Novel corrosion protection method by microbial extracellular polymeric substances (EPS)

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Objectives

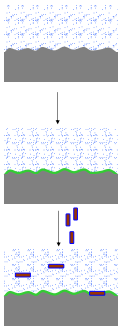
Corrosion of metal substrates in aqueous media often is demonstrably induced or sped up by bacterial biofilm /1,2/. ***Microbially influenced corrosion (MIC)*** is discussed to contribute about 20% of the entire costs of corrosion failures /3/. Among harmful microorganisms one finds so called ***sulphate reducing bacteria (SRB)*** – a group of anaerobically existing bacteria producing hydrogen sulphide.

On the other hand, some microorganisms can release chemical substances that were found to influence the adhesive properties of microbial cells - ***extracellular polymeric substances (EPS)*** – in general highly heterogeneous mixtures of different substance classes /4/. Appropriate EPS are promising candidates for the inhibition of cell adhesion thus for the prevention of biofilm formation. Also biofilms can exhibit protective properties /5/.

The aims of this project are to identify appropriate EPS and to establish a system for corrosion protection by application of EPS – biogenic and renewable substances – as protecting films.

Fundamentals

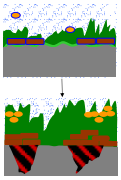
Biofilms and Biocorrosion



Any surface exposed to a liquid or gaseous phase will quickly be covered by substances present in the phase. The result is called **conditioning film** and can influence further adhesion of microorganisms.

Microbial cells - wrapped in **EPS** - can adhere on the surface and establish stable bondings depending on the forces of interaction: The first step of **biofilm-formation.** EPS are constantly released into the evolving biofilm. Whereas EPS are discussed to mediate these forces, no doubts exist on the high importance of EPS for the stability of the biofilm.

The biofilm grows due to adhesion of **other species** and cell division. Inside the biofilm one also finds metabolites, corrosion products etc.

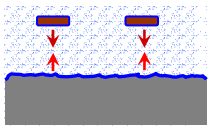


The high number of synergistically existing microbial cells and the diversity of metabolites can attack the substrate and severely damage the entire system: biocorrosion and **MIC**.
A typical failure mode of microbial attack is local corrosion

Protection

EPS as biosurfactants /6/:
Desorption of cells is induced by addition of small amounts of special EPS of surface active properties.

Footprint effect /7/:
Upon desorption some bacteria leave patches of substances on the surface. Other cells avoid to adhere on these patches.



Suitable EPS must comply with the following premises:
• resistance against microbial degradation
• non-corrosive behaviour
• sufficient adhesiveness

Experimental and Results

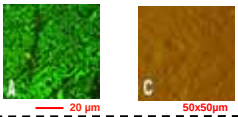
Substrate metals:
• pure iron (ARMCO)
• carbon steel (St 37)
• high alloyed steel (DIN 1.4301)
Abrasion with emery paper (P180), rinsing in distilled water, ultrasonication in ethanol, rinsing in acetone,

Setup for mimicking MIC:
Desulfovibrio vulgaris as representative sulphate reducing bacteria (SRB) in Postgate C growth medium: mainly sulphate and lactate.
• in-situ Inoculation after mounting of the substrates
• Pre-inoculated solutions: approx. $5*10^8$ cells*ml^{-1}.

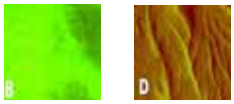
EPS were harvested from planktonic biofilms by centrifugation and purified. Freeze-dried EPS are re-suspended.

