Hydrogen Sulfide Generation in Shipboard Oily-water Waste: Part 2. Microbiological Aspects

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Ship Structures and Materials Division
Aeronautical and Maritime Research Laboratory

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ABSTRACT

A study has been undertaken to identify the microbiological and environmental factors which influence the formation of hydrogen sulfide in oily-water wastes in ships of the Royal Australian Navy (RAN). In particular, methods were developed for isolation and enumeration of sulfate-reducing bacteria which produce the hydrogen sulfide in the waste. These methods were then used to find the important environmental features which contribute to the growth and metabolism of these bacteria.

Bilge specimens obtained from RAN and foreign naval vessels were examined in the laboratory. One or more species of sulfate-reducing bacteria were found in most samples of these wastes, with population levels as high as 800,000 per mL. Investigation of the bacteria isolated from these samples showed that growth of sulfate-reducing bacteria from shipboard oily-water wastes occurred at significant rates in the temperature range 12-45°C and in the pH range 6-8. Growth of these bacteria was also influenced by the availability of organic and inorganic nutrients and compounds known to inhibit the growth of sulfate-reducing bacteria.

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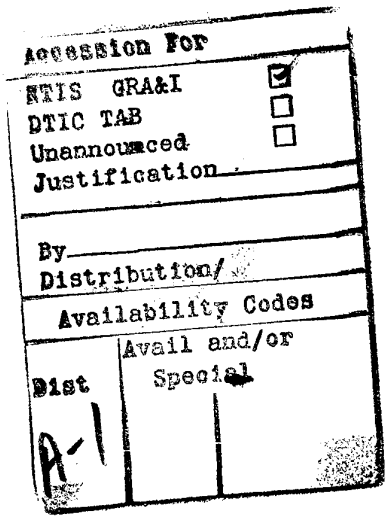

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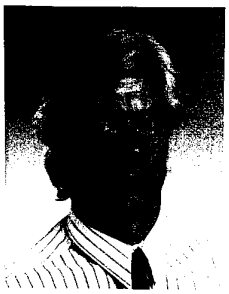

John Upsher, BSc Hons (Bath), MSc (La Trobe), joined Aeronautical and Maritime Research Laboratory as a microbiologist in 1966 then for 20 years investigated different problems associated with microbial deterioration of materials and equipment in storage and in the tropical environment. Responding to increasing concern within Defence on environmental matters, he has more recently investigated the bacterial generation of hydrogen sulfide in naval oily water wastes and the disposal of wastes including sewage and plastics.

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Contents

List of Tables

List of Figures

1. Introduction

The Royal Australian Navy has been experiencing problems with hydrogen sulfide forming in oily-water wastes in ships and submarines. Since hydrogen sulfide is a toxic gas, its presence in the closed environment of a ship is cause for concern, particularly as high concentrations of this gas can be fatal. If we are to eliminate this hazard from ships we require a detailed understanding of the mechanism by which the gas is produced. DSTO-AMRL has investigated the chemical and microbiological factors in ships which contribute to hydrogen sulfide formation in the oily-water wastes. The results of this investigation should enable design of rational approaches to removal of the hazard.

In the first report in this series [Hodgeman et al., 1995a], the involvement of sulfate-reducing bacteria in the process was discussed. In this report we describe in detail the microbiological aspects of the investigation. Specifically, we have cultured the bacteria associated with sulfide generation in bilge in the laboratory. These cultures were then used to investigate the factors which influence the metabolism and growth of sulfate-reducing bacteria in a marine oily-water waste environment. Subsequent reports in this series discuss ship factors [Hodgeman et al., 1995b] and recommendations for minimising the formation of hydrogen sulfide in oily-water wastes [Hodgeman et al., 1995c].

2. Background

On board HMAS Stalwart, on the 22nd October 1985, three seamen lost their lives through hydrogen sulfide asphyxiation. Toxic quantities of this gas were generated in oily-water waste held in storage and were released when a large quantity of the waste escaped into a compartment of the ship [Royal Australian Navy, 1986]. Although the toxicity of hydrogen sulfide is well known [Gosselin et al., 1976; Postgate, 1984], deaths from asphyxiation by this gas occur all too frequently in many occupational environments [Suruda and Agnew, 1989]. The oil and gas industries, for example, often experience high concentrations of hydrogen sulfide at well heads and in refineries and many fatalities have been recorded [Suruda and Agnew, 1989]. Oily-water wastes on ships, however, are not an obvious source of dangerous quantities of this gas.

Hydrogen sulfide is a familiar gas, easily recognised by its foul smell of rotten eggs. Ships' crews have generally accepted that bilges and holding tanks produce the smell of hydrogen sulfide although the cause of the smell has generally been uncertain. Usually the atmospheric concentration of hydrogen sulfide is well below toxic concentrations so that the gas has become a familiar part of the environment. The potential for generation of a toxic atmosphere in a ship by this process, however, has not been widely appreciated by crews. The problem has also been exacerbated by the need to store oily-water wastes on the ship to meet current environmental regulations.

On HMAS Stalwart at the time of the accident, the oily-water holding tank in the stern section of the ship contained 82 tonne of waste. This consisted largely of sea-water containing various cleaning and passivating agents with a layer of diesel oil [Royal Australian Navy, 1986]. Although it was later recognised that the conditions in the holding tank were suitable for growth of bacteria, the cause of hydrogen sulfide generation was not unequivocally linked with the growth of sulfate-reducing bacteria. There were, however, indications that sulfate-reducing bacteria were involved. In particular, no pathway to hydrogen sulfide by a purely chemical process could be found. In addition, the only adequate source of sulfur for generation of toxic levels of hydrogen sulfide was the sulfate present in the sea-water component of the waste. Reduction of sulfate to hydrogen sulfide by a chemical process would require a particularly strong chemical reducing agent. Sulfate-reducing bacteria, on the other hand, can do this without difficulty if the environment is suitable for their growth.

The waste in the holding tank of HMAS Stalwart was probably typical of that stored in other ships complying with environmental regulations. This waste was probably also similar to the many industrial wastes which commonly produce hydrogen sulfide from a combination of organic matter, sulfate and other nutrients in the presence of sulfate-reducing bacteria. The prominent feature of the wastes in ships, however, is the large amount of sulfate originating from sea-water in combination with biodegradable detergents used for cleaning machinery spaces [Hodgeman et al., 1995b].

Sulfate-reducing bacteria are widely distributed in the marine environment [Postgate, 1984] and their presence in ships cannot be considered unusual. These bacteria will grow only in environments depleted of oxygen (i.e., anaerobic) and in which the redox potential (Eh) has been lowered to at least -100 mV [Postgate, 1984]. Oxygen levels in the waste are readily lowered by the growth of aerobic bacteria which consume the oxygen if sufficient organic nutrient is present. In addition, the sulfate-reducing bacteria will maintain the redox potential at levels favourable to their growth by release of hydrogen sulfide [Cypionka et al., 1985]. Diffusion of oxygen from the bulk waste is restricted when the cells of the sulfate-reducing bacteria are present within sludge or surface biofilms. Thus, populations of these bacteria may be flourishing in localised regions of an environment which is aerobic and superficially appears unsuitable for their growth [LaMotta, 1976; Jorgensen, 1977]. Growth of sulfate-reducing bacteria in sludge and surface films will ensure inoculation of any new oily-water waste entering the bilge or holding tank.

