Microbial Ecology of Anaerobic Biosystems Treating Mining Influenced Waters

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Outline

- Anaerobic biozones
- Assessment approach
- Microbial ecology results
- Summary

Anaerobic reactions

sulfate removal

sulfide production

formation of MeS

Fe, Cu, Cd, Zn, Cd, Pb, Hg, Ni

formation of As_2S_s,Sb_2S_3, MoS_2,

increase pH

formation of $Me(OH)_3$

Al, Fe

Anaerobic reactions (con't)

increase alkalinity

formation of $MeCO_3$

Zn, Mn, Fe

reduce redox active metals

As, Cr, Se, Mo, Sb, U, Hg

transform nitrogen

nitrate, nitrite, cyanide

sorption/co-precipitation

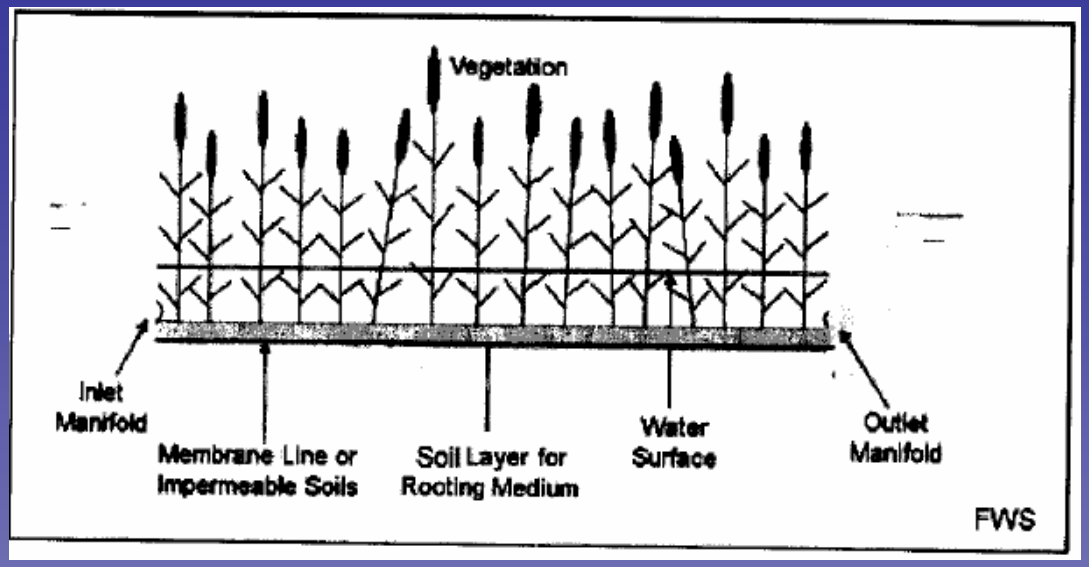
Fe, Cu, Cd, Zn, Mn, Cd, Pb, Hg, Ni

Anaerobic biozones

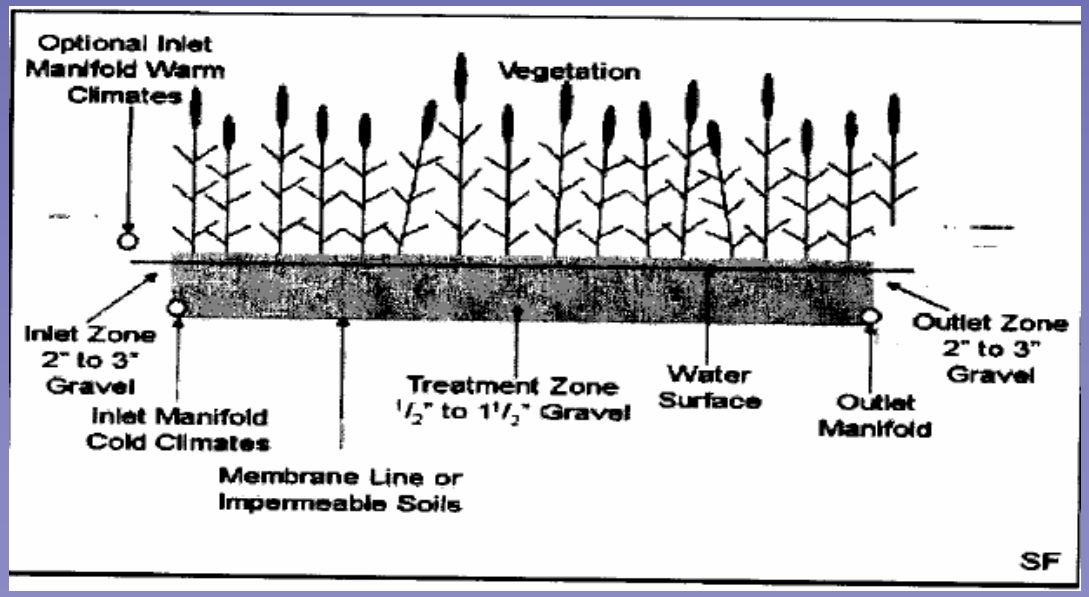
- Wetlands
- Sulfate reducing bioreactors
  - Compost
  - Compost free
- Permeable reactive barriers
- Permeable reactive zones

Wetlands

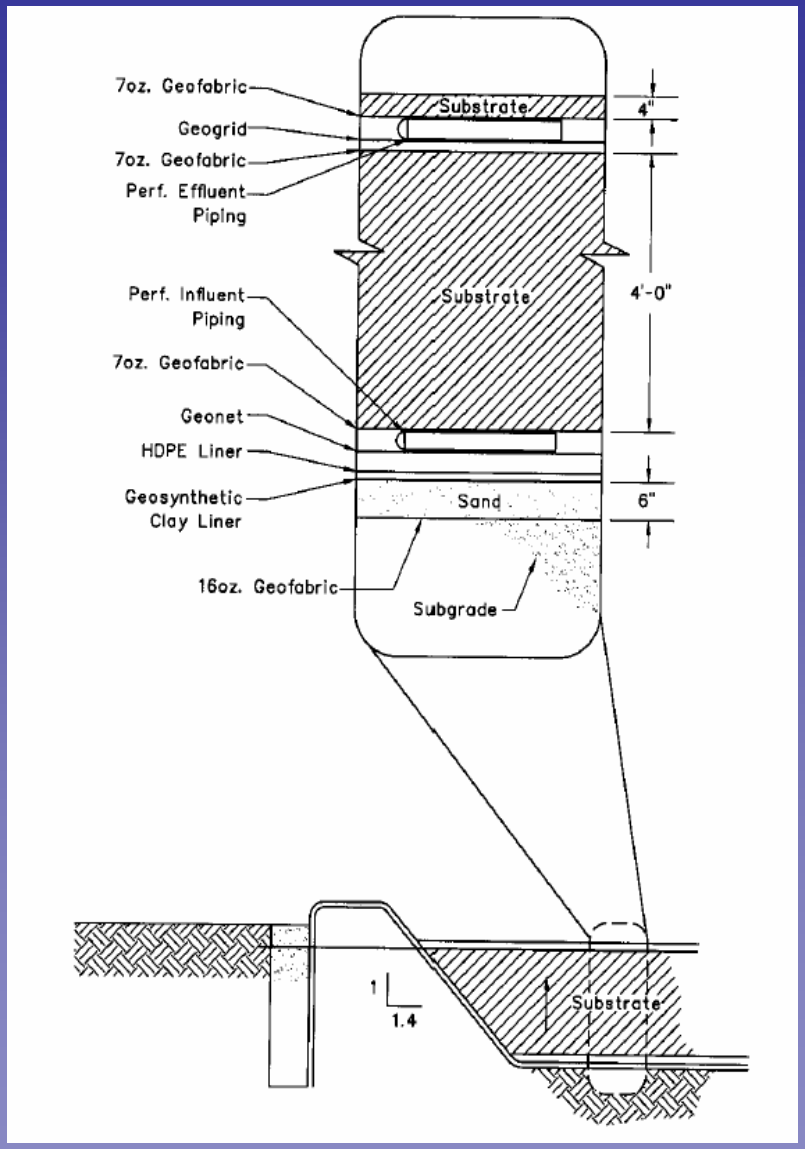
Free water surface



Subsurface flow



Passive bioreactor constructed at Burleigh Tunnel in Silver Plume, Colorado



EPA/540/R-02/506
Dec 2002

Compost free bioreactor system(THIOPAQ)

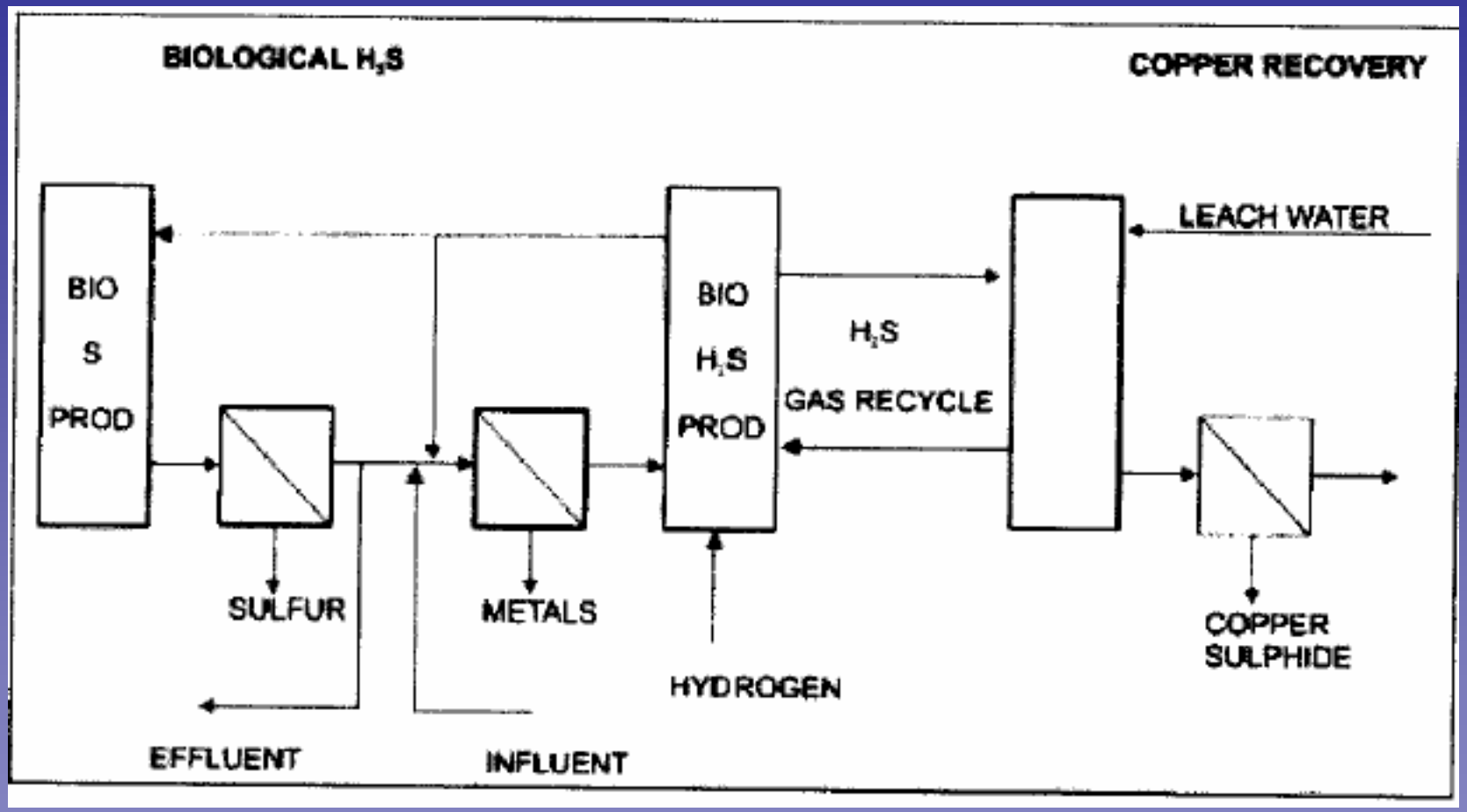
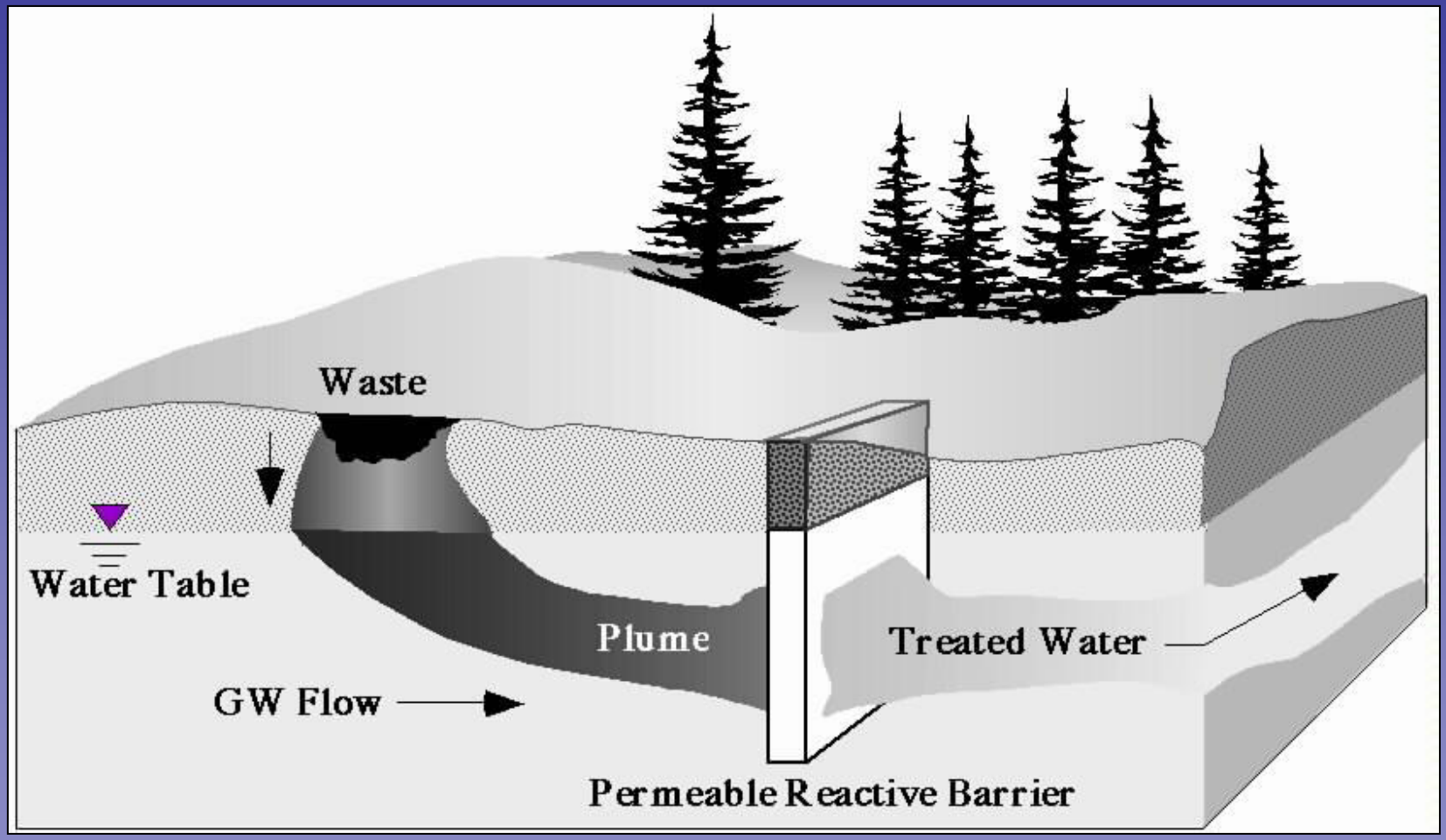


