# NUTRIENT CYCLING IN FRESH-WATER LAKES ON SIGNY ISLAND, SOUTH ORKNEY ISLANDS

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ABSTRACT. Samples of mud and water from three fresh-water lakes on Signy Island, South Orkney Islands, have been examined for bacteria capable of cycling nitrogen and sulphur compounds. The data obtained indicate that, with the exception of purple and green photosynthetic sulphur bacteria, all the bacteria necessary for carrying out these reactions are present in the lakes investigated. Furthermore, the results show that these organisms will grow and perform these reactions at the low temperatures (*c.* 5° C) commonly encountered in these lakes following disappearance of the ice cover.

SIGNY ISLAND (lat. 60°43′S., long. 45°38′W.) is one of the South Orkney Islands group which lie on the Scotia Ridge, within the maritime Antarctic (Holdgate, 1964). The general climatic conditions prevailing in this region have been well documented (Holdgate, 1964), and Heywood (1967, 1968) has described in considerable detail the morphology and physico-chemical characteristics of the Signy Island lakes. The lakes are frozen for 8–9 months of the year and maximum water temperatures are rapidly attained following disappearance of the ice cover. Due to extreme cloud cover, frequent precipitation and strong winds, the water temperatures rarely, if ever, exceed 5° C.

Although the presence of bacteria in the Antarctic has been known for many years (Ekelöf, 1908; Sieburth, 1963; Stanley and Rose, 1967; Baker, 1970), the information available is still fragmentary. Much of the early work was concerned with the isolation of bacteria from Antarctic soils and little attention was paid to the effects of the environment upon the microflora. Indeed it was not until Stanley and Rose (1967) pertinently pointed out that standard microbiological techniques were lethal to obligately psychrophilic bacteria that these organisms were found in reasonable numbers in the Antarctic. With the exception of the work of Stanley and Rose (1967), little is known regarding the bacteriology of Antarctic fresh waters. The aim of this present investigation has been to study in detail the bacteriology of certain Signy Island fresh-water lakes in relation to the cycling of nitrogen and sulphur compounds. Three lakes were chosen for study. Lakes 2 and 10 receive some effluent drainage from a gull colony and a seal wallow, respectively, whilst lake 4, a cirque lake, receives only ice melt water.

## METHODS

Samples of mud and fresh water were obtained from lakes 2, 4 and 10 in March 1972. They were maintained at 0° C during storage and transport to the United Kingdom. Subsequent transport to Dundee was in refrigerated containers which ensured that the samples were never exposed to lethal temperatures. The samples were finally stored at 0° C.

Samples of sediment and water from the three lakes have been examined for specific bacterial groups capable of transforming nitrogen and sulphur compounds using liquid culture-enrichment techniques. The liquid media were equilibrated at the temperature at which they were to be subsequently incubated (either 5° or 20° C) prior to inoculation with either 1 g. wet weight of sediment or 10 ml. of water. The samples were then incubated at 5° ± 1° C for 10 days or at 20° ± 1° C for 5 days. The following enrichment media were used:

i. *Nitrogen-free media.* The enrichment medium for *Azotobacter* sp. was that devised by Norris (1959). It was dispensed into 100 ml. conical flasks to a depth of 10 mm. before autoclaving at 121° C for 15 min. The mud and water samples were also examined for *Clostridium pasteurianum* by inoculating into the medium described by Veldkamp (1970). The flasks were then incubated under nitrogen in a desiccator.

ii. *Denitrifying bacteria.* The medium used was that of Bollag and others (1970). It was dispensed into 1 oz. McCartney bottles and a pH indicator was added. This consisted of 0·5 ml. of a 1 per cent solution of ethanolic bromothymol blue per litre of medium; a colour change to green (acidic) or more intense blue (alkaline) registered the effect of the growth of the cultures on the pH of the medium.

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iii. *Nitrifying bacteria.* The medium described in Skerman (1967) was used to enrich for these bacteria. Ammonium sulphate was added to the medium when enriching for *Nitrosomonas* sp. and sodium nitrate for *Nitrobacter* sp. The media were dispensed in 10 mm. layers in 100 ml. conical flasks and autoclaved at 121° C for 15 min. Appearance of nitrite and nitrate in the media was determined using Griess-Llosvay reagent according to the method described by Cowan and Steel (1966).

iv. *Proteolytic activity.* Serial ten-fold dilutions of 1 ml. volumes of the lake water samples and 1 g. wet weight amounts of sediment were prepared in cold sterile phosphate buffered saline. Aliquot volumes (0·5 ml.) of each dilution were plated in duplicate on to gelatin agar. Zones of proteolysis were determined by the addition of a 12 per cent acid mercuric chloride solution to the plates (Cowan and Steel, 1966).

v. *Desulfovibrio medium.* The modified medium of Starkey as described by Skerman (1967) was used to enrich for these bacteria. The medium was dispensed into 4 oz. glass bottles which, after the addition of the inoculum, were aseptically filled to the neck with additional sterile medium and sealed with screw caps. The enrichments were incubated at 20° C for 10 days and at 5° C for 30 days.

vi. *Aciduric and non-aciduric Thiobacillus media.* These media were prepared according to the methods described in Skerman (1967) and dispensed in shallow layers into 100 ml. conical flasks before autoclaving at 121° C for 15 min.