The mode of respiration also governs the type of environment in which sulfate-reducing bacteria will grow. As with aerobic bacteria, an organic carbon source is required and is catabolysed to generate adenosine triphosphate (ATP), the biological energy-storage substance. Carbon dioxide and water are the by-products of this process. However, unlike aerobic bacteria, which dispose of the unwanted electrons from this energy-producing process by reduction of oxygen to water, the sulfate-reducing bacteria reduce sulfate to hydrogen sulfide. Some Desulfovibrio species of sulfate-reducing bacteria can grow without sulfate by using pyruvate or fumarate as alternative electron acceptors [Postgate, 1952; Widdell, 1988]. However, since sulfate is present in sea-water at a concentration of 0.27% [Sverdrup et al., 1942], it is unlikely to be a limiting factor in the shipboard oily-water waste environment where sea-water is a common component.

3. Results and Discussion

3.1 Development of Culture Media

Our first attempts to isolate and enumerate these bacteria from oily-water wastes were with Postgate's medium C [Postgate, 1984; see Appendix, Medium #2]. This medium provided the inorganic requirements, the organic nutrients and the redox poising agent (ascorbate). Tests were done to find the optimum concentrations of sodium chloride, organic nutrients, and vitamins required for growth of the bacteria in this medium. The results of these experiments are discussed below. We varied the concentration of other components (such as calcium, iron and redox poising agents) but only minor effects on growth of the bacteria were observed.

The sulfate-reducing bacteria inhabiting bilges and holding tanks are most likely to be of marine or littoral origin and well adapted to a saltwater environment. Since bilge specimens typically have a sea-water content of 10-80% [Hodgeman et al.,1995b], we considered it important to establish the optimum salt content for the culture medium. Table 1 shows the influence of salt concentration on growth of the bacteria from several ships. All cultures grew well in the presence of 1% sodium chloride while three of the four cultures (those from Stalwart, Aris and Caio Duilio) were inhibited by 5% sodium chloride. The culture from Godavari, which grew well at 5% sodium chloride, failed to grow in the medium with no added salt. This suggested the bilge specimen from Godavari may have contained the species Desulfovibrio salexigens or Desulfovibrio desulfuricans subsp. aestuarii [Postgate & Campbell, 1966]. Our observation that two of the cultures were retarded with no added salt suggested that 1% sodium chloride was adequate for the species encountered. All subsequent media used in this work had sodium chloride added to give a 1% concentration.

Lactate is the organic electron donor usually employed in media for culturing sulfate-reducing bacteria in the laboratory. It provides energy for growth and organic carbon for cell construction. For most bacteriological purposes, however, the remainder of the organic metabolic requirements are supplied as protein hydrolysates such as peptone, tryptone, or yeast extract. Yeast extract is also a good source of B vitamins which are necessary for the growth of some sulfate-reducing bacteria [Postgate, 1984]. We conducted an experiment to find the influence of tryptone, yeast extracts and B group vitamins on the growth of sulfate-reducing bacteria from bilge. The results of this experiment (Table 2) show that added vitamins had only marginal effect on growth. The best growth was observed with 0.1% yeast extract and 0.4% tryptone (Table 2, medium No. 9). We subsequently employed these additive concentrations in all routine media.

3.2 Standard Method for Enumeration

Our initial attempts to develop a method for finding the populations of sulfate-reducing bacteria in oily-water waste focused on colony counting approaches. Unfortunately, the soluble ferrous salt used as a sulfide indicator rapidly coloured the whole medium and masked individual colonies. Attempts to restrict the zone of black colouration by increasing the iron concentration were unsuccessful so colony counting was replaced by an extinction method based on serial dilution of the original culture (Plate 1).

Replicate estimates of decimal dilutions of culture suspensions is the basis of the "most probable number" method for measuring populations of free living bacteria. Two main factors affect the accuracy of this method with sulfate-reducing bacteria. First, many sulfate-reducing bacteria are tactophilic, (i.e., they attach to suspended particles [Postgate, 1984], or form aggregates), so that colonies in the culture may not be derived from a single bacterial cell. Secondly, recovery of bacterial cells from aqueous sources is not always quantitative. It has, for example, been reported that actual populations can exceed those measured by up to two orders of magnitude [Baker et al., 1983; Ferguson et al., 1984; Colwell et al., 1985]. Thus, use of a "most probable number" method for sulfate-reducing bacteria generates a population index and not the actual count of bacterial cells. Throughout this work we refer to this index as the sulfate-reducing bacteria population.

3.3 Bacteriological Assessment

Bilge specimens collected from ships were transported to the laboratory packed in ice and refrigerated on arrival to minimize the effects of changes which occurred after collection [Hodgeman et al., 1995b]. Bacteriological assessment was generally done within three days of collection. In addition to the sulfate-reducing bacteria, we also measured the total aerobic bacteria count and the population of coliform bacteria. The total count was made to obtain an indication of the bacterial load of the samples. The coliforms were of significance because of their ability to produce lactate and other low molecular weight metabolites which are suitable nutrients for sulfate-reducing bacteria. Results of these measurements are presented in Table 3. These data show the wide range of bacteria populations which are found in oily-water waste. Sulfate-reducing bacteria counts ranged from effectively zero to 800,000 per mL. Total aerobe and coliform counts, where data was available, also showed wide variations.

3.4 Effect of Temperature

Sulfate-reducing bacteria occupy environmental niches with a wide range of prevailing temperatures. Species of these bacteria which grow well at 0°C (psychrophilic bacteria) have been recorded [Barghoorn & Nichols, 1961] and sulfate reduction has been observed in sediments of antarctic pools [Franzmann et al., 1985]. Sulfate-reducing

bacteria are also found in environments of elevated temperature (thermophilic bacteria). Desulfovibrio thermophilus, for example, has an optimum growth temperature of 65°C and has been isolated from subterranean waters at temperatures as high as 84°C [Volikov et al., 1983].

The sulfate-reducing bacteria from oily-water wastes in ships are more likely to grow in the middle temperature range (mesophilic bacteria) of 10-45°C [Postgate, 1984]. This was confirmed by measurement in the laboratory of the temperature range over which sulfate-reducing bacteria from several ships would grow. Upper and lower temperature limits were found and are presented in Table 4. None of these specimens showed any growth at temperatures below 12°C, although some variation was observed in the upper temperature limits. Thus, the sulfate-reducing bacteria from HMAS Sydney did not grow above 40°C while those from the holding tank of HMAS Stalwart continued to grow at temperatures up to 48°C. The optimum temperature for most bacteria is usually only a few degrees below the upper limit. All grew well between 20°C and 38°C and we found no evidence for the presence of thermophilic or psychrophilic sulfate-reducing bacteria in any of the oily-water waste specimens.

For sulfate-reducing bacteria, an increase in temperature in the range from the lower limit to the optimum growth temperature will result in a corresponding increase in the rate of sulfate reduction. Widdell has reported that the rate of sulfate reduction by bacteria of marine origin increases from 2.0 to 3.9 times for each 10°C rise in this temperature range [Widdell, 1988]. Thus, the rate of hydrogen sulfide generation in oily-water waste will depend on the bilge temperature. Bilge temperatures measured for ships berthed in Sydney varied from 14 to 22°C [Hodgeman et al., 1995b]. Since bilge temperature reflects that of the surrounding sea, higher temperatures would be expected in tropical waters where the sea temperature can exceed 30°C. Thus, for ships operating in tropical waters, hydrogen sulfide could be generated at several times the rate at which it is produced in cooler regions.

