

DETECTION OF SULPHATE-REDUCING BACTERIA USING NACE SERIAL
DILUTION AND SRB RAPIDCHECK II METHODS

NORHAFIZA BINTI ROSLAN

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To my beloved mother and father,
and to all friends.
Thank you for your support.

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ABSTRACT

Microbiologically Influenced Corrosion (MIC) or bacterial corrosion can be a major problem in process equipment exposed to different types of waters in the production, processing and handling of crude oil and gas. Sulphate-reducing bacteria (SRB) are among the most destructive environmental organism and their industrial impact is widespread. They cause corrosion and stress corrosion cracking of metals and alloys used in petroleum production, cooling water system, waste water treatment and all aqueous process. Hence, there is a constant need to monitor bacterial proliferation in such systems in order to take remedial action. The most commonly used methodology to determine bacterial contamination is the Serial Dilution Technique which is culturing the sample in a laboratory until the numbers of SRB were sufficiently high to be detected by observation of general blackening of the sample. However, this method though very reliable, is quite involved and time consuming as it takes 28 days to analyze the result. So, another method is proposed to speed up this culturing process or bypass culturing altogether and monitor the organisms directly. This method is known as SRB Rapidcheck II. Results from the evaluation indicate that the SRB Rapidcheck II method provided higher SRB-population estimates in this case and results were available in 20 minutes instead of 28 days required by the NACE serial dilution method. Due to alarm activity of sulphate-reducing bacteria in the pipeline, it is recommended to conduct biocide treatment to kill SRB which is anaerobic.

ABSTRAK

Mikroorganisma penyebab karat atau bakteria kakisan boleh menjadi satu masalah yang besar dalam peralatan proses yang terdedah kepada pelbagai jenis air dalam pengeluaran, pemprosesan dan pengendalian minyak mentah dan gas. Bakteria pengurangan sulfat (SRB) adalah antara organisma paling merosakkan alam sekitar dan perindustrian di mana ia akan memberi kesan dan impak yang meluas. Ia menyebabkan pengurangan dan tekanan terhadap retakan karat logam dan aloi yang digunakan dalam pengeluaran petroleum, penyejukan system air, dan rawatan sisa air. Oleh itu, adalah perlu untuk memantau pembiakan bakteria dalam sistem tersebut bagi mengambil tindakan pemulihan yang berterusan. Kaedah yang paling biasa digunakan untuk menentukan pencemaran bakteria adalah teknik kecairan bersiri yang iaitu kultur sampel di makmal sehingga berjaya meraih bilangan yang cukup tinggi bagi dapat dikesan oleh pemerhatian melalui warna sampel yang menjadi hitam. Walaupun kaedah ini sangat boleh dipercayai, ia memakan masa kerana mengambil masa 28 hari untuk menganalisis hasilnya. Jadi, satu lagi kaedah dicadangkan untuk mempercepatkan proses kultur ini dan memantau organisma secara langsung. Kaedah ini dikenali sebagai SRB Rapidcheck II. Hasil dari penilaian menunjukkan bahawa kaedah SRB Rapidcheck II menyediakan populasi SRB yang lebih tinggi dan keputusan tersedia dalam masa 20 minit daripada 28 hari yang diperuntukkan oleh kaedah bersiri pencairan NACE. Disebabkan oleh adanya aktiviti bakteria pengurangan sulfat dalam saluran paip, adalah disyorkan untuk menjalankan rawatan biocide untuk menghapuskan SRB yang bersifat anaerobik.

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LIST OF ABBREVIATION

APS	-	Adenosin-5-phosphosulfate
ATP	-	Adenosin-5-triphosphate
IETS	-	Industrial Effluent Treatment System
GAB	-	General Activated Bacteria
MIC	-	Microbiologically Influenced Corrosion
MPN	-	Most Probable Number
NACE	-	National Association of Corrosion Engineers
SRB	-	Sulphate-Reducing Bacteria
TCOT	-	Terengganu Crude Oil Terminal
TDS	-	Total Dissolved Solids

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Microbiologically Influenced Corrosion (MIC) or bacterial corrosion can be a major problem in process equipment exposed to different types of waters in the production, processing and handling of crude oil and gas. MIC is the degradation of a material under the influence of environmental factors complicated by the metabolic activities of microorganisms, particularly bacteria. The attachment of bacteria to reservoir rock and the subsequent trapping of particulate matter in the biopolymer they produce can result in substantial reductions in the volume of water that can be injected into a formation. Sulfides generated in these bio-films can further reduce injectivity by precipitation as iron sulfides.