Figure 3-18: Simplified Flow Diagram of the Demonstration Plant at Kennecott

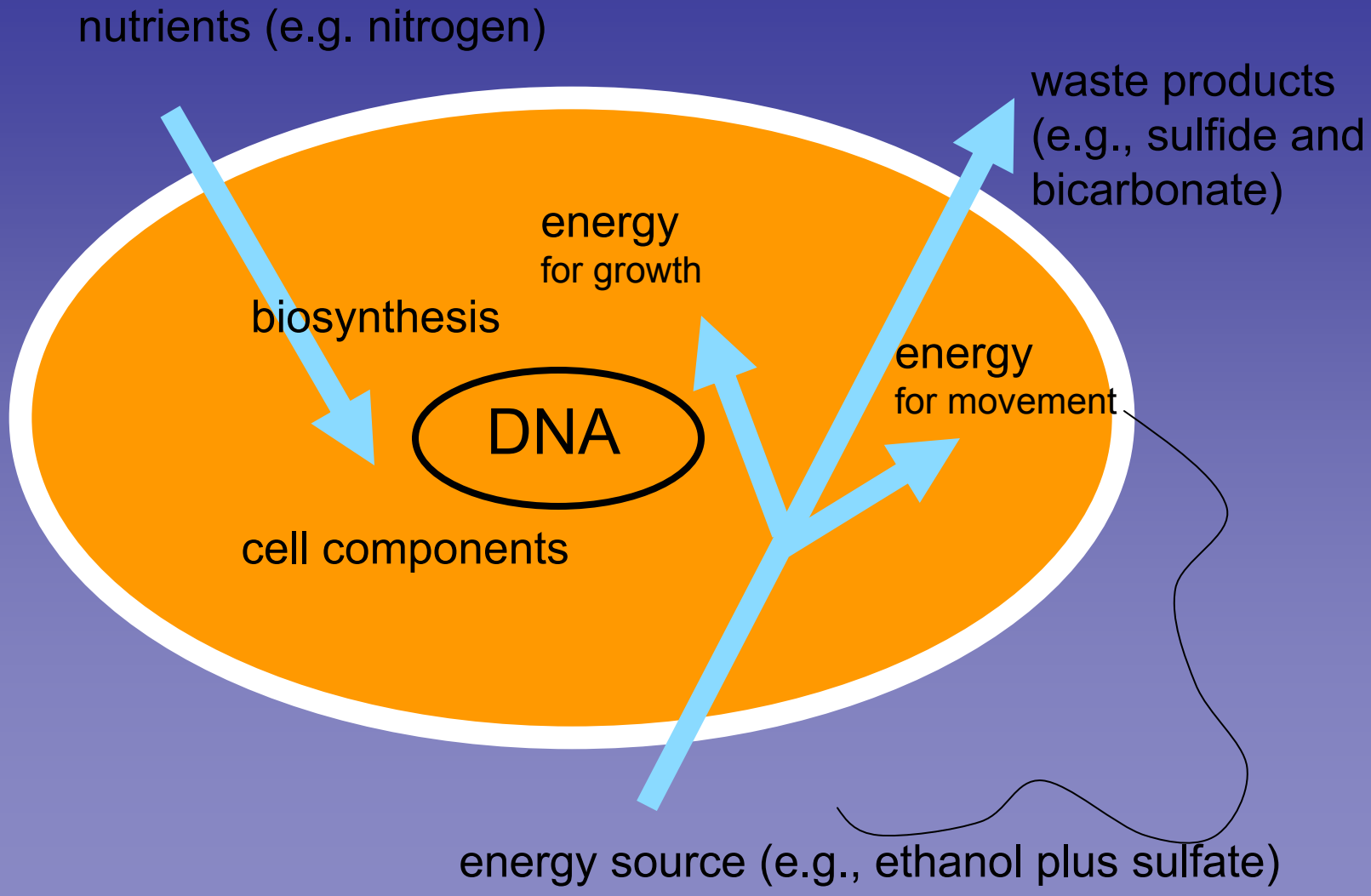
Treatment of Sulphates in Mine Effluents, INAP, Oct 2003

Permeable reactive barriers

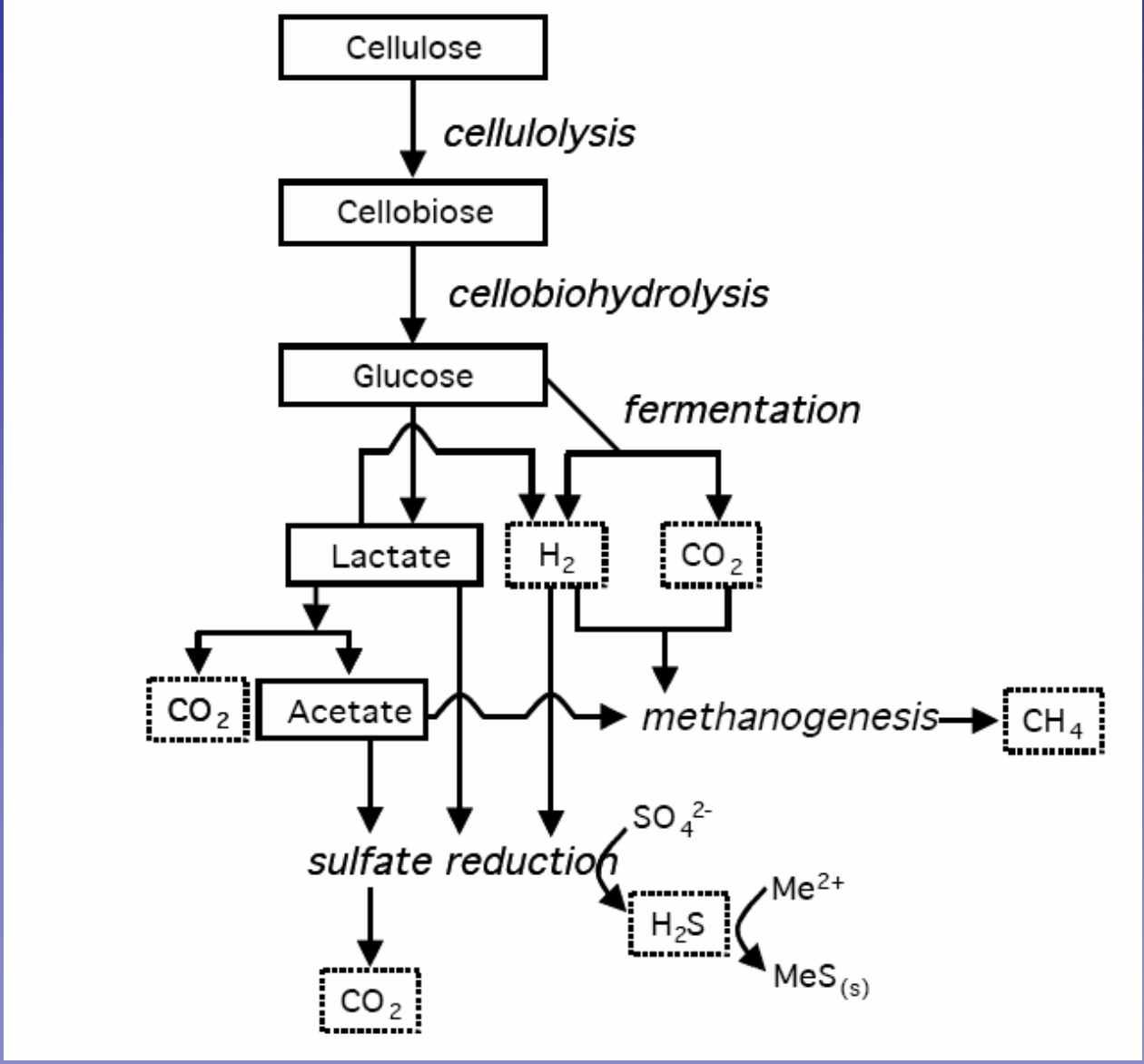


(www.powellassociates.com/sciserv/3dflow.html)

Microbial processes



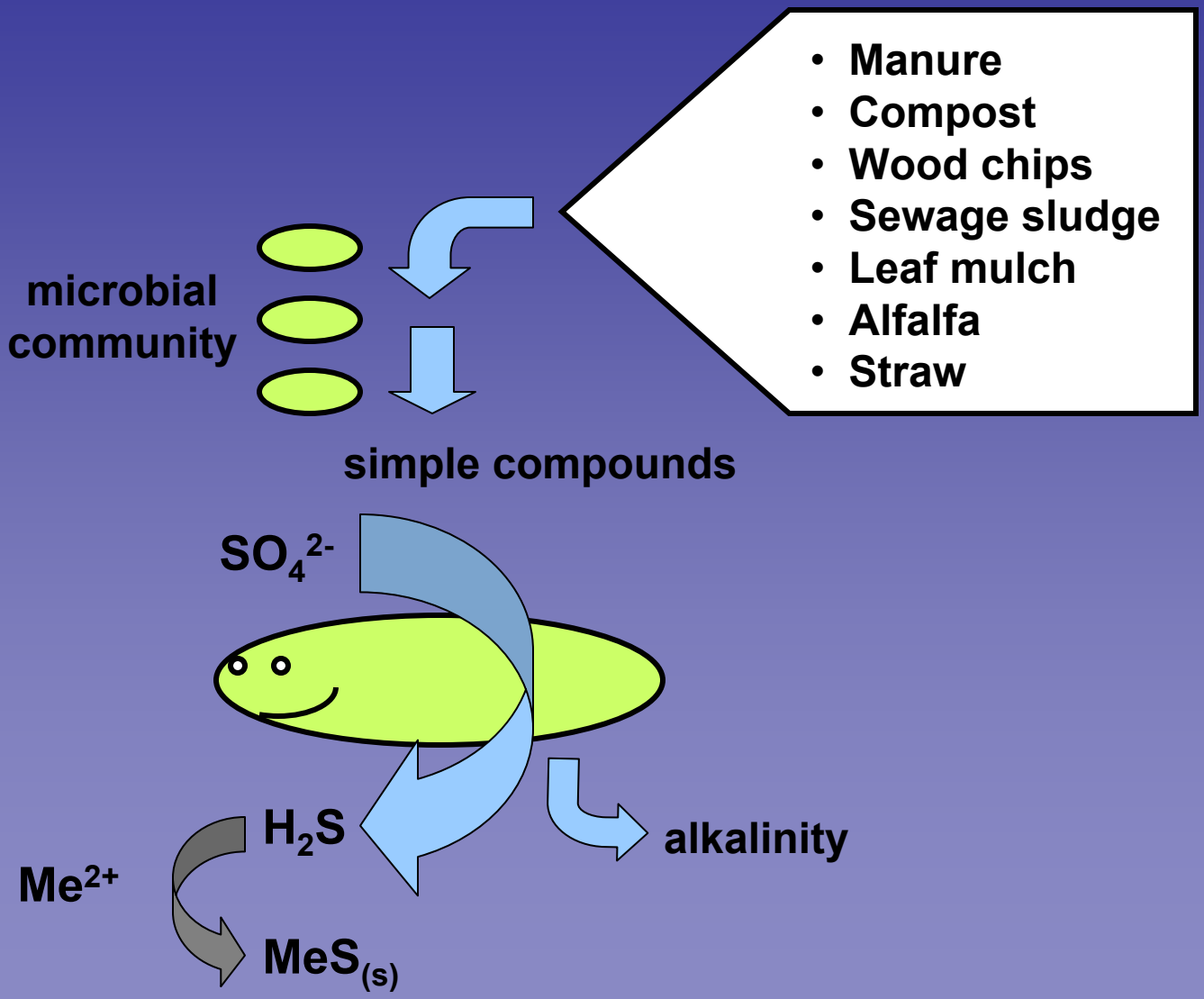
Microbial ecology model



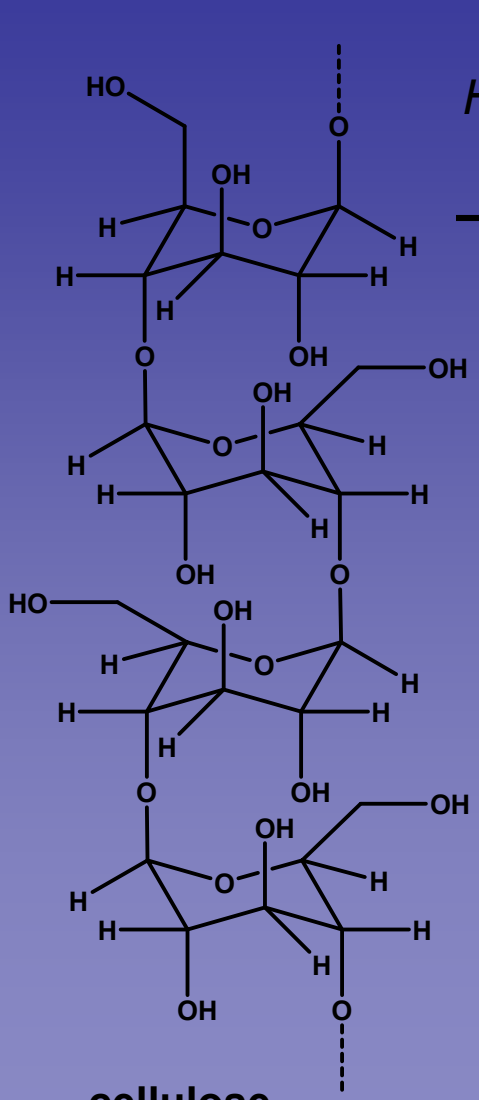
Tools for assessment

- Measurement of reactants and product
  - Solution phase
  - Solid phase
  - Gas phase
- Selective plating
- Analysis of DNA
  - ribosomes
    - broad screening
    - diversity
  - functional genes

