Perchlorate Degradation in Fixed Bed Bioreactors- 1

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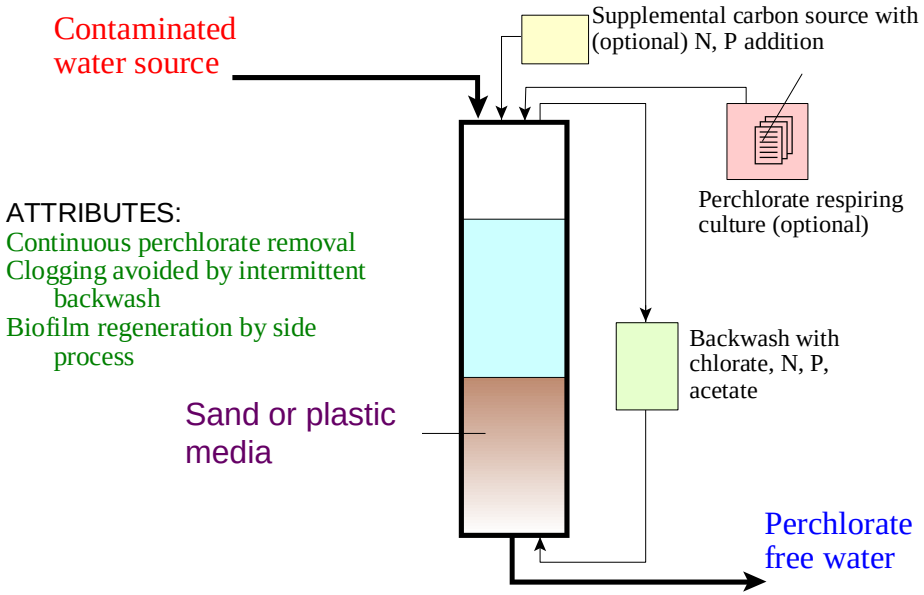


Perchlorate Bioremediation…

Is it Rocket Science?

Perchlorate Research in the Logan Laboratory

- Bioremediation research idea circa 1992, University of Arizona, Tucson, AZ
- Search for funding– failures (USAF, DOE, private companies)
- National Science Foundation (NSF) exploratory grant in 1994– to investigate important and emerging topics: chlorate biodegradation
- 1994-1997: Research on chlorate degradation continues
- Search for funding– failures continue
- CA Dept of Health: 1997, new IC method developed; study shows perchlorate in 1/3 of well sampled

Perchlorate Research in the Logan Laboratory

- July 1997: Move to Penn State Univ.
- November 1997: Phone calls…

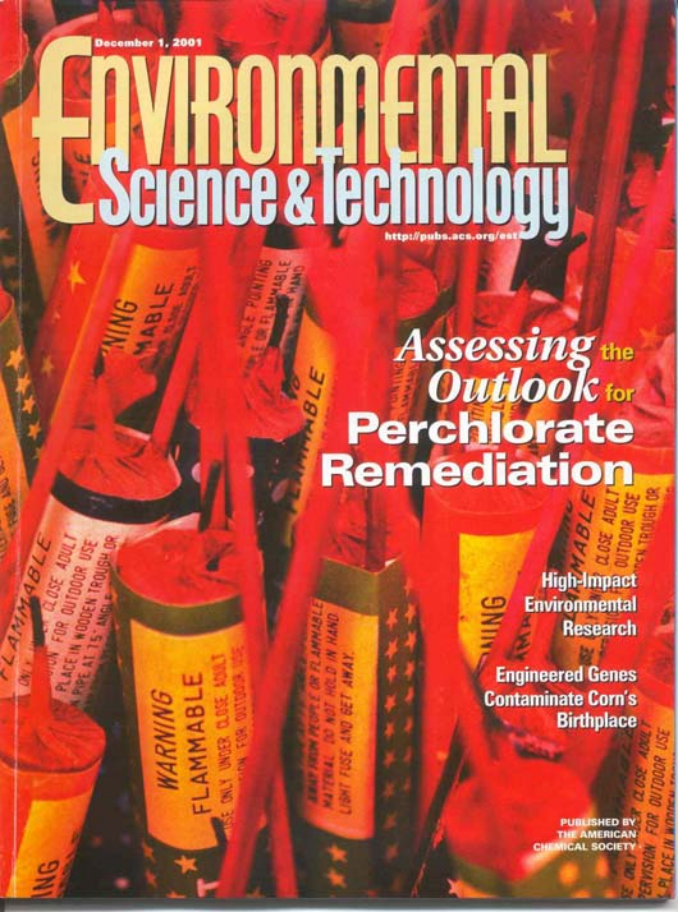
- Research grants finally obtained…
  - May 1998: NSF grant for research on chlorate- and perchlorate-respiring bacteria
  - November 1998: AWWARF Phase 1 Grant for bench scale studies
  - September 2000: NSF grant on respiratory enzymes used by perchlorate-respiring bacteria
  - November 2000: AWWARF Phase 2 Grant for pilot scale studies