3.5 Effect of pH

Sulfate-reducing bacteria prefer a pH near pH 7. Growth of these bacteria is usually inhibited outside the pH 6-9 range [Widdell, 1988], although sulfate reduction has been observed in an environment of pH 3-4. The bacteria isolated from this low pH environment were, however, not active below pH 6 in the laboratory. This implies they had come from microniches of higher pH than that of the bulk environment [Tuttle et al., 1969].

An experiment was conducted to find the pH range in which sulfate-reducing bacteria from oily-water waste would grow. The results are shown in Table 5 and confirm Widdell's generalisation. Two cultures grew below pH 6.0 and two at a little above pH 8.0. In the sloppy agar environment used, however, it is possible that microenvironments of more favourable pH existed.

In the study made of wastes obtained from ships' bilges [Hodgeman et al., 1995b], both pH and sulfate-reducing bacteria counts were recorded. The pH of these wastes ranged from 4.3 to 9.0 (mean 7.2, standard deviation 0.8) and sulfate-reducing bacteria counts ranged from effectively zero (<10 per mL) to 800,000 per mL. The measured bacterial populations are plotted against pH in Figure 1. Although these data do not show any direct relationship between pH and bacteria counts, the clustering of growth in the pH range 6-8 is obvious. Two samples with pH levels above 8.0 showed moderately high bacterial counts. These could be attributed, however, to the rise in pH expected on evaporation of hydrogen sulfide from the waste [Postgate, 1984]. Growth of sulfate-reducing bacteria in ships' bilges therefore occurs within the normal pH range expected for these bacteria.

3.6 Effect of Organic Substrates

We examined the effects of several organic substrates on growth of sulfate-reducing bacteria from oily-water wastes. The results of these experiments are shown in Table 6. Lactate was included in all media after preliminary experiments showed it gave the best recovery of sulfate-reducing bacteria from oily-water waste samples. This agrees with its suitability for most species of these bacteria [Postgate, 1984; Widdell, 1988]. Acetate was used because it has been proposed as a major electron donor for some marine sulfate-reducing bacteria [Skyring, 1987; Dicker and Smith, 1985; Balba and Nedwell, 1982]. Other organic substrates were included because of their known use by at least some strains or species of sulfate-reducing bacteria and their possible occurrence in oily-water waste.

Microscopic examination of the cultures revealed the presence of motile, gram negative vibrioforms (Plate 2) in all samples which showed sulfate reduction. This suggested that Desulfovibrio [Kluyver & van Neil, 1936] was of widespread occurrence in Australian ships and those of other nations. The abilities of sulfate reducers to oxidise the low-energy, small organic substrates lactate, formate, pyruvate and ethanol was confirmed in this experiment. However, this behaviour is so widespread that it was of no assistance in identifying the species of Desulfovibrio present.

Acetate is oxidised by only one species of Desulfovibrio (and by members of the other sulfate-reducing bacteria genera). However, many sulfate-reducing bacteria species are known to use acetate in the presence of carbon dioxide. Since carbon dioxide was likely to be present in the experimental media, the ability of bilge cultures to oxidise acetate in these tests is not indicative of any particular species.

The only species of Desulfovibrio to utilise propionate is D. baarsii and only D. sapovorans can use palmitate [Widdell, 1981]. Although propionate is used by other sulfate-reducing genera [Widdell & Pfennig, 1984], D. baarsii is considered a possible inhabitant of the ships sampled because its natural habitat is anaerobic mud of fresh and brackish water environments. It also has an affinity for fatty acids up to C18 [Postgate, 1984].

The only species of sulfate-reducing bacteria to utilise choline is Desulfovibrio desulfuricans (Beijerinck) [Kluyver & van Neil, 1936], which is common in fresh and marine waters, particularly when polluted with organic substances [Postgate, 1984]. The results in Table 6 show that all cultures tested used choline.

It thus appears that Desulfovibrio desulfuricans was present in all of the waste samples examined, except that from HMAS Jervis Bay. D. baarsii and D. sapovorans were also possible inhabitants of some oily-water waste specimens. Other species may also have been present but were not apparent from these tests.

3.7 Effect of Phosphate

Phosphate, which is necessary for respiration and the storage and transfer of energy, is an essential component for the growth of microorganisms. A deficiency of phosphate in the environment can limit microbial replication and metabolic activity. In any aqueous environment, the microbiota is continually removing phosphate so the amount of free phosphate will be low [Burkholder et al., 1990]. This is true for ocean waters and many fresh water bodies. Ocean water contains phosphate in concentrations from 0.001 to 0.10 ppm [Sverdrup et al., 1942] and similar low levels have been recorded in freshwater bodies [Moeller & Wetzel, 1988]. The deficiency of phosphate in sea-water has been identified as a factor limiting the activity of petroleum-metabolising bacteria [Atlas & Bartha, 1972] and the respiration and growth of sulfate-reducing bacteria [Liu & Peck, 1981]. Of 28 bilge specimens that we examined, only one showed a detectable concentration of phosphate (2.3 ppm) [Hodgeman et al., 1995b].

In an experiment to find the effects of low phosphate concentrations on sulfate-reducing bacteria growth we prepared a basal medium without added phosphate. The phosphate contents of the tryptone and yeast extracts used to prepare the medium, however, gave final phosphate concentrations which were at least tenfold those found in bilge samples. We observed good growth of sulfate-reducing bacteria at all phosphate concentrations tested. Any attempt to obtain a base medium with less than about one ppm of phosphate would have resulted in the depletion of other essential nutrients. The only conclusion we can draw from these results is that the lowest level of phosphate tested (i.e., 10.7 ppm) is adequate for sustained growth of the sulfate-reducing bacteria in oily-water waste.

3.8 Effect of Nitrogen Sources

A source of nitrogen for synthesis of nucleic acids and enzymes is as essential for the sulfate-reducing bacteria as it is for other types of bacteria. Nitrogen-containing substances most often used by bacteria include ammonium salts, nitrates and amino acids. Nitrate is not a suitable source of nitrogen for sulfate-reducing bacteria, possibly because of its oxidising potential [Postgate, 1984]. Sulfate-reducing bacteria utilise several amino acids [Stams et al., 1985] and media are available for cultivation of these

bacteria which provide nitrogen only as organic substrates [Postgate, 1984]. Sea-water contains less than one ppm of dissolved nitrogen as nitrate, ammonium and organic nitrogen compounds [Sverdrup et al., 1942]. The nitrogen content of oily-water wastes, however, is probably much more variable than that of sea-water although sufficient nitrogen from all sources is clearly available for support of considerable bacterial populations.

An experiment was conducted to find the ability of sulfate-reducing bacteria from a waste specimen to use ammonium ions as a source of nitrogen. In the test medium tryptone, yeast extract and sodium sulfate were replaced by ammonium sulfate. No sulfate-reducers from oily-water waste grew in this medium though the control produced a population of more than one million per mL. We concluded that the bacterial culture used in this experiment was unable to use ammonium ions as a nitrogen source. In view of observations elsewhere, this might be unusual.

