

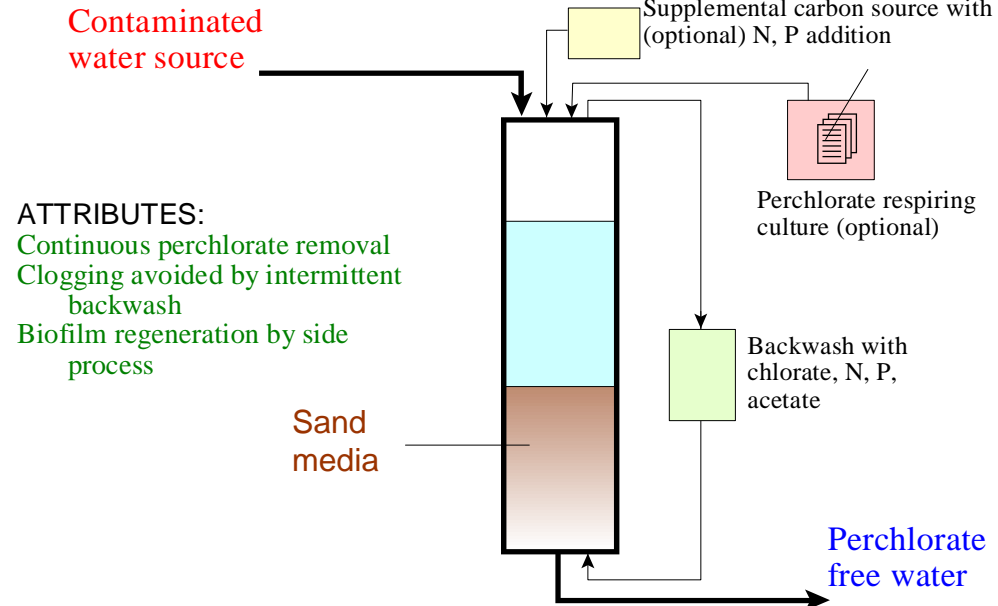
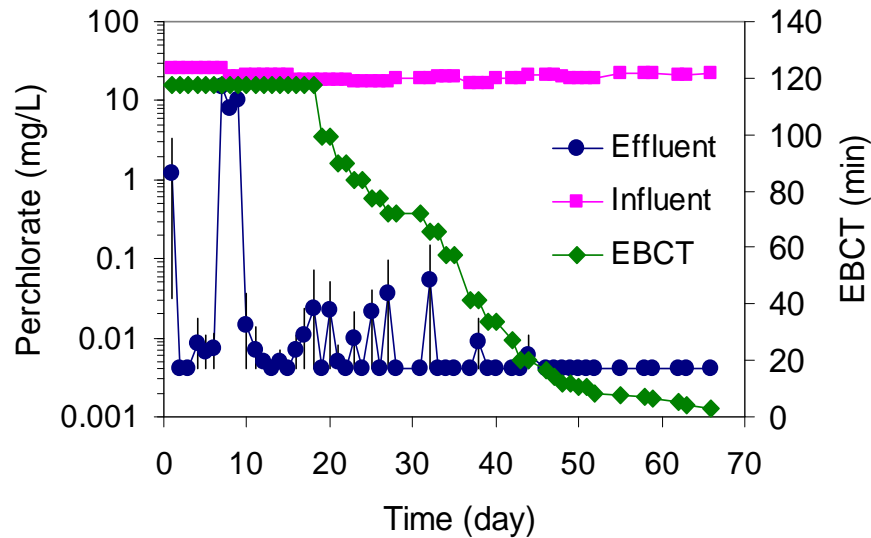
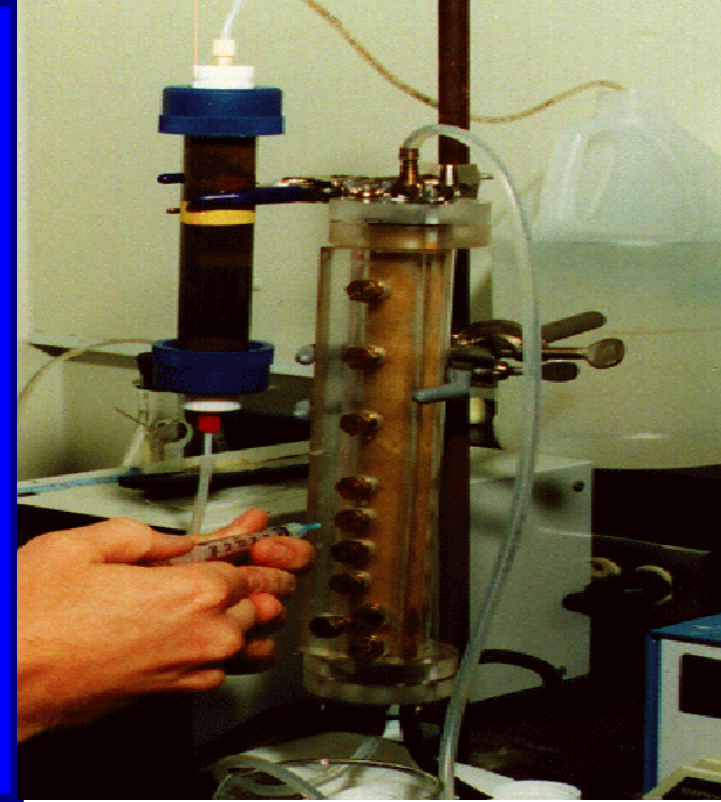
Perchlorate Degradation in Acetate-fed Bioreactors

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Kijung Kim, Bruce Logan

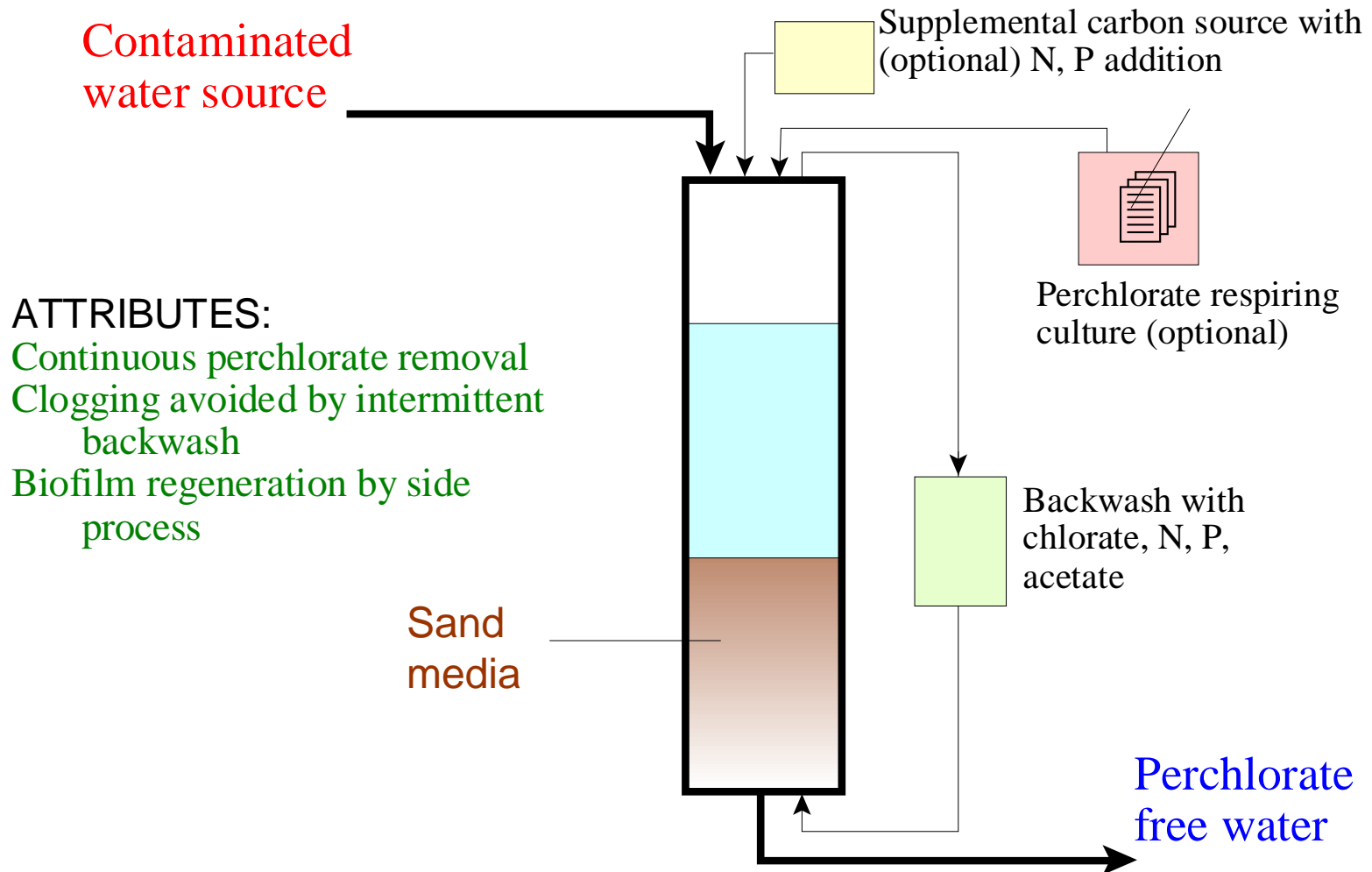
Department of Civil & Environ. Eng.

Penn State University



PERCHLORATE FIXED-BED REACTOR

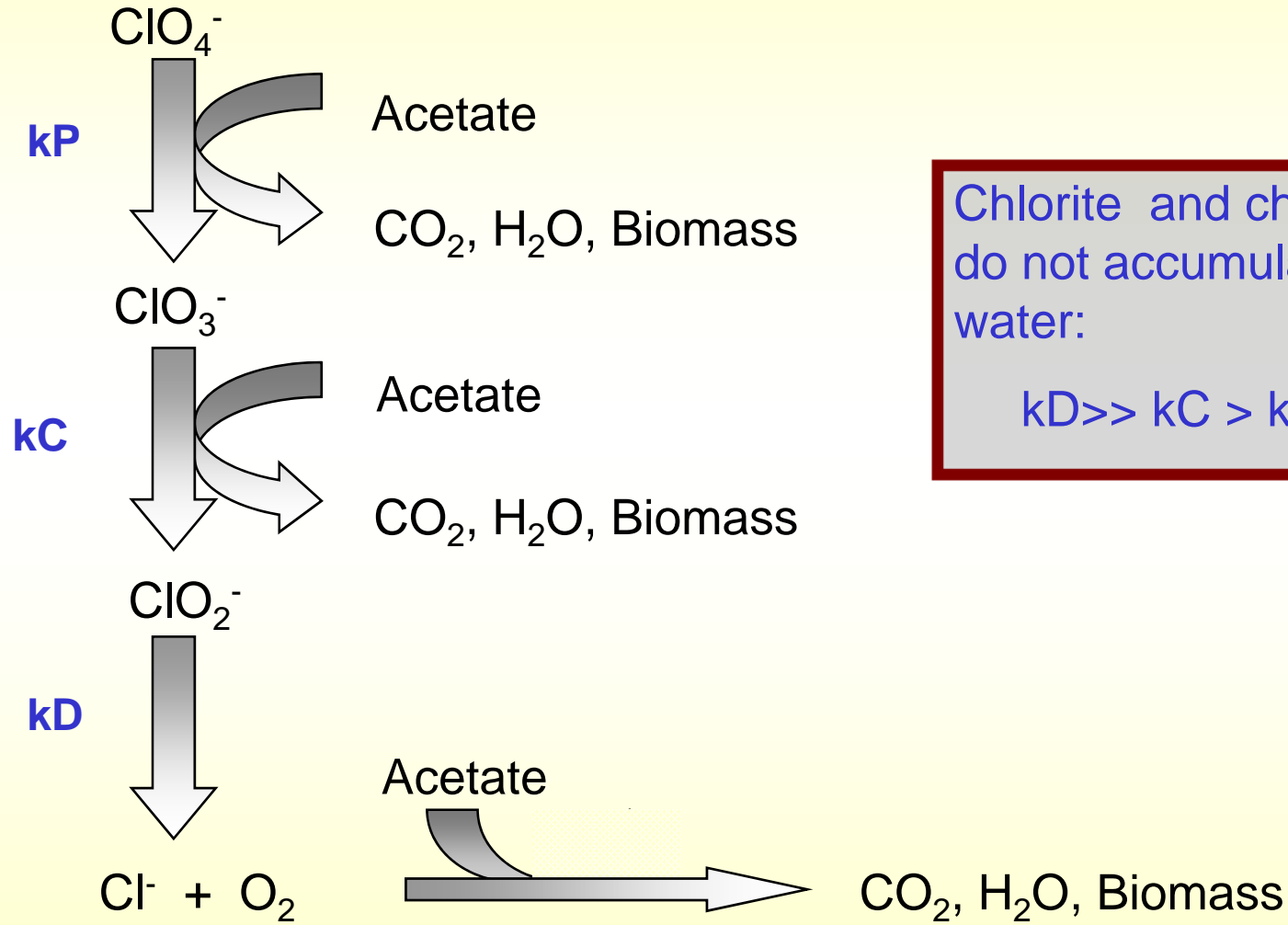
[Penn State Patent/Invention Disclosure No. 1887]



