# Molecular Analysis of Aramid Polymer Film Impressions of Corrosion Coupon Biofilms

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## Molecular analysis for microbiologically influenced corrosion (MIC)

- Advances in molecular biological tools have allowed us to overcome the culture bias
- DNA is readily extracted from field samples to allow both qualitative and quantitative analysis of taxonomic and functional genes indicative of MIC related organisms in biofilms associated with surface corrosion
  - DGGE
  - qPCR

## Molecular analysis for microbiologically influenced corrosion (MIC)

- Using these tools we can:
  - Detect MIC bacteria to provide timely corrective action
  - Quantify MIC bacteria to determine the appropriate aggressiveness of treatment
  - Monitor remedial efficacy in a corrosive environment

## Using molecular biological tools to analyze biofilms

- A sampling bias remains in our analysis of biofilms
  - MIC biofilms are microbiologically diverse and structurally complex
  - Microbes active at the interface are most likely to initiate corrosion
  - Standard sampling of a biofilm on a corrosion coupon is a swab or whole coupon extraction
  - A sampling method that accounts for spatial heterogeneity of biofilms can improve the diagnosis of MIC

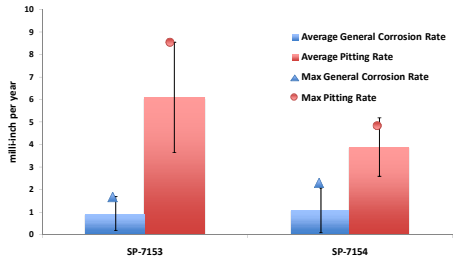
## Using molecular biological tools to analyze biofilms

- To address this spatial bias in sampling of corrosion coupon biofilms we have developed a method to sample biofilms in layers
- A solution of aramid polymer in DMAc is applied to the surface in a thin layer. The coupon is then immersed in sterile distilled water. The aramid polymer then precipitates entrapping a layer of the biofilm matrix. The process is then repeated until all of the biofilm has been removed.

## Experimental procedure

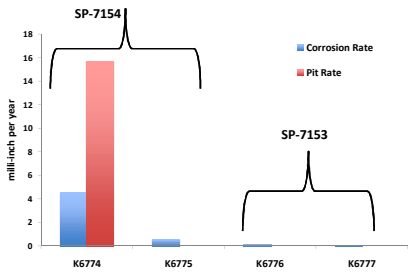
- Duplicate corrosion coupons installed in two natural gas well line “drips” for 100 days
- Surface deposits collected from one set with sterile swabs
- Aramid polymer layers were used to collect discrete layers of biofilm from the other set
- Coupons were examined for corrosion
- DNA was extracted from swabs and biofilm layers for microbial analysis

## Historic coupon monitoring

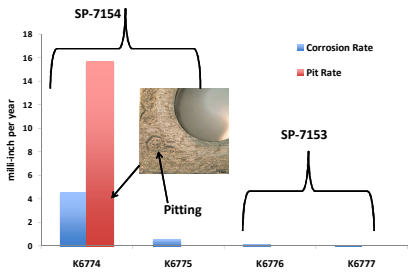


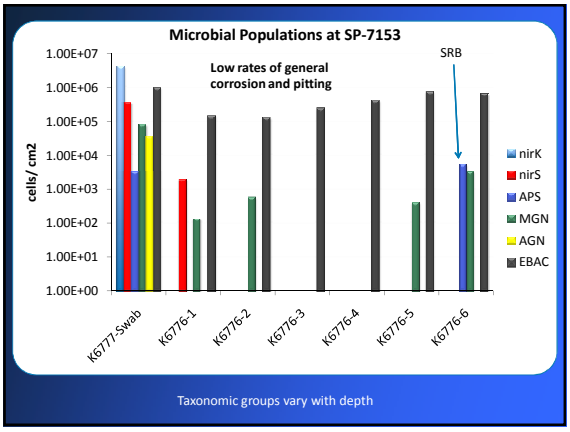

## Collecting a layer of biofilm from a coupon

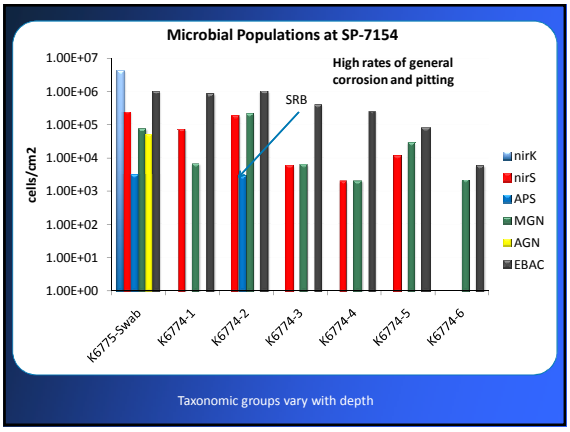
## Corrosion analysis results

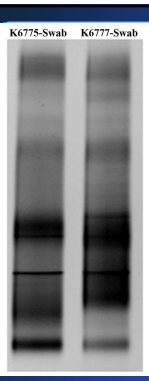



## Corrosion Analysis Results



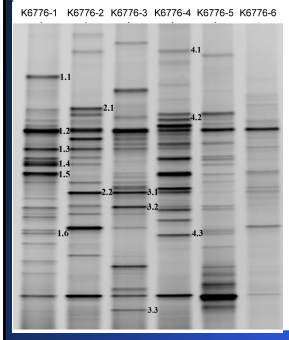



## DGGE profiles of DNA extracted from swab samples

- Smeared, indistinct bands
- No usable sequence data obtained




## DGGE profiles from K6776 polymer layers

- Banding patterns appear to represent unique and diverse microbial communities
- Bands from K6776-5 and -6 did not produce phylogentic matches

## Genera identified in K6776 polymer layers

- K6776-1
  - *Staphylococcus (promotes adhesion to metallic surfaces)*
  - *Cloacibacterium*
  - *Variovorax (implicated in corrosion of copper pipes*
  - *Nitrospira (isolated from corroded iron pipe)*
- K6776-2
  - *Delftia (implicated in corrosion of Ni-Cu and Ni-Zn coatings)*
- K6776-3
  - *Massilia (highly adhesive to metallic pipe, detected in corroded lead pipe)*
  - *Propionivibrio, Alcaligenes*
- K6776-4
  - *Staphylococcus, Euzebya, Phenylobacterium*

## Conclusions

- Discrete layers of biofilm collected using aramid polymer
- DNA extracted from small aramid polymer samples
- qPCR indicated the microbial community varied between aramid polymer layers, especially K6776.
- Sequence analysis provided insight into microorganisms that may play a role in MIC

**In a 2nd case study in a sea water injection system from a corrosion coupon with high rates of pitting corrosion**

- Aerobes, facultative anaerobes
- Facultative anaerobes, anaerobic hydrocarbon degraders, iron II and iron III reducers
- Facultative anaerobes, anaerobic hydrocarbon degraders, iron II and iron III reducers
- Strict anaerobes, SRB, iron reducers (including SRB known to directly uptake electrons from metallic iron)




# Questions ???