerobic cellulose degradation
Lentisphaerae	3	*Lentisphaeria*	Petroleum-hydrocarbon degradation
Nitrospirae	2	*Nitrospirales*	Acid tolerant fermentation
Planctomycetes	3	-----	Aromatic hydrocarbon degradation & denitrification
TM7	2	-----	Acidic mine drainage
Unknown Bacteria	2-6	-----	Related to soil/ sediment biofilms
Archaea	2	*Methanoregula*	Degradation of C7-8 iso-alkanes (methanogenic conditions)

Edwards et al, 2016 (In prep)

Influence of Biofilms?

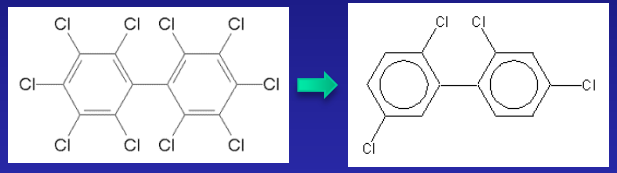
Taxon	Fold increase	Example organism/family	Function
Acidobacteria	2-35	*Holophagaceae*	Anaerobic cellulose degrader
Actinobacteria	3	*Micromonosporaceae*	Sediment bacterium
Alphaproteobacteria	2-10	*Rhodospirillaceae*	Metal (Se, Zn) reduction in soil
Betaproteobacteria	16	*Zoogloea*	Remediation of pharmaceutical and personal care products
Deltaproteobacteria	2-5	*Desulfobulbaceae*	Toluene-Degradation
Gammaproteobacteria	4	*Methylomonas*	Anaerobic methane oxidation
Armatimonadetes	3-5	-----	Anaerobic ammonium oxidation
Bacteroidetes	2-3	*Niabella*	Soil/Rhizosphere bacterium
Chloroflexi	2-8	*Anaerolineae*	Anaerobic degradation of oil-related compounds
Cyanobacteria	2-4	*Nostocaceae*	Nitrogen transformation
Elusimicrobia	3-4	*Elusimicrobiales*	Degradation of aromatic compounds
Firmicutes	4	*Ruminococcaceae*	Anaerobic cellulose degradation
Lentisphaerae	3	*Lentisphaeria*	Petroleum-hydrocarbon degradation
Nitrospirae	2	*Nitrospirales*	Acid tolerant fermentation
Planctomycetes	3	-----	Aromatic hydrocarbon degradation & denitrification
TM7	2	-----	Acidic mine drainage
Unknown Bacteria	2-6	-----	Related to soil/ sediment biofilms
Archaea	2	*Methanoregula*	Degradation of C7-8 iso-alkanes (methanogenic conditions)

Edwards et al, 2016 (In prep)

Effect of Biofilm Augmentation?

Summary:

- Enhanced PCB dechlorination



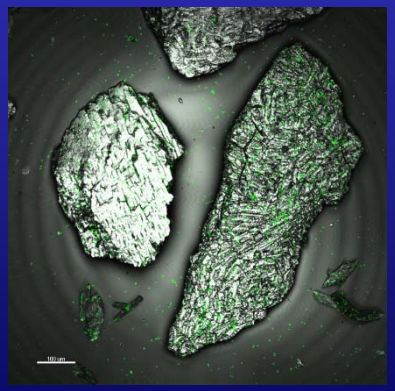
- *Sediment Population analysis:*

- Other *Chloroflexi* than "usual suspects" are involved (*Dehalococcoides* and DF1)
- 18 groups of bacteria show 2+ fold upregulation
  $\rightarrow$ Related to contaminated sediment/soil (anaerobic)

What does this mean?

Biofilms impact the overall sediment population, NOT only the PCB dechlorinating population.

Mechanism?

