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A green biocide enhancer for the treatment of sulfate-reducing bacteria (SRB) biofilms on carbon steel surfaces using glutaraldehyde

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ABSTRACT

Generally speaking, a much higher concentration of biocide is needed to treat biofilms compared to the dosage used to for planktonic bacteria. With increasing restrictions of environmental regulations and safety concerns on large-scale biocide uses such as oil field applications, it is highly desirable to make more effective use of biocides. In this paper a green biocide enhancer ethylenediaminedisuccinate (EDDS) that is a biodegradable chelator, was found to enhance the efficacy of glutaraldehyde in its treatment of sulfate-reducing bacteria (SRB) biofilms. Experiments were carried out in 100 ml anaerobic vials with carbon steel coupons. The ATCC 14563 strain of *Desulfovibrio desulfuricans* was used. Biofilms on coupon surfaces were visualized using scanning electron microscopy (SEM). Experimental results showed that EDDS reduced the glutaraldehyde dosages considerably in the inhibition of SRB biofilm establishment and the treatment of established biofilms on carbon steel coupon surfaces.

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1. Introduction

Biofilms cause various problems such as medical infections, fouling of water cooling system, product contamination, and microbiologically influenced corrosion (MIC) (Carpentier and Cerf, 1993; Diosi et al., 2003; Raad et al., 2003; Kjellerup et al., 2006). MIC accounts for as much as 20% of all forms of corrosion, amounting to billons of dollars in losses each year (Flemming, 1996). A major Alaska Prudhoe Bay oil field pipeline had to be shut down due to a leak in 2006, which caused a turmoil in the global oil market. MIC was suspected to be one of two major factors (Jacobson, 2007) in the leak. MIC typically causes localized corrosion due to the patchy biofilms formed on a metal surface. Among aerobic and anaerobic bacteria related to MIC, sulfate-reducing bacteria (SRB) are most often blamed (Kobrin, 1993; Feio et al., 2000). SRB reduce sulfate to sulfide and produce hydrogen sulfide. Pitting may be initiated underneath biofilms. With further development of the biofilms, metabolic products may lower the local pH, thus deepening the pits (Videla and Herrera, 2005).

Based on their success in using ethylenediaminetetraacetic acid (EDTA) with antibiotics to eradicate biofilms on catheters, Raad and Sherertz (2001) patented the idea of treating SRB biofilms using chelators in combination with biocides. Initial experimental results showed that EDTA enhanced the glutaraldehyde's and THPS' inhibition of planktonic SRB growth (Zhao et al., 2005; Wen and Gu, 2007). EDTA's slow biodegradability has sparked a call for its replacement in various industrial applications with green chelators (European Commission, 2004). A popular biodegradable chelator is ethylenediaminedisuccinate (EDDS). The work below demonstrated that EDDS enhanced glutaraldehyde's treatment of SRB biofilms on carbon steel surfaces.

2. Materials and methods

2.1. Bacteria and culture conditions

Desulfovibrio desulfuricans subsp. *aestuarii* ATCC 14563 was used in this work. It is a marine strain SRB that favors a liquid medium with a salinity equivalent to 0.5–6 wt% NaCl (Ollivier et al., 1994). Enriched artificial seawater (ASW) and modified ATCC 1250 medium with 25 ppm Fe^{2+} were used for SRB growth. The composition of enriched ASW medium included a salt mix (Instant Ocean®[1] salt mix intended for marine aquariums) 36 g, $Fe(NH_4)_2(SO_4)_2$ 125 mg, sodium lactate (60 wt% syrup) 4.5 ml and yeast extract 1 g, in 1 L of water. The comparison between ASW and the typical natural

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[1] Instant Ocean is a registered trademark of Spectrum Brands, Atlanta, GA, USA.

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seawater was reported by Atkinson and Bingman (1996). Culture media were sterilized using an autoclave before use.

2.2. Substratum for biofilm growth

Disk shaped C1018 coupons with a top surface area of 1.12 cm^2 were used as the substratum for biofilm growth in 100 ml vials. The bottom surface and the side surface were coated with Teflon to prevent exposure. Before immersion into the vials, the exposed surface was polished using sand papers with 200, 400 and 600 grits successively.

