Using Geochemical Data to Predict the Dominant Microbial Processes in Deep Aquifers Targeted for Aquifer Storage and Recovery (ASR)

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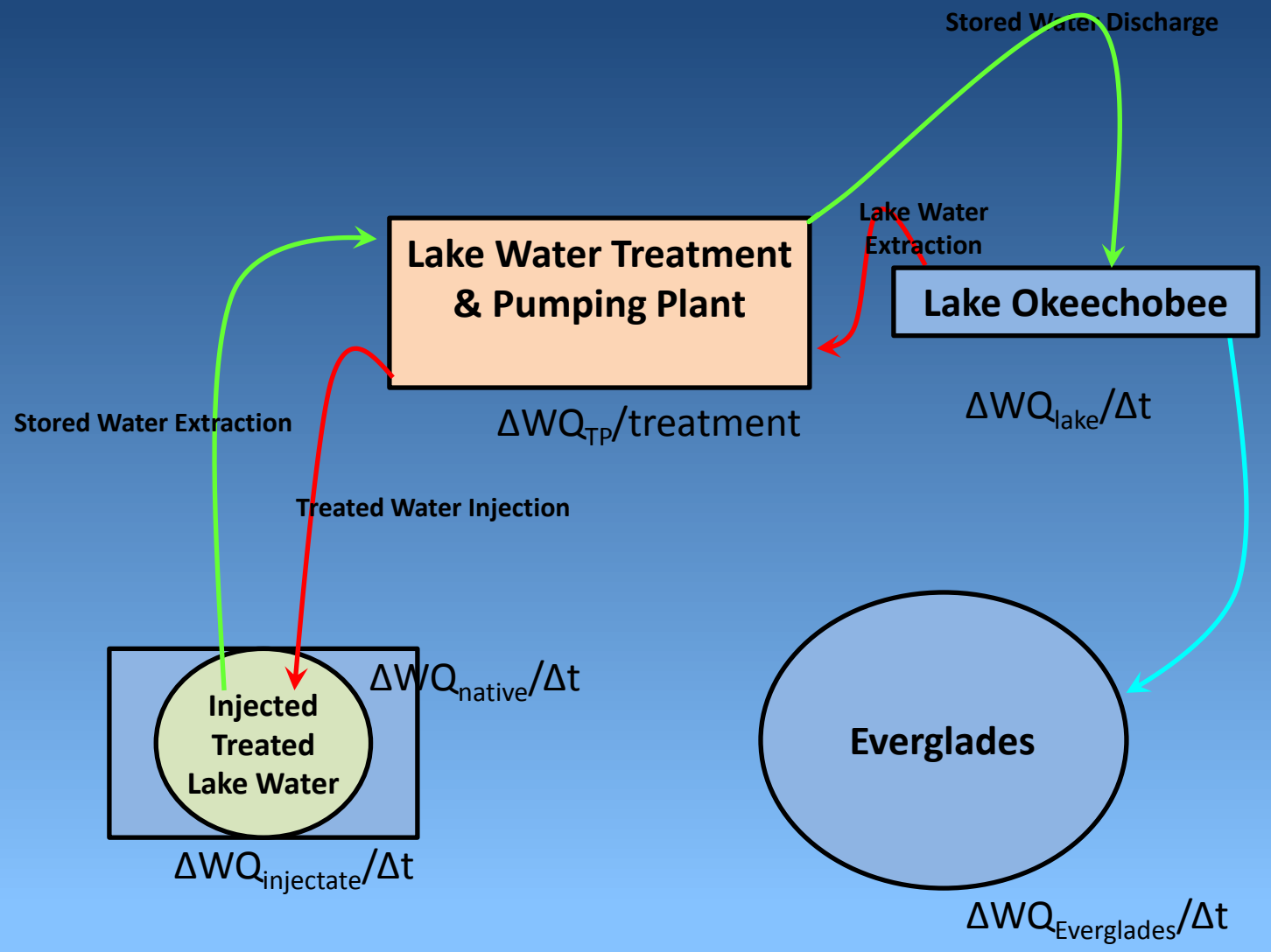
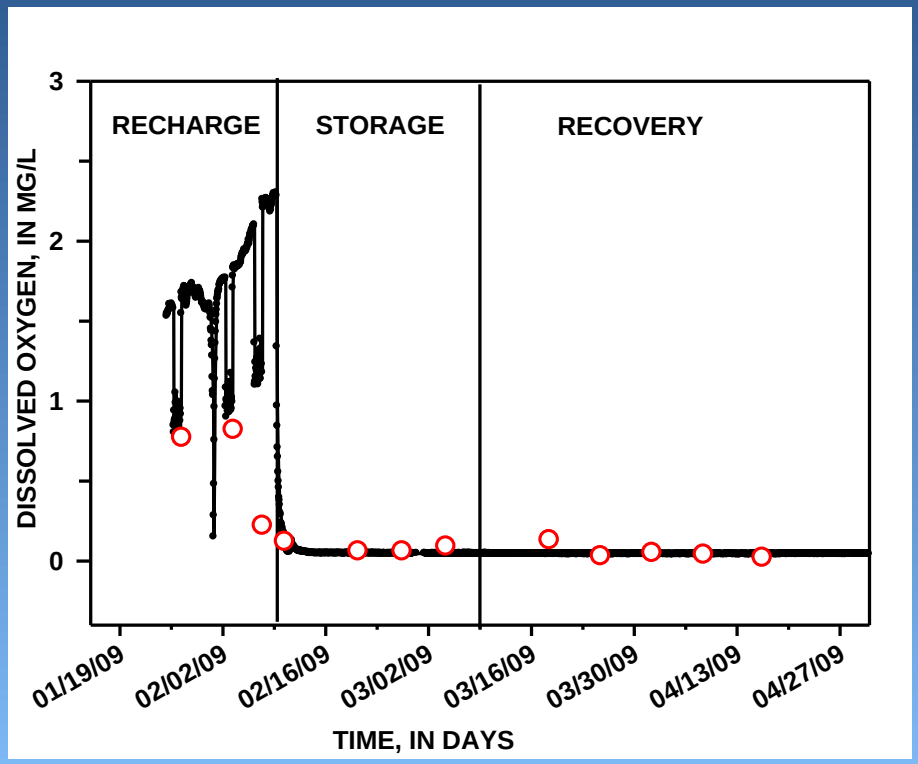
Conceptual Diagram of the Comprehensive Everglades Restoration Plan's Utilization of ASR