Perchlorate Degradation: Topics

- Bacterial isolate used in tests: KJ
- Bench-scale acetate-fed reactors
 - Mixed culture
 - Pure culture (KJ)
- Effect of O₂ exposure on perchlorate reduction

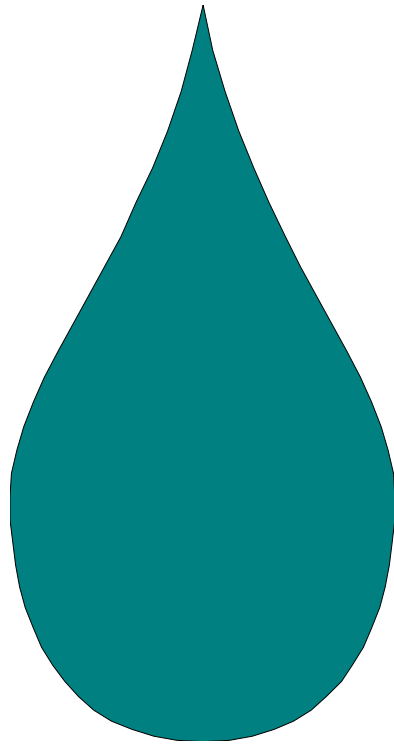
Perchlorate Reducing Pathway



Chlorite and chlorate do not accumulate in water:

$$kD \gg kC > kP$$

Are perchlorate-respiring bacteria abundant in nature?



One milliliter (1 mL) of water
(about one teaspoon) contains:

~ 10^6 bacteria

~1 perchlorate-degrading
bacterium

PRM Abundance: observations

- PRMs present in natural waters at $\sim 1000/10^9$
- PRMs in “pristine” soils, that have no evidence of perchlorate contamination: $\sim 1/10^9$
- At sites where perchlorate is present, have a more abundant perchlorate reducing population, $\sim 100/10^9$

Several Perchlorate Respiring Bacteria Isolated in Our Laboratory

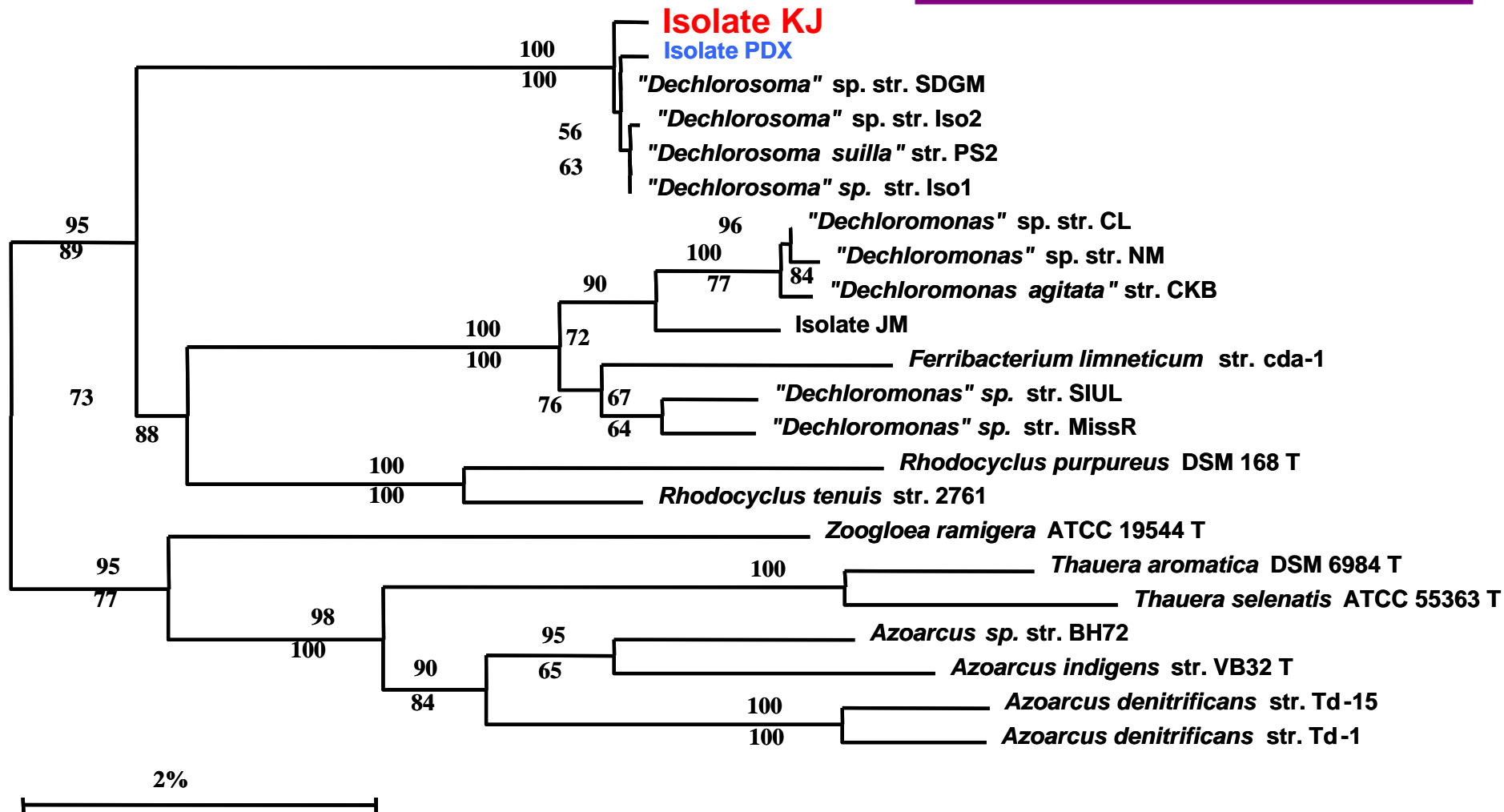
- *Dechlorosoma* sp. KJ: isolated from a perchlorate-degrading packed bed bioreactor;
- *Dechlorosoma* sp. PDX: isolated from primary digester wastewater;
- *Dechloromonas* sp. JM: isolated from a hydrogen-oxidizing consortium in a autotrophic packed-bed biofilm reactor;
- *Dechloromonas* sp. HZ: isolated from a gas-phase anaerobic packed-bed biofilm reactor.

Phylogentic analysis of KJ

(courtesy of Dr. Ian Head, Univ. of New Castle)

Classification:

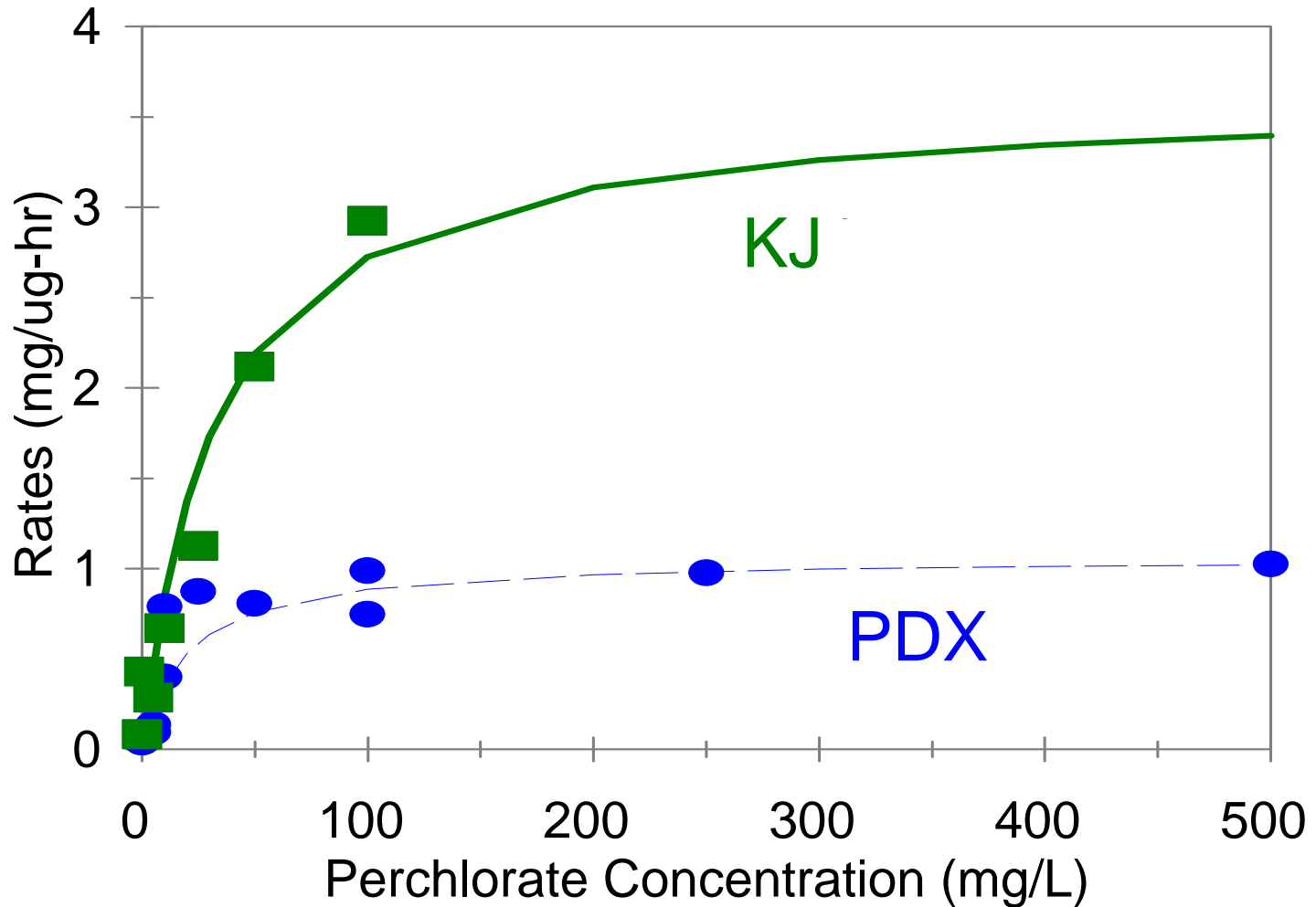
Dechlorosoma sp. KJ



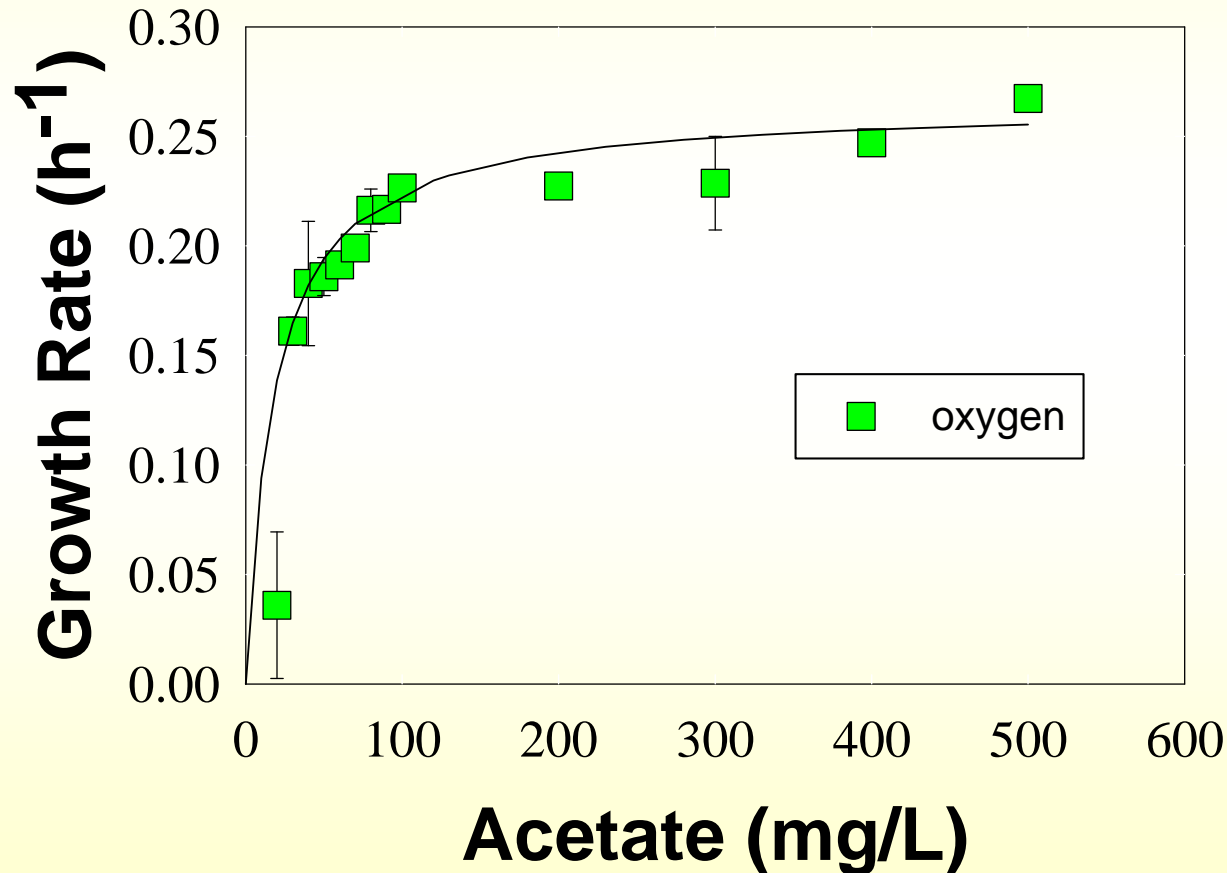
From: Logan et al. (2001)

KJ Perchlorate kinetics: $K_s=33$ mg/L

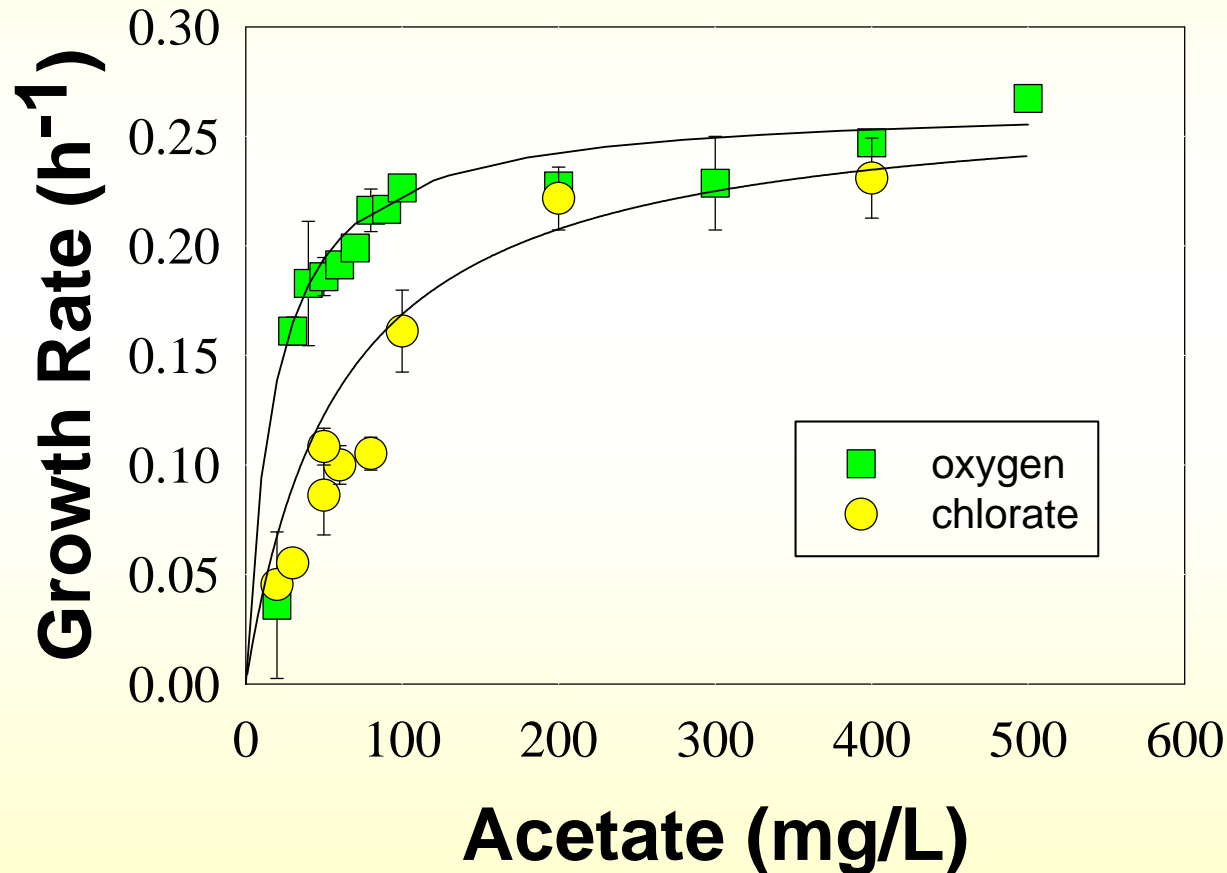
(lactate grown cell suspensions)



