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Enhanced Mitigation of Pipeline Biocorrosion Using A Mixture of D-Amino Acids with A Biocide

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Introduction

MIC Costs

- MIC accounts for about 20% of all corrosion (Flemming,1996).
- MIC has been recognized as a significant problem in the oil and gas industry since 1980s. It is also a problem in water utilities, etc.
- New law signed by Obama on Jan. 3, 2012 (H. Con. Res. 93) doubles the maximum fine for safety violations on oil and gas pipelines.

Flemming, H. C. "Biofouling and microbiologically influenced corrosion (MIC)-an economic and technical overview." In: Heitz, E., Sand W., and Flemming, H.-C. (eds.), Microbial Deterioration of Materials, Springer, Heidelberg, pp. 5-14 (1996).

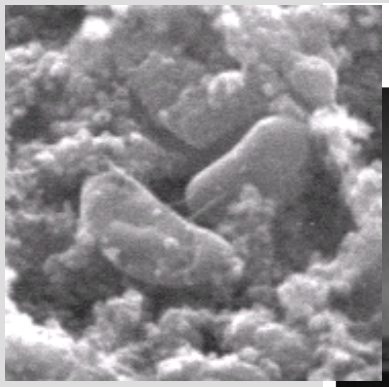
Introduction

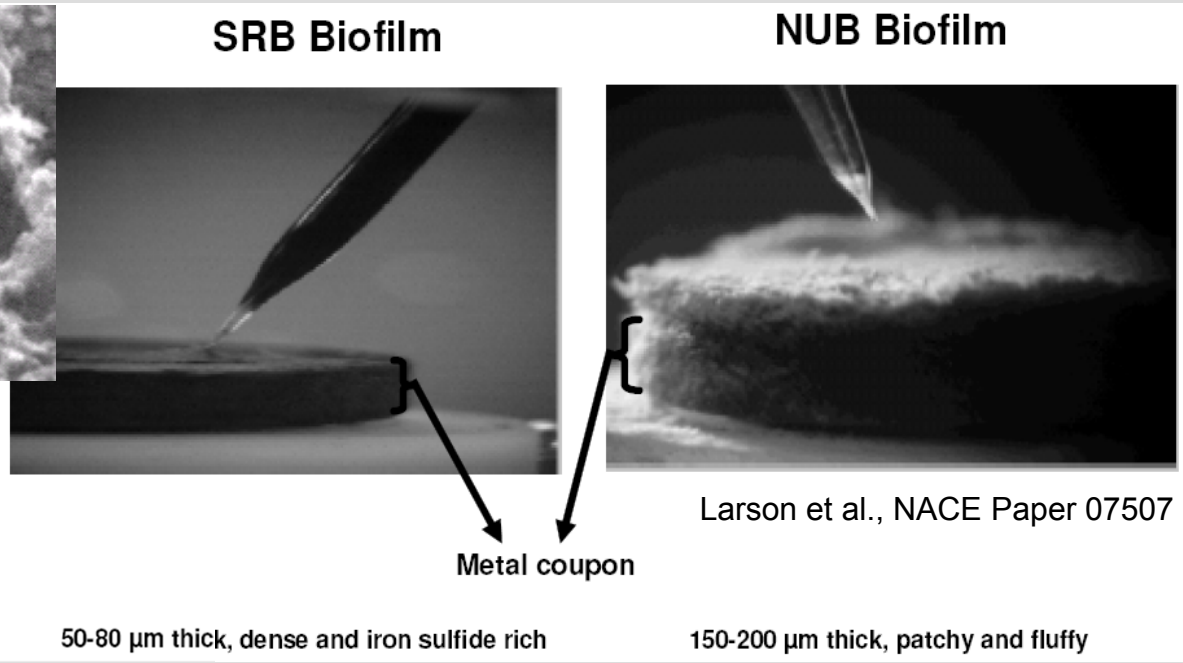
Water-Wetting Impact on MIC

1. Some microbes can live in oily matter with just a little moisture. However, a far greater diversity of microbes can live in aqueous environments.
2. A line with continuous water-wetting is far more prone to MIC than one seeing oil-wetting or intermittent water-wetting.
3. MIC is becoming a bigger problem because overall water-wetting is becoming more prevalent. (Aging infrastructure is another a major factor.)