Layer preparation on 1.4301

Dip-coating for 2 hours in EPS suspension (5 mg*ml^{-1}), sterile and anaerobic conditions. Afterwards drying in air or nitrogen atmosphere



Droplet-coating:
50 µl of EPS-containing suspension are applied on the substrate surface, sterile and anaerobic conditions. Liquid evaporates in air or nitrogen atmosphere



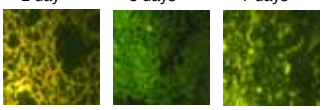
A, B: Fluorescence microscopic images after Lektin staining
C, D: Atomic force microscopic images

Dip-coating: often not evenly shaped layer, dissolves within hours.
Droplet: thicker and more uniform layer exhibiting trenches, more stable against desorption

Adhesion of *D.Vulgaris* on 1.4301 modified with *D.Vulgaris*-EPS

Dip-coated surface, in-situ inoculation

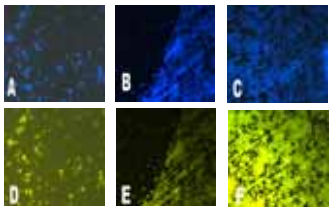
1 day 3 days 7 days



Fluorescence microscopic images after Lektin staining

Uncovered regions (dark spots) even after 7 days!

Droplet-coated surface, pre-inoculated, after 5h



Fluorescence microscopic images after staining:
Upper row: after DAPI staining; lower row: after Lektin staining. A, D: coated regions; B, E: boundary uncoated-coated; C, F: uncoated region

Single cells clearly distinguishable.
Low cell number in regions covered by EPS!

Test of resistance against microbial degradation

SRB-growth medium Postgate C was prepared replacing lactate as carbon source by EPS.

The planktonic cell densities of *D.Vulgaris* – measured after 4 days of incubation – are used as indicator.

Result:
Only xanthan and EPS of *Lactobacillus Fermentum* can be used as carbon source.

Medium	Cells*ml^{-1}
Reference Experiments	
Postgate C, no lactate, sterile conditions	0
Postgate C, no lactate, inoculum	$2,8x10^7$
Postgate C, lactate, inoculum	$2,2x10^8$
No lactate, EPS, sterile conditions	
Postgate C + EPS *L. fermentum*	$1,3x10^7$
Postgate C + Xanthan	$\mathbf{3,8x10^8}$
Postgate C + EPS *P. fluorescens* (009)	nd[b]
Postgate C + EPS *P. fluorescens* (4358)	nd
Postgate C + EPS *P. flava*[a]	
Postgate C + EPS *P. cichorii*	nd
Postgate C + EPS *P. fragi*	nd
Postgate C + EPS *R. opacus*	nd
Postgate C + Dextran	nd
No lactate, EPS, inoculum	
Postgate C + EPS *L. fermentum*	$2,8x10^8$
Postgate C + Xanthan	$\mathbf{6,2x10^8}$
Postgate C + EPS *P. fluorescens* (009)	$3,0x10^7$
Postgate C + EPS *P. fluorescens* (4358)	$3,9x10^8$
Postgate C + EPS *P. flava*	$1,4x10^8$
Postgate C + EPS *P. cichorii*	$2,2,x10^7$
Postgate C + EPS *P. fragi*	$2,6x10^7$
Postgate C + EPS *R. opacus*	$2,4x10^7$
Postgate C + Dextran	$2,9x10^7$

a: not enough substance available
b: nd = not detectable, cell numbers too low

Conclusions

Only few EPS can be metabolized by Desulfovibrio vulgaris as carbon source

Corrosion due to EPS under anaerobic conditions only was observed for *D.Vulgaris*-EPS and *D.Indonesiensis-EPS* (AiF 173 ZN).

Up to now poorly adhesive properties! Most EPS quickly desorb or dissolve in aqueous media within a couple of days or even hours.

Influence on cell adhesion:
Lower cell density and irregularly formed biofilm in case of *D.Vulgaris*-EPS when compared to untreated surface.
Positive effect of binary EPS by sequential deposition of xanthan and *D.Vulgaris*-EPS has to be further examined.

Prospectives

Chemical analysis of EPS-composition
Microscopic analysis of interactions EPS | substrate
Amelioration of adhesive properties of EPS
Electrochemical investigations on the influence of EPS on (bio-)corrosion

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