The growth of bacteria at the well-bore of water injection wells or in producing formations can result in the generation of substantial amounts of H₂S. This H₂S can create corrosion problems, safety hazards and result in reduced value of petroleum products. This souring is insidious since it often takes a long period of time before the extent of the problem is obvious. By the time H₂S is detected in the system, irreversible damage may have taken place, necessitating changes in metallurgy and additional processing of the petroleum products (Reinsel et al., 1996).

In order for bacteria problems to occur, the environmental conditions must be viable to allow growth of bacteria and the required nutrients have to be present. Bacteria require several different nutrients in order for microbial growth to occur. These materials may be naturally present or added in treating chemicals. Algal blooms in surface waters and marine environments can produce rich sources of nutrient for microbial growth (Dunsmore, 2003). When corrosion occurs in a system, the cathodic corrosion product is sometimes hydrogen. This hydrogen is available to microorganisms that can produce the enzyme hydrogenase. The bacteria can then make use of the corrosion process as a nutrient source.

In general, MIC will occur where the bulk solution pH ranges from 5 to 9 and total dissolved solids are less than 200,000 ppm (Boivin, 2002). Frequently, the elements for a corrosion cell are present before the bacteria become involved in the corrosion process. The presence and activity of microorganisms greatly accelerates, and often concentrates, the corrosion phenomenon.

Bacteria can be seen to intensify rather than act as the original cause of many corrosion incidents. A number of different types of bacteria strains have been implicated in the MIC process. These include among others, sulfate reducing bacteria (SRB), acid producing bacteria, and iron fixing bacteria, sulphur bacteria, sulfide generating bacteria, and slime forming bacteria. However, it may be noted that SRB are the best known agents of MIC and were the first to be implicated in the MIC process. Because they are so commonly found in corrosion situations, they are used as a marker organism to assess the risk of bacterial corrosion in a system (Sooknah et al., 2007)

1.1.1 Sulfate Reducing Bacteria (SRB)

SRB are indigenous to the oil field and cause severe operational problems that increase costs significantly throughout the petroleum industry. In addition, the presence of hydrogen sulfide produced by SRB leads to safety concern. These problems can be prevented or alleviated by controlling SRB populations. Quick, accurate SRB population estimates can reduce operating costs significantly, improve oilfield safety, and decrease sulfide releases into the environment.

Biological sulfate reduction by SRB is the only known process by which, in aquatic environments of moderate temperatures (0 to 75°C [32 to 167F]), H₂S is formed from sulfate. In sediments of ponds, lakes, and marine environments, SRB are usually part of the indigenous community of microorganisms and are rather inconspicuous in non polluted waters. In oilfield water systems, however, SRB cause serious problems: (1) corrosion of iron in the absence of air (anaerobic corrosion), (2) precipitation of amorphous ferrous sulfide that, by plugging, diminishes the injectivity of water injection wells, (3) contamination of fuel gas with H₂S, and (4) contamination of stored fuel oil with H₂S. Furthermore, H₂S is extremely toxic if inhaled; it easily escapes from contaminated waters and may accumulate, under poorly ventilated conditions. It is usually recognized by its distinctive, unpleasant odor, but high concentrations anesthetize the sense of smell (Cord-Ruwisch et al., 1987).

1.1.2 Types of SRB

SRB are not homogenous. It is very likely that many more types of SRB occur in nature. The cell forms of SRB most commonly found by light microscopy are curved and oval to rod-shaped; their diameters usually range from 0.5 to 2 μm , their lengths from 1 to 5 μm (Postgate, 1984). Many SRB are actively motile by flagella. Other forms are spheres and long multicellular filaments. Several types of SRB tend to grow in clumps or cell aggregates and stick to surfaces.

