A Journey Through the World of Applied Aquatic Ecology

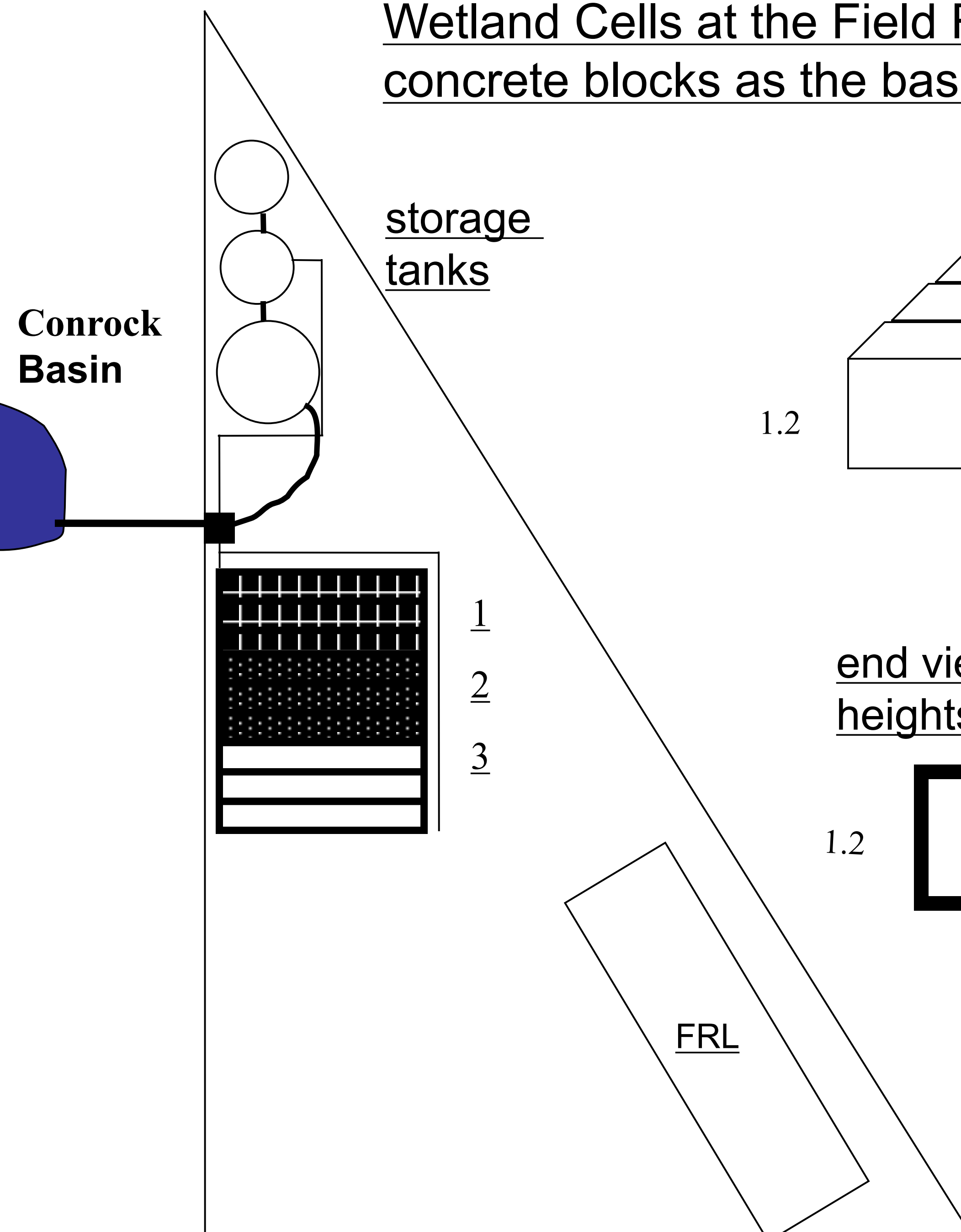
Stephen Lyon, Ph.D.

Part 2

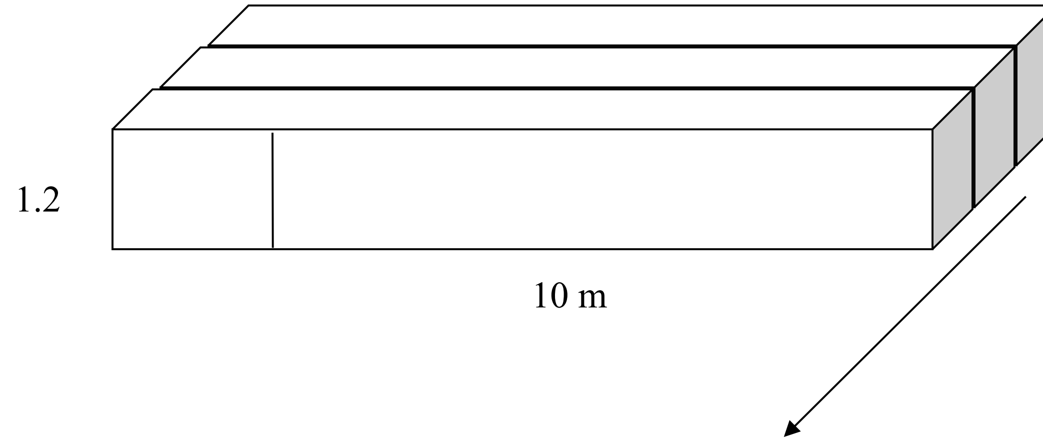
“The secrets of nature are in knowing their details”

OCWD Field Research Laboratory (FRL), Anaheim, CA, established 2001

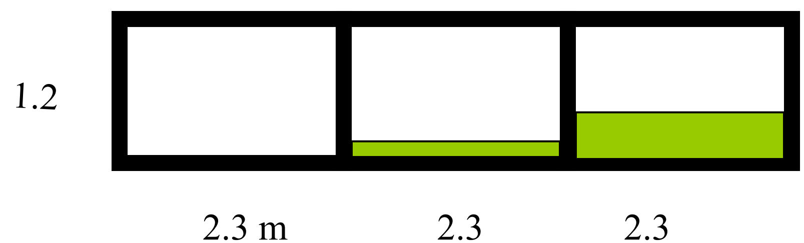
Wetland Cells at the Field Research Laboratory using 2’x2’x6’ concrete blocks as the base material



3D view of a 3-cell unit

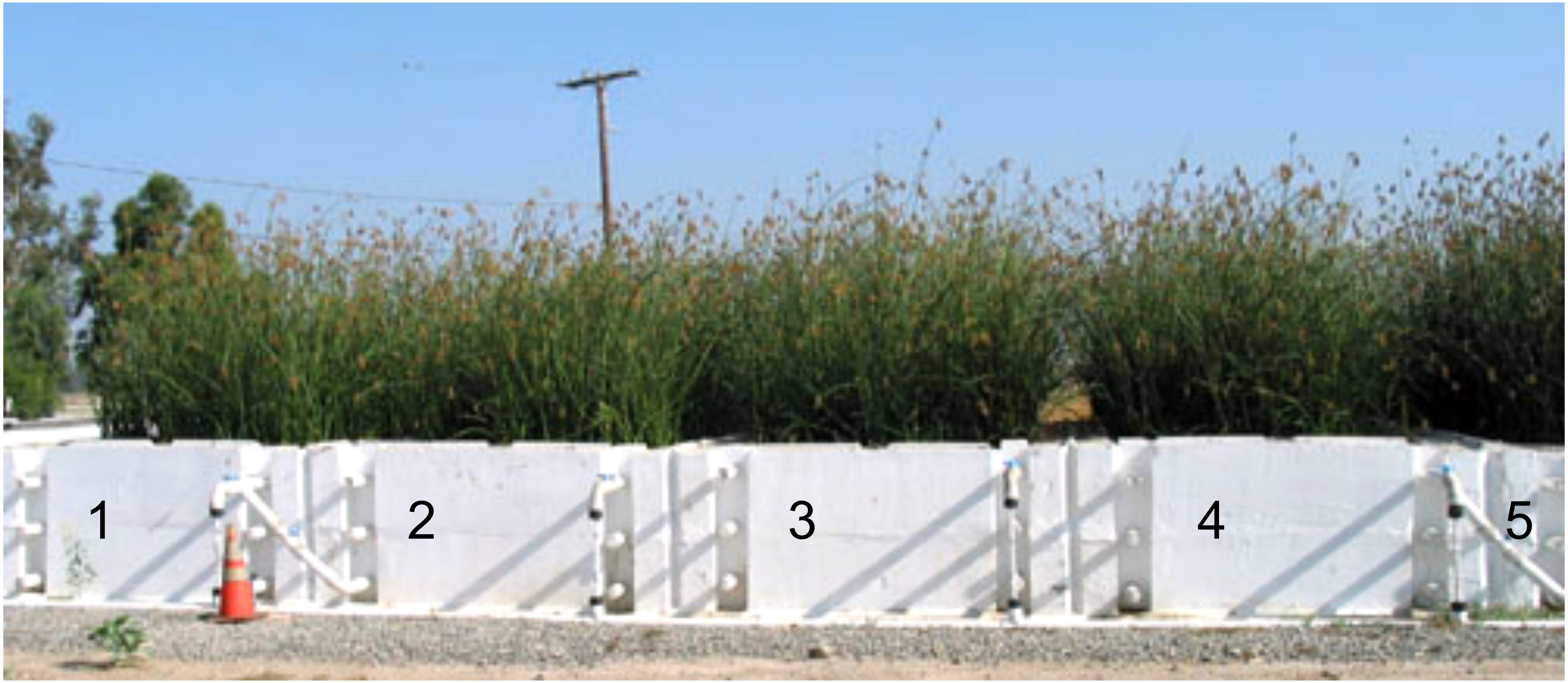


end view of three cells with different bed heights to allow for flow from cell to cell




July 2003


October 2003



SSF Constructed Wetlands at the OCWD FRL, July 2004. Cells 1-6 received 2, 4, 8, 12, 15 L/minute of Santa Ana River Water.

Summary of SSF Nutrient Data

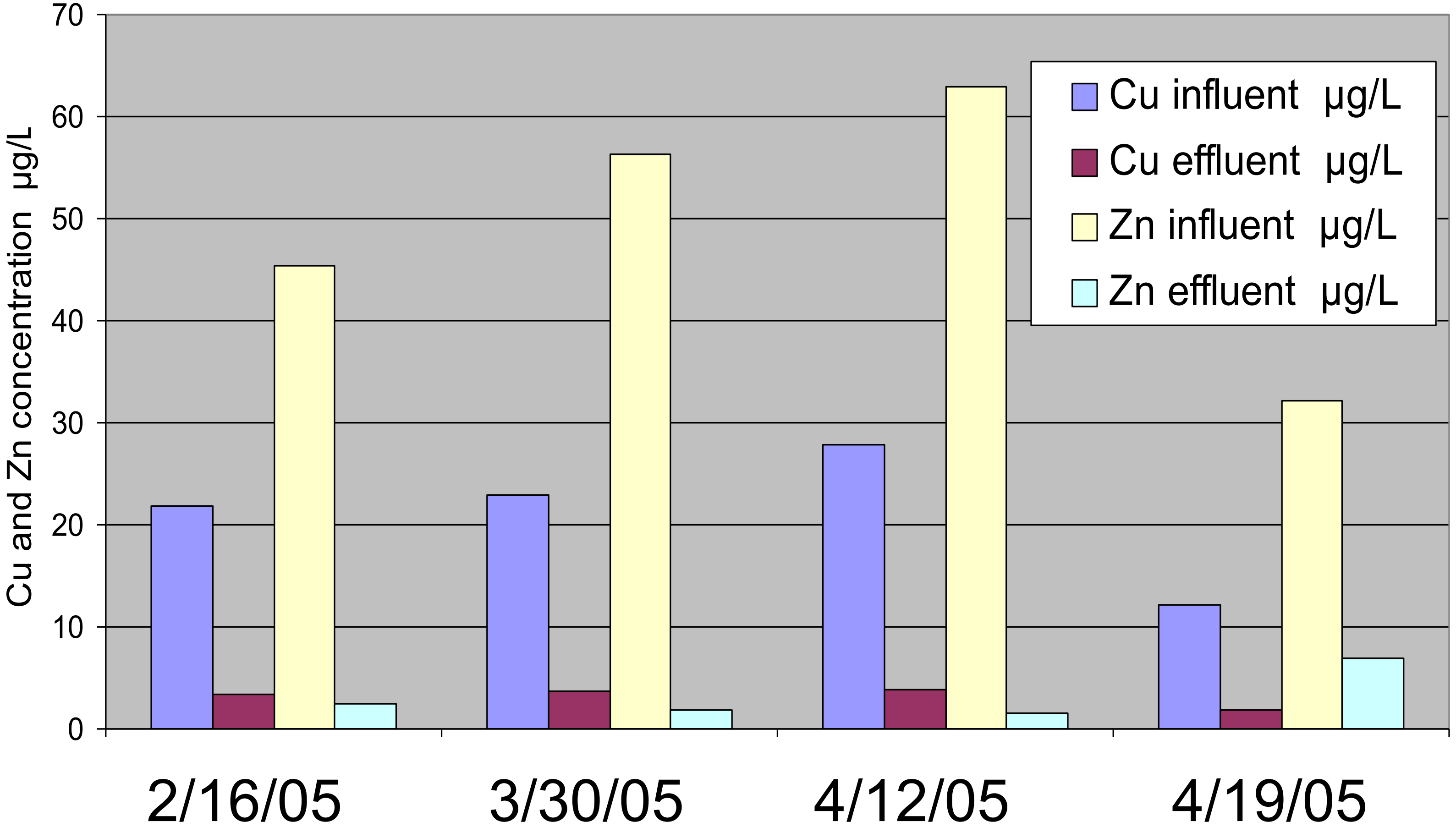
	average % Nitrate removal	average % Phosphate removal
5 gpm	16.9	19.2
4 gpm	19.6	23.0
3 gpm	27.4	30.9
2 gpm	39.6	39.3
1 gpm	59.5	56.5
0.5 gpm	**87.5**	**85.8**

average inflow (mg/l)

