

**Effect of Antimicrobial Halophilic Plant Extracts
on Microbiologically Influenced Corrosion (MIC)**

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ABSTRACT

Offshore oil production is susceptible to internal corrosion, which can occur through microbiologically influenced corrosion (MIC) caused by biofilm-forming sulfate-reducing prokaryotes (SRP). The oil and gas industry relies primarily on biocides and mechanical cleaning to mitigate MIC. Halophytes (salt-tolerant plants) produce a variety of bioactive compounds, some of which have antimicrobial activity. MIC was studied on UNS S31600 stainless steel coupons in flasks with three extract types, from four halophytes, at three different concentrations. Flasks were inoculated batch-wise with anaerobic sediment from the Wadden Sea (Denmark) to mimic North Sea oil production MIC. Using H₂S as activity indicator for SRPs initial trials showed a >99.5% reduction in H₂S concentration in samples treated with 20% (v/v) Type B extracts compared to extract-free controls, indicating a significant reduction of SRP. ATP concentrations were only lowered by ≥15% (v/v) concentrations of extracts. Next-generation 16S rRNA amplicon sequencing of DNA from Bacteria and Archaea showed a significant shift away from SRPs in the microbial composition in inoculations with extracts. Visual reduction in corrosion was observed on the coupons treated with 20% Type B extract from halophytes 3 and 4.

Keywords: Microbiologically influenced corrosion, MIC, corrosion, halophyte, mitigation, biorefining

INTRODUCTION

Microbiologically influenced corrosion (MIC)

Offshore oil production facilities are subject to internal corrosion, potentially leading to human and environmental risk and significant economic losses. Microbiologically influenced corrosion (MIC) and reservoir souring are important factors for corrosion-related maintenance costs in the petroleum industry.¹ MIC is caused by sulfate-reducing prokaryotes (SRP), which can be Bacteria (SRB) or Archaea (SRA), with the main focus in literature being on SRB.²⁻⁵ The microorganisms most frequently reported in literature to be responsible for MIC are the SRB; *Desulfovibrio*, *Desulfobacter*, *Desulfomonas*, *Desulfotomaculum*, *Desulfobacterium*, *Desulfobotulus*, and *Desulfotignum*, and methanogens.^{2,5} MIC occurs mainly due to seawater pumped in during enhanced (or secondary-) oil recovery, where the seawater contains large amounts of sulfate (up to 25-30 mM⁴). However, SRB are a widespread group of organisms that can use a variety of organic electron donors, including low-molecular-weight aliphatic and aromatic hydrocarbons, alcohols, carboxylic acids, and volatile fatty acids.² MIC is often seen as localized pitting attacks that are generally associated with the presence of microbial communities embedded in a protective, glue-like, extracellular polymer (often with embedded bioinorganic matrixes) referred to as biofilms. Avoiding the initial formation of biofilms is often thought of as the most crucial step in MIC control.⁶

Several publications highlight the attempts made to simulate biofilm formation in the laboratory, both for a better understanding of its formation under certain conditions³ and testing the effect of biofilm inhibitors and biocides such as Tetrakis-hydroxymethyl phosphonium Sulfate (THPS) and glutaraldehyde (GA).^{5,7} The effectiveness of THPS and GA against established biofilms is much lower than against planktonic cells.^{6,8} Previous studies show that THPS cannot effectively mitigate SRB growth in general.⁶ Therefore, physical removal of biofilm is sometimes required, which can, in some cases, prevent production while maintenance is being performed.¹ The bio-corrosive processes involving SRB have similarly been studied in the laboratory by exposing metal coupons to the corrosive environment containing SRB previously grown in specially designed culture media, such as different variations on the Postgate medium.^{7,9} The formation of a black iron sulfide precipitate can indicate the presence of SRB in these laboratory setups. Other reported methods to detect bacterial activity and presence include analytical procedures, such as; analyzing reduction in sulfate and nitrate (colorimetrically or HPLC);^{2,4} cultivation with metabolic product indicators (such as phenol red-dextrose reagent);⁴ identification of metabolic products by HPLC;⁴ or DNA extraction and sequencing.⁷

