International Biodeterioration & Biodegradation 143 (2019) 104173

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Examination of archived rusticles from World War II shipwrecks

Brenda J. Little [a], Jason S. Lee [a, *], Brandon R. Briggs [b], Richard Ray [c], Andrew Sylvester [d]

[a] Naval Research Laboratory, Stennis Space, Center, MS 39529, United States
[b] Department of Biological Sciences, University of Alaska-Anchorage, Anchorage, AK 99508, United States
[c] NVision Solutions Inc. Bay St. Louis, MS 39520, United States
[d] Department of Geology and Environmental Earth Science, Miami University, Middletown, OH 45042, United States

ARTICLE INFO

Article history:
Received 29 September 2016
Accepted 7 December 2016
Available online 23 December 2016

ABSTRACT

The authors examined the physiochemical and microbiological properties of archived rusticles from World War II shipwrecks in the Gulf of Mexico. Rusticles, iron (Fe)-rich accumulations on shipwrecks in marine environments, have long been assumed to be the result of low alloy steel corrosion. In many cases the assumed corrosion has been attributed to biodeterioration because of the presence of specific types of bacteria associated with the rusticles. However, archived rusticles from WWII shipwrecks in the Gulf of Mexico (GOM) do not have the mineralogical layering typical of iron corrosion products. Moreover, spatial relationships between bacteria and rusticles cannot be interpreted as biodeterioration. The authors concluded that environmental Fe plays a role in rusticle formation and differences in Fe concentrations can be used to explain differences in rusticle size and distribution with depth in the GOM. Both biotic and abiotic mechanisms for Fe accumulation are provided.

Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

UNESCO (2003) estimates that there are three million shipwrecks in the world's oceans. The Northern Shipwrecks Database (2016) contains loss records for more than 100,000 shipwrecks for North America over the past 400 years. Shipwrecks in oxygenated marine waters are characterized by iron (Fe)-rich accumulations on metal hulls, railings and machinery, containing low alloy steels, including carbon steel, wrought and cast iron (Cook and Peterson, 2005). The Fe-rich accumulations, first observed on the RMS Titanic, were referred to rusticles, based on shape (icicle-like) and color (reddish-orange) (Ballard, 1987). Since that original observation the term "rusticle" has been used to describe all shapes and sizes of metal-rich accumulations on marine shipwrecks (Cullimore et al., 2002).

Rusticles, from multiple shipwrecks and of varying ages, have been consistently described as porous Fe oxides/oxyhydroxides, having distinct microbial communities and containing accumulations of metal cations from seawater (Cullimore and Johnston, 2008). The spatial relationship between bacteria and rusticles has been assumed to be an indication that bacteria caused the deterioration of the low alloy steel. Cullimore and Johnston (2008) concluded that rusticles are corrosion products produced by microbiologically influenced corrosion, referred to as both biological extraction (Cullimore et al., 2002) and biodeterioration (Cullimore and Johnsen, 2001).

It follows from that conclusion that the quantity of Fe in rusticles can be used to predict hull deterioration. Cullimore and Johnsen (2001) estimated that approximately 650 tons (dry weight) of rusticles had accumulated on the bow section of the RMS Titanic. They equated that to a mass loss between 0.13 and 0.20 tons of Fe daily and concluded a remaining lifetime of 280–420 years for the wreck. Such calculations ignore the role of water chemistry, particularly dissolved and particulate environmental Fe, in the formation and stabilization of rusticles.

World War II shipwrecks in the Gulf of Mexico (GOM) provide a unique sample set to develop a comprehensive understanding of rusticle formation. The ships were sunk within months of each other at differing depths (554–1965 m) (Church et al., 2009), (Table 1). Therefore, exposure times are comparable, but exposure sites have the possibility of differing environmental conditions (Table 2).

In 2004, a multidisciplinary team surveyed six GOM wreck sites as part of the National Oceanographic Partnership Program sponsored by the US Department of the Interior, Minerals Management Service and the National Oceanic and Atmospheric Administration

* Corresponding author.
E-mail address: Jason.Lee@nrlssc.navy.mil (J.S. Lee).

http://dx.doi.org/10.1016/j.ibiod.2016.12.005
0964-8305/Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Table 1
World War II shipwrecks in the Gulf of Mexico.[a]

Structure	Depth (m)	Dates in Service	Vessel Type	Cargo	Observations
Virginia	87	1941 to 1942	Tanker	180 k barrels gasoline	Fish and invertebrates count incomplete due to poor visibility; vermilion snapper and various corals
Halo	143	1920 to 1942	Tanker	63 k barrels crude oil	Few brown rusticles, corals, invertebrates, reef fish
Gulfpenn	554	1921 to 1942	Tanker	90 k barrels gasoline	Some microbial concretions; abundant *Lophelia pertusa*, high invertebrate diversity, reef fishes
U-166	1256	1942 to 1942	U-Boat	Mines and torpedoes	Brown and white rusticles, Venue flytrap anemones, abundant red deep-sea crab, squat lobsters and other deepwater demersal species
Robert E. Lee	1490	1924 to 1942	Passenger Freighter	Passengers	Abundant brown rusticles, Venus flytrap anemones, red deep-sea crabs, squat lobsters and other deepwater demersal species
Alcoa Puritan	1964	1941 to 1942	Cargo Freighter	10 k tons bauxite	Greatest density of rusticle formations, predominant invertebrate was deep-sea crab, other demersal species

[a] Reproduced from Church et al. (2009) with permission from the Oceanography Society.

