MICROBIALLY-INFLUENCED CORROSION IN AN UNSATURATED REPOSITORY

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Microbes are known to be involved in many types of corrosive processes

* Pitting
* Hydrogen embrittlement
* Tubercle formation
* Dissolution

Why microorganisms are involved in the corrosion process

* They are ubiquitous
* Beijerenck - "Everything is everywhere, nature selects"
* They are small
* They are metabolically versatile
* They withstand extreme environmental conditions
  Some require "extreme" environmental conditions
* Some microbial processes are destructive to materials
  but what is the alternative?

Common Microbial Metabolic Groups Involved in MIC

Sulfate-reducing bacteria (SRB) - produce H_2S, consume H_2

Iron oxidizing bacteria - Fe(II) —> Fe(III) cause rusting

Polymer producing bacteria - many genera under many conditions form biofilms

Acid producing bacteria -

- Inorganic acids
  - *Thiobacillus* sp.
- Organic acids
  - Fermentative heterotrophs
  - Fungi

Biofilm Development

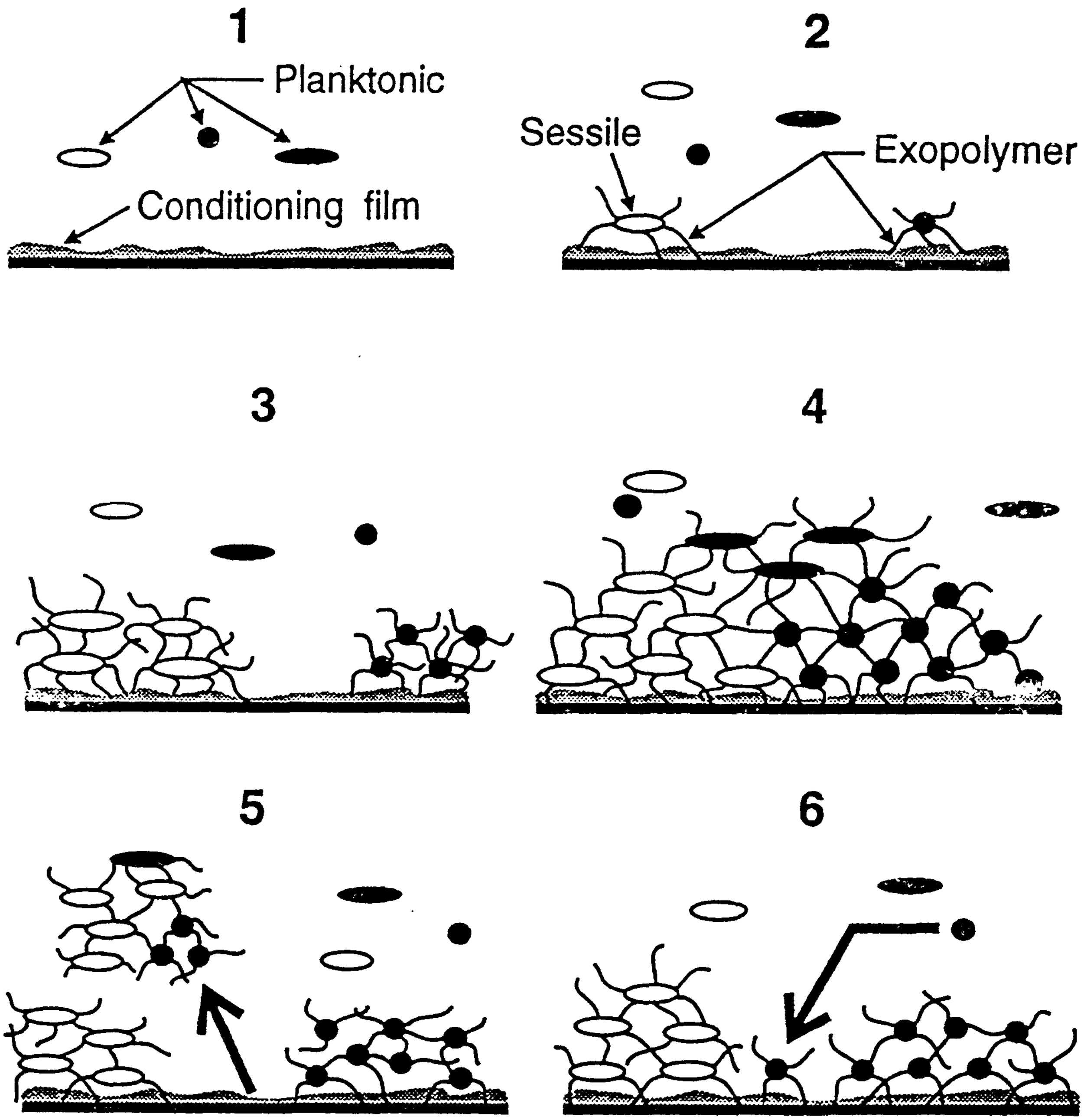
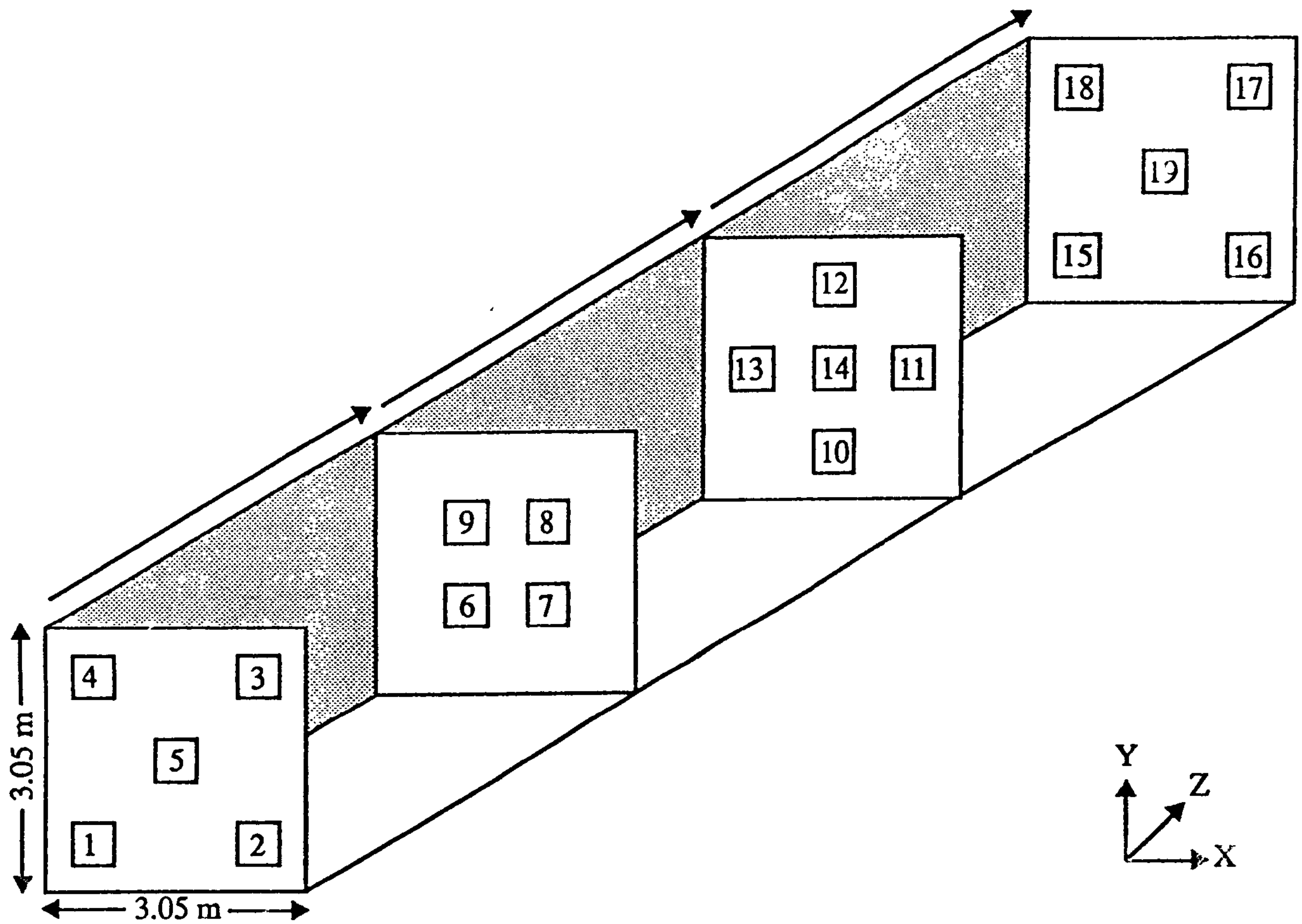


