**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/. Microbiologically 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 Bio corrosion**

Any surface exposed to a liquid or gaseous phase will quickly be covered by substances present in the phase. The exposed surface 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 the 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.

## Experimental and Results

**Layer preparation on 1.4301**

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

50 µl of EPS-containing suspension are applied on the substrate surface, sterile and anaerobic conditions.

Liquid evaporates in air or nitrogen atmosphere

**Drop-coating:**

After 2 hours in EPS suspension, the EPS layer is dried in air or nitrogen atmosphere.

50 µl of EPS-containing suspension are applied on the substrate surface, sterile and anaerobic conditions. Afterwards drying in air or nitrogen atmosphere.

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

Dip-coated surface, in-situ inoculation

1 day 3 days 7 days

Fluorescence microscopic images after staining

Uncovered regions (dark spots) even after 7 days!

**Drop-coated surface, pre-inoculated, after 5h**

Fluorescence microscopic images after staining


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

## 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

## Conclusions

**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.

**Corrosion due to EPS under anaerobic conditions only was observed for D. Vulgaris-EPS and D. Inodenonesi-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 D. Vulgaris-EPS and P. fluorescens 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

**References**