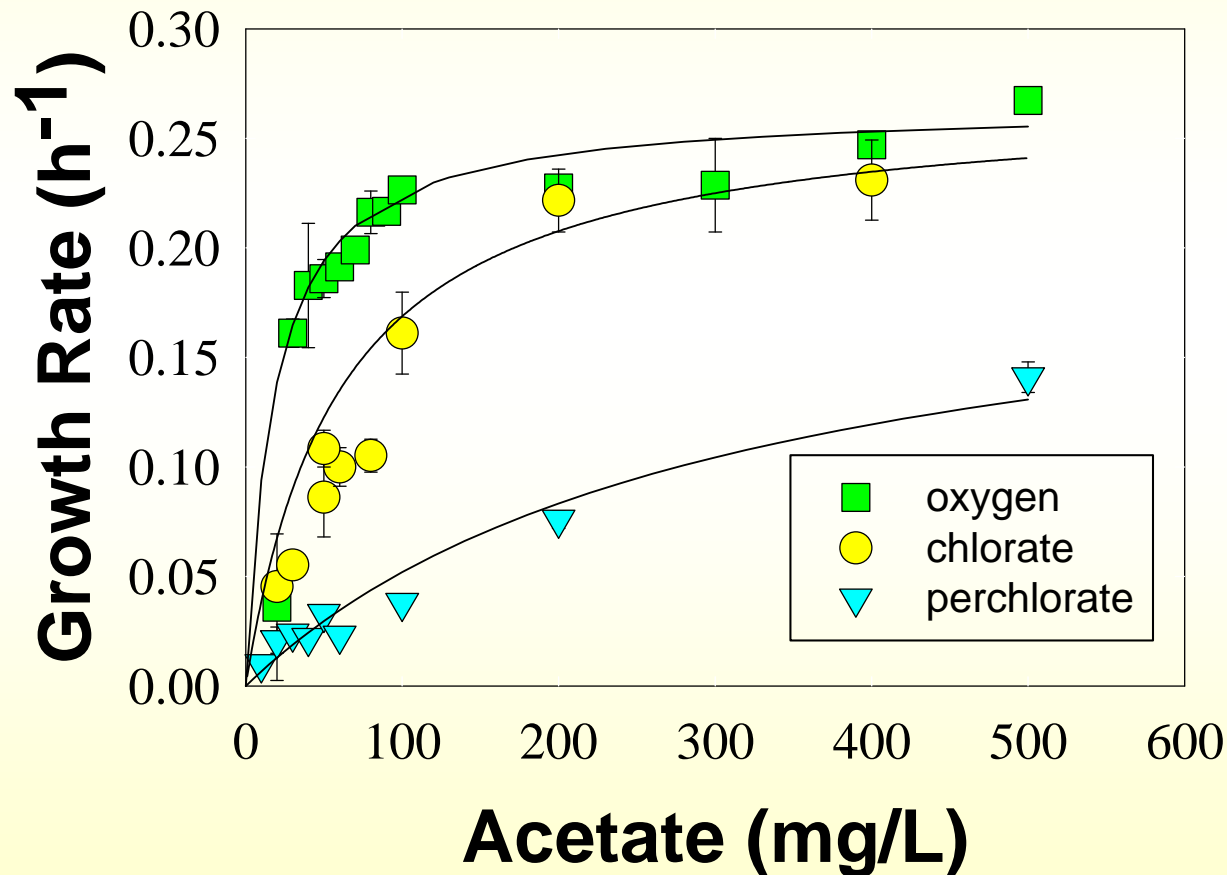
Substrate kinetics: acetate



Substrate kinetics: acetate



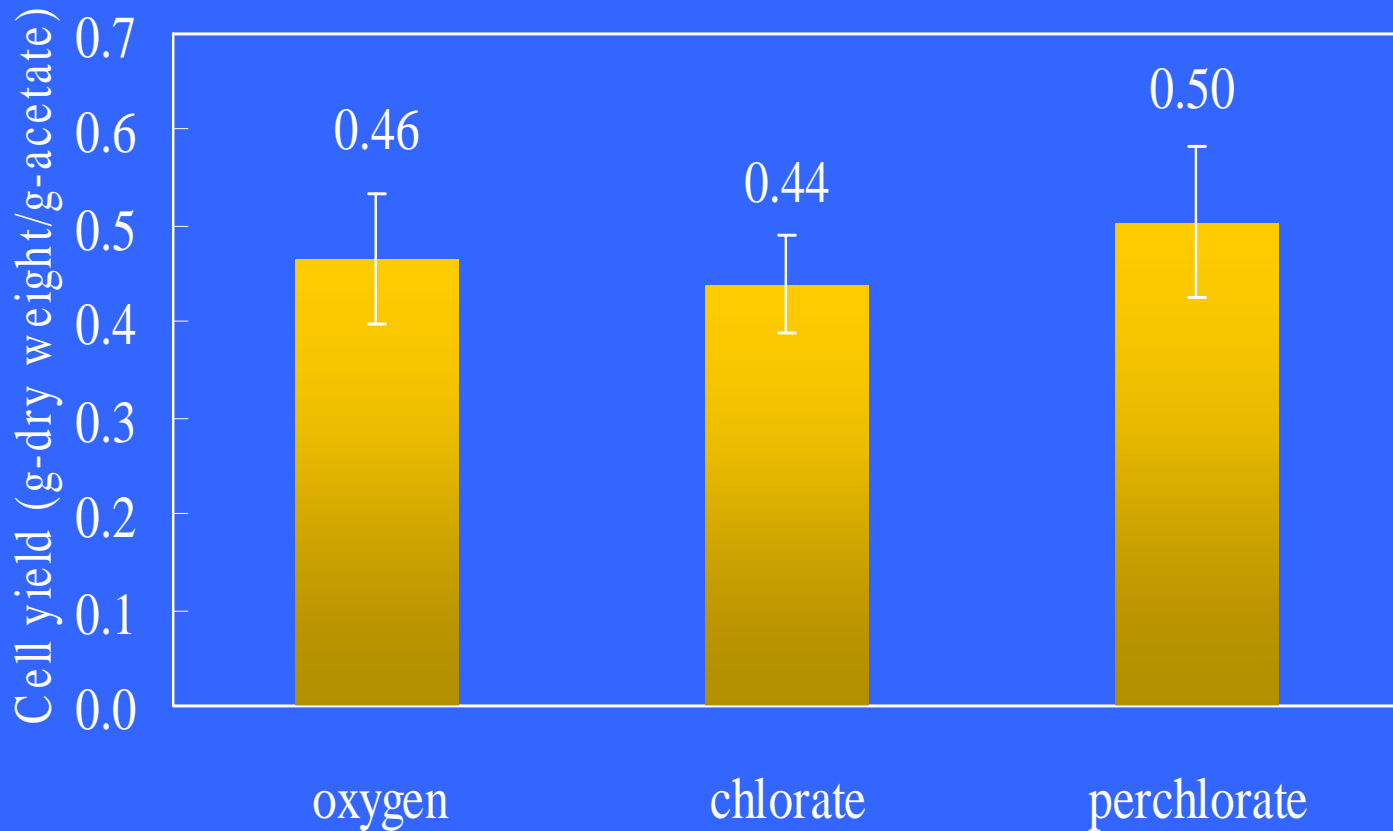
Substrate kinetics: acetate



Kinetic constants for growth of KJ on acetate

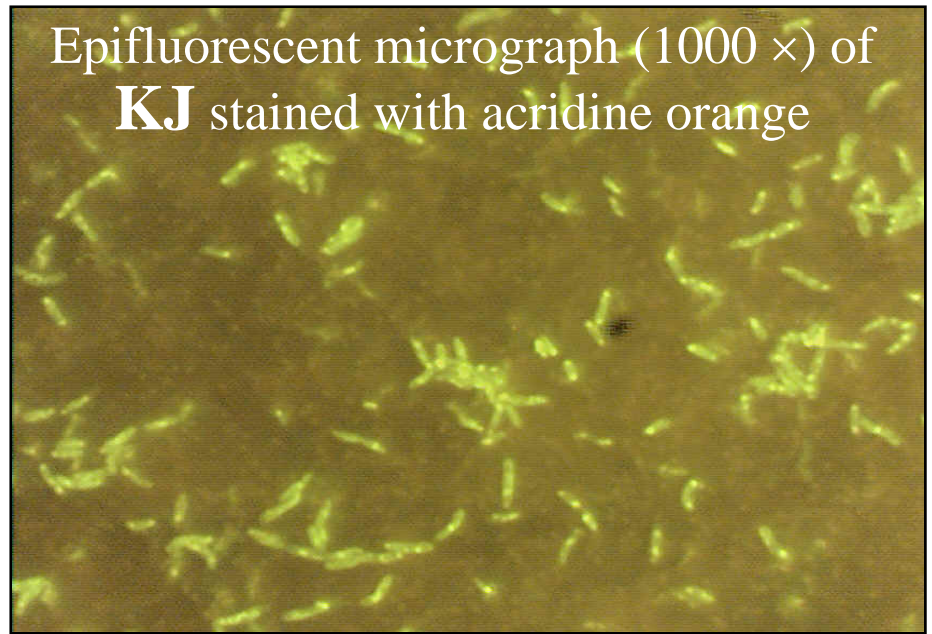
Electron Acceptor	μ_m (h ⁻¹)	K_S (mg/L)
Oxygen	0.27 ± 0.02	14 ± 1
Chlorate	0.27 ± 0.03	60 ± 25
Perchlorate	0.14 ± 0.01	470 ± 290

Cell yield- KJ

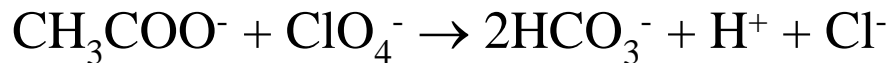


Overall Stoichiometry

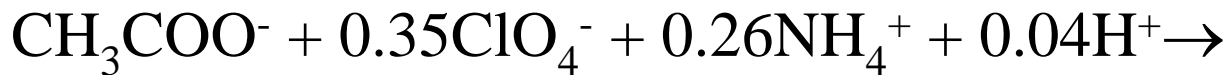
Epifluorescent micrograph (1000 ×) of
KJ stained with acridine orange



Without biosynthesis: acetate/perchlorate = 1:1 (mol/mol)



With biosynthesis: acetate/perchlorate = 2.9:1 (mol/mol)

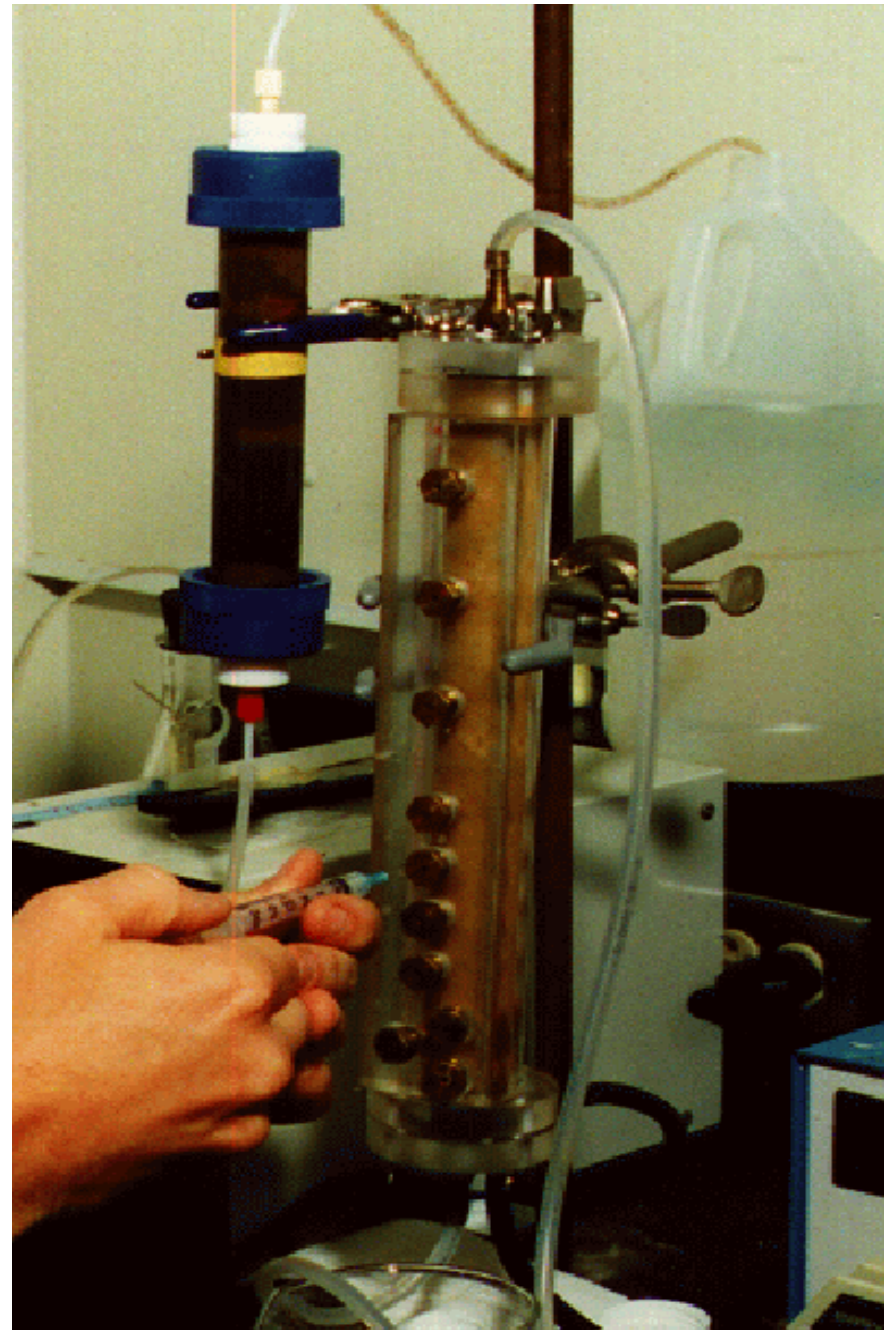


Perchlorate Degradation: Topics

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Acetate-Fed Sand Packed-bed Column Experiments