Table 2
Environmental conditions of seawaters exposed to shipwrecks.

Structure	Depth (m)	Salinity ($^{0/00}$)	Temp. (°C)	$[O_2]$ (mg L^{-1})	Phosphate (μM)	Silicate (μM)	Nitrate (μM)
Robert E. Lee	554	35	8	4.6	0.1	1.5	1.5
Gulfpenn	1490	34.96	4.2	3.1	0.8	5	13
Alcoa Puritan	1965	34.98	4.2	2.9	1.1	7	17

Office of Ocean Exploration and Research (Church et al., 2009). A remotely operated vehicle was used to collect rusticles from the wrecks of *Alcoa Puritan, Gulf Penn, and Robert E. Lee* (Fig. 1).

The following study examined the physiochemical properties and microbial communities of archived rusticles collected from the three GOM shipwrecks in 2004, using a combination of physiochemical characterization techniques including synchrotron-based *in-situ* micro X-ray diffraction (μ-XRD), scanning electron microscopy (SEM), transmission electron microscopy (TEM), inductively coupled plasma – optical emission spectroscopy (ICP-OES) and DNA sequence analysis. Data are used to challenge the hypothesis that rusticles are corrosion products.

2. Methods and Materials

2.1. Sample history

Dr. Roy Cullimore (Droycon Inc., Regina, Saskatchewan, Canada) supplied rusticles that had been collected in 2004 from *Alcoa Puritan, Gulfpenn, and Robert E. Lee.* Materials were received at the Naval Research Laboratory in Stennis Space Station, MS in 2008 and stored in a desiccator until their examination in 2009 and 2014–2016. Digital images were obtained for each piece of material prior to processing.

2.2. Scanning electron microscopy/energy dispersive spectroscopy (SEM/EDS)

Pieces of as-received rusticles were encased in EpoThin® low viscosity epoxy (Buehler, Lake Bluff, IL) and a sub-sample was cut from each using an IsoMet™ low speed diamond saw (Buehler, Lake Bluff, IL). Thin sections (~100 nm) were also prepared for synchrotron μ-XRD analysis. A Quanta™ 200 SEM (FEI Company, Hillsboro, OR) was used to collect images of epoxy embedded cross sections. Elemental analysis was performed by energy dispersive spectroscopy (EDS) using a Noran™ System 7 X-ray microanalysis system.

2.3. Synchrotron-based in-situ micro X-ray diffraction (μ-XRD)

Thin sections of epoxy embedded rusticles were polished to 1200 grit and ~35 nm thickness. Synchrotron-based *in-situ* micro X-ray diffraction (μ-XRD) analyses were performed at Sector 13IDE X-ray microscopy beamline Advanced Photon Source, Argonne National Laboratories (Argonne, IL). A MAR 345 image plate area detector was used for microcrystallography studies and was positioned at approximately 450 mm from the sample. Two-dimensional μ-XRD patterns were collected for 100 s at 16 KeV with a wavelength (λ) of 0.6850 Å. Two-dimensional diffractograms were converted to one-dimensional 2θ scans using the software package DioptasWin64. Crystalline phase identifications were made on the basis of peak position and peak intensities following the protocol of Gerke et al. (2013).

2.4. Inductively coupled plasma – optical emission spectroscopy (ICP-OES)

Rusticles from each of the three shipwrecks were ground by hand, with an agate mortar and pestle. Aliquots of each were used for inductively coupled plasma – optical emission spectroscopy. An Agilent 720ES ICP-OES with axial viewing was used to analyze all samples.