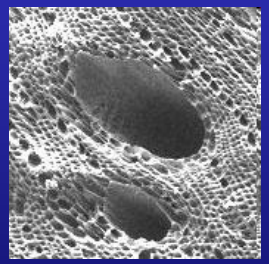


Edwards et al, 2016 (In prep)

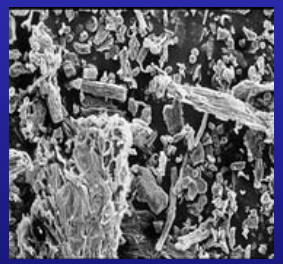
Biofilm based delivery system

Ongoing research activities:
Identification of the **mechanism** responsible for increased activity of GAC-Biofilm based bioaugmentation

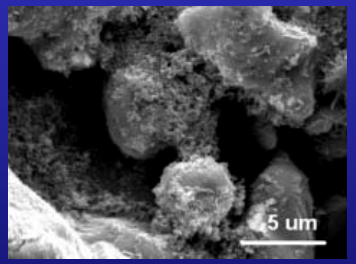
- Electrical conductivity?
- Sorption (kinetics)?
- Surface area/porosity?
- Other?



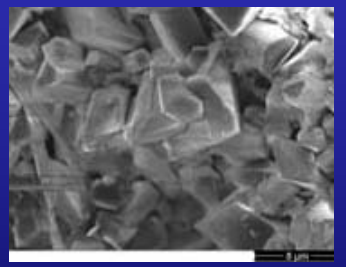
Activated carbon



Biochar



Fe covered AC

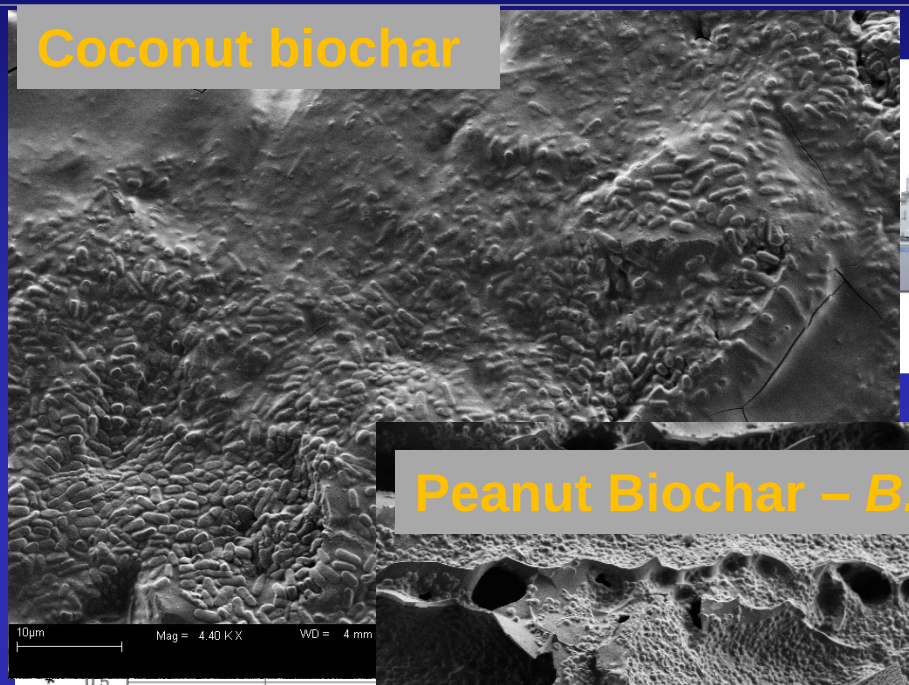


Zeolite

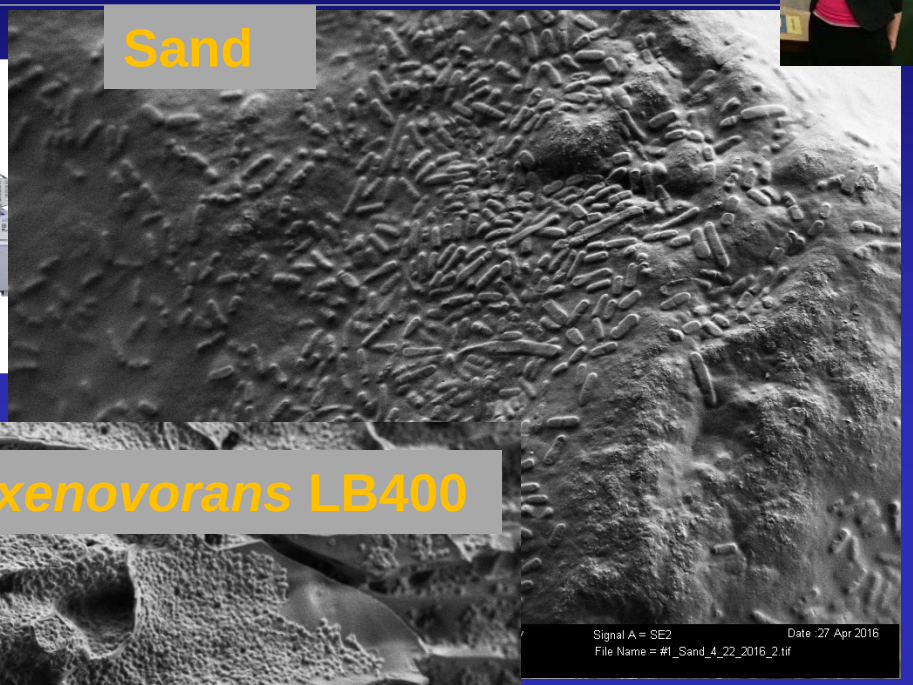