Figure 3-1. Schematic showing successive stages in biofilm development

From Geesey 1994 NRC Report



18
17
19
15
16
12
13
14
11
10
9
8
6
7
4
3
5
1
2
3.05 m
3.05 m
Y
Z
X

Tunnel Survey and 3-D Mining for Spatial Heterogeneity

1. Microbial communities were heterogeneous on scales from approximately 1.5 m to > 10 km.

2. Both Gram positive and negative bacteria were found, actinomycetes and few fungi.

3. Even though enrichments for autotrophs capable of NH_4^+ and S^o oxidation were prepared, only heterotrophic bacteria were found.

4. These organisms were like the well documented survivors of the marine environment and soil, i.e., *Pseudomonas* sp. and *Arthrobacter* sp.

Starvation-Survival of Subsurface Isolates

1. Typical subsurface microorganisms show individual patterns of survival in an artificial pore water solution.

2. Placed on crushed rock and compared to planktonic microorganisms, some survive much better.

3. Many unknown factors (such as surface effects) must influence the survival of microbes for very long periods of time.

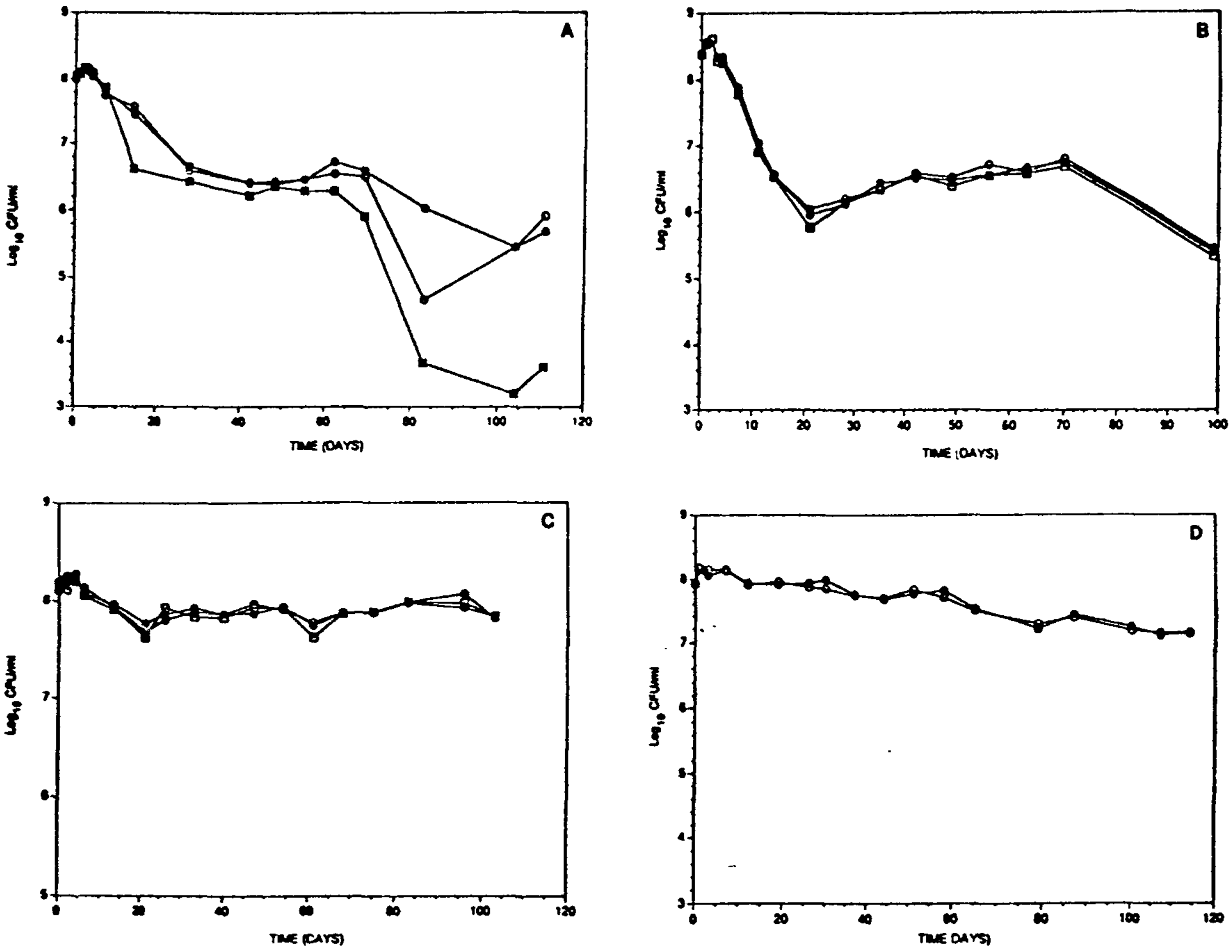


Fig. 1. Starvation-survival curves demonstrating changes in viable cell counts with time. Viable cells were recovered on R2A (○), D2A (●), ampicillin-containing R2A (■), or nalidixic acid-containing R2A (□). A = isolate N05R4; B = isolate N05R5a; C = isolate N05R8; D = isolate N05R7.

Sampling

Sampling with minimal perturbation is the goal

Great strides have been made in the DOE Subsurface Science Program to develop low/no contamination drilling (argon, air) - provides for an extensive vertical profile