J. Bioremed., 2(2), 1998

Perchlorate Remediation-- Reviews in 1997 (published in 1998)

→ Not much known…

- Chlorate was known to be readily biodegraded, but perchlorate persisted in groundwater.
- Perchlorate in water was known to be extremely stable (reviewed by Urbansky, 1998).
- Very few bacteria were known to degrade perchlorate; One treatment technology had been developed to biodegrade perchlorate at high concentrations to only < 500 ppb (reviewed by Logan 1998, Frankenberger et al. 1998)



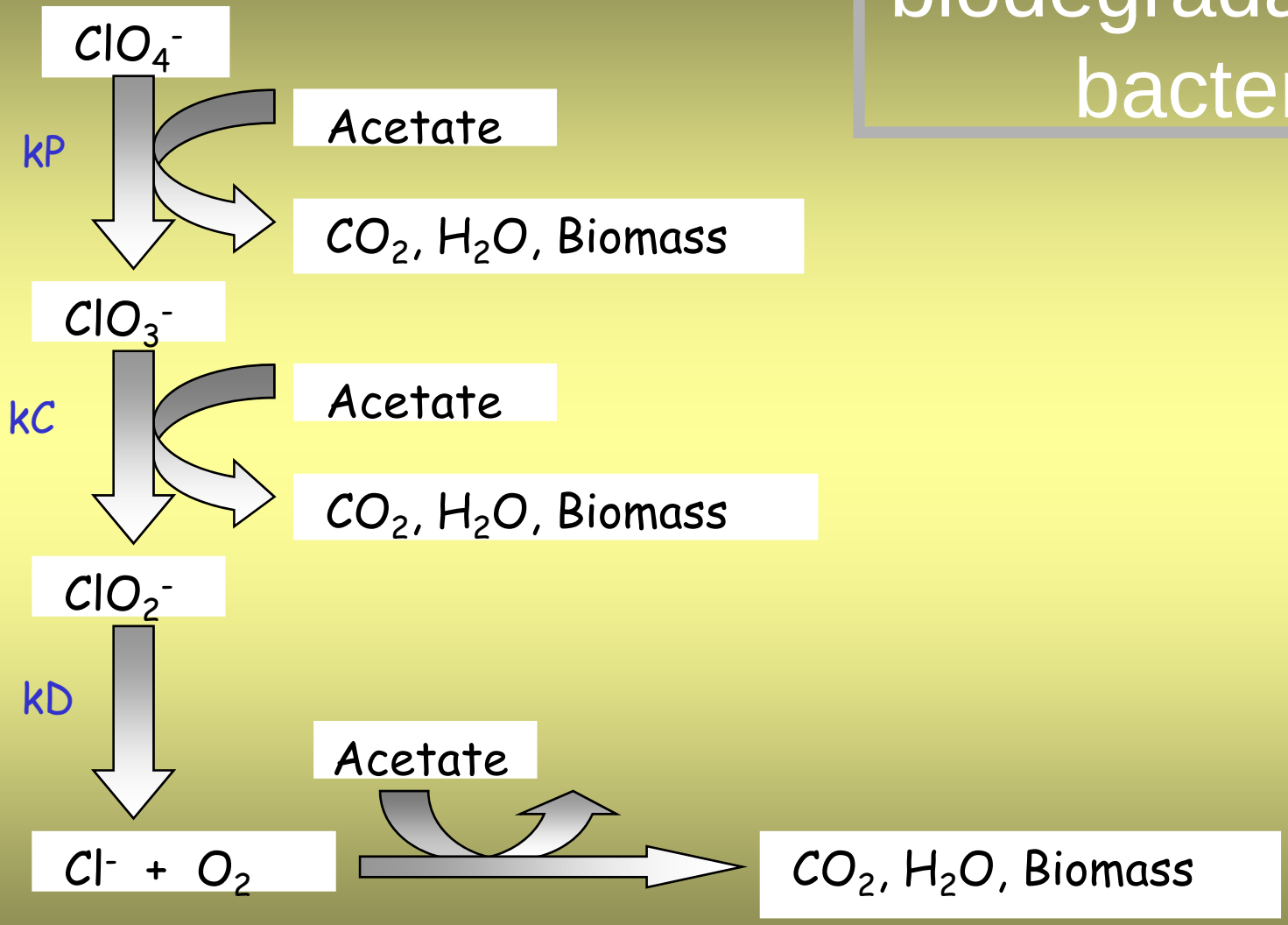
ES&T Cover, December 2001 issue

Outlook for Perchlorate Remediation in 2002?

→ *Quite Promising!*

- In only a few years, a variety of technologies have been developed to remove (physical/chemical) and degrade (chemical and biological) perchlorate in ex-situ systems
- *In-situ* perchlorate degradation is being demonstrated as well.