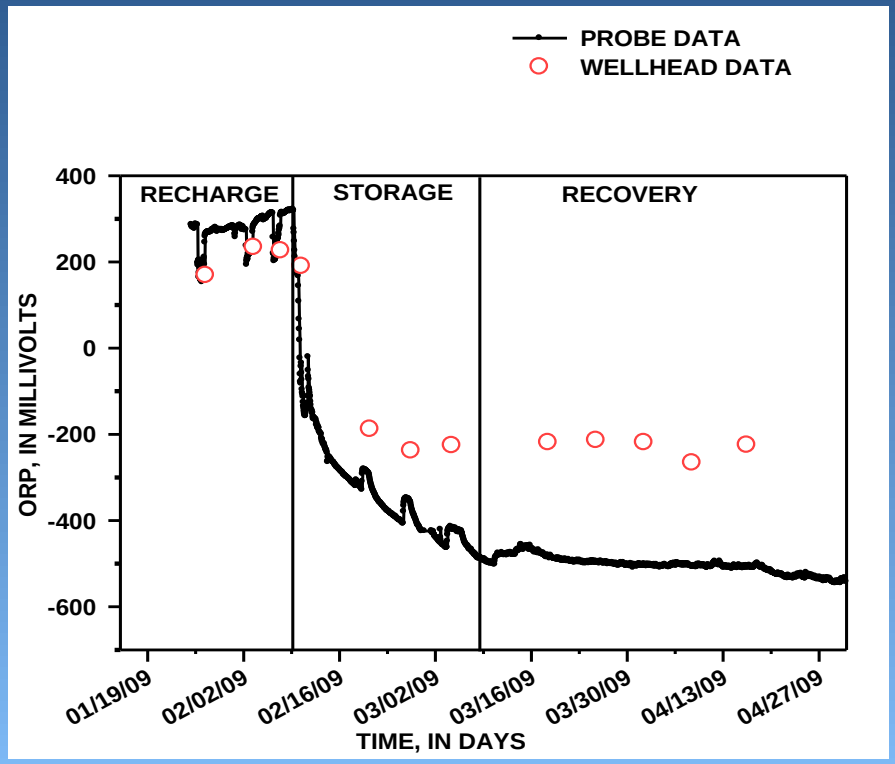


Kissimmee River ASR Pilot System, Okeechobee, FL

- 5 MGD pumping rate
- Kissimmee River source water – filtered, UV disinfected

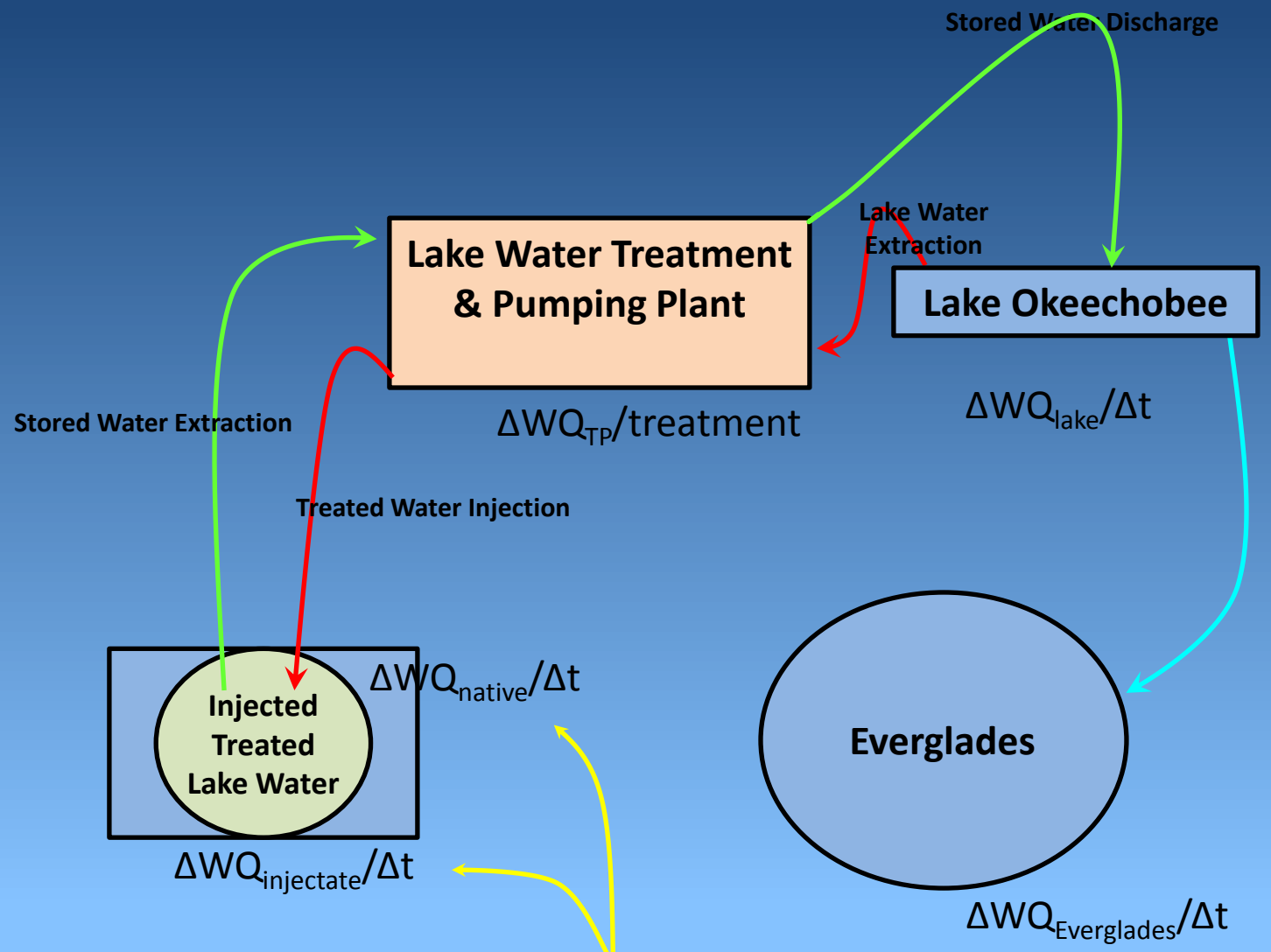
How Long Do Oxic Conditions Persist After ASR Recharge?





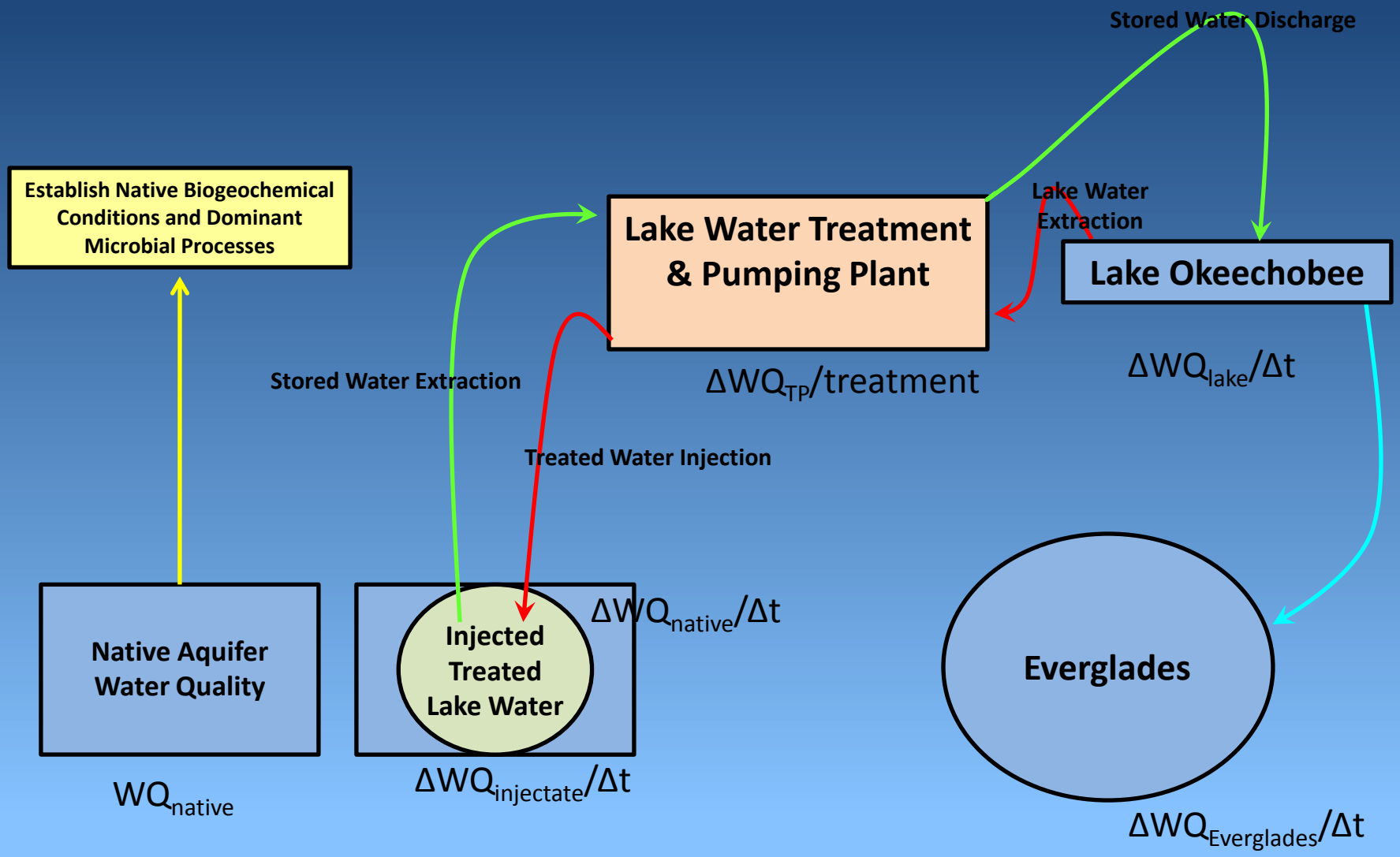
***Monitoring well ~350ft from injection well.**

Conceptual Diagram of the Comprehensive Everglades Restoration Plan's Utilization of ASR



Why won't geochemical modeling reliably predict these changes in water quality?