Halophyte antimicrobials

Halophytes are plant species that grow in saline water or salty soil. These plants have been shown to produce a wide variety of very active phytochemicals that exhibit potent antimicrobial properties, which have been shown to be easily extractable.¹⁰⁻¹² The halophyte extracts contain an array of different antimicrobial compounds which work synergistically, making it less likely that the microorganisms develop Tolerance. In our previous studies,¹³ we have extracted and enhanced these natural halophyte antimicrobials for efficient contamination control in anaerobic fermentation (ethanol fermentation with yeast).¹⁴ Halophyte extracts have been shown to inhibit methanogens and volatile fatty acids-producing bacteria significantly.¹⁵⁻¹⁸ Halophytes specialize in growing under saline conditions in the very competitive aquatic environment, protecting themselves from attacks by seawater, bacteria, and archaea. The plants also have an ability to synthesize compatible solutes and to deal with reactive oxygen species, e.g., by producing strong antioxidants, especially phenolic compounds, which often also have antimicrobial activity.¹⁹⁻²² This paper examines the effect of halophyte-based phytochemicals on MIC.

Further research is currently underway to expand on the results presented in this paper. Future research will also assess the weight loss of the coupons, include 3D surface scans, and investigate further how the microbial community changes as different plant extracts are added.

EXPERIMENTAL PROCEDURE

Overview of experiments carried out

Four different halophyte species were extracted using three extraction methods (Extract A, B, and C) (Figure 1). 100 mL serum bottles were filled with halophyte extract diluted to different concentrations (5, 10, 15, and 20%), Postgate medium,⁹ and a UNS S31600 stainless steel coupon was added. The bottles were flushed with nitrogen and were inoculated with 1% (v/v) of a preculture of Postgate and sediment obtained from the Wadden Sea, Denmark, and then incubated for 28 days at room temperature.

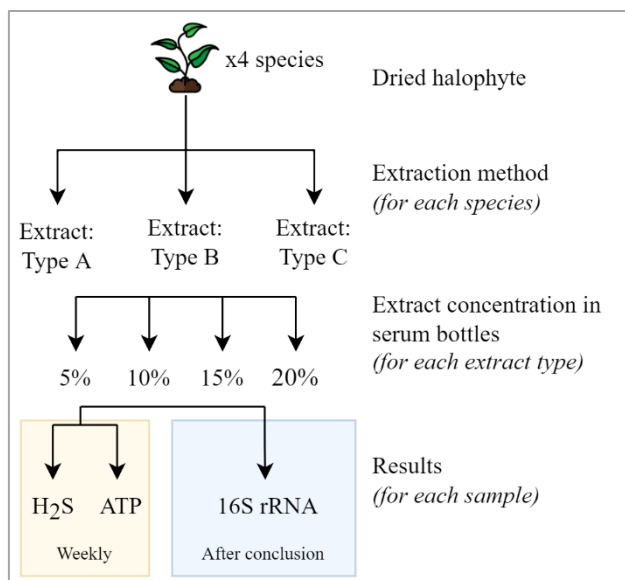


Figure 1: Experimental plan. From four halophyte species, three types of extracts were made. Each of these was tested in inoculated serum bottles at four concentrations (v/v%)

Each flask had its H₂S and ATP concentrations measured weekly over 28 days. At the time the experiments were terminated, liquid samples from each flask were frozen at -20°C. Selected samples were later sent for 16S rRNA Amplicon Sequencing at a commercial laboratory following their protocols. Coupons were collected, rinsed, and inspected for visible corrosion but were not weighed or 3D scanned in this experiment.

Extractions of plant extracts

The experiments in this paper describe the use of three different water-based extracts obtained by different extraction methods. The extraction methods are currently part of a pending proprietary patent application. Each of the three extraction methods was applied to the four plant species under investigation in this study. The extracts are identified in the paper as Type A, B, and C, as per Figure 1. Type C extract is a 50:50 mixture by volume of Type A and Type B

Collection and enumeration of SRB

A mixed bacterial culture (MBC) was obtained from the Danish Wadden Sea (N 55.31050, E 8.64248) using a hand-held sediment sampler ~20 cm deep into the waterlogged sediment three random sites within 50 meters of each other. After collecting the sediment, it was submerged in a 10 L plastic container with an addition of sterilized 0.9% (v/v) saline water. The container was sparged with 100% N₂, and the lid was closed tight and left in a fume hood at room temperature (22 °C).

A fresh preculture of the MBC was produced three days prior to the serum flask inoculation. A 250 mL blue cap flask containing 150 mL of Postgate medium was inoculated with a small amount (<10% v/v) of the Wadden Sea sediment from the sparged container and was left for three days at room temperature (22 °C), after which it was used to inoculate the serum flasks.