3.9 Effects of Molybdate and Copper

Molybdate is a specific inhibitor of microbial sulfate reduction and is widely used experimentally for this purpose [Dicker & Smith, 1985; Ivanov et al., 1989; Haggblom & Young, 1990]. It inhibits sulfate-reducing bacteria by blocking the respiratory process. Copper, on the other hand, is toxic for many microbiological systems and its effects on sulfate-reducing bacteria have been described [Norqvist & Roffey, 1983]. In a medium undergoing sulfate reduction, however, the precipitation of insoluble copper sulfide would effectively remove the toxicant from the medium, although its effects on a fresh culture can still be observed. We examined both inhibitors in experiments aimed at finding their toxicity towards sulfate-reducing bacteria from oily-water waste. Sulfide production in inoculated media containing different concentrations of each inhibitor was investigated and the results are shown in Table 7. The bacterial culture showed much greater sensitivity to copper than to molybdate and failed to grow at all concentrations of copper from 0.01 to 3.00 mM. Molybdate, on the other hand, only inhibited growth at concentrations above 1 mM. Inhibition of other sulfate-reducing bacteria cultures has been reported with 20 mM [Nedwell & Ibrahim, 1981] and 50 mM [Skyring, 1988] solutions of molybdate.

Although copper effectively prevented growth of sulfate-reducing bacteria from oily-water waste, its use cannot seriously be considered for control of these bacteria since it will inevitably be precipitated as insoluble copper sulfide. It serves here as a reference with which to compare the efficiency of molybdate as an inhibitor of hydrogen sulfide generation. The results show molybdate to be less effective than copper as a sulfide inhibitor by at least two orders of magnitude. It would require 240 grams of molybdate to maintain a concentration of 10 mM in a tonne of oily-water waste.

4. Conclusions

We have shown that several different species of anaerobic sulfate-reducing bacteria are present in the liquid wastes in bilges and holding tanks of many ships of the Royal Australian Navy. The sulfate-reducing bacteria populations in the waste were influenced by several environmental factors but conditions are often adequate to support >100,000 per mL. The presence of these bacteria in shipboard oily-water waste cannot in any way be considered unusual. However, we conclude that the presence of any sulfate-reducing bacteria shows the potential for generation of hydrogen sulfide in the waste. Furthermore, if conditions in the waste are very favourable to the growth of these bacteria then the potential for production of large quantities of hydrogen sulfide exists.

5. Experimental Section

5.1 Optimisation of Sodium Chloride Content

Medium #1 was modified by omission of thioglycollate and used as the basal medium. Test media were prepared from the basal medium by addition of 1% or 5% sodium chloride. The sterilised test media were then inoculated with a bilge specimen (0.2 mL / 25 mL) from one of four ships (see Table 1). The inoculated specimens were incubated at 30°C and examined for growth of sulfate-reducing bacteria by formation of black ferrous sulfide after 7 and 28 days. Triplicate specimens of each culture were used.

5.2 Optimisation of Organic Nutrient

Medium #1, modified by omission of yeast extract and thioglycollate and addition of sodium chloride (1% w/v), was used as the basal medium. Test media, with selected additions of yeast extract, tryptone and the vitamins biotin, p-aminobenzoic acid and thiamine hydrochloride, were prepared (see Table 2 for concentrations of additives). These were inoculated with a bilge specimen (0.2 mL / 25 mL) from one of two ships (see Table 2). The inoculated media were incubated at 30°C and examined for growth of sulfate-reducing bacteria after 7 days. Triplicate specimens of each culture were used.

5.3 Standard Method for Enumeration

Serial dilutions of the oily-water waste specimens were prepared in a redox-poised diluting fluid containing sodium chloride, sodium sulfate and ascorbic acid (see Appendix). The first dilution was made by aseptic addition of the bilge specimen (1.0 mL) to the diluting fluid (9.0 mL). The mixture was shaken for one minute to ensure the breakup of sulfate-reducing bacteria aggregates. This procedure was repeated (excluding the shaking step) until a 10^5-fold or 10^6-fold dilution of the original bilge specimen had been achieved.

The diluted oily-water waste specimens (1.0 mL) were aseptically added to warm, molten, freshly prepared Medium #3 (25.0 mL) and mixed. The samples were incubated at 30°C for up to 14 days (no new colonies were observed after this period). Growth of sulfate-reducing bacteria in the medium was assessed from the extent of blackening (ferrous sulfide) or, where possible, by counting the number of individual black colonies in the medium (see Plate 1). The sulfate-reducing bacteria population was estimated from the extinction dilution, i.e., the dilution at which any blackening first failed to appear. All tests were conducted in triplicate.Since this method is likely to underestimate the true population of sulfate-reducing bacteria, (see above), blackening of just one tube of the three was taken as a positive reading.

5.4 Effect of Temperature

Sulfate-reducing bacteria from oily-water waste samples, grown in Medium #3 at 30°C for 4 to 7 days, were used for inoculation. Test specimens were prepared by adding 0.1 mL of the culture to 25.0 mL of Medium #3. The inoculated specimens were then packed in styrofoam beads and incubated at various temperatures in bacteriological incubators which maintained the set temperature to within 1°C. The tubes were examined daily until blackening occurred. At the higher temperatures the experiment was ended after 14 days while at the lower temperatures 28 days was allowed to obtain a positive reading. All tests were conducted in triplicate.

5.5 Effect of pH

Medium #3 (110% strength) was used as the basal medium. Test media were prepared by adjusting the pH of the basal medium with dilute sodium hydroxide or hydrochloric acid followed by sufficient distilled water to reduce the nutrient concentrations to their normal level. The pH of each test medium was remeasured after sterilisation and samples (25.0 mL) inoculated with 0.1 mL of the pregrown cultures described in the previous section. The specimens were incubated at 30°C for 7 days and the growth of sulfate-reducing bacteria estimated from the extent of blackening. All tests were done in triplicate.

5.6 Effect of Organic Substrates

The potential organic substrates were added to the basal medium (Medium #3) to give a final concentration of approx. 0.5% w/v. Organic acids were added as the corresponding sodium salt and the pH adjusted to 7.0 with dilute sodium hydroxide or hydrochloric acid. Ethanol was introduced after sterilisation by membrane filtration to avoid loss by evaporation. The sulfide indicator, ammonium ferrous sulfate, was also introduced by membrane filtration to avoid precipitation. The warm, molten test media were inoculated with sulfate-reducing bacteria cultures grown in Medium #1 with 1% sodium chloride added (0.2 mL) and incubated at 30°C. Growth of sulfate-reducing bacteria was estimated from the extent of blackening of the medium. All tests were done in triplicate and a two-thirds majority was required to record a positive influence of the additive on the growth of the bacteria. The basal medium, treated the same, but without the organic substrate, was used as the control.

5.7 Effect of Phosphate

Bilge specimens were grown in Medium #3 at 30°C for 7 days. 0.1 mL of each culture was suspended in 10.0 mL of a minimal-phosphate diluting fluid prepared according to Medium #4, but from which agar, tryptone, yeast extract and lactate were omitted. Test media were prepared from Medium #4 and, after inoculation with 0.2 mL of the suspension, incubated at 30°C for 7 days. The control medium was found to have a phosphate content of 10.9 ppm. Growth of sulfate-reducing bacteria was estimated from the extent of blackening of the media.