Nutritionally, SRB may be divided into two major groups. Species of the first group carry out an incomplete oxidation of organic substrates with acetate as an end product. Species of the second group oxidize organic substrates, including acetate, completely to CO_2 (Widdel & Pfennig, 1984). Most incompletely oxidizing SRB may grow rather fast under optimum conditions and reach doubling times of about 3 hours. The best-studied representatives are *Desulfovibrio* species that can be easily isolated from nearly every aquatic sediment. For most, lactate, is an excellent substrate that is oxidized to acetate and CO_2 . Many *Desulfovibrio* species also grow well with H_2 and sulfate as energy source; they require acetate and CO_2 as carbon source for cell synthesis. *Desulfovibrio sapovorans* and some similar, as yet unnamed SRB oxidize long-chain fatty acids to acetate. *Desulfobulbus* species oxidize propionate to acetate. Most of the known spore-forming *Desulfotomaculum* species resemble the commonly found *Desulfovibrio* species (Brandis & Thauer, 1981).

1.1.3 Impacts of SRB in oil field environment

Sulphate reducing bacteria proliferation can seriously impact operating costs of a facility because of a number of reasons which include:

- 1) Cost of line or equipment failures due to SRB and resultant repair or replacement.
- 2) Increased energy costs due to increased line friction within flowlines due to microbial fouling.
- 3) Increased energy costs due to reduced heat transfer caused by fouling.
- 4) Increased pumping requirements to overcome injection well plugging.
- 5) Cost of clean-up of fouled and plugged exchangers, filters and injection wells.
- 6) Loss in product quality for produced oil and gas, gas and oil in storage, and refined products.
- 7) Cost of chemicals (biocides) for treating the system.
- 8) Cost of monitoring effectiveness of biocide treatment.

Another major cost, and integrity risk, arises where facilities are built for sweet service but are subsequently subjected to sour conditions. Hence, there is a constant need to monitor bacterial contamination in operating systems in order to take remedial action.

1.2 Statement of Problem

Sulfate-reducing bacteria (SRB) are among the most destructive environmental organism and their industrial impact is widespread. They cause corrosion and stress corrosion cracking of metals and alloys used in petroleum production, cooling water system, waste water treatment and all aqueous process.

In general, SRB produced hydrogen sulfide which is more lethal than hydrogen cyanide. The sulfide is sometimes used by other organisms to manufacture sulfuric acid, which in turn is rapidly destroying oilfield pipeline. The symptoms of these SRB related problems are obvious; hydrogen sulfide odor, blackening of waters, and black sulfide corrosion products. By the time these symptoms appear, it may be difficult to deal with the problem. Thus, effective treatment is much easier if the infestation is detected at an early stage.

In this project, serial dilution method is used for SRB detection, which is culturing the sample in a laboratory until the numbers of SRB were sufficiently high to be detected by observation of general blackening of the sample. But, this method is time consuming as it takes 28 days to analyze the result. So, another method is proposed to speed up this culturing process or bypass culturing altogether and monitor the organisms directly. This method is known as SRB Rapidcheck II which will be able to convey the result in 20 minutes.

1.3 Objectives

The objectives of the project are:

- 1) Describe the impact of the presence of sulphate reducing bacteria to the operating cost of a facility
- 2) Detect and quantify the population of sulphate reducing bacteria (SRB) at Terengganu Crude Oil Terminal (TCOT).
- 3) Compare the accuracy of two methods which are Rapidcheck II method and NACE Serial Dilution method to quantify the population of sulphate reducing bacteria.
- 4) Discuss the advantages and limitations of some of these field kits compared to the Serial Dilution Technique.

1.4 Scope of Study

The main scope of the study is to detect the presence of sulphate reducing bacteria at Terengganu Crude Oil Terminal pipelines.

The scope covers of the project are as follows:

- 1) Employ water samples from different locations at Terengganu Crude Oil Terminal (TCOT).
- 2) Perform the parameter of water samples such as pH, Cl^- , SO_4^{2-} , total hardness and total dissolves solid in laboratory.
- 3) Perform the Rapidcheck II test and quantify the population of sulphate reducing bacteria.
- 4) Perform the NACE Serial Dilution test and quantify the population of sulphate reducing bacteria.
- 5) Evaluate the accuracy of Rapidcheck II method over NACE Serial Dilution method.

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