A. Prieto et al (in prep), 2016

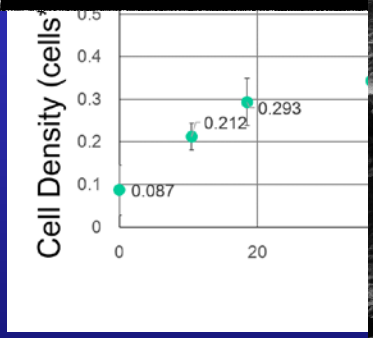
Biofilm based delivery system

Coconut biochar

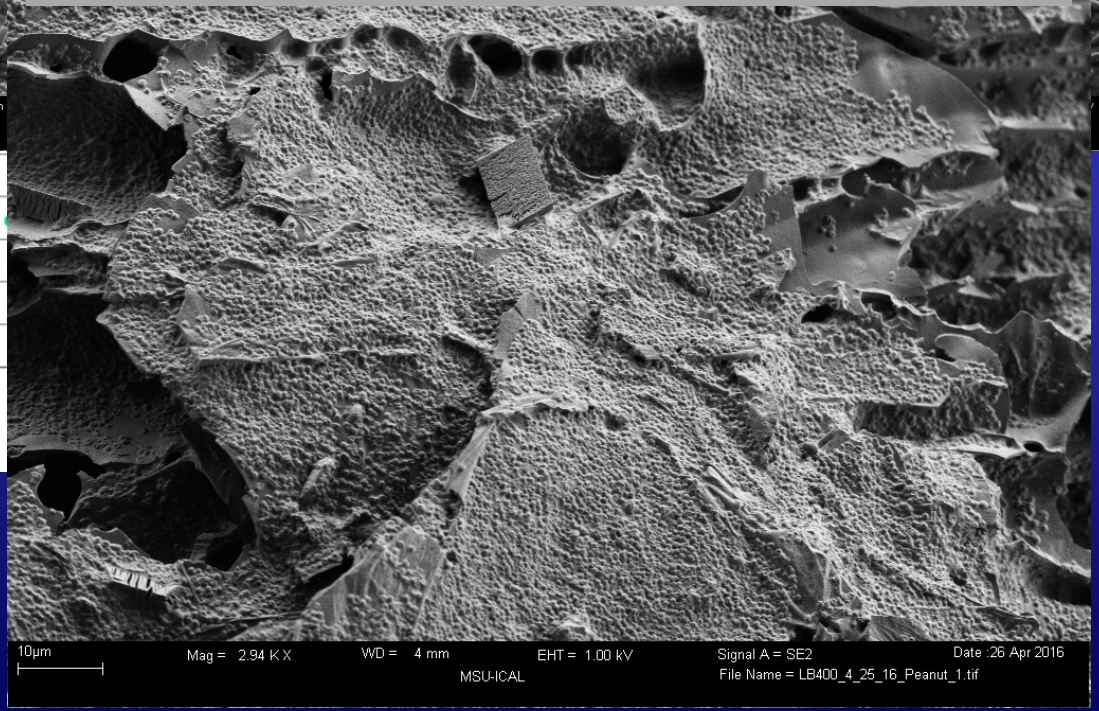


Sand



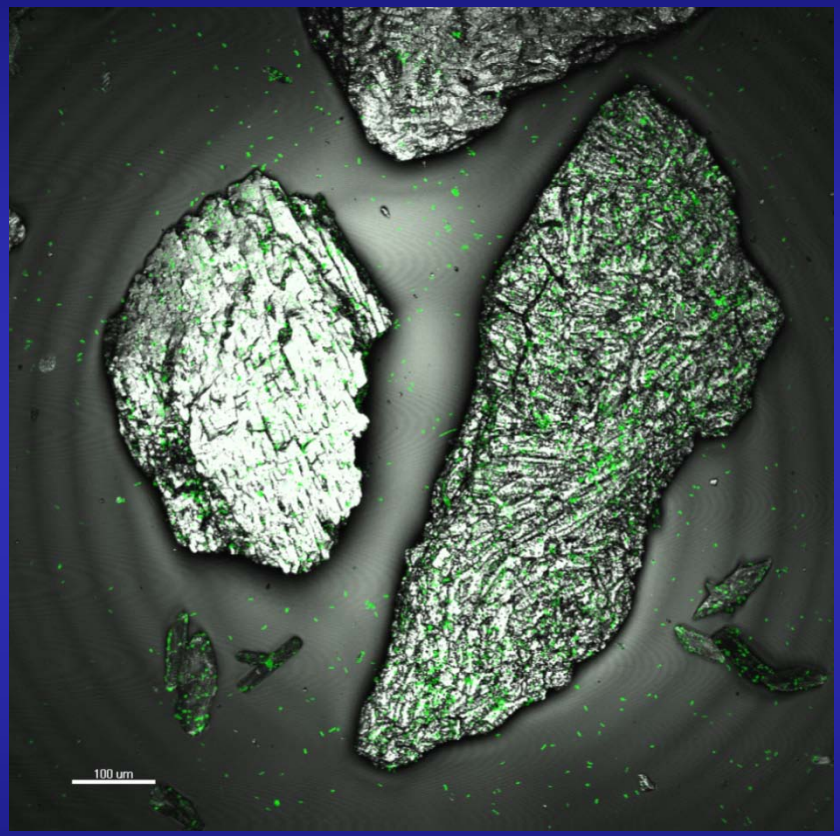


Peanut Biochar – *B. xenovorans* LB400



Summary

