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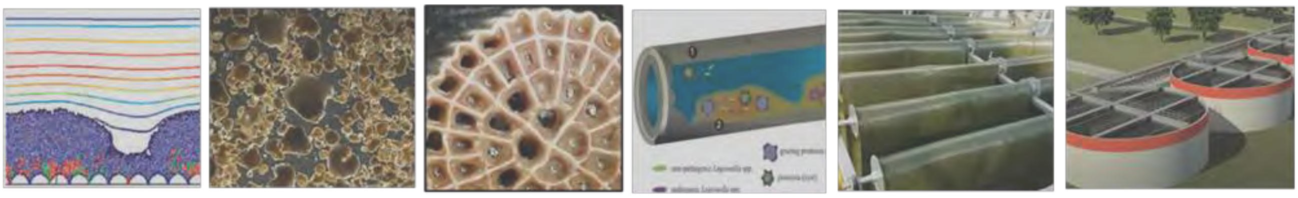

Clean Biocide Project: Halophilic plant extracts for prevention of microbiologically influenced corrosion (MIC)

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Summary: Studying the use of extractives from halophilic (salt tolerant) plants to mitigate the formation of biofilms and biocorrosion (MIC). Initial screening of four different plants have shown promising results. At the highest concentration tested (20%) the extracts were able to reduce H_2S production caused by sulfate-reducing bacteria, by 2 times. 16S rRNA amplicon sequencing results shows a significant reduction in the abundance of sulfate-reducing organisms in particular.

Keywords: Microbiologically influenced corrosion (MIC); Halophyte Extract; Reactor Testing

Introduction

Many water systems are susceptible to internal corrosion caused by or enhanced by bacteria; microbiologically influenced corrosion (MIC). One of the main groups of bacteria responsible of such corrosion is anaerobic sulphate reducing bacteria (SRB). (Skovhus, Enning and Lee, 2017). Stagnant water in fire sprinkler systems, nutrient rich sewage water, water used in from oil production, and cooling water for various industrial applications are all examples of systems in which biofilm formation and MIC have favorable conditions. All of the above also have the potential for catastrophic failure if a leak is caused by untended corrosion. To mitigate MIC, corrosion resistant coatings, biocides, and mechanical cleaning are used.

Halophytes are salt tolerant plants that can thrive in salt marshes, beaches and intertidal zones where other plants cannot. They do this by producing a variety of bioactive compounds such as antioxidants, biocides and many other. (Cybulska *et al.*, 2014). Initial trials have shown a reduction in corrosion when extractives from some halophyte species are introduced into an MIC prone environment

Materials and methods

MIC effects was studied on carbon steel coupons in serum flasks and laboratory scale biofilm reactors. 100 ml serum bottles filled with “Postgate desulfovibrio medium” (DSMZ GmbH, 2017), halophyte extract, a coupon and inoculated with anaerobic sediment from the Wadden sea in Denmark. Four different halophytes were investigated, each of them extracted in three different ways. Four different concentrations of each extract were tested in flasks over the course of four weeks. *The halophyte types and the extraction methods are part of a pending patent and cannot be disclosed at present time.*

Biofilm reactors’ media was inoculated with the produced water from the oil and gas industry. The medium was recirculated between a flask and the reactor and from changed twice per week. Extracts were added with the medium after 2 weeks. One was dosed with extracts for 4 hours twice per week, and one was dosed continuously.

During the experiments weekly samples of H_2S and tATP was measured; H_2S to assess activity of SRB and tATP to assess activity of all microbial activity. Coupons were cleaned and weighted before and after experiments and the weight loss was noted

Results and discussion

The experiments show a significant reduction in H2S, indicating a reduction of SRB species; the effect becoming more pronounced as concentration increases (***Figure 10***). Visual degradation of the biofilm was observed during the experiment after addition of extracts and Biofilm formation on carbon steel coupons from a bioreactor was reduced by two-thirds.

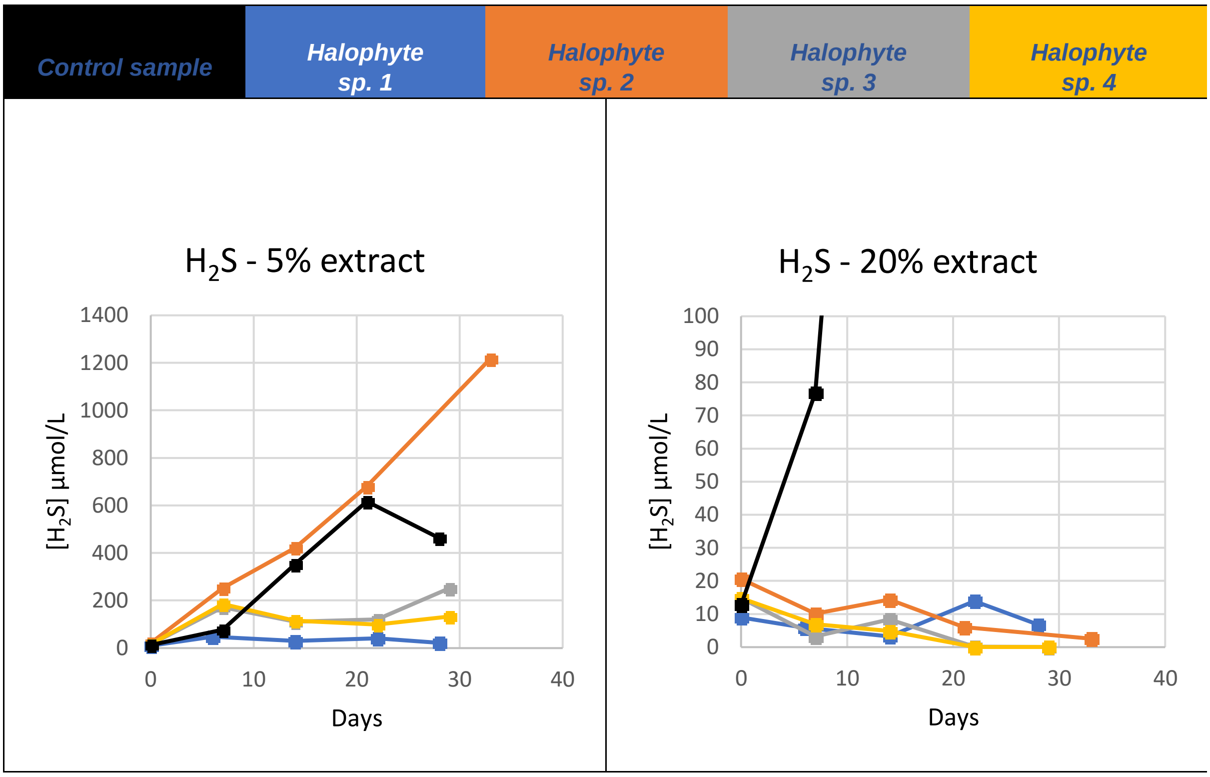

Figure 10: Serum bottle H_2S concentration in bottles with 5% (a) and 20% (b) (v/v%) extract. Black line is concentration observed in controls without added halophyte extracts Note that the Control (black) are the same, but the Y-scale is changed.

Furthermore, next generation 16S rRNA amplicon sequencing of DNA from Bacteria and Archaea, proved a significant shift away from SRBs (

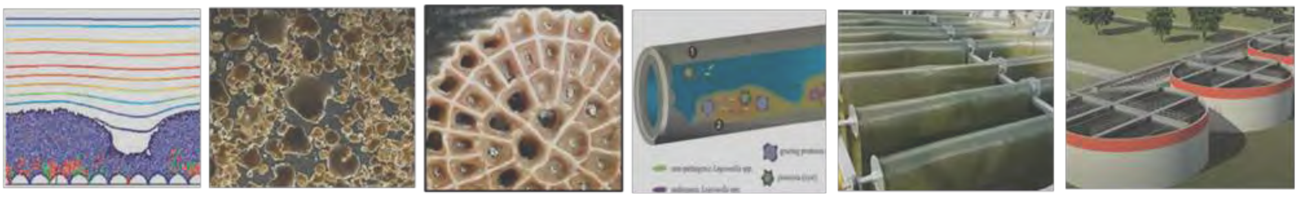

Table 2) in the microbial composition when compared to samples not treated with extracts. Lastly, visual and measurable reduction in corrosion was observed with 3D surface scanning. Long-term solutions to prevent MIC using natural antimicrobial compounds from halophyte plants are discussed and proposed in this study.

Table 2: 16S rRNA amplicon sequencing results comparing the most common species in two samples. Highlighted in bold are species identified as causes for MIC. Arrows indicate an increase or decrease in relative abundance in the sample. Note that a relative increase could be caused by a decrease in overall bacteria concentration.

BACTERIA GENERA	CONTROL	20% EXTRACT	
Clostridium sensu stricto 13	18%	5%	↓
Methanosarcina	8%	0%	↓
Photobacterium	8%	6%	↓
Terrisporobacter	7%	1%	↓
Fusobacteriaceae	6%	1%	↓
Vibrio	6%	23%	↑
Desulfosporosinus	6%	0%	↓
Clostridium sensu stricto 1	4%	6%	↑
(FAMILY) Lachnospiraceae Spp.	4%	0%	↓
Paraclostridium	3%	4%	↑
(ORDER) Clostridiales Spp	3%	1%	↓
Shewanella	3%	2%	↓
Epulopiscium	2%	2%	→
Bacillus	1%	1%	→
Clostridium sensu stricto 7	1%	38%	↑
OTHER	18%	10%	↓

In just a few days a biofilm had formed in both bioreactors. After two weeks when the extracts were added it was clear that adding the extracts continuously (***Figure 11-B: right***) was the superior method of the two and proved very effective at removing the established biofilm. H_2S readings were taken continuously and showed low SRB activity in the bioreactor (***Figure 11-B: right)*** Atempts at measuring the other reactor was made, but readings results were distorted by a thick biofilm layer covering the sensor.

Figure 11: (A) Bioreactor which was continuously treated with the halophyte extracts in growth medium for after 1 week (B) Comparison between bioreactors after experiment concluded and bioreactors were emptied. From left, bioreactor treated with halophyte extracts for 4 hours twice per week, and a bioreactor with halophyte extract added to growth medium. The first exhibit no disernable difference from before extracts were added.

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Is the presenting author an IWA Young Water Professional? Y/**N**
(i.e. an IWA member under 35 years of age)

Bio: Jakob Lykke Stein, M.Sc. in Chemical Engineering from Aalborg University Esbjerg in 2019. For the past year I have been working in a biorefining-focused research group. My focus is environmentally friendly biocides from halophytes (salt-tolerant plants) for the oil and gas industry, but our findings extend to other fields where biocorrosion happens. Starting November, I will be continuing my research as part of my Ph.D.