Review

Biofilms are responsible for MIC





Larson et al., NACE Paper 07507

Anodic: $4Fe \rightarrow 4Fe^{2+} + 8e^-$

Cathodic: $SO_4^{2-} + 9H^+ + 8e^- \rightarrow HS^- + 4H_2O$

1. Biofilms transfer electrons across cell walls while planktonic cells cannot.
2. Biofilms secrete locally high concentrations of organic acids

Review

Current Mitigation Methods – Spray and Scrub

- Biocides/Biostats (THPS and glutaraldehyde, etc.)

  Problems with toxicity, resistance, high costs, strict environmental regulations

- Physical scrubbing (pigging)

  Some pipelines cannot be pigged.

- Microbial competition

NRB can be used to mitigate souring, but not necessarily MIC.

- Sulfate removal, UV radiation, ultrasound: Expensive or ineffective
- "Protective" biofilm: Possible only in very defined setting. Wishful thinking. Bugs will attack.

Review

Treatment Is Expensive

- Downtime is costly
- Biocides are expensive at large scales
- Discharge problem
- Some pipelines cannot be pigged.

Review

Evaluation Efficacy

1. To evaluate biocide efficacy, 2 or 3 log reduction required (99% or 99.9% kill). (1 log reduction may be due to MPN error.)
2. Antibiotic treatment of humans does not need a large log reduction because humans have an immune system that will take over once an upper hand is gained.
3. A 5 log reduction (99.999% kill) is much better than a 3, because it takes much longer for the bugs to recover. Time gap is much large between treatment cycles. (No help from an immune system.)

Review

Sessile Cell Count

1. Scrub off the cells from a biofilm first
2. Count the cells in a liquid suspension just like counting planktonic cells.
3. Need MPN if cell count too low

Review

Problems with Outcome Assessment

1. It is easy to assess efficacy against planktonic cells.
2. No so for biofilms. They can be hard to locate.
3. Biofilms are far more difficult to eradicate. 10X or higher doses. 1,000X reported.

Review

How A Biofilm Protects Itself - Mechanism 1

Diffusional limitation

Many people think this is likely the primary mechanism.

However, this may not be true in some cases in which antimicrobials are observed rather evenly distributed throughout the biofilm.

Review

How A Biofilm Protects Itself - Mechanism 2

Lowered Metabolic Rates

Sessile cells in a biofilm are smart. When they sense a biocidal threat, they become less active. Thus, they are less prone to the biocidal effects.

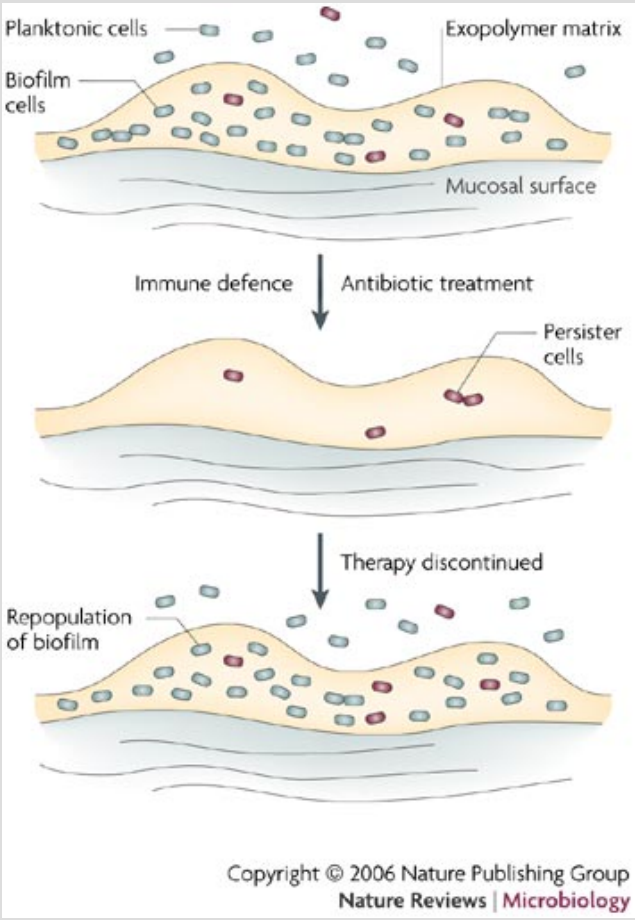
Review

How A Biofilm Protects Itself - Mechanism 3

Formation of Persister Cells

Persister cells are tougher than others.
They survive while their neighbors in the same biofilm community die. They even benefit from the nutrients released by dead cells.