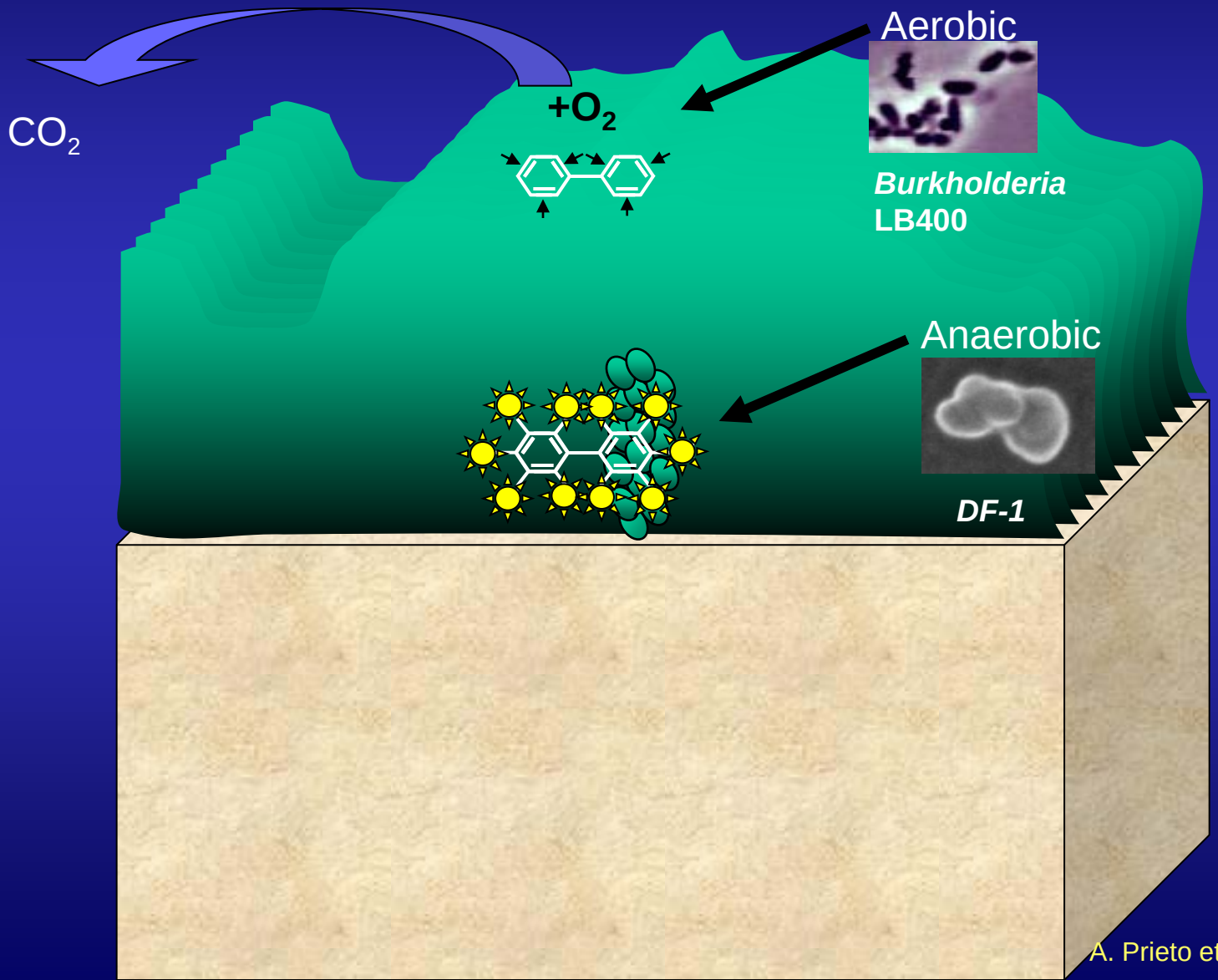
- Dechlorinating **biofilms** can effectively be cultured under anaerobic conditions
- Application of anaerobic biofilms as a **delivery vehicle** enhances dechlorination of PCBs in sediment
- Biofilms are **robust** and can be maintained in sediment