2.3. EDDS

Octaquest®[2] E30, a trisodium salt of EDDS known as [S,S]-EDDS (Fig. 1), was used. It is a biodegradable chelator having similar chelation ability as EDTA, with no persistent metabolites formed during biodegradation (Schowanek et al., 1997).

2.4. Efficacies of biocide enhancers for inhibition of planktonic SRB growth

Planktonic SRB growth was carried out in 100 ml anaerobic vials. An anaerobic chamber with a nitrogen environment was used to provide an anaerobic environment for inoculation. After distributing 50 ml of the medium into each vial and adding an appropriate amount of a biocide and a biocide enhancer, it was inoculated with a two-day old SRB seed culture. The initial SRB cell concentrations were 1.42×10^6 cells/ml right after inoculation. The vials were sealed and then placed in an incubator at 37 °C. SRB growth was monitored by counting the (viable) motile planktonic cells using a hemocytometer under an optical microscope at 400× magnification in broth samples drawn from the vials using a syringe.

2.5. SEM observations

To study the SRB biofilm on a C1018 carbon steel coupon under SEM, the coupon's exposed surface was exposed to 2.5 wt% glutaraldehyde for 8 h and subsequently washed with a graded series (30%, 50%, 70%, 100% v/v) of ethanol for dehydration. The sample was then critical point dried and coated with a gold alloy prior to SEM observations. The entire surface area of coupons was examined under SEM to locate sessile SRB.

2.6. EDDS and glutaraldehyde in inhibiting SRB biofilm formation

Laboratory experiments were carried out in 100 ml anaerobic vials at 37 °C. Nitrogen sparging was used to remove oxygen in liquids. In some cases, 0.5 g/L of cysteine as an oxygen scavenger was used to prevent contamination by trace amount of oxygen. Different concentrations of glutaraldehyde and EDDS were added to vials before inoculation (Table 1). 1 ml of two-day old SRB stock culture was used to inoculate each vial. The SRB cell concentration right after inoculation was about 4.3×10^5–5.1×10^5 cells/ml. Initial pH was around 6.8 in modified ATCC 1250 medium and around 7.8–8.1 in ASW. Coupons were taken out after around 8 days for SEM examination.

2.7. EDDS and glutaraldehyde in treating established SRB biofilms

Biofilms were pre-grown in ATCC 1250 medium. One coupon was taken out for SEM analysis 4 days after inoculation to validate that the coupon surface was indeed covered by a biofilm. Coupons covered with biofilms were washed with sterilized distilled water, and then transferred into vials with fresh modified ATCC 1250 medium. EDDS and glutaraldehyde at different concentrations were then added (Table 1). All the procedures were conducted in a nitrogen-filled anaerobic chamber. Effects of EDDS and glutaraldehyde treatment were checked 8 days after they were introduced using SEM analysis.

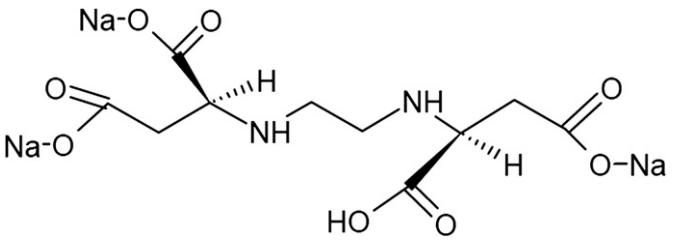

Fig. 1. Structure of [S,S]-EDDS.

3. Results and discussion

Glutaraldehyde is a widely used biocide in oil fields as well as tetrakis hydroxymethyl phosphonium sulfate (THPS), quaternary ammonium compounds (QAC), bromo-nitropropanediol (BNPD), etc. Because of its broad-spectrum and biodegradability, glutaraldehyde was selected in this study. Because of its interaction with the SRB culture medium, glutaraldehyde exhibited inhibition (i.e., suppression or delay) of SRB cell growth rather than killing them (Von Rege and Sand, 1998; Gardner and Stewart, 2002; De Savaria and de Mele, 2005; Cetin et al., 2007). Gardner and Stewart (2002) reported that 50 ppm of glutaraldehyde retarded the SRB planktonic growth to 143 h in Postgate C medium. Due to its ability to cross-link proteins, glutaraldehyde is also a common fixative (Hayat, 2000). Since the experimental duration was around 8 days to examine the existence of SRB, the surface morphology comparison would not be affected by the addition of glutaraldehyde as a fixative (2.5 wt%) at the end of experiments while the highest concentration used in the inhibition study was 500 ppm. The standard protocol using 2.5 wt% assures that all cells are immobilized by killing them.