The "regrowth rates of stressed biofilms are truly phenomenal when the stress is removed" (p. 58, The Biofilm Primer by Costerton, 2007).



Review

How A Biofilm Protects Itself - Mechanism 4

Upregulation of resistance genes

Upregulation of antimicrobial genes for enzyme inactivation of antimicrobial agents will counter biocides

Bacteria are known to pass resistance genes (in a plasmid) to each other.

Long-term use of a particular biocide may lead to resistant bugs (because biocide use promotes them).

Review

How A Biofilm Protects Itself - Mechanism 5

Efflux pumps

Bacteria use energy to perform active transport against diffusion to pump out unwanted toxic substances.

They are classified into five major superfamilies (Wikipedia)

1.The major facilitator superfamily (MFS)

2.The ATP-binding cassette superfamily (ABC)

3.The small multidrug resistance family (SMR)

4.The resistance-nodulation-cell division superfamily (RNI

5.The Multi antimicrobial extrusion protein family (MATE

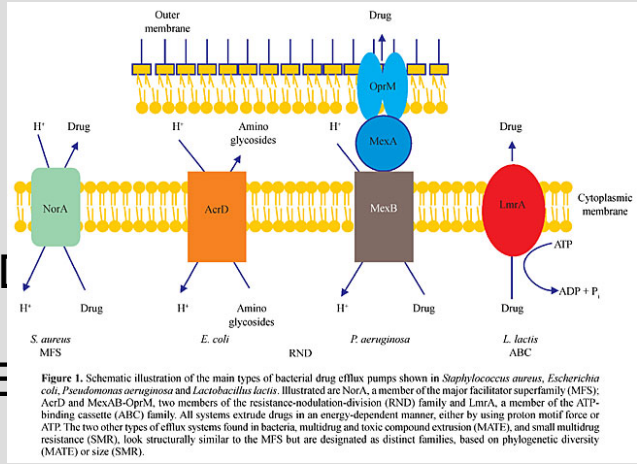


Figure 1. Schematic illustration of the main types of bacterial drug efflux pumps shown in *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Lactobacillus lactis*. Illustrated are NorA, a member of the major facilitator superfamily (MFS); AcrD and MexAB-OprM, two members of the resistance-nodulation-division (RND) family and LmrA, a member of the ATP-binding cassette (ABC) family. All systems extrude drugs in an energy-dependent manner, either by using proton motif force or ATP. The two other types of efflux systems found in bacteria, multidrug and toxic compound extrusion (MATE), and small multidrug resistance (SMR), look structurally similar to the MFS but are designated as distinct families, based on phylogenetic diversity (MATE) or size (SMR).

Genet. Mol. Res. 2 (1): 48-62 (2003)

(In eukaryotes, efflux pumps are also known since 1976.)

Review

Environmentally Friendly Biocides

They must be readily biodegradable (not just biodegradable).

- THPS (very acidic, slightly more effective at acidic pH)
- Glutaraldehyde (mildly acidic, more effective at basic pH)

There are dozens of industrial biocides (including bleach). Due to cost, safety, efficacy, and environmental concerns, only these two are widely used at large scales in O & G.

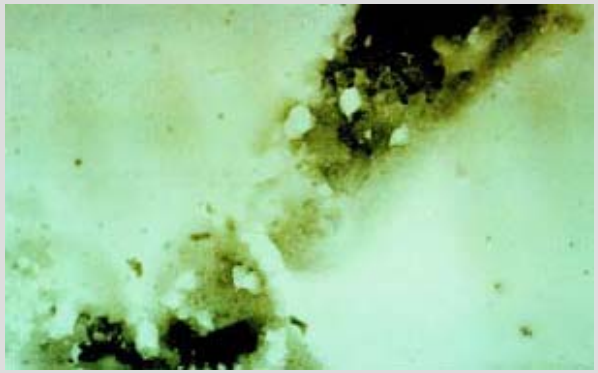
Downside: Biodegradability will render a biocide less effective over time.