Perchlorate biodegradation by bacteria



This morning's session

- Short review of perchlorate degradation technologies
- Emphasis here on AWWARF studies:
  - Bench scale bioreactors
  - Pilot scale studies in Redlands, CA
  - Economic analysis of full-scale, fixed bed (PSU-O4) system

OVERVIEW- Part 1

- 8:30 Perchlorate bioreactor study-- Overview and introduction: Bruce Logan
- 8:45 Review of perchlorate remediation technologies: Jianlin Xu
- 9:10 Bench scale studies (20 min each)
  - » Acetate fed bioreactors: Yanguang Song
  - » Hydrogen-fed (autotrophic) bioreactors: B. Logan
  - » Membrane bioreactors: Jaci Batista (UNLV)
- 10:10 – 10:30: BREAK

Review of perchlorate remediation technologies

J. Xu, Y. Song, B. Min, L. Steinberg, and B.E. Logan

Dept of Civil and Environmental Engineering
The Pennsylvania State University, University Park, PA

OUTLINE OF PRESENTATION

- General introduction
- Microbial remediation technologies
- Other remediation technologies
- Microbial processes in conjunction with other technologies
- Enzymes used for perchlorate degradation
- Conclusions and outlook

General Introduction

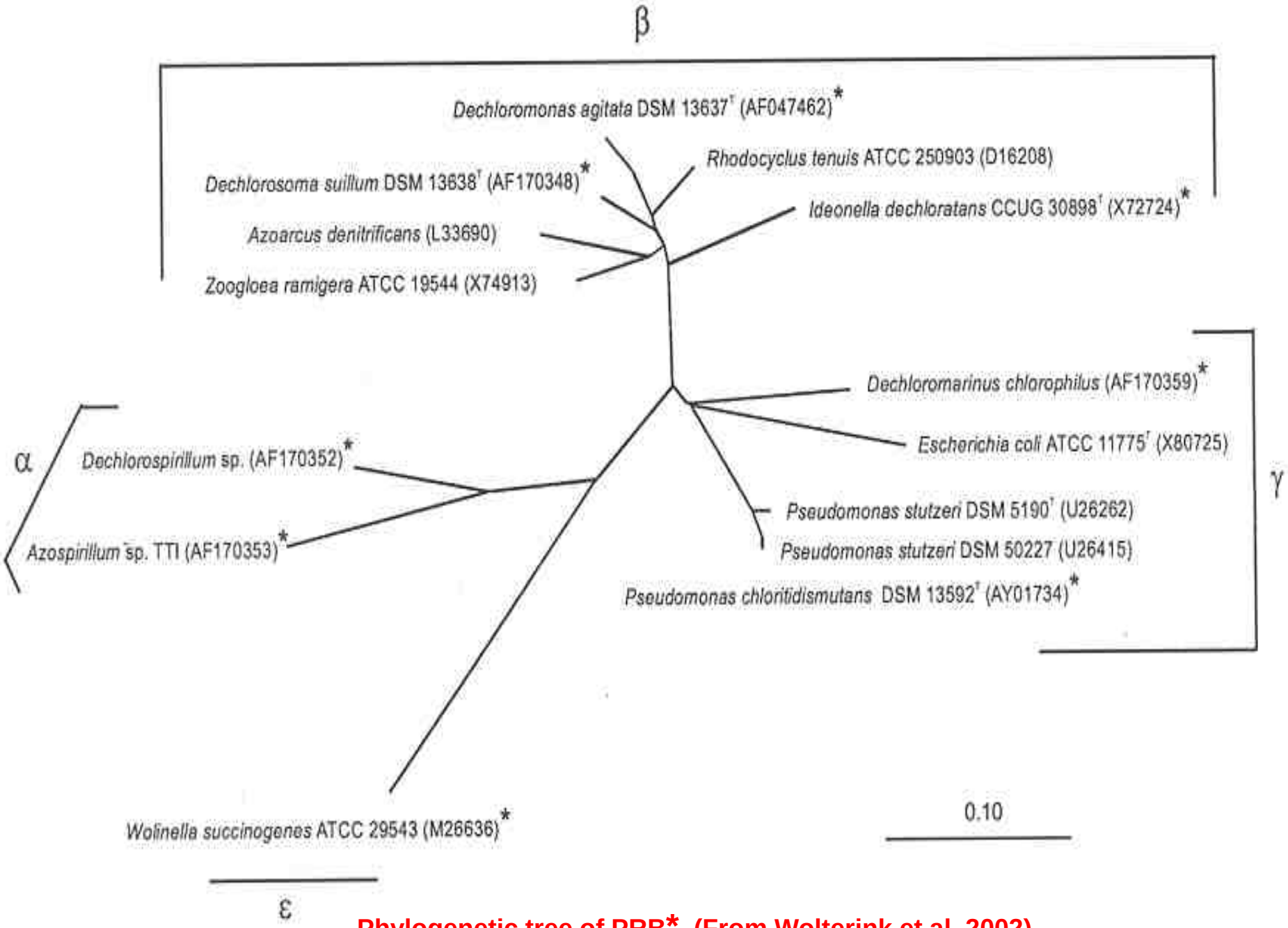
- Perchlorate (ClO_4^-) characteristics
  - Man-made chemical: solid propellants for missiles, rockets, explosives and air bags (Only natural source: Chilean caliche)
  - Perchlorate is a strong oxidizing agent:

    $ClO_4^- + 8H^+ + 8e^- \rightarrow Cl^- + 4H_2O$, $E^\circ = 1.287$ V
  - Perchlorate is very resistant to degradation in natural environments
- Toxicity
  - Perchlorate affects hormone production by the thyroid
  - Action guideline in California: 18 ppb —> 4 ppb (2002)
- Perchlorate contamination: at least 20 states, 15 million people
- Perchlorate respiring bacteria (PRB) are abundant in nature: 1-1000 per g soil or water

PRB isolates

- *Dechlorosoma* sp. KJ and PDX, *Dechloromonas* JM and HZ (Logan PSU, 2000-2002)
- *Dechlorosoma suillum* JPLRND (Hatzinger et al. Envirogen, 2002)
- *Dechloromonas agitata* CKB, etc. (>13 strains) (Coates and Achenbach SIU, 1999-2001)
- Strain pec1ace (Frankenberger UC Riverside, 1999)
- Strain GR-1 (van Ginkel Netherlands, 1996)
- *Wolinella succinogenes* HAP-1 (Wallace USAF, 1996)
- *Vibrio dechloraticans* (Korenkov USSR, 1976)

- All isolates are members of *Proteobacteria*



Phylogenetic tree of PRB* (From Wolterink et al. 2002)

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Microbial treatment processes: Bench scale

Heterotrophic reactor studies

- PRB isolates:
  - *Vibrio dechloraticans* (Korenkov, USSR, 1976)
  - HAP-1 (Walace et al., ARA/USAF, 1998)
  - GR1 (van Ginkel et al. Netherlands, 1998)
  - KJ (Kim and Logan, PSU, 2001)
  - Perc1ace (Frankenberger, UC Riverside, 2002)

- Enriched Mixed cultures:
  - Attaway et al. (USAF, 1993)
  - Giblin et al. (UC Riverside, 2000)
  - Kim and Logan (PSU, 2000)
  - Hatzinger et al. (Envirogen, 2000)
  - Brown et al. (U. Illinois, 2002)

Microbial treatment processes: Bench scale

Hydrogen reactor studies

-Enriched/Mixed cultures:

Logan (PSU, 2000-2002)

Frankenberger (UC Riverside, 2000)

Rittmann (Northwestern, 2002) (autotrophic denitrifier biofilm)

-PRB isolates:

Dechloromonas spp. HZ, JM (PSU, Logan 2000 and 2002)

Dechloromonas sp. JDS5 (U. Iowa, Parkin 2002)

GR1 (Netherlands, van Ginkel 1998)

HAP-1 (ARA, Wallace, 1996)

Microbial treatment processes: Pilot and full scale processes

- Ex-situ bioremediation

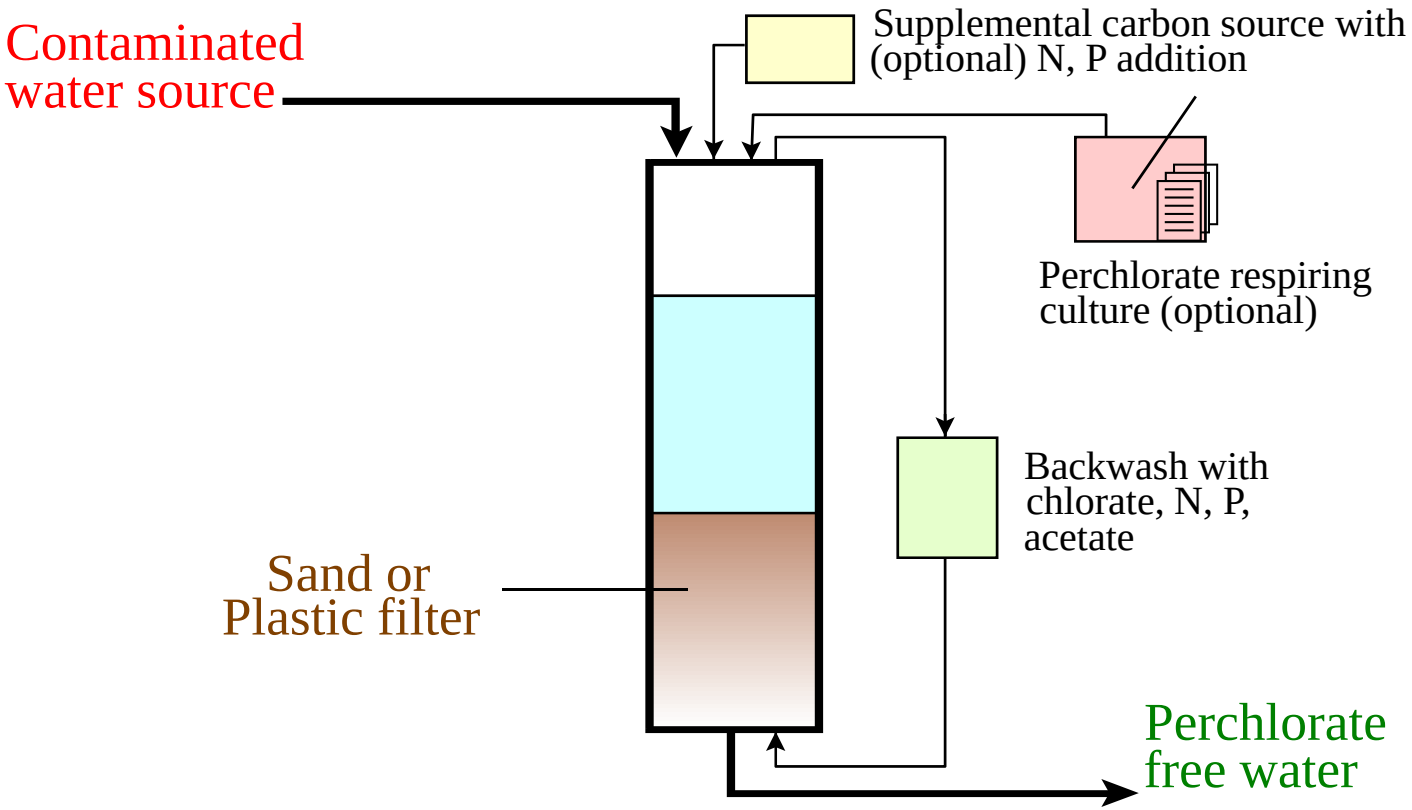
* Packed bed reactor – Pilot scale for groundwater (PSU UC Riverside, and JPL)