Table 2 (data from Wen and Gu, 2007) shows the effects of EDDS on the enhancement of the inhibition of planktonic SRB growth. 30 ppm of glutaraldehyde combined with 2000 ppm of EDDS is effective to control the SRB growth, while biocide alone lost its inhibition on SRB growth after 5 days of inoculation. Since a chelator itself cannot inhibit planktonic SRB growth, it has to combine with a biocide to take effect. The treatment of 20 ppm of glutaraldehyde combined with 2000 ppm of EDDS is as good as or better than found with 30 ppm glutaraldehyde alone.

Biofilms protect sessile bacteria from biocide attacks (Denyer, 1995; Morton et al., 1998). Stoodley et al. (1999) showed that dense biofilms with sessile cells glued together by extracellular polymeric substances (EPS) increases mass transfer resistances. The limited nutrition supply decreases the bacteria metabolic activity and increases the resistance to biocides. Others suggested that a biofilm may change the physiology of sessile bacteria, which improve their

Table 1
Test matrix for EDDS enhancement of glutaraldehyde treatment of SRB biofilms on carbon steel surfaces in ASW.

		Inhibiting biofilm formation	Treating established biofilms
Medium	ASW	Modified ATCC 1250	Modified ATCC 1250
Glutaraldehyde (ppm)	25	0, 30	30, 500
EDDS (ppm)	0, 2000	0, 2000	0, 2000

[2] Octaquest is a registered trademark of Octel Performance Chemicals, Cheshire, United Kingdom.

Table 2
Time to attain stationary phase for planktonic SRB growth in ASW with and without EDDS and glutaraldehyde treatments.

Treatment	Time to attain stationary phase[a] (h)
2000 ppm of EDDS, no glutaraldehyde	21
10 ppm of glutaraldehyde, no EDDS	21
10 ppm of glutaraldehyde, 2000 ppm of EDDS	76
20 ppm of glutaraldehyde, no EDDS	76
20 ppm of glutaraldehyde, 2000 ppm of EDDS	116
30 ppm of glutaraldehyde, no EDDS	116
30 ppm of glutaraldehyde, 2000 ppm of EDDS	>212

[a] Starting from inoculation time. Cell concentration right after inoculation was around 1.4×10^6 cells/ml. Cell concentration at stationary phase was around 2.2×10^8 cells/ml.

biocide resistance (Morton et al., 1998; Fux et al., 2005). A much higher concentration of biocide may be needed to remove the sessile bacteria, compared to the dosage for treating planktonic bacteria (Davies, 2003; Meyer, 2003). This is supported by the results in Fig. 2 that indicates that even with a glutaraldehyde concentration as high as 500 ppm for the treatment of a pre-grown SRB biofilm, sessile SRB cells were still visible. A more effective treatment is desired to reduce the dosage.

Trace metals such as manganese, zinc, iron are necessary for bacteria metabolism and biofilm growth (Dunne and Burd, 1992). Calcium was reported to be essential in the bonding of polymer molecules in biofilms (Carpentier and Cerf, 1993). Researchers found that bacterial adhesion is sensitive to chelating agents, which have been introduced to treat biofilms during sanitization of medical instruments (Taweechaisupapong and Doyle, 2000; Banin et al., 2006; Chudzik et al., 2007). Raad et al. (2003) reported that EDTA combined with minocycline is effective in eradicating biofilms on catheter surfaces. They also found that the eradication of *Staphylococcus aureus* and *Candida parapsilosis* biofilms was sped up greatly by using 25% (v/v) ethanol with minocycline–EDTA (Raad et al., 2007). Weinberg (2004) pointed out that biofilm formation was suppressed by iron chelators.