5.8 Effect of Nitrogen Sources

Medium #5, modified by an addition of B-group vitamins by membrane filtration, was used as the test medium. The cultures were prepared by serial dilution of an actively growing culture (from HMAS Perth) in redox-poised diluting fluid. The test medium was inoculated with the diluted culture (10^4-fold to 10^8-fold dilutions) and incubated at 30°C for 14 days. Growth of sulfate-reducing bacteria was estimated from the extent of blackening of the medium.

5.9 Effect of Molybdate and Copper

Medium #3 was used as the base medium. Test media were prepared by addition of 1.0 mL of appropriately diluted solutions of copper sulfate ($CuSO_4.5H_2O$) or sodium molybdate ($NaMoO_4.2H_2O$) to give additive concentrations of 0, 0.01, 0.03, 0.1, 0.3 and 1.0 mM. The test media were inoculated with the culture from HMAS Perth described in the previous section (0.1mL) using a dilution with a count of approx. 10,000 sulfate-reducing bacteria/mL. The tests were done in triplicate.

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7. References

Atlas, R.A. and Bartha, R. (1972). "Degradation and mineralisation of petroleum in sea water: limitation by nitrogen and phosphorus", Biotechnology and Bioengineering, 14, 309-318.

Balba, M.T. and Nedwell, D.B. (1982). "Microbial metabolism of acetate, propionate and butyrate in anoxic sediment from Colne Point salt marsh, Essex, UK", Journal of General Microbiology, 128, 1415-1422.

Baker, R.M., Singleton, F.L. and Hood, M.A. (1983). "Effects of nutrient deprivation on Vibrio cholerae", Applied and Environmental Microbiology, 46, 930-940.

Barghoorn, E.S. and Nichols, R.L. (1961). "Sulfate reducing bacteria in pyritic sediments in Antarctica", Science, 134, 190.

Burkholder, J.M., Wetzel, R.G. and Klomparens, K.L. (1990). "Direct comparison of phosphate uptake by adnate and loosely attached microalgae within intact biofilm matrix", Applied and Environmental Microbiology, 56, 2882-2890.

Colwell, R.R., Brayton, P.R., Grimes, D.J., Roszak, D.B., Huq, S.A. and Palmer, L.M. (1985). "Viable but non-culturable Vibrio cholerae and related pathogens in the environment: implications for release of genetically engineered microorganisms", Biotechnology, 3, 817-820.

Cypionka, H., Widdell, F. and Pfennig, N. (1985). "Survival of sulphate-reducing bacteria after oxygen stress and growth in sulfate-free oxygen-sulfide gradients", FEMS Microbiology Ecology, 31, 39-45.

Dicker, H.J. and Smith, D.W. (1985). "Metabolism of low molecular weight organic compounds by sulfate-reducing bacteria in a Delaware salt marsh", Microbial Ecology, 11, 317-335.

Ferguson, R.L., Buckley, E.N. and Palumbo, A.V. (1984). "Response of marine bacterioplankton to differential filtration and confinement", Applied and Environmental Microbiology, 47, 49-55.

Franzmann, P.N., Skyring G.W., Burton, H.R. and Deprez, P.P. (1985). "Sulfate reduction rates in and aspects of the limnology of four lakes and a fjord of the Vestfold hills, Antarctica", Hydrobiologia, 165, 25-33.

Gosselin, R.E., Hodge, H.C., Smith, R.P. and Gleason M.N. (1976). "Clinical Toxicology of Commercial Products", Fourth Edition, Williams and Wilkins, Baltimore.

Haggblom, M.M. and Young, L.Y. (1990). "Chlorophenol degradation coupled to sulphate reduction", Applied and Environmental Microbiology, 56, 3255-3260.

Hodgeman, D.K.C., Fletcher, L.E., and Upsher, F.J. (1995b). "Hydrogen Sulfide Generation in Shipboard Oily-water Waste. Part 1. Origin of the Hydrogen Sulfide", DSTO-TR-0053.

Hodgeman, D.K.C., Fletcher, L.E., and Upsher, F.J. (1995b). "Hydrogen Sulfide Generation in Shipboard Oily-water Waste. Part 3. Ship Factors", DSTO-TR-0055.

Hodgeman, D.K.C., Fletcher, L.E., and Upsher, F.J. (1995c). "Hydrogen Sulfide Generation in Shipboard Oily-water Waste. Part 4. Minimization of the Problem", DSTO-TR-0056.

Ivanov, M.V., Lein, A.Y., Reeburgh, M.S.and Skyring, G.W. (1989). "Interaction of sulphur and carbon cycles in marine sediments", pp 125-179 in P. Brimblecombe and A. Y. Lein (eds) "Evolution of the Global Biogeochemical Sulphur Cycle", SCOPE, J. Wiley, Chichester.

Jorgensen, B.B. (1977). "Bacterial sulfate reduction within reduced microniches of oxidized marine sediments", Marine Biology, 41, 7-17.

Kluyver, A.J. and van Neil, C.B. (1936). "Prospects for a natural system of classification of bacteria", Zentrabl. Bakteriol. Parastitenkd. Infektionskr. Hyg. Abt., II 94, 396-403. (Cited in N.R. Kreig and J.G. Holt (eds) "Bergey's Manual of Systematic Bacteriology", p666, Williams & Wilkins, Baltimore).

LaMotta, E.J. (1976). "Internal diffusion and reaction in biological films", Environmental Science and Technology, 10 (8), 765-769.

Liu, C. and Peck, H.D. (1981). "Comparative bioenergetics of sulfate reduction in Desulfovibrio and Desulfotomaculum spp", Journal of Bacteriology, 145, 966-973.

Moeller, R.E and Wetzel, R.G. (1988). "Littoral vs profundal components of sediment accumulation: contrasting roles as phosphorus sinks", International Association of Theoretical and Applied Limnology, Verhandlungen, 23, 386 - 393.

Nedwell, D.B. and Ibrahim, M.B. (1981). "Hydrogen as an electron donor for sulfate-reducing bacteria in slurries of salt marsh sediment", Microbial Ecology, 7, 305-313.

Norqvist, A. and Roffey, R. (1983). "Alternative method for monitoring the effect of inhibitors on sulfate reduction", Journal of Applied Microbiology, 29, 335-344.

Postgate, J.R. (1952). "Growth of sulphate reducing bacteria in sulphate-free media.", Research , 5, 189-190.

Postgate, J.R. (1984). "The sulphate reducing bacteria. 2nd ed", Cambridge University Press, Cambridge.

Postgate, J.R. and Campbell, L.L. (1966). "Classification of Desulfovibrio species, the non-sporulating sulfate-reducing bacteria", Bacteriological Reviews, 30, 732-738.

Royal Australian Navy (1986). Naval Board of Inquiry into HMAS Stalwart. HMAS Stalwart Toxic Gas Accident 22nd October 1985 - Summary of Board of Inquiry Report.

Skyring, G.W. (1987). "Sulfate reduction in coastal ecosystems", Geomicrobiology Journal, 5, 295-373.