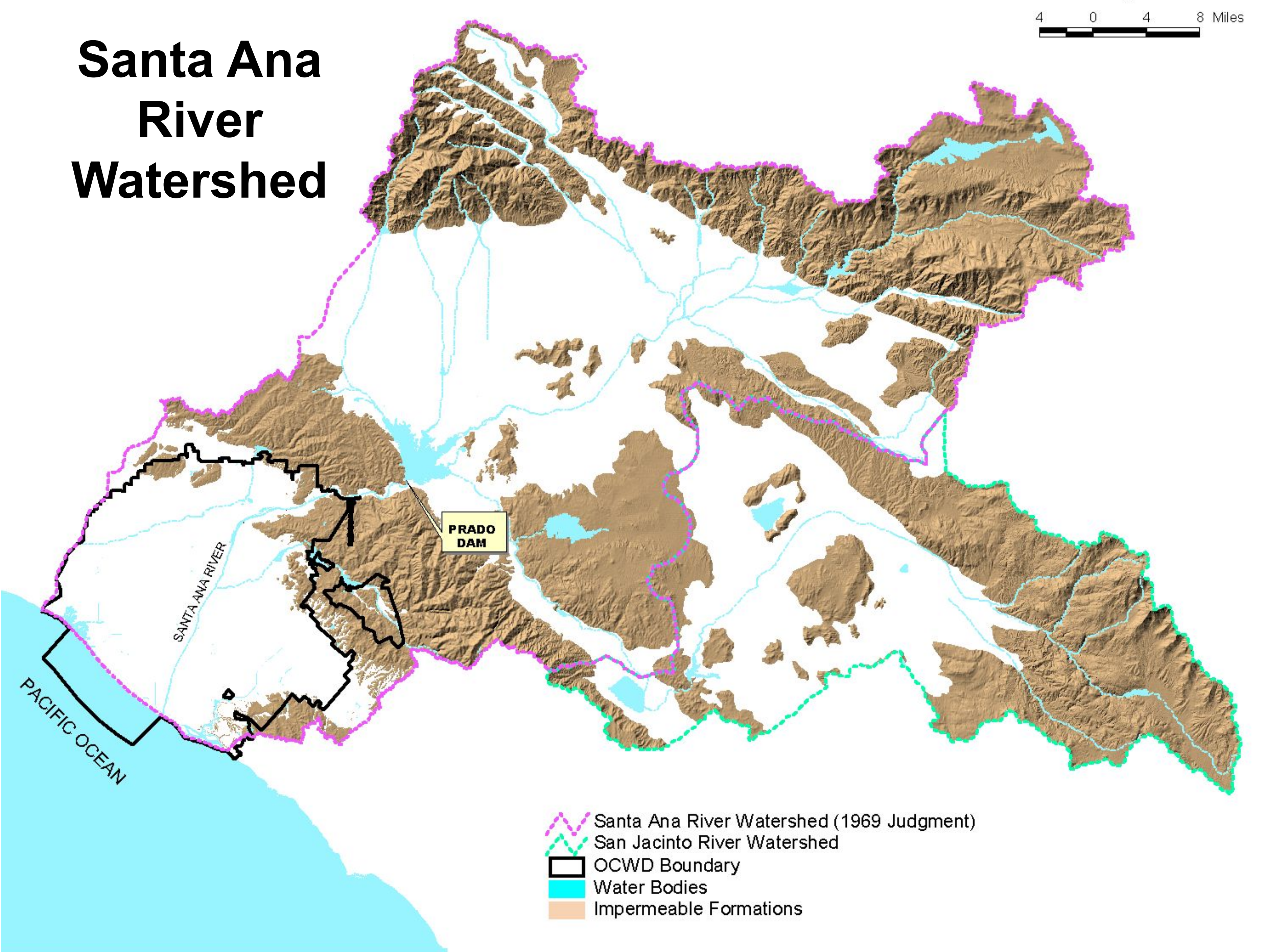
Nitrate :3.73

Phosphate: 1.78

Removal of Copper and Zinc by SSF Wetlands



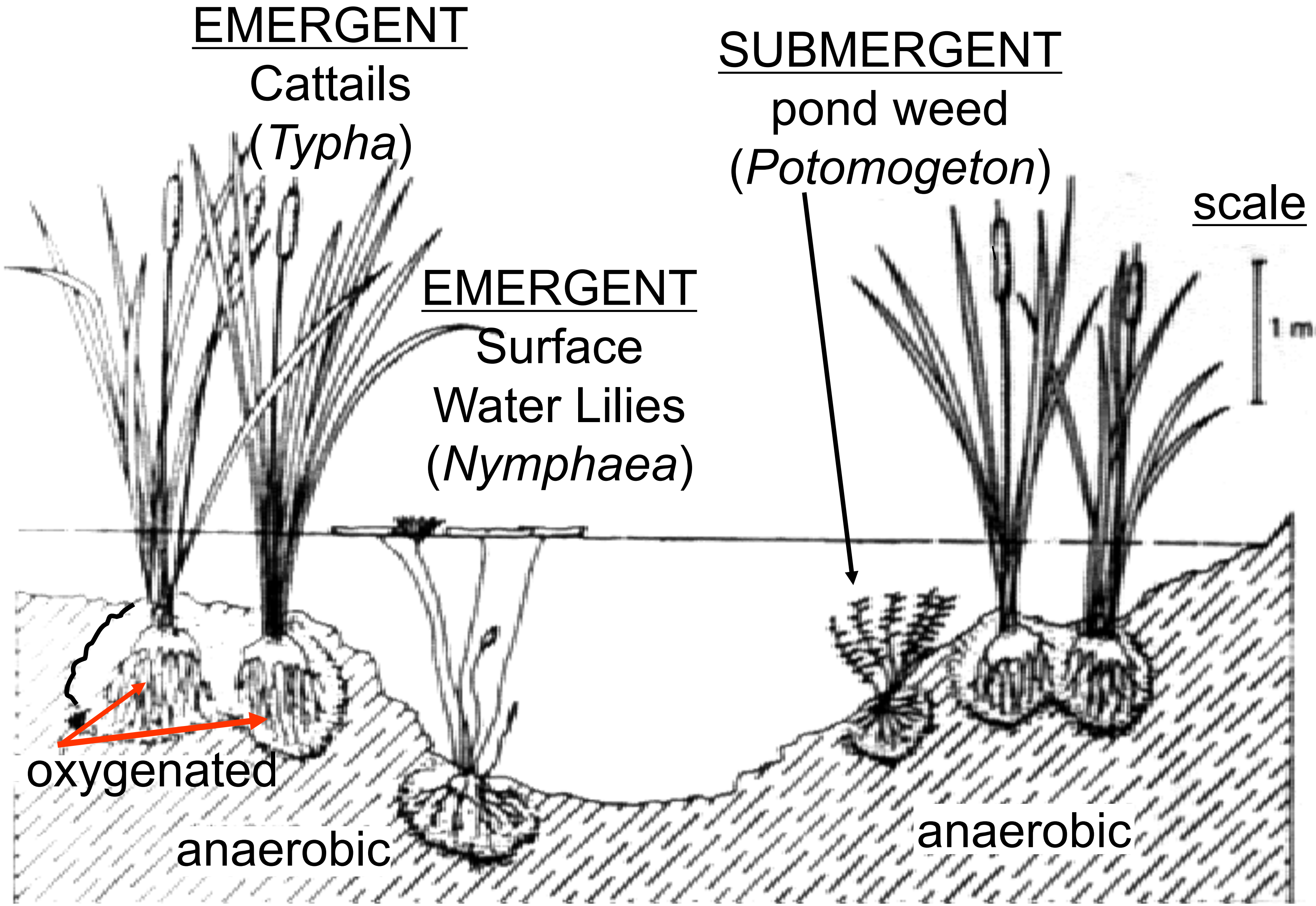
Santa Ana River Watershed



The Great Wetlands Toolbox

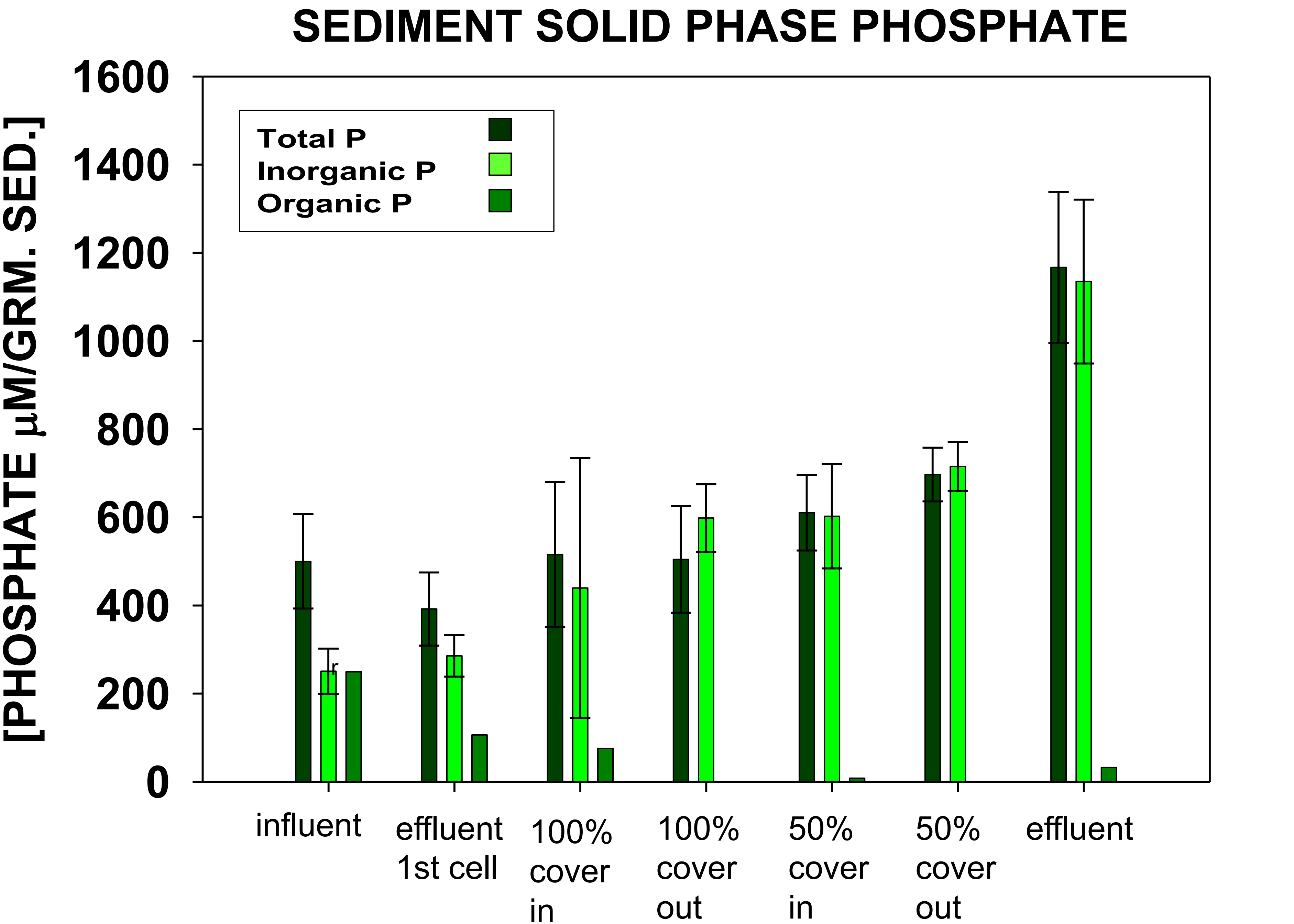
- Aquatic Ecology
- Environmental Chemistry
- Classical Microbiology
- Sediment Enzymes
- Stable Isotope Analysis
- Microbial Community Analysis using molecular methods

Free Water System (FWS) Constructed Wetlands in Prado Basin – 180 hectares - 240 ML/D



In a natural or open wetland the majority of treatment occurs as a function of microbial processes at the sediment/water interface

OCWD Prado Wetlands (188 hectares) treats 240 ML/D of Santa Ana River Water. Shown above are the current sampling sights that are monitored on a weekly basis and % plant cover.


SEDIMENT SOLID PHASE PHOSPHATE
[PHOSPHATE μM/GRM. SED.]
1600
1400
1200
1000
800
600
400
200
0
Total P
Inorganic P
Organic P
influent
effluent 1st cell
100% cover in
100% cover out
50% cover in
50% cover out
effluent

Summary of Prado Nutrient Data

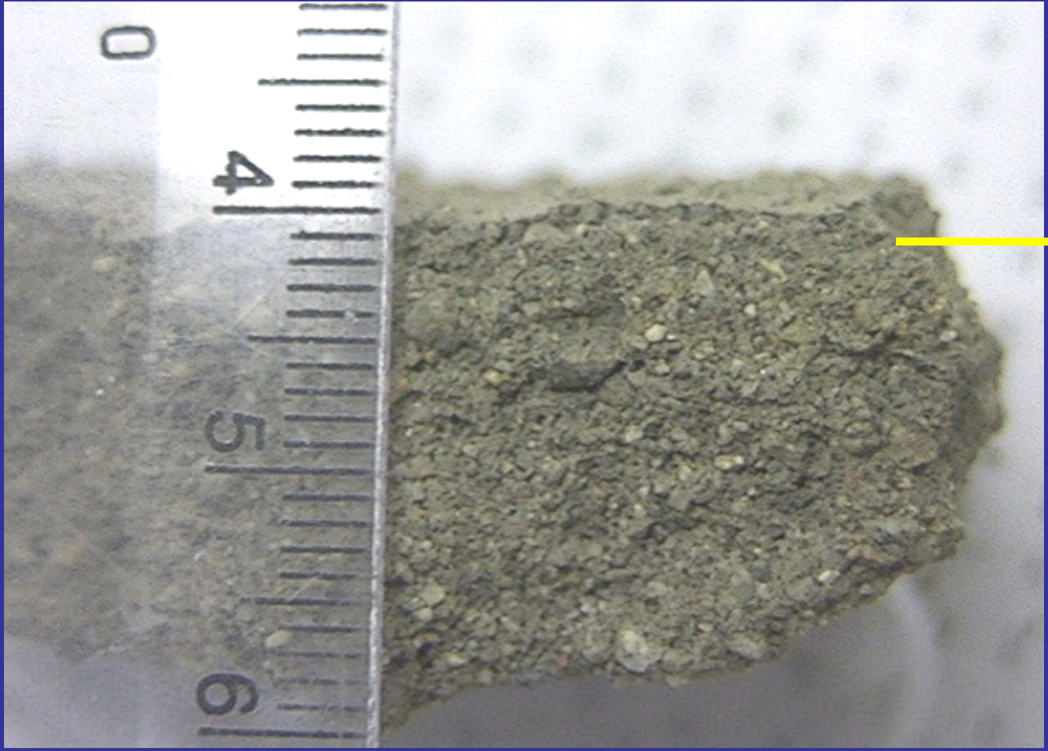
	average % Nitrate removal	average % Phosphate removal
outflow	64.3	**39.3**
25% cover	76.0	**33.0**
75%cover	**89.0**	28.4
50%cover	**96.3**	22.3
100%cover	11.4	16.1

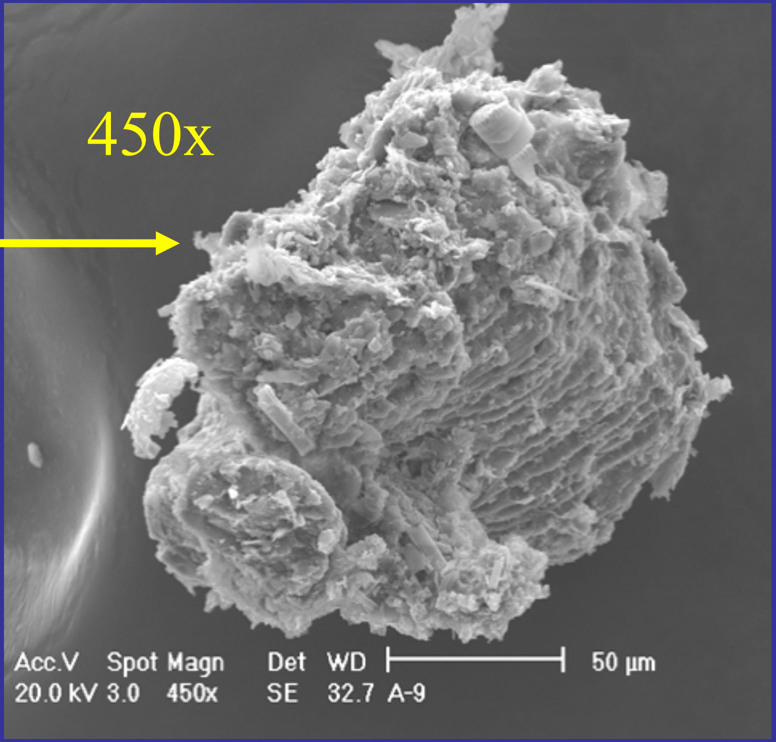
average inflow (mg/l)

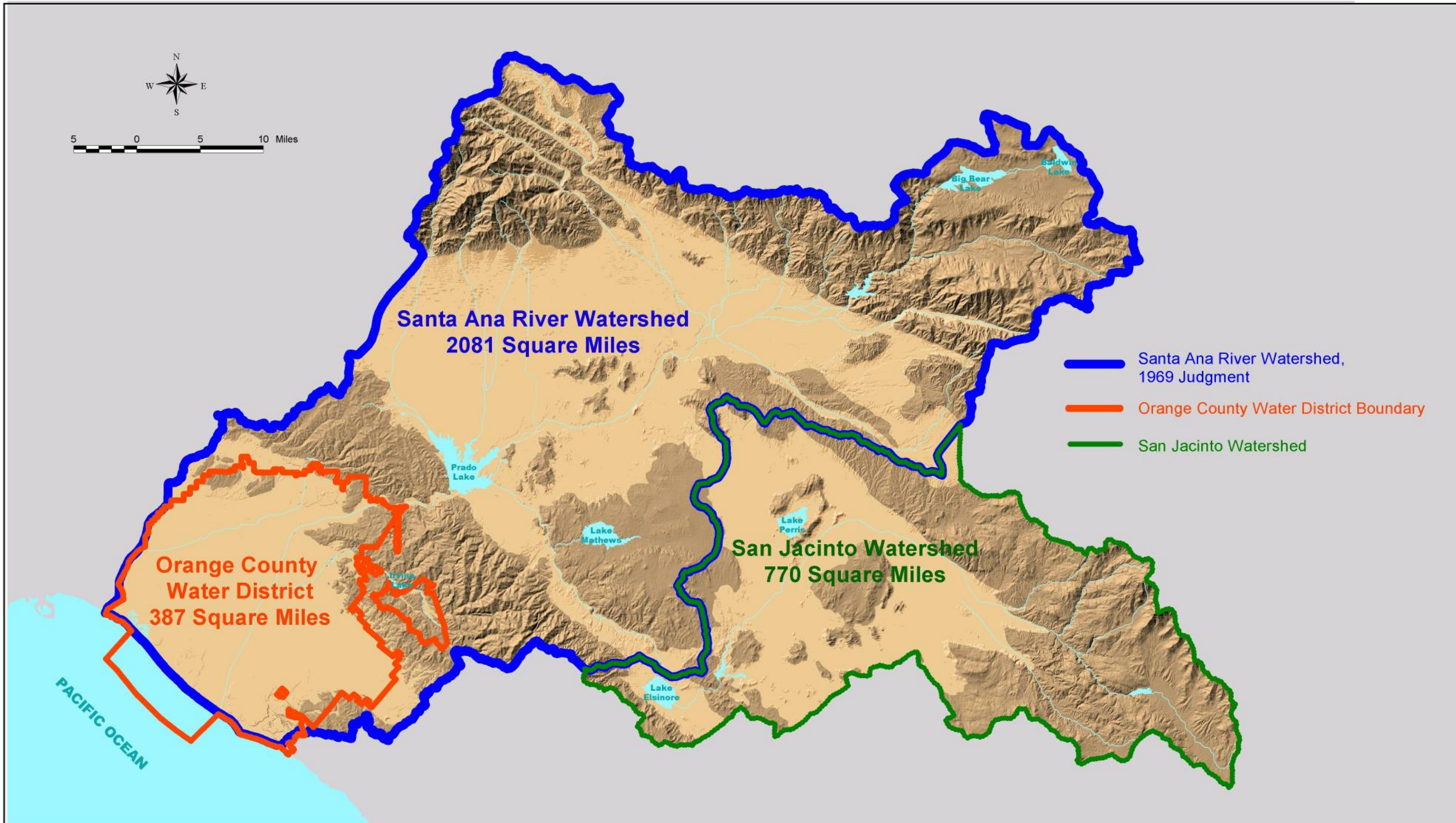
Nitrate :6.57

Phosphate: 3.13

Hardened Crust Layer Formation in Percolation Basin Sediments





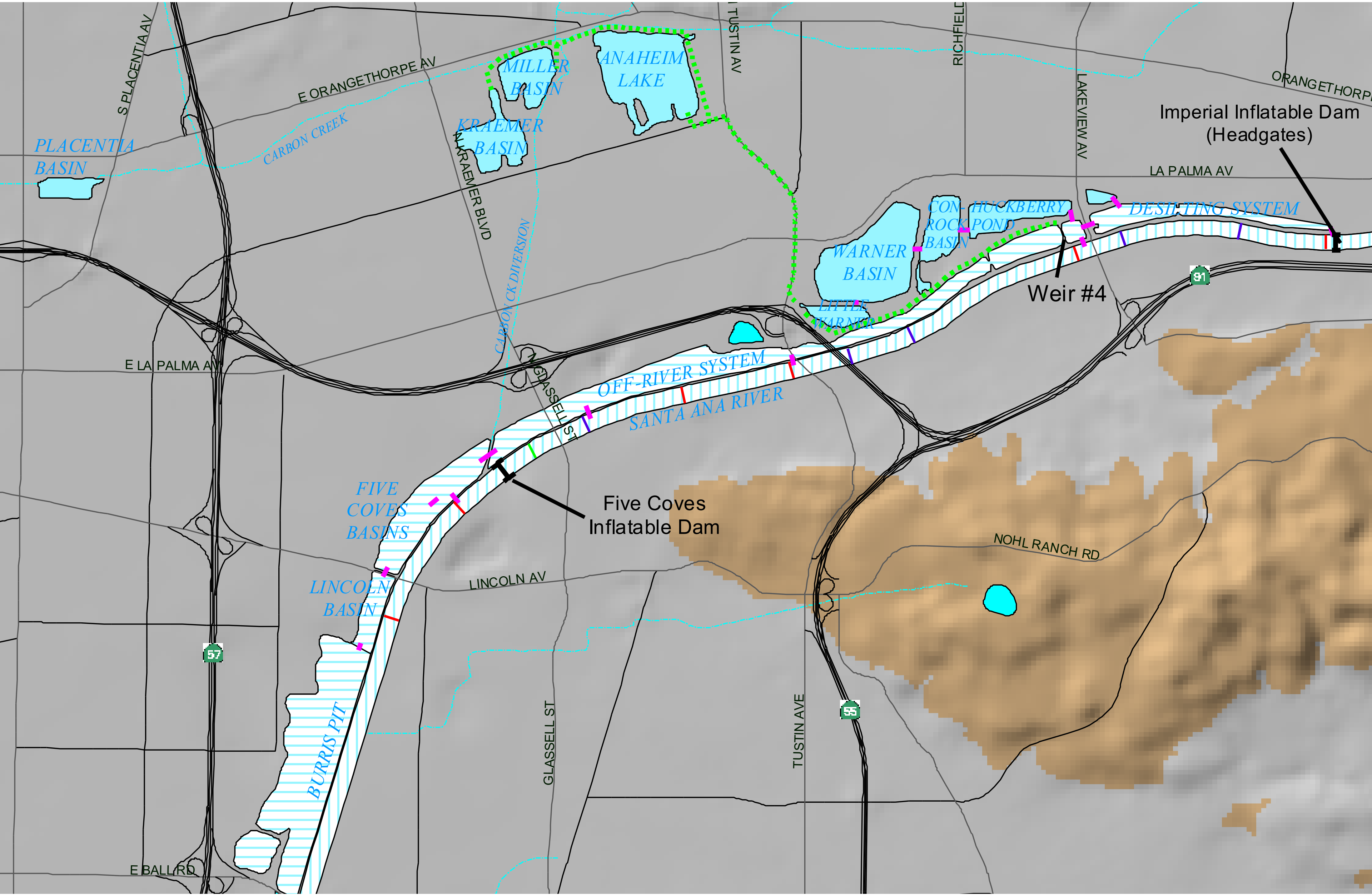


ORANGE COUNTY
WATER DISTRICT

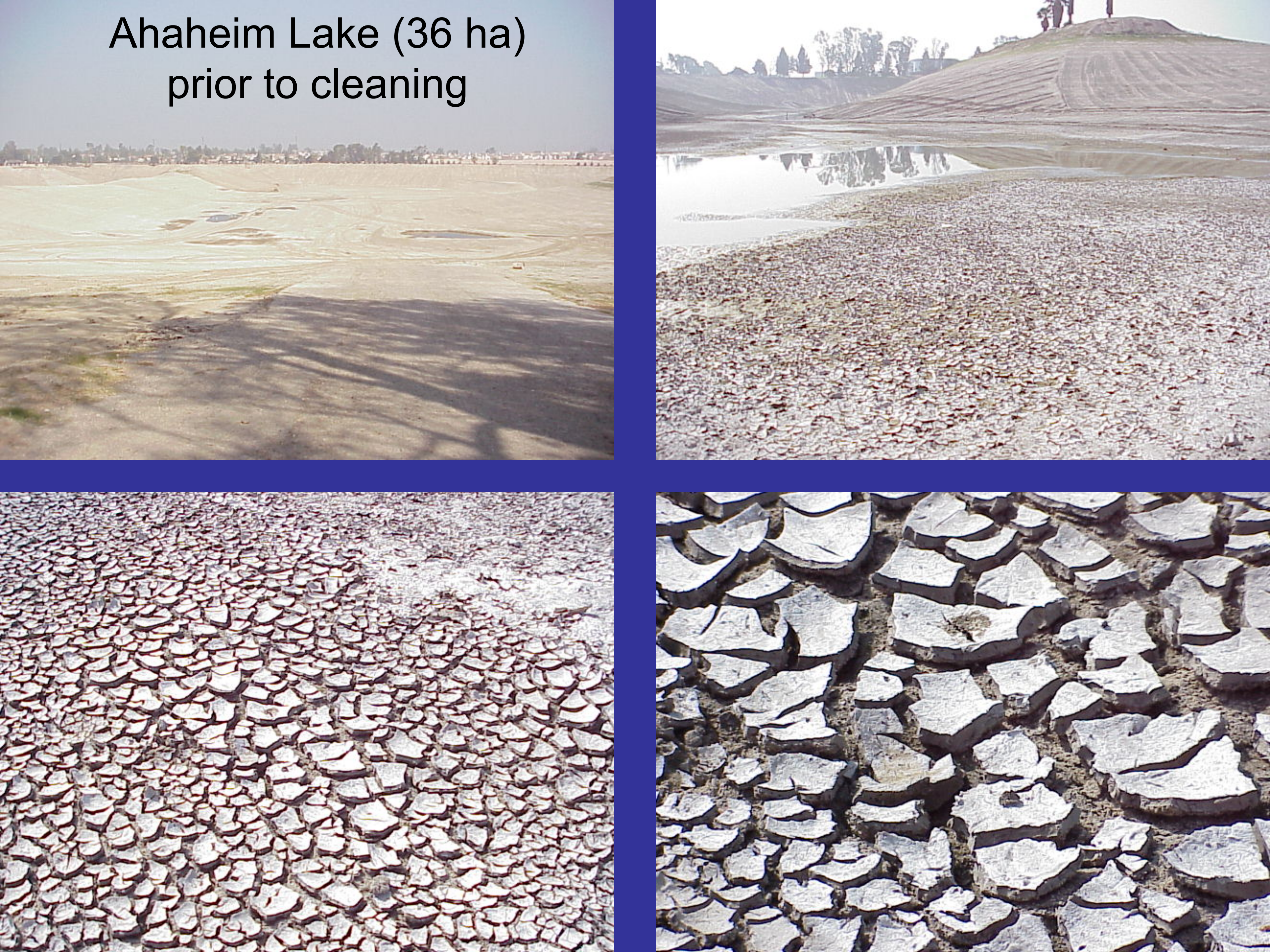
WATERSHED AND DISTRICT BOUNDARIES

Santa Ana River is Critical for Replenishment of Orange County's Groundwater Basin

- Over 2 million residents in Orange County depend on groundwater for 75% of their water supply
- Any factor in the watershed which degrades the river impacts the drinking water supply