Assessment of SRBRs (Sulfate-Reducing Bioreactors)

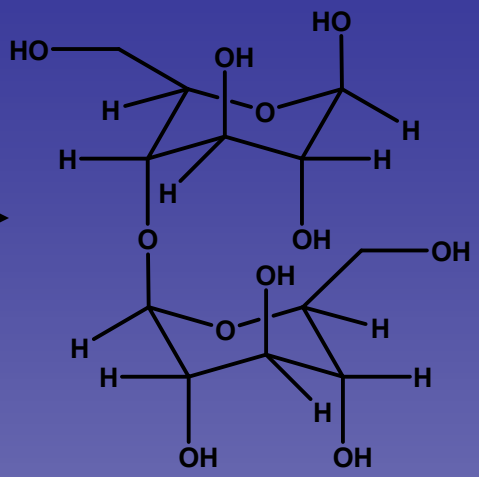


Microbial ecology of SRBRs



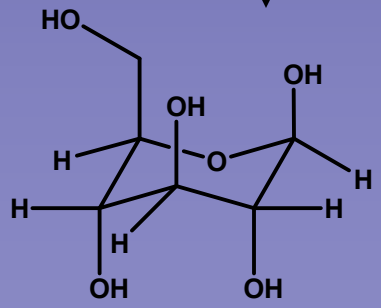
cellulose

Hydrolytic Bacteria



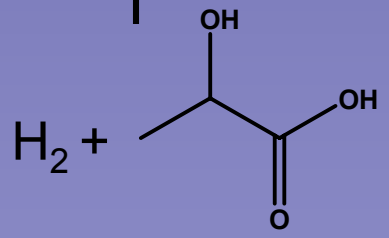
cellobiose

Hydrolytic Bacteria



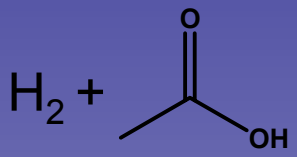
glucose

Fermenters



small organic acids (lactate)

Sulfate Reducers, Fermenters



smaller organic acids (acetate)

Methanogens, Sulfate Reducers, Fermenters

CO_2, CH_4

Column and Batch Experiments

50 mg/L Mn^{2+}

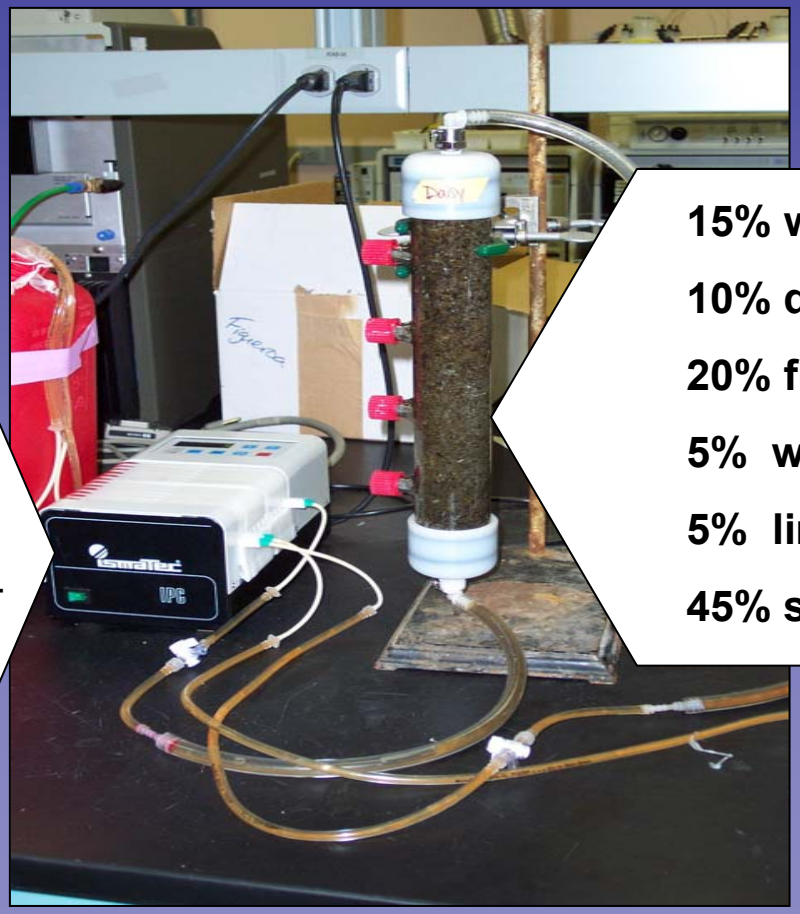
50 mg/L Zn^{2+}

(50 mg/L Ni^{2+})

1000 mg/L SO_4^{2-}

pH 6

30 ml/d



15% walnut wood

10% dried alfalfa

20% fresh dairy manure

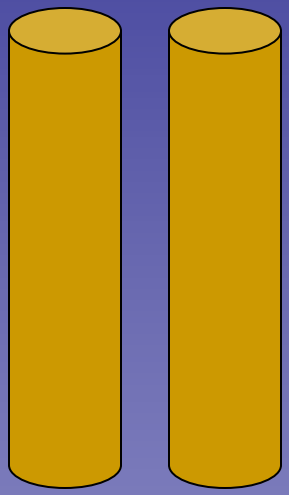
5% wetland sediment

5% limestone

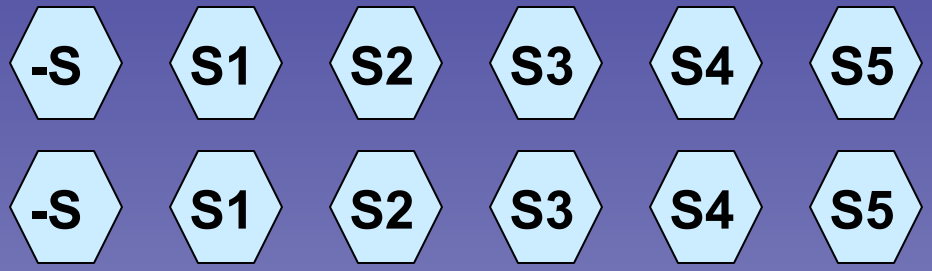
45% silica sand

Experimental Design

Columns



Batch Studies

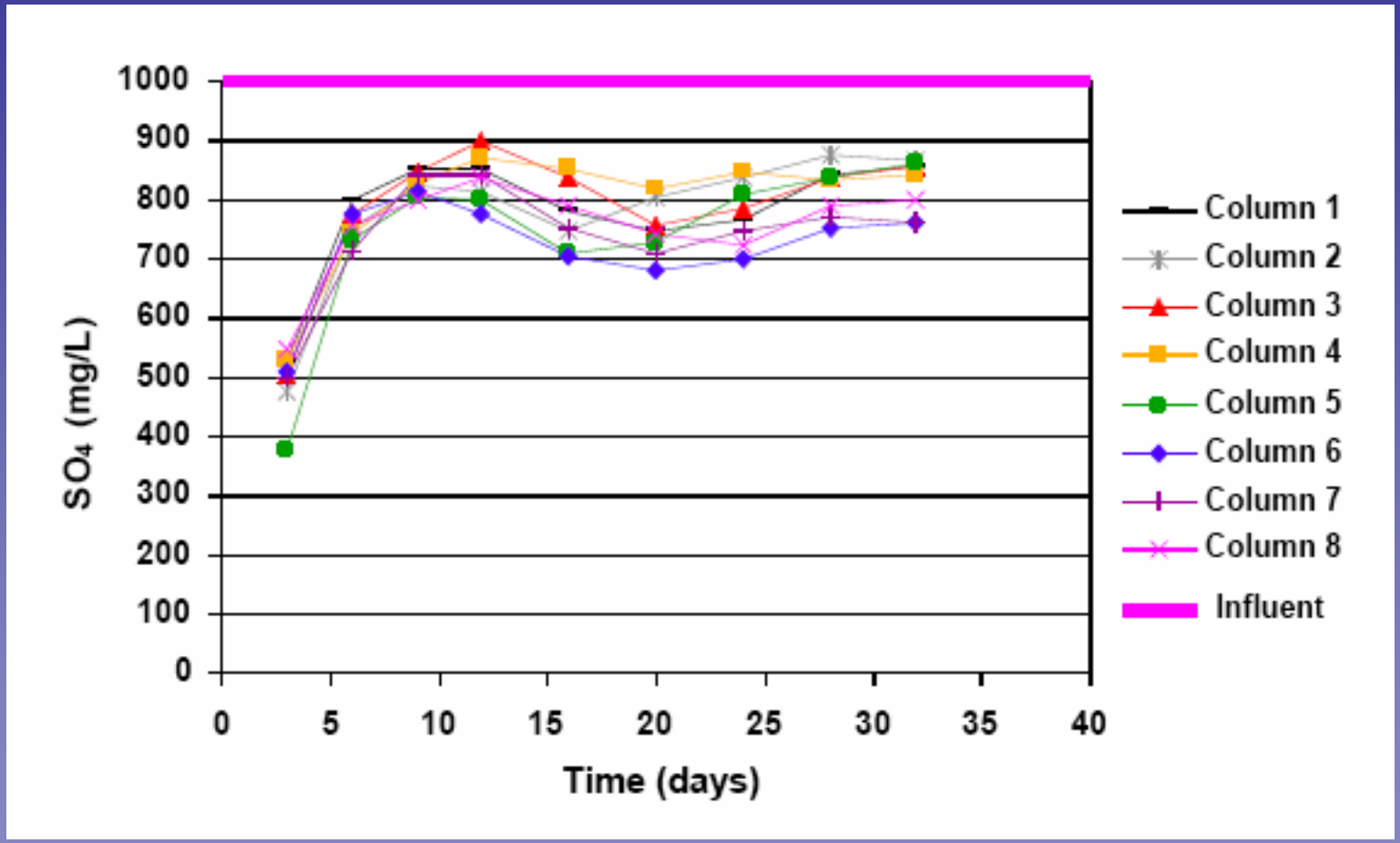


- SO_4^{2-} (IC)
- Mn^{2+}, Zn^{2+} (ICP-AES)
- pH (probe & meter)
- Alkalinity (HACH titration)

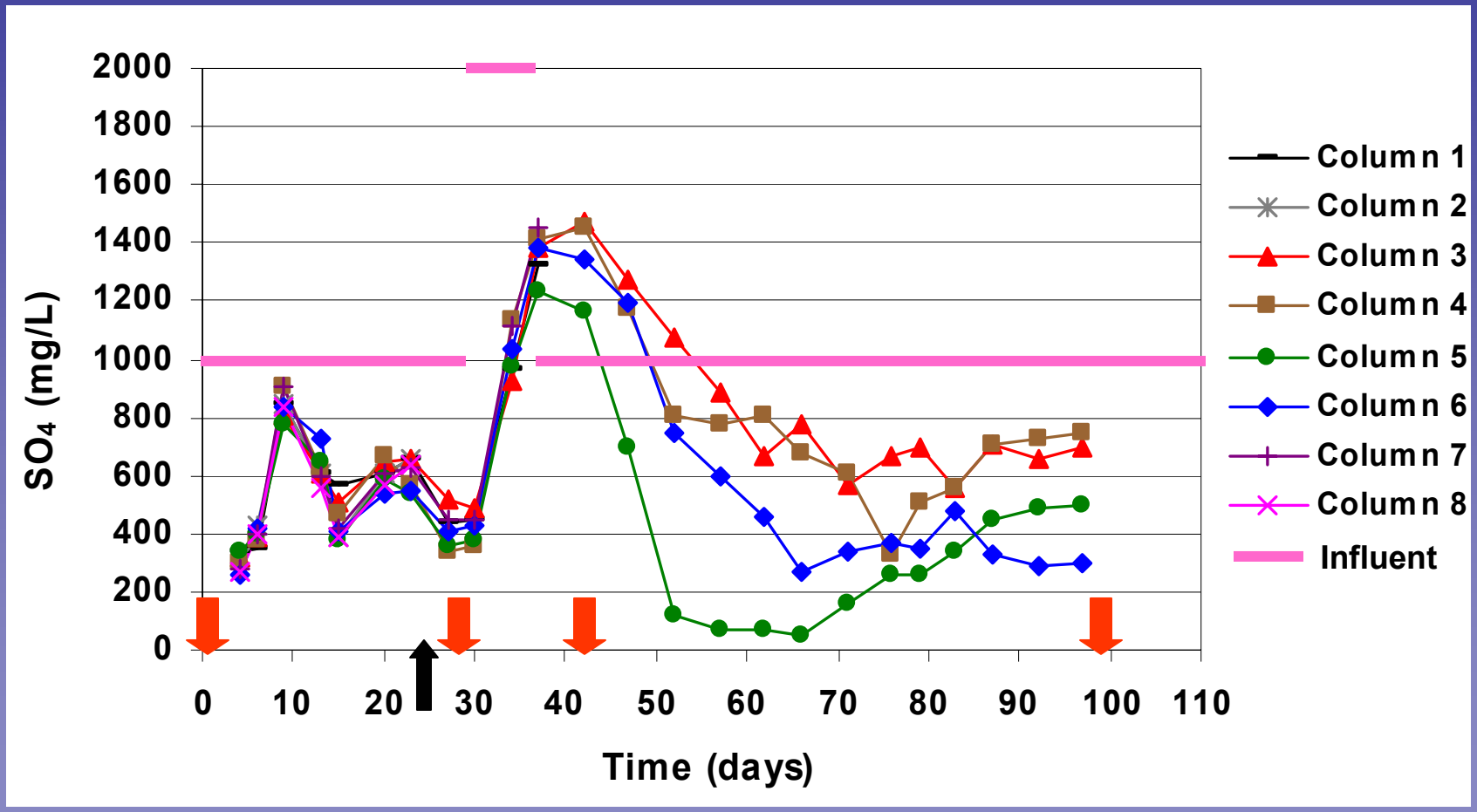
- SO_4^{2-}
- Mn^{2+}, Zn^{2+}, Ni^{2+}
- pH