Skyring, G.W. (1988). "Acetate as the main energy substrate for the sulfate reducing bacteria in Lake Eliza (South Australia) hypersaline sediments", FEMS Microbiology Ecology, 53, 87-94.

Stams, A.J.M., Hansen, T.A. and Skyring, G.W. (1985). "Utilization of amino acids as energy substrates by two marine Desulfovibrio strains", FEMS Microbiological Ecology, 31, 11-15.

Suruda, A. and Agnew, J. (1989). "Deaths from asphyxiation and poisoning at work in the United States 1984-6", British Journal of Industrial Medicine, 46, 541.

Sverdrup, H.U., Johnson, M.W. and Flemming, R.H. (1942). "The Oceans: Their Physics, Chemistry and General Biology", Prentice-Hall, New Jersey, p. 176.

Tuttle, J.H., Dugan, P.R., Macmillan, C.B. and Randles, C.I. (1969). "Microbial dissimilatory sulfur cycle in acid mine water", Journal of Bacteriology, 97, 594-602.

Volikov, I.I. and Rosanov A.G. (1983) "The sulfur cycle in oceans. Part 1, Reservoirs and fluxes", pp 357-423 in M.V. Ivanov and J.R. Freney, (eds), "The Global Biogeochemical Sulfur Cycle", SCOPE, 19, John Wiley, Chichester.

Widdell, F. (1981). In "Validation of the publication of new names and new combinations previously published outside the IJSB", List No. 7, International Journal of Systematic Bacteriology, 31, 382-383.

Widdell, F. (1988). "Microbiology and ecology of sulfate- and sulfur-reducing bacteria", In "Biology of Anaerobic Microorganisms", Zehnder, G.F., and Alexander, J.B., (eds), Wiley, New York, pp. 469-585.

Widdell, F. and Pfennig, N. (1984). "Desulfococcus, Desulfobacter, Desulfobulbus and Desulfosarcina", in N.R. Krieg and J.G.Holt (eds), "Bergey's Manual of Systematic Bacteriology", Vol. 1(pp 673-679), Williams & Wilkins, Baltimore.

Table 1. Effects of additional sodium chloride on growth of sulfate-reducing bacteria

Culture	At 7 days			At 28 days		
	0%	1%	5%	0%	1%	5%
Stalwart	+	+	-	+	+	-
Godavari (India)	-	W	+	-	+	+
Aris (Greece)	W	+	-	+	+	-
Caio Duilio (Italy)	W	W	-	+	+	-

Symbols:
(+) Dense growth with individual colonies obscured.
(W) Weak sulfate-reducing bacteria growth with few colonies present.
(-) No growth of sulfate-reducing bacteria.

Table 2. Effects of different nutrient combinations

Medium No.	Nutrient Additions (%)			Source	
	Yeast extract	Tryptone	Vitamins	Stalwart	Jervis Bay
1	0	0	0	-	-
2	0.1	0	0	-	-
3	0	0.1	0	-	+
4	0.1	0.1	0	++	++
5	0	0	YES	-	-
6	0.1	0	YES	-	-
7	0	0.1	YES	+	+
8	0.1	0.1	YES	++	++
9	0.1	0.4	0	++++	++++
10	0.4	0.1	0	+++	++

Vitamins: Biotin (BDH) 5 microgram/100mL
p-Aminobenzoic acid (BDH) 25 microgram/100mL
Thiamine hydrochloride (BDH) 50 microgram/100mL

Symbols: -, +, ++, etc., are arbitrary assessments based on average number and size of colonies.

Table 3. Microbiological assessment of oily-water waste samples.

Source	Location		SRB	Total Count		Coliforms
Bayonet	ER		100	NA		NA
Aris (Greece)	ER	<	10	NA		NA
Godavari (India)	ER		1,000	NA		NA
Caio Duilio (Italy)	BR		100	NA		NA
Caio Duilio (Italy)	ER		1,000	NA		NA
Waikato (NZ)	BR	>	10	NA		NA
Waikato (NZ)	ER	>	10,000	NA		NA
Colbert (France)	ER	<	10	NA		NA
Success	ER	<	10	NA		NA
Sydney	ER	>	100,000	NA		NA
Sydney	AMR 1	>	100,000	NA		NA
Sydney	AMR 2		10	NA		NA
Success	ER		100	NA		NA
Success	NA		100,000	NA		NA
Canberra	ER	>	100,000	NA		NA
Canberra	AMR 2	>	100	NA		NA
Warrnambool	ER	<	10	1,000,000	>	10,000
Darwin	ER	>	100	NA	>	100
Darwin	AMR 2	>	100	NA	>	100
Darwin	AMR 3	<	10	NA	<	10
Parramatta	ER		800,000	NA	>	1,000
Cook	ER		2,000	NA	>	100
Cook	AMR		300,000	NA	>	100
Sydney	ER	>	10,000	1,600,000	>	10,000
Brisbane	BR 1		10	NA		100
Brisbane	BR 2		10	NA		100

Table contd.

Table 3 (cont.). Microbiological assessment of oily-water waste samples.

Source	Location	SRB	Total Count	Coliforms
Perth	ER 1	20,000	NA	100 (1)
Perth	ER 1/2	< 1,000	NA	1,000
Perth	ER 2	3,000	NA	10,000 (2)
Canberra	ER	1,000	5,400,000	< 100
Canberra	ER	200	1,500,000	100
Perth	BR 2	400	NA	< 100
Perth	ER 1	20,000	NA	10
Adelaide	AMR 2	> 100,000	11,300,000	10
Adelaide	ER	100	6,000,000	< 10
Success	ER P	> 1,000	5,600,000	100
Success	ER S	> 1,000	2,200,000	100
Cook	ER 1	10,000	1,600,000	10,000 (3)
Cook	ER 2	1,000	1,200,000	1,000

Locations:

(ER) Engine Room, (BR) Boiler Room, (AMR) Auxilliary Machinery Room, (NA) Not available.

Coliforms identified:

(1) Perth engine room 1; Enterobacter sakazaki, Escherichia coli.
(2) Perth engine room 2; Providencia rettgeri, Acinetobacter anitratus.
(3) Cook engine room 1; Enterobacter agglomerans.
Also, Stuart engine room; Klebsiella pneumoniae.

Table 4. Temperature ranges for growth of sulfate-reducing bacteria obtained from naval bilge.

Source	Minimum degC	Maximum degC
Aris (Greece), engine room	12	45
Godavari (India), engine room	12	45
Jervis Bay, engine room	12	45
Stalwart, engine room	12	45
Stalwart, holding tank sludge	12	48
Success, engine room	12	45
Sydney, engine room	12	45
Sydney, oily waste	12	40
Tobruk, engine room	12	46
Tobruk, generator room	12	45
Waikato (NZ), engine room	12	42

Table 5. Growth of sulfate-reducing bacteria at different pH.

pH	Jervis Bay	Stalwart	Success
5.3	-	-	-
5.7	+	-	+
6.3	+	+	+
6.6	+	+	+
7.0	+	+	+
7.3	+	+	+
8.0	+	+	+
8.6	weak	+	-
9.1	-	-	-

Observed ranges:

Jervis Bay	5.7 - 8.6
Stalwart	6.3 - 8.6
Success	5.7 - 7.3 (<8.0)

Table 6. Use of organic electron donors by sulfate-reducing bacteria from oily-water waste.