Mining by Alpine miner (or other device) followed by hand sampling provides for minimally impacted samples and large quantities of rock can be removed for 3-D comparison, geochemical analyses, and replication.

Storage and Perturbation of Samples

1. Microbial culturable numbers increase, but not the total or direct counts.

2. The microbial diversity often diminishes as some "weed" species out-grow.

3. Individual microbes may be found solely at the initial work-up, throughout storage, or only after perturbation and storage - not only does out-growth take place, but new organism types are "resuscitated" by the process as well.

4. The change in microbial communities probably begins at the time of sampling, but is documented within the first 24 hrs.

5. Many microbes appear to remain dormant as vegetative cells under starvation conditions for long periods of time. Some may be capable of immediate reactivation yet others require environmental factors that favor their slower reactivation (resuscitation).

COMPARISON OF PHYSICAL AND COMMUNITY LEVEL ANALYSIS OF RAINIER MESA AND YUCCA MOUNTAIN, NTS

SITE	RAINIER MESA, NTS			YUCCA MOUNTAIN, NTS		
SAMPLE	ROCK	CLAY	WATER	YM	YC	QA
% MOISTURE	ND[c]	ND	ND	3.9	4.3	4.3
pH	ND	ND	ND	8.0	8.7	8.8
CULTURABLE COUNTS[a]	5.09	4.41	1.40	4.65	3.58	5.55
DIVERSITY	2.04	2.33	1.95	2.06	2.00	1.30
EQUITABILITY	0.76	0.81	0.81	0.68	0.72	0.53
% EPS PRODUCERS[b]	30.6	43.5	31.5	4.25	2.7	8.2
IRON-OXIDIZER MPN[f]	.54	BLD[e]	7.0	2.29	BLD	1.28
SULFATE REDUCER MPN[f]	2.2	BLD	BLD	NC[d]	NC	NC

KEY:

[a] log transformed organisms/ml for Rainier Mesa samples.
log transformed organisms/g dry weight for Yucca Mountain samples.