Conceptual Diagram of the Comprehensive Everglades Restoration Plan's Utilization of ASR



Research Site Locations

Study Site Location and Well Designations

Location	Well	Aquifer Zones	Depth (fbs)	Casing Size (inches)
Paradise Run	HIF-42U	Upper Floridan	1040	24
	HIF-42L	Avon Park Producing Zone	1540	14
Palm Beach	15U	Upper Floridan	1144	18
	15M	Avon Park Producing Zone	1583	12
LaBelle	MZ1	Upper Floridan	873	18
	MZ3	Avon Park Producing Zone	1759	7

Paradise Run Site (HIF-42): Wells 42U and 42L

Study Objectives

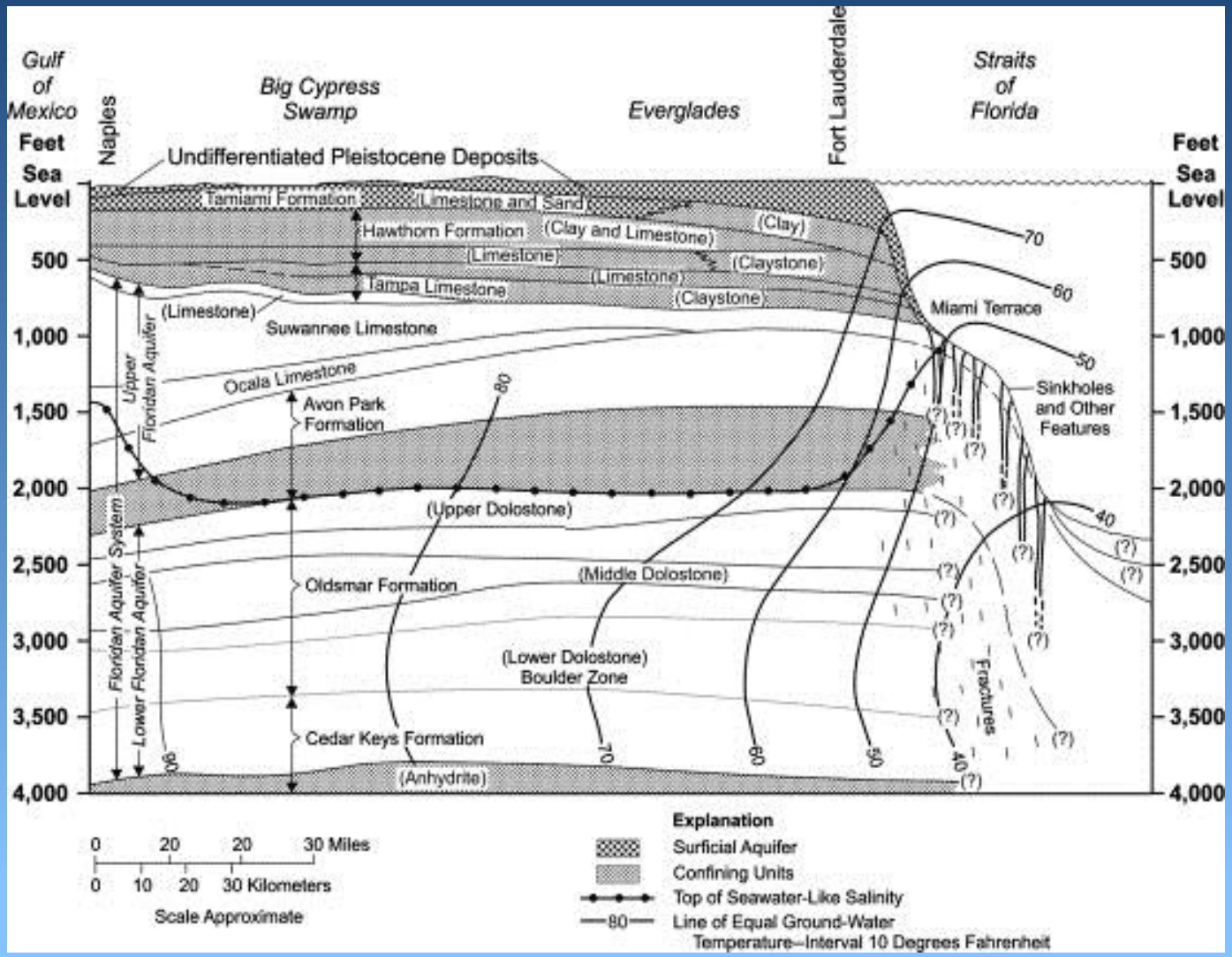
- Compare & contrast the geochemical data from the six wells in the Upper Floridan Aquifer (UFA).
- Quantify the die off rates of two microbial indicators (*E. coli* & *Pseudomonas aeruginosa*) of fecal contamination using diffusion chambers and above ground flow through mesocosms connected to the UFA
- Development of human health related quantitative microbial risk assessment model for each indicator

Study Objectives

- Quantify the microbial diversity in the UFA using a high throughput sequencing technology, the *G3 PhyloChip*

- Use geochemical and molecular approaches to assist in characterizing water quality factors that influence indicator bacterial survival and persistence and predict the most probable native microbial biogeochemical processes in each well
  - Predicting the most probable native microbial processes that dominate in each aquifer based on thermodynamic principles
  - Determination of carbon mineralization and biomass production (^{14}C-acetate) and biomass turnover (^{3}H-leucine) rates under native conditions

Hydrogeologic Cross Section Through South Florida

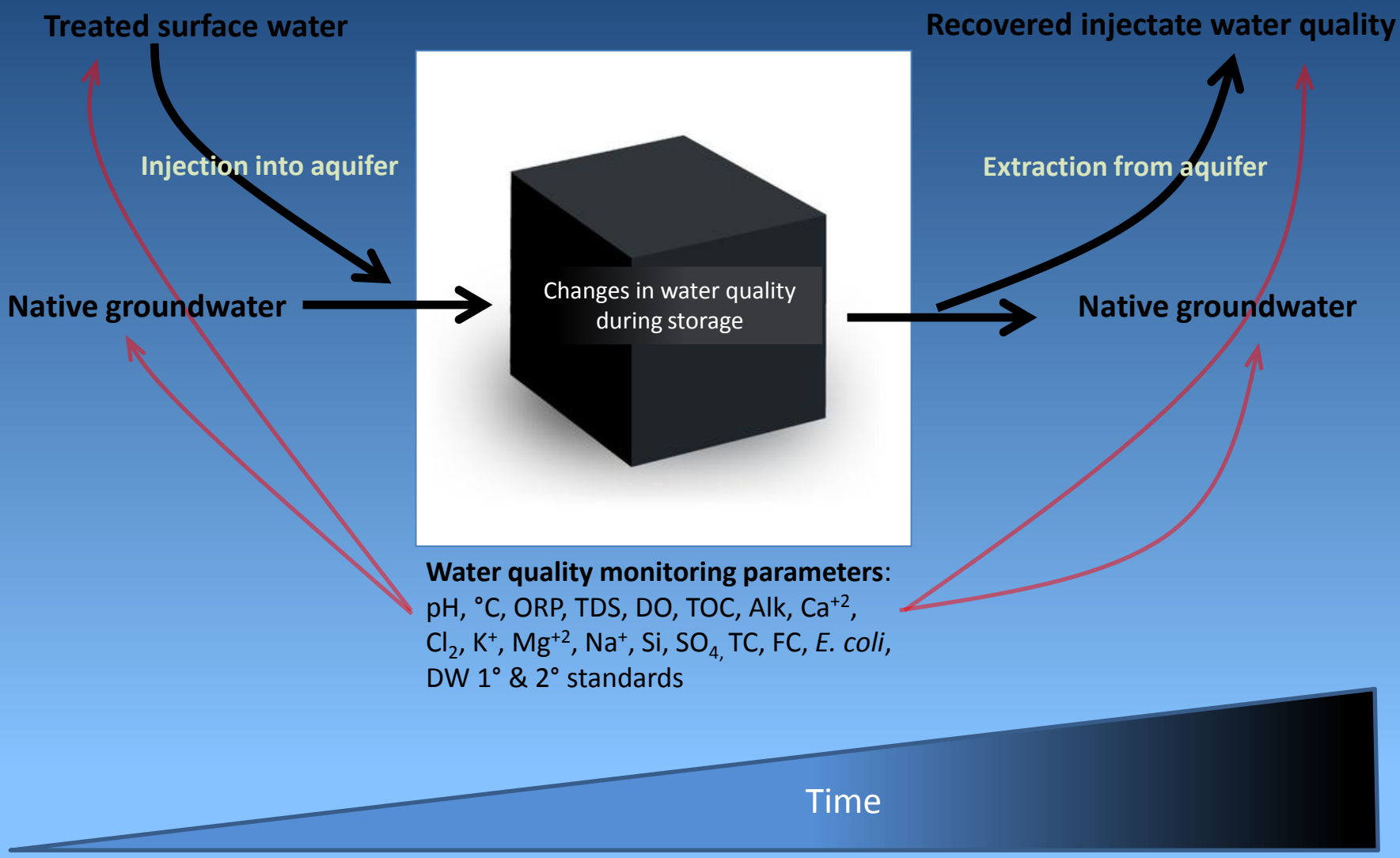


http://dx.doi.org/10.1016/S0167-5648(05)52008-2

Characteristics of the Upper Floridan Aquifer

- These ground waters were last exposed to the surface of Earth 20K-25K years ago.
  - No photosynthetically fixed carbon since leaving surface of Earth
- Extremely reduced (ORP < -300 mV)
- Anoxic with varying concentrations of sulfides
- Survival in this zone of the UFA is constrained by processes that function at the biogeochemical boundaries of known life.
- Microbial processes dominate and control native geochemical processes and responses to perturbations, like recharge events.
  - Biogeochemical processes

Monitoring the Water Quality of Native Ground Waters and Injected & Recovered Surface Waters