Review

THPS

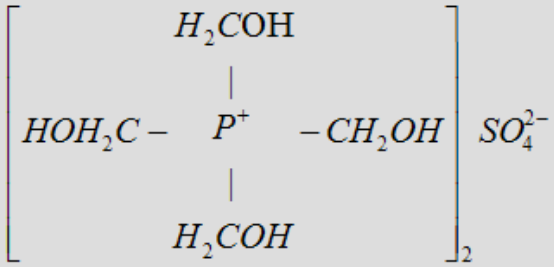
THPS is a broad-spectrum biocide effective at a broad pH range.

- Cell lysis
- Interfering w/ ADP-ATP energy cycle
- Inhibiting/denaturing lactate dehydrogenase
- Inhibiting sulfate reduction
- "Short-circuit" of proton flux within the cell



THPS "shotgun" effect damaging cell membrane/wall

(Jones et al., NACE Paper #10257)



Tetrakis Hydroxymethyl Phosphonium Sulfate

Review

Glutaraldehyde

Glutaraldehyde is a broad-spectrum biocide. Mildly acidic. Stable at acidic pH. Not stable at basic pH. Buffered to pH 7.5-8.5 just before use.

- Amine-reactive crosslinker (a potent cross-linking fixative). Thus, it is not compatible with D-aa.
- Two aldehyde groups are reactive (particular with proteins)

Review

Biocide Blend/Cocktail

Biocides are usually mixed (blended) with other chemicals (scale-remover, corrosion inhibitor, oxygen scavenger, surfactant, etc.)

THPS is more effective at acidic pH, while glutaraldehyde the opposite.

THPS degrades faster when pH increases.

THPS is corrosive.

Acrolein is biodegradable and very effective, but it was used as a chemical weapon in WWI.

Review

Repeated Biocide Dosing

The field system is usually not fully sterilized (even if so, contamination will make it non-sterile again). Bugs bounce back. Treatment cycles required.

Similar pattern occurs in souring mitigation.

Different from antibiotic treatment in humans with a healthy immune system that can take over the control.

Review

Biocide Dosing Strategy

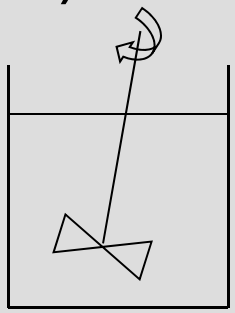
1. A initial heavy dose (shock treatment)
2. Lower maintenance dose

Slug treatment (concentrated, short exposure)

Biocide slug in pipe

Batch treatment (diluted, long exposure)

Continuous-flow treatment (diluted, continuously injection)



Batch treatment

Results and Discussion

New Biocide Enhancer Technology

1. Oil-field biocide use is very expensive (large scale)
2. More restrictive environmental regulations desire lower dosages
3. No new blockbuster green biocides are expected
4. We need to rely on biocide cocktails (as in cocktail treatment for HIV)

Results and Discussion

Smart Ways to Achieve Better Biofilm Removal

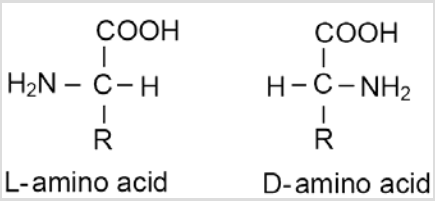
Planktonic cells are much easier to treat. Let's make sessile cells planktonic!

1. Use quorum-quenching

   Too species-specific. Chemicals too expensive. Impractical to start with for field biofilm consortia! A non-starter.