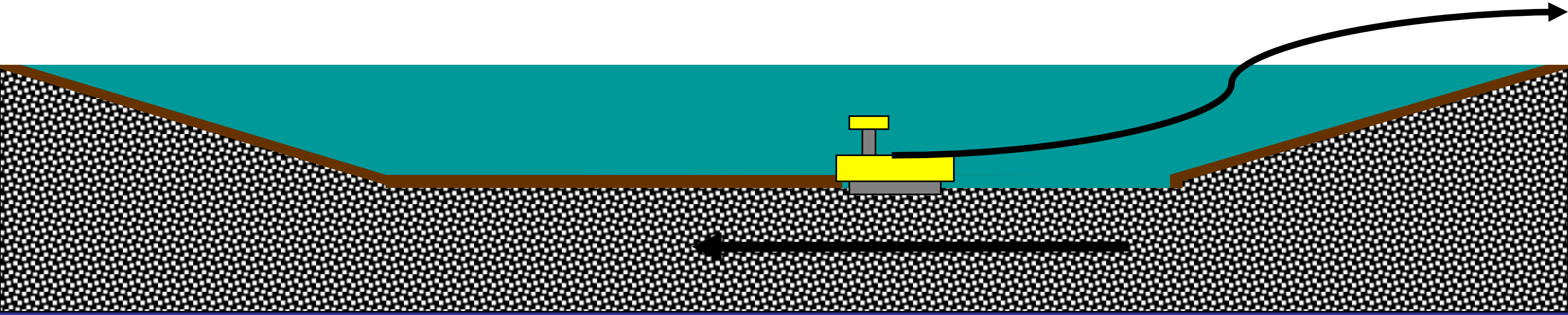


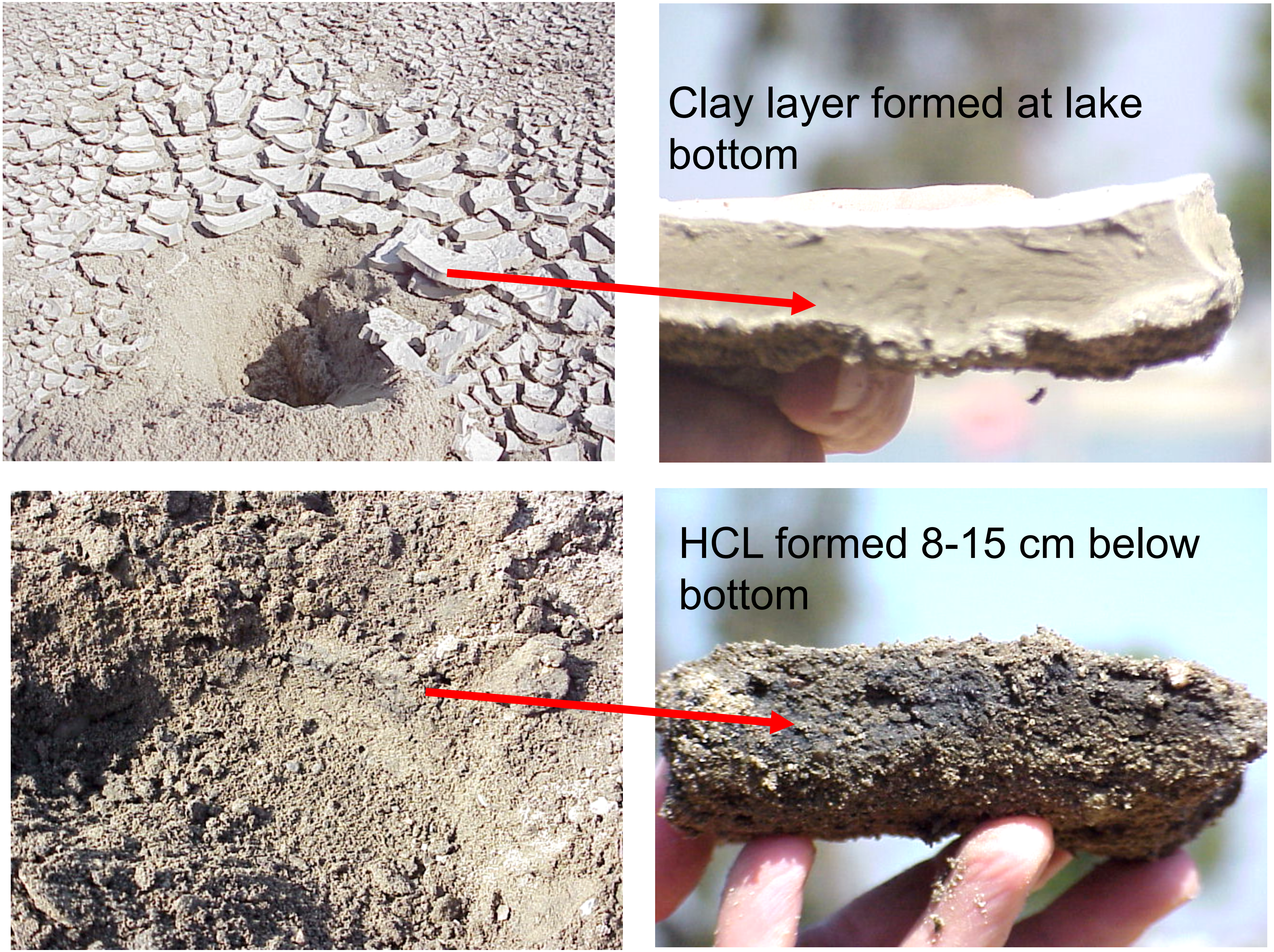
6 Km^2 groundwater recharge zones that percolates 480 ML/D


Ahaheim Lake (36 ha)
prior to cleaning

Basin Cleaning Vehicle (BCV-II)

BCV Removing Sediment from recharge basin



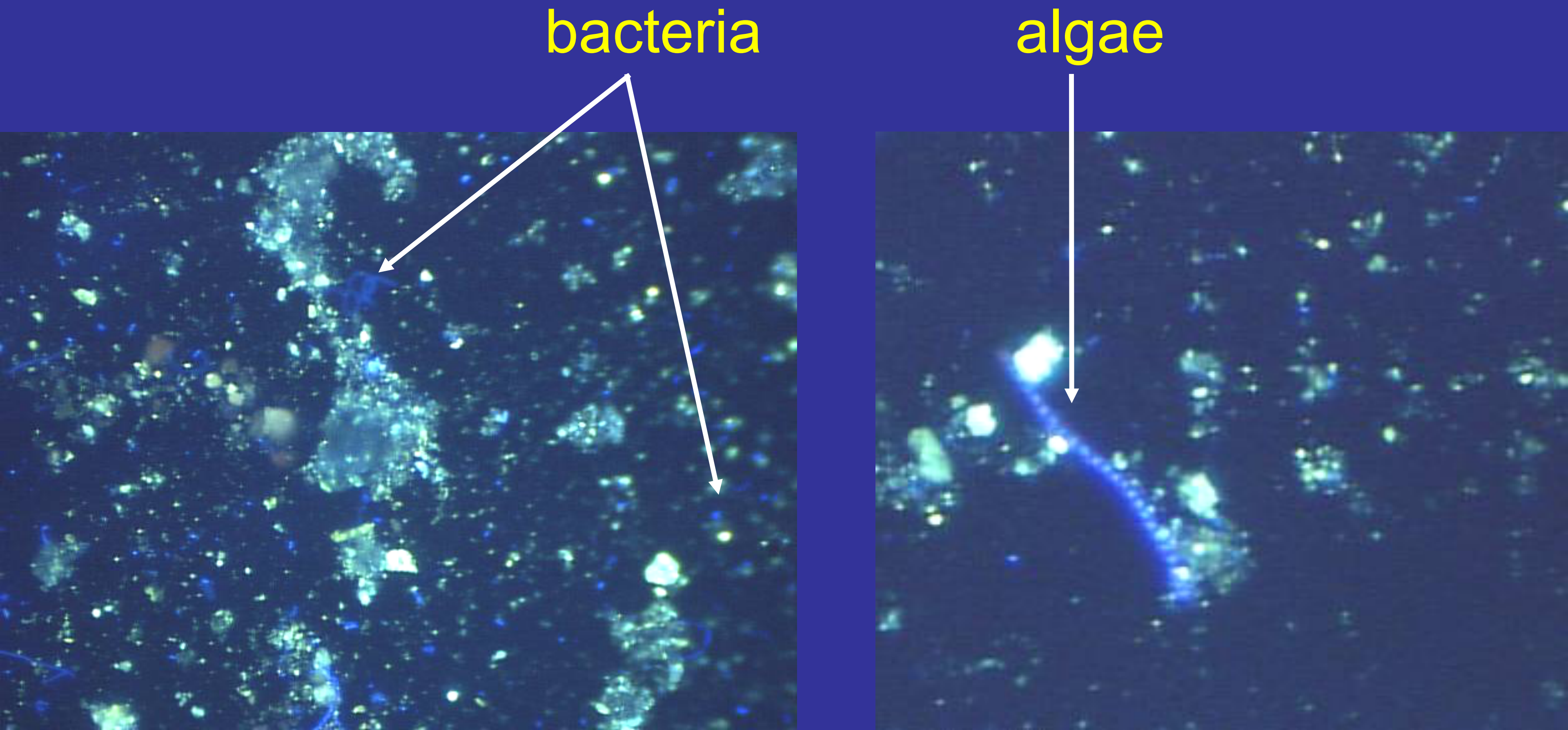

Clay layer formed at lake bottom
HCL formed 8-15 cm below bottom

BCV effectiveness is reduced by hard crust formation in the bottom of the recharge basins

Fine crust, 1mm

Loose sand found below crust layer

The source water for the recharge basins contains organic and inorganic particulate matter of varying size and dissolved solids



Light microscopy of sand grains coated with silt and clay

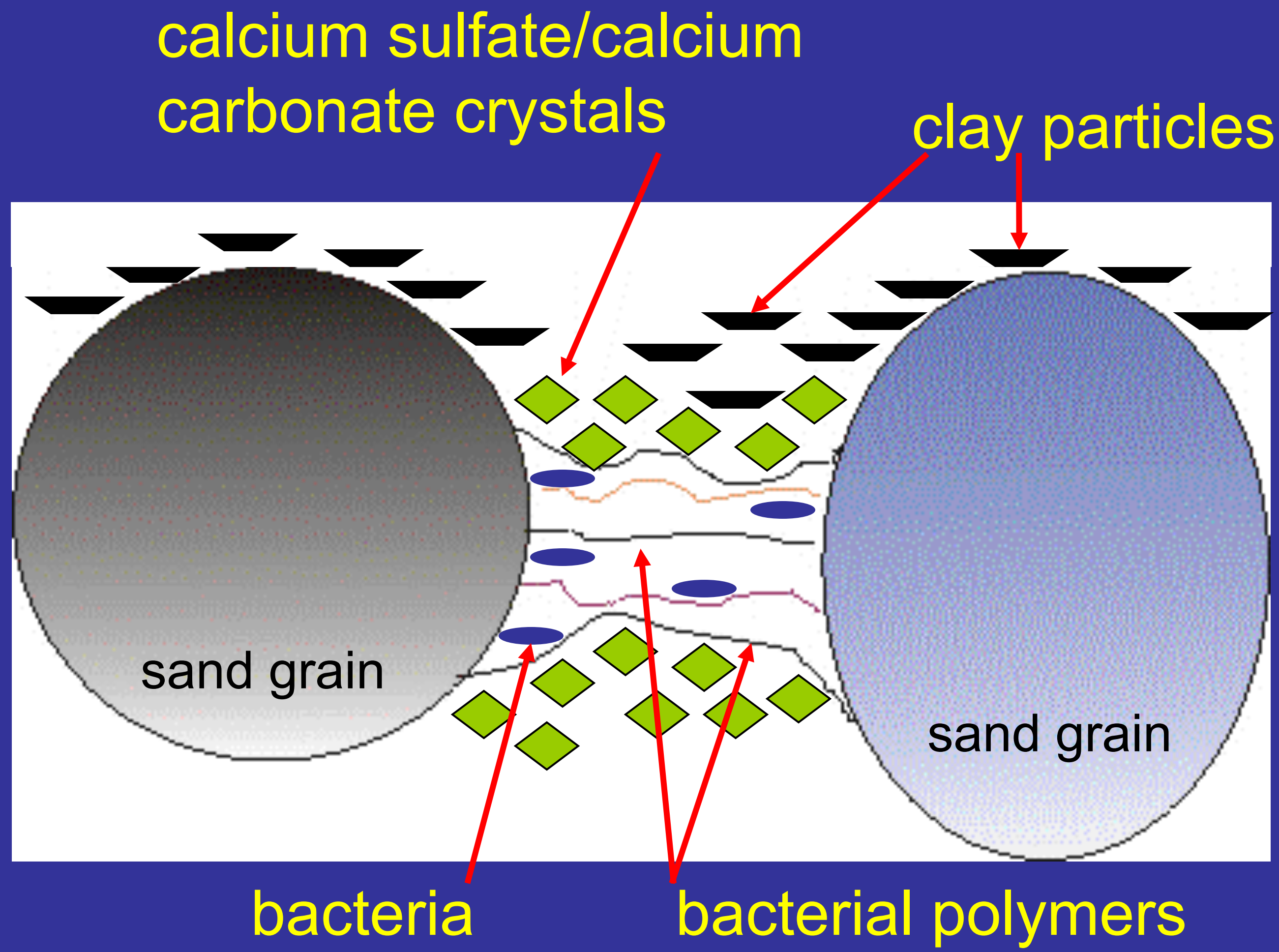
Organic components of crust layer

Protein content: 1300 ug/g soil

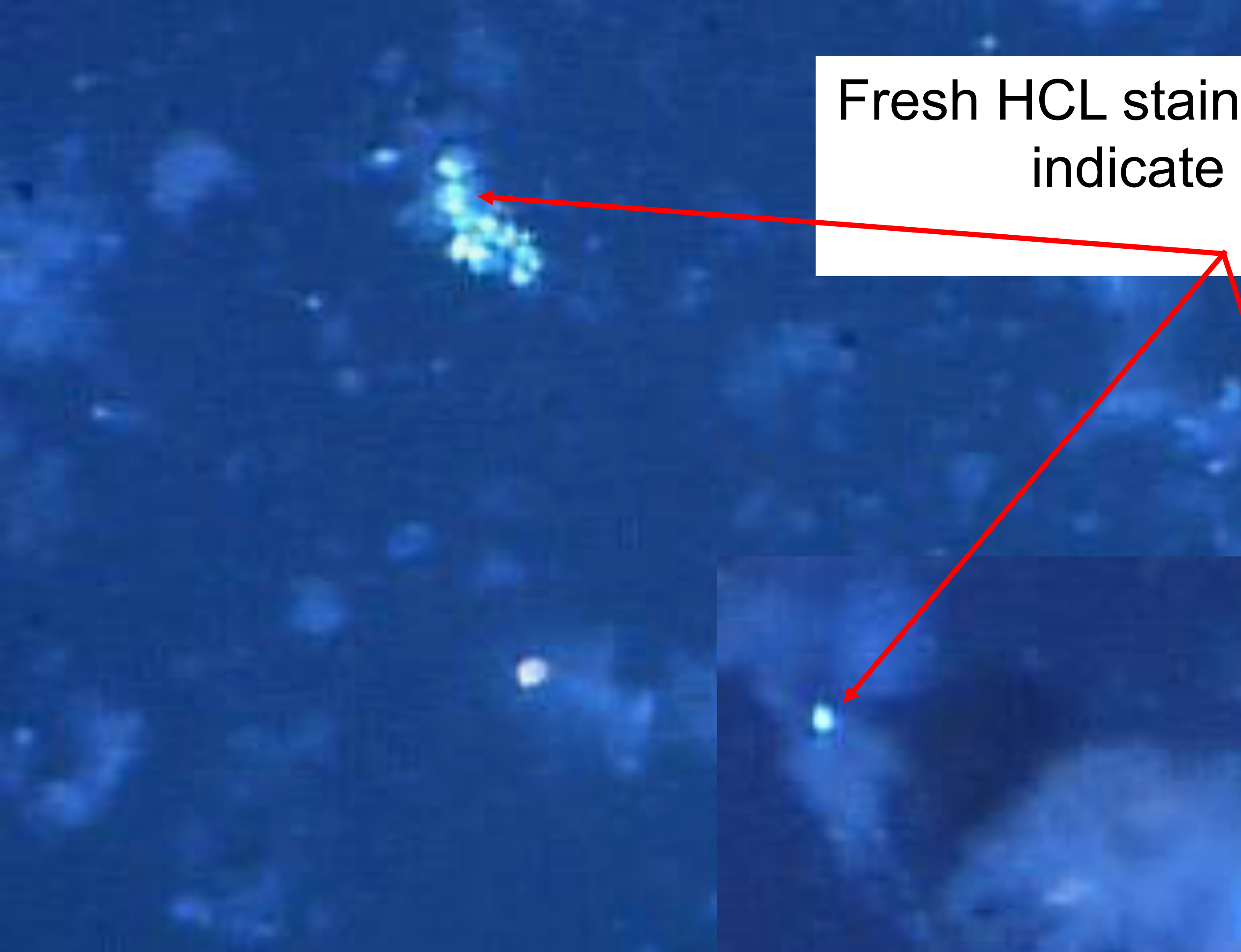
Carbohydrate content: 1300 ug/g soil

Chlorophyll : 10.3 ug/g soil

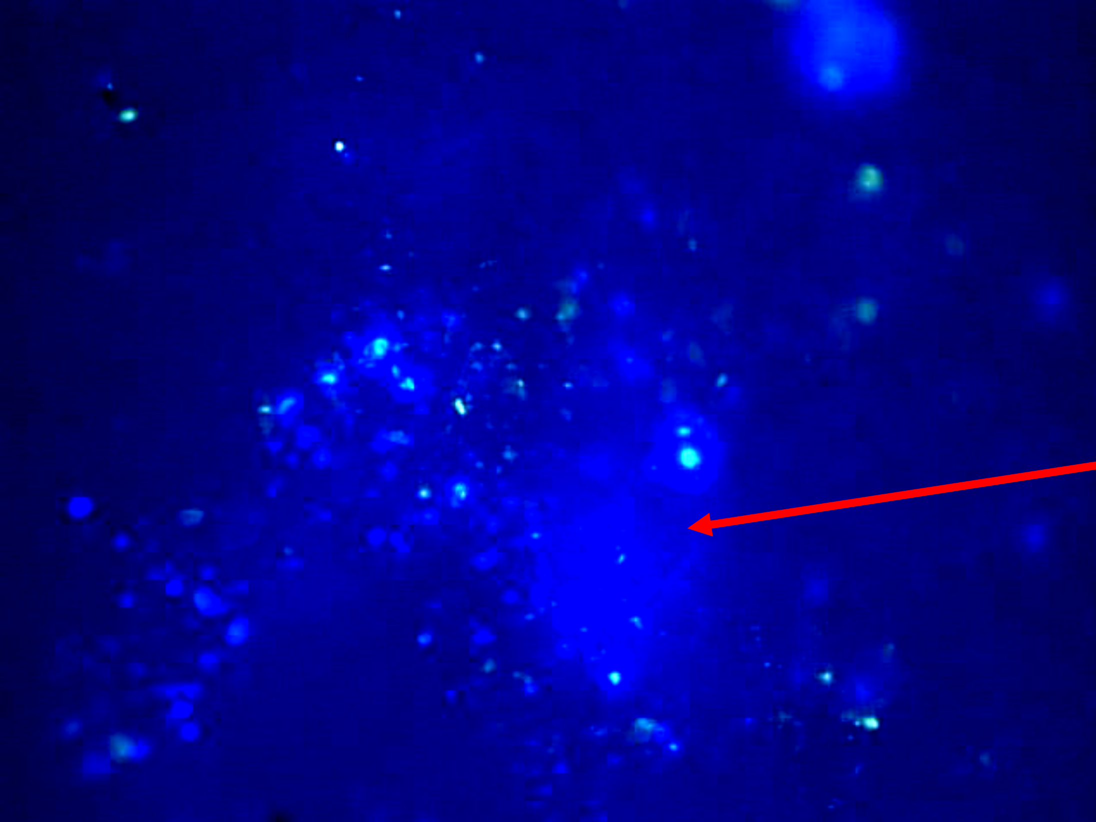
Proposed model of crust formation including the adsorption of fine crystals to the negatively charged bacterial polymers and the sedimentation of clay particles onto the crystals and sand particles.