- CO_2 (Micro GC)
- H_2S
- CH_4
- O_2
- H_2

Sulfate removal with influent Ni^{2+}

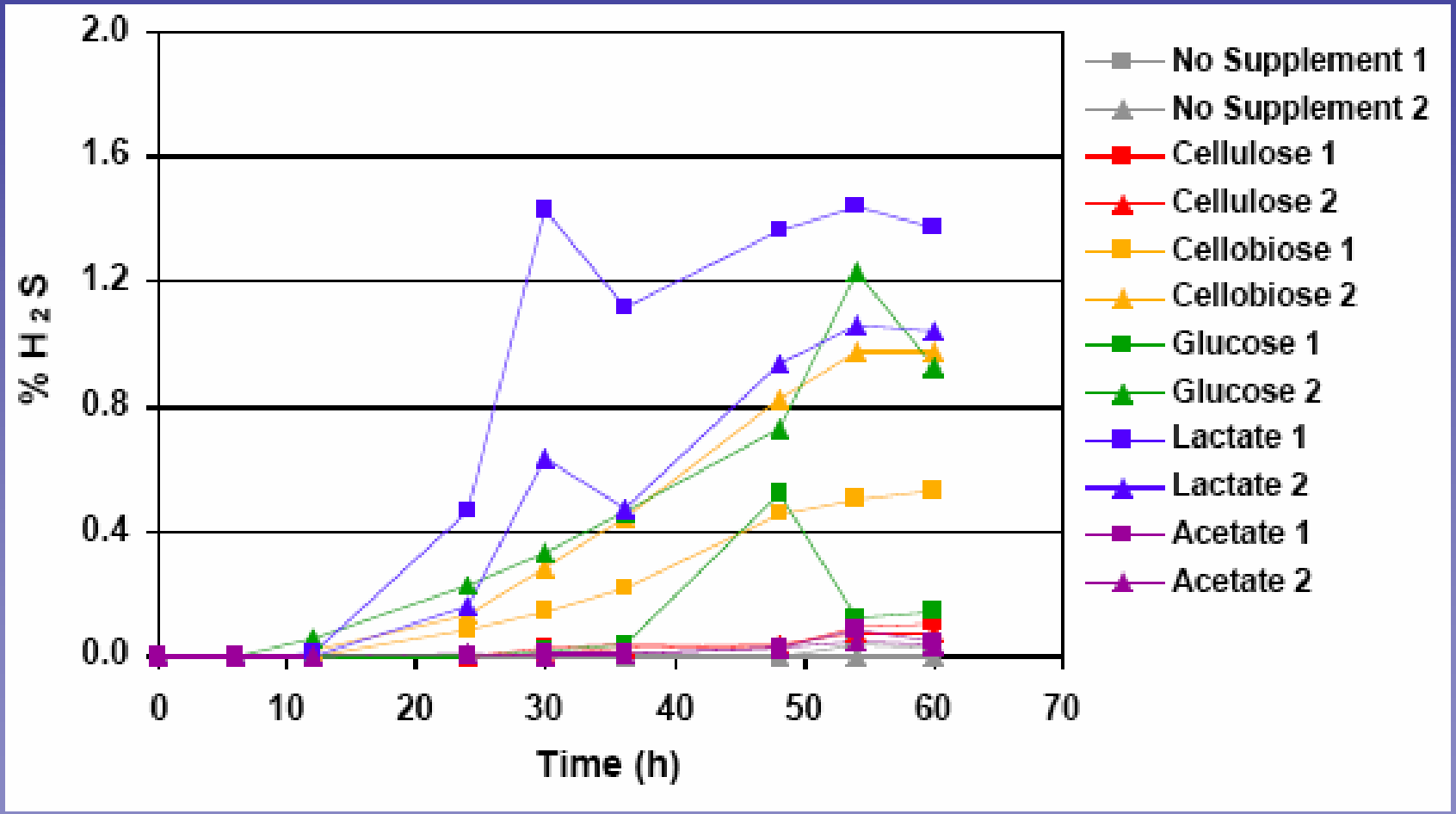


Sulfate removal w/o influent Ni^{2+}



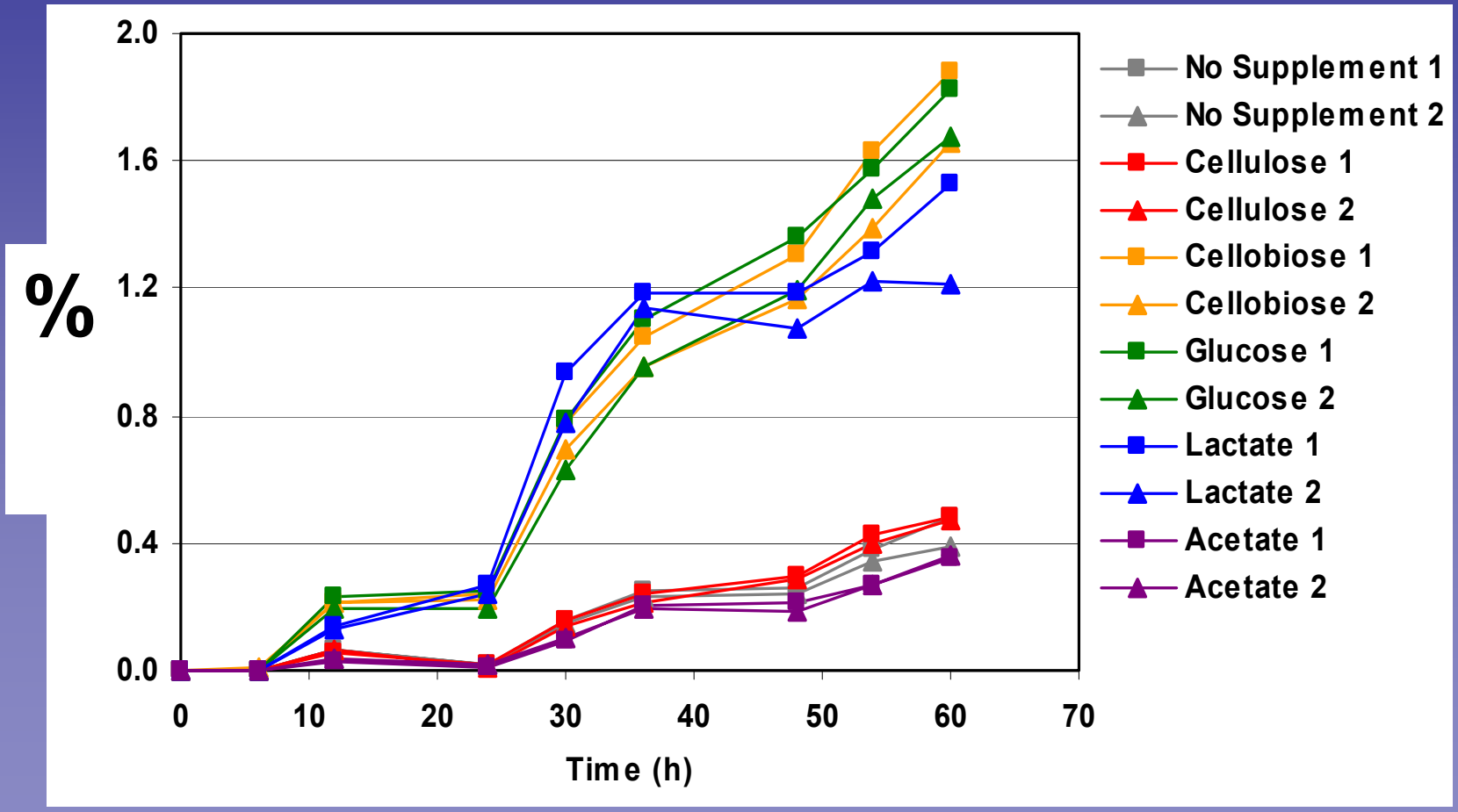
H_2S comparison

set 1 w/ Ni, day 38

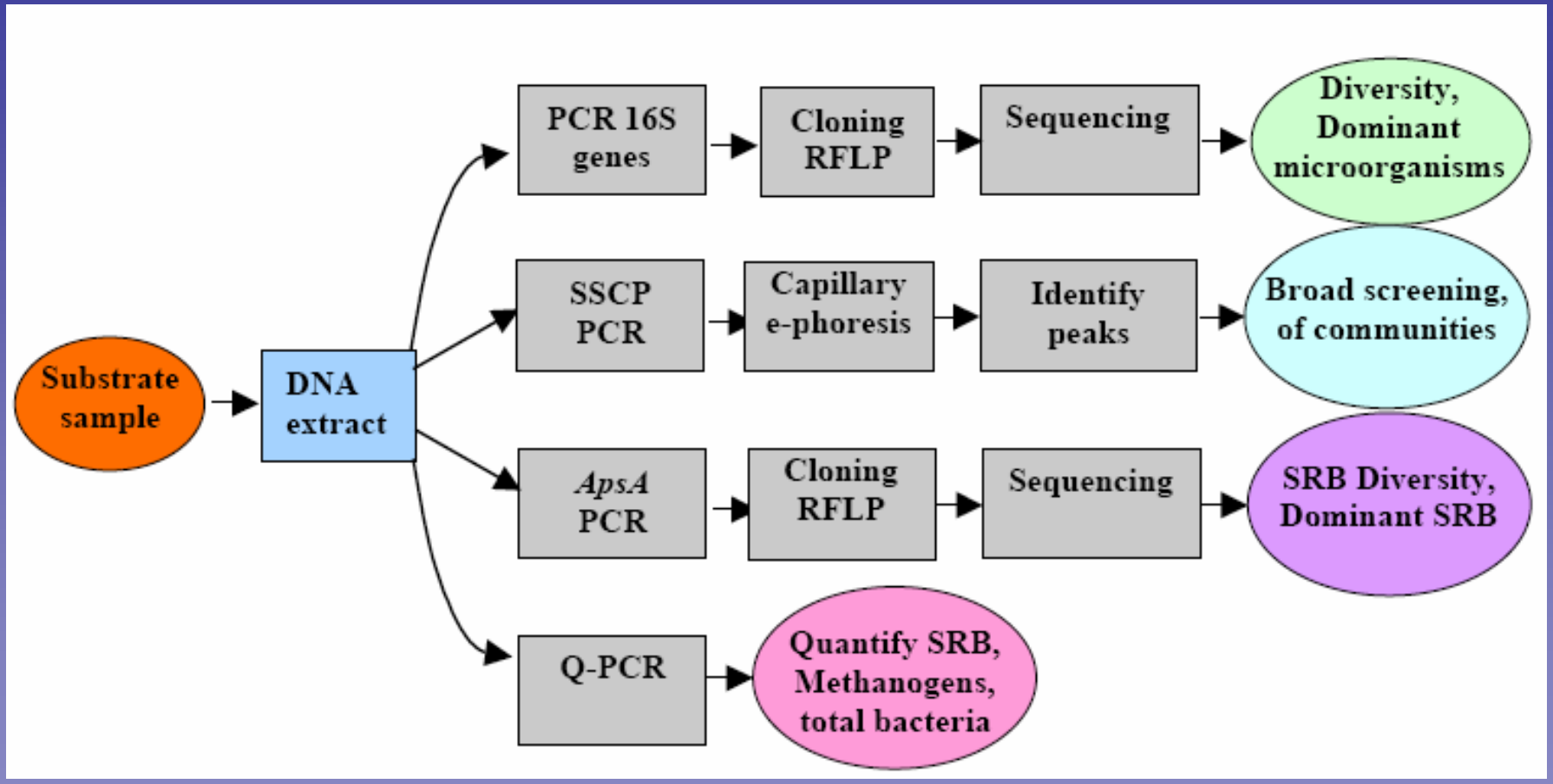


H_2S comparison

set 2 w/o Ni, day 41



Flow-diagram overview of DNA analysis
(developed by Pruden)