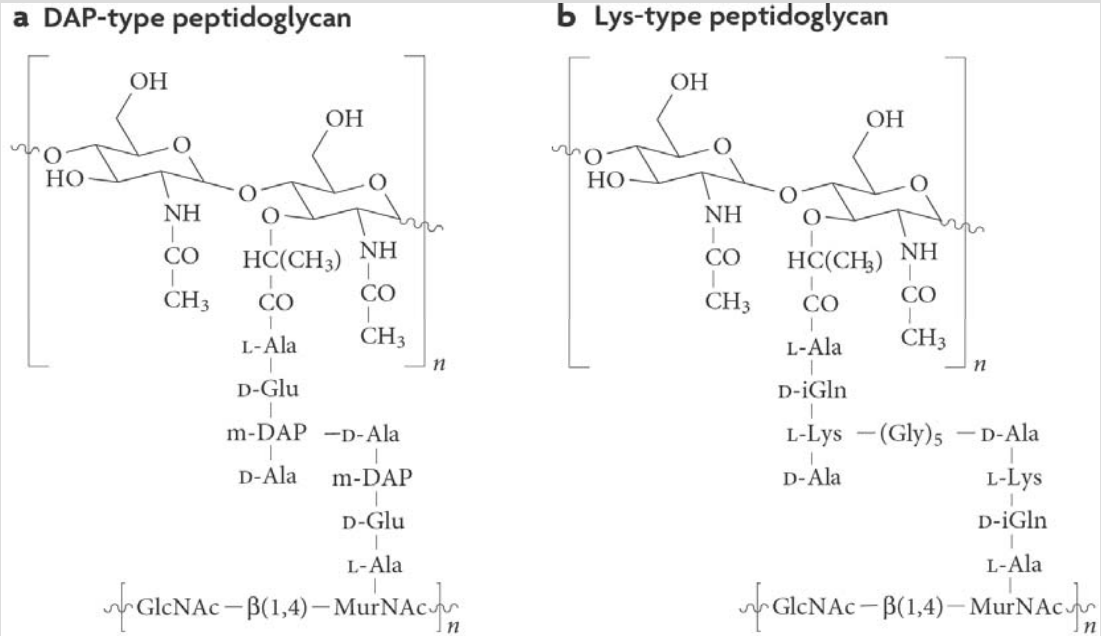
2. D-amino acids

   Common feature in bacterial cell walls: peptidoglycan containing D-amino acids that are signal molecules. D-alanine substitution signals biofilm dispersal. D-amino acids are cheap! Some of them work very well at very low concentrations!



Results and Discussion

D-Amino Acids Are Biocide Enhancers



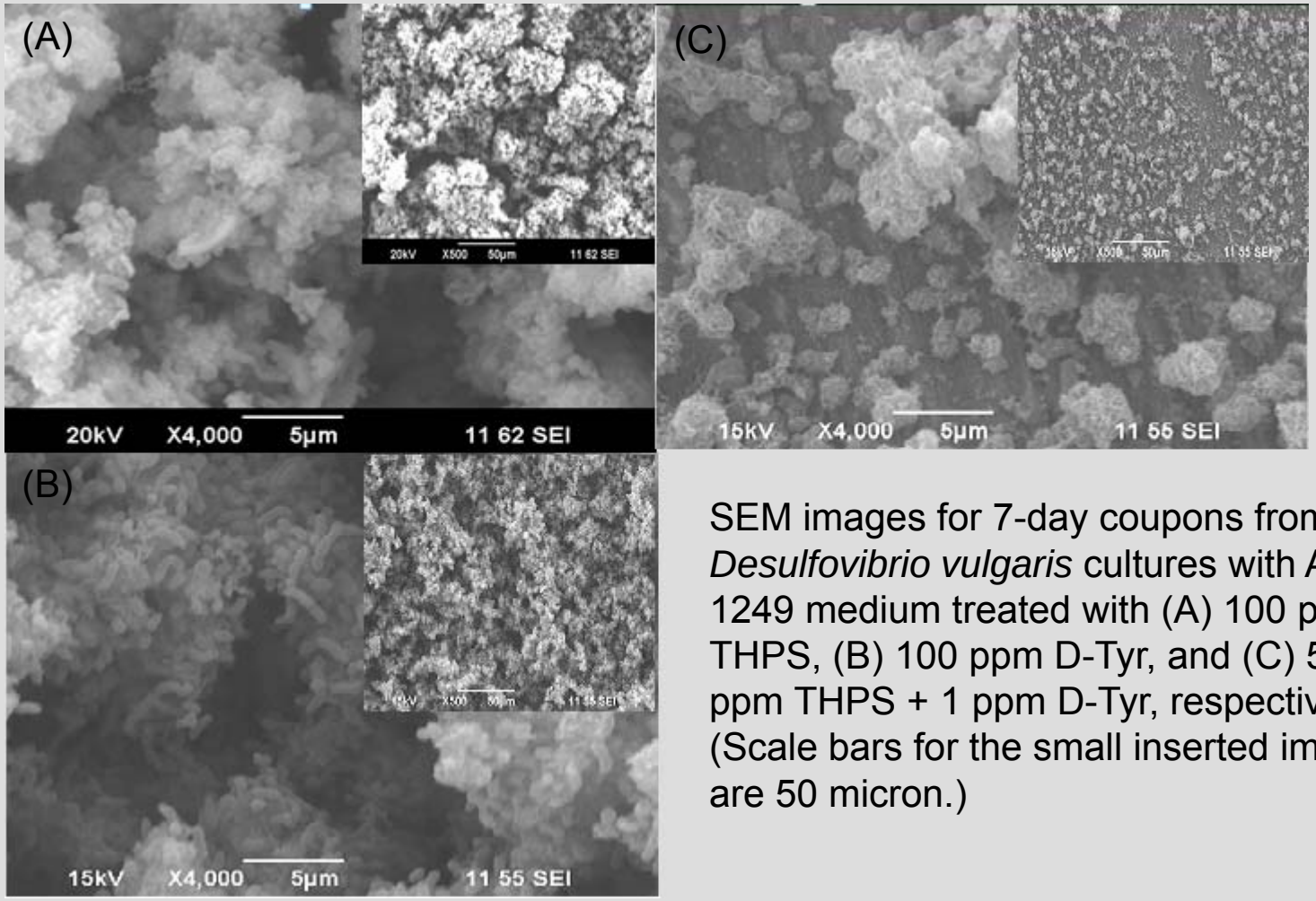
Royet, J., Dziarski, R., 2007. Nat. Rev. Microbiol. 5, 264–277.