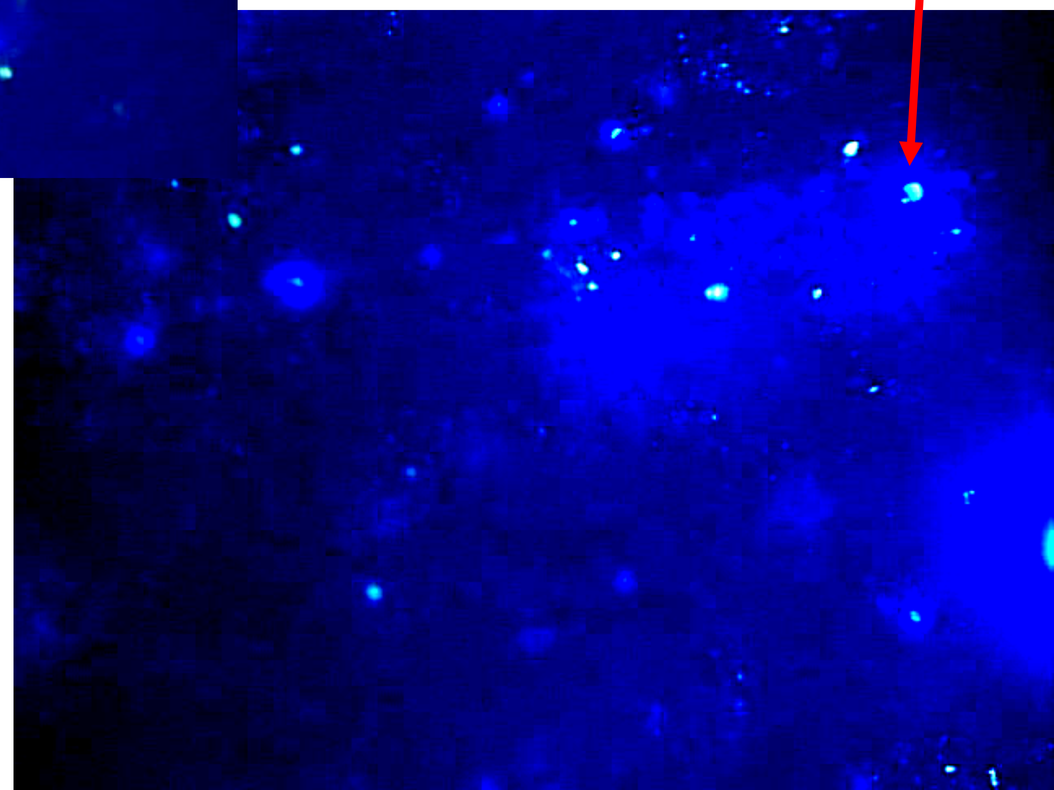
Fresh HCL stained with DAPI to indicate the presence of bacteria

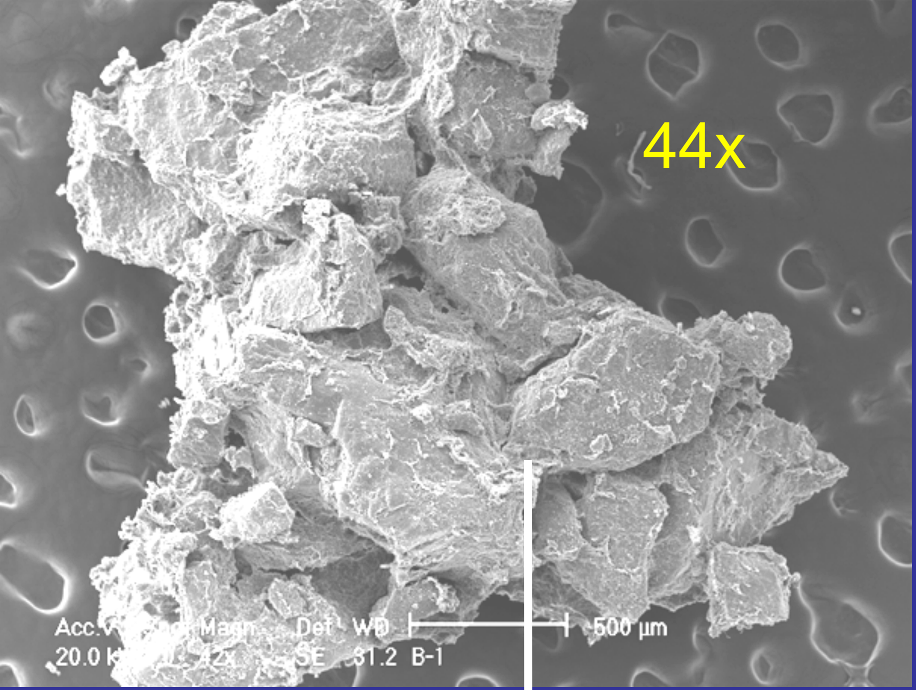


600x magnification

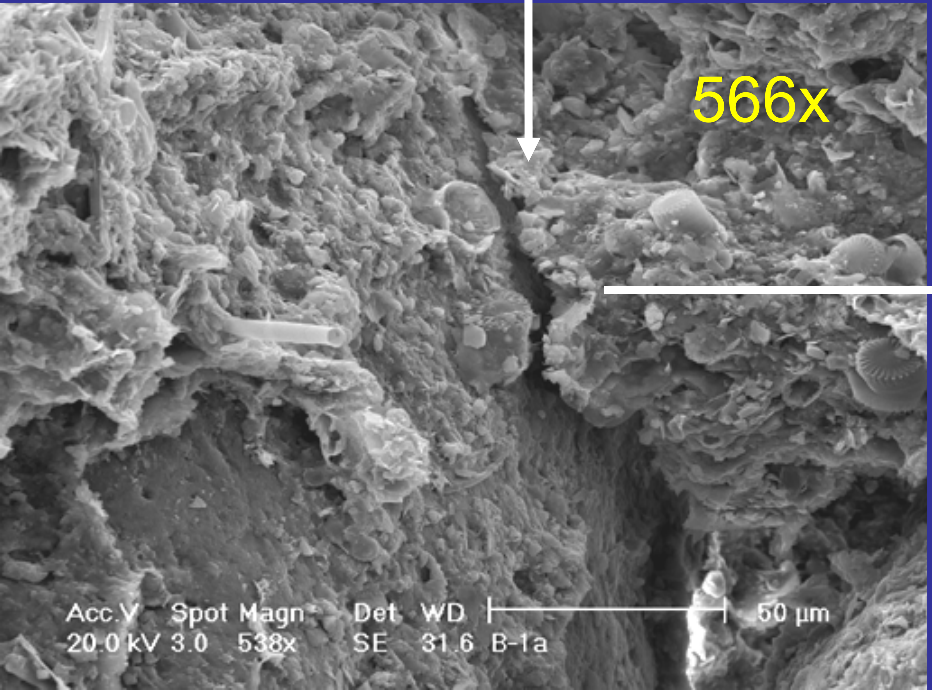


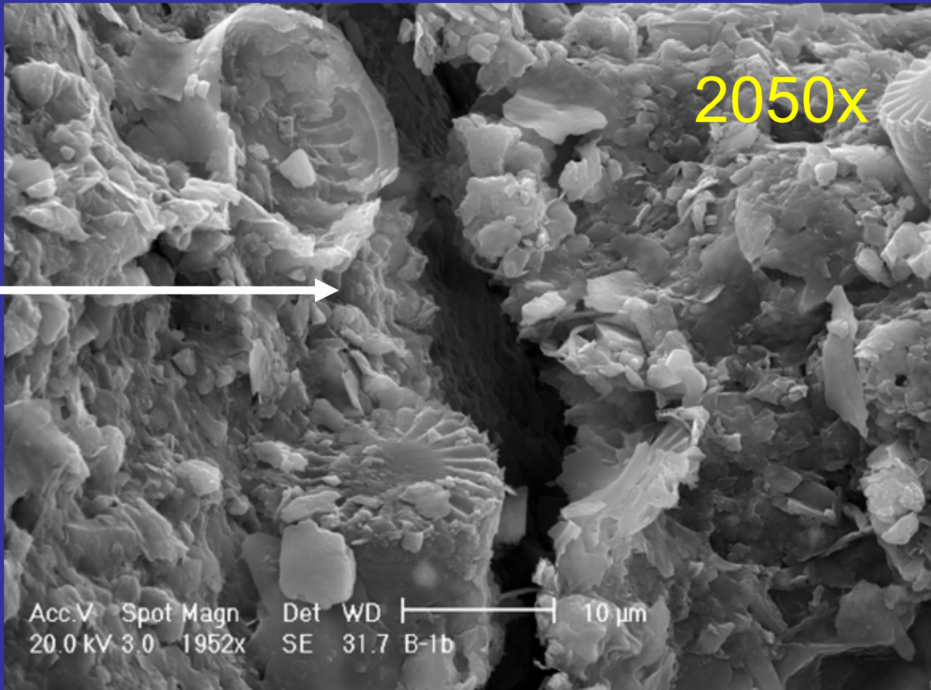
HCL stained to indicated the presence of bacteria and bacterial biofilms



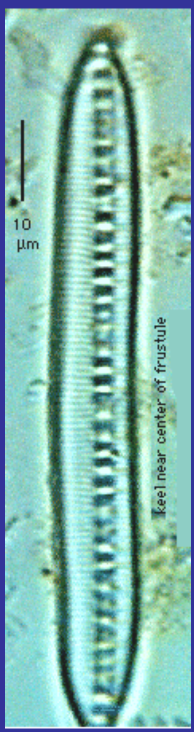


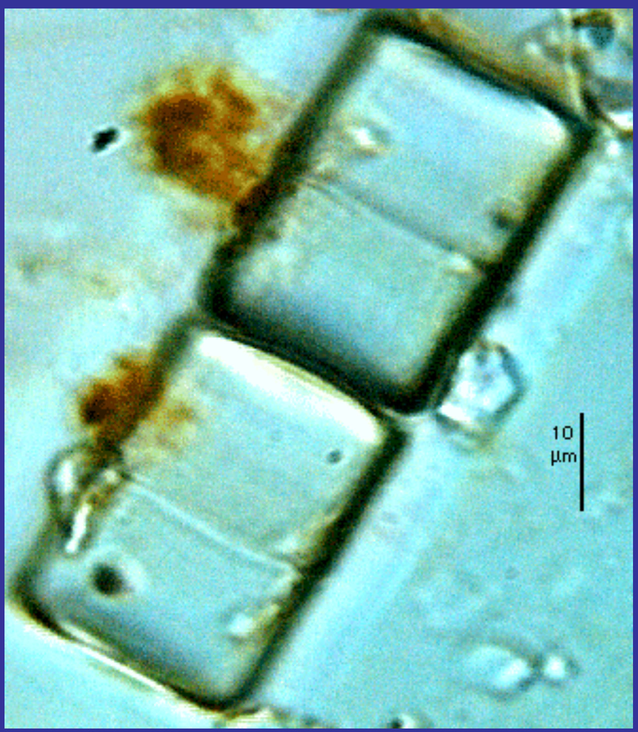
Scanning Electron Microscopy (SEM) of Crust