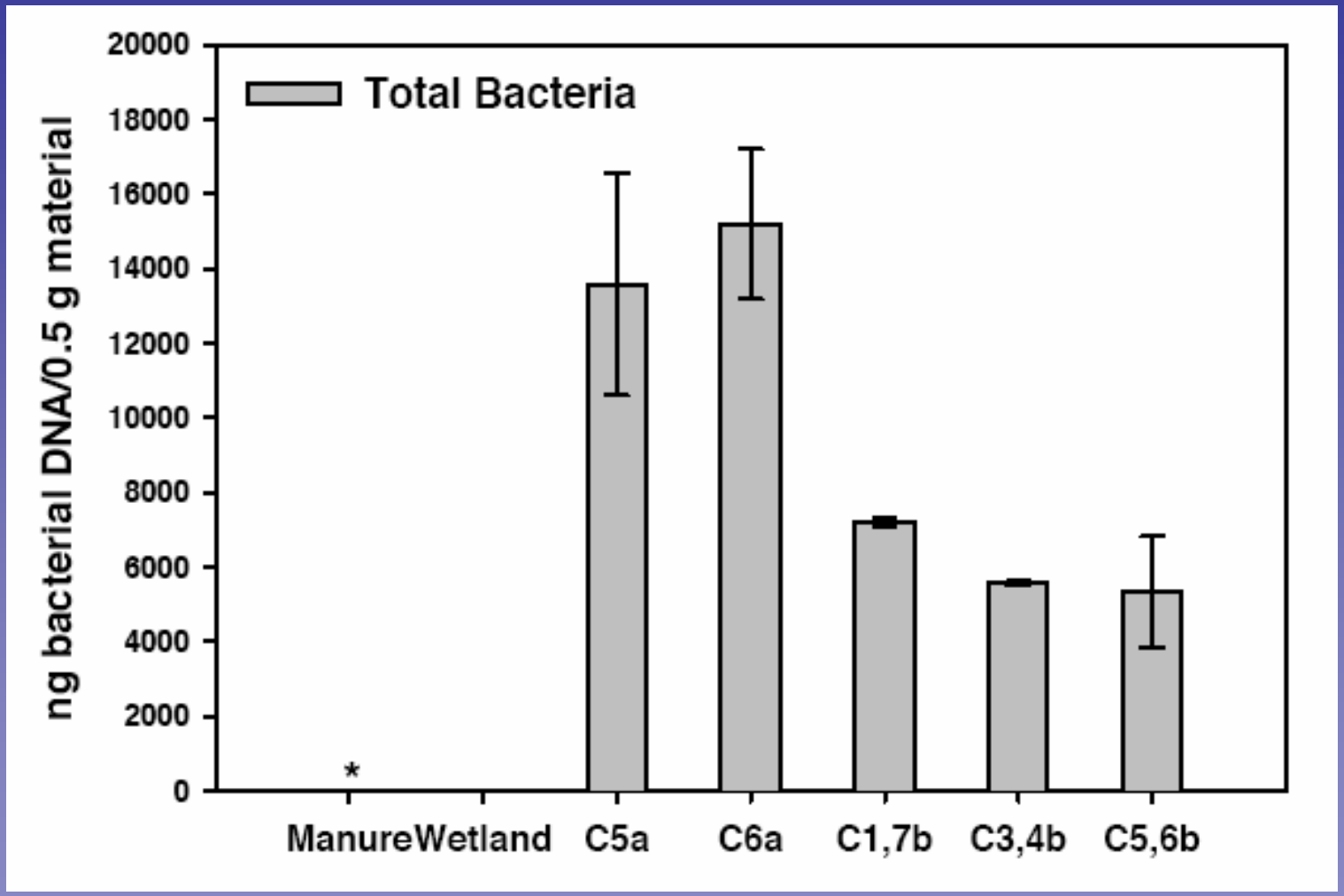
Diversity based on ribosomal DNA and broad screening

Bacteria
Bacterioides- (99%) (CFB- Elbe River snow isolate 9_3)
Bacterioides sp. (99%) (Bacteriodetes- Elbe River snow isolate 9_3)
Clostridium sp. (98%) (Firmicutes, Clostridium- UnID anoxic Eubacterium)
Bacterioides sp. (99%) (CFB- uncultured bacterium mle1-2)
Clostridium sp. (93%) (Firmicutes, Clostridium, clone GOUTA13)
Clostridium sp. (96%) (Firmicutes, Clostridium, clone p-2506-18B5)
Clostridium glycolicum (98%) (Firmicutes, Clostridium)
Acetivibrio cellulolyticus (98%) (Firmicutes, Clostridium)

Archae
Methanobrevibacter sp. (Euryarchaeota, Methanobacterium Group)
Methanosarcina (Euryarchaeota, Methanosarcinales Group)
Methanosarcina Euryarchaeota (Euryarchaeota, Methanosarcinales Group)
Methanobrevibacter sp. (Euryarchaeota, Methanobacterium Group)

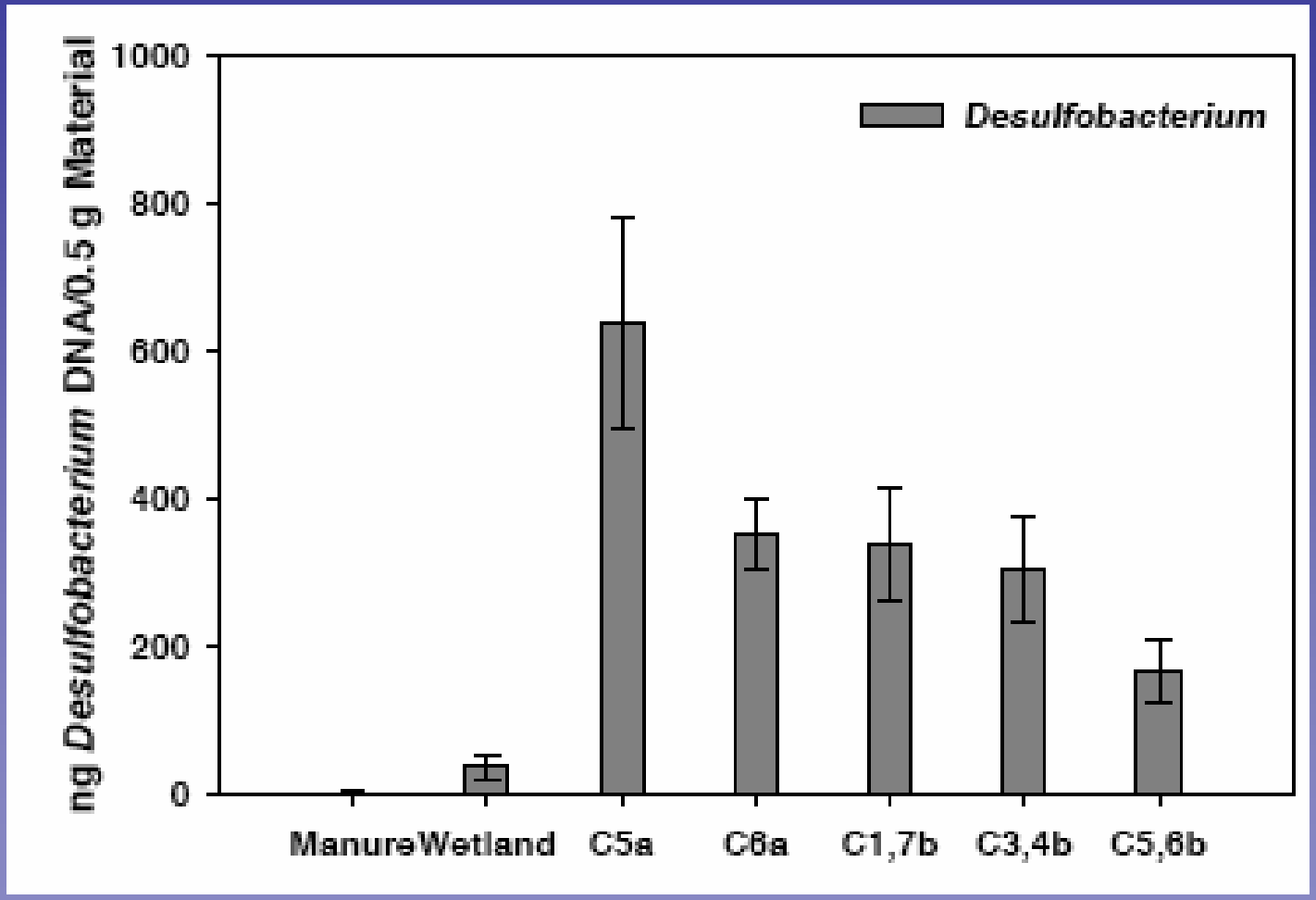
Pruden et al. 2005

Results for total DNA



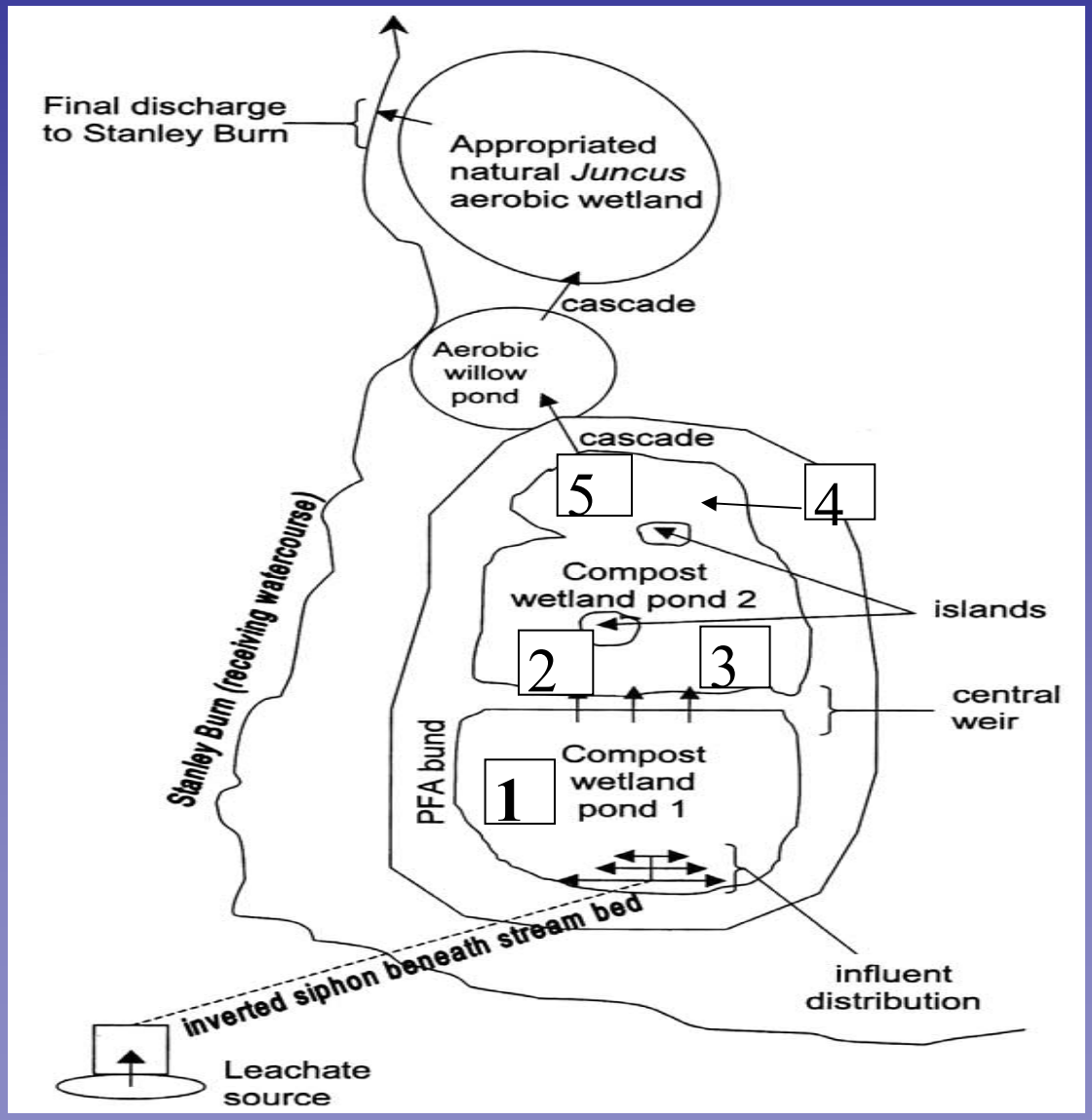
Pruden et al. 2005

Results for SRB functional gene



Pruden et al. 2005

Quaking House wetland



Hallberg and Johnson 2005

Dominant Microbial Community Members In Quaking House Compost Wetland

Iron-reducing neutrophiles (*Geobacter* and *Shewanella* spp.)