All bacterial cell walls contain D-alanine. The D-alanine terminus is a trigger. Replacement of it with another D-amino acid triggers biofilm dispersal (Science 328:627–629, 2010).

However, for recalcitrant biofilms such as SRB biofilms, this trigger is not convincing enough. We discovered that adding a biocide stress will do the trick (DOI 10.1007/s11274-012-1116-0).

Results and Discussion

New D-aa + Biocide Technology For Biofilm Prevention

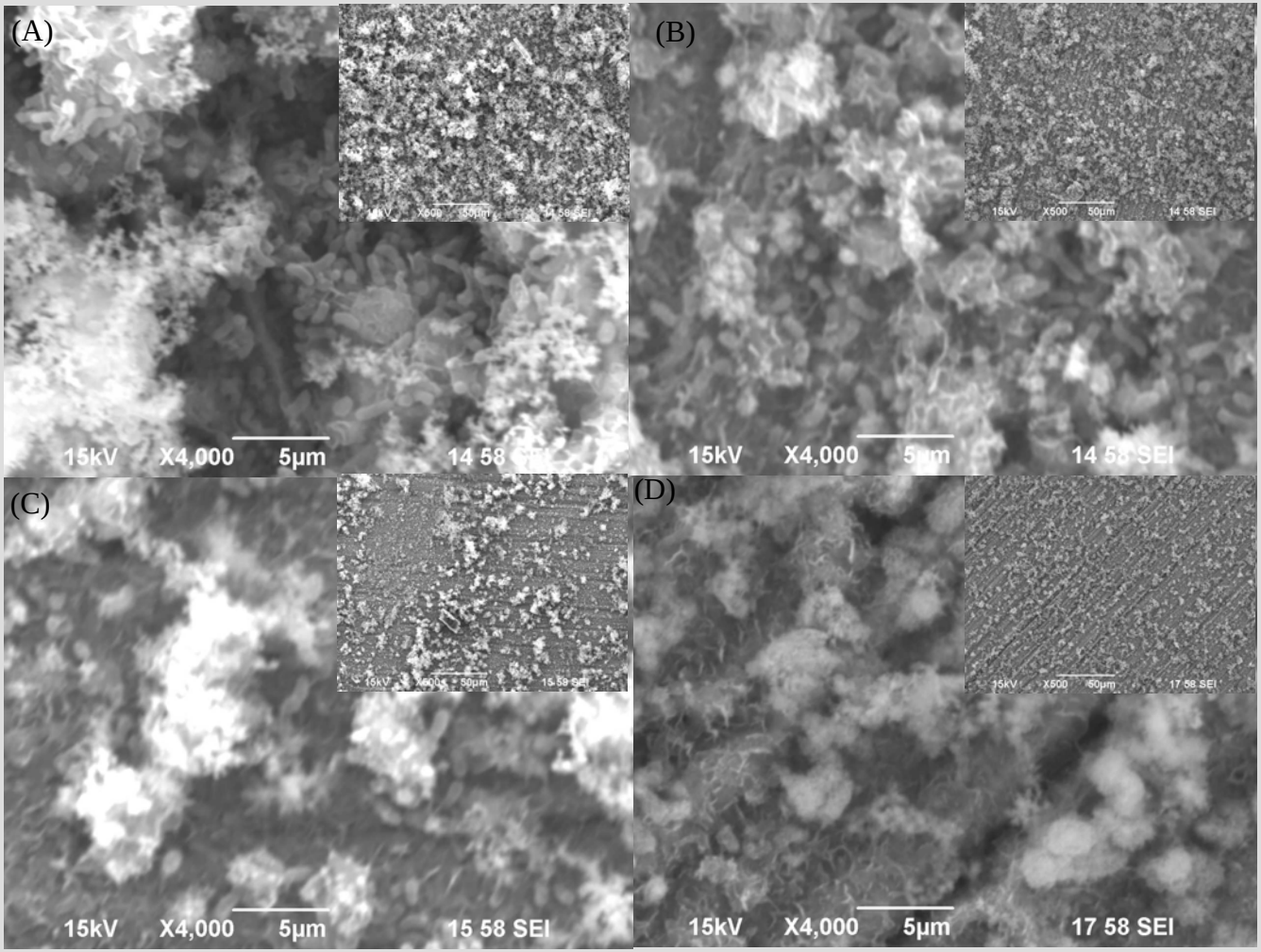


SEM images for 7-day coupons from *Desulfovibrio vulgaris* cultures with ATCC 1249 medium treated with (A) 100 ppm THPS, (B) 100 ppm D-Tyr, and (C) 50 ppm THPS + 1 ppm D-Tyr, respectively. (Scale bars for the small inserted images are 50 micron.)