Fig. 3 shows the comparison of coupon surfaces with and without biocide enhancer. Surface morphology is different between Fig. 3a (without EDDS) and Fig. 3b (with EDDS). In Fig. 3a sessile SRB cells are clearly visible, while in Fig. 3b they are absent. Figs. 4 and 5 show the comparison of coupon surfaces with different EDDS and glutaraldehyde concentrations in modified ATCC 1250 medium.

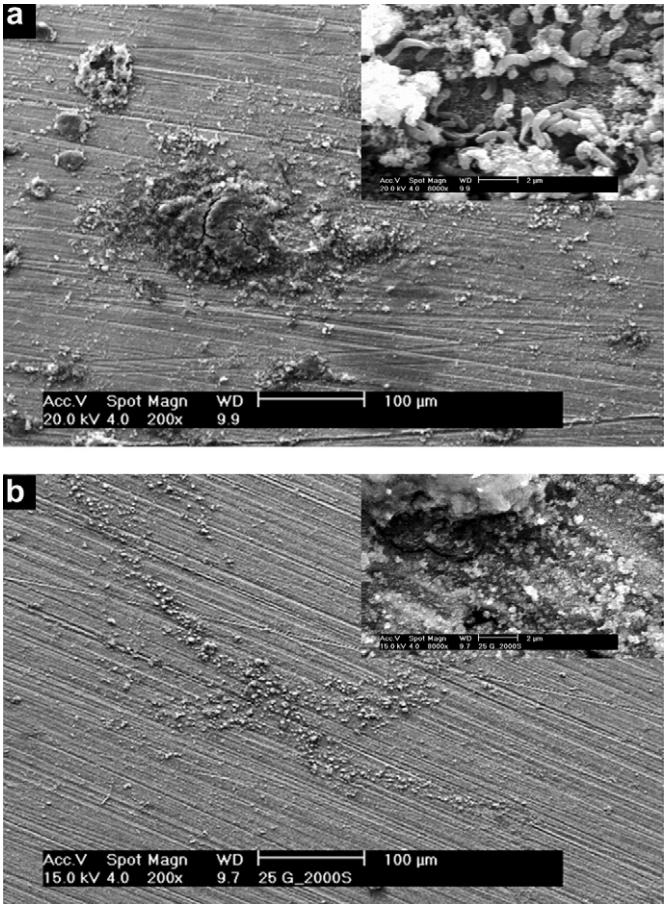

Fig. 3. Effects of EDDS enhancement of glutaraldehyde on preventing SRB biofilm establishment using 25 ppm of glutaraldehyde in ASW for 8 days: (a) without EDDS; (b) with 2000 ppm of EDDS. The figure is representative of about 0.3% of the total exposed coupon surface. Scale bar in the insert is 2 µm.

Sessile SRB grew better in ATCC 1250 that is much more nutritious than ASW. EDDS alone did not prevent SRB biofilm establishment (Fig. 4). In contrast, sessile SRB were absent on coupon surface with 30 ppm of glutaraldehyde and 2000 ppm EDDS (Fig. 5b), while the

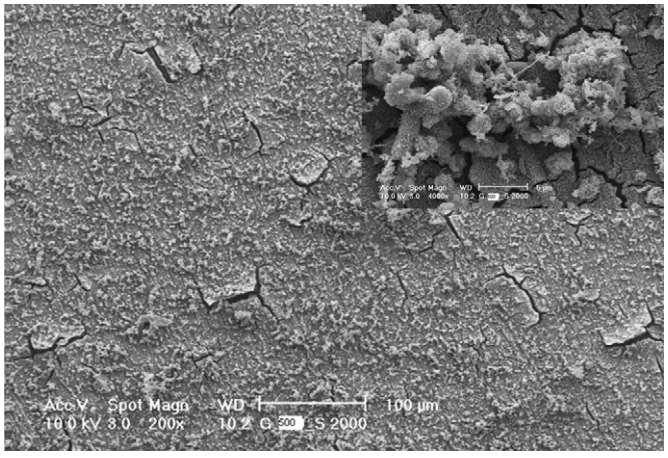

Fig. 2. Pre-grown SRB biofilm treated with 500 ppm glutaraldehyde in modified ATCC 1250 medium for 8 days. The figure is representative of about 0.3% of the total exposed coupon surface. Scale bar in the insert is 5 µm.