2.5. Transmission electron microscopy (TEM)

Individual rusticles from each shipwreck were fixed overnight in 0.1 M sodium cacodylate buffer (pH 7.0), 2.0% formaldehyde, and 2.5% glutaraldehyde; rinsed with distilled water and post-fixed 45 min in cacodylate buffered (pH 7.0) 1% osmium tetroxide; dehydrated with an ethanol series (50, 70, 85, 95, and 100%) followed by acetone; infiltrated in 50/50% by volume ERL 4206® resin [resin contains nonenyl succinic anhydride (26 g), vinyl cyclohexene dioxide (10 g), diglycidyl ether (6 g), and dimethylaminoethanol (0.2 g)]; and cured at 70 °C for 36 h. Ultrathin sections (~90 nm) were taken with a Porter-Blum MT-2B® microtome (Ivan Sorvall, Inc., Newton, CT) and a diamond knife (Diatome-U.S., Fort

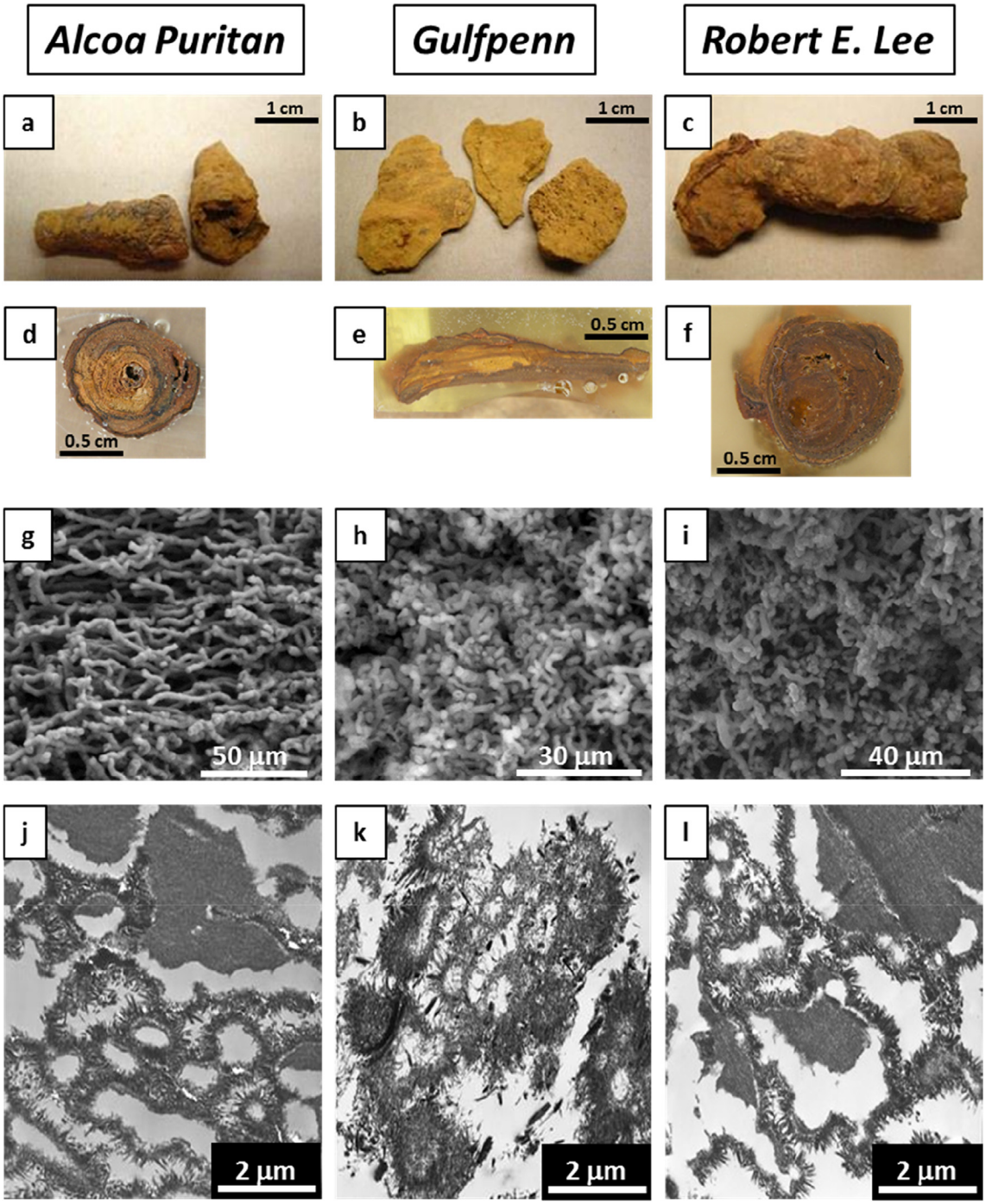

Fig. 1. Rusticles collected (2004) from *Alcoa Puritan* (a), *Gulfpenn* (b) and *Robert E. Lee* (c). Cross-sectioning illustrated layered structures in both cylindrical [*Alcoa Puritan* (c); *Robert E. Lee* (e)] and plate-like morphologies [*Gulfpenn* (d)]. Fe-encrusted bacterial shapes were located in the inner-most structures of all rusticles (g–i) where high-resolution cross-sectioning (j–l) illustrated voids with dimenions appropriate for bacteria, surrounded by fine-grained, amorphous Fe oxyhydroxides.