vii. *Thiobacillus denitrificans medium.* The medium of Baalsruud and Baalsruud (1954) was used and made up in 1 oz. McCartney bottles, each containing a Durham tube for gas collection.

viii. *Purple and green sulphur bacteria media.* The media of van Niel (1931) and Larsen (1952) were used to enrich for these two groups of photosynthetic bacteria. The inoculated media were incubated at both 5° and 20° C illuminated by 25 W tungsten filament lamps placed at a distance of 250 and 350 mm., respectively, from the cultures.

ix. *Estimation of $H_2S$-producing bacteria.* Serial ten-fold dilution of water and sediment were prepared as described previously. Aliquot volumes (0·5 ml.) of each dilution were plated out in duplicate on to Bacto peptone-iron agar (Difco). After incubation at 20° C for 4 days and at 5° C for 10 days, counts were made of the number of black-pigmented colonies.

Samples from the liquid enrichments were removed aseptically by Pasteur pipette and wet-mount preparations made. These preparations were examined by phase-contrast microscopy. Cultures were also Gram stained using Burke's (1922) modifications and examined for motility by the hanging drop technique. Identification was based on the key devised by Skerman (1967).

## Results

Examination of the aerobically incubated nitrogen-free liquid enrichments showed that no nitrogen-fixing bacteria were present in any of the water samples from lakes 2, 4 and 10. Enrichment cultures using mud from the three lakes as the inoculum all yielded nitrogen-fixing bacteria. Nitrogen-fixing ability was determined using the acetylene reduction technique (Stewart and others, 1967) Aliquot volumes (0·5 ml.) of serial ten-fold dilution from the positive liquid enrichments were plated on to a medium of the same composition solidified by the addition of 1 per cent Ionagar No. 2 (Oxoid Ltd.). After 4 days incubation at 20° C small colourless colonies, which later became yellowish grey, appeared. Examination of wet-film and Gram-stained preparations from single colonies showed that the cells were spherical to rod shaped, large (4–6 $\mu$m. in diameter), motile and Gram negative. The pure cultures were checked for nitrogen-fixation by the acetylene-reduction method and all proved positive. The ability of these organisms to fix nitrogen together with their characteristic morphology suggest that these bacteria are *Azotobacter* sp. Growth of these organisms occurred at both 5° and 20° C, albeit more slowly at the former temperature. All attempts to isolate the N-fixing anaerobe *Clostridium pasteurianum*, a commonly occuring N-fixing bacterium in soil, have failed.

Denitrifying bacteria were found to be present in all the mud and water samples from lakes 2, 4 and 10, and grew rapidly at both incubation temperatures. Examination of wet-film and

Gram-stained preparations from the enrichment cultures showed the presence of Gram-negative, asporogenous, motile rods and Gram-positive cocci. Preliminary biochemical tests on 25 isolates of these Gram-negative rods show that they are oxidase and catalase positive, and with the exception of one isolate, utilize glucose oxidatively only. The data so far available suggest that these Gram-negative rods are most probably *Pseudomonas–Achromobacter* sp., whilst the Gram-positive cocci are *Micrococcus* sp. Enrichment cultures for *Thiobacillus denitrificans* showed bacterial growth and gas production from the mud samples from lakes 2, 4 and 10. Serial dilutions from the positive enrichments were prepared and 0·5 ml. volumes plated on to the liquid enrichment medium solidified by the addition of 1 per cent Ionagar No. 2. Small translucent colonies appeared after 3–4 days at 20° C and examination of slide preparations showed the presence of small, motile Gram-negative rods. Inoculation of pure cultures from the dilution plates into the liquid medium resulted in growth and gas production. These data together with the energy source utilized provide positive evidence for the presence of *Thiobacillus denitrificans* in the muds from the three lakes.

Bacterial growth was observed in liquid enrichments for nitrifying bacteria in both the water and sediment samples from lakes 2 and 4. No growth of nitrifying bacteria was observed in enrichments inoculated with sediment or water samples from lake 10. Growth of these organisms was extremely slow, taking 10 days at 20° C and 24 days at 5° C before a pellicle was visible on the surface of the medium. Tests with Griess-Llosvay reagent showed the presence of nitrite and nitrate in the respective positive enrichment media at the end of the incubation period but not in the uninoculated controls. Slide preparations from the *Nitrosomonas* and *Nitrobacter* positive enrichments were prepared and examined under the microscope. The predominant bacteria present in the *Nitrosomonas* enrichments were Gram-negative, motile, cocco-bacillary rods occasionally in pairs but usually single and grew aerobically. No growth was observed when 1 ml. volumes from the enrichments were inoculated into nutrient broth. Similarly, examination of the positive *Nitrobacter* enrichments showed the predominance of motile Gram-negative, small rods (0·8–1·0 $\mu$m.), usually single but occasionally occurring as aggregates of three or four cells. No growth was observed in nutrient broth. These features, together with the energy sources utilized, the formation of nitrite and nitrate in the respective media, and the absence of growth in organic media provide evidence that the organisms isolated were *Nitrosomonas* sp. and *Nitrobacter* sp.

Many of the bacteria present in the water and mud samples were actively proteolytic as measured by gelatin hydrolysis and counts are presented in Table I. Slide preparations showed that these organisms were Gram-negative motile rods. No attempt has been made to classify these organisms. Higher counts