* Fluidized bed reactor – Pilot and full scale for groundwater (Envirogen/US Filter)

* Suspended growth reactor - full scale for high strength perchlorate wastes (USAF/ARA)

- In-situ bioremediation

PSU: Fixed bed reactor



☞ **The pilot scale of fixed bed reactor successfully treating about 3000 gallons per day of perchlorate contaminated groundwater**

(After Logan 2001, US patent; Logan 2002)

Microbial treatment processes: Pilot and full scale processes

- Ex-situ bioremediation

* Packed bed reactor – Pilot scale for groundwater (PSU and JPL)

* Fluidized bed reactor – Pilot and full scale for groundwater (Envirogen/US Filter)

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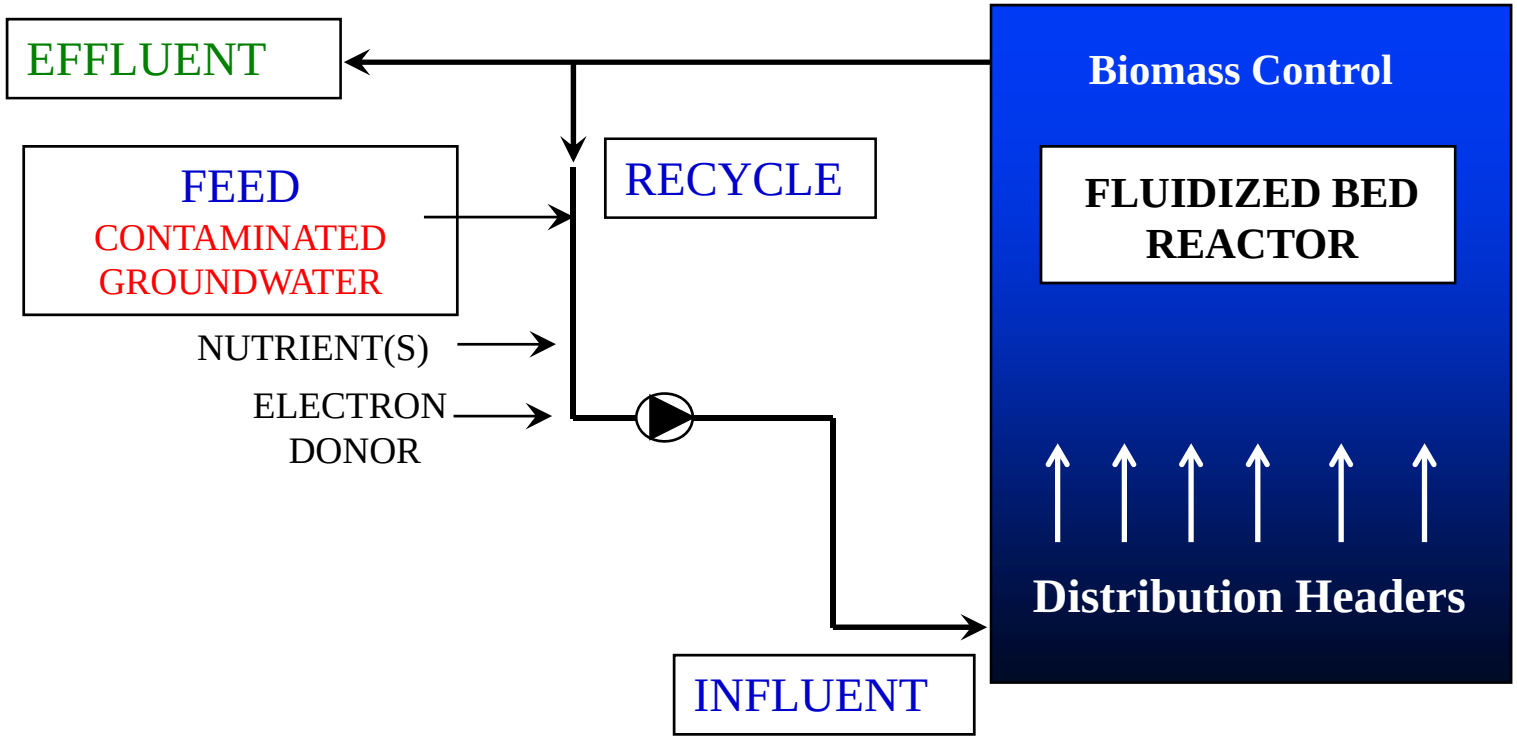
- In-situ bioremediation