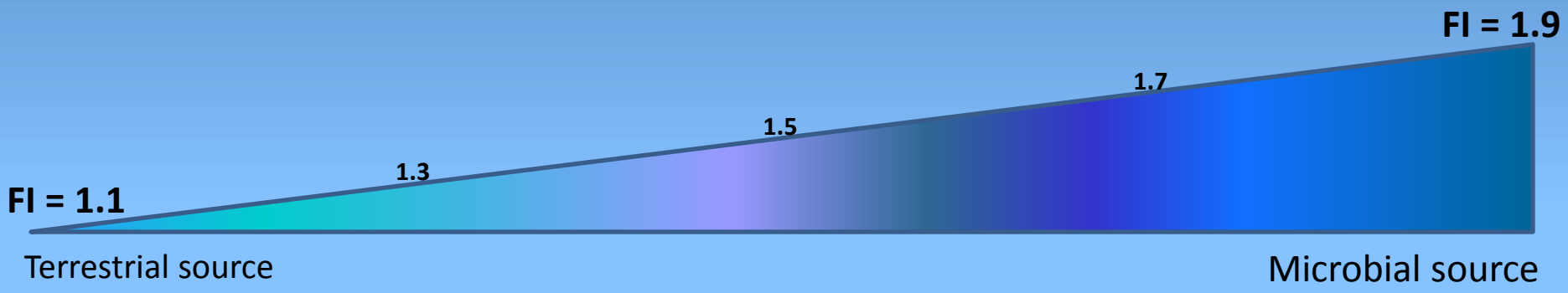


Geochemical Data

Parameter (mg/L)	*MZ-1*	*MZ-3*	*42-U*	*42-L*	*15-U*	*15-M*
ORP (mV)	-312	-309	-338	-351	-355	-365
Chloride	640	9700	160	1600	1600	1300
Sodium	440	4700	98	800	890	740
Fluoride	0.78	*BDL*	0.57	0.29	0.97	1.10
Calcium	80	550	44	200	120	110
Potassium	24.0	230.0	5.5	40.0	36.0	29.0
Magnesium	75	650	33	140	130	120
Manganese	0.013	0.035	0.007	0.006	0.011	0.010
Bromide	2.0	34.0	*BDL*	5.1	4.8	4.3
Barium	0.039	0.035	0.034	0.040	0.015	0.028
Silica	9.8	9.1	14.0	12.0	13.0	13.0
Ferrous iron	0.034	0.030	0.025	0.025	0.055	0.021
Ferric iron	0.140	0.190	0.095	0.170	0.280	0.380
Ammonia (as N)	0.19	0.28	0.20	0.26	0.44	0.33
Nitrogen, total	0.25	0.38	0.21	0.29	0.52	0.53
Sulfide	2.1	1.6	1.4	1.6	3.7	4.2
Sulfate	330	1800	160	450	400	330
Methane	0.032	0.015	0.011	0.009	0.016	0.017
Hydrogen (nM)	120.0	1.2	95.0	6.4	62.0	35.0
Total inorganic carbon	160	200	210	190	310	330
Acetic acid	0.120	0.070	0.095	0.070	0.120	0.070
i-Pentanoic acid	0.056	*BDL*	*BDL*	*BDL*	*BDL*	*BDL*
Lactic acid	*BDL*	3.8	*BDL*	*BDL*	*BDL*	*BDL*
Propionic acid	0.071	*BDL*	*BDL*	*BDL*	*BDL*	0.190
Pyruvic acid	0.067	*BDL*	*BDL*	*BDL*	*BDL*	*BDL*
Bicarbonate	151.50	189.95	199.07	181.71	296.44	315.80
Carbonate	6.79	4.15	8.77	3.37	5.25	6.04
Carbon dioxide	1.66	5.90	2.16	4.92	8.31	8.17

DOC Quantification and Characterization

Sample Source	DOC (mg/L)	FI (470nm/520nm)	VFA:DOC (%)
MZ1	1.2	1.73	25.8
MZ3	1.1	1.73	351.8
42U	1.1	1.71	9.1
42L	1.2	1.70	5.8
15U	1.7	*	7.1
15M	1.9	*	13.7
UFA (Miami)	1.2	1.77	
Biscayne Aquifer (Miami)	3.1	1.49	
Lake Okeechobee	19.7	1.32	

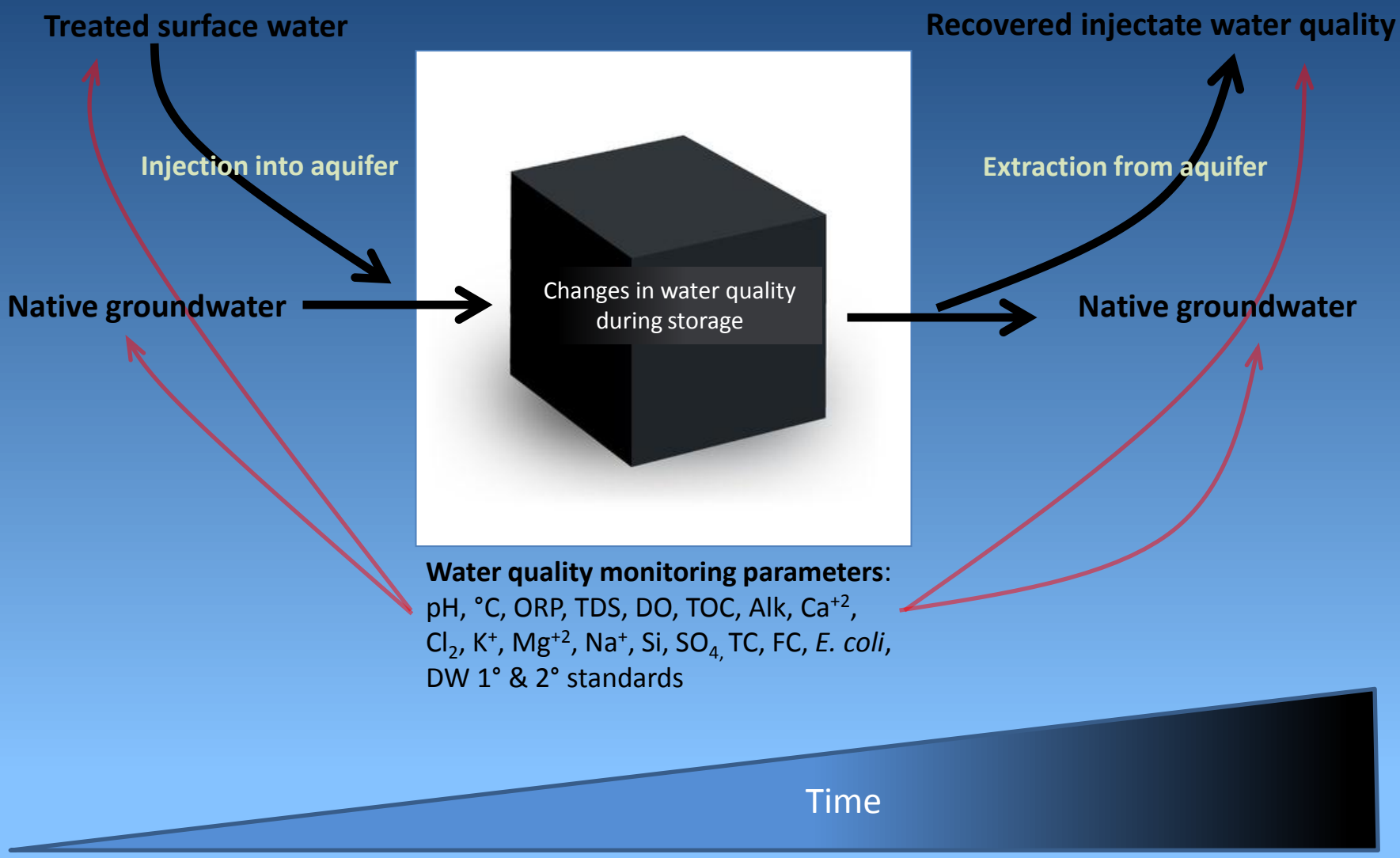


Water Quality Comparisons

Recharge vs. Native Aquifer

Parameter	*Unit*	*Recharge Water*	*UFA*
Dissolved O_2	mg/L	4.4 ± 2.4	0.0
Color	PCU	122 ± 138	nd
Sulfate	mg/L	16 ± 7.3	160-1800
Sulfide	mg/L	0.1 ± 0.3	1.4-4.2
DOC	mg/L	15.7 ± 1.6	1.1-1.9
TOC	mg/L	17.4 ± 2.9	nd
Iron	µg/L	256 ± 141	120-401
Nitrate	mg/L	0.15 ± 0.115	BDL
Nitrite	mg/L	0.016 ± 0.006	BDL
Ammonia	mg/L	0.09 ± 0.07	0.19-0.44
Phosphorus	mg/L	0.067 ± 0.042	BDL

