

Scanning Electron Microscopic Study of Slime Formations in a Water Injection Station of Oil India Limited in Assam, India

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Microorganisms specifically groups of bacteria exhibiting physiological activities of production of acids are a major cause of concern because of their ability to induce corrosion in oil field pipelines and metal systems involved in water handling. Water Injection Stations as a means of secondary recovery from existing oil producing reservoirs, are often employed in most upstream oil and gas industries to ensure replenishment of voidage, maintenance of reservoir pressure and optimization of crude emulsion throughput. In the present study, scanning electron microscopy of macroscopic orange coloured slime formations sampled from leaking valves on the flow-lines of a Water Injection Stations of Oil India Limited revealed the presence of filamentous bacterial mats in association with diatoms. The species composition of the acidic slime formations from the sampled locations reveal the possible role of acid producing iron oxidizing bacteria (IOB) like *Acidithiobacillus ferrooxidans* in association with *Gomphonema* sp. in creating conditions for bio-corrosion.

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INTRODUCTION

Microorganisms that inhabit the most extreme acidic niches on our planet are highly diverse in terms of their physiologies and phylogenetic relationships (Hallberg & Johnson, 2001; Baker & Banfield, 2003). Bacteria are known to produce conspicuous biofilms or macroscopic structures such as streamers, snottites and microbial stalactites. Electron Microscopic examination of these macroscopic structures which are commonly encountered in acidic environments (Johnson, 2009), reveal that extracellular polymeric substances (EPS) produced by the bacterial cells are responsible for the phenomenon (Johnson et al., 2014). Industrial process water is not sterile, so there is biofilm in all water handling systems. However, problems occur when the biofilm builds up ("bio-fouling"), creating dead biomass and therefore a nutrient source that leads to re-growth of organisms in the water. Slime-forming acid producing bacteria grow in a patchy distribution over the metal surface and exclude oxygen via

respiration; the slime impedes oxygen diffusion, creating an oxygen concentration cell. This ultimately leads to the subsequent macro-fouling of the system (visualized as orange coloured slime) and concomitant enhancement of microbially induced corrosion. Although the economic implications of bio-fouling in industrial water systems are high the phenomenon is often overlooked by corrosion engineers who often fail to accept, recognize and mitigate the problem (Coetser & Cloete, 2005). Macroscopic microbial growth that appear as gelatinous orange coloured slime in acidic metal-rich environments have been reported to be composed of mixed bacterial communities of iron- and sulphur-oxidizing acidophiles. Acidophilic microorganisms primarily bacteria play a major role in enhanced bio-corrosion of the pipelines and metal systems involved in oil field water handling systems. In addition, water injection systems of upstream oil and gas industries infested heavily with bacteria also experience a drastic loss of injectivity, formation damage and filter plugging (Penkala et al., 2002). Scanning electron microscopy

(SEM) is used extensively to study the ultrastructure and morphological features of biological samples (Kim, 2012). The present investigation therefore aimed to ascertain the role of microorganisms in the formation of macroscopic acid slime in metal surfaces of leaking valves on the injection-lines using SEM.

MATERIALS AND METHODS

Description of Study Site

Water injection as secondary recovery scheme for the Nahorkatia oil field of Oil India Limited located in the North-Eastern state of Assam in India, was commenced in the year 1966. Water is taken out from deep tube wells from a depth of 100 to 120 m and sent to Water Injection Stations at a surface pressure of 6 to 8 kg/cm². Eight water injection pumps are being used to boost injection water pressure to around 125 kg/cm² and after boosting the water is, injected to nine water injection wells at depths of about approximately 2,700 m, through two different discharge manifolds. All the water injection lines are 4 inch carbon steel.

Sample Collection

Macroscopic slime and water samples in replicates of three, were collected from two identified locations namely Source Well Manifold and Deep Tube Well NBS (DTW NBS) (Fig. 1, 2) in the Water Injection Stations under study in sterile glass containers as per the guidelines given in NACE Standard TM0194-2004 (field monitoring of bacterial growth in oil and gas systems). The water samples for iron test were collected carefully and acidulated using hydrochloric acid (to pH 4~5) to avoid precipitation of Fe(OH)₃ and Fe₂O₃ during

transportation to laboratory. The Iron content as Fe²⁺ (in ppm) was determined calorimetrically by using iron test kit containing thioglycolic acid and ammonium thioglycolate (MColorTest; Merck KGaA, Germany) in a Shimadzu UV-Vis spectrophotometer (UV-1700, software: UV probe version 2.31; Shimadzu, Japan). P^H of the water samples was measured using a calibrated Hanna HI 2215 P^H meter (Hanna Instruments, USA).

Scanning Electron Microscopy of Slime Samples

Each of the replicate samples collected from the sampling locations were prepared by making thin sections with a sharp knife from which 5×10 mm sections were attached to a 15 mm specimen stub using a carbon tape. The sample was the air-dried in ambient conditions. The samples were then sputter coated with gold in a SEM coating system SC 502 (Bio-Rad, USA). SEM S-3600N (Hitachi High-Technologies Corporation, Japan) was used to analyze the specimens, using a secondary electron detector and an accelerating voltage of 15 kV. Images were captured with scan speed of 80/100 seconds (50/60 Hz). A total of ten SEM micrographs for each sample were generated at varying magnifications for image analysis and microorganism identification.