Fluidized bed reactor (Envirogen)



☞ **The full scale of FBR system successfully treating more than 5.8 million gallons per day of groundwater containing perchlorate**

(After Hatzinger et al. 2002)

Microbial treatment processes: Pilot and full scale processes

- Ex-situ bioremediation

* Fixed bed reactor – Pilot scale for groundwater (PSU and JPL)

* Fluidized bed reactor – Pilot and full scale for groundwater (Envirogen/US Filter)

* Suspended growth reactor - full scale for high strength perchlorate wastes (USAF/ARA)

-In-situ bioremediation

* Multiple field studies (**Geosyntec/Aerojet)**

Microbial treatment processes: Pilot and full scale processes

- In situ bioremediation: Trenches (**Envirogen**)

(After Togna and Hatzinger 2002)

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Physical and chemical processes

- **Anion exchange**
  - * Selective removal of perchlorate
  - * Effective regeneration of resin
  - * Chemical destruction of perchlorate in brine wastes
- **Active carbon**
  - * Absorption
- **Membrane filtration**
  - * Non-selective removal of all anions

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Microbial processes in conjunction with other technologies

- Perchlorate removal in high saline wastes:

* Difficult to degrade thoroughly in original brines (Logan PSU, Frankenberger UC Riverside, Batista U. Nevada)

* HAP-1: dilution and degradation (Coppolla ARA)

- Biological activated carbon

* Absorption and degradation (Brown et al. U. Illinois)

- Phyto-remediation

* Plant tissues: uptake and destruction (Naengung, U. Georgia; Schnoor, U. Iowa)

* Bacteria associated with plants: strong ability to reduce perchlorate

* Potential application for in situ remediation

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- Enzymes used for perchlorate degradation
  - Recent results from Penn State
- Outlook

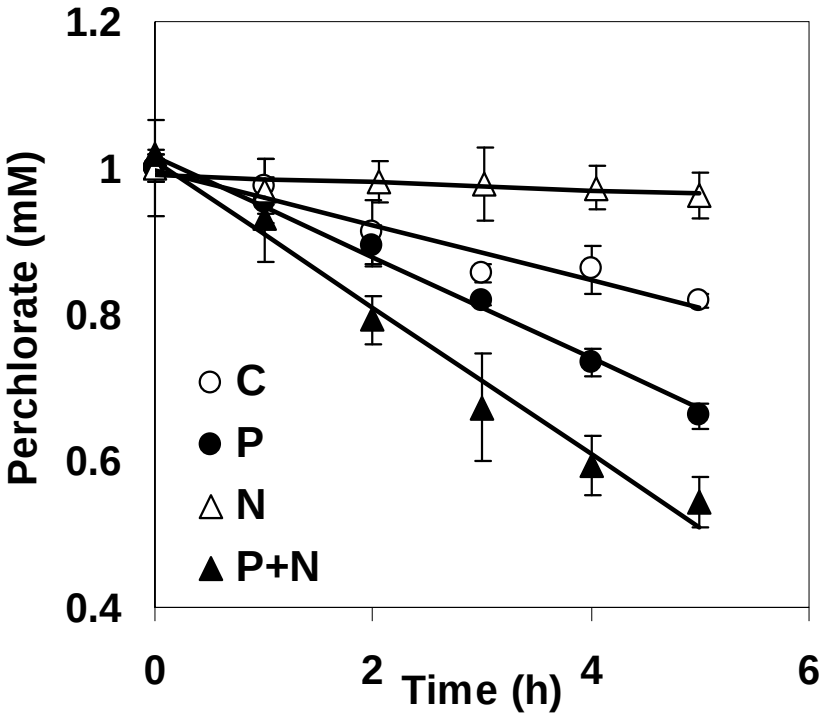
PRB degrade both nitrate and perchlorate– what is the effect of nitrate on perchlorate reduction?