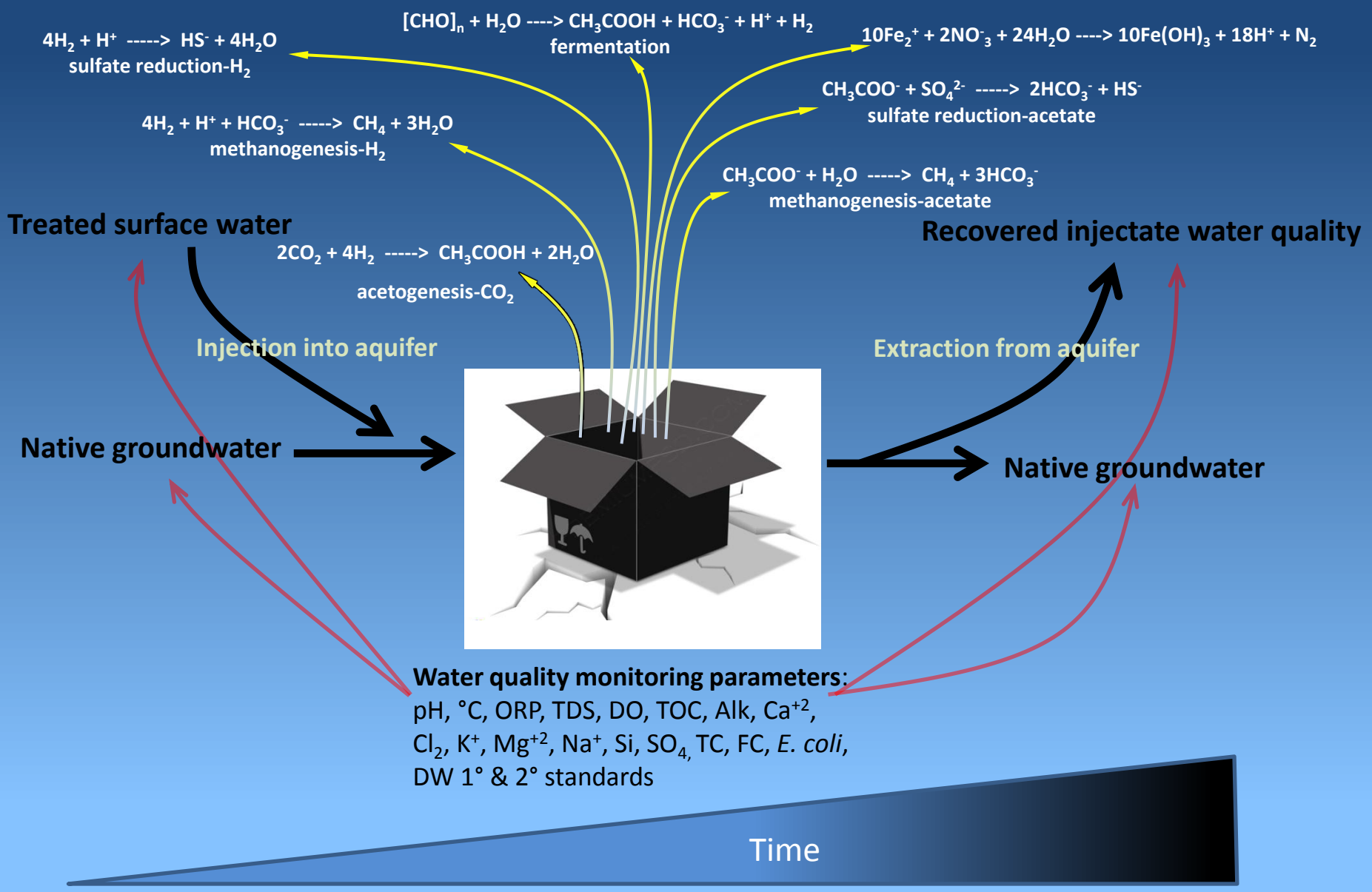
Monitoring the Water Quality of Native Ground Waters and Injected & Recovered Surface Waters



How Native & Injected Microorganisms Survive in Regional Aquifer Systems

What's in the Black Box?

Monitoring the Water Quality of Native Ground Waters and Injected & Recovered Surface Waters



Aerobic (Oxygenic) Reactions

$CH_4 + 2O_2 \rightarrow HCO_3^- + H^+ + H_2O$

Acetate $+ 2O_2 \rightarrow 2HCO_3^- + H^+$

$CH_4 + 2O_2 \rightarrow CO_2^- + 2H_2O$

$2H_2S + 0.5O_2 + Fe^{2+} \rightarrow$ Pyrite $+ 2H^+ + H_2O$

$Fe^{+2} + 0.25O_2 + 1.5H_2O \rightarrow$ Goethite $+ 2H^+$

$2H^+ + O_2 \rightarrow 2H_2O$

$2H_2 + O_2 \rightarrow 2H_2O$

$2CO + O_2 + 2H_2O \rightarrow 2HCO_3^- + 2H^+$

$NH_3 + 1.5O_2 \rightarrow NO_2^- + H^+ + H_2O$

$2NO_2^- + O_2 \rightarrow 2NO_3^-$

$HS^- + 2O_2 + H_2O \rightarrow SO_4^{2-} + 2H^+$

$S_2O_3^{2-} + 2O_2 + H_2O \rightarrow 2SO_4^{2-} + 2H^+$

$S + 1.5O_2 + H_2O \rightarrow SO_4^{2-} + 2H^+$

$2HS^- + 2O_2 \rightarrow S_2O_3^{2-} + H_2O$

$2HS^- + O_2 + 2H^+ \rightarrow 2S + 2H_2O$

$4Fe^{2+} + O_2 + 10H_2O \rightarrow 4Fe(OH)_3 + 8H^+$

$2Mn^{2+} + O_2 + 2H_2O \rightarrow 2MnO_2 + 4H^+$

Fermentation Reactions

$4\text{Formate} + H^+ + H_2O \rightarrow CH_4 + 3HCO_3^-$

$\text{Acetate} + H_2O \rightarrow CH_4 + HCO_3^-$

$S_2O_3^{2-} + H_2O \rightarrow SO_4^{2-} + H^+ + HS^-$

$S_2O_3^{2-} + 4H_2 \rightarrow 3H_2O + 2HS^-$

$\text{Propanoate} + 3H_2O \rightarrow \text{Acetate} + HCO_3^- + H^+ + 3H_2$

Mn & NO_3 Reduction Reactions

$Acetate + 4MnO_2 + 7H^+ \rightarrow 4Mn^{2+} + 4H_2O + 2HCO_3^-$

$4H_2 + NO_3^- + H^+ \rightarrow NH_3 + 3H_2O$

$5H_2 + 2NO_3^- + 2H^+ \rightarrow N_2 + 6H_2O$

$Acetate + NO_3^- + H_2O \rightarrow 2HCO_3^- + NH_3$

$Acetate + 1.6NO_3^- + 0.6H^+ \rightarrow 2HCO_3^- + 0.8H_2O + 0.8N_2$

$4CO + NO_3^- + 5H_2O \rightarrow 4HCO_3^- + NH_3 + 3H^+$

$2.5CO + NO_3^- + 2H_2O \rightarrow 2.5HCO_3^- + 1.5H^+ + 0.5N_2$

$S_2O_3^{2-} + NO_3^- + 2H_2O \rightarrow 2SO_4^{2-} + H^+ + NH_3$

$HS^- + NO_3^- + H_2O \rightarrow SO_4^{2-} + NH_3$

$2S + 1.5NO_3^- + 3.5H_2O \rightarrow 2SO_4^{2-} + 2.5H^+ + 1.5NH_3$

$4Mn^{2+} + NO_3^- + 5H_2O \rightarrow 4MnO_2 + 7H^+ + NH_3$

$5Fe^{2+} + NO_3^- + 12H_2O \rightarrow 5Fe(OH)_3 + 9H^+ + 0.5N_2$

$NO_2^- + H^+ + NH_3 \rightarrow 2H_2O + N_2$

$CH_4 + 4NO_3 \rightarrow CO_2 + 4NO_2 + 2H_2O$

$H_2S + 4NO_3^- \rightarrow SO_4^{2-} + 4NO_2^- + 2H^+$

$H_2S + NO_3^- \rightarrow Sulfur + NO_2^- + H_2O$

$2H_2S + NO_3^- + Fe_2^+ \rightarrow Pyrite + NO_2^- + 2H^+ + H_2O$

$2Fe^{2+} + NO_3^- + 3H_2O \rightarrow 2Goethite + NO_2^- + 4H^+$

$H_2 + NO_3^- \rightarrow NO_2^- + H_2O$

Ammonification Reactions

$3CH_4 + 4NO_2^- + 8H^+ \rightarrow 3CO_2 + 4NH_4^+ + 2H_2O$

$3H_2S + 4NO_2^- + 2H^+ + H_2O \rightarrow 3SO_4^{2-} + 4NH_4^+$

$3H_2S + NO_2^- + 2H^+ \rightarrow 3\text{Sulfur} + NH_4^+ + 2H_2O$

$6H_2S + NO_3^- + 3Fe_2^+ \rightarrow 3\text{Pyrite} + NH_4^+ + 4H^+ + 2H_2O$

$6Fe_2^+ + NO_3^- + 10H_2O \rightarrow 6\text{Goethite} + NH_4^+ + 10H^+$

$3H_2 + NO_2^- + 2H^+ \rightarrow NH_4^+ + 2H_2O$

Fe(III) Reduction Reactions

$$\text{Acetate} + 4\text{Hematite} + 15H^+ \rightarrow 8Fe^{2+} + 8H_2O + 2HCO_3^-$$