Washington, PA) and collected on 200-mesh copper grids. Grids were stained with lead citrate and 2% aqueous uranyl acetate and viewed using a Zeiss EM 109-T transmission electron microscope (Carl Zeiss, Inc., Thornwood, NY).

2.6. Molecular microbiological methods

Deoxyribonucleic acid (DNA) was extracted from 0.5 g samples of rusticles using the FastDNA™ Spin Kit (MP Biomedical, OH, USA) according to manufacture recommendations. Bead beating was used to increase extraction efficiency (Lu et al., 2014). The extracted DNA was amplified using universal bacterial and archaeal primers 515F and 806R, respectively (Hou et al., 2013) with Illumina adaptors and barcodes attached following the protocol of Caporaso et al. (2010) The amplified DNA was sequenced on an Illumina MiSeq using the 300 cycle V2 reagent kit. Sequences were demultiplexed and quality filtered using QIIME (Caporaso et al., 2010). Sequences that had an average Phred score <20 were removed from

subsequent analysis. Operational taxonomic unit (OTU) clusters at 97% sequence identity were determined using the UCLUST algorithm (Edgar, 2010). Taxonomy was assigned to a representative sequence from each OTU using the Greengenes database (McDonald et al., 2012).

3. Results

3.1. Gross morphology

Rusticles from the *Alcoa Puritan* (Fig. 1a) and *Robert E. Lee* (Fig. 1c) were cylindrical and composed of reddish brown to black layers. Each cylindrical piece contained a central cavity (Fig. 1d and f). Rusticles from the *Gulfpenn* had plate-like morphologies (Fig. 1b) made up of distinct layers ranging in color from reddish brown to black (Fig. 1e). No cavities were found in the plates.

3.2. Mineralogy

Alcoa Puritian and *Gulfpenn* rusticles were primarily α-FeOOH (goethite). The *Robert E. Lee* sample was primarily α-FeOOH with minor amounts of γ-FeOOH (lepidocrocite).

3.3. Chemistry

Fe concentrations for rusticles ranged from 52.7 to 64.7 percent by weight (% wt.) with sulfur (S) concentrations ranging from 0.47 to 6.31(% wt.) as quantified by ICP-OES (Table 3). A wide range of trace elements, including barium (Ba), cobalt (Co), chromium (Cr), copper (Cu), molybdenum (Mo), nickel (Ni), lead (Pb), rubidium (Rb), tin (Sn), strontium (Sr), vanadium (V), zinc (Zn) and zirconium (Zr) were detected in the rusticles (Table 4).

3.4. Micromorphology

Fe-encrusted bacterial shapes were located with SEM within well-defined regions adjacent to the cavity in case of the cylindrical samples and in the innermost layer in the plates (Fig. 1 g-i). In addition TEM micrographs for all three samples (Fig. 1 j-l) indicated the presence of voids with appropriate dimensions for bacteria, surrounded by fine-grained, amorphous Fe oxyhydroxides.

3.5. Microbiology

A total of 6449, 5981, and 4048 DNA sequences were obtained from the *Gulfpenn*, the *Robert E. Lee*, and the *Alcoa Puritian* rusticles, respectively. Archaea were present and accounted for 1–5% of the total community. The metal oxidizer *Metallosphaera sedula* was the most abundant Archaea followed by the ammonia oxidizer *Nitrosopumilus* sp. The bacterial phylum *Proteobacteria* dominated all samples. Putative sulfate or sulfur reducing bacteria consisted of *Desulfurobacteria, Desulfurovibro, Sulfurospirillum, Sulfurimonas*, and *Acidithiobacillus* sp. These organisms accounted for 34, 30 and 39% of the community for *Alcoa Puritian*, *Gulfpenn*, and *Robert E. Lee*, respectively. The Zetaproteobacteria, *Mariprofundus,* was detected in the *Gulfpenn* at 1.4% of the total community. Putative Fe reducing bacteria (FeRB) (i.e. *Caldithrix, Geobacter*) were also detected but consisted less than 1% of the total community for all rusticles.

Table 3
Major elements (% by weight) in rusticles collected in 2004 using ICP-OES.

Structure	Si	S	Al	Fe	Mn	Mg	Ca	Na	K	P
Robert E Lee	0.11	0.47	0.03	52.72	0.02	0.04	0.02	0.26	0.02	0.11
Gulfpenn	0.12	0.46	0.01	64.68	0.03	0.25	0.08	1.57	0.05	0.08
Alcoa Puritan	0.06	6.31	0.01	56.90	0.02	0.18	0.11	0.65	0.03	0.00

4. Discussion

Church et al. (2009) reported that the size and distribution of GOM rusticles varied with depth (Table 1). They reported that rusticles covered approximately 35 percent (%) of observable surfaces on the *Alcoa Puritan* (at 1965 m); rusticles on the *Robert E. Lee* (at 1490 m) were up to 2 m long, 250 mm thick and covered about 20% of observable surfaces; and no rusticles were reported for the *Gulfpenn* (at 554 m). Church et al. (2009) however, did indicate that microbial concretions were observed on approximately 30% of visible surfaces of the *Gulfpenn*. The environmental parameters that influence corrosion of carbon steel, i.e. temperature, salinity and oxygen, do not vary significantly among these locations and cannot be used to explain the observed differences in rusticle size and distribution (Table 2).