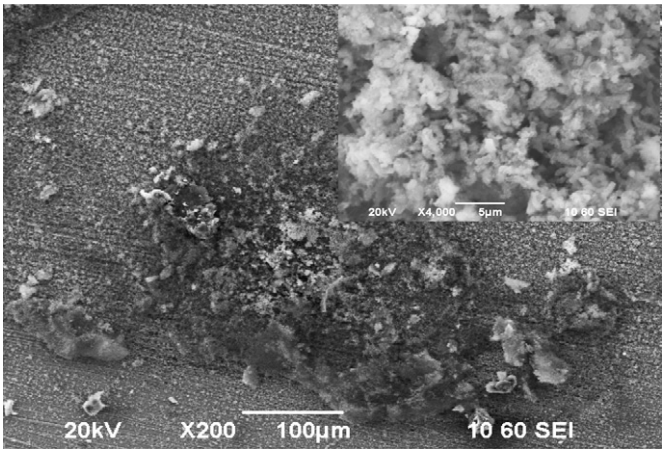

Fig. 4. Control sample with 0 ppm of glutaraldehyde and 2000 ppm of EDDS in modified ATCC 1250 medium for 8 days. The figure is representative of about 0.3% of the total exposed coupon surface. Scale bar in the insert is 5 µm.

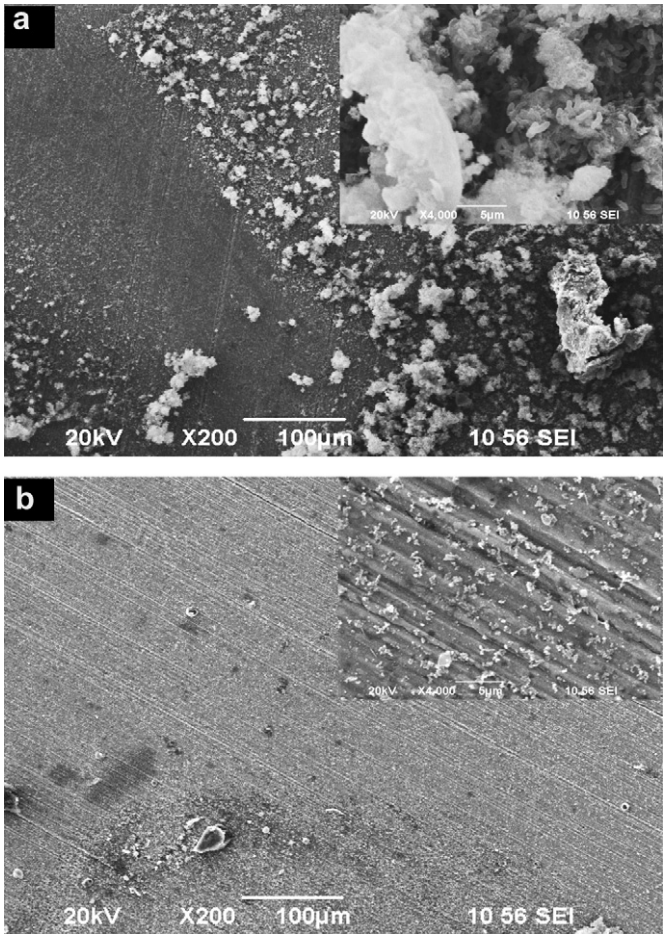

Fig. 5. Effects of EDDS enhancement of glutaraldehyde on preventing SRB biofilm establishment using 30 ppm of glutaraldehyde in modified ATCC 1250 medium for 8 days: (a) without EDDS; (b) with 2000 ppm of EDDS. The figure is representative of about 0.3% of the total exposed coupon surface. Scale bar in the insert is 5 μm.