[b] determined by morphology on R2A.

[c] test not done.

[d] test not completed.

[e] below levels of detection.

[f] organisms/ml for Rainier Mesa samples.
organisms/g dry weight for Yucca Mountain samples.

EPS PRODUCTION ON A VARIETY OF CARBON-CONTAINING MEDIA

SAMPLE	YM	YC	QA
1% PTYG	33%	50%	30%
R2A	83%	50%	80%
LURIA AGAR	58%	67%	70%
CASAMINO ACIDS	67%	50%	70%
CITRATE	8.3%	50%	30%
ACETATE	17%	83%	30%
STARCH	50%	67%	70%
TWEEN 80	42%	33%	30%
1% GLUCOSE	58%	83%	100%
1% GALACTOSE	50%	50%	100%
1% GLUC./GALACTOSE	58%	50%	100%

NOTE: The isolates tested in this analysis represent the following percentages of organisms in the community that were EPS-producers on one or more types of media:

YM: 17.6%
YC: 30.0%
QA: 50.0%

IMPLICATIONS

I. Heterogeneity

A. A wide variety of microbes is present in ashfall tuff.

B. Metabolic types involved in MIC have been demonstrated.

C. Geochemistry and geology do not predict microbial numbers nor metabolic types.

D. The natural rock environment supports organisms capable of both survival and growth when limiting nutrients are supplied.

II. Survival

A. Tested isolates appear to survive well in pore water and the time is probably lengthened by factors such as the presence of surfaces and consistently oligotrophic conditions.

B. The best microbial survivors come from oligotrophic environments such as the open ocean and subsurface rock.

C. We now believe that these and other subsurface bacteria have been in place for millions of years; even the vadose supports the survival of large communities of microbes.

D. A recent publication in AEM documents the increased survival of microbes through the desiccation resistance of EPS.

E. Areas of rock may be sterilized but surrounding rock serves as inoculum for recolonization when conditions are supportive.

III. Sampling and Perturbation

A. The goal must be to minimize the impact by exogenous nutrients and microbial contamination.

B. Communities change when perturbed - important to the backfill process.

C. After perturbation, more EPS producers are found, more metabolically versatile bacteria predominate, and the generation time of isolates becomes shorter.

D. Any microbiological testing needs to be done on or near the site and ASAP.

IV. EPS and Biofilms

A. EPS producers and biofilms are highly implicated in MIC.

B. There are reports that EPS alone may be corrosive.

C. Its production is stimulated by perturbation and the communities are enriched for these types after perturbation.

D. EPS may serve as a carbon source for future bacterial growth - little is known of its presence and persistence in the subsurface.

V. Transport

A. Water and microbes can be transported through fractures and macropores, even in zeolitized tuff.

B. Microbes only require a thin film of water for growth.

C. Even vadose rock has enough water for microbial survival and growth.

Future Interests

EXOPOLYMER PRODUCTION AND BIOFILM EFFECTS

1. Environmental conditions which stimulate/retard EPS production, including nutrient additions, surfaces, environmental shock.

2. Comparison of EPS composition obtained from rock and representative isolates - collaboration with Dr. Anders Sonneson (Center for Environmental Biotechnology, Oak Ridge/ U. of Tennessee).

3. Consortia in biofilms and corrosion - collaboration with Dr. Denny Jones (University of Nevada, Reno).

4. Effect of pH, dessication and (perhaps) radiation in the survival of Yucca Mountain isolates.

5. Persistance of EPS in subsurface rock - EPS as a carbon and energy source for heterotrophic bacterial growth.

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Publications

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