All GOM rusticles in this study were predominantly Fe (52.7–64.7%) and S (0.4–6.3%). Rusticles from the *Alcoa Puritian* and *Gulfpenn* were almost exclusively α-FeOOH. Similarly, the rusticle from the *Robert E. Lee* was primarily α-FeOOH with minor amounts of γ-FeOOH. The physiochemical properties and microbial content of the GOM rusticles were consistent with those previously reported for other shipwrecks. For example Stoffyn-Egli and Buckley (1992) indicated that the interior of the aggregates were α-FeOOH and the surface γ-FeOOH. They also examined flakes of Fe corrosion products from the *RMS Titanic* and demonstrated that the flakes had the same bulk mineralogy as the rusticles, i.e., a mixture of α-FeOOH and γ-FeOOH.

In the present study three approaches were used to identify microorganisms in the archived rusticles: SEM and TEM identification of Fe-encrusted micron scale structures (fossils, e.g., sheaths and stalks) and DNA sequencing. Bacterial shapes and voids in GOM rusticles, identified by SEM and TEM, were consistent with Fe-encrusted microorganisms (Fig. 1g-l) including Fe-oxidizing bacteria (FeOB). DNA sequencing, capable of detecting live and dead cells, identified FeOB and Fe-reducing bacteria, sulfate-reducing bacteria (SRB) and heterotrophic bacteria in the GOM samples. In previous investigations, bacteria in rusticles have been detected using liquid culture techniques, specific for viable microorganisms. Using liquid culture, Cullimore and Johnston (2008) evaluated the microbiological communities in rusticles and concluded that Fe-related bacteria (category includes both FeOB and FeRB), SRB and heterotrophic bacteria were present in rusticles collected from five shipwrecks in locations other than the GOM. Interestingly, results of the DNA testing from the GOM rusticles that had been stored for 9 years was similar to on-site liquid culture testing of rusticles from other locations.

FeOB derive energy from the oxidation of ferrous (Fe^{2+}) to ferric (Fe^{3+}) at/near neutral pH and in some cases the result is the formation of dense deposits of Fe oxides. Some FeOB are microaerophilic, requiring low concentrations of oxygen (O_2) for growth (Druschel et al., 2008). Lee et al. (2013) demonstrated that FeOB were among the first colonizers on corroding carbon steel in the marine environment. In that study, FeOB were not responsible for the corrosion, but grew at the microaerophilic conditions created at the corroding metal interface. FeRB reduce ferric (insoluble Fe) to ferrous (soluble) and are typically co-located with FeOB.

SRB are a group of ubiquitous, diverse anaerobes that use sulfate as the terminal electron acceptor, producing sulfide (Postgate, 1979). Under anaerobic conditions SRB couple the oxidation of organic carbon or molecular hydrogen (H_2) with reduction of sulfate (concentration in seawater 2 g l^{-1}) to sulfide that then reacts with metal ions, including Fe^{+2} (Little and Lee, 2007). Under these

Table 4
Trace elements (parts per million[a]) in rusticles collected in 2004 using ICP-OES.

Structure	Ba	Co	Cr	Cu	Mo	Ni	Pb	Rb	Sn	Sr	V	Zn	Zr
Robert E. Lee	8.41	3.98	9.13	5.70	58.4	4.5	11.1	21.2	7.89	6.48	101	132	14.4
Gulfpenn	2.96	7.19	11.9	0.80	97.2	2.1	16.6	67.0	3.99	33.6	63.9	72.4	14.8
Alcoa Puritan	2.07	6.83	0.64	BD	7.10	7.15	12.4	13.6	2.72	29.6	19.2	58.9	14.2

[a] Parts per million x 10^{-4} = **% by weight**.

circumstances metal sulfides precipitate around SRB. As more sulfides are produced by SRB the sulfide-deficient mineral is converted to a sulfide-rich mineral, e.g. in the case of Fe sulfides, $(Fe,Ni)S_{0.9}$ (makinawite) to FeS_2 (pyrite). Despite the identification of SRB in rusticles from shipwrecks around the world and the global 2 g L^{-1} sulfate in natural seawater, there have been no reports of sulfide minerals associated with SRB in rusticles. The similarity between mineralogical data collected from archived and freshly collected samples may be related to inherent instability. Steger and Desjardins (1978) reported that the predominant oxidation products of $Fe_{1-x}S$ were α-FeOOH and elemental S. In the GOM samples, α-FeOOH dominated the mineralogy and large concentrations (0.4–6.3 wt%) of S were detected by ICP-OES, suggesting that unstable Fe sulfides could have formed in the rusticles.

