MIC in LLW/ILW repository

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Novel method to study microbe- steel interaction

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Background

The deterioration of metals or metal alloys due to microbial activity is termed microbially-induced corrosion (MIC). The participation of microorganisms in the process induces several effects, the most significant being local changes in the electrochemistry at the metal-solution interface under the microbial biofilm. Microbially-induced corrosion in deep bedrock environment is important when evaluating the long-term safety of the disposal of low and intermediate level radioactive waste that contains several steel materials[1]. Biofilm formation is crucial for the initiation of MIC. For a complex ecosystem like a natural biofilm, determining the ecology of each species and their potential effect on corrosion is challenging and the underlying mechanisms of MIC are complex and insufficiently understood. It is known that distinct microbial community enriches on the corrosion pits or at the grain boundaries of steel material, but what is the function of these microorganisms remains unknown[1,2]. These microorganisms may be attracted at the location after the pit initiation due to local environmental conditions, or they might be in role of pit initiation. HCR-FISH technique[3] provides more insight of microbial distribution on the corroding surfaces, i.e. what species are accumulated inside the corrosion pits or at the grain boundaries. Combining the cell visualization methods to existing surface characterization methods, new knowledge of microbially induced corrosion can be gained.

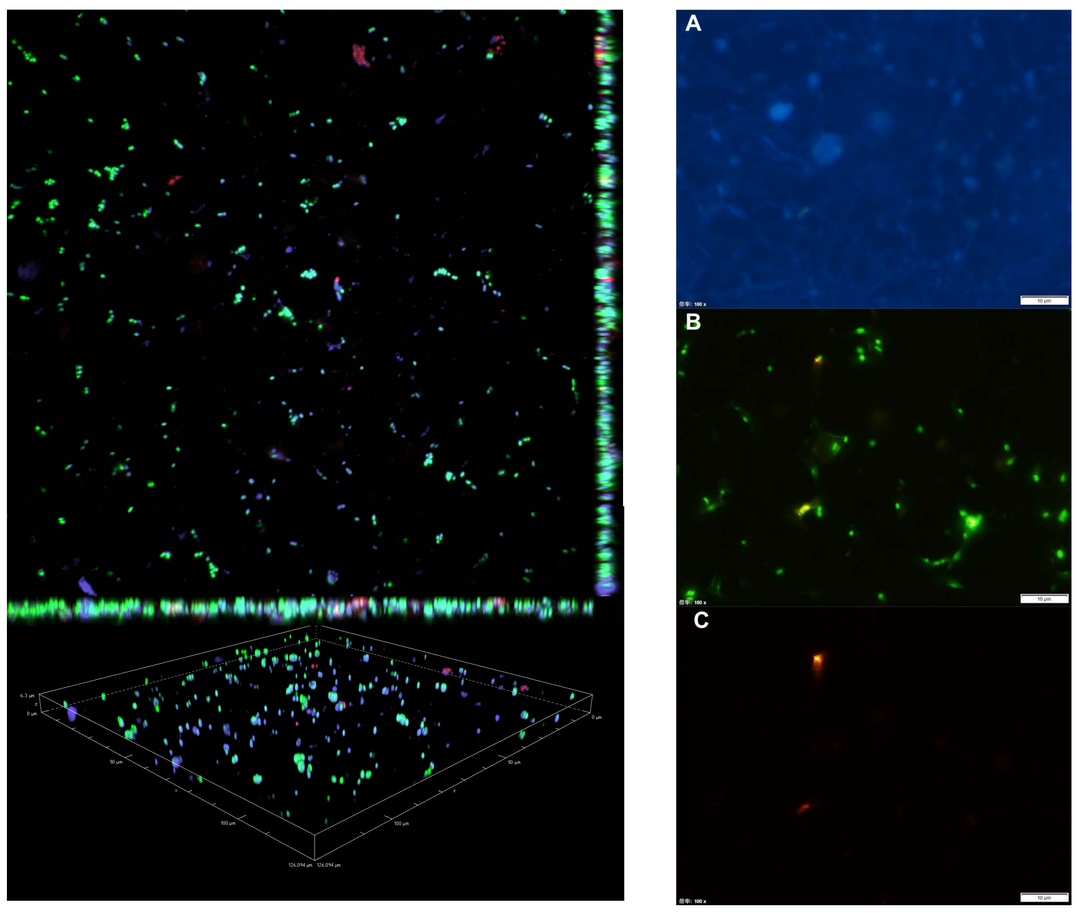
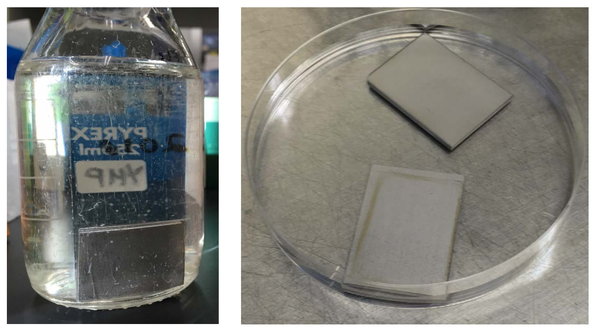


Figure 2. *Microbes on surface of stainless steel. Blue - DAPI stained cells (non specific), Green - bacteria specific fluorescent probes, Red - Archaea specific fluorescent probes. Left panel, 3 dimensional confocal microscopy view of stainless steel surface, right panel epifluorescent views of A) all microbes, B) Bacteria, C) Archaea.*

Method

1. Stainless steel coupons were immersed in natural ground water from repository site for 6 months (Fig 1).
2. Biofilm was fixed on surface of steel and cell walls of microbes forming biofilm were made permeable to probes.
3. Fluorescent probes specific for either archaea or bacteria was hybridized to 16S rRNA sequences of corresponding microbes.
4. The biofilms were counter stained using dye that stains all cells.
5. Coupons were imaged using confocal microscope that allows 3D detection of fluorescent cells on the surface of stainless steel.

Figure 1. *Stainless steel coupons immersed in ground water for 6 months.*



Results

HCR-FISH technique[3] designed at JAMSTEC was successfully further modified to be applicable to detect microbes on steel surface. The probes tested here allowed the detection of bacteria and archaea on steel surface. Combined to confocal microscopy, the method allows detailed 3D detection of microbes in relation to surface morphology of steel. Biofilm forming microbial community was dominated by bacteria (green) and archaea (red) were detected only occasionally (Fig. 2). In addition the un-hybridized community (blue) was large, likely due issues of probe permeabilization or probe misfit. Microbes were mainly attached to grain boundaries indicating possible role of microbes in opening of grain boundaries during immersion in groundwater (Fig 2).

Conclusions

- **The HCR-FISH methods proved to be efficient capturing the location of single microorganism on steel surfaces.**
- **Method allows us to investigate the relationship between the microorganism and the surface morphology (pitting, grain boundaries).**
- **By using specific probes we can get detailed knowledge of role of individual microbial species on MIC**

References

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