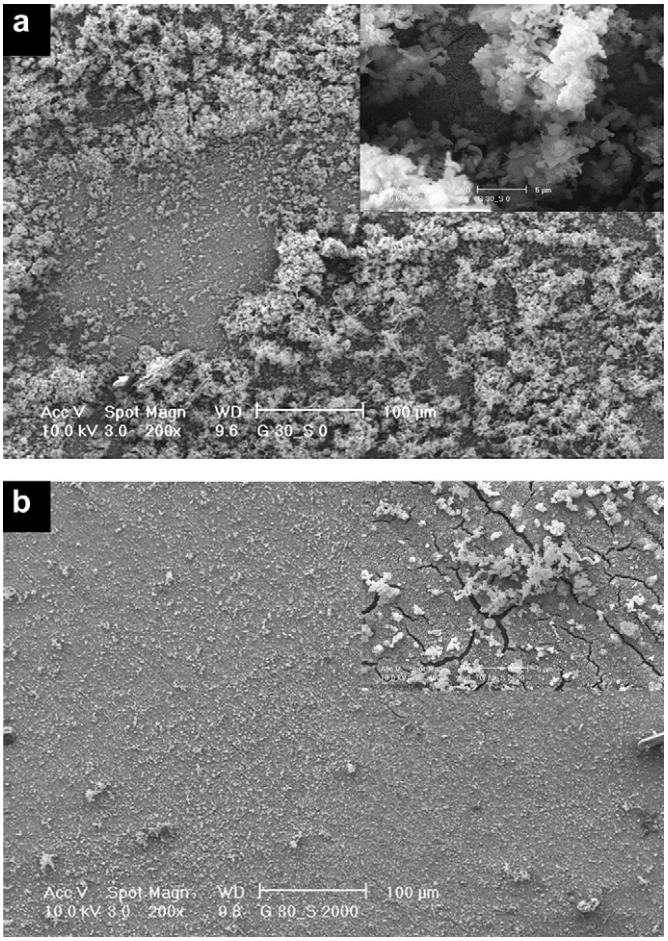

Fig. 6. Effects of EDDS enhancement of glutaraldehyde on treating established SRB biofilm using 30 ppm of glutaraldehyde in modified ATCC 1250 medium for 8 days: (a) without EDDS; (b) with 2000 ppm of EDDS. The figure is representative of about 0.3% of the total exposed coupon surface. Scale bar in the insert is 5 μm.

same concentration of glutaraldehyde alone was not effective against the sessile SRB (Fig. 5a).

Piet and Rossmoore (1984) reported the synergistic effects of monocopper citrate (II) (MCC) with antimicrobial agents. MCC as a chelator by itself did not inhibit bacterial growth. The authors stated that the possible working mechanism was that toxic copper ions form complexes with MCC in the outside liquid medium at a high pH and they are released inside the microbial cells at pH 7. Rosemoore (1987) later patented its uses. One of the specified applications was in metalworking fluids. Allison et al. (2000) discussed the possible antimicrobial resistance mechanisms of biofilms. The reaction-diffusion-limitation theory may explain the SRB biofilm behavior. In addition to its barrier to antimicrobial/biocide, EPS itself will react with biocides, thus reduce the effectiveness of biocides. As discussed previously, EDDS may inhibit the bacterial adhesion and then biofilm formation by chelating several cations which are keys for biofilm matrices, enhancing the penetration of glutaraldehyde to SRB biofilm.

Figs. 2 and 6 show the EDDS enhancement of glutaraldehyde treatment of established SRB biofilms. Sessile SRB cells were obviously present on coupon surfaces with 30 ppm glutaraldehyde treatment alone (Fig. 6a). With the added 2000 ppm of EDDS, sessile SRB cells were hardly noticeable (Fig. 6b). The effect of EDDS enhanced 30 ppm glutaraldehyde treatment is better than 500 ppm glutaraldehyde treatment without EDDS shown in Fig. 2. Vaara (1992) mentioned that chelators can remove the divalent cations such as Mg^{2+} and Ca^{2+} from lipopolysaccharide resulting in increased permeability on the outer membrane for Gram-negative cells. With the help of EDDS, it might be easier for glutaraldehyde to penetrate through the SRB biofilms and the outer membrane of SRB, achieving the higher efficacy on inhibition of sessile SRB growth.