Origin	Con	Lac	For	Pyr	Ace	Pro	Pal	Suc	Mal	Ben	Cho	Eth	Gly
Jervis Bay	0	+	+	+	+	nt	-	-	+	-	nt	nt	-
Stalwart	0	+	+	+	+	+	-	nt	nt	nt	+	nt	
Cook	0	+	+	+	+	+	-	nt	nt	nt	+	+	nt
Parramatta	0	+	+	+	weak	weak	-	nt	nt	nt	weak	+	nt
Sydney	0	+	+	+	+	+	+	nt	nt	nt	+	nt	nt
Perth	0	+	+	+	nt	nt	nt	nt	nt	nt	+	+	nt
Godavari (India)	0	+	+	+	+	+	weak	nt	nt	nt	weak	weak	nt
Aris (Greece)	0	+	+	+	+	+	-	nt	nt	nt	nt	nt	nt
Waikato (NZ)	0	+	+	+	+	+	+	nt	nt	nt	+	nt	nt

Symbols:
(0), zero growth assessed on medium with no additive;
(+), growth greater than control;
(-), growth less than control;
(weak), growth slightly greater than control, indeterminate;
(nt), not tested.

(Con), control (Lac), lactate (For), formate
(Pyr), pyruvate (Ace), acetate (Pro), propionate
(Pal), palmitate (Suc), succinate (Mal), malate
(Ben), benzoate (Cho), choline (Eth), ethanol
(Gly), glycerol

Table 7. Effect of molybdate and copper salts on growth of sulfate-reducing bacteria.

Concentration (mM)	Observations Day 3	Day 8
Molybdate		
0.00	+++	+++
0.01	++-	+++
0.03	++-	++-
0.10	++-	+++
0.30	++-	++-
1.00	---	+--
3.00	---	---
Copper		
0.00	+++	+++
0.01	---	---
0.03	---	---
0.10	---	---
0.30	---	---
1.00	---	---
3.00	---	---

Symbols: + = sulfide production, observed as black FeS
- = no sulfide production

(Each symbol represents observation of one tube)

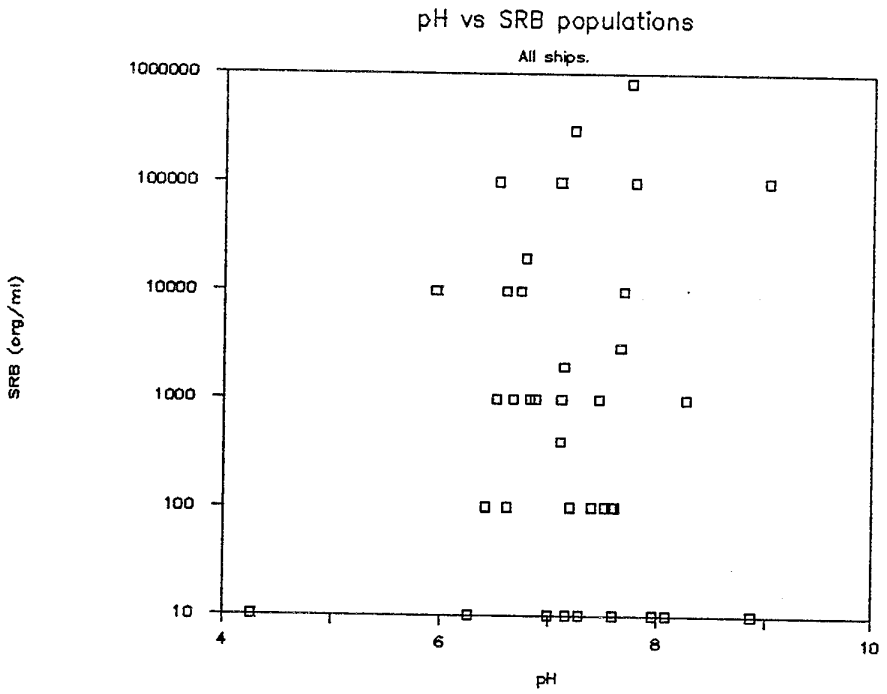

Figure 1. pH vs sulfate-reducing bacteria populations.

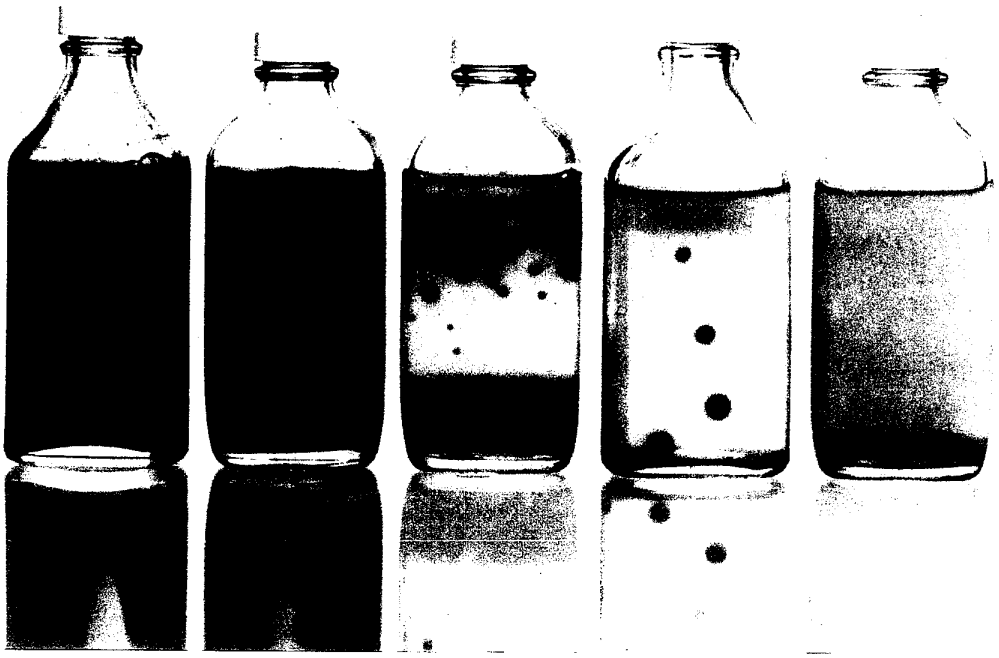

Plate 1. Serial dilution of sulfate-reducing bacteria in oily-water waste sample.

The dilution series shown here was prepared in 100ml medical flat bottles which give better separation of SRB colonies than the McConkey tubes that were regularly used. The bottles show the appearance after four days incubation; the dilutions were from 10^{-1} to 10^{-5} (L to R).

In the 10^{-1} and 10^{-2} dilutions sufficient sulfide was produced to colour the entire medium, obscuring the individual colonies.

In the 10^{-3} and 10^{-4} dilutions the colonies were well separated but with further incubation the whole medium would blacken and any colonies developing later would not be recognised.

The assessment of this sample would have been '>10 000/ml'.