The SEM micrographs included the images of the macroscopic slime formations at the two sampling locations were compared with available web based resources (classification databases with images and previous scientific reports) like DIATOMS of the United States (<http://westerndiatoms.colorado.edu/about>), The ANSP Algae Image Database (https://diatom.ansp.org/algae_image/), European Diatom Database (<http://craticula.ncl.ac.uk/Eddi/jsp/index.jsp>), The Automatic Diatom Identification And Classification



Fig. 1. Macroscopic orange coloured (acidic) slime formation in a leaking valve of water flow line in Source Well Manifold.

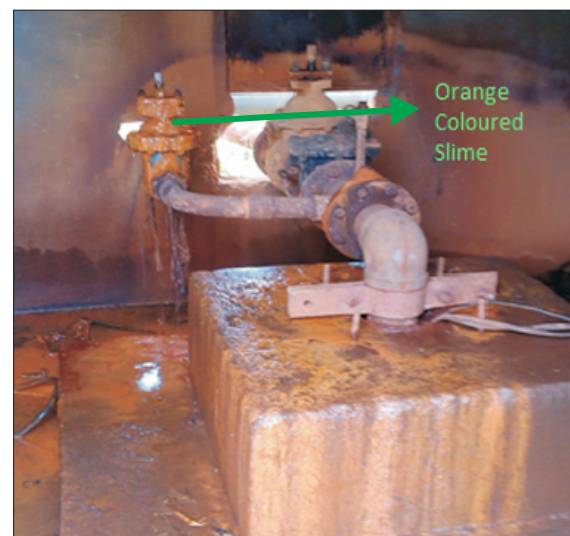
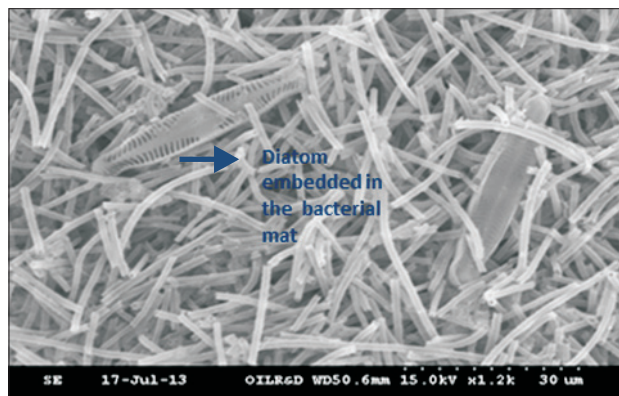
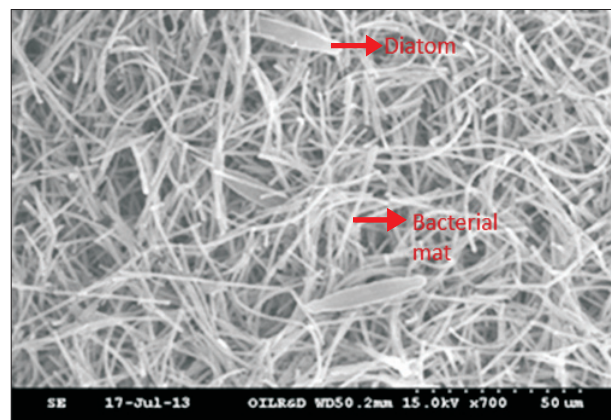


Fig. 2. Macroscopic orange coloured (acidic) slime formation in water flow line of Deep Tube Well NBS.

Table 1. Sample characteristics and summary of inferences

Sample No.	Description	pH	Iron content of water from the sampling point (ppm)	Species composition of biofilm
1	Orange coloured slime (Source Well Manifold)	2.8	6	<i>Acidithiobacillus ferrooxidans</i> and <i>Gomphonema</i> sp.
2	Orange coloured slime (Deep Tube Well NBS)	3.0	7	<i>Acidithiobacillus ferrooxidans</i> and <i>Gomphonema</i> sp.

**Fig. 3.** Scanning electron microscopy micrograph of orange coloured (acidic) slime formation sample (Source Well Manifold).**Fig. 4.** Scanning electron microscopy micrograph of orange coloured (acidic) slime formation sample (Deep Tube Well NBS).

(ADIAC) Diatom Image Database (<http://rbg-web2.rbge.org.uk/ADIAC/db/adiacdb.htm>), The Prokaryotes (eds by Gu JD and Mitchell R (ISBN: 978-3-642-31330-1), Description of *Acidithiobacillus ferrooxidans* in Microbewiki (<http://microbewiki.kenyon.edu/index.php/Thiobacillus>), Description of Iron Bacteria (https://pl.wikipedia.org/wiki/Bakterie_%C5%BCelazowe) to arrive upon the most probable species identities for the bacterial and diatom species.