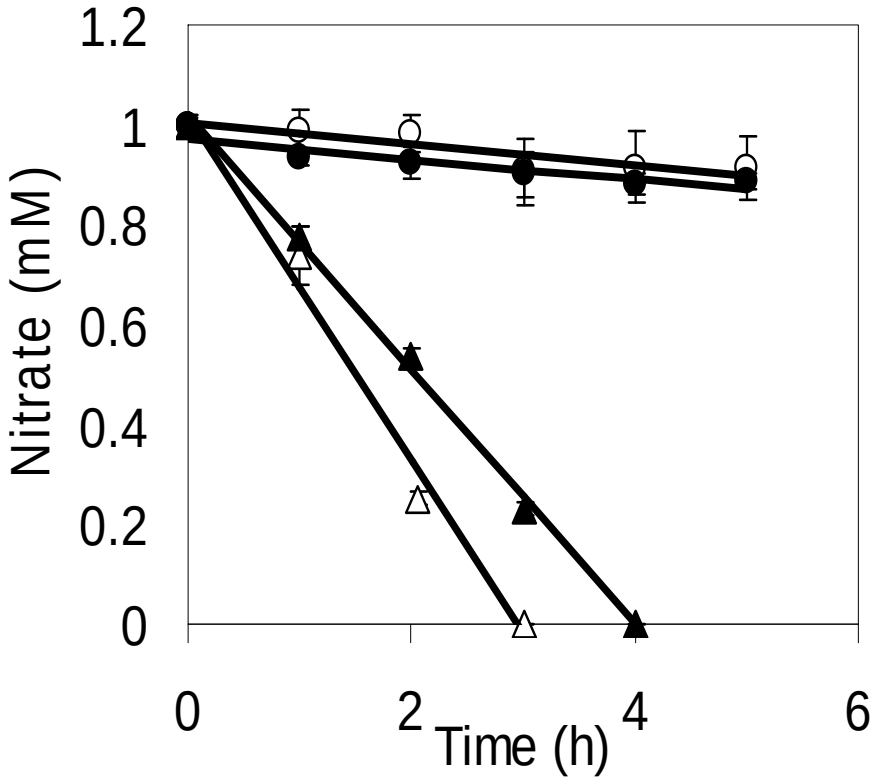
- Nitrate is often present in perchlorate contaminated groundwater
- Nitrate is usually degraded earlier than perchlorate, and often competitively inhibits perchlorate reduction
- PRB maintain a basal nitrate reductase activity (GR1, perc1ace, *Dechlorosoma suillum*, and *Dechloromonas agitata* CKB)
- Nitrate reductase reduces chlorate to chlorite

QUESTION: Are perchlorate and nitrate reduction pathways separate in strain KJ?

- Examine uptake kinetics of ClO_4^- and NO_3^- by KJ cells grown on:
  ClO_4^-, ClO_3^-, NO_3^-, and
  NO_3^-+ClO_4^-

Perchlorate and nitrate degradation by washed cells of *Dechlorosoma* sp. KJ originally grown on P=ClO_4^-, C=ClO_3^-, N=NO_3^-





Perchlorate and nitrate reductases are inducible and separate in *Dechlorosoma* sp.KJ

What is the effect of dissolved oxygen on perchlorate reducing enzymes?

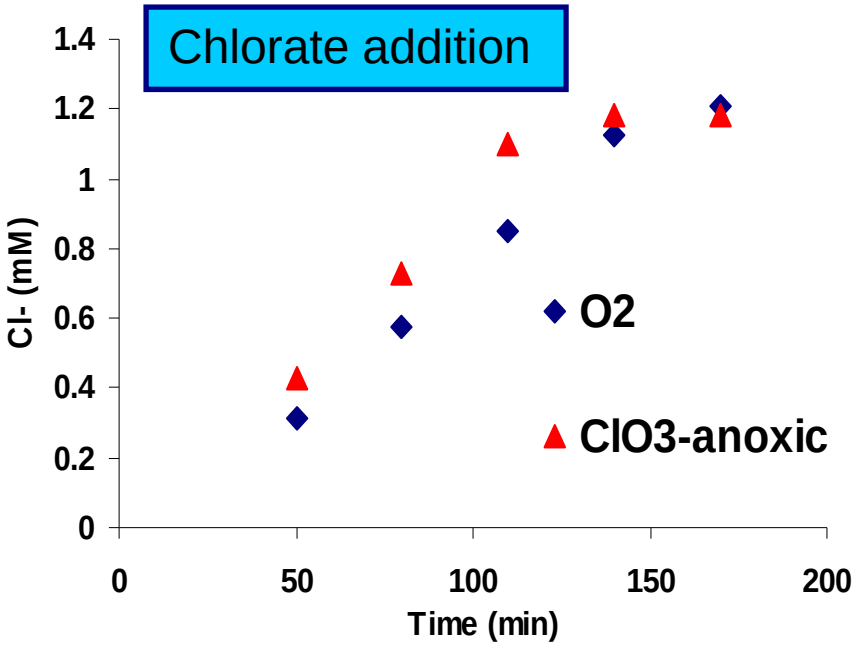
- Oxygen is usually present in perchlorate contaminated groundwater, and oxygen inhibits perchlorate reduction.
- Some bacteria can degrade chlorate, but not perchlorate