$$CO + \text{Hematite} + 3H^+ \rightarrow 2Fe^{2+} + H_2O + HCO_3^-$$

$$H_2 + \text{Hematite} + 4H^+ \rightarrow 2Fe^{2+} + 3H2O$$

S & SO_4 Reduction Reactions

$H_2 + S \rightarrow HS^- + H^+$

Acetate $+ 4S + 4H_2O \rightarrow 5H^+ + 2HCO_3^- + 4HS^-$

$4H_2 + H^+ + SO_4^{2-} \rightarrow HS^- + 4H_2O$

Acetate $+ SO_4^{2-} \rightarrow 2HCO_3^- + HS^-$

$4CO + SO_4^{2-} + 4H_2O \rightarrow 4HCO_3^- + HS^- + 3H^+$

$CH_4 + SO_4^{2-} \rightarrow H_2O + HCO_2^- + HS^-$

$CH_4 + SO_4^{2-} + 2H^+ \rightarrow CO_2 + H_2S + 2H_2O$

$7CH_4 + 8SO_4^{2-} + 8H^+ + 4Fe^{2+} \rightarrow$ 4Pyrite $+ 7CO_2 + 18H_2O$

$7H_2S + SO_4^{2-} + 4Fe^{2+} \rightarrow$ 4Pyrite $+ 6H^+ + 4H_2O$

$15Fe^{2+} + 2SO_4^{2-} + 20H_2O \rightarrow$ Pyrite + 14Goethite $+ 26H^+$

$7H_2 + 2SO_4^{2-} + Fe^{2+} + 2H^+ \rightarrow$ Pyrite $+ 8H_2O$

$4H_2 + SO_4^{2-} + 2H^+ \rightarrow H_2S + 4H_2O$

CO_2 Reduction Reactions

$$4H_2 + H^+ + 2HCO_3^- \rightarrow \text{Acetate} + 4H_2O$$

$$4H_2 + H^+ + HCO_3^- \rightarrow CH_4 + 3H_2O$$

Pathway and stoichiometry of reaction	ΔG^0 (kJ mol^{-1})
Oxic respiration:	
$[CH_2O] + O_2 \text{ ---> } CO_2 + H_2O$	-479
Denitrification:	
$5[CH_2O] + 4NO_3^- \text{ ---> } 2N_2 + 4HCO_3^- + CO_2 + 3H_2O$	-453
Mn(IV) reduction:	
$[CH_2O] + 3CO_2 + H_2O + 2MnO_2 \text{ ---> } 2Mn_2^+ + 4HCO_3^-$	-349
Fe(III) reduction:	
$[CH_2O] + 7CO_2 + 4Fe(OH)_3 \text{ ---> } 4Fe_2^+ + 8HCO_3^- + 3H_2O$	-114
Sulfate reduction:	
$2[CH_2O] + SO_4^{2-} \text{ ---> } H_2S + 2HCO_3^-$	-77
$4H_2 + SO_4^{2-} + H^+ \text{ ---> } HS^- + 4H_2O$	-152
$CH_3COO^- + SO_4^{2-} + 2H^+ \text{ ---> } 2CO_2 + HS^- + 2H_2O$	-41
Methane production:	
$4H_2 + HCO_3^- + H^+ \text{ ---> } CH_4 + 3H_2O$	-136
$CH_3COO^- + H^+ \text{ ---> } CH_4 + CO_2$	-28
Acetogenesis:	
$4H_2 + 2CO_3^- + H^+ \text{ ---> } CH_3COO^- + 4H_2O$	-105
Fermentation:	
$CH_3CH_2OH + H_2O \text{ ---> } CH_3COO^- + 2H_2 + H^+$	10
$CH_3CH_2COO^- + 3H_2O \text{ ---> } CH_3COO^- + HCO_3^- + 3H_2 + H^+$	77

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Fermentation:	
$CH_3CH_2OH + H_2O \text{ ---> } CH_3COO^- + 2H_2 + H^+$	10
$CH_3CH_2COO^- + 3H_2O \text{ ---> } CH_3COO^- + HCO_3^- + 3H_2 + H^+$	77

- ***Competing reactions?***

  How can there be SO_4 , CH_4 & acetate in same sample?

- ***Biofilms on walls of aquifer flow path?***

  After injection of treated surface water the biofilms on walls of aquifer in region of displaced native groundwater act as solid phase bioreactors.

Surviving in Deep Groundwater Systems

Life At The Lower Limits of Disequilibrium

$$aA + bB \rightarrow cC + dD$$

Assuming all reactants are at 1M and system is at 1 atm

$$\Delta G^0 = \sum \Delta G_f^0(\text{products}) - \sum \Delta G_f^0(\text{reactants})$$

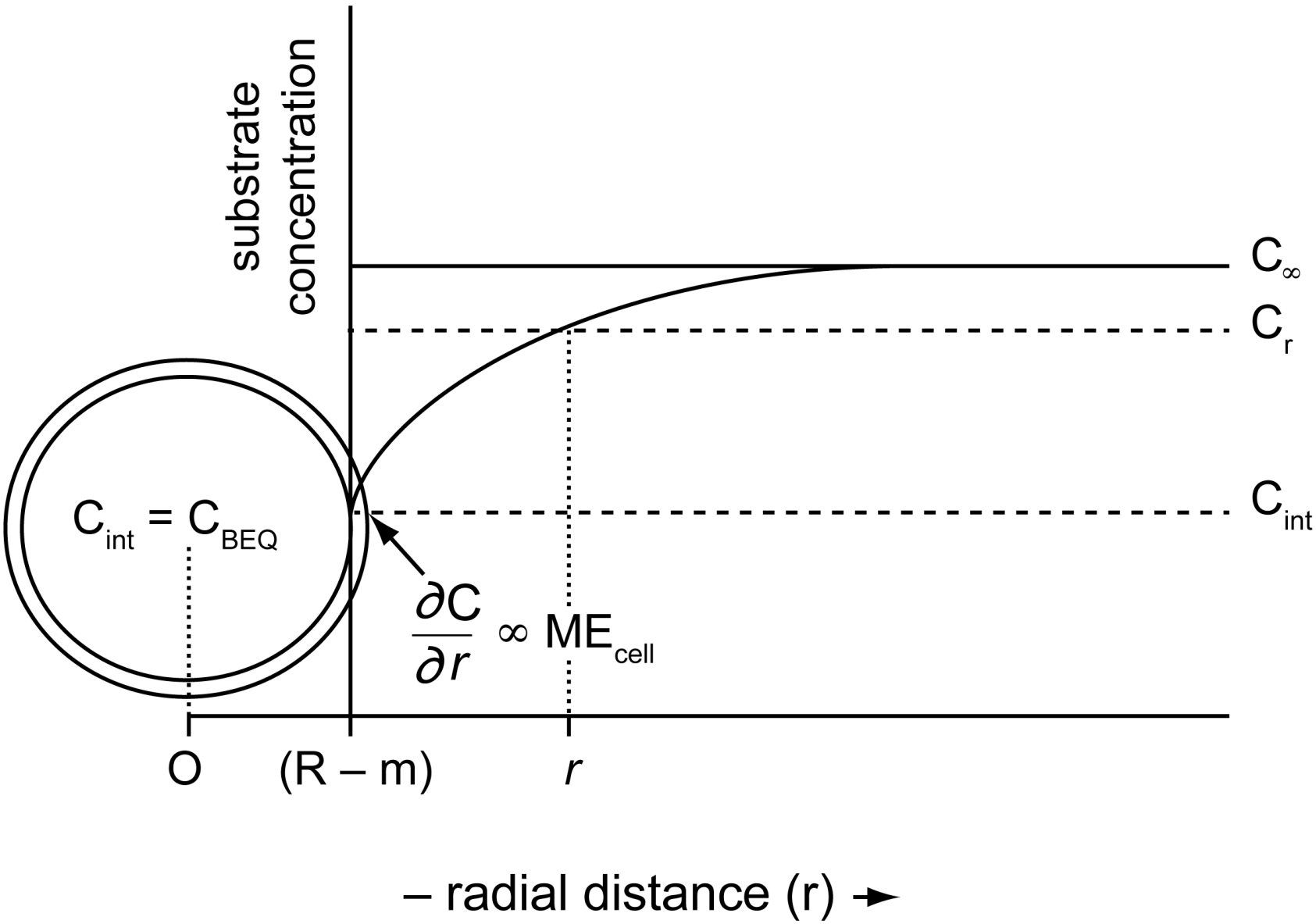
However, standard conditions DO NOT prevail in natural systems!