→ **Good solution for anaerobic bioremediation**

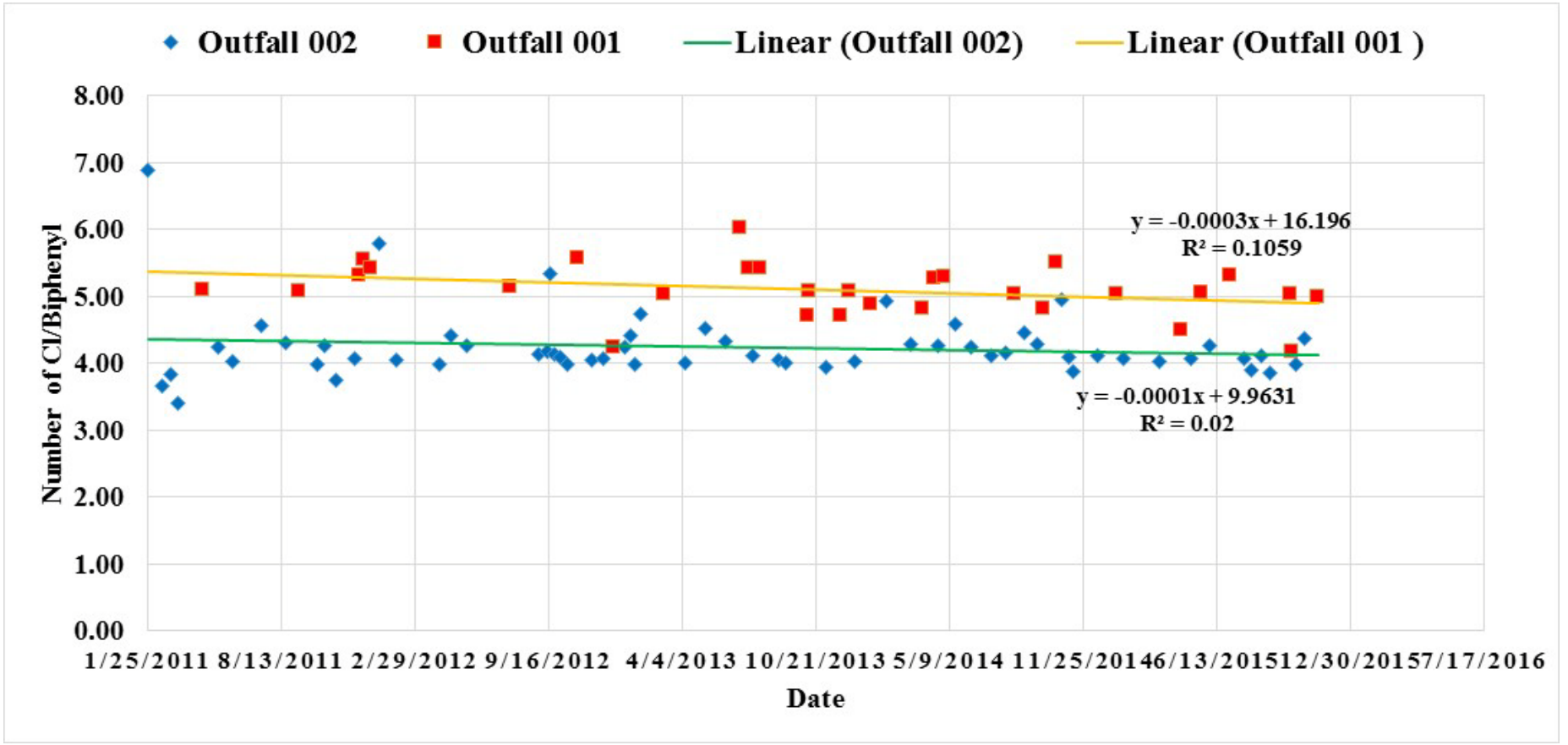
→ **Complete mineralization?**

Fate of PCBs in Mixed Biofilms



A. Prieto et al (in prep), 2016

Recontamination of sediment from wastewater?



	Outfall 002	Outfall 001
Average	**4.25**	**5.09**
STDEV	0.51	0.39

UNIVERSITY OF MARYLAND

Acknowledgements

Financial support for Project ER 2135

Kjellerup Lab

Terp Service Day

Students:

Freshta Akbari, Sarah Edwards

Chiara Draghi (Graduated)

Kirstie Coombs, BS (2017)

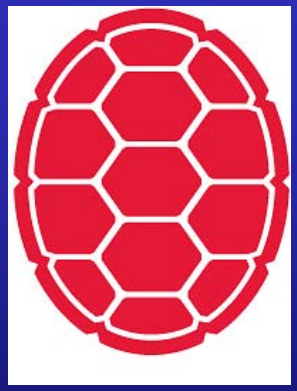
Raymond Jing, Ph.D. (2019)

Dr. Ana Prieto, Post Doc.

Others involved:

- Kevin R. Sowers, Ph.D. IMET, UMBC
- Ms. Betsy Pitts, M.Sc., Center for Biofilm Engineering, Montana State University
- Dr. Recep Avci, Ph.D., Image and Chemical Analysis Laboratory, Montana State University
- Natasha Andrada & Upal Ghosh, Ph.D., UMD/UMBC

Thanks for your attention.



Contact – Please email: bvk@umd.edu