Xu et al., World J. of Microbiology and Biotechnology, 28, 3067-3074 (2012).

Results and Discussion

New D-aa + Biocide Technology For Biofilm Removal



SEM images for coupons (initially covered with mature *D. vulgaris* biofilms) after undergoing 1-hour shock treatment in 1/4 strength medium treated with (A) 50 ppm THPS, (B) 100 ppm D-Tyr, and (C) 30 ppm THPS + 1 ppm D-Tyr, (D) 50 ppm THPS + 1 ppm D-Tyr, respectively. (Scale bars for the small inserted images are 50 μm.)

Xu et al., World J. of Microbiology and Biotechnology, 28, 3067-3074 (2012).

Results and Discussion

New D-Amino Acid + Biocide Technology

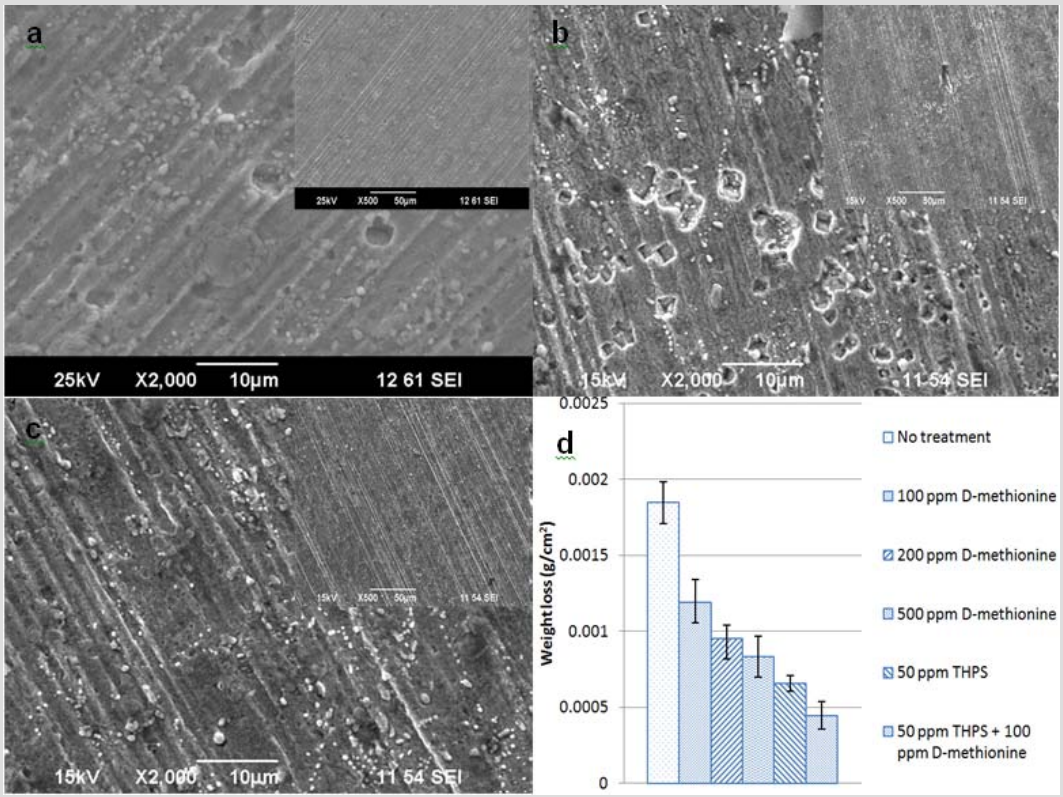
Sessile cell counts on coupons (initially covered with mature SRB biofilms) after undergoing shock treatment in 1/4 strength medium.