$$\Delta G = \Delta G^0 + RT \cdot \ln \{[C]^c[D]^d / [A]^a[B]^b\}$$

Microbial Physiologies & Their Likelihood of Occurring in the UFA Based on Available Free Energy (ΔG_r)

Microbial Process	Process Equation	Available Gibbs Free Energy (ΔG_r)					
		MZ-1 (kJ/mol)	MZ-3 (kJ/mol)	15U (kJ/mol)	15M (kJ/mol)	42U (kJ/mol)	42L (kJ/mol)
H_2-sulfate reduction	$4H_2 + H^+ + SO_4^{2-} \rightarrow HS^- + 4H_2O$	43.95	85.57	48.63	55.39	46.90	69.45
Acetate-sulfate reduction	$CH_3COO^- + SO_4^{2-} \rightarrow 2HCO_3^- + HS^-$	-54.89	-57.17	-50.26	-47.83	-51.79	-53.80
H_2-methanogenesis	$4H_2 + H^+ + HCO_3^- \rightarrow CH_4 + 3H_2O$	-67.15	-60.66	-71.64	-69.98	-70.99	-66.09
Acetate methanogenesis	$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$	-45.90	-46.15	-45.76	-44.11	-47.10	-47.24
H_2-acetogenesis	$4H_2 + H^+ + 2HCO_3^- \rightarrow CH_3COO^- + 4H_2O$	98.74	142.64	98.79	103.12	98.59	123.2
CO_2-acetogenesis	$2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O$	-41.07	1.18	-39.48	-34.92	-37.56	-14.98

* Negative ΔG_r values < -20 kJ/mol cannot sustain microbial life


substrate concentration
C_∞
C_r
C_{int}
$C_{int} = C_{BEQ}$
$\frac{\partial C}{\partial r} \propto ME_{cell}$
O
(R – m)
r
– radial distance (r) →

Maximum Energy Acquisition Rate for the Limiting Substrate

(moles·cell^{-1}·sec^{-1})

$$EAR_{max} = 4 \pi D r C$$

where:

D is the diffusion coefficient for the limiting substrate

r is the radius of the microbial cell

C is the concentration of the limiting substrate in the water

Maximum Acquisition Rate for Limiting Substrate

In Situ Concentrations

Limiting Substrate	MZ1 (mol cell^{-1} sec^{-1})	MZ3 (mol cell^{-1} sec^{-1})	15U (mol cell^{-1} sec^{-1})	15M (mol cell^{-1} sec^{-1})	42L (mol cell^{-1} sec^{-1})	42U (mol cell^{-1} sec^{-1})
Acetate	6.15×10^{-15}	2.58×10^{-15}	5.74×10^{-15}	3.40×10^{-15}	3.32×10^{-15}	5.24×10^{-15}
H_2	1.68×10^{-15}	1.70×10^{-17}	8.69×10^{-16}	4.91×10^{-16}	8.98×10^{-17}	1.33×10^{-15}

Maximum Utilization Rates for the Limiting Substrate
In Situ Concentrations

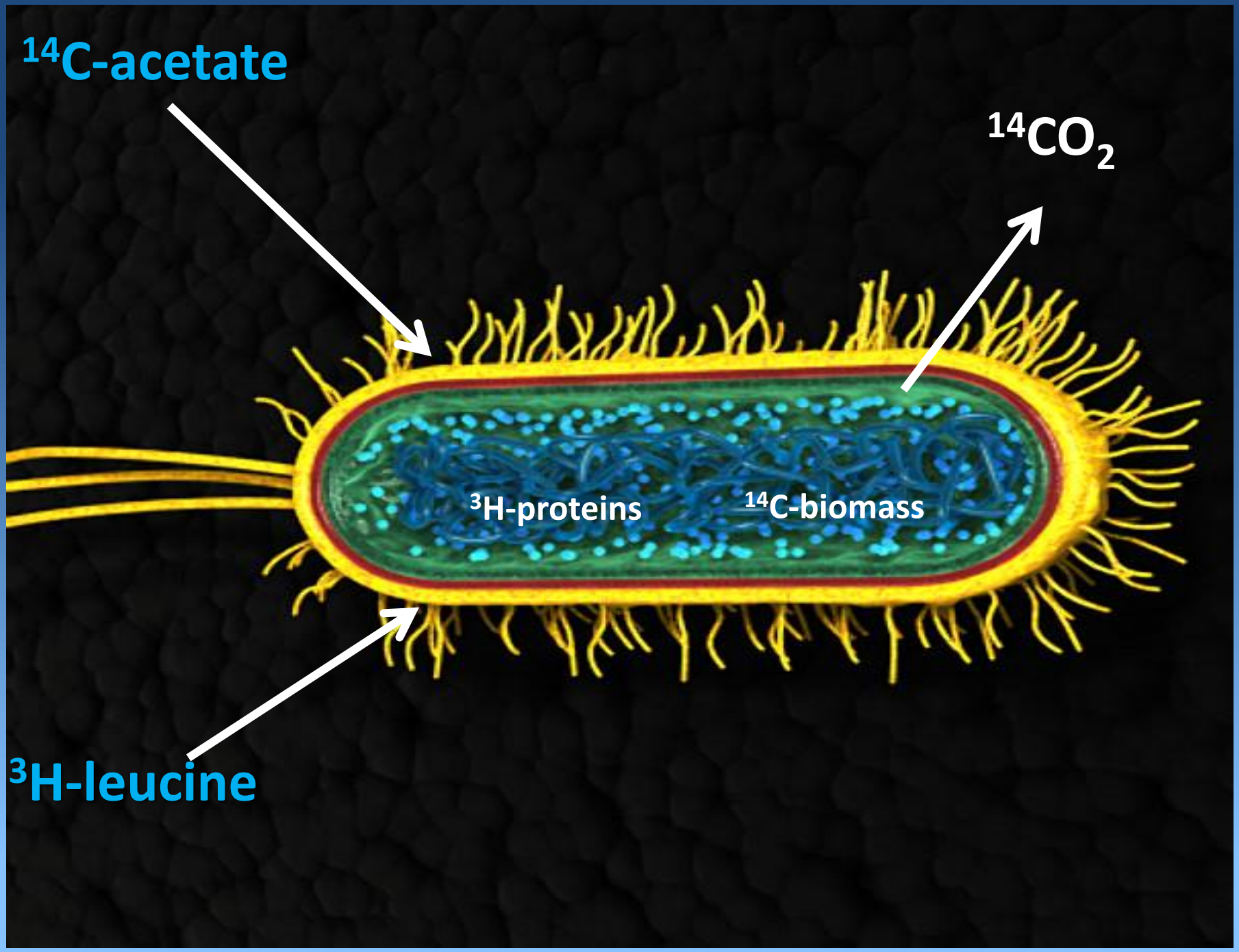
Limiting Substrate	MZ1 (mol L^{-1} d^{-1})	MZ3 (mol L^{-1} d^{-1})	15U (mol L^{-1} d^{-1})	15M (mol L^{-1} d^{-1})	42L (mol L^{-1} d^{-1})	42U (mol L^{-1} d^{-1})
Acetate	2.66×10^{-2}	1.12×10^{-2}	2.48×10^{-2}	1.47×10^{-2}	1.43×10^{-2}	2.27×10^{-2}
H_2	7.26×10^{-3}	7.36×10^{-5}	3.75×10^{-3}	2.12×10^{-3}	3.88×10^{-4}	5.74×10^{-3}

* Cell concentration: 5×10^{7} cells/L

Application of Maximum Energy Acquisition and Flux Rate Data

Acetate-sulfate reduction $CH_3COO^- + SO_4^{2-} \rightarrow 2HCO_3^- + HS^-$

*Knowing the concentration and acquisition rate for the limiting substrate, which is acetate in this example, the rates and concentrations at which the products (bicarbonate and hydrogen sulfide) are produced can be used in reactive transport models, thereby adding a microbial component to the model. These types of data would be useful for estimating the production of geochemical constituents in the stored water that may be detrimental to well production (e.g., clogging) and water quality (e.g., high concentrations of hydrogen sulfide and highly reduced and aggressive water).