Heterotrophic bacteria all require organic carbon for growth. A specific aerobic, heterotrophic bacterium, *Halomonas titanicace* was isolated from the RMS Titanic (Sanchez-Porro et al., 2010). The popular press speculated that *H. titanicace* was "eating" the Titanic. Noel et al. (2016) working with *H. titanicae,* evaluated corrosion of freshly exposed carbon steel in the presence and absence of the bacterium. In their laboratory tests (Noel et al., 2016), corrosion in the presence of the bacterium was less than that observed in sterile controls under all exposure conditions. To date there have been no definitive experiments to support the claims that microorganisms are influencing the rate of degradation of marine shipwrecks. In referring to conclusions related to the Titanic, McCarty and Foecke (2008) challenged the notion that bacteria "… could extract iron from the ship …" Their objection to the claims of biodeterioration of the wreck is based on the absence of specific mechanisms for the proposed biological processes.

GOM rusticle properties (i.e., Fe oxides/hydroxides, porosity, microbial community accumulation of ions from seawater) are typical of some corrosion products, but cannot be used to conclude that the samples are, in fact, the result of biotic and or abiotic corrosion processes. Corrosion requires anodic and cathodic reactions, i.e., the anodic dissolution of Fe cannot continue in the absence of a cathodic reaction. No plausible, sustainable cathodic reaction for rusticle formation has been identified to account for anodic dissolution of shipwreck hulls. FeOOH within Fe corrosion products can accept electrons (act as a cathode) and be reduced to Fe_3O_4 (magnetite), a mixed valence (i.e., Fe^{2+} and $Fe^{3+)}$ oxide (McEnaney and Smith, 1980). The result is a three-layered deposit (Ray et al., 2010) that persists during storage. The layering which is found universally in Fe corrosion products formed in oxygenated environments (Gerke et al. 2008, 2010, 2012, 2013; Herro, 1998, Ray et al., 2009, 2010; Sarin et al., 2001, 2004a,b; Stone, 2007), with and without microorganisms, have not been identified in rusticles and challenges the notion that the rusticles on shipwrecks are corrosion products.

Rusticle growth on shipwrecks in GOM does not require continuous corrosion reactions. Environmental microbiology and chemistry can be used to explain the observations of Church et al. (2009) that rusticle size and distribution increased with depth. Nutrients increase with depth (Table 4). Global data indicate that the vertical concentration profiles for dissolved and particulate Fe [DFe and PFe, respectively] in marine environments are also nutrient-like, with low concentrations in the surface layers and increasing concentrations (nmol kg^{-1}) with depth (Johnson et al., 1997). In global surveys, [DFe] values at the surface were <0.2 nmol kg^{-1}, while below 500 m, the average [DFe] was typically 0.76 nmol kg^{-1}. Similar observations were made for PFe ranging from 0.1 nmol kg^{-1} in the first 500 m to 0.2–0.8 at depths >1000 m. FeOB, ubiquitous in marine environments and consistently identified in rusticles, can oxidize DFe and accumulate PFe, resulting in the formation of rust-colored accumulations of fine-grained Fe oxides/hydroxides. Many microorganisms produce negatively charged EPS that form metallic complexes with multivalent cations (Yue et al., 2015). Tourney and Ngwenya (2014) reported a clear association between EPS and Fe-rich precipitates. In addition, fine-grained Fe oxides, including goethite and lepidocrocite, have large surface areas and can act as abiotic sorbents of dissolved metal ions (Hua et al., 2012; Smith, 1999). A wide range of major and trace elements, including silicon (Si), titanium (Ti), aluminum (Al), manganese (Mn), magnesium (Mg), calcium (Ca), sodium (Na), potassium (K), phosphorus (P), Ba, Co, Cr, Cu, Mo, Ni, Pb, Rb, Sn, Sr, V, Zn and Zr were detected in the GOM rusticles (Tables 3 and 4). The concentrations of trace elements varied among the samples, but were in all cases consistent with ions in seawater (Wiesenburg and Little, 1988).

5. Conclusions

Rusticles are iron-rich accumulations that contain bacteria. In general, the microbiological and mineralogical data derived from archived GOM WWII shipwreck rusticles are in agreement with previous data reported for newly collected rusticles, e.g., FeOB, FeRB and SRB in association with α-FeOOH (goethite) and minor to trace amounts of γ-FeOOH (lepidocrocite). There are several reasons to reject the notion that rusticles are exclusively corrosion products. No one has identified a cathodic reaction that could sustain anodic dissolution of the encrusted shipwrecks. The physiochemical properties of rusticles from GOM shipwrecks are not consistent with the mineralogical layering typical of Fe corrosion products. Proposed mechanisms for rusticle formation must consider both biotic and abiotic processes for accumulation of environmental iron in the marine environment.