Methylotrophic bacteria, e.g., methane oxidizers

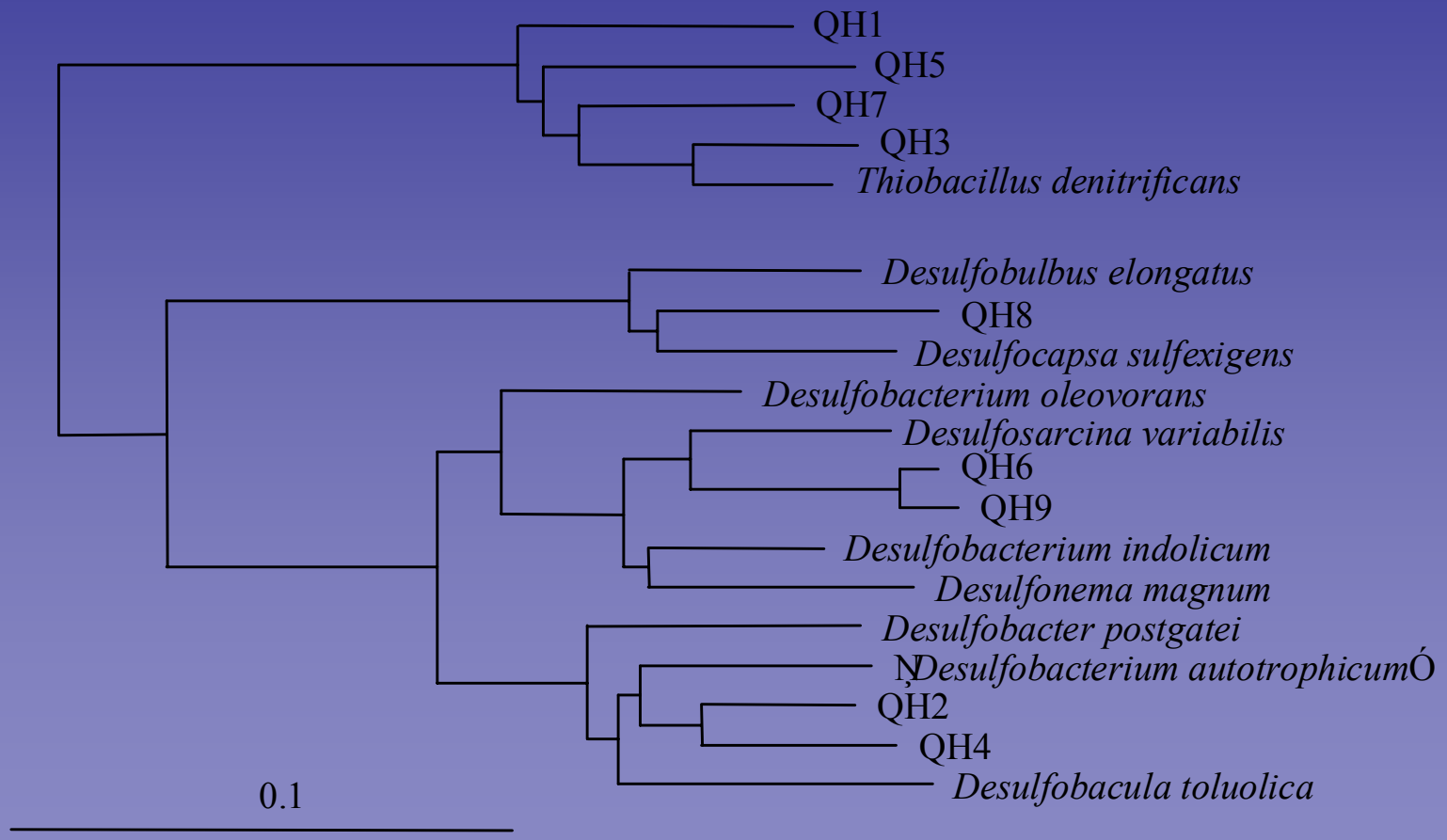
Sulfur-oxidizers such as *Achromatium* spp

Cellulolytic microbes of the genus *Clostridium*

Sulfate Reducing Bacteria of the δ-*Proteobacteria* division

Hallberg and Johnson 2005

Sulfate reducers
In Quaking House Compost Wetland



Hallberg and Johnson 2005

Archea
In Quaking House Compost Wetland

Archea

Dominant populations related to microbes found in sulfide containing waters and anaerobic digesters

Methanogens

Methanoculleus thermophilus (96% similarity)
Methanosarcina thermophila (98% similarity).

Hallberg and Johnson 2005

Summary

Compost based sulfate reducing bioreactors dominated by non-sulfate reducing bacteria

Decline in bioreactor sulfate reduction did not correlate with a decline in sulfate reducer population

Methanogens did not compete with sulfate reducers in bioreactor columns

Glucose utilizers (fermenters and cellulolytic/fermenters) appear to be more sensitive to metal toxicity

Diversity of bacteria is large

Archea less diverse than bacteria

Implications

Sulfate reduction is controlled by cellulose degradation

- Cellulose degradation is rate-limiting step
- Cellulolytic/fermenters more sensitive to metal toxicity
- Availability and sustainability of food source for cellulolytic/fermenters is critical

Microbial communities in anaerobic biozones are diverse

- Competition for substrates required by sulfate reducers may occur

Acknowledgements

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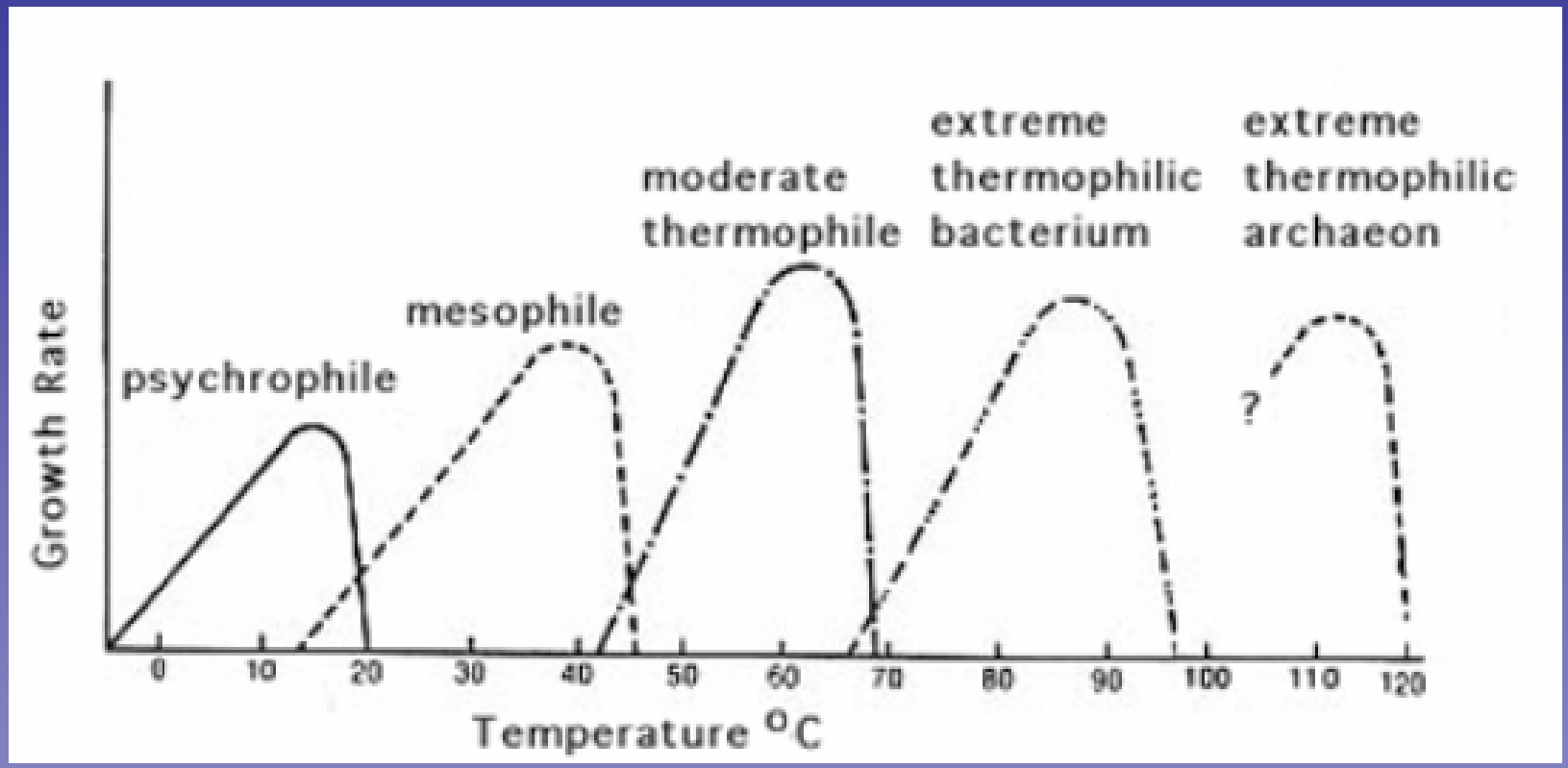
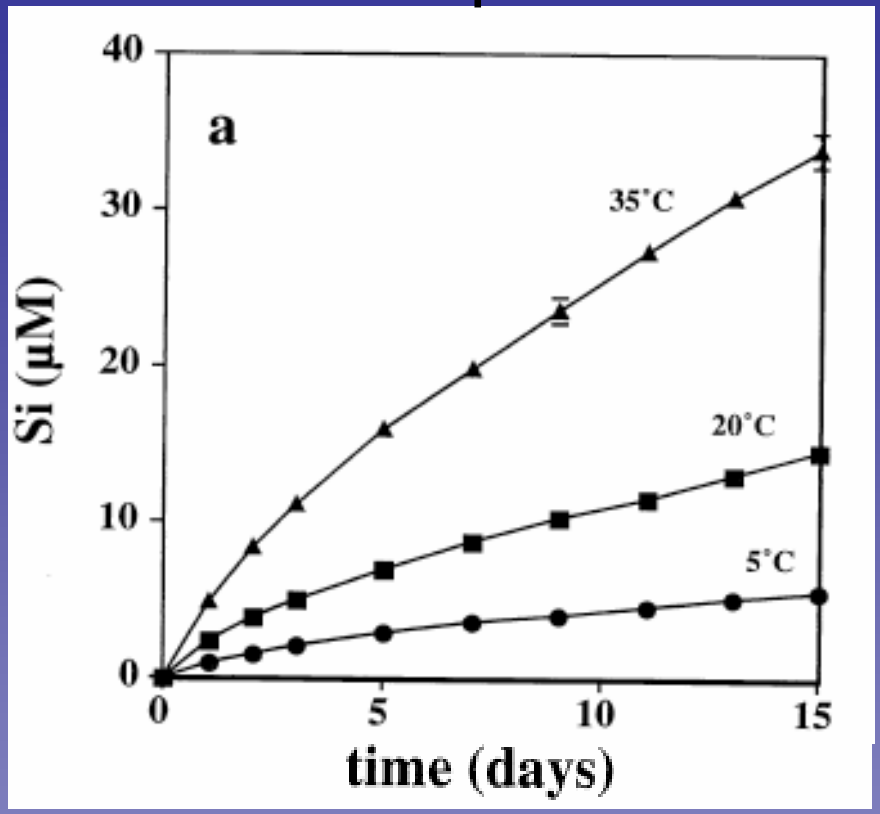
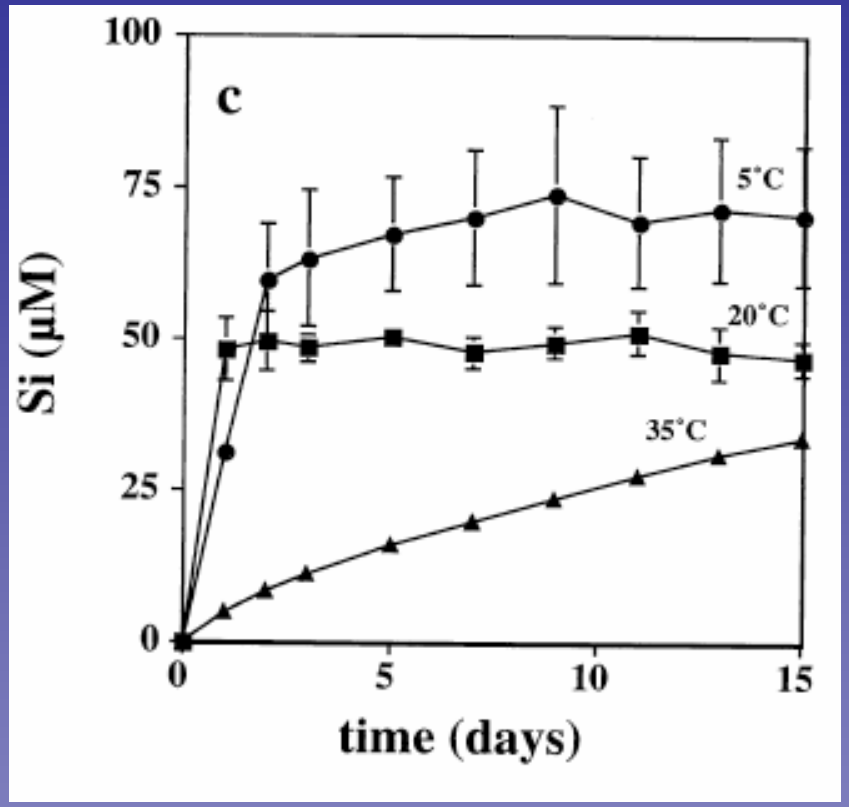
Questions?



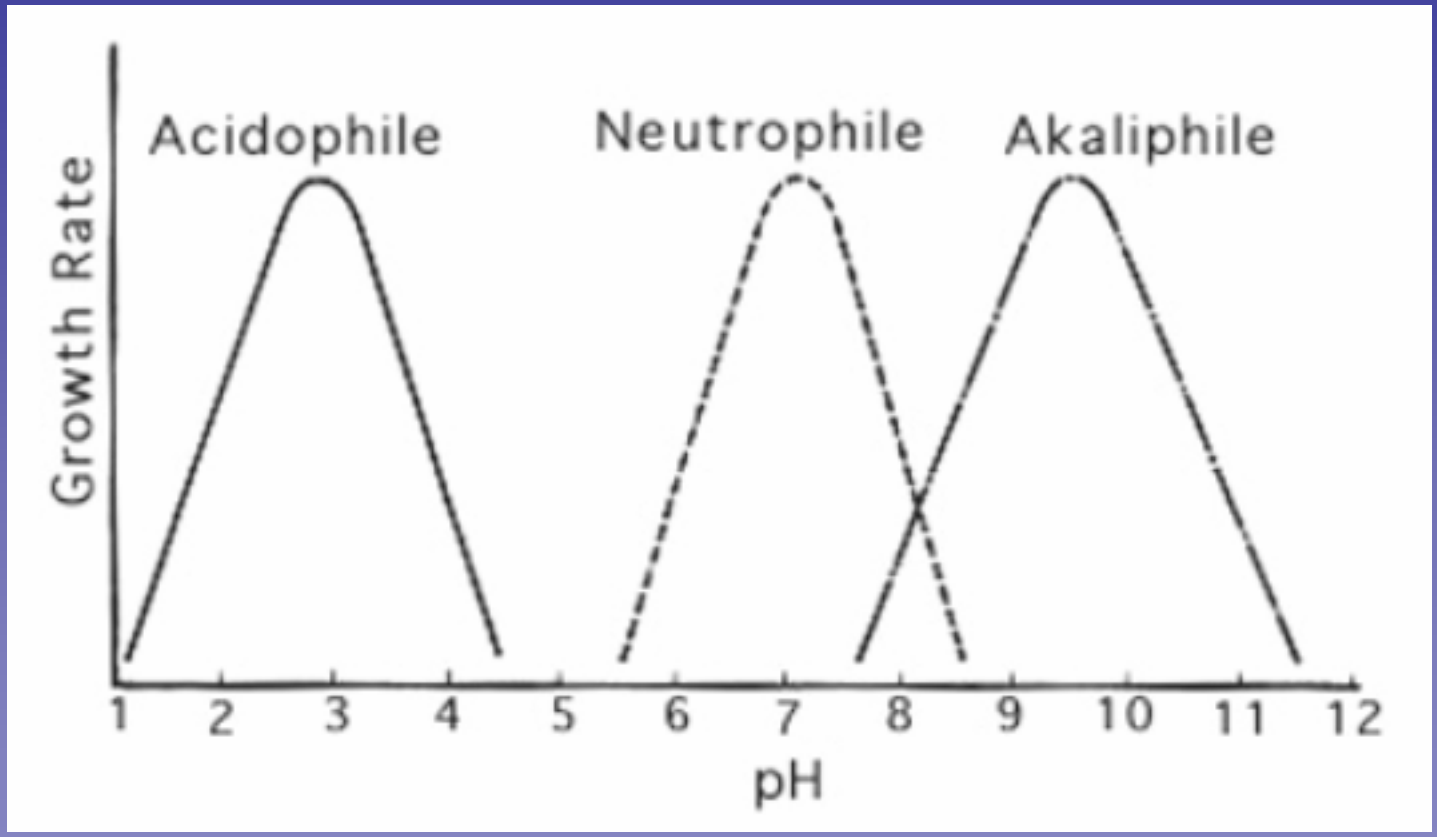
Todar 2004 Online Textbook of Bacteriology