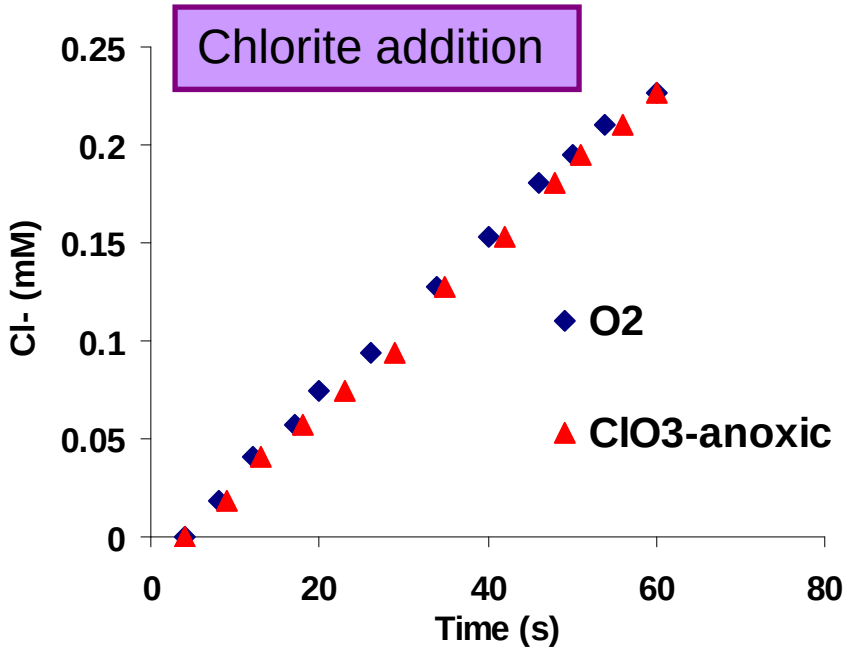
  Example: Pseudomonas sp. PDA, isolated from a primary digestor sludge, can degrade chlorate but not perchlorate

- Are chlorate reductase and chlorite dismutase synthesized under aerobic conditions in PDA?
- Examined chlorate reductase and chlorite dismuatse activities PDA
  - Both Chlorate Reductase and Chlorite Dismutase (CD) measured by chloride formation

ClO_3^- reduction and Chlorite dismutation by washed cells of *Pseudomonas* sp. PDA

(grown on either O_2 or ClO_3^- as an e^- acceptor)





Chlorate reductase and chlorite dismutase are constitutively expressed under both aerobic and anaerobic conditions, in the presence and absence of chlorate

Is chlorite dismutase activity present in all PRB under aerobic conditions?

- Although many PRB have been isolated, chlorite dismutase (CD) activities have been measured by only one PRB under aerobic conditions
- A dissolved oxygen (DO) probe was used by others to measure CD activity based on: $ClO_2^- \rightarrow O_2 + Cl^-$
- We examined three different methods to compare CD activity
  - DO probe
  - Chloride probe
  - Ion chromatography used to quantify chloride
- Chloride probe was easiest and worked the best.

CD activities (U/mg-DW) measured with Cl^- electrode for different PRB grown either anaerobically on ClO_3^- or aerobically

Strain	CD activities		Ratio
	Anaerobic	Aerobic	
Dechlorosoma sp. KJ	52.3	1.02	51
Dechlorosoma sp. PDX	34.9	3.90	9
Dechloromonas sp. HZ	35.6	3.03	12
Dechloromonas sp. JM	24.5	0.51	48
Perc1ace	37.2	0.92	40

All aerobically-grown PRB had CD activity, with a range of: 0.5-4 U/mg-DW

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CONCLUSIONS- Enzyme Studies

- Perchlorate degradation and denitrification pathways are inducible and separate in PRB strain KJ
- ClO_3^- degradation enzymes, ClO_3^- reductase and chlorite dismutase, are constitutively expressed in strain PDA
- All PRB tested have CD activities for both aerobic and anaerobic cultures

OUTLOOK

- Microbial remediation is promising
  - Perchlorate can be removed in contaminated groundwater and industrial wastes
  - Dissolved oxygen inhibits perchlorate reduction
  - Nitrate reduced prior to perchlorate degradation
  - Acetate is most often used e^- donor
- Perchlorate removal with H_2 as e^- donor
  - Less biomass produced than with organic substrates
  - No residue (versus organic substrates)
  - Pilot and full scale tests are needed for membrane-bioreactor process
- Anion exchange technology also shows great potential, but brines are a major concern

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