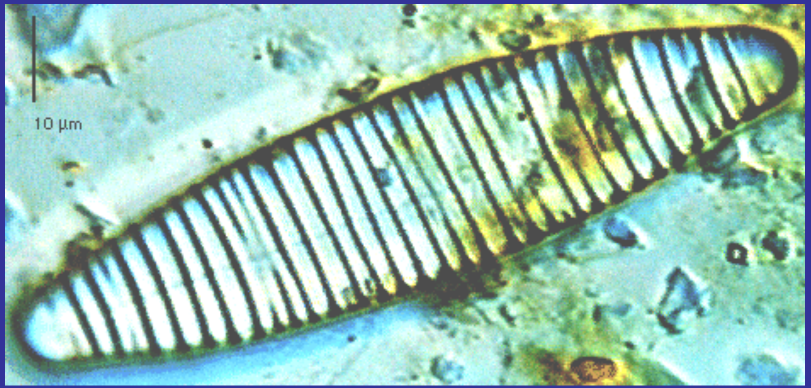


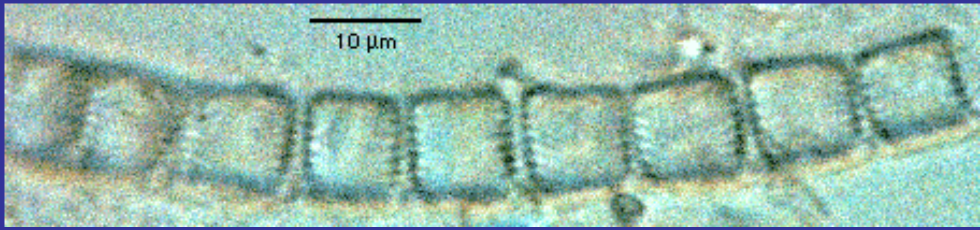


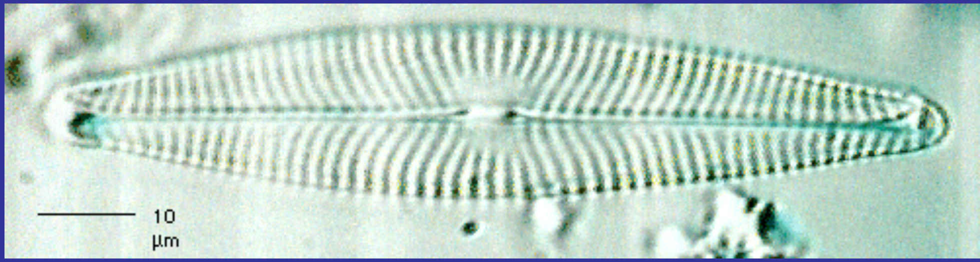
Common diatoms from fresh water samples

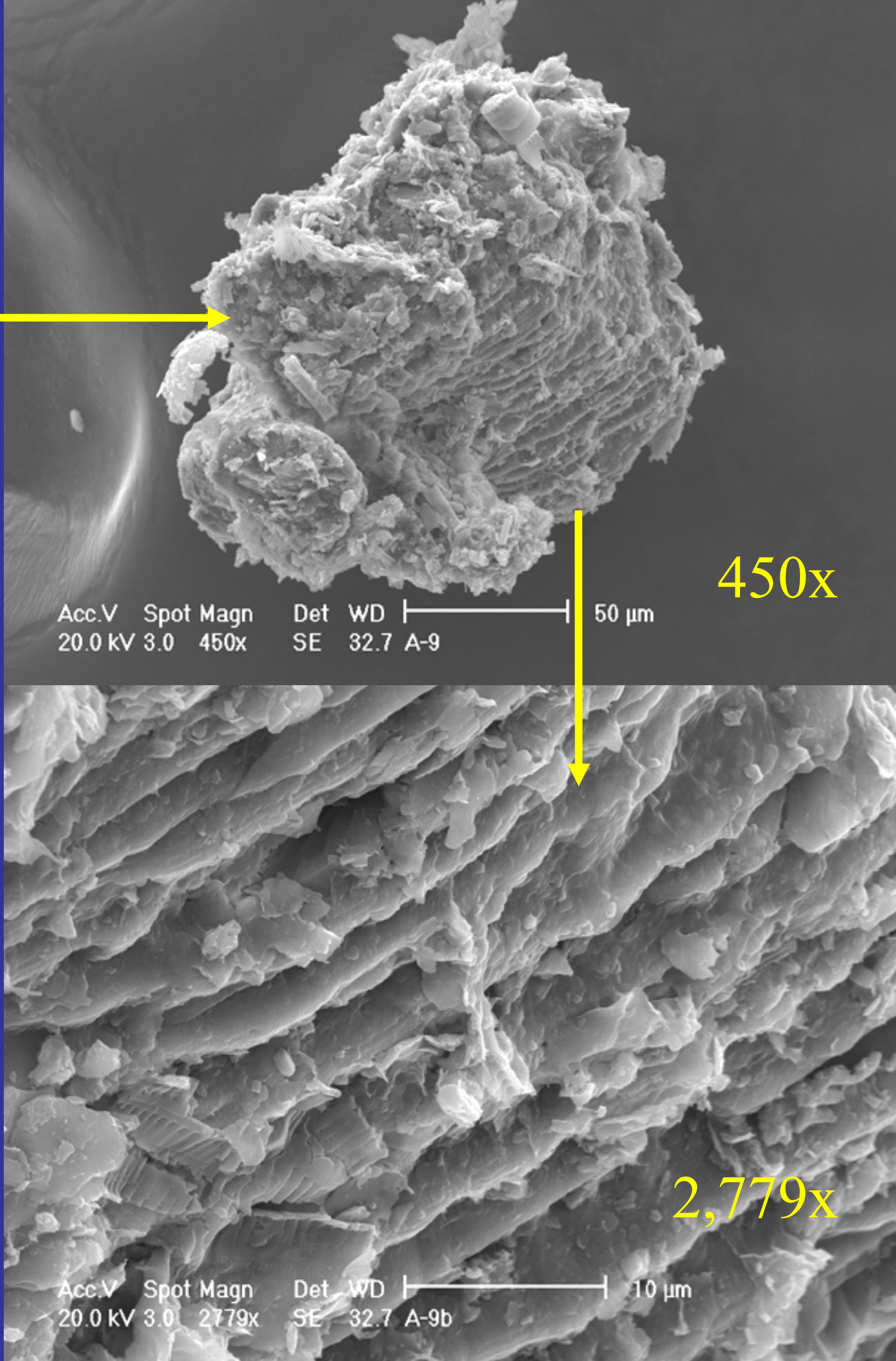








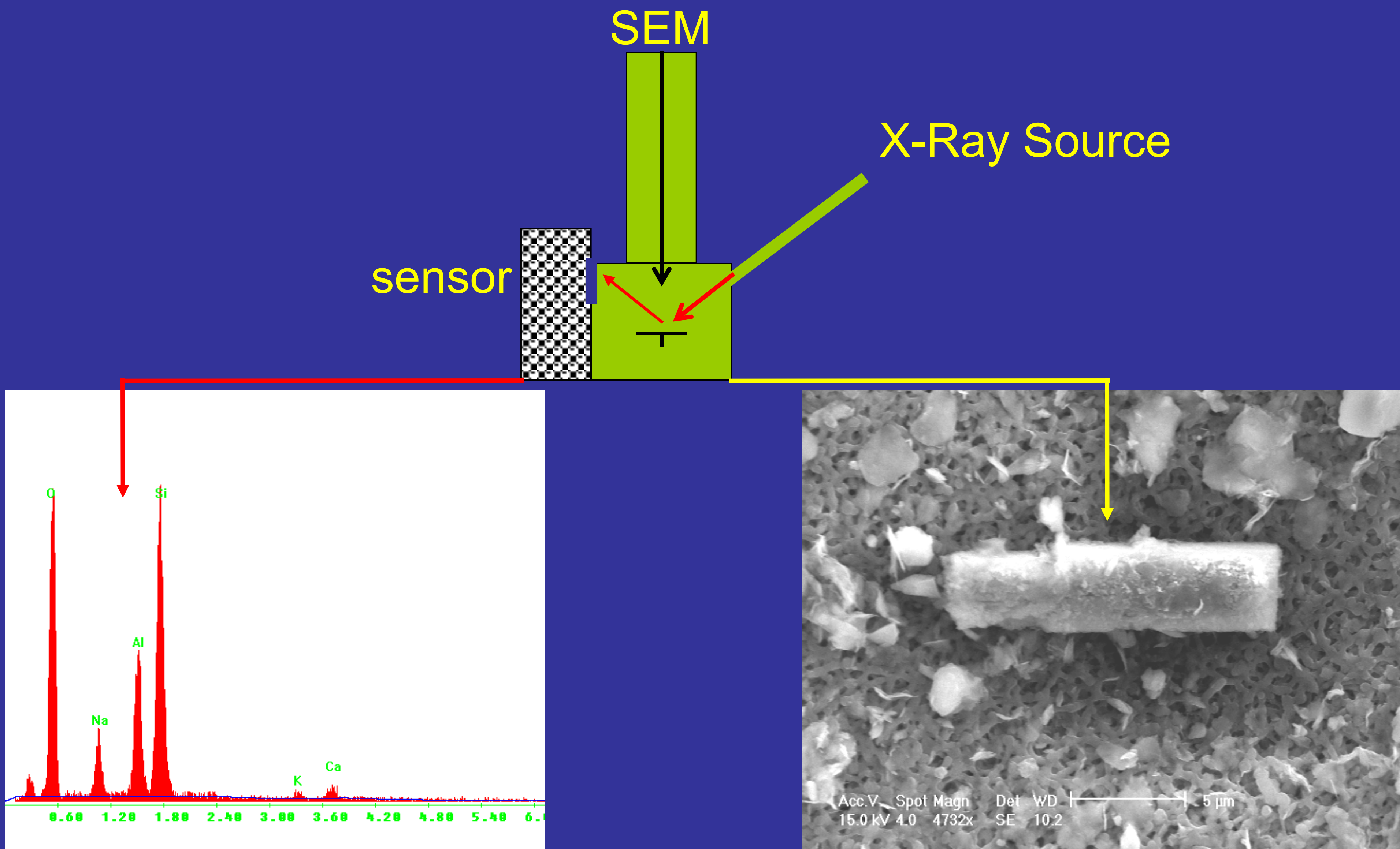


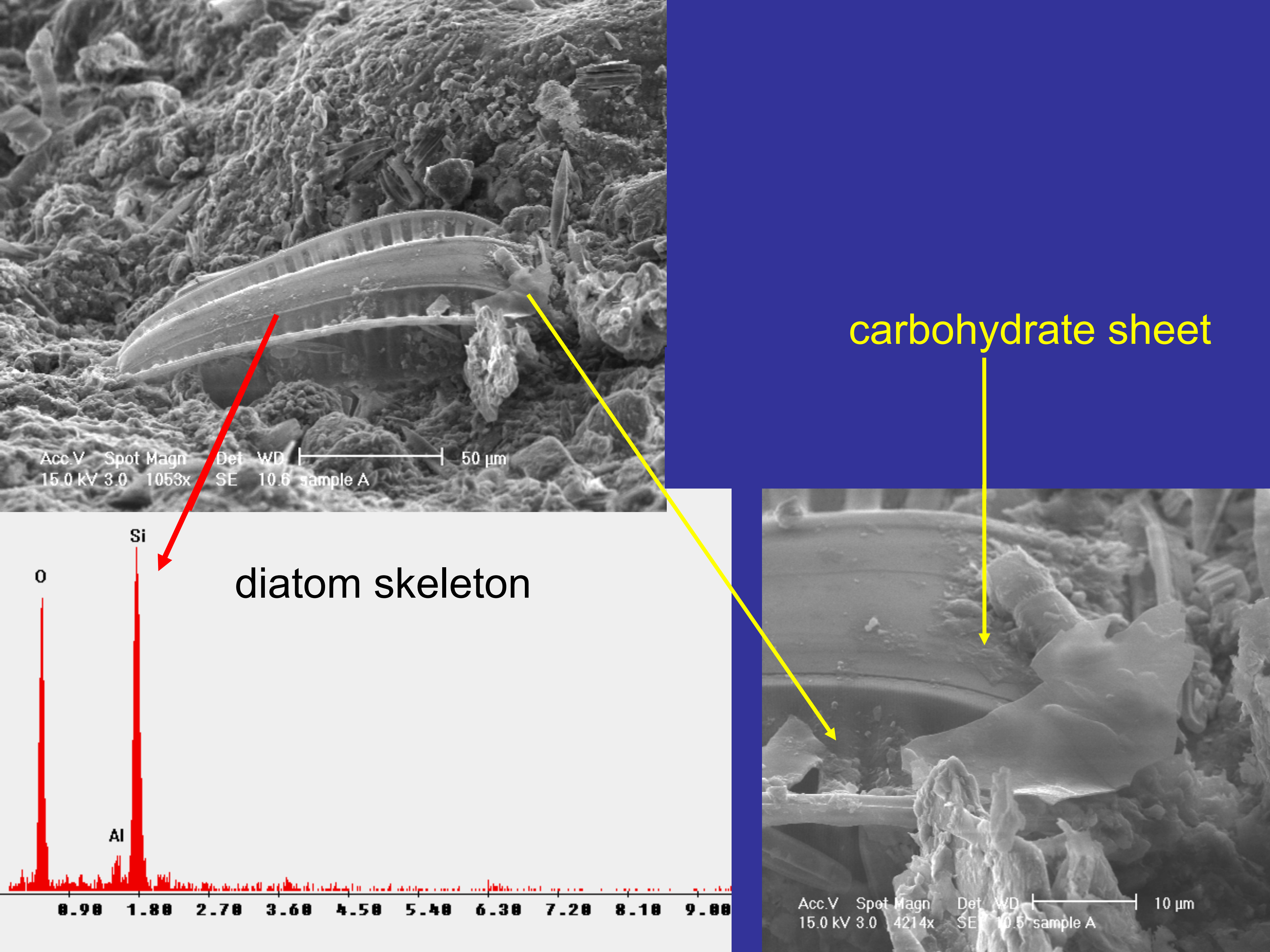


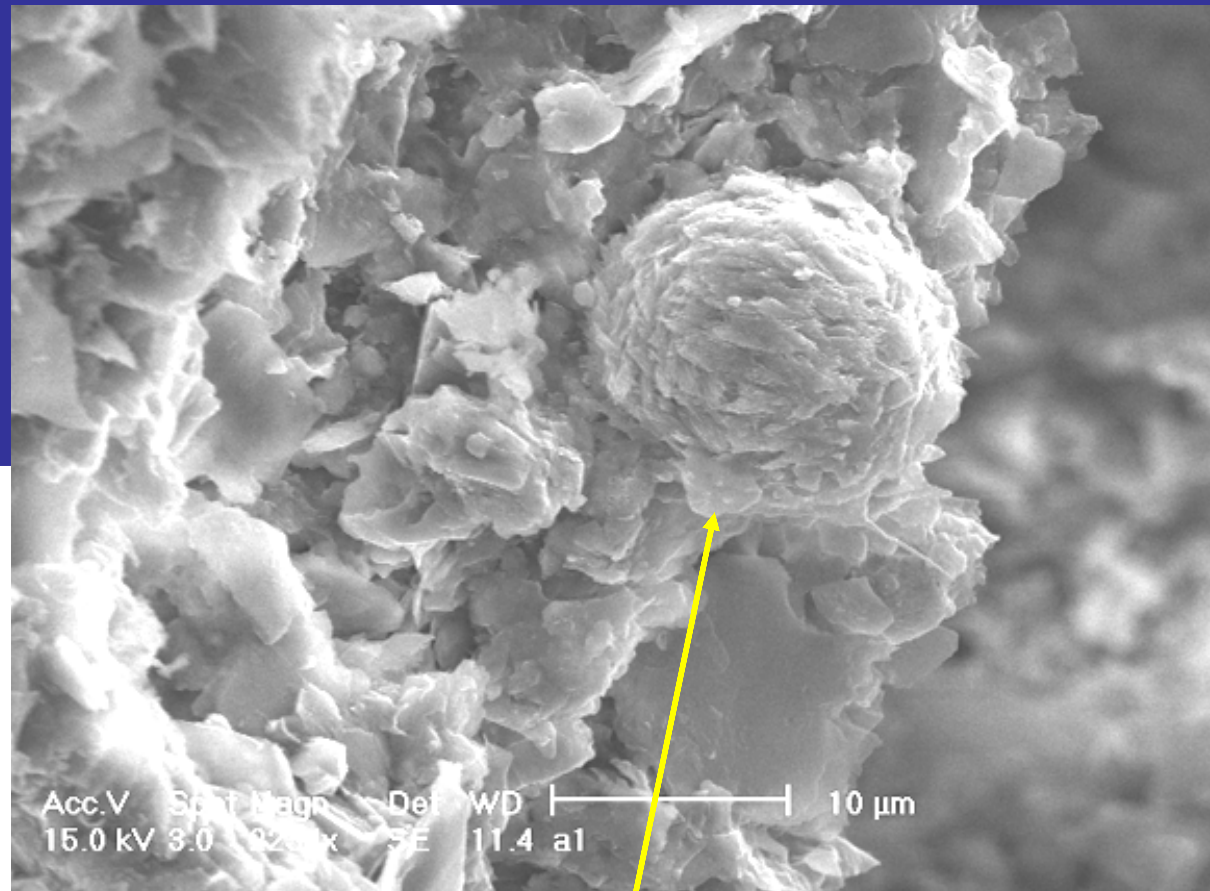
Note the stacking of clay particles from a fragment taken at the surface of the crust

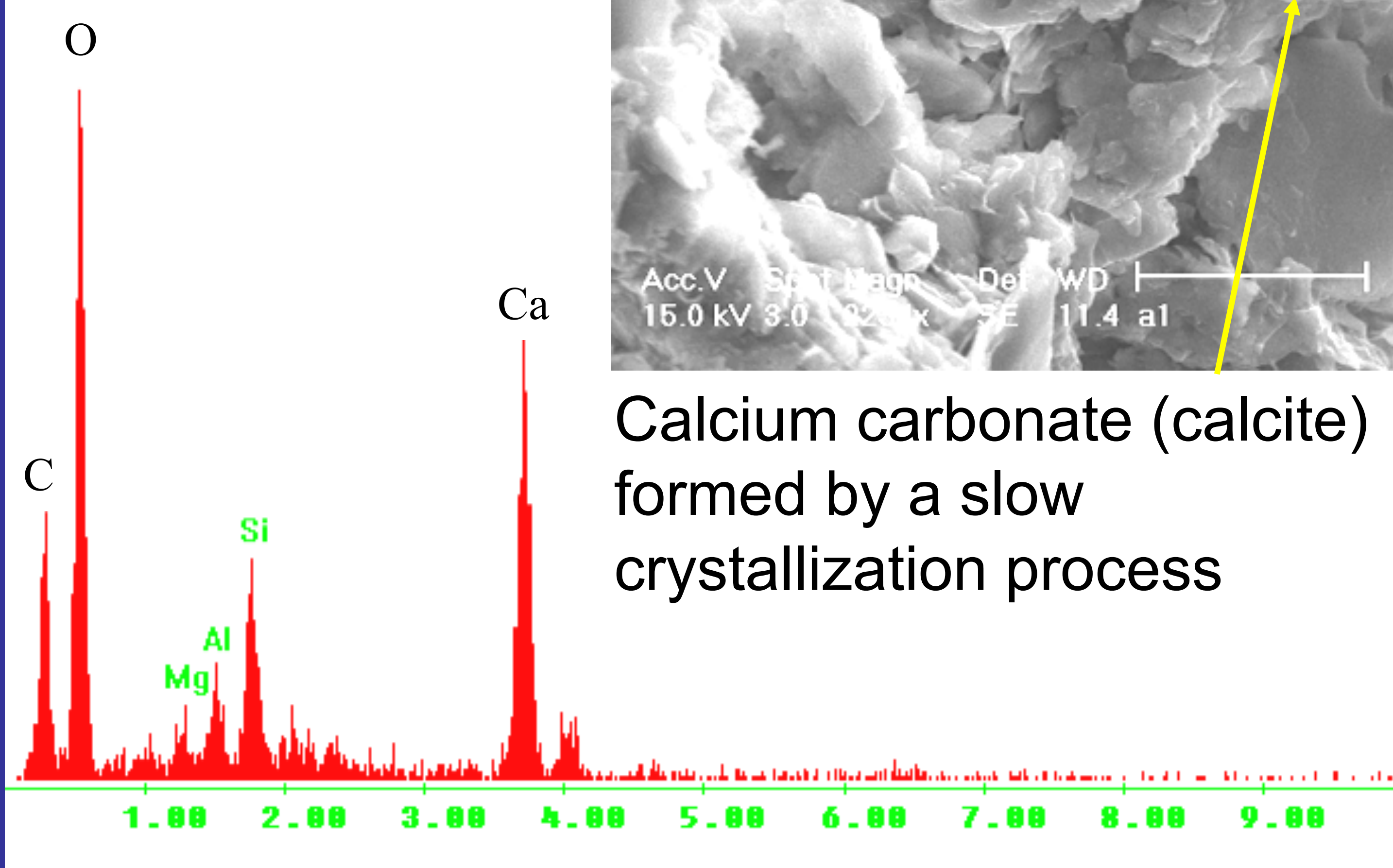
Scanning Electron Microscopy

Electron Dispersive X-ray Spectroscopy(SEM-EDX)

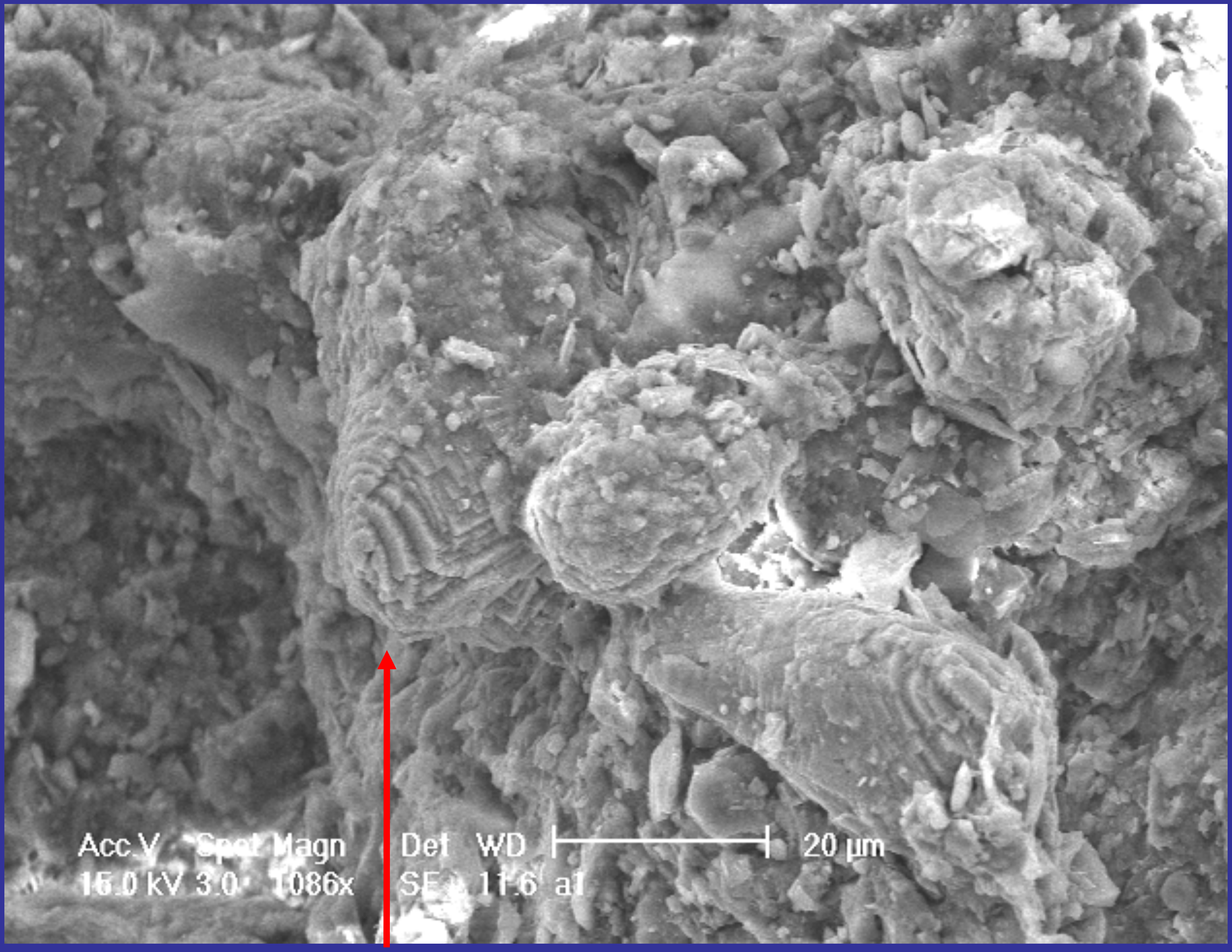



carbohydrate sheet
diatom skeleton
Acc.V Spot Magn Det WD 50 µm
15.0 kV 3.0 1053x SE 10.6 sample A
O
Si
Al
0.90 1.80 2.70 3.60 4.50 5.40 6.30 7.20 8.10 9.00
Acc.V Spot Magn Det WD 10 µm
15.0 kV 3.0 4214x SE 10.5 sample A

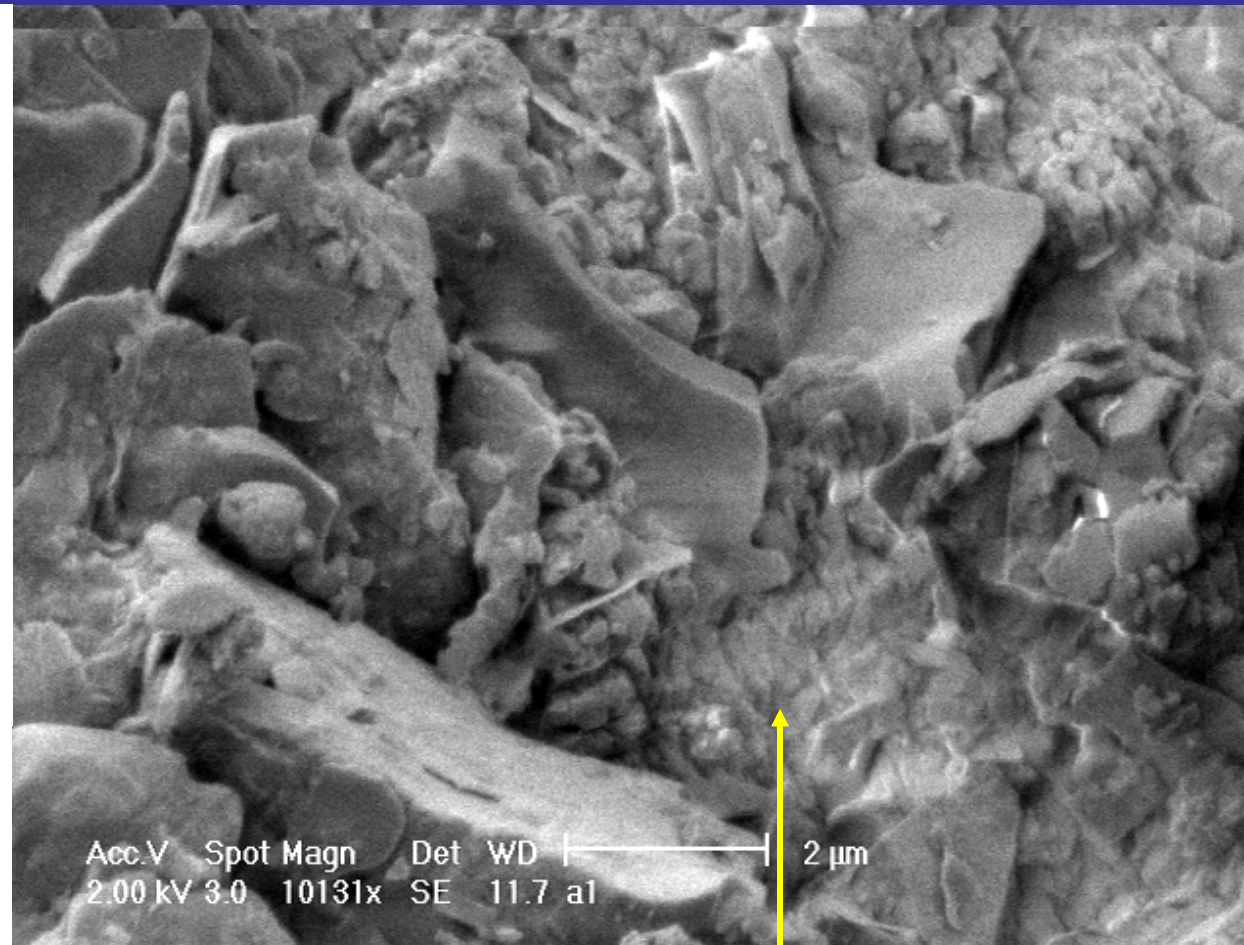


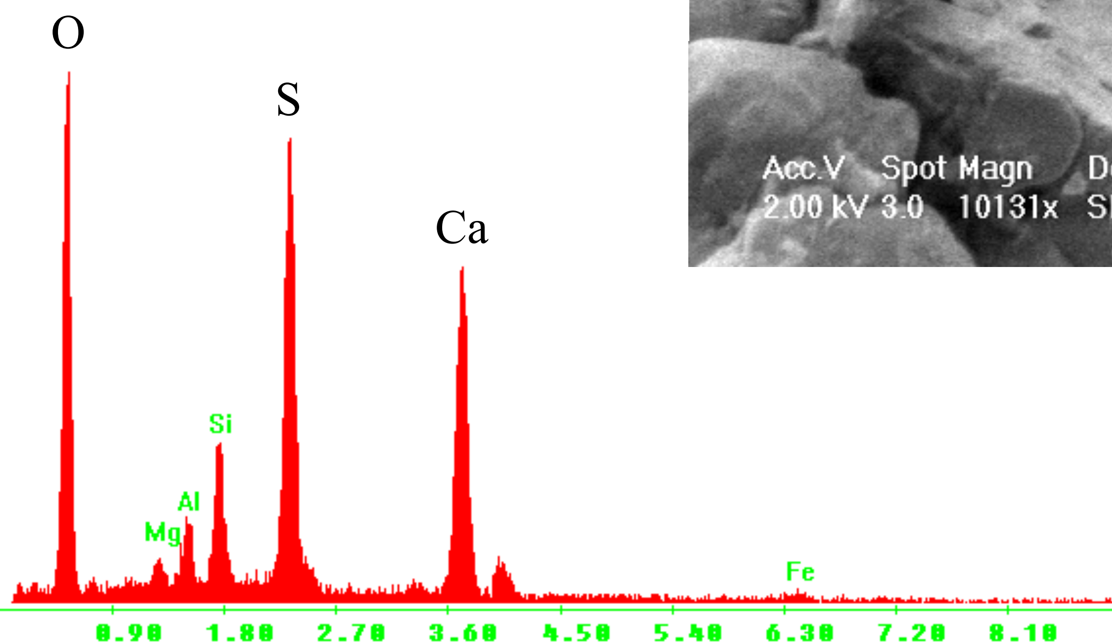


Calcium carbonate (calcite) formed by a slow crystallization process



Calcium carbonate (calcite)





Calcium sulfate

PROJECT MILESTONES (BCV-3):

- 1999- OTI was contracted to modify BCV-2 into BCV-3.
- 2001 (March) – District received delivery of BCV-3.

