Kinetics study of *Acidiphilium cryptum* in glucose media

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Outline

- Relevance to Acid Mine Drainage (AMD) prevention and remediation
- Materials and Methods
- Results
- Conclusions

AMD and bacteria

- AMD results from the oxidation of sulfide minerals– usually pyrite (FeS_2)
  - $FeS_2(s) + 3.5\ O_2 + H_2O$? $Fe^{2+} + 2SO_4^{2-} + 2H^+$ (1)
  - $2Fe^{2+} + 0.5O_2 + 2H^+ \rightarrow 2Fe^{3+} + H_2O$ (2)
  - $FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 16H^+ + 2SO_4^{2-}$ (3)
- Autotrophic iron oxidizing bacteria catalyze reactions (1) and (2)
  - Increase rates of oxidation by factor of 10^6
  - Inhibition of activity decreases amount of AMD

Current Techniques

- Oxygen barriers around mines and mine tailings
- Neutralizing AMD and precipitating metals via application of alkaline materials
- Bactericides

Most prevention and remediation techniques require continual maintenance and aren't very practical

Biostimulation

- AMD environments usually have a lot of other "stuff" besides acidophilic autotrophic bacteria
  - Heterotrophic bacteria
  - Fungi
  - Etc.

Stimulating biological activity of other organisms will create a deficiency of oxygen for autotrophs

- Only works if organisms stimulated ***grow faster*** or ***contain more biomass*** than organisms to inhibit

Acidiphilium Cryptum

- Found in populations of *At. ferrooxidans* (Harrison et al., 1980)
- Objective: Determine if biostimulation of *A. cryptum* would be a significant component in the inhibition of autotrophs in AMD environment
  - How fast does *A. cryptum* grow?
    - Kinetics studies will elucidate growth rates and substrate depletion rates

Objective

- Determine parameters of rate expressions based on Monod equation
  - Yield [Y(# bacteria / mg Oc)]
  - Maximum specific growth rate [μ_{max}(hr^{-1})]
  - Rate constant [K_{oc}]
- Two differential equations were used to determine parameters
  - for biomass approximation through optical density (OD) and most probable number (MPN) data

$$\frac{d[Ac]}{dt} = \mu_{max}[Ac]\left(\frac{[Oc]}{K_{oc} + [Oc]}\right)$$

  - for substrate depletion approximation through total organic carbon (TOC) data

$$\frac{d[Oc]}{dt} = \frac{-\mu_{max}}{Y}[Ac]\left(\frac{[Oc]}{K_{oc} + [Oc]}\right)$$

Materials and Methods

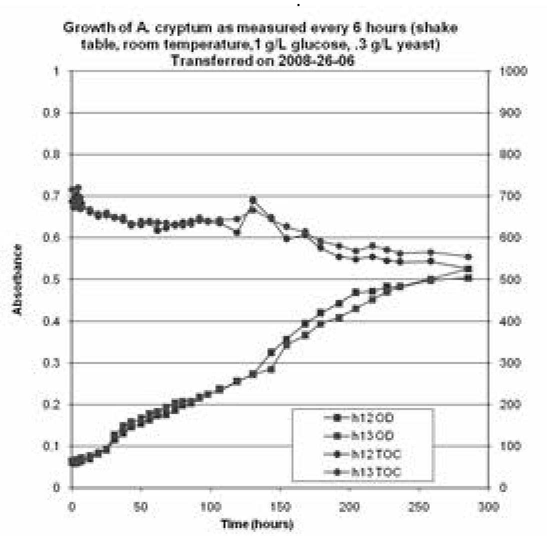
- Grew cultures in media of glucose (or sucrose), yeast extract, and salts
- Sampled at regular intervals
  - Total organic carbon (TOC) measurements were used to determine rates of substrate depletion
  - Used most probable number (MPN) analysis to determine approximate # bacteria/ mL
  - Optical density analysis was used to assess increases in turbidity
    - Paired with MPN data to make a standard curve

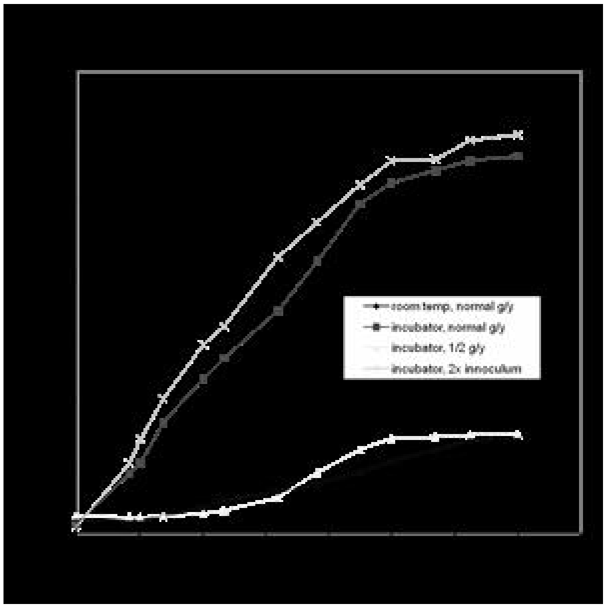
Initial Results and problems in working with *A. cryptum*:

1st Kinetics Experiment

•**Problem:**

- **Growth curve does not follow trend of Monod equation…**





Solution:

•Modify conditions in which bacteria is grown

- **Glucose concentration**
- **Temperature**
- **Innoculum content**

•Temperature plays significant role in growth rate of *A. cryptum*

•Finding pivotal in *A. cryptum* research thus far

Results (cont'd):

3rd Kinetics Experiment

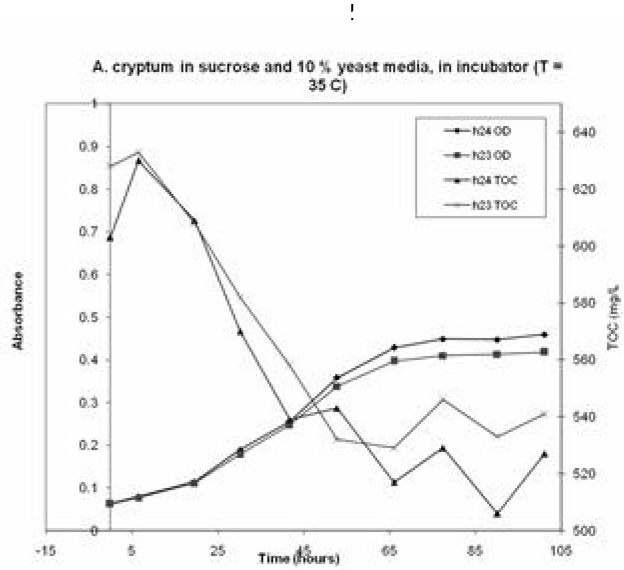
•Media

- 1 g/l sucrose
- .08 g/L yeast extract

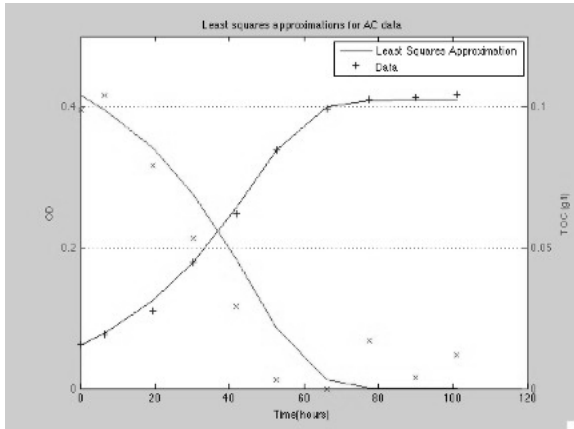
•Sampled at in intervals of 12-24 hours

•Limited data set used for non-linear regression analysis

•Data for sucrose as carbon substrate proved to be best fitting

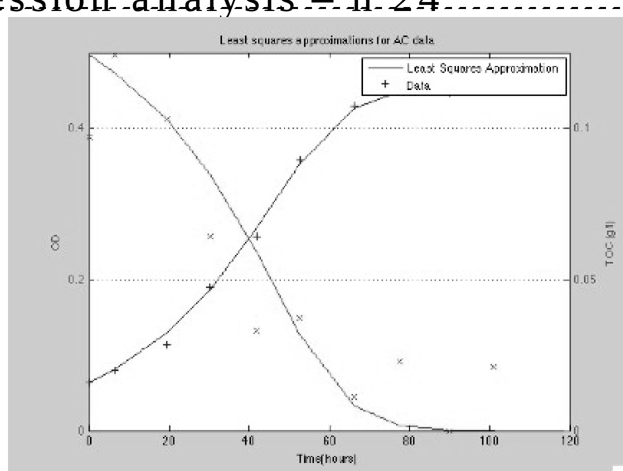


Regression analysis -h23



Least squares approximation of OD and TOC data from experiment h23 ,for optical density $R^2 = 0.999869$, for TOC $R^2 = 0.996472$

Regression analysis – h 24



Least squares approximation of OD and TOC data from experiment h24 ,for optical density $R^2 = 0.994753$, for TOC $R^2 = 0.999832959$

Parameters

Parameter	Experiment h23	Experiment h24	*At. ferrooxidans*	*L. ferrooxidans*
μ_{max} (hr^{-1})	.0521	.0452	0.11	0.04
Y (#bacteria/ mg TOC)	5.2111E+07	4.897E+07		
K_{oc} (mg/L)	.0478	.0255		

•Relative to its primary "competitor" *At. Ferrooxidans*, *A. cryptum* is slow growing

•For prevention or bioremediation strategies, this might require a lot more biomass to be effective

Conclusions

- *A. cryptum* is slow growing
  - Success of biostimulation may depend on large biomass
- Growth rates sensitive to temperature
- TOC may not be the best measure for glucose or sucrose consumption
  - Need to develop a way to measure individual chemical components
- May not be the best model heterotroph for AMD environments

Future Research

- Further investigation on the effects of temperature on growth rates
- Mixed (vs. pure) culture kinetics
- Growth rates of other heterotrophic microorganisms
  - Other bacteria, fungi, etc

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References

Harrison, A. P., Jarvis, B. W., & Johnson, J. L. (1980). Heterotrophic bacteria from cultures of autotrophic *thiobacullus ferrooxidans*: Relationships as studied by means of deoxyribonucleic acid homology. *Journal of Bacteriology, 143*(1), 448-454.

Marchand, E. A., & Silverstein, J. (2003). The role of enhanced heterotrophic bacterial growth on iron oxidation by acidithiobacillus ferrooxidans. *Geomicrobiology Journal, 20*(3), 231-244.