4. Conclusions

Preliminary experimental data in this work demonstrated that EDDS has efficacy in enhancing glutaraldehyde for the inhibition of SRB biofilm establishment and for the treatment of established SRB biofilms. EDDS can be used to cut down the glutaraldehyde dosage considerably. The data above showed at least an order of magnitude dosage reduction could be achieved. These initial tests used a rather high concentration of EDDS to prove the concept. Tests using lower dosages of EDDS and combination with biocides like THPS, BNPD and QAC are desired in the future.

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References

Allison, D.G., Maira-Litran, T., Gilbert, P., 2000. Antimicrobial resistance of biofilms. In: Evans, L.V. (Ed.), Biofilms: Recent Advances in Their Study and Control. Harwood Academic Publishers, Amsterdam, Netherlands, pp. 149–166.

Atkinson, M., Bingman, C., 1996. The composition of several synthetic seawater mixes. Journal of Aquariculture and Aquatic Science 8, 39–43.

Banin, E., Brady, K.M., Greenberg, E.P., 2006. Chelator-induced dispersal and killing of *Pseudomonas aeruginosa* cells in a biofilm. Applied and Environmental Microbiology 72, 2064–2069.

Carpentier, B., Cerf, O., 1993. Biofilms and their consequences with particular reference to hygiene in the food industry. Journal of Applied Bacteriology 75, 499–511.

Cetin, D., Bilgic, S., Donmez, G., 2007. Biocorrosion of Low alloy steel by *Desulfotomaculum* sp. and effect of biocides on corrosion control. ISIJ International 47, 1023–1028.

Chudzik, B., Malm, A., Rajtar, B., Polz-dacewicz, M., 2007. *In vitro* inhibitory activity of EDTA against planktonic and adherent cells of *Candida* sp. Annals of Microbiology 57, 115–119.

Davies, D., 2003. Understanding biofilm resistance to antibacterial agents. Nature Reviews Drug Discovery 2, 114–122.

Denyer, S.P., 1995. Mechanisms of action of antibacterial biocides. International Biodeterioration and Biodegrading 36, 227–245.

De Savaria, S.G., de Mele, M.F.L., 2005. Enhancement of glutaraldehyde biocidal efficacy by the application of an electric field. Effect on sessile cells and on cells released by the biofilm. World Journal of Microbiology & Biotechnology 21, 1077–1081.

Diosi, G., Telegdi, J., Farkas, Gy., Gazso, L.G., Bokori, E., 2003. Corrosion influenced by biofilms during wet nuclear waste storage. International Biodeterioration and Biodegradation 51, 151–156.

Dunne, W.M., Burd, E.M., 1992. The effects of magnesium, calcium, EDTA, and pH on the in *vitro* adhesion of *Staphylococcus epidermidis* to plastic. Microbiology and Immunology 36, 1019–1027.

European Commission, 2004. Edetic acid (EDTA) (CAS No: 60-00-4): European Union Risk Assessment Report Vol. 49. EINECS No: 200-449-4. European Chemicals Bureau, Luxembourg, online. Available at: http://ecb.jrc.ec.europa.eu/DOCUMENTS/Existing-chemicals/RISK_ASSESSMENT/REPORT/edtareport061.pdf (accessed 02.01.09).

Feio, M.J., Rainha, V., Reis, M.A., Lino, A.R., Fonseca, I.T.E., 2000. The influence of *Desulfovibrio desulfuricans* 14 ATCC 27774 on the corrosion of mild steel. Materials and Corrosion 51, 691–697.

Flemming, H.C., 1996. Economical and technical overview. In: Heitz, E., Flemming, H.C., Sand, W. (Eds.), Microbially Influenced Corrosion of Materials. Springer Verlag, Berlin, New York, pp. 5–14.

Fux, C.A., Costerton, J.W., Stewart, P.S., Stoodley, P., 2005. Survival strategies of infectious biofilms. Trends in Microbiology 13, 34–40.

Gardner, L.R., Stewart, P.S., 2002. Action of glutaraldehyde and nitrite against sulfate-reducing bacterial biofilms. Journal of Industrial Microbiology & Biotechnology 29, 354–360.

Hayat, M.A., 2000. Chemical fixation. In: Hayat, M.A. (Ed.), Principles and Techniques of Electron Microscopy: Biological Applications, fourth ed. Cambridge University Press, Cambridge, UK, pp. 28–45.