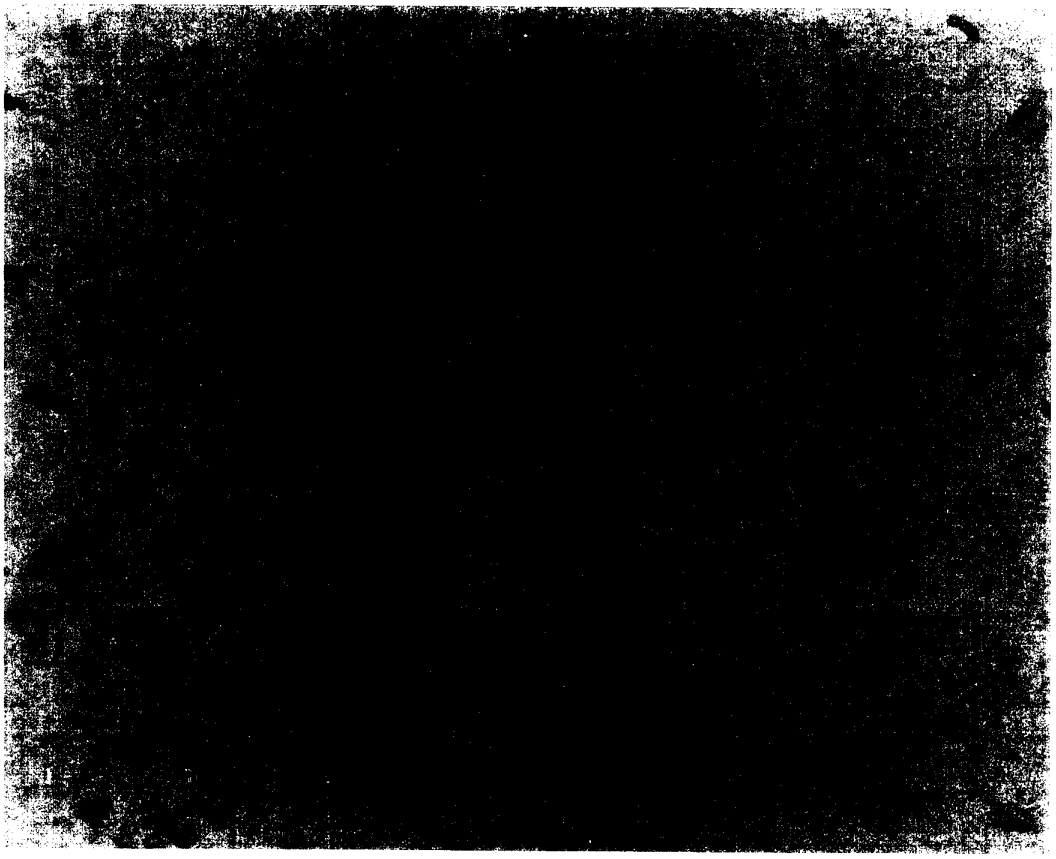

Plate 2. Gram stain of sulfate-reducing bacteria enrichment culture from oily-water waste.

The more strongly stained, orange-pink curved and sigmoid cells are Desulfovibrio sp..

Appendix

Medium #1: Postgate's Medium B.

KH_2PO_4	0.5 g/L
NH_4Cl	1.0 g/L
$CaSO_4$	1.0 g/L
$MgSO_4.7H_2O$	2.0 g/L
Sodium lactate	3.5 g/L
$FeSO_4.7H_2O$	0.5 g/L
Yeast extract	1.0 g/L
Ascorbic acid	0.1 g/L
Thioglycollic acid	0.1 g/L
Water, tap	1.0 L
pH adjusted to	7.0-7.5

Medium #2: Postgate's Medium C.

KH_2PO_4	0.5 g/L
NH_4Cl	1.0 g/L
Na_2SO_4	4.5 g/L
$CaCl_2.2H_2O$	0.06 g/L
$MgSO_4.7H_20$	0.06 g/L
Sodium lactate	6.0 g/L
Yeast Extract	1.0 g/L
$FeSO_4.7H_20$	0.04 g/L
Sodium citrate	0.3 g/L
Water, distilled	1.0 L

Medium #3: AMRL sulfate-reducing bacteria culture medium.

K_2HPO_4	0.5 g/L
$MgSO_4.7H_20$	1.0 g/L
NaCl	10.0 g/L
Na_2SO_4	1.0 g/L
K_2CO_3	0.5 g/L
$CaCl_2.2H_2O$	0.1 g/L
Ascorbic acid	0.2 g/L
Tryptone	0.4 g/L
Yeast extract	0.1 g/L
Sodium lactate (70%)	4.0 g/L
$(NH_4)_2FeSO_4.6H_2O$ (added after medium sterilisation)	0.2 g/L
Water, tap, filtered	1.0 L

Medium #4: Low Phosphate Medium.

$(NH_4)_2SO_4$	0.5 g/L
KCl	0.5 g/L
$MgSO_4.7H_2O$	1.0 g/L
$CaCO_3$	0.1 g/L
NaCl	10.0 g/L
Ascorbic acid	0.1 g/L
Tryptone, (Oxoid)	0.5 g/L
Yeast extract (Oxoid)	0.1 g/L
Sodium lactate 70%	5.0 g/L
$(NH_4)_2FeSO_4.6H_2O$ (added after medium sterilisation)	0.1 g/L
Agar, (Ionagar No 2 Oxoid)	4.0 g/L
Water, deionised	1.0 L

Medium #5: Ammonium-substituted medium.

NaCl	10.0 g/L
$MgSO_4.7H_2O$	1.0 g/L
$(NH_4)_2SO_4$	1.0 g/L
K_2HPO_4	0.5 g/L
K_2CO_3	0.5 g/L
$CaCl_2.2H_2O$	0.1 g/L
Ascorbic acid	0.1 g/L
Sodium lactate,70%	4.0 g/L
$(NH_4)_2FeSO_4.6H_2O$ (added after medium sterilisation)	0.1 g/L
Agar,(Ionagar No 2)	4.0 g/L
Water, deionised	1.0 L
pH adjusted to	7.0

Redox-poised diluting fluid.

NaCl	10.0 g/L
Na_2SO_4	1.0 g/L
Ascorbic acid	0.1 g/L
Water, filtered tap	1.0 L

Hydrogen Sulfide Generation in Shipboard Oily-water Waste:
Part 2: Microbiological Aspects

F.J. Upsher, L.E. Fletcher and D.K.C. Hodgeman

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Hydrogen Sulfide Generation in Shipboard Oily-water Waste Part 2: Microbiological Aspects

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ABSTRACT

A study has been undertaken to identify the microbiological and environmental factors which influence the formation of hydrogen sulfide in oily-water wastes in ships of the Royal Australian Navy (RAN). In particular, methods were developed for isolation and enumeration of sulfate-reducing bacteria which produce the hydrogen sulfide in the waste. These methods were then used to find the important environmental features which contribute to the growth and metabolism of these bacteria.

Bilge specimens obtained from RAN and foreign naval vessels were examined in the laboratory. One or more species of sulfate-reducing bacteria were found in most samples of these wastes, with population levels as high as 800,000 per mL. Investigation of the bacteria isolated from these samples showed that growth of sulfate-reducing bacteria from shipboard oily-water wastes occurred at significant rates in the temperature range 12-45°C and in the pH range 6-8. Growth of these bacteria was also influenced by the availability of organic and inorganic nutrients and compounds known to inhibit the growth of sulfate-reducing bacteria.