Temperature Effects

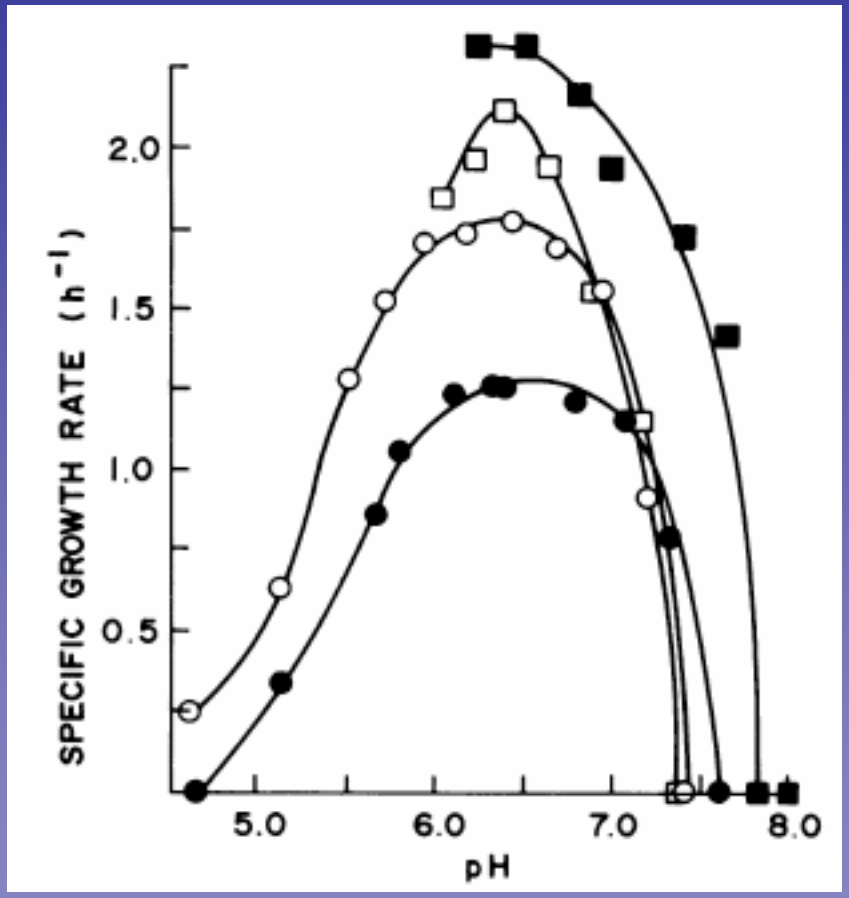


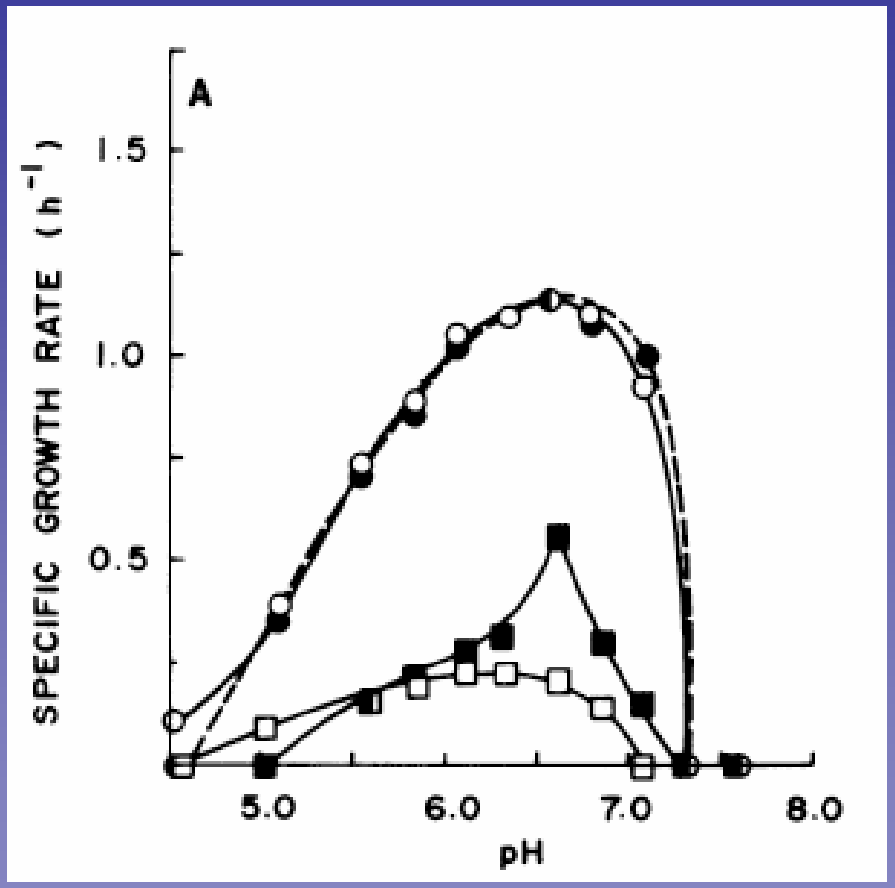


Welch and Ullman 1999
Dissolution of bytownite feldspar


Growth Rate
Acidophile
Neutrophile
Akaliphile
1
2
3
4
5
6
7
8
9
10
11
12
pH

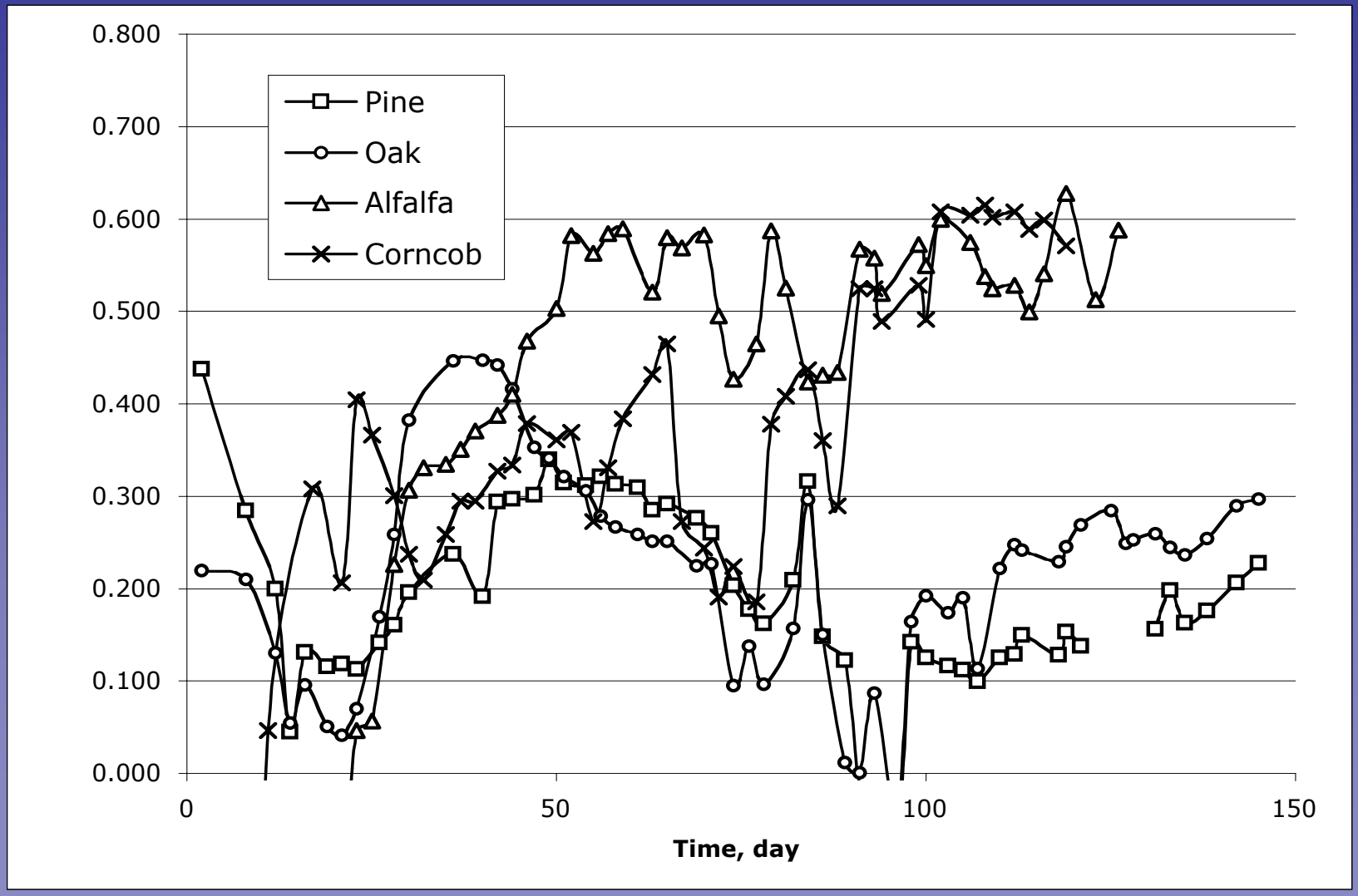
pH Effects





Therion et al. 1982

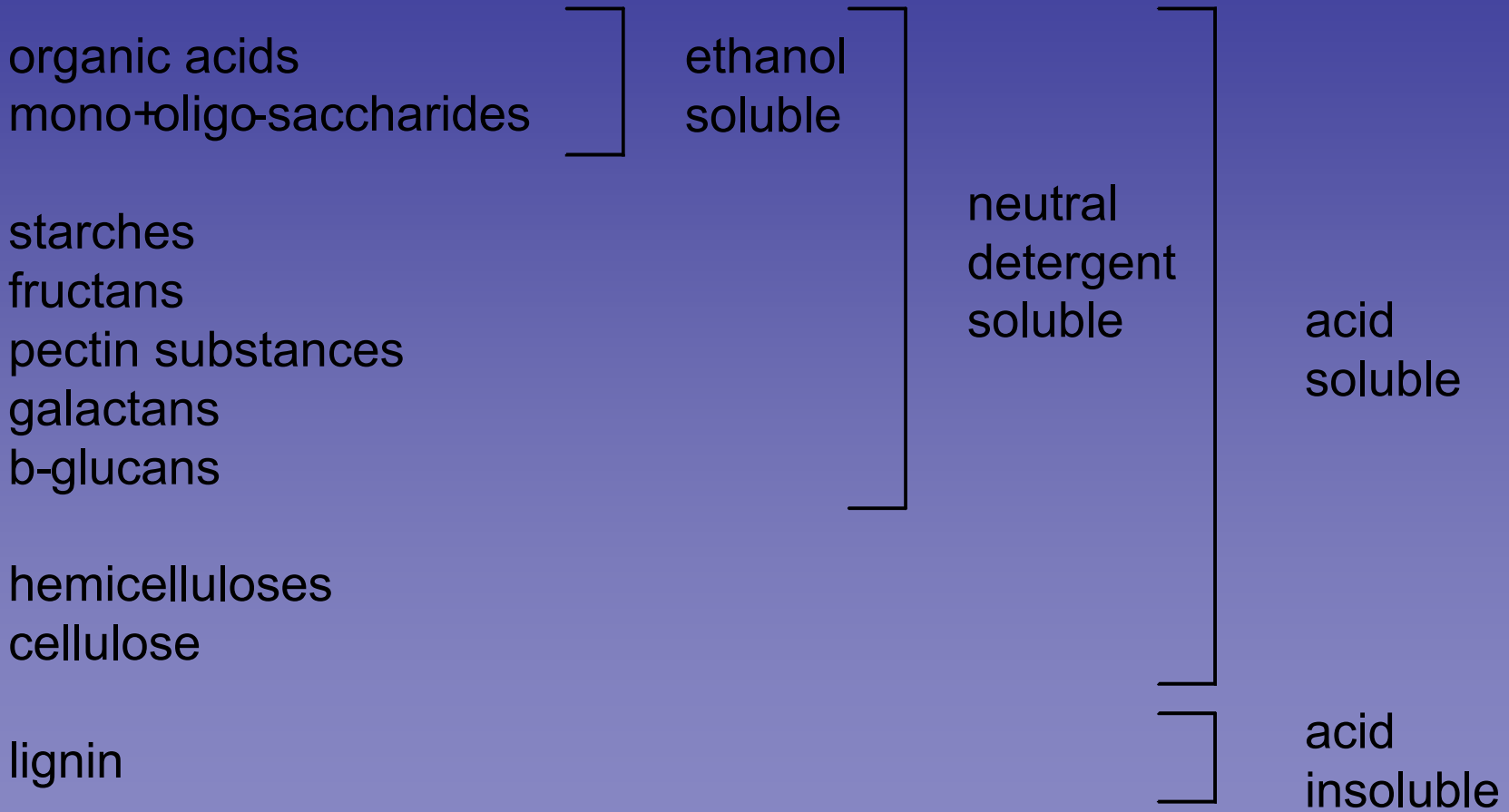
Sulfate reduction rates



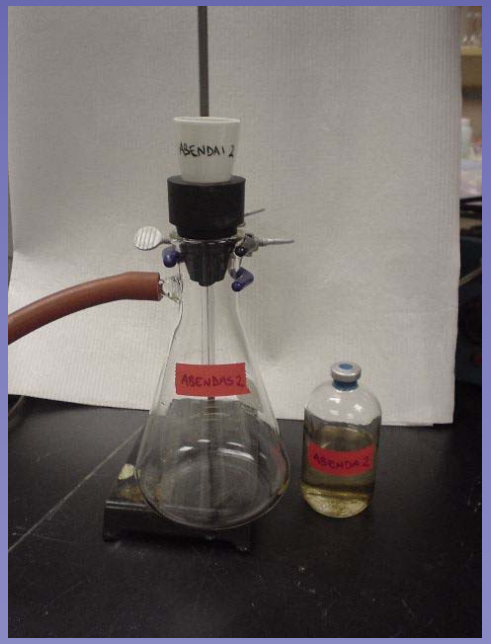
Microbial Activity (substrates)