^{14}C-acetate
$^{14}CO_2$
^{3}H-proteins
^{14}C-biomass
^{3}H-leucine

Measured vs. Geochemical Models for Substrate Utilization Rates

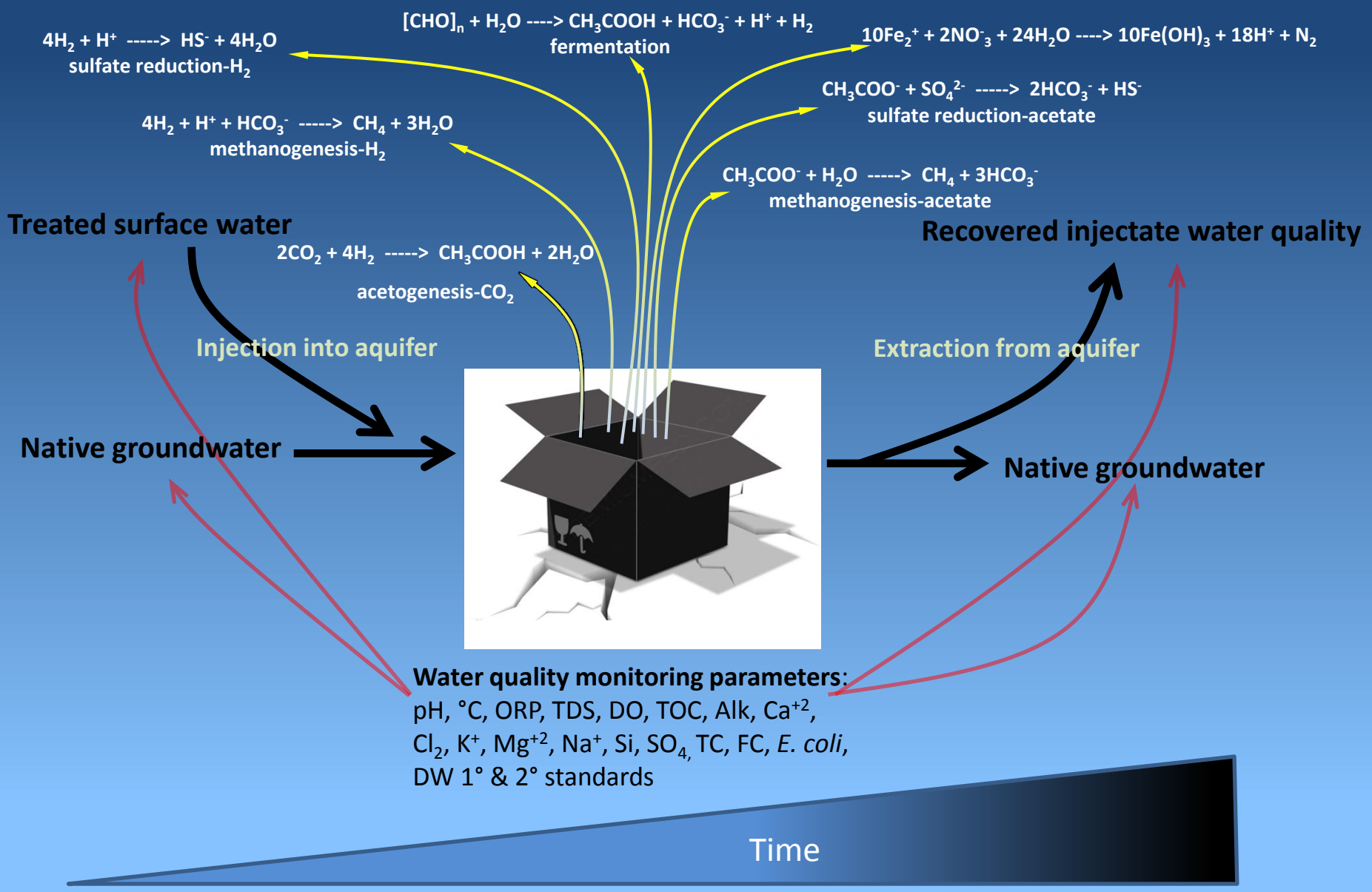
In Situ Concentrations of Acetate

Well	Measured [1] ^{14}C-acetate CO_2 + Biomass Utilization Rate *(moles/day)*	Geochemical [1] Acetate Maximum Utilization Rate [2] *(moles/day)*	Geochemical : Measured Utilization Rate Ratio
42U	2.08×10^{-7}	0.0227	1.1×10^{5}
42L	1.68×10^{-7}	0.0143	8.5×10^{4}
15U	2.89×10^{-7}	0.0248	8.6×10^{4}
15M	1.70×10^{-7}	0.0147	8.7×10^{4}
MZ1	1.66×10^{-7}	0.0266	1.6×10^{5}
MZ3	2.92×10^{-7}	0.0112	3.8×10^{4}

[1] Total community = 5×10^{7} cells/L

[2] Assumed 100% of bacterial community is active and utilizing acetate at a BGE of 100%

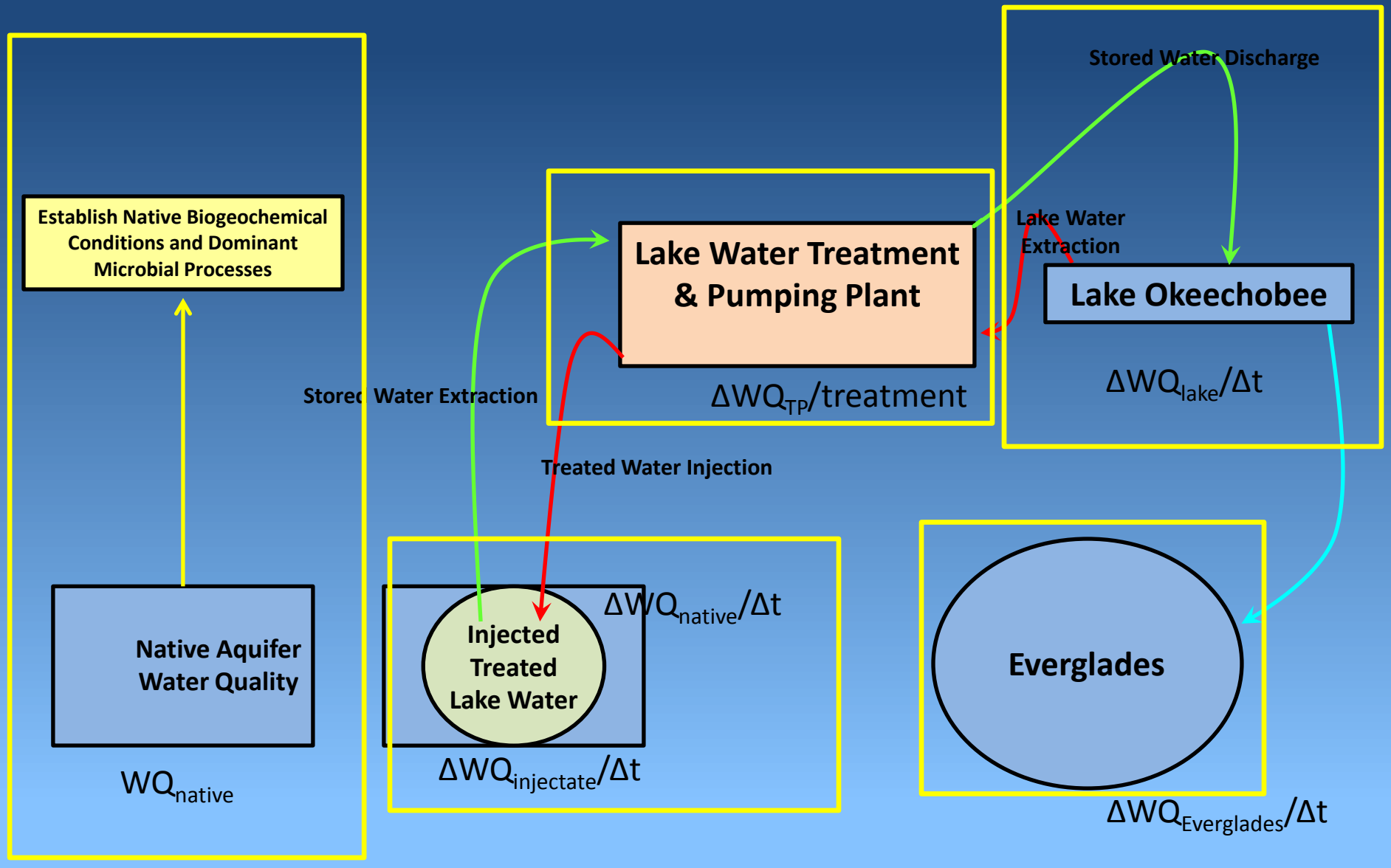
Monitoring the Water Quality of Native Ground Waters and Injected & Recovered Surface Waters



Summary

- The geochemistry of ground waters from the UFA are significantly different on a local to regional scale.
- Multiple microbial processes that influence the geochemistry are present in the native UFA and will drive this ecosystem's response to recharge events.
  - Using thermodynamic and microbial ecology principles more reliable biogeochemical models of changes in the quality of recharge water during storage can be achieved (e.g., reactive transport models).

Managing Groundwater Resources as Functional Ecosystems: A Biogeochemical Approach



Data Gaps

- There are no comparable data sets as described for this study for:
  - Lake source water
  - Recharge water prior to injection
  - Recovered water
  - The areas of Lake Okeechobee that receive the recovered water.

- The contribution of subsurface biofilm communities to biogeochemical processes that influence the chemical and microbiological quality of the stored waters.
  - Positive and/or negative influence?

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