All the single electrons: Do anaerobes put them on a ring?

Pamela Weisenhorn
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Introduction

- Humus is abundant in soils and sediments
  - Especially under anaerobic conditions

- Humic acids can serve as both electron donors or electron acceptors

- Ability to use humics as an electron acceptor is widespread

- Use of humics as electron acceptor can shift the CO2:CH4 emissions at the ecosystem scale
Question and Approach

Q: What effects do humics have on the metabolism of different functional groups of bacteria from a non-peat environment?

A: Used enrichments started during our group projects from Trunk River.

Focused on sulfate reducers, homoacetogens, and methanogens.
Experimental Design

Continued using same media conditions as before:
Basal anaerobic freshwater media with CO2 + H2
PLUS:
  Sulfate reducers: BES + sulfate
  Homoacetogens: BES
  Methanogens: Rif

Applied four treatments:
Control: No changes
Low humics: Humics extracted from ca. 1g of soil
AQDS: 10mM (AQDS acts as a humic analogue)
High humics: Humics extracted from ca. 5g of soil

Three replicates of each
Methods

Sulfide: Cline assay

Methane: GC

Acetate: HPLC

Minor products: HPLC

(Citrate, Formate, Propionate, Lactate Succinate)
Overview of variation throughout incubation

Enrichment:
- ACE
- MET
- SRB
- Neg

Treatment:
- Control
- Low humics
- AQDS
- High humics
Overview of variation throughout incubation
Overview of variation throughout incubation

Day 1

Day 5

Day 7

Enrichment
- ACE
- MET
- SRB
- Neg

Treatment
- Control
- Low humics
- AQDS
- High humics
One day after treatment initiation

Enrichment
- □ ACE
- ▲ MET
- ● SRB
- ◇ Neg

Treatment
- Red: Control
- Blue: Low humics
- Green: AQDS
- Violet: High humics
Five days after treatment initiation
Seven days after treatment initiation

Enrichment
- □ ACE
- ▲ MET
- ● SRB
- ◆ Neg

Treatment
- ■ Control
- ▲ Low humics
- ● AQDS
- △ High humics
Ordination results

- NMDS is best
- Some variation in initial conditions
- Overlapping variation between Day 5 and Day 7
- Metabolic products separate by enrichment type
- AQDS tend to separate from other treatments within enrichments
Sulfate Reducing Bacteria
Effects on sulfate reducers

- AQDS suppressed sulfide production; humic treatments had little effect
- AQDS initially suppressed formate consumption and led to an increase in formate
- AQDS suppressed succinate accumulation
Homoacetogens
Methane concentration

Control
Low Humics
AQDS
High Humics
Effects on homoacetogens

- AQDS suppressed acetate production; humic treatments had little effect
- Didn't have the same effect on formate as found in the sulfate reducing bacteria
- Also suppressed succinate formation
Methanogens
Acetate concentration

Control | Low Humics | AQDS | High Humics

mM
Effects on methanogens

- AQDS suppressed methane production; humics had little effect
- AQDS inhibited formate consumption and led to its accumulation.
- AQDS led to accumulation of lactate
Conclusions

- AQDS showed the predicted effects:
  - Reduced production of sulfide by sulfate reducers
  - Reduced production of acetate by homoacetogens
  - Eliminated production of methane by methanogens
- Neither humic treatment had a significant effect
  - Very pale – possibly low concentration or low aromaticity
- Some evidence for fermentations