- Measure organic substrate characteristics and select substrates for column tests
- Relate sulfate removal in columns to organic substrate characteristics
- Measure consumption of substrates with time

Nutritional evaluation



Extraction methods



Nutritional characterization

Sample ID	Organic Acids	Saccha - rides	NDSF & Starch	NDSC	Cellulose	Lignin
Alfalfa	10.9	3.0	33.4	47.3	28.5	8.2
Brew Waste	4.7	11.5	16.4	32.7	38.3	15.5
Beet Pulp	1.8	9.6	39.2	50.6	32.5	3.6
Corncobs	9.2	0.8	15.5	25.5	56.4	11.5
Wetland	3.5	0.0	ND*	3.5	6.0	2.7
Manure	3.5	0.2	0.0	3.7	27.7	5.1
Maple	0.0	0.7	7.2	7.9	68.5	21.2
Oak	3.6	1.6	11.6	16.8	59.7	20.7
Pine	1.2	1.0	9.1	11.4	64.5	22.8
Poplar	0.0	0.4	14.1	14.5	66.2	17.5
Walnut Hulls	0.5	0.5	23.2	24.2	40.8	26.6
Walnut	2.0	1.7	6.8	10.5	67.1	19.9

Column Experiments

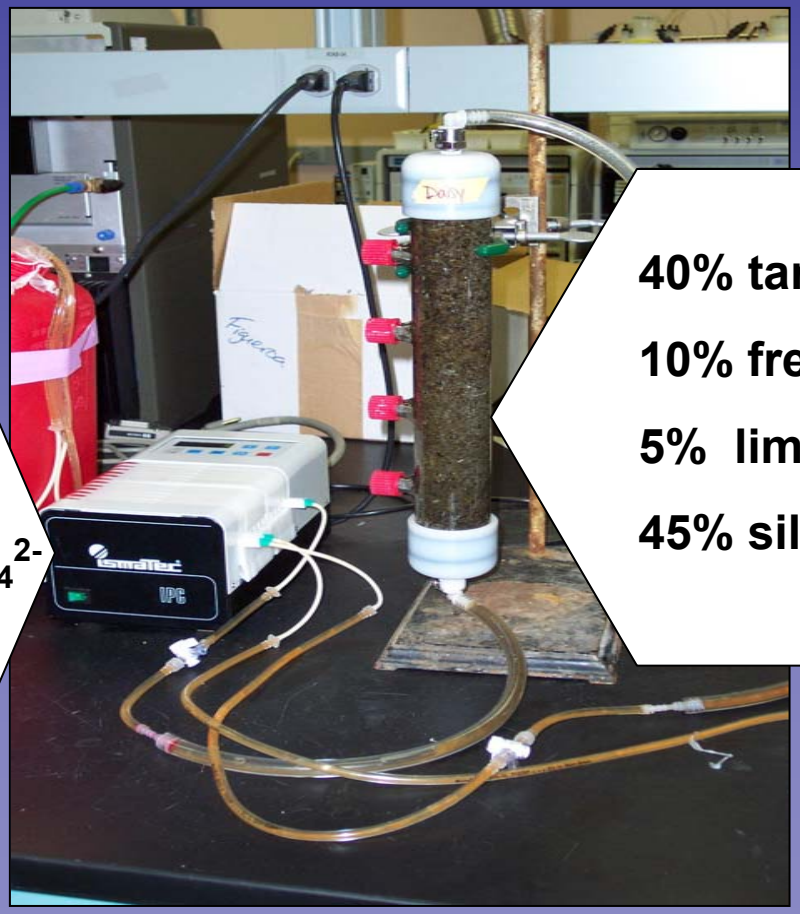
50 mg/L Mn^{2+}

50 mg/L Zn^{2+}

1000 mg/L SO_4^{2-}

pH 6

30 ml/d



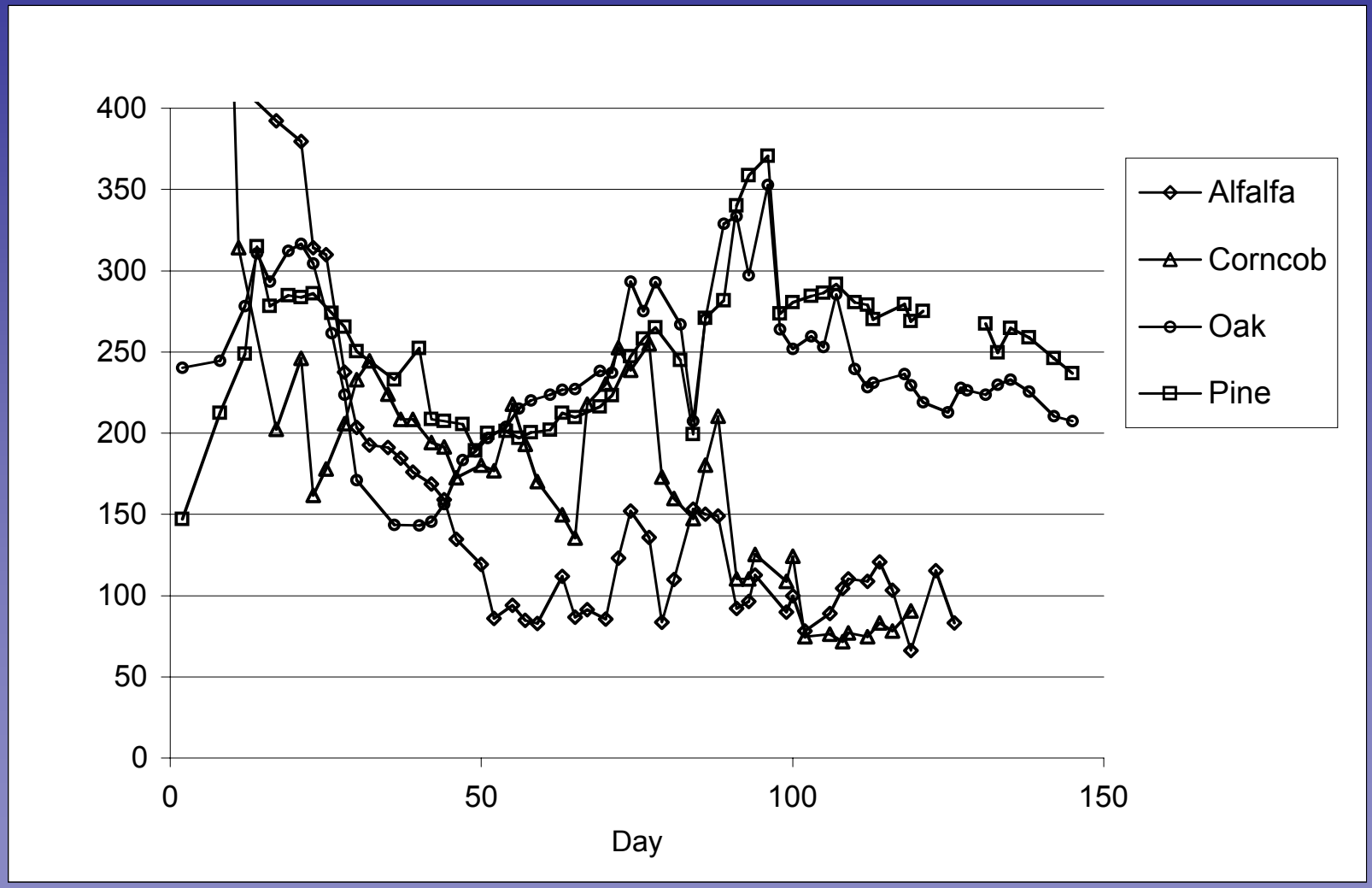
40% target substrate

10% fresh dairy manure

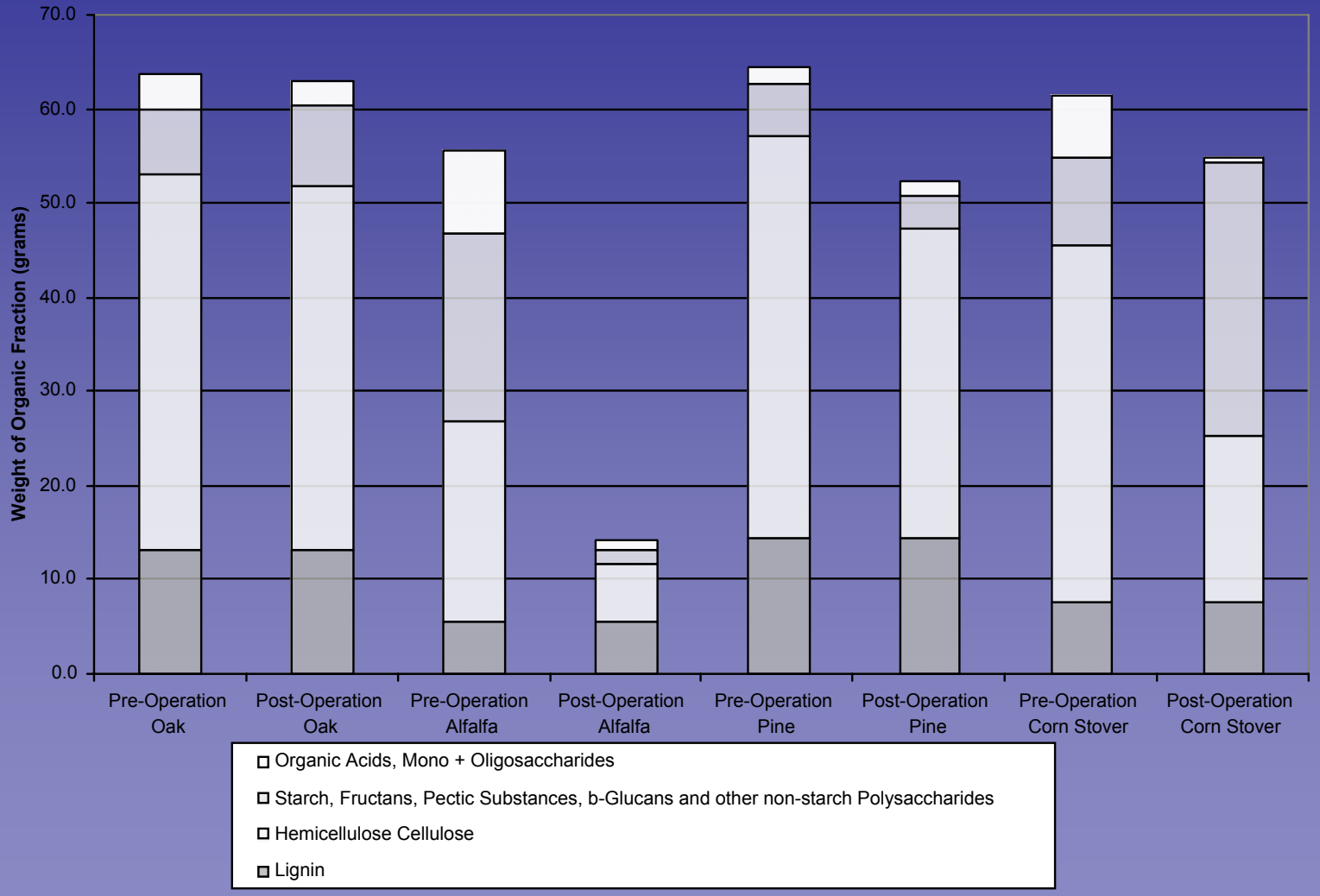
5% limestone

45% silica sand

Column sulfate effluent



Substrate consumption



Implications of substrate analysis experiments

The high sulfate reduction rates observed were related to high fractions of readily degradable components

Relative degradability: alfalfa > corn stover > wood

Highly soluble solid phase substrates do not provide long term release of carbon

Modeling

Modeling the Influence of Decomposing Organic Solids on Sulfate Reduction Rates for Iron Precipitation

PAULO S. HEMSI,[†]
CHARLES D. SHACKELFORD,[*,†] AND
LINDA A. FIGUEROA[‡]

Environmental Science and Technology
39(9): 3215-3225.

Summary

Existing biotechnology tools underutilized

Stoichoimetry tools can improve
- estimates of reagent requirements
- give insight into monitoring requirements

Microbial assessment tools provide important information on the reaction catalyst (bacteria)
- who's there (potential)
- what can they do(activity)

Available models provide useful tools for design