Serum flask fermentations

Table 1: The liquids added to each 100 mL flask. The total liquid volume in each flask was 50 mL at the start of the experiment

Sample	Postgate	Extract	Preculture
Control	48.0 mL	-	2.0 mL
5%	45.5 mL	2.5 mL	2.0 mL
10%	43.0 mL	5.0 mL	2.0 mL
15%	40.5 mL	7.5 mL	2.0 mL
20%	38.0 mL	10.0 mL	2.0 mL

Postgate medium was added to the flasks according to Table 1. A round UNS S31600 stainless steel coupon (Ø9 x 4 mm) was dropped into each flask. The flasks were flushed with nitrogen gas, capped with a butyl rubber stopper and clamped shut with an aluminum lid, as seen in Figure 2, and autoclaved for 15 minutes at 121 °C. After cooling to room temperature overnight, the flasks were inoculated with a 2 mL mixed microbial culture from a preculture as described above. Extracts were added immediately after, according to Table 1. The flasks were covered in aluminum foil and placed in a fume hood for four weeks at room temperature (22 °C). Each condition was run in duplicate containing an increasing percentage of halophyte extract from left to right.

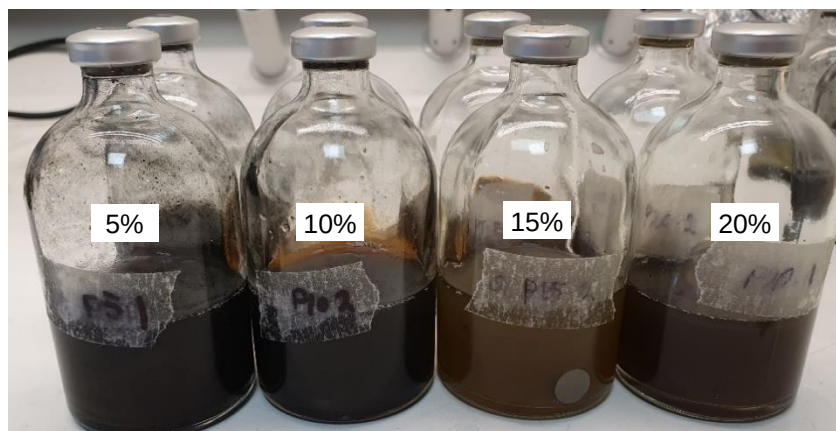


Figure 2: 100 mL serum flasks with stainless steel coupons and inoculated with MBC. Active volume was 50 mL of halophyte extracts/Postgate medium and 2 mL of inoculum.

Sample Analysis

Over four weeks, a 2 mL sample was taken every week from each serum flask with a sterile syringe and needle. Prior to sampling, the bottles were shaken. Samples were diluted with 8 mL of hyper-filtered water inside the syringe. H₂S concentration was measured by injection into an H₂S sensor⁽¹⁾, and ATP was measured immediately after taking a sample, using an RLU-based approach⁽²⁾. OD and cell mass were not measured due to large amounts of black FeS precipitated during fermentation.

After the experiment was terminated, liquid samples were selected for 16S rRNA amplicon sequencing at a commercial laboratory following their protocols to investigate how the microbial community changed in the presence of the halophyte extracts.^{23,24} More samples with extracts from one extraction method (Type B) were chosen than the other two types since the Type B extracts appeared to perform better at inhibiting MIC. As per the laboratory's protocols, the analysis targeted the bacteria 16S rRNA gene variable regions 3-4 (bV34-A) in combination with taxonomic classification against the SILVA 132 database.²³

The harvested steel coupons were rinsed with a mild soap solution (in demineralized water) and a soft brush to remove loose FeS precipitation. Then they were rinsed in demineralized water, dried by submersion in 99% ethanol, and inspected for visible corrosion.

RESULTS AND DISCUSSION

H₂S Generation

The graphs in Figure 3 show H₂S concentrations in the serum flasks over 28 days. The charts each show results from one type (denoted by a letter) of an extract from one halophyte species (denoted by a number) and the control sample. For each extract, the results from the highest and lowest concentrations are shown for each halophyte species as solid and dotted lines, respectively.

⁽¹⁾ *UniSense SULF microsensor*

⁽²⁾ *EnSURE™ Touch along with Hygiena AquaSnap Total*

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