Consequences of HCL studies

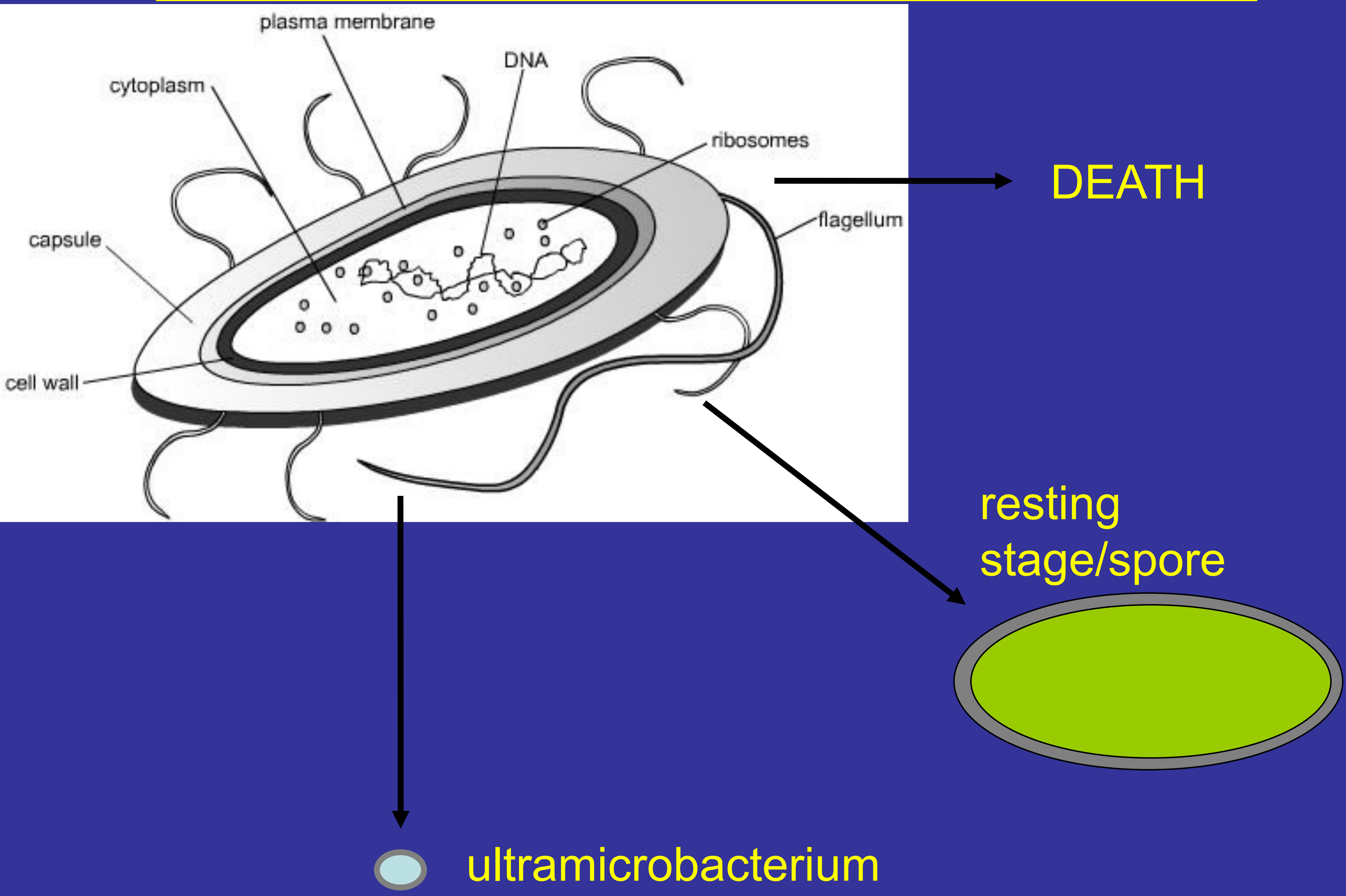
- Better understanding of the clogging process in the shallow and deep basins

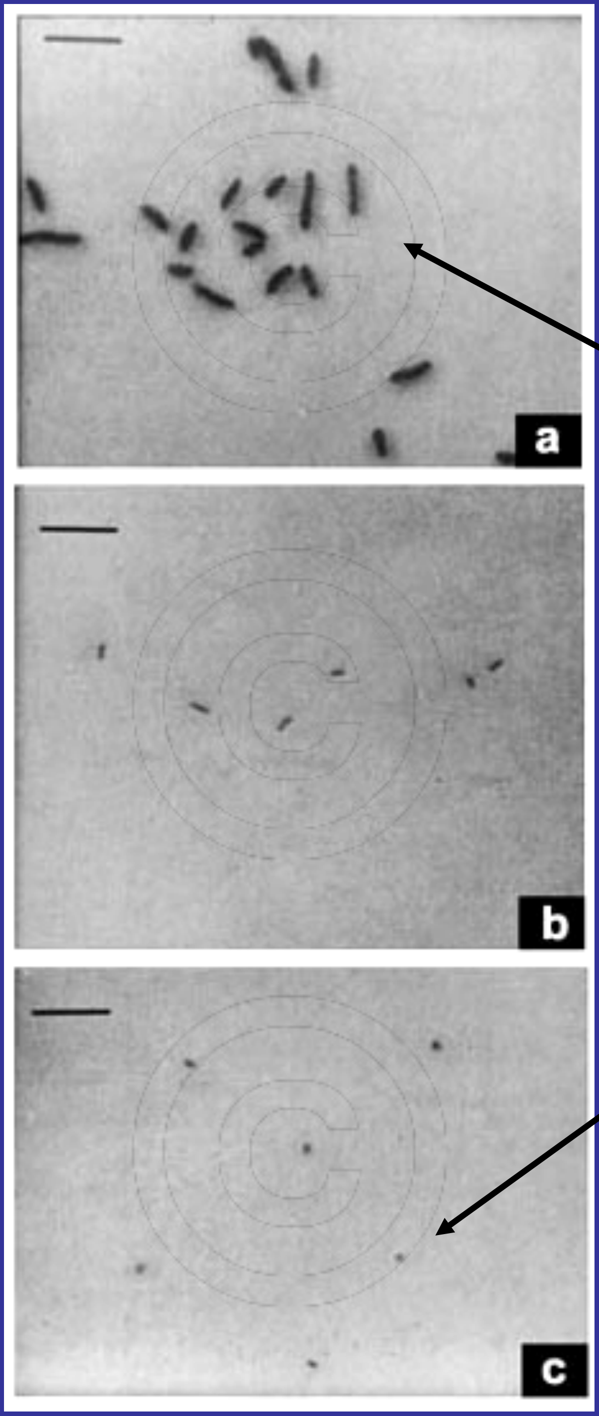
- Development of the next generations of Basin Cleaning Vehicles (BCV-III & BCV-IV) with cutter heads and strong vacuum hoods

Characterization of Hardened Crust Layer in Groundwater Recharge Basins and Utilization of Ultramicrobacteria for Soil Stabilization and Prevention of Salt Water Intrusion

Ultramicrobacteria

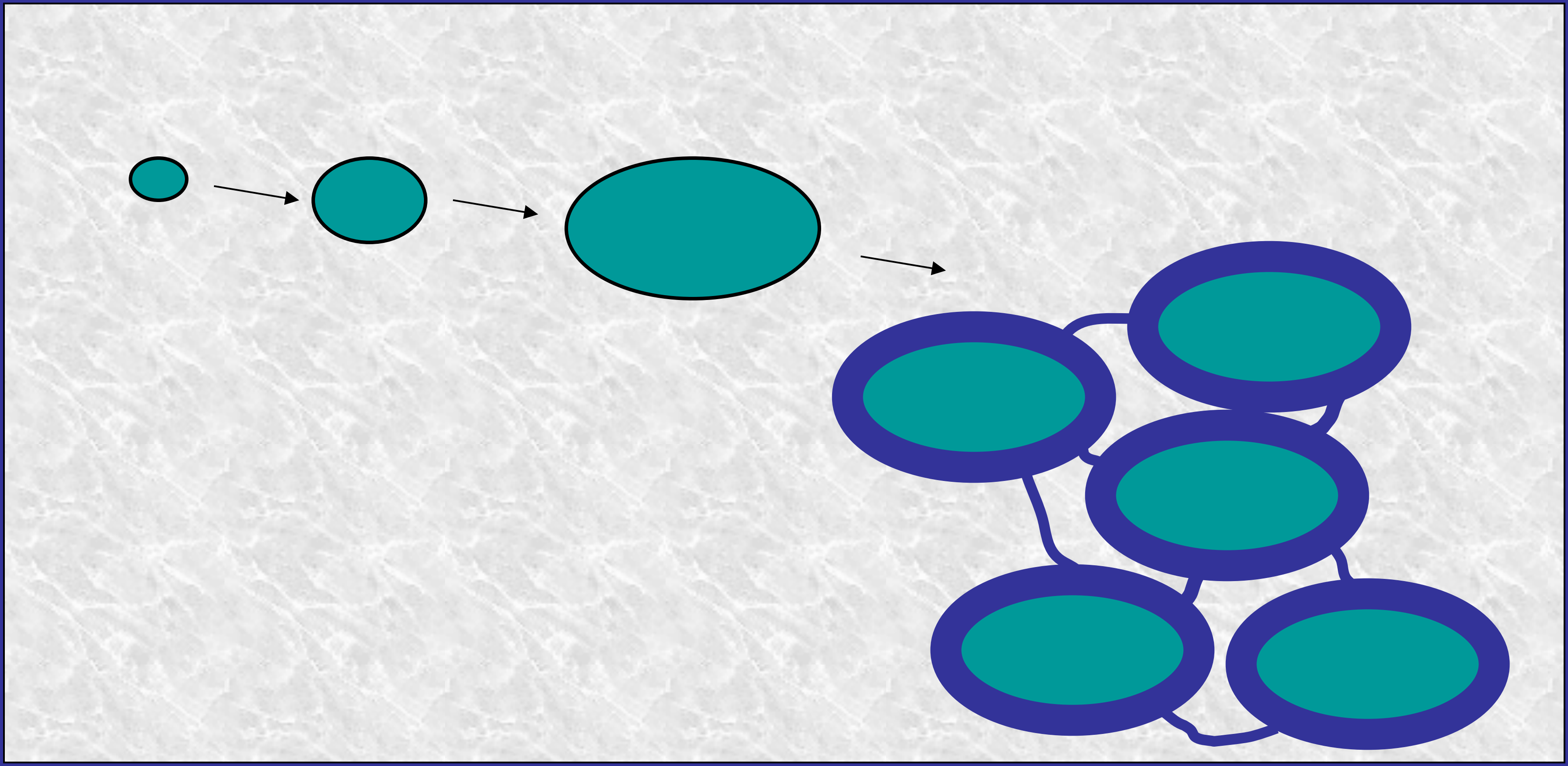
Fate of bacteria as a result of starvation



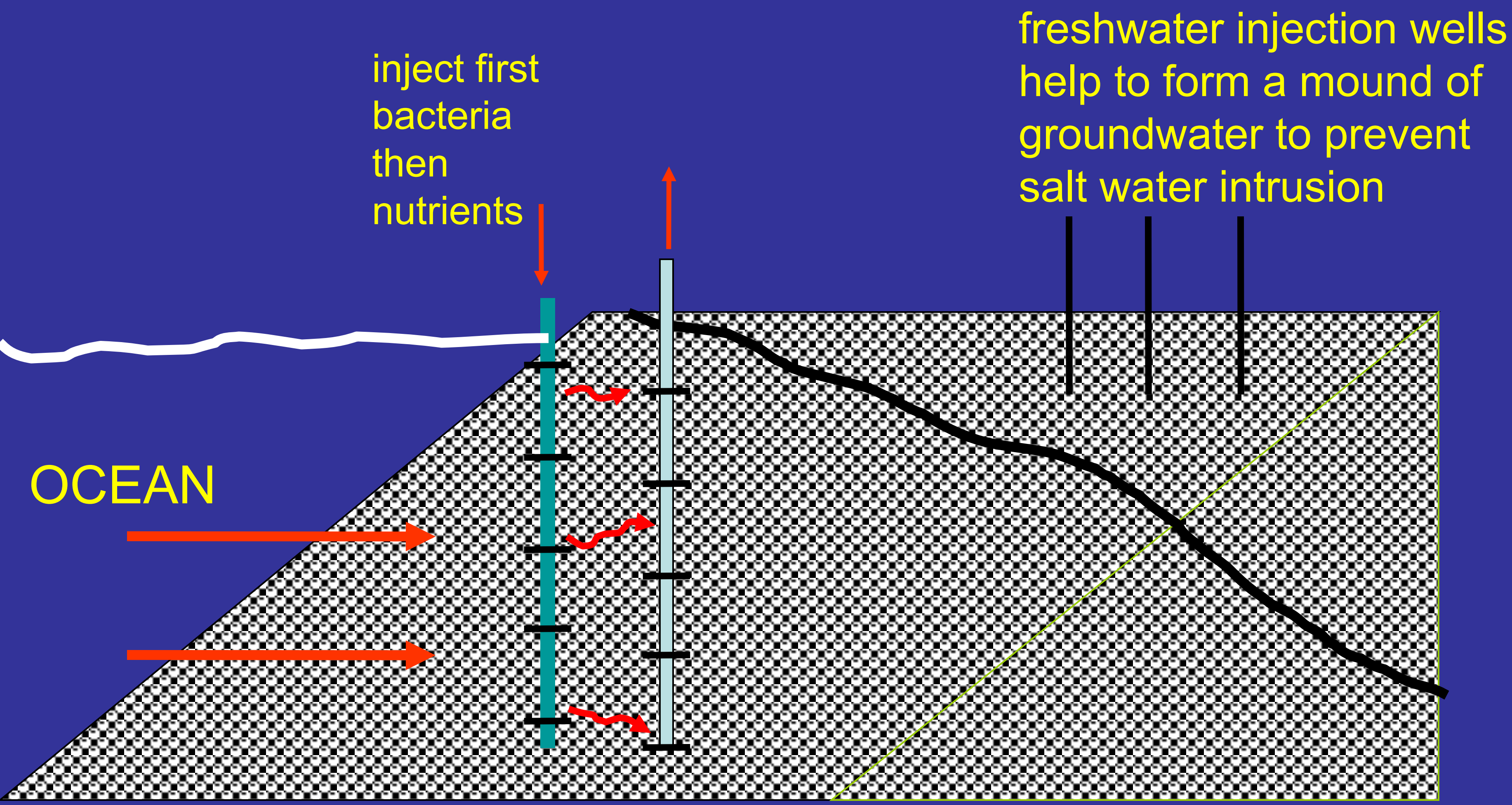


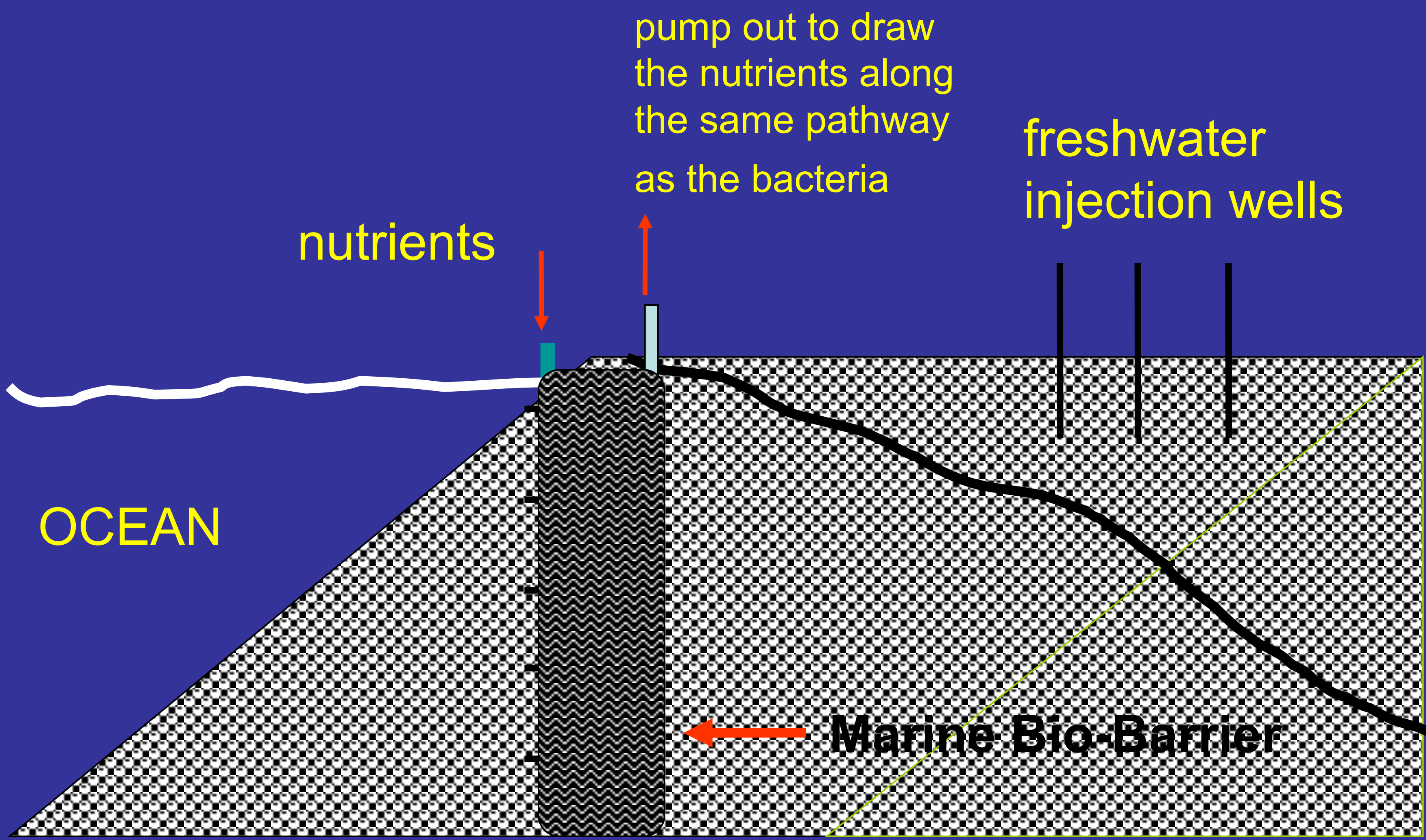
Development of ultramicrobacteria from normal size to final size (<0.1 μm^3)

Transformation of ultramicrobacteria to a microbial biofilm



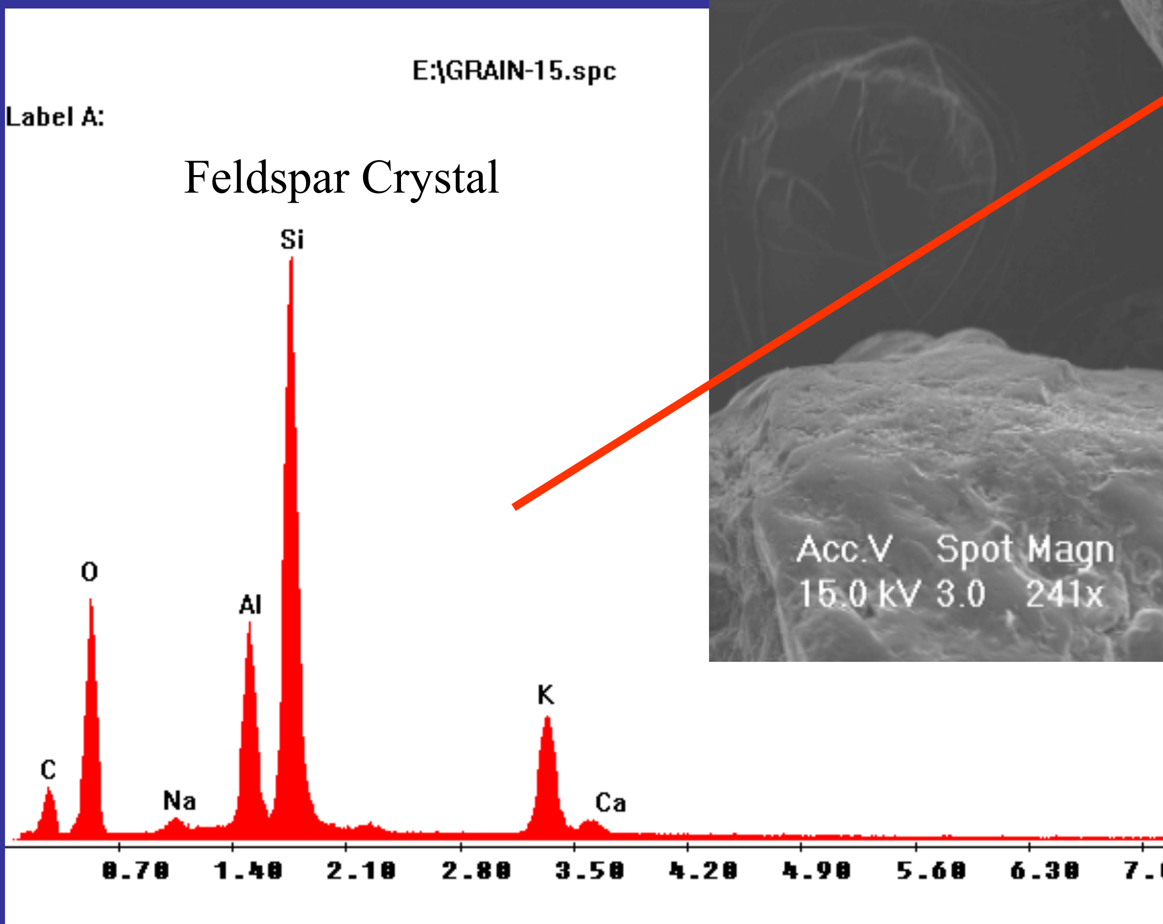
criteria for bacteria selection: 1. lives only in marine environment 2. produces polymer 3. turns into “ultra micro bacteria” when starved