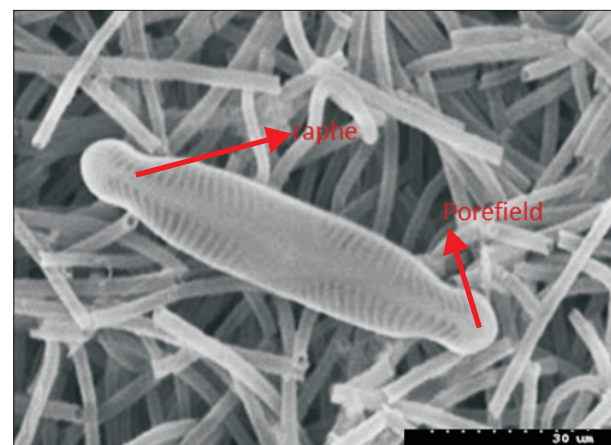
RESULTS

The macroscopic acid slime formations in both the sampling locations are shown in Fig. 1 and 2. SEM micrographs of gelatinous orange coloured slime sampled from the leaking valves of flowlines at both the sampling locations i.e., a Source Well Manifold and DTW NBS, shows the presence of cylindrical rod like structures that appear to be filamentous bacterial mats. These bacterial biofilms are seen to be interspersed with structures typical of diatoms (Fig. 3-5).

Table 1 summarizes the inferences from the SEM micrographs for both the sampling locations where the probable identity of the microbial species involved in the formation of macroscopic orange coloured acid slime formations is suggested.

DISCUSSION

The pattern of the microbial mats in both the sampling locations as visualized in the SEM micrographs are similar, with the likelihood of the presence of only a single morphotype of

**Fig. 5.** Diatom species involved in the biofilm formation.

bacteria (cylindrical rod like filaments) and diatom species in both the cases (Fig. 3 and 4). Similar observations about the limited biodiversity of the microbial communities of the streamers have been made by Hallberg and his co-workers in 2006, while studying the macroscopic streamer growths in acidic and metal-rich mine waters in North Wales (Hallberg et al., 2006). Further they reported that all of the iron-oxidizing autotrophic bacterial isolates from the two sampling sites under study i.e., an abandoned copper mine and a chalybeate spa were isolates of *A. ferrooxidans* rather than commonly encountered *Leptospirillum* spp. Wakao et al. (1985) isolated only iron and sulphur oxidizing bacteria from acid streamers

draining an iron sulfide mine in Japan (pH 1.8~2.2) and concluded that the streamers were composed (predominantly) of the chemolithotroph *A. ferrooxidans*, which was embedded in a gelatinous matrix.

The results clearly reveal the microbial origin of the macroscopic orange coloured acidic slime formations where biofilms of filamentous aerobic acid producing iron oxidizing bacteria (IOB) (*A. ferrooxidans*) that are able to utilize the oxidized iron available in the water can be visualized. Acid producing bacteria were also detected in high numbers in the water samples collected from the flow lines of both the locations where the acid slime formations were observed (data not shown). Ferrous iron-oxidizing filamentous bacterial isolates have been reported to form macroscopic slime (streamer-like growths) in laboratory conditions also (Johnson et al., 1992). The presence of diatoms (Fig. 5, Table 1) with the bacterial species in the biofilms is interesting as diatoms are now known to contribute to biocorrosion in association with acidophilic bacteria (Landoulsi et al., 2011). Cooksey (1981) while studying the adhesion of a fouling diatom to glass observed that the primary biofilm is generally dominated by bacteria, whereas the first major accumulation of biomass is attributed to diatoms. The adhesion of diatoms on metal surfaces itself is related with the secretion of mucilaginous material (EPS) (Hoagland et al., 1993). Diatom EPS which are mostly carbohydrate-based polymers with some protein content, provides diatoms with the ability to bind to both hydrophilic and hydrophobic substrata (Landoulsi et al., 2011). *Gomphonema* sp. have been reported from urban wetlands in India (Alakananda et al., 2013) and have been reported as the dominant biofouling diatom species on

stainless steel surface biofilms after immersion in natural waters (Andrewartha et al., 2010). Recently, Yang et al. (2015) while studying the seasonal variations in fouling diatom communities on the Yantai coast of China also reported *Gomphonema* sp. to be one of the dominant biofouling diatom communities that developed on glass slides immersed in seawater.

CONCLUSIONS

The present investigation successfully establishes the microbial origin of the macroscopic acid slime formations in the leaking valves on the flow-lines of a Water Injection Stations of Oil India Limited. The presence of diatoms in association with acid producing iron bacterial biofilms as revealed by the SEM micrographs indicate that a symbiotic relationship exists between the constituent microflora of these macroscopic slime formations for creating conditions for bio-corrosion. Currently research is undergoing to ascertain the identity of the microorganisms with molecular biological tools and in studying their role in enhancing bio-corrosion.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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