Acknowledgements

Micro-XRD analyses were performed at GeoSoilEnviroCARS (Sector 13), Advanced Photon Source (APS), Argonne National Laboratory. GeoSoilEnviroCARS is supported by the National Science Foundation - Earth Sciences (EAR-1128799) and Department of Energy - GeoSciences (DE-FG02-94ER14466). This research used resources of the Advanced Photon Source, a U.S. Department of Energy (DOE) Office of Science User Facility operated for the DOE Office of Science by Argonne National Laboratory under Contract No. DE-AC02-06CH11357. We would like to also thank undergraduate students Steven Marshall and Andrew Sylvester from Miami University, Middletown Ohio for their assistance with sample

preparation for and data collection at Sector 13 and microbiological analyses. NRL publication number NRL/JA/7303—15-2610. NRL efforts funded through NRL Base Program.

References

Ballard, R.D., 1987. The Discovery of the Titanic. Warner Books, New York.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K., Gordon, J.I., et al., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335–336.

Church, R.A., Warren, D.J., Irion, J.B., 2009. Analysis of deepwater shipwrecks in the Gulf of Mexico: artificial reef effect of six World War II shipwrecks. Oceanography 22, 50–63.

Cook, D.C., Peterson, C.E., 2005. Corrosion of submerged artifacts and the conservation of the USS *Monitor*. AIP Conf. Proc. 765, 91–96.

Cullimore, D.R., Johnston, L.A., 2008. Microbiology of concretions, sediments and mechanisms influencing the preservation of submerged archaeological artifacts. Int. J. Hist. Archaeol. 12, 120–132.

Cullimore, D.R., Pellegrino, C., Johnston, L., 2002. RMS Titanic and the emergence of new concepts on consortial nature of microbial events. Rev. Environ. Contam. Toxicol. 173 (173), 117–141.

Cullimore, R., Johnsen, R., 2001. Biodeterioration of the RMS Titanic [Online]: Encyclopedia Titanica. Available from: http://www.encyclopedia-titanica.org/rms-titanic-biodeterioration.html.

Druschel, G.K., Emerson, D., Sutka, R., Suchecki, P., Luther, G.W., 2008. Low-oxygen and chemical kinetic constraints on the geochemical niche of neutrophilic iron(II) oxidizing microorganisms. Geochim. Cosmochim. Acta 72, 3358–3370.

Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460–2461.

Gerke, T.L., Little, B.J., Luxton, T.P., Scheckel, K.G., Maynard, J.B., 2013. Strontium concentrations in corrosion products from residential drinking water distribution systems. Environ. Sci. Technol. 47, 5171–5177.

Gerke, T.L., Maynard, J.B., Schock, M.R., Lytle, D.L., 2008. Physiological characterization of five iron tubercles from a single drinking water distribution system: possible new insights on their formation and growth. Corros. Sci. 50, 2030–2039.

Gerke, T.L., Scheckel, K.G., Maynard, J.B., 2010. Speciation and distribution of vanadium in drinking water iron pipe corrosion by-products. Sci. Total Environ. 408, 5845–5853.

Gerke, T.L., Scheckel, K.G., Ray, R.I., Little, B.J., 2012. Can dynamic bubble templating play a role in corrosion product morphology? Corrosion 68, 025004–025001-025007.

Herro, H.M., 1998. MIC Myths - Does Pitting Cause MIC? CORROSION/98. NACE International, Houston, TX.

Hou, W.G., Wang, S., Dong, H.L., Jiang, H.C., Briggs, B.R., Peacock, J.P., Huang, Q.Y., Huang, L.Q., Wu, G., Zhi, X.Y., et al., 2013. A comprehensive census of microbial diversity in hot springs of Tengchong, Yunnan Province China using 16S rRNA gene pyrosequencing. PLoS One 8, e53350.

Hua, M., Zhang, S.J., Pan, B.C., Zhang, W.M., Lv, L., Zhang, Q.X., 2012. Heavy metal removal from water/wastewater by nanosized metal oxides: a review. J. Hazard. Mater. 211, 317–331.

Johnson, K.S., Gordon, R.M., Coale, K.H., 1997. What controls dissolved iron concentrations in the world ocean? Mar. Chem. 57, 137–161.

Lee, J.S., McBeth, J.M., Ray, R.I., Little, B.J., Emerson, D., 2013. Iron cycling at corroding carbon steel surfaces. Biofouling 29, 1243–1252.

Little, B.J., Lee, J.S., 2007. Microbiologically Influenced Corrosion Hoboken. John Wiley and Sons, Inc, New Jersey.