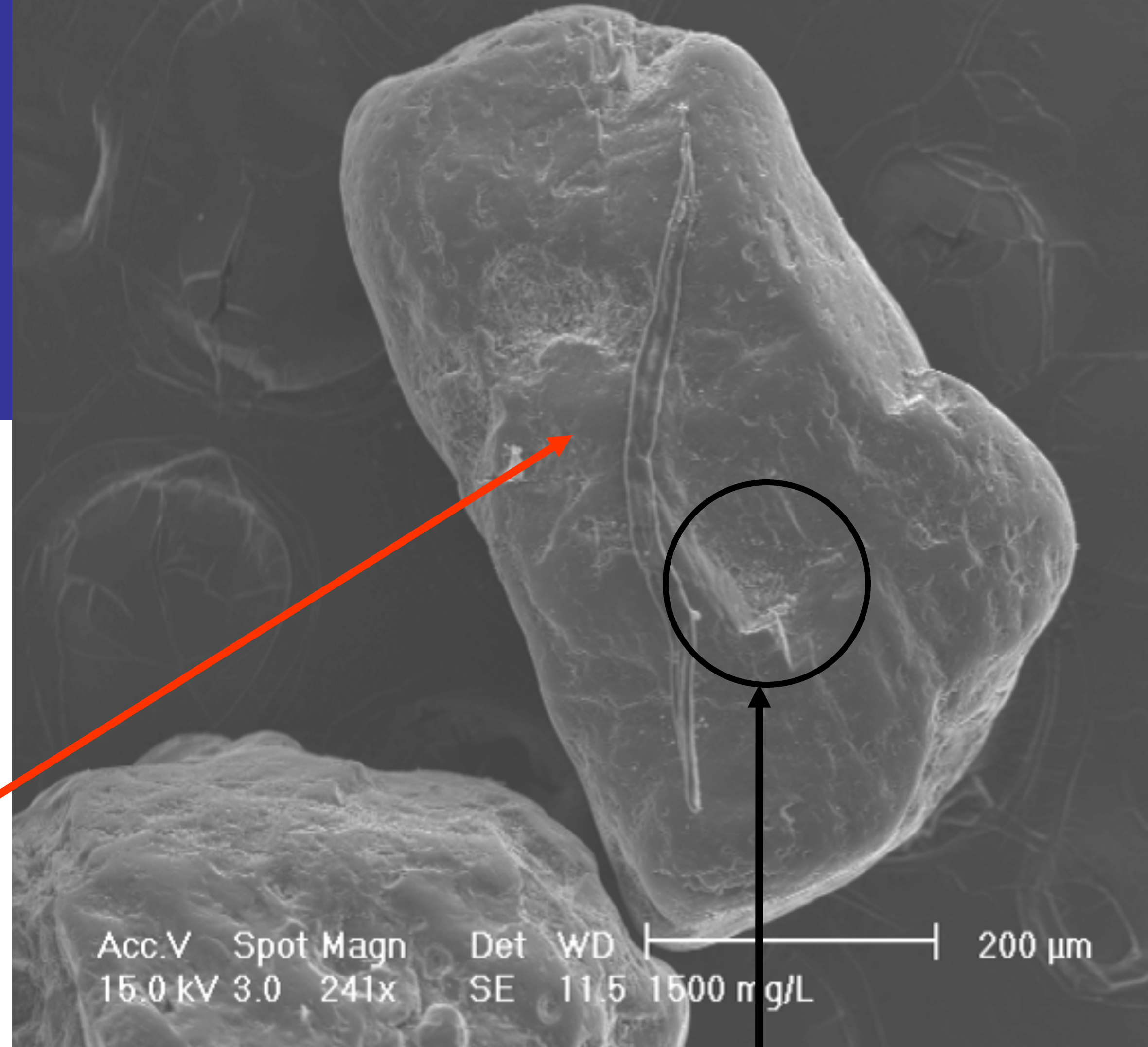




The addition of nutrients and salts to the bio-barrier could facilitate the deposition of minerals within the barrier similar to what took place in the Hardened Crust Layer

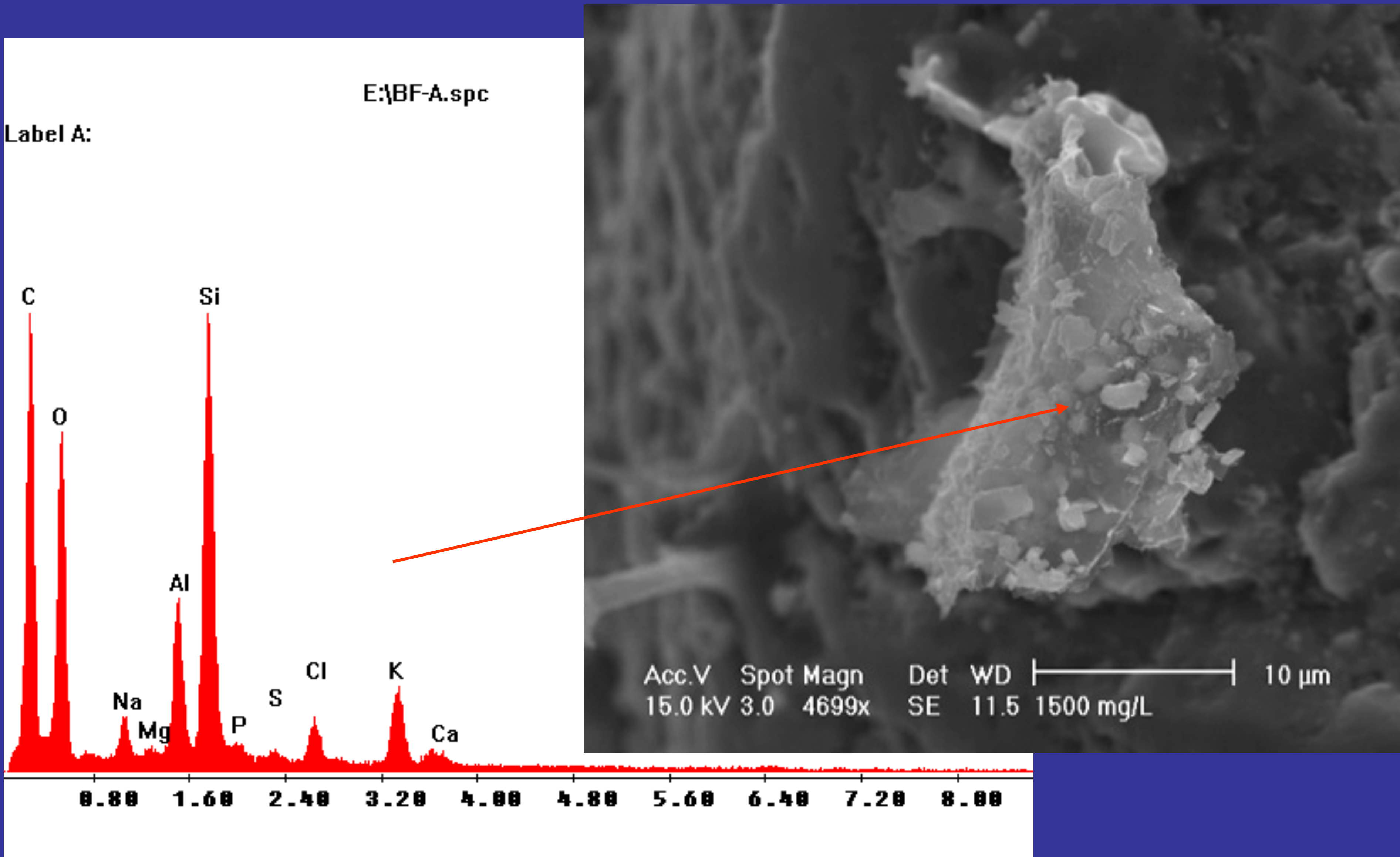
UC Irvine undergraduates conducting hydraulic conductivity and biofouling studies



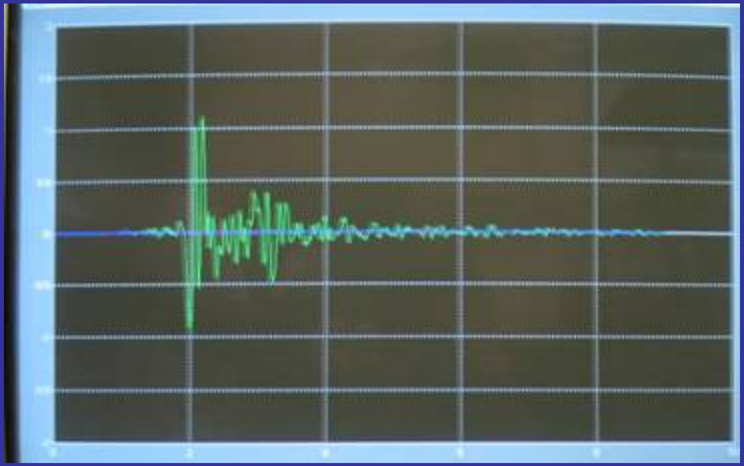


mineral and microbial development

Microbial and Mineral Formation Results (cont’d)



Earthquake Simulations

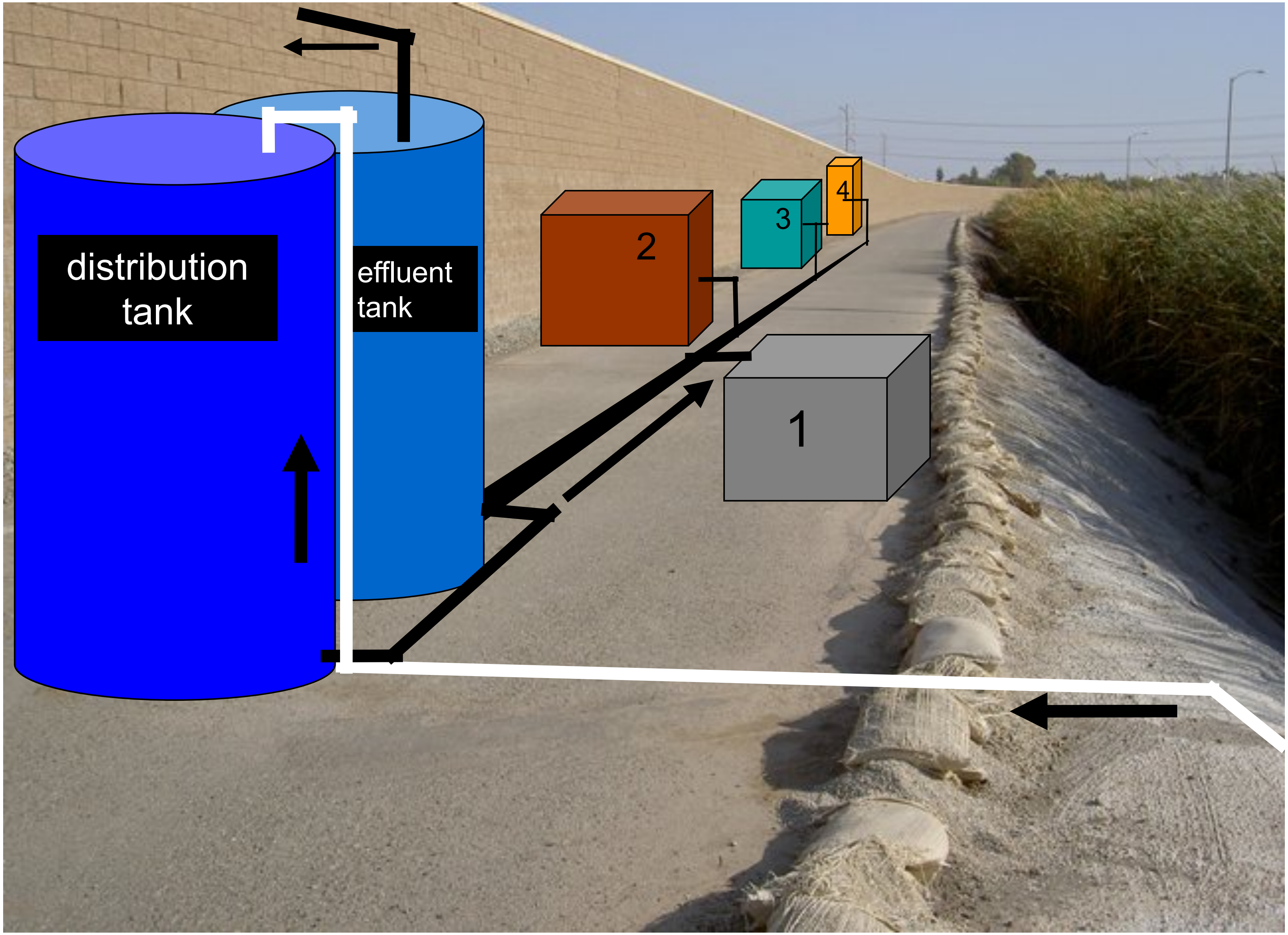


In addition to examining the minerals formed in the columns, we further investigated the effect the mineral formation has on the sand column stability under earthquake conditions.

10 Cycles of Northridge Earthquake simulations were run at the UCI Structures Lab using the UCIST shake table. There was no change in the column structure after the simulations.

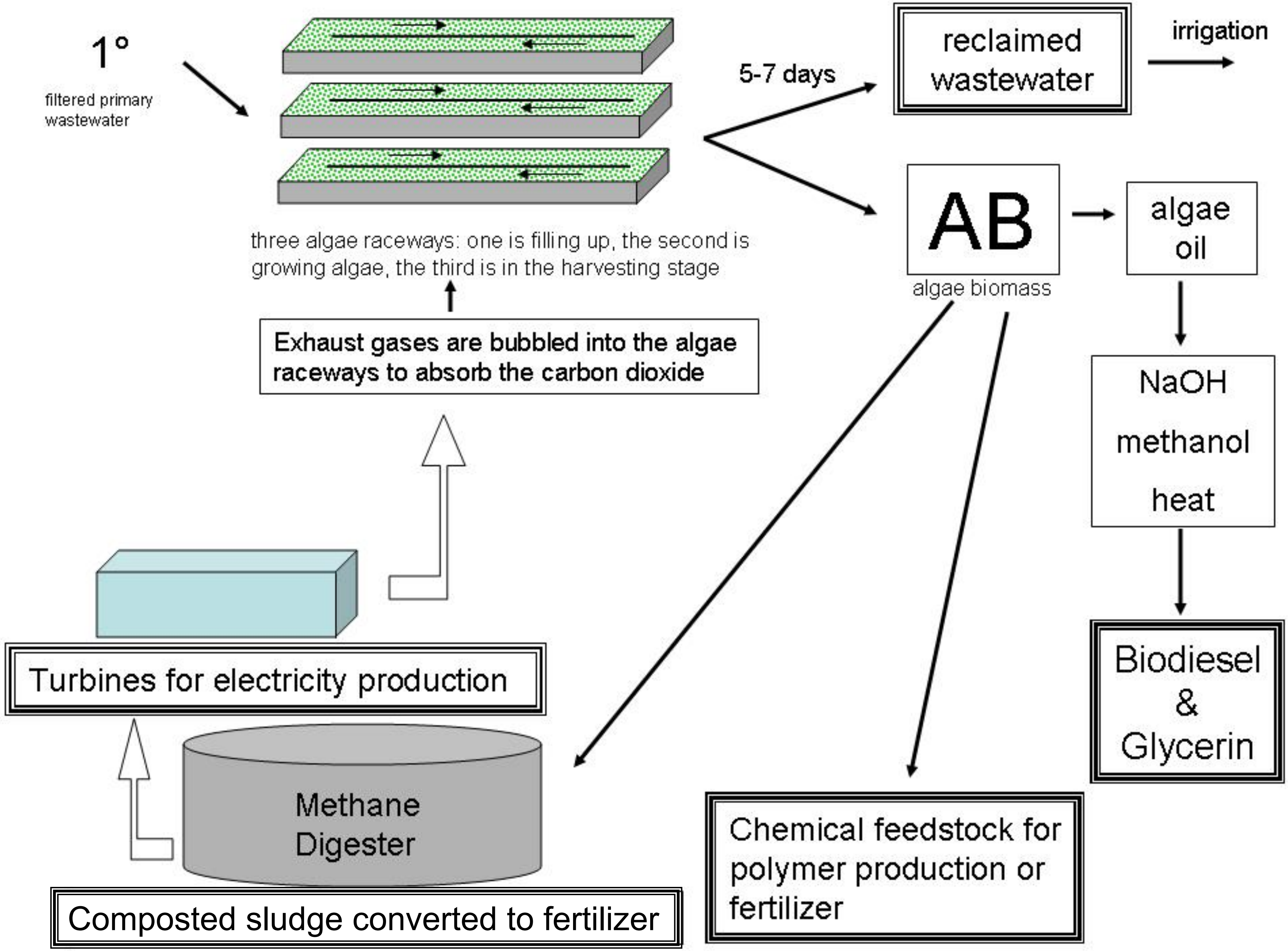
Mobile Environmental Solutions



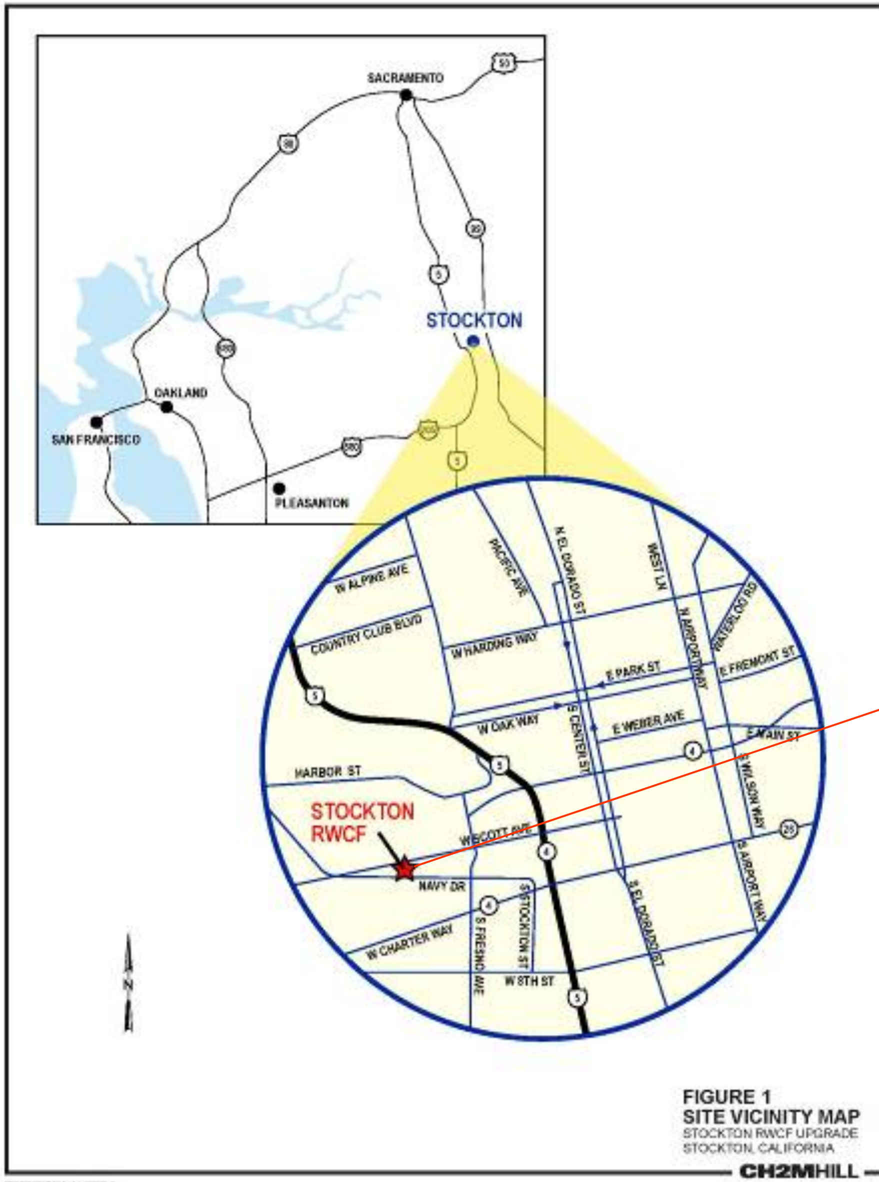
Schematic of the test site where 4 different processes were used to remove Selenium and Nitrate from surface waters.