Treatment	Sessile cell count for 1 hour treatment (cells cm^{-2})	Sessile cell count for 3 hour treatment (cells cm^{-2})
No treatment (control)	$\geq 10^6$	$\geq 10^6$
100 ppm D-Tyr	$\geq 10^5$	$\geq 10^5$
50 ppm THPS	$\geq 10^4$	$\geq 10^3$
50 ppm THPS + 1 ppm D-Tyr	<10	<10
100 ppm THPS	<10	<10

Xu et al., World J. of Microbiology and Biotechnology, 28, 3067-3074 (2012).

Results and Discussion

New D-Amino Acid + Biocide Technology



SEM images of coupon surfaces after *D. vulgaris* biofilm removal for coupons obtained after 7 days of incubation at 37°C from ATCC 1249 medium with the addition of (a) 50 ppm THPS, (b) 500 ppm D-methionine, (c) with 50 ppm THPS + 100 ppm D-methionine, respectively, accompanied by normalized weight loss data shown in (d). Scale bars for the small inserted images are 50 µm.

Xu et al., Materials and Corrosion, in press (2012). DOI: 10.1002/maco.201206894

Research Plan

Task 1 – Testing D-Tyr and D-Met Against Field Biofilm Consortia

Biofilms	Two different biofilm consortia
Biocide cocktail	THPS + D-tyrosine, THPS + methionine
Temperature	37^{o}C and actual pipeline temperature
Test duration	15, 30 days for prevention of biofilm establishment; 1, 3 hours for biofilm removal
Coupon	X65 (pipeline) carbon steel

Research Plan

Task 2 – Screening Additional D-Amino Acids

Bacteria	*D. vulgaris* (ATCC 7757)
Biocide cocktail	THPS + D-amino acid
Temperature	37^{o}C
Test duration	1, 3 hours for biofilm removal
Coupon	X65 carbon steel

Research Plan

Task 3 – Using D-aa Mixture + Biocide

Biofilms	Two different biofilm consortia
Biocide cocktail	THPS + D-amino acids (various choices and dosages)
Temperature	37^{o}C and actual pipeline temperature
Test duration	1, 3 hours for biofilm removal
Coupon	X65 carbon steel

Research Plan

Task 4 – Field Testing

Biocide cocktail	THPS + D-amino acids (various choices and dosages)
Temperature	pipeline temperature
Test duration	1, 3 hours for biofilm removal
Coupon	X65 carbon steel

Research Plan

Milestones

Milestones	**Time**
Finish collecting and testing of field biofilm consortia using D-tyrosine and D-methionine + THPS (*Task 1*)	End of 2014
Finish selection and testing of additional D-amino acids + THPS (*Task 2*)	End of 2014
Mid-term report to PHMSA	End of 2014
Finish laboratory testing of D-amino acid mixtures + THPS (*Task 3*)	Mid 2015
Identifying a field test location (*Task 4*)	End of 2014
Finish field testing (*Task 4*)	End of 2015
Final project report to PHMSA	End of 2015

Thank You!