Lu, J.R., Gerke, T.L., Buse, H.Y., Ashbolt, N.J., 2014. Development of an *Escherichia coli* K12-specific quantitative polymerase chain reaction assay and DNA isolation suited to biofilms associated with iron drinking water pipe corrosion products. J. Water Health 12, 763–771.

McCarty, J.H., Foecke, T., 2008. What really sank the titanic: new forensic discoveries New York. Citadel Press.

McDonald, D., Price, M.N., Goodrich, J., Nawrocki, E.P., DeSantis, T.Z., Probst, A., Andersen, G.L., Knight, R., Hugenholtz, P., 2012. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. ISME J. 6, 610–618.

McEnaney, B., Smith, D.C., 1980. The reductive dissolution of gamma-FeOOH in corrosion scales formed on cast-iron in near-neutral waters. Corros. Sci. 20, 873–886.

Noel, N., Kranenburg, M., Klijnstra, J., 2016. *Halomonas Titanicae*- How Corrosive Is that Microorganism Actually Compared to a Sulfate Reducer Such as *Desulfovibrio Indonesiensis*, 18th International Congress on Marine Corrosion and Fouling.

Northern Shipwrecks Database, 2016. Northern Maritime Research. Available from: www.NorthernMaritimeResearch.com.

Postgate, J.R., 1979. The Sulfate Reducing Bacteria. Cambridge University Press, New York, NY.

Ray, R.I., Lee, J.S., Little, B.J., 2009. Factors contributing to corrosion of steel pilings in Duluth-Superior Harbor. Corrosion 65, 707–717.

Ray, R.I., Lee, J.S., Little, B.J., Gerke, T.L., 2010. The anatomy of tubercles: a corrosion study in a fresh water estuary. Mater. Corros. 61, 993–999.

Sanchez-Porro, C., Kaur, B., Mann, H., Ventosa, A., 2010. *Halomonas titanicae* sp nov., a halophilic bacterium isolated from the RMS Titanic. Int. J. Syst. Evol. Microbiol. 60, 2768–2774.

Sarin, P., Snoeyink, V.L., Bebee, J., Jim, K.K., Beckett, M.A., Kriven, W.M., Clement, J.A., 2004a. Iron release from corroded iron pipes in drinking water distribution systems: effect of dissolved oxygen. Water Res. 38, 1259–1269.

Sarin, P., Snoeyink, V.L., Bebee, J., Kriven, W.M., Clement, J.A., 2001. Physico-chemical characteristics of corrosion scales in old iron pipes. Water Res. 35, 2961–2969.

Sarin, P., Snoeyink, V.L., Lytle, D.A., Kriven, W.M., 2004b. Iron corrosion scales: model for scale growth, iron release, and colored water formation. J. Environ. Eng.-ASCE 130, 364–373.

Smith, K.S., 1999. Metal sorption on mineral surfaces: an overview with examples relating to mineral deposits. In: Plumlee, G.S., Logsdon, M.J. (Eds.), Reviews in Economic Geology, the Environmental Geochemistry of Mineral Deposits: Processes, Techniques and Health Issues. Society of Economic Geologists, Littleton, CO, pp. 161–182.

Steger, H.F., Desjardins, L.E., 1978. Oxidation of sulfide minerals, 4. pyrite, chalcopyrite and pyrrhotite. Chem. Geol. 23, 225–237.

Stoffyn-Egli, P., Buckley, D.E., 1992 August 16-21. The Titanic 80 years later: initial observations on the microstructure and biogeochemistry of corrosion products. In: Bailey, G.W., Bentley, J., Small, J.A., edtiors (Eds.), Proceedings of the 50th Annual Meeting of the Electron Microscopy Society of America, 27th Annual Meeting of the Microbeam Analysis Society and the 19th Annual Meeting of the Microscopical Society of Canada. San Francisco Press, Inc, San Francisco, CA.

Stone, D.A., 2007. The Aggrgated Precipitation of Iron Minerals in Three Systems: Tubular Growth, Liesegang Patterns and Interfacial Cementation. Ph. D. Dissertation. University of Arizona, Tucson.

Tourney, J., Ngwenya, B.T., 2014. The role of bacterial extracellular polymeric substances in geomicrobiology. Chem. Geol. 386, 115–132.

UNESCO, 2003. Underwater Cultural Heritage: United Nations Educational, Scientific and Cultural Organization. Available from: http://www.unesco.org/new/en/culture/themes/underwater-cultural-heritage/the-underwater-heritage/wrecks/.

Wiesenburg, D.A., Little, B.J., 1988. A synopsis of the chemical physical properties of seawater. Ocean Phys. Eng. 12, 127–165.

Yue, Z.B., Li, Q., Li, C.C., Chen, T.H., Wang, J., 2015. Component analysis and heavy metal adsorption ability of extracellular polymeric substances (EPS) from sulfate reducing bacteria. Bioresour. Technol. 194, 399–402.