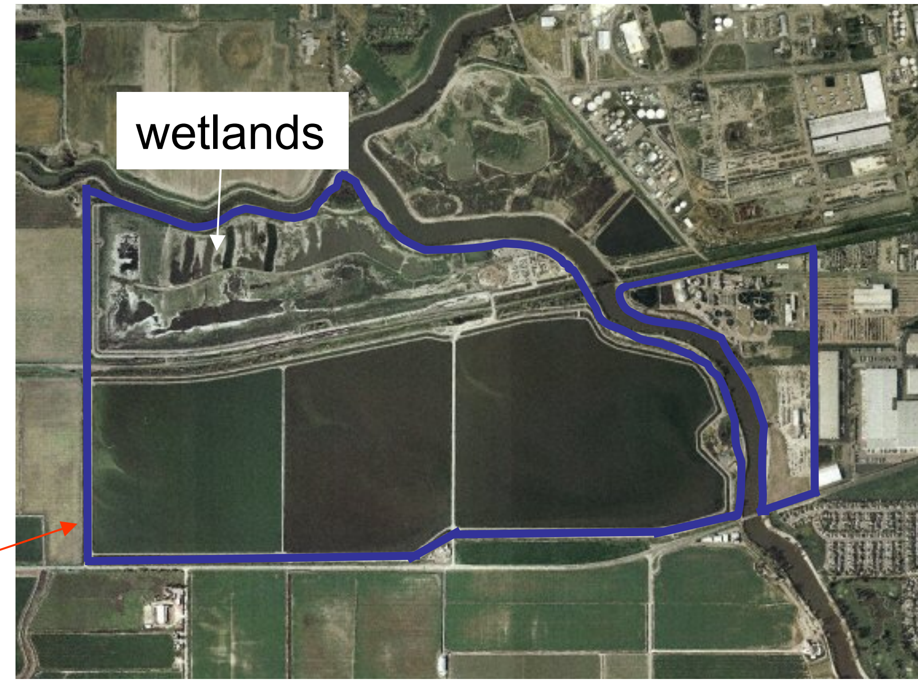
An Algae-Based Wastewater Treatment System with Multiple Benefits


1°
filtered primary wastewater
three algae raceways: one is filling up, the second is growing algae, the third is in the harvesting stage
5-7 days
reclaimed wastewater
irrigation
AB
algae biomass
algae oil
NaOH
methanol
heat
Biodiesel & Glycerin
Exhaust gases are bubbled into the algae raceways to absorb the carbon dioxide
Turbines for electricity production
Methane Digester
Composted sludge converted to fertilizer
Chemical feedstock for polymer production or fertilizer

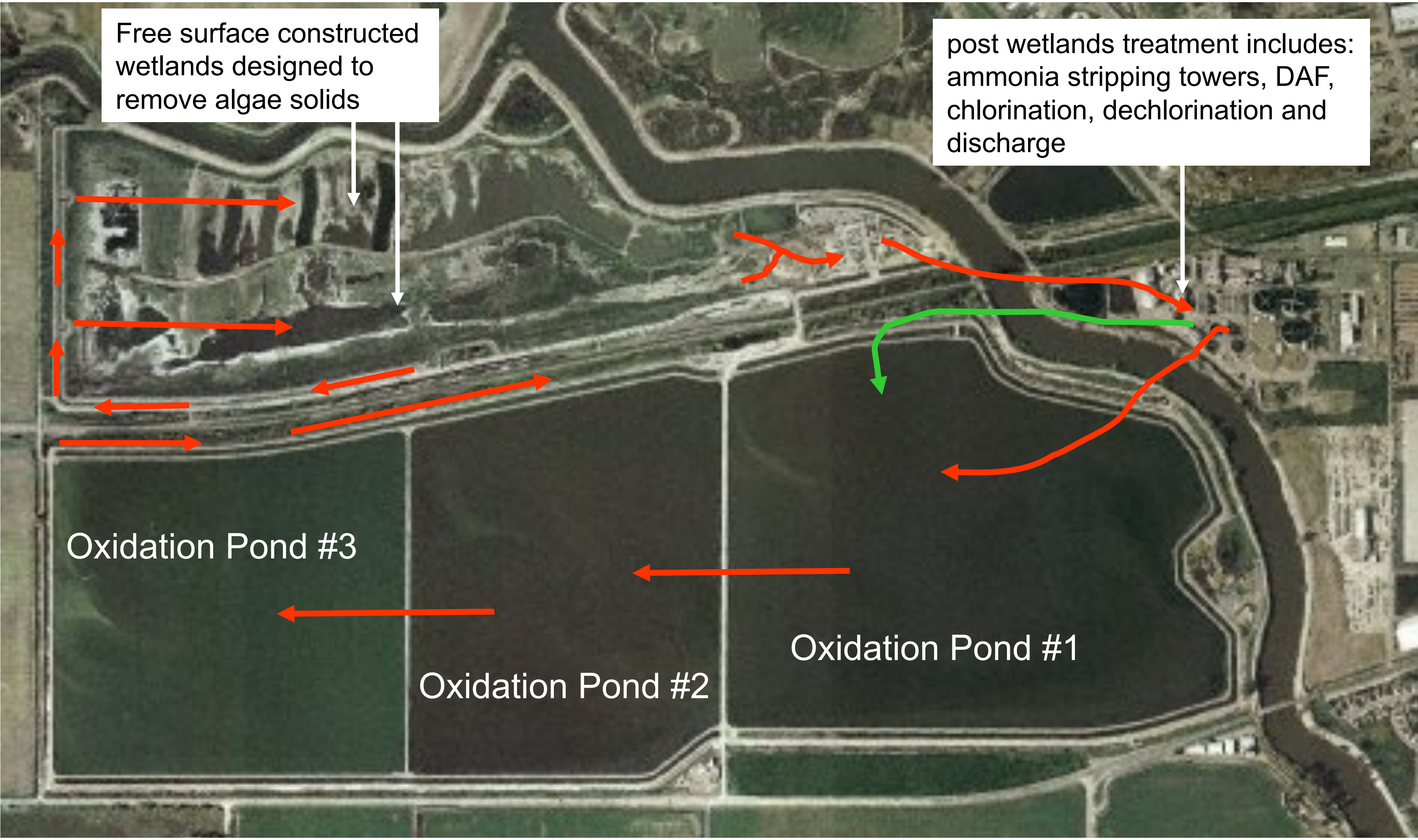
Jason Selwitz was a graduate student in Regenerative Studies at the California Polytechnic University-Pomona shown here with different strains of algae growing on primary wastewater. This research was conducted at the John T. Lyle Center for Regenerative Studies.

Possible Reconfiguration of the Stockton Regional Wastewater Control Facility to Yield Multiple Benefits

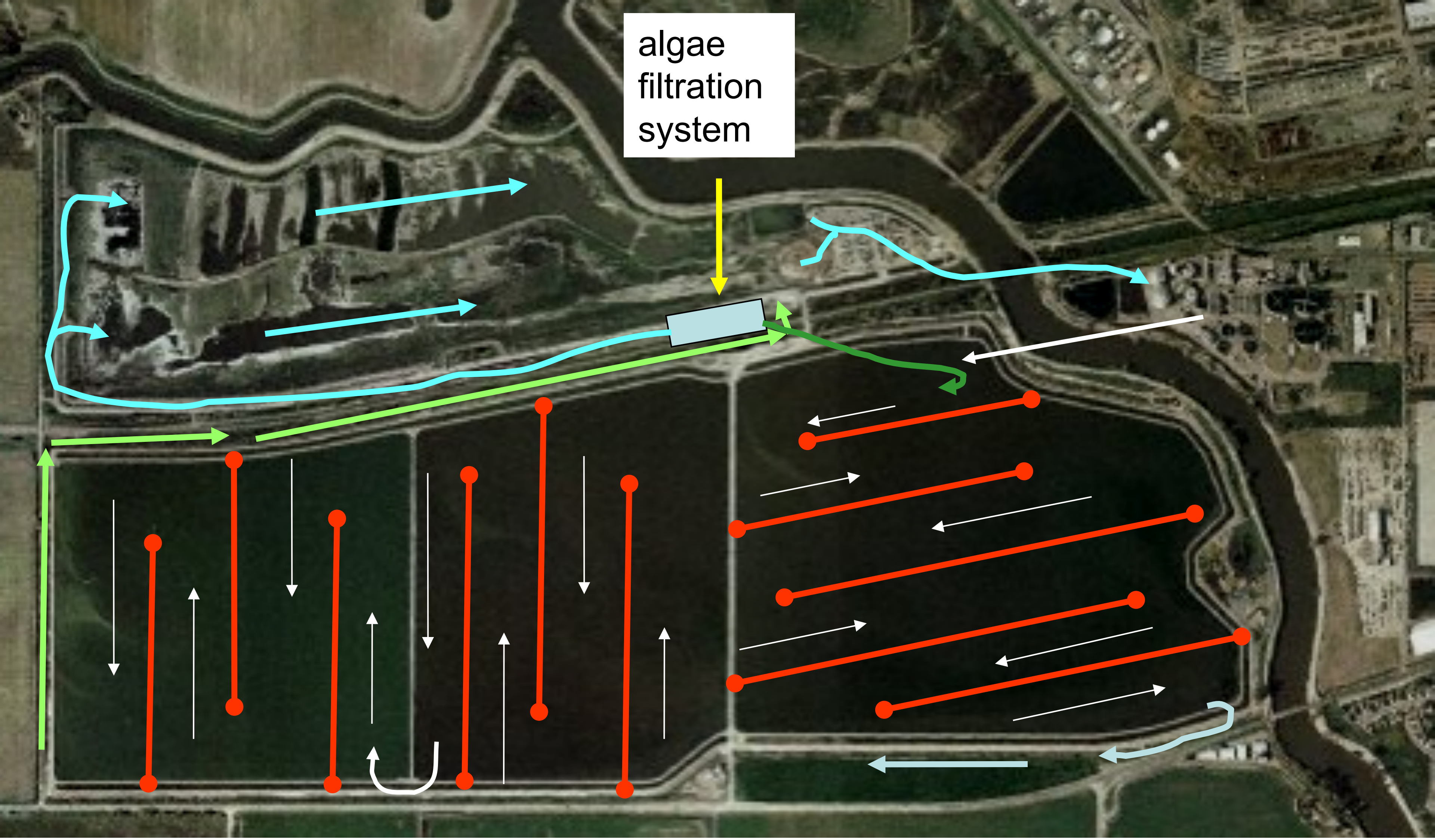




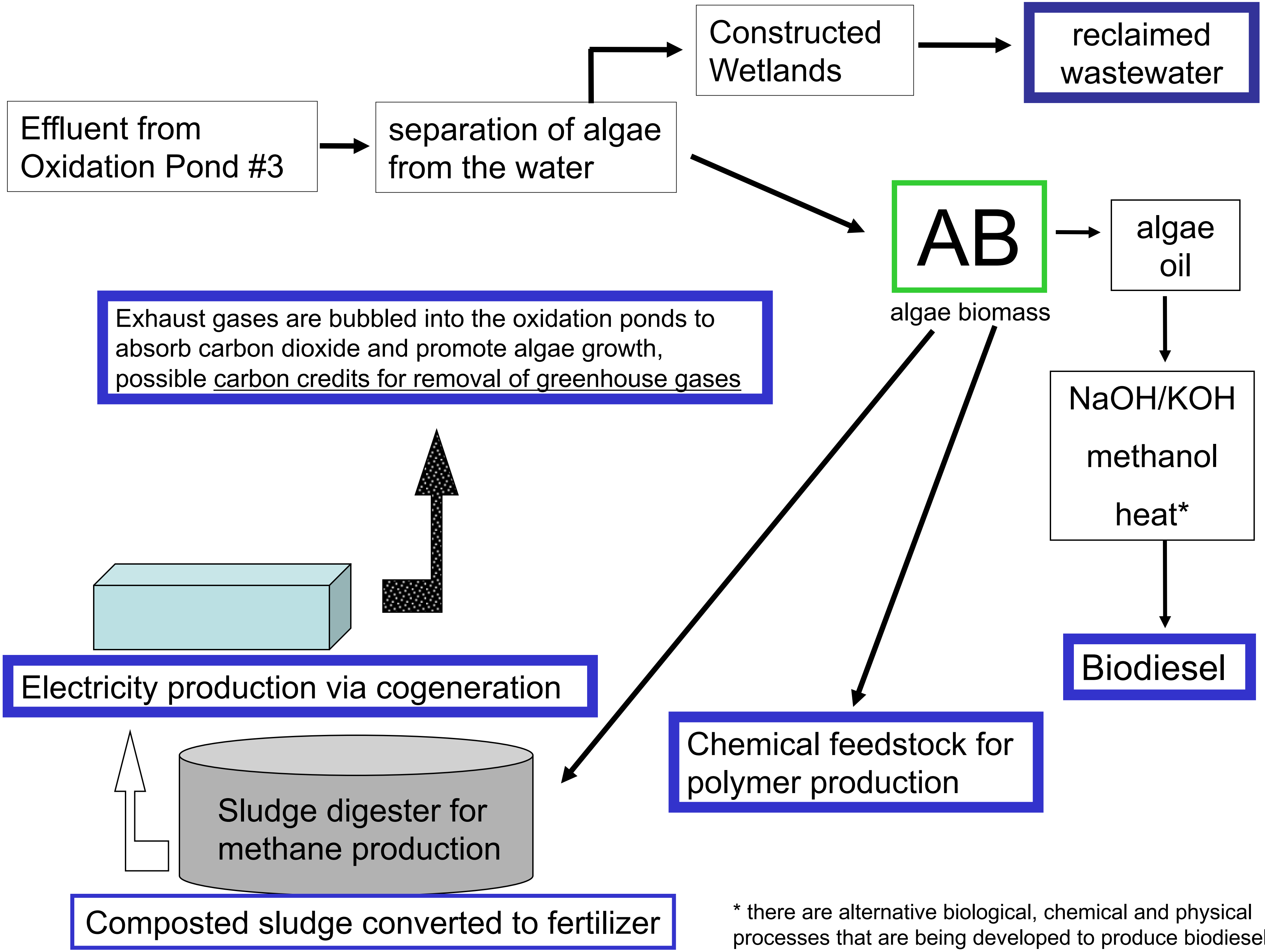
Stockton Regional Wastewater Control Facility discharges 120 ML/D into the San Joaquin River



Current flow patterns of the water from the Stockton treatment facility through the oxidation ponds (approx. 40 hectares each), wetlands and to the dissolved air flotation (DAF) tanks. The green line indicates the flow of the concentrated algae solids back to the first oxidation pond.



Theoretical reworking of the oxidation ponds where Hypalon curtains with floating tops and weighted bottoms allow for serpentine flow within each pond to develop more of a plug flow through the three ponds. This allows for more thorough nutrient stripping by the algae. The algae are filtered off after leaving oxidation pond #3 and the water goes through a final polishing stage in the constructed wetlands. Part of the filtered algae is returned to the inflow of oxidation pond #1. Advanced treatment using the algae may make the ammonia towers and DAF tanks unnecessary.


Effluent from Oxidation Pond #3
separation of algae from the water
Constructed Wetlands
reclaimed wastewater
AB
algae biomass
algae oil
NaOH/KOH
methanol
heat*
Biodiesel
Exhaust gases are bubbled into the oxidation ponds to absorb carbon dioxide and promote algae growth, possible carbon credits for removal of greenhouse gases
Electricity production via cogeneration
Chemical feedstock for polymer production
Sludge digester for methane production
Composted sludge converted to fertilizer
* there are alternative biological, chemical and physical processes that are being developed to produce biodiesel

Chapter 6
Algae-Based Wastewater Treatment for Biofuel Production: Processes, Species, and Extraction Methods

Stephen R. Lyon, Hossein Ahmadzadeh and Marcia A. Murry

Abstract This chapter develops the principles and rationale for an algae-based biofuel production coupled to bioremediation of municipal and agricultural wastewaters. A synergistic model for algal wastewater treatment is proposed, which addresses several economic bottlenecks to earlier algal systems and promotes value-added products, including a high-quality effluent in addition to biodiesel to improve the economic feasibility of algal biofuels. Finally, we review candidate species for full-scale algae production ponds based on algal structure, physiology and ecology, and methods for extraction of algal oils for biodiesel production and coproducts. The dominant strains of algae that are commonly found in wastewater ponds, including *Euglenia*, *Scenedesmus*, *Selenastrum*, *Chlorella,* and *Actinastrum*, are suggested as candidates for large-scale culturing based on their ability to strip nutrients and organic matter from wastewater, grow rapidly, and produce a significant level of algal oil. Oil extraction by supercritical fluid extraction (SFE) is discussed as an efficient means of isolating algal oil and other commercially important high-value compounds from algal biomass. Together with water and CO_2 reclamation, such products may shift the economics of algal biomass production to allow production of low-value commodities including biodiesel and biogas.

Lyon, S.R., Ahmadzadeh, H. and Murry, M.A. 2015. "Algae-Based Wastewater Treatment for Biofuel Production: Processes, Species and Extraction Methods" in *Biomass and Biofuels from Microalgae, Biofuel and Biorefinery Technologies 2*, Springer International Publishing, Switzerland. N.R. Moheimani et al. (eds.), DOI 10.1007/978-3-319-16640-7_6. pp 95-115.

Contents lists available at ScienceDirect

Algal Research

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Wastewater treatment and microbial communities in an integrated photo-bioelectrochemical system affected by different wastewater algal inocula

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ABSTRACT

Integrated photo-bioelectrochemical (IPB) systems are a newly emerging technology for sustainable wastewater treatment through synergistic cooperation between microbial fuel cells (MFCs) and algal bioreactors. This study aimed to advance our understanding of the IPB performance in contaminant removal and bioenergy production and how IPB function is affected by different algal–bacterial inocula. Fed with a synthetic solution, the IPB system could achieve more than 90% removal of solution organic compounds and nearly 100% of ammonium nitrogen. Production of algal biomass was significantly different among three inocula, varying from 5.9 to 53.3 mg L^{-1}. The highest energy production was 0.089 kWh m^{-3}, including direct electrical energy of 0.055 kWh m^{-3} and indirect electrical energy of 0.034 kWh m^{-3} from biomass conversion, and a positive energy balance could be achieved. The natural algal inocula resulted in the cyanobacteria *Leptolyngbya* and green alga *Acutodesmus* as dominant photoautotrophs in cathode suspension and biofilms, providing oxygen for MFC function. Differences in IPB efficiency could be related to microbial composition; one inoculum resulted in absence of Xanthomonadaceae bacteria, while another had more γ-proteobacteria. Specific taxa identified could be important for optimizing electricity generation and algal biomass for biofuel production.

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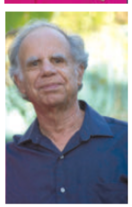
An Introduction to Microbial Biofilms



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We live in a microbial soup. We *are* a microbial soup; the human body contains an estimated 10 times more bacterial cells than mammalian cells.[1]

Those of us outside of the biotechnology or pharmaceutical arena may not focus on the potential for living organisms to impact surfaces of controlled environments or even on surfaces of the product itself. However, microbes can grow exponentially if there is warmth, a food source, and moisture. These three conditions are readily found in controlled environments and as part of manufacturing processes, including critical cleaning.[2]

While 150 years have been devoted to the study of single species of microbes growing in culture media, most microbes live in multi-species, organ-like, societal-structures termed biofilms.[3] Biofilms are a potential problem in manufacturing facilities, including in controlled environments. Trying to manage contamination without understanding biofilms would be akin to making decisions about human physiology based on in vitro studies alone.

Evolution

Biofilms have an evolutionary advantage over discrete colonies; they are designed to survive. In forming biofilms, microbes make their own protective armor — extracellular polymeric substances (EPS). EPS contains polysaccharides and proteins as well as other biobased materials. EPS provides protection from the environmental challenges such as UV radiation, pH changes, and osmotic shock. EPS can absorb and retain moisture, preventing desiccation. Channels within the biofilm increase the availability of water and nutrients. When biofilms contain multiple organisms there can be what ecologists call “symbiosis” or mutually beneficial societal behavior. This can mean a greater quantity and diversity of food production; if fermentation happens, the biofilm can have its own on-site gastropub. EPS can contain extracellular DNA (eDNA)[4]. eDNA is considered to have a role in biofilm formation and development — a recent study demonstrated that eDNA has the ability to bind several classes of antibiotics.[5] The main point to consider is that the biofilm has multiple components, polysaccharides, proteins, eDNA, and lipids. Each of the components can play different roles in the structure and function of the biofilm.

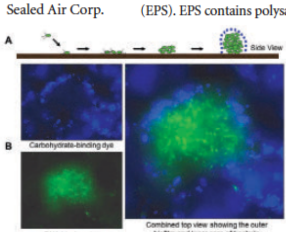

Fig. 1A. The sequential development of a biofilm showing initial attachment, growth, secretion of EPS and maturation. Fig. 1B. Fluorescent micrographs showing the top view of the biofilm column in 1A where the carbohydrate and bacterial portions of the biofilm fluoresce in the presence of carbohydrate and DNA binding dyes. (Microscopic images courtesy of B. Glembocki)

Persistent problem, holistic approach

Overall, the effect of biofilms is undoubtedly positive, or life on earth would be problematic or at least quite different. However, biofilms are persistent, and some are harmful. Dental plaque is a biofilm. Some implant-based infections are associated with biofilms. Because the living portion of biofilms are protected by the EPS armor, disinfection become more of a challenge. Biofilms have been reported in systems for drinking water. Biofilms have been found on produce and in the grout in tiles floors (e.g. kitchens, bathrooms, and industrial facilities). In the manufacture of critical products, the implication is that biofilms have the potential to compromise the controlled environment, the process, and even the product.

Battin calls for “a holistic approach that integrates biology, physics, and chemistry” to understand systems with a “biodiversity equal or superior to the biodiversity of plants in a tropical rainforest or fish in the ocean.”[6] By analogy, cleaning and sanitation processes have to be coordinated in controlled environments and in critical manufacturing applications, and the processes have to be rigorously demonstrated and defined. Since cleaning of controlled environments is typically aqueous-based, cleaning has to combine the cleaning agent with an appropriate level of physical cleaning action. Particularly in dealing with biofilms, if the cleaning is incomplete, disinfection will also be incomplete.[7]

Designing to deter biofilms is also important.[8] Hygienic design is a common practice in the food and beverage (F&B) industry where food processing equipment and rooms are designed not to have the nooks and crannies where bacteria could hide, grow, and create a protective biofilm. The machines have smooth surfaces that are easy to clean. Grinstead points out that there is a high degree of similarity between the conventional

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“soils” in the F&B industry and structural components of biofilms (i.e. proteins, lipids, and other organic polymers). Alkaline cleaners are good for removing most carbohydrates, and chlorinated alkaline cleaners are used in protein removal.[9] These are the kind of lessons learned in the F&B world that could readily be applied to industrial and biomedical cleanrooms.

A rose by any other name

It should also be pointed out that there are a number of definitions of biofilms. Definitions have evolved and become more refined along with our own understanding of biofilms. We occasionally hear the term used to describe microbes that are trapped in “stuff” (e.g. machining oil, cleaning agent residue) where the “stuff” is not actually produced by the microbe itself. Even if microbes trapped in “stuff” would not be considered a biofilm by most experts, people involved with controlled environments still need to be concerned about appropriate cleaning and disinfection. An understanding of biofilm and biofilm management is likely to help, even with mixed soils that are not true biofilms.

The purpose of this article is to introduce the community to the complex world of microbial biofilms. In an upcoming article, we plan to describe the current state of regulations, test methods for growing biofilms, and validation of “biofilm kill.”

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Thank You!

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Questions??