Analysis and Modeling of Urea Hydrolysis by Ureolytic Bacteria for Use in Subsurface Calcium Carbonate Precipitation

Christopher Neighbor, Megan Kaufman, Rick Colwell

Oregon State University, College of Engineering

Subsurface Biosphere Initiative
Research and education focused on life below Earth's surface.

U. S. Department of Energy Office of Science
Subsurface Biogeochemical Research

Abstract

The research was conducted to model the kinetics of urease activity in various bacteria. The organisms were grown in a controlled flask environment which was sampled at various time points for pH, ammonia, urea, and cell concentrations. From this data a quantitative analysis of the rate of urea hydrolysis by the organisms was determined using an enzyme kinetics model.
The values found for urease activity will aid in further testing of the organisms to model *in situ* ureolysis within aquifers.

Intro

^{90}Sr is a radioactive element which can replace calcium in the bones if consumed through drinking water. Groundwater which has been contaminated with ^{90}Sr can be remediated through the use of cells which cause calcium carbonate precipitation and precipitation of Sr.
A strain of *Pseudomonas aeruginosa* has been altered to make urease which then hydrolyzes urea to produce ammonium and bicarbonate. The ammonium induces desorption of Sr and Ca from the soil minerals and the bicarbonate promotes $CaCO_3$ to precipitate onto nearby minerals encasing the ^{90}Sr. The microbe can be used in a porous media reactor in the lab to simulate and optimize the calcium carbonate precipitation that would occur in an aquifer. Various other ureolytic organisms will also be tested.
The ureolytic organisms which are being tested are *Sporosarcina pasteurii* (S.past), *E.coli pURE*, and *Pseudomonas aeruginosa AH298urease* as well as *Pseudomonas aeruginosa AH298* and *E. coli DH5α* as negative control organisms.

Objective:

To determine enzyme kinetic parameters for the urease of selected model bacteria that can be used in experiments to investigate the urea-mediated calcium carbonate precipitation in aquifers.

pH Modeling and Effect on Activity

The enzyme activity of urease has been found to be strongly related to the pH of the solution in which it is acting[1]. In order to determine the V_{max} of the cell's urease, the pH of the solution in the flasks for the different organisms was modeled.

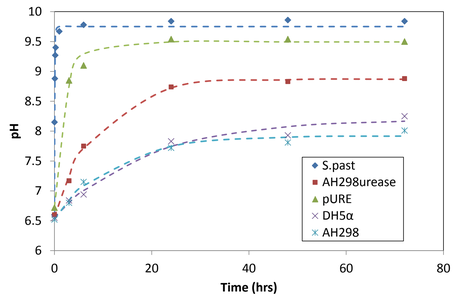


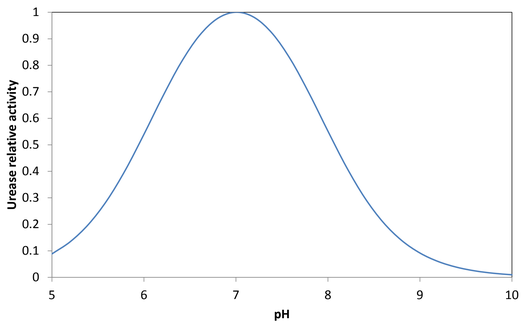
Fig.1 The recorded pH data of the flasks is represented by the points and the model for each flask is represented by the dashed line.

The constants were found using the following equation and least squares fitting $pH = -C_1 e^{-kt} + C_2$

C_1, k, and C_2 are calculated pH model constants

	S.past	pURE	AH298urease	DH5α	AH298
C_1	9.75	9.49	8.87	8.20	7.92
k (1/hr)	13.185	0.436	0.115	0.053	0.087
C_2	3.12	2.76	2.31	1.65	1.40

pH Effect on Urease Activity



$$f(pH) = \frac{1}{1 + \frac{10^{-pH}}{K_{es,1}} + \frac{K_{es,2}}{10^{-pH}}}$$

Fig. 2 This curve represents the relative activity of urease at the pH values which occurred during the flask study. The of urease is strongly related to the pH

The K_{es} values determine the relative curve and optimum pH of ~7 for urease

$K_{es,1}$(mol/L)	7.57E-07
$K_{es,2}$(mol/L)	1.27E-08

These K_{es} values were taken from an article[1] on urease kinetics and were considered constant for all organisms.

Determining Kinetic Parameters

From the urea concentrations at different time points, the rate at which the urea is hydrolyzed can be determined using the following model equation. The model includes non-competitive product inhibition by the ammonium and the functional activity of the urease at a recorded pH.

$$V = \frac{V_{max}[S]}{K_m + [S]} * \frac{1}{1 + \frac{[P]}{K_p}} * f(pH)$$

V_{max} represents the max rate of urea hydrolysis for a certain enzyme concentration, K_m indicates the substrate affinity for the enzyme, and K_p calculates non-competitive product inhibition.

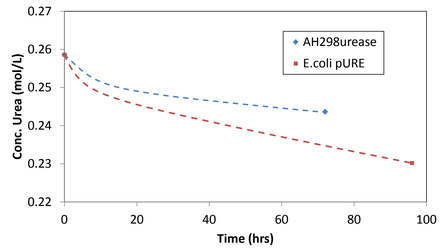


Fig. 3 The urea concentration was measured using HPLC and fit by the enzyme model which is plotted as a dashed line.

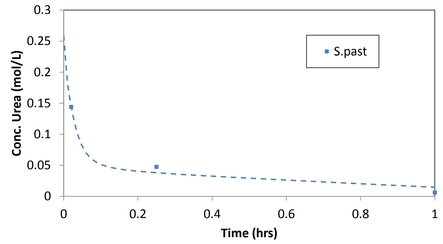


Fig. 4 The urea hydrolysis of *Sporosarcina pasteurii* was found to be significantly larger than for the other organisms. The product concentration was determined stoichiometrically and was used to determine the K_p value.

	S. past	pURE	AH298urease
V_{max} (mol/L*hr)	7.987	0.0026	0.00093

K_m (mol/L)	0.003
K_p (mol/L)	0.143

V_{max} is given for the urease at 7pH and 20°C
The K_m and K_p values are assumed to be constant amongst the organisms. The K_m value represents was taken from literature[1] and is found to not have a significant effect at these operating substrate concentrations.

Conclusions:
From these data and modeling a quantitative value for the rate of urea hydrolysis for various ureolytic bacteria was determined. Further urea data acquisition is planned and will allow for more accurate analysis using the enzyme model.

Acknowledgements:
DOE Subsurface Biogeochemical Research Program,
The calcium carbonate precipitation team from MSU, INL, and OSU

References:
[1] M. Fidaleo, et al. (2003). Kinetic Study of Enzymatic Urea Hydrolysis in the pH Range 4-9. *Chemical and biochemical engineering quarterly* , 17(4) 311-318.