Sampling from the side
ports of the column



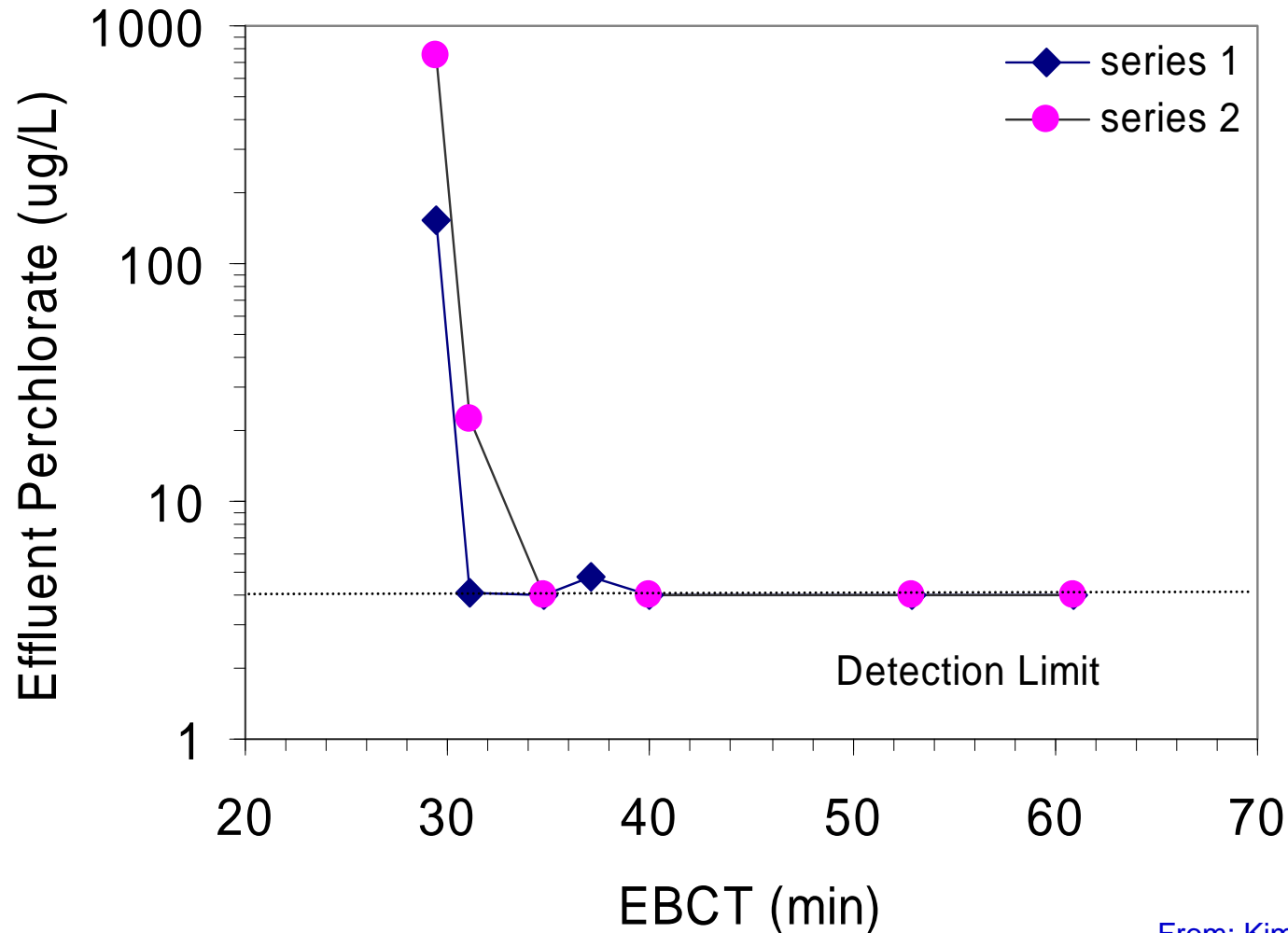
Acetate-fed Bioreactors

- **Columns:** 14 cm or 28 cm long.
- **Mixed cultures:** enriched on perchlorate (activated sludge samples)
- **Feed:** Artificial groundwater (AGW) containing 20 mg/L perchlorate and nutrients

Mixed cultures

(14 cm sand column)

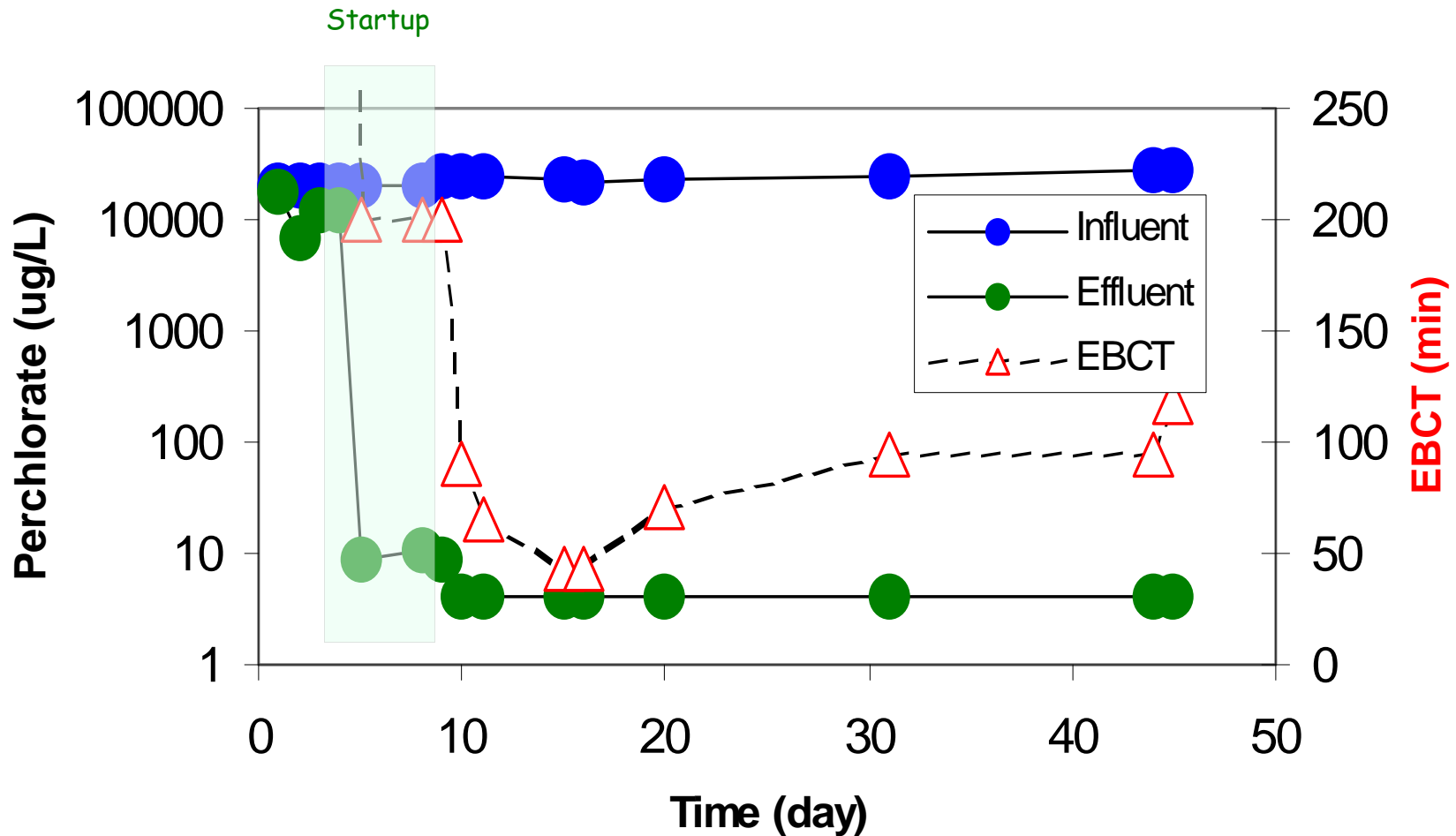
Critical
EBCT was
34 minutes.



Mixed cultures

(28 cm sand column)

Critical
EBCT was
~40 minutes.



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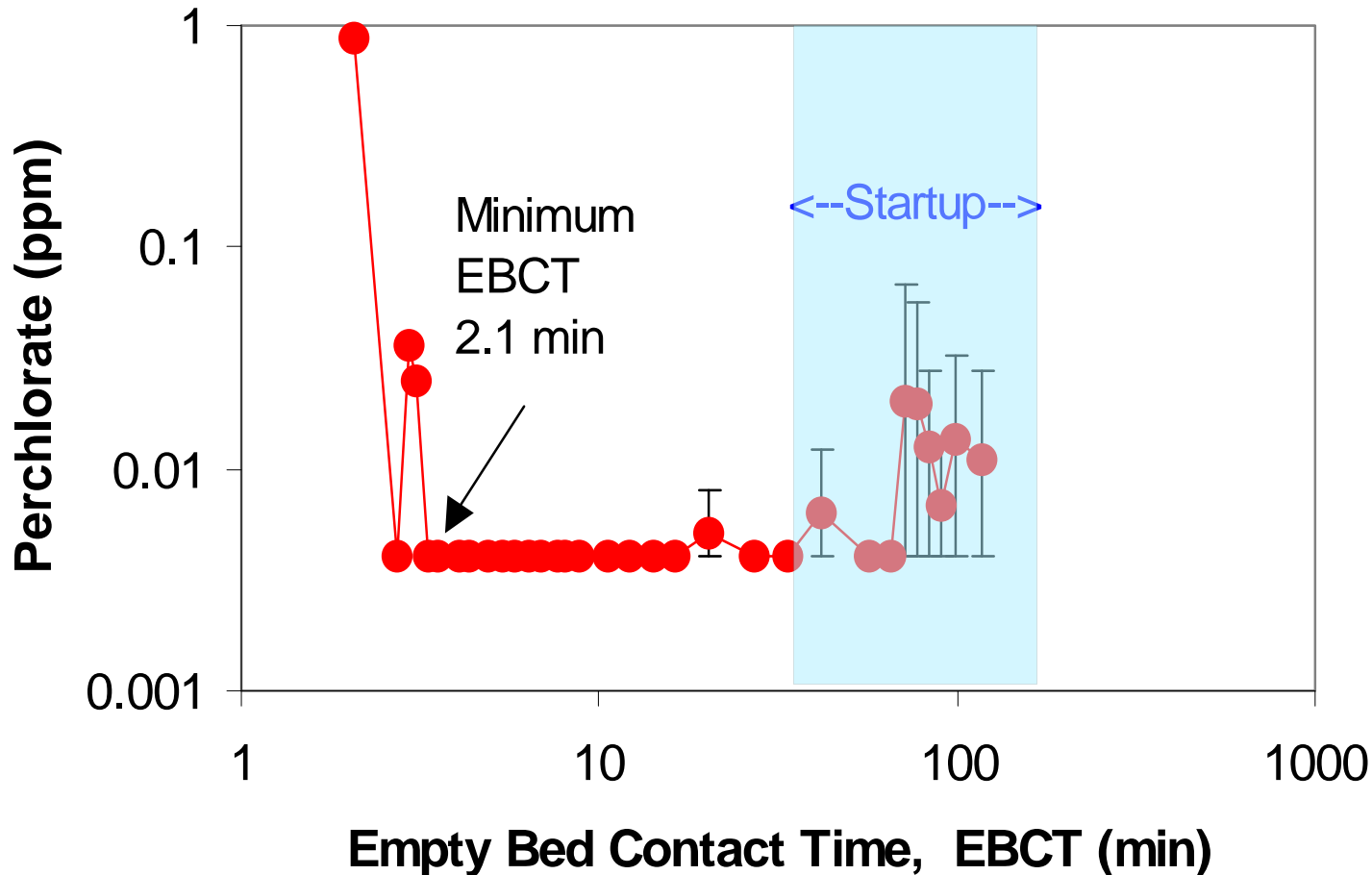
Acetate-fed Bioreactors

- **Columns:** 14 cm or 28 cm long.
- **Pure culture:** *Dechlorosoma* sp. **KJ**
- **Feed:** Artificial groundwater (AGW) containing 20 mg/L perchlorate and nutrients; water from the City of Redlands

Pure culture: KJ

(28 cm-sand column)

Critical EBCT
was ~2.1
minutes.

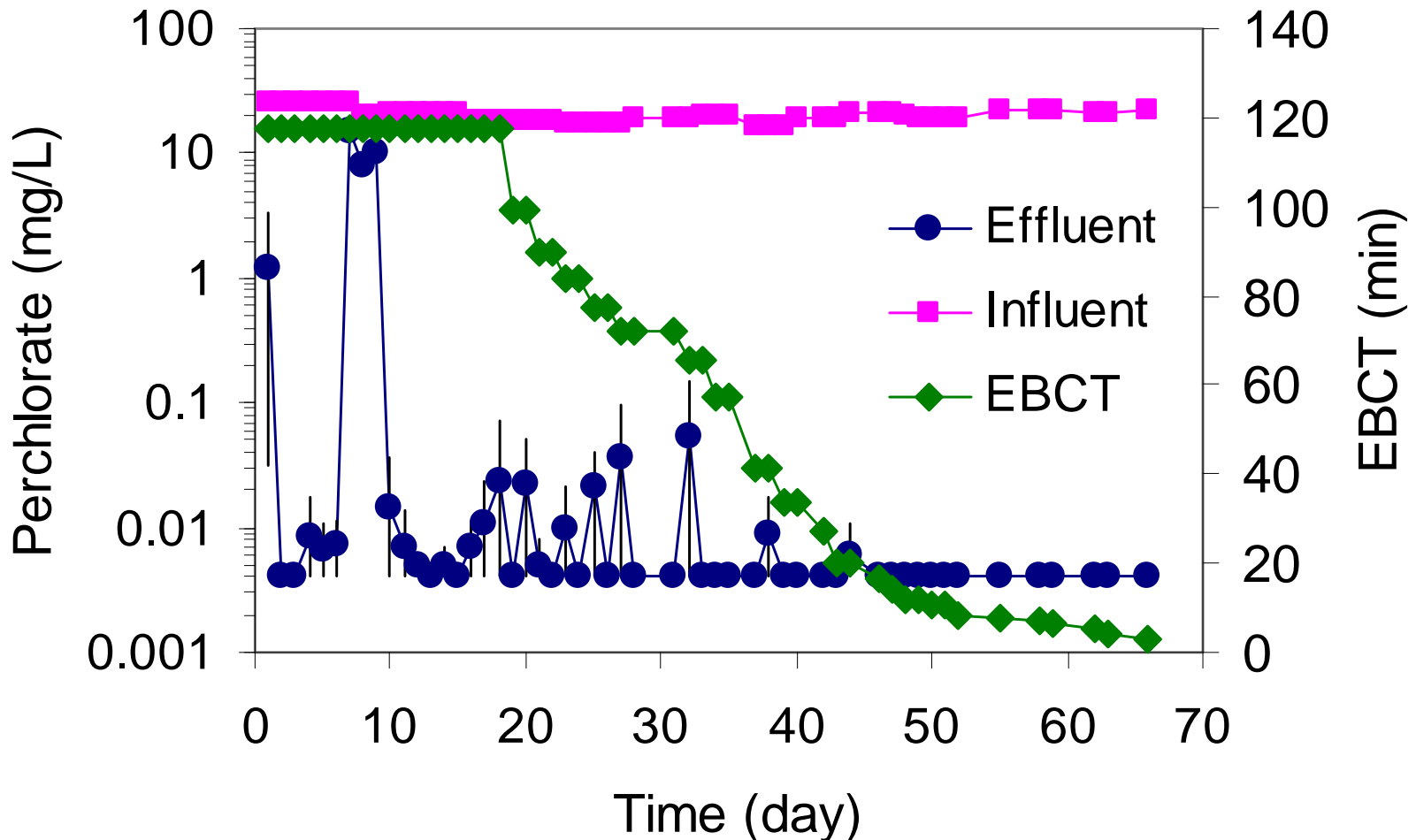


Pure culture: KJ

(28 cm-sand column)

As EBCT decreased:

Perchlorate was non-detectable for 84% of samples (n=147)



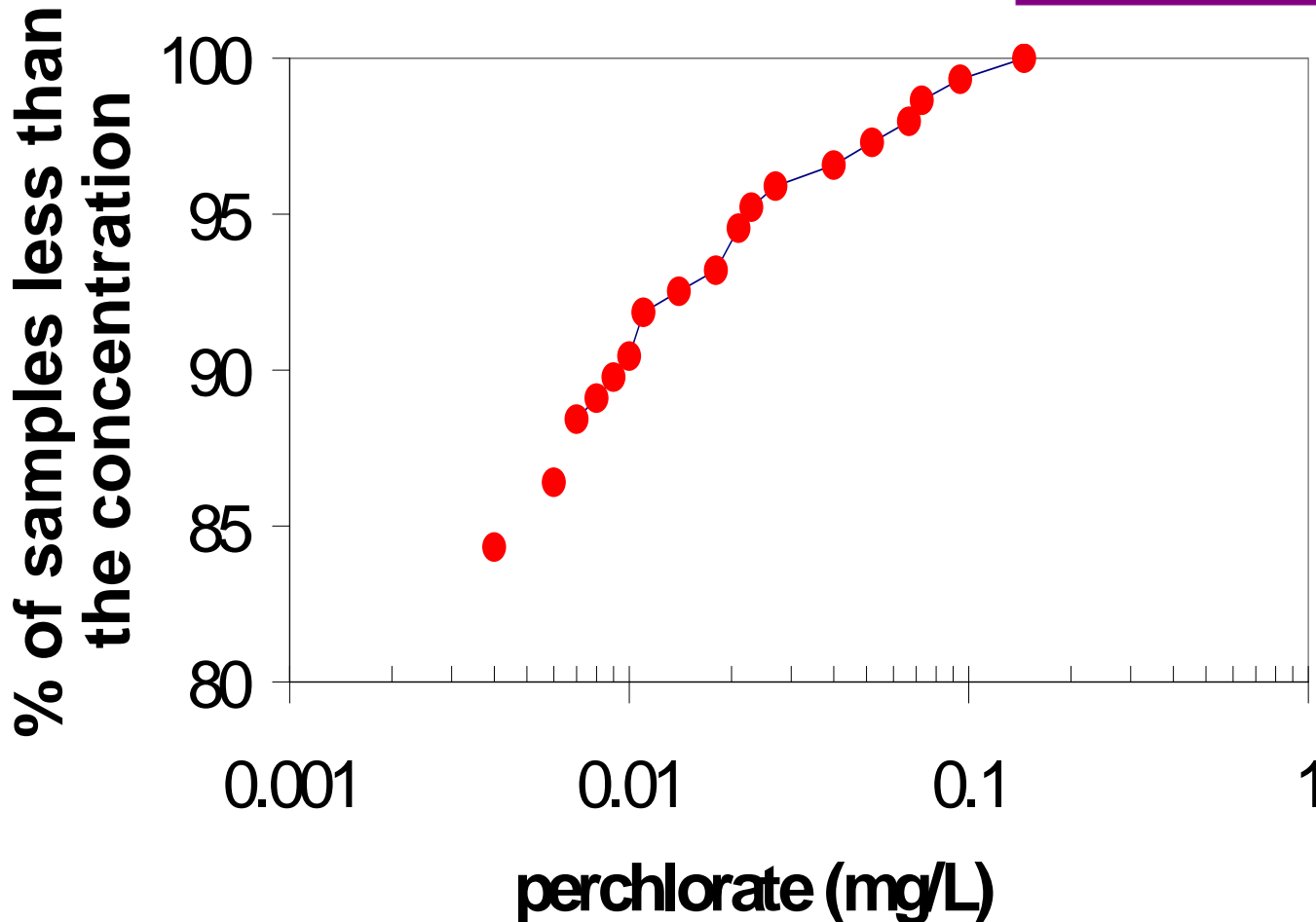
Pure culture: KJ

(28 cm-sand column)

**Reliability: After start up,
perchlorate removal was
stable at:**

95% of time: <23 ppb

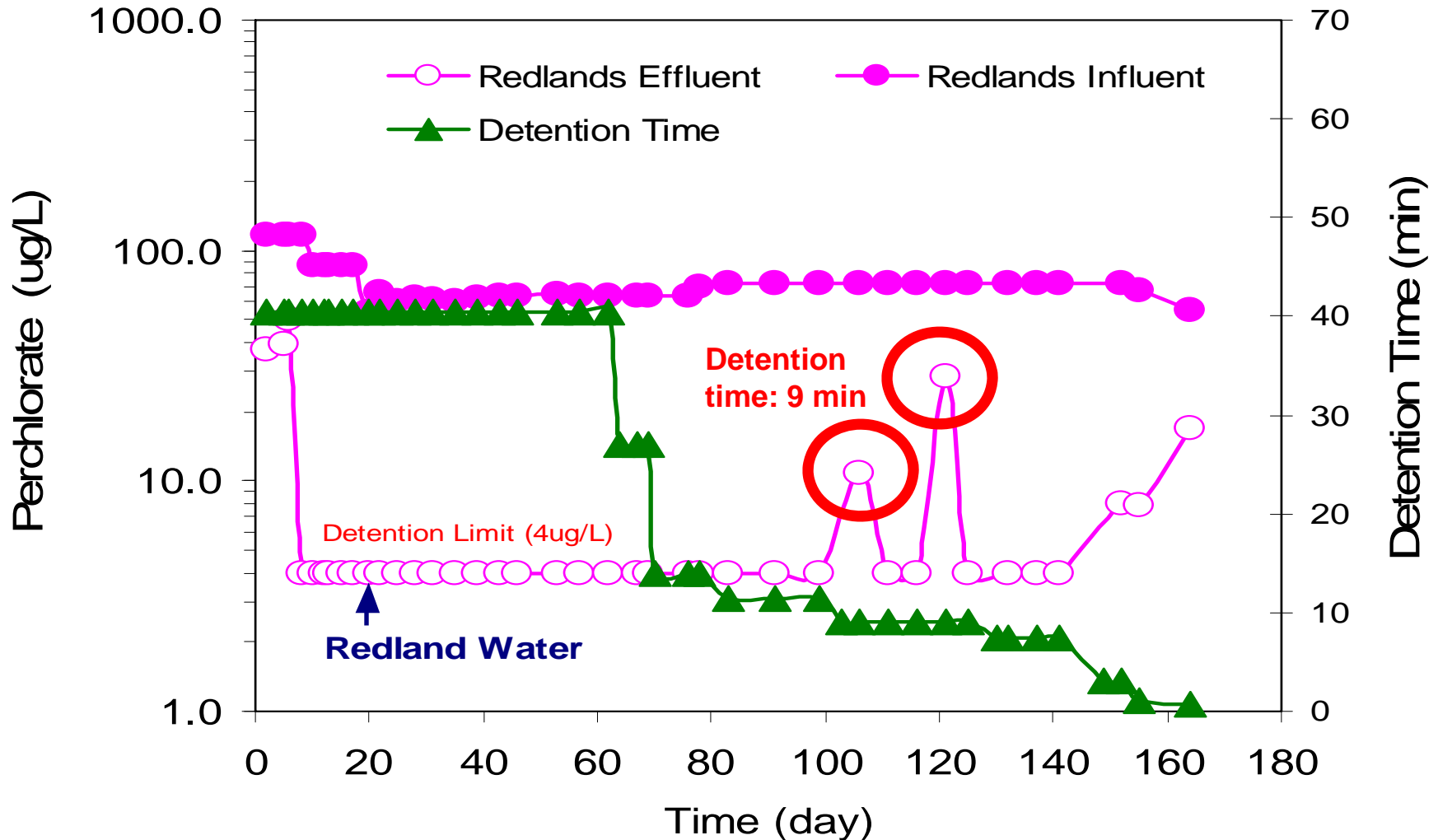
84% of time: <4 ppb



Redlands Water

Pure culture KJ, (28 cm-sand column)

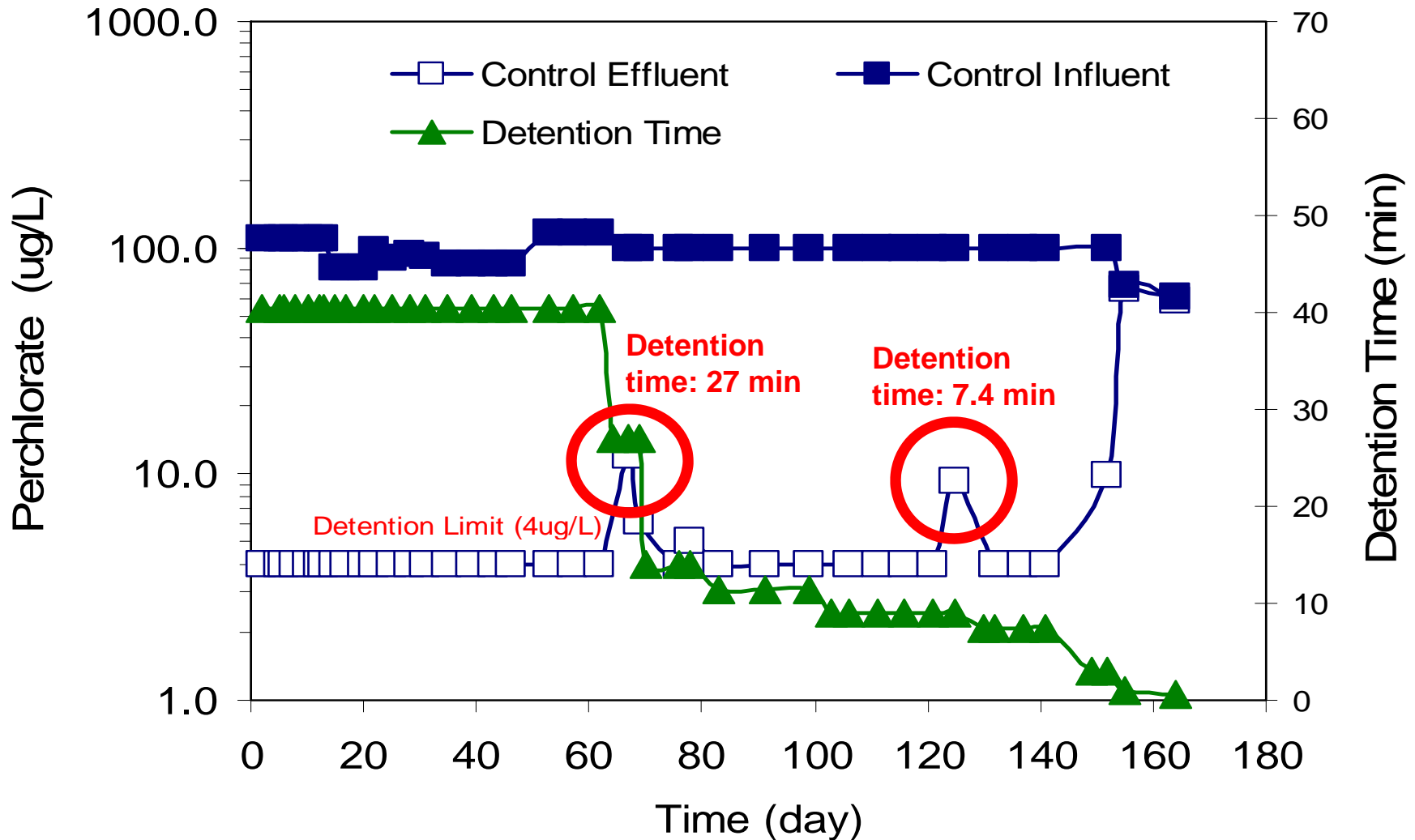
Perchlorate is completely removed using Redlands water (containing NO_3^-) for detention time >11 min.



Control w/o Nitrate

Pure culture KJ, (28 cm-sand column)

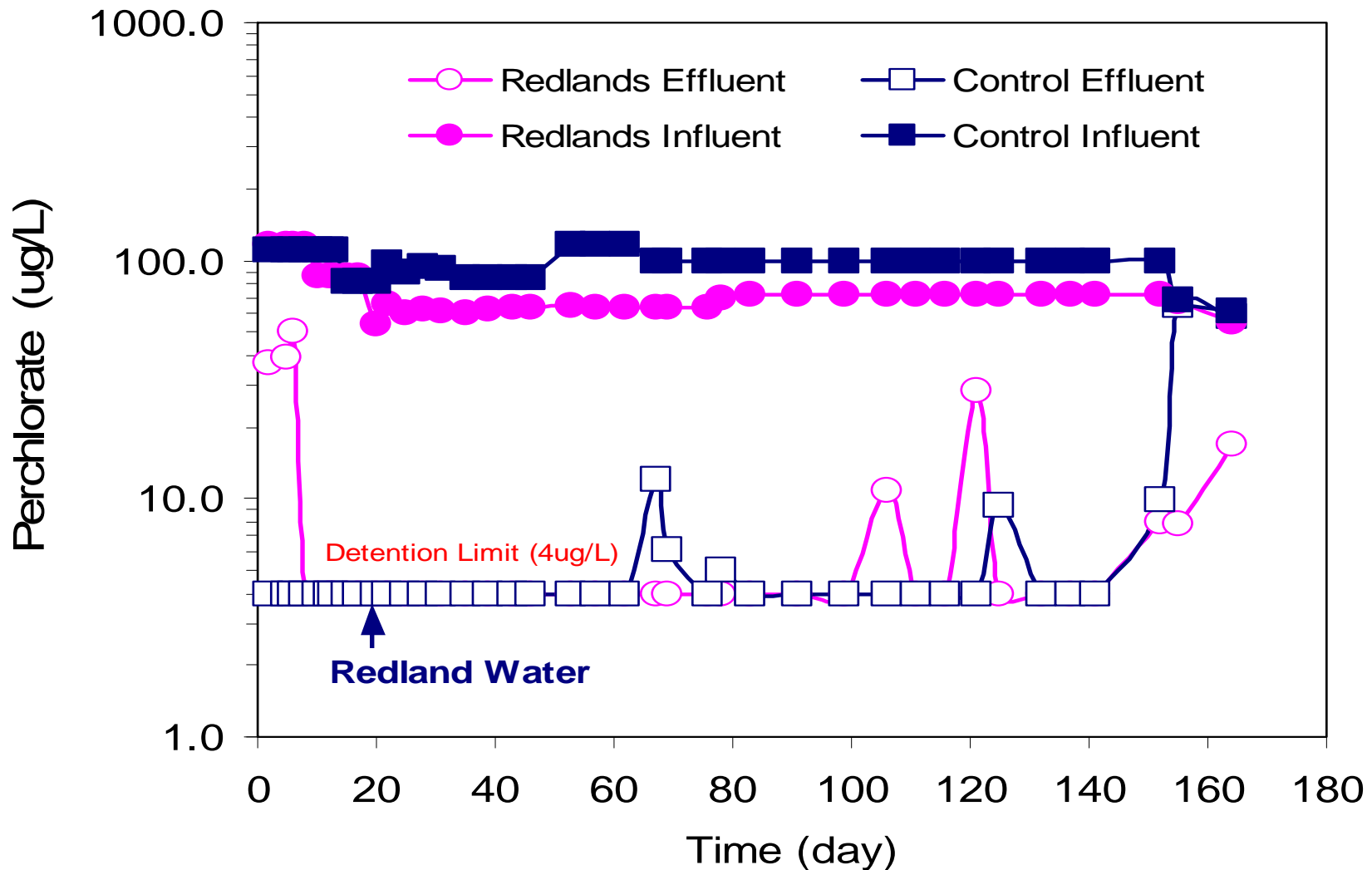
Perchlorate is completely removed using artificial groundwater (no NO_3^-) for detention time >8 min.



Effect of Nitrate

Pure culture KJ, (28 cm-sand column)

Perchlorate degradation is more stable for groundwater with nitrate.



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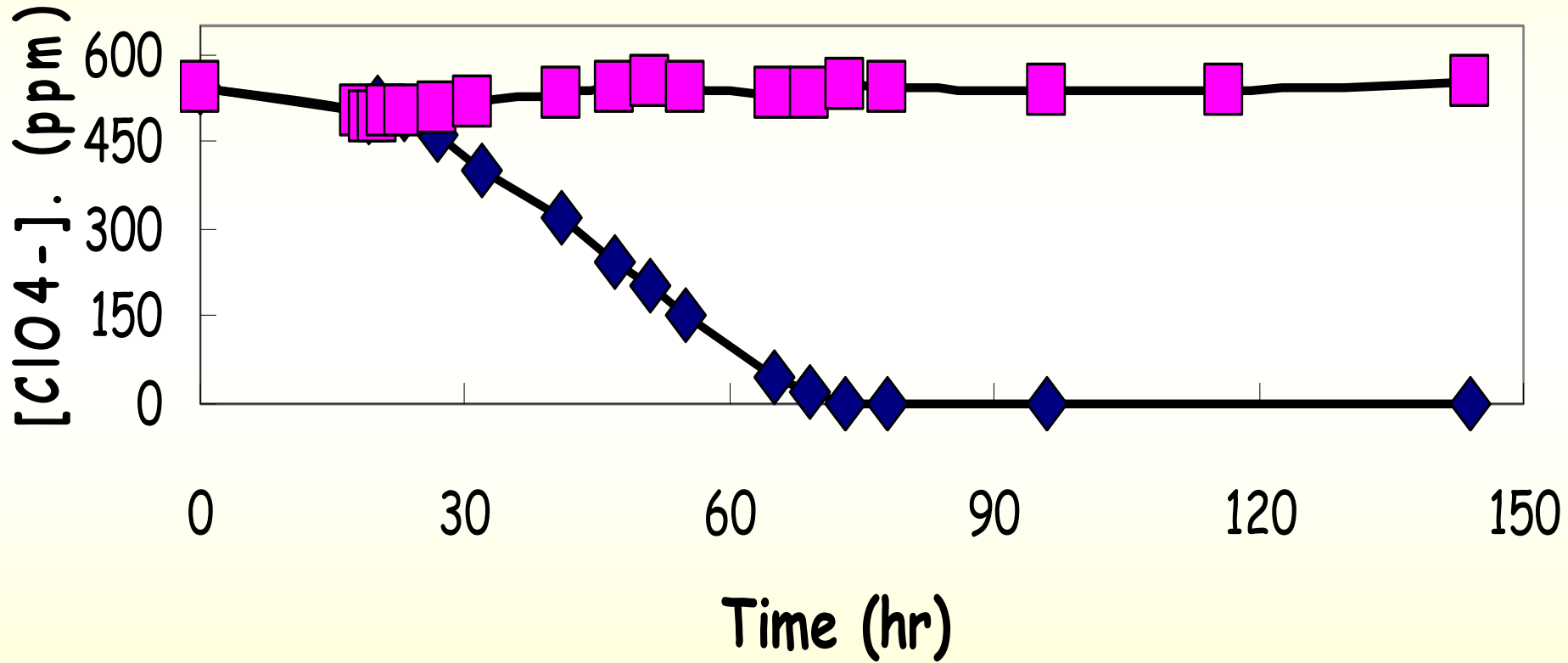
Problem...

When a reactor is shut down (either for maintenance or due to mechanical problems), perchlorate respiring bacteria can be exposed to high concentrations of DO.

High DO concentrations inhibit perchlorate reduction. Our question was:

How long can an anaerobic culture be exposed to oxygen before losing the ability to reduce perchlorate?

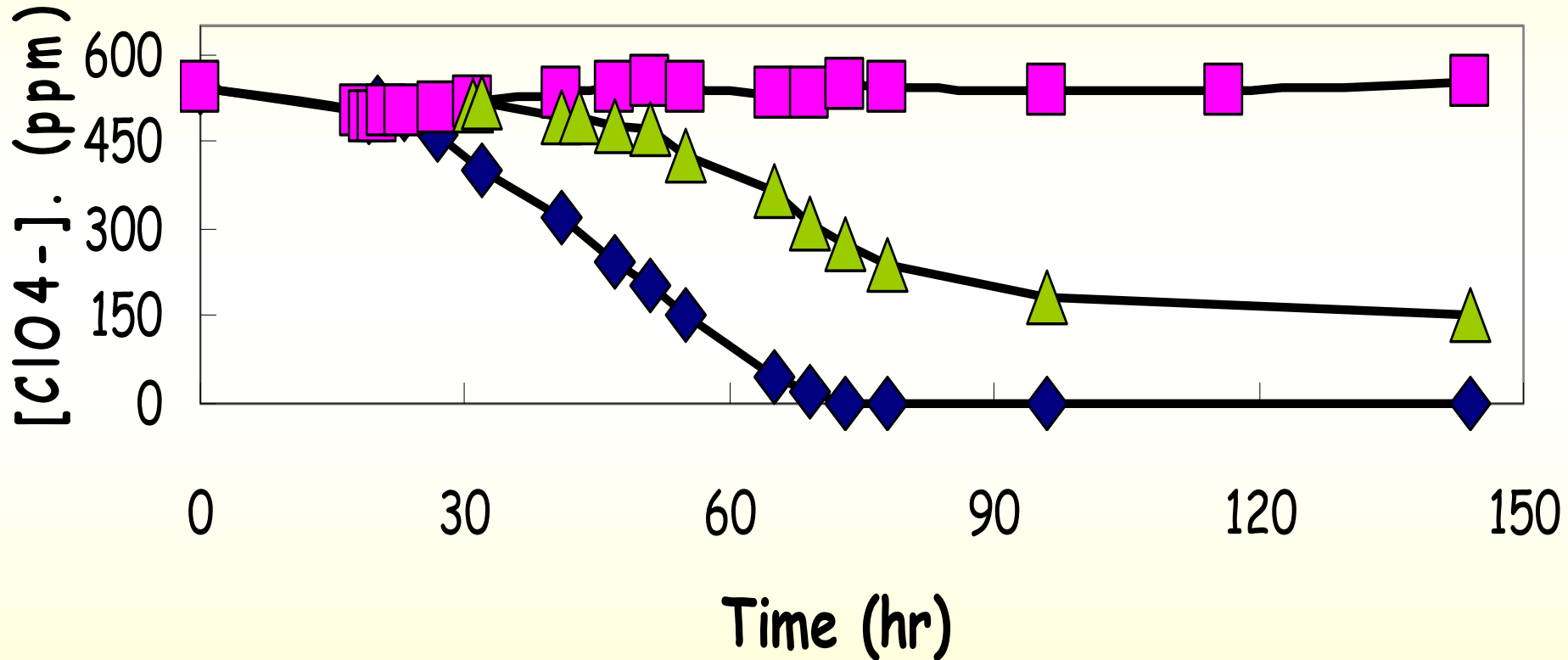
Oxygen Exposure



◆ Anaerobic Control

■ Ana-aer Control

Oxygen Exposure

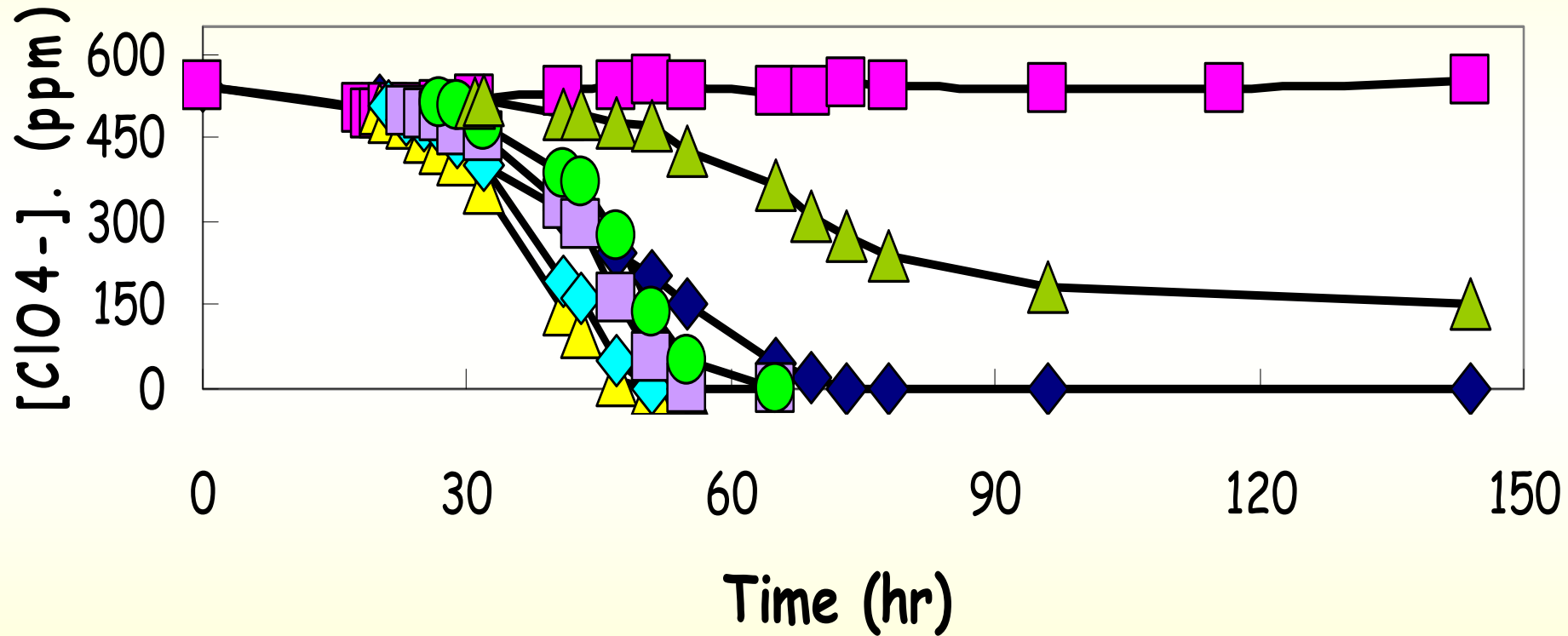


◆ Anaerobic Control

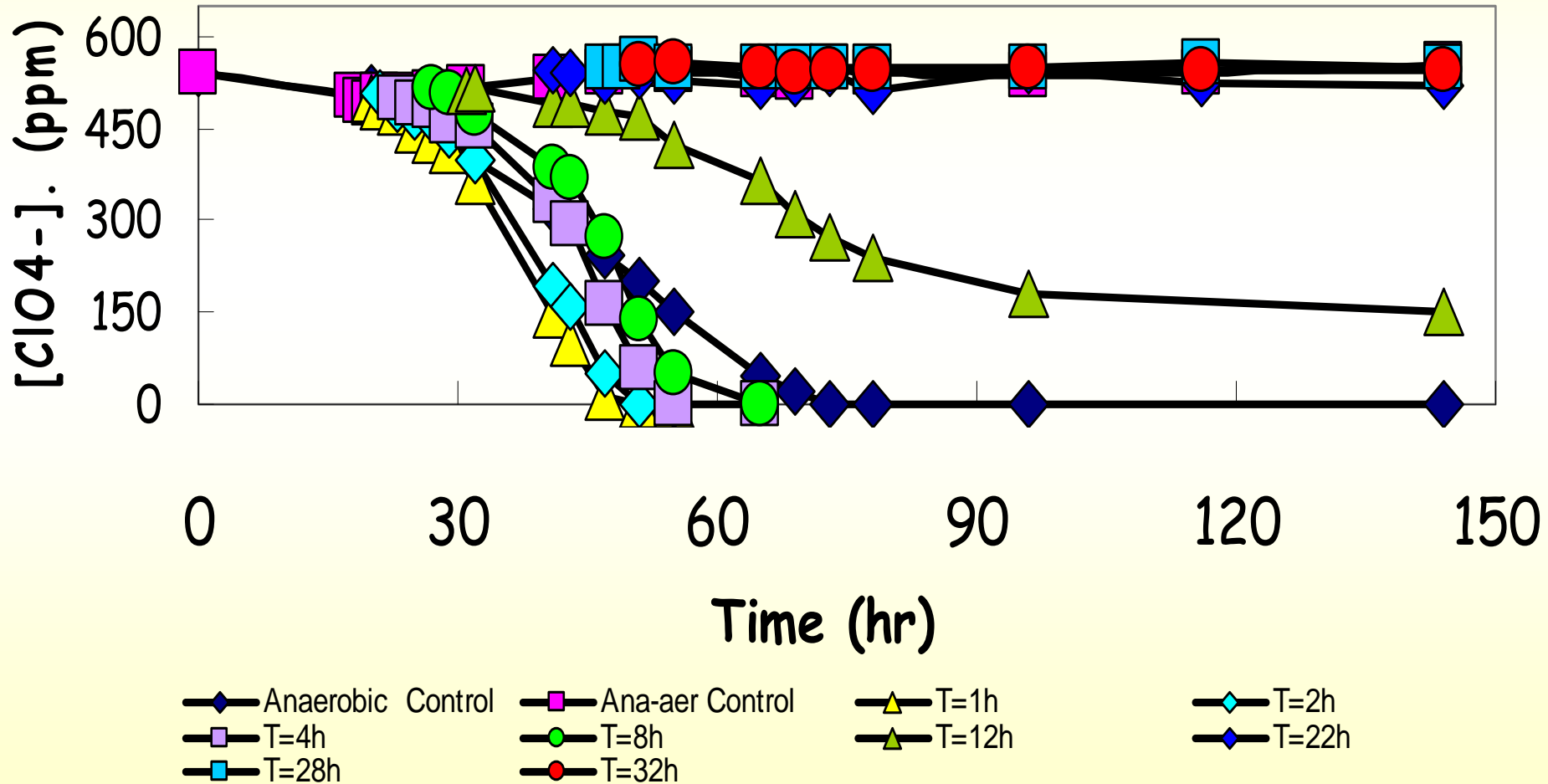
■ Ana-aer Control

▲ T=12h

Oxygen Exposure



Oxygen Exposure



Solution...

- There should be no problems for short exposure times (<12 h) of the bacteria to oxygen.
- Avoid oxygen exposure for longer shut down times → Do not drain the reactor. This will keep air out of the system to maintain the culture under anaerobic condition.
- Stop feeding the electron donor (this will lower pH) and nutrients (N, P).

CONCLUSIONS

- It is possible to obtain high perchlorate removal rates, with EBCT as low as 2.1 min, in packed bed reactors inoculated with pure culture (KJ). Mixed cultures require longer times (>11 min).
- Perchlorate removal was achieved in the laboratory using groundwater from the Redlands site, containing dissolved oxygen and nitrate.
- Perchlorate reduction by strain KJ can be recovered after exposure to oxygen for less than 12 hours.

ACKNOWLEDGMENTS

Funding

AWWARF: Project manager Frank Blaha

National Science Foundation

Penn State University

Thank You!

Q's and A's