Jacobson, G.A., 2007. Corrosion at Prudhoe Bay – a lesson on the line. Materials Performance 46, 27–34.

Kjellerup, B.V., Gudmonsson, G., Sowers, K., Nielsen, P.H., 2006. Evaluation of analytical methods for determining the distribution of biofilm and active bacteria in a commercial heating system. Biofouling 22, 145–151.

Kobrin, G., 1993. A Practical Manual on Microbiologically Influenced Corrosion. NACE International, Houston, TX.

Meyer, B., 2003. Approaches to prevention, removal and killing of biofilms. International Biodeterioration and Biodegradation 51, 249–253.

Morton, L.H.G., Greenway, D.L.A., Gaylarde, C.C., Surman, S.B., 1998. Consideration of some implications of the resistance of biofilms to biocides. International Biodeterioration and Biodegradation 41, 247–259.

Ollivier, B., Caumette, P., Carcia, J.-L., Mah, R.A., 1994. Anaerobic bacteria from hypersaline environments. Microbiological Reviews 58, 27–38.

Piet, L., Rossmoore, H.W., 1984. The evaluation of monocopper (II) citrate as a potential antimicrobial metal complex. Developments in Industrial Microbiology 25, 531–536.

Raad, I., Chatzinikolaou, I., Chaiban, G., Hanna, H., Hachem, R., Dvorak, T., Cook, G., Costerton, W., 2003. In vitro and ex vivo activities of minocycline and EDTA against microorganisms embedded in biofilm on catheter surfaces. Antimicrobial Agents and Chemotherapy 47, 3580–3585.

Raad, I., Hanna, H., Dvorak, T., Chaiban, G., Hachem, R., 2007. Optimal antimicrobial catheter lock solution, using different combinations of minocycline, EDTA, and 25-Percent ethanol, rapidly eradicates organisms embedded in biofilm. Antimicrobial Agents and Chemotherapy 51, 78–83.

Raad, I., Sherertz, R., 2001. Chelators in combination with biocides: treatment of microbially induced biofilm and corrosion. US patent 6,267,979.

Rosemoore, H.W., 1987. Synergistic antimicrobial or biocidal mixtures. US patent 4,708,808.

Schowanek, D., Feijtel, T., Perkins, C., Hartman, F.A., Federle, T.W., Larson, R.J., 1997. Biodegradation of [S, S], [R, R] and mixed stereoisomers of ethylene diamine disuccinic acid (EDDS), a transition metal chelator. Chemosphere 34, 2375–2391.

Stoodley, P., Dodds, I., Boyle, J.D., Lappin-Scott, H.M., 1999. Influence of hydrodynamics and nutrients on biofilm structure. Journal of Applied Microbiology Symposium Supplement 85, 19–28.

Taweechaisupapong, S., Doyle, R.J., 2000. Sensitivity of bacterial coaggregation to chelating agents. Immunology and Medical Microbiology 28, 343–346.

Vaara, M., 1992. Agents that increase the permeability of the outer membrane. Microbiological Reviews 56, 395–411.

Videla, H.A., Herrera, L.K., 2005. Microbiologically influenced corrosion: looking to the future. International Microbiology 8, 169–180.

Von Rege, H., Sand, W., 1998. Evaluation of biocide efficacy by microcalorimetric determination of microbial activity in biofilms. Journal of Microbiological Methods 33, 227–235.

Weinberg, E.D., 2004. Suppression of bacterial biofilm formation by iron limitation. Medical Hypotheses 63, 863–865.

Wen, J., Gu, T., 2007. Evaluations of a green biocide and a green biocide enhancer for the mitigation of biocorrosion using an electrochemical bioreactor. In: AIChE (American Institute of Chemical Engineers) (Ed.), Annual Meeting Conference Proceedings. Salt Lake City, UT, USA 4–9 November 2007. AIChE, New York.

Zhao, K., Wen, J., Gu, T., Nesic, S., 2005. Effects of biocides and a biocide enhancer on SRB growth. In: AIChE (American Institute of Chemical Engineers) (Ed.), Annual Meeting Conference Proceedings. Cincinnati, OH, USA, 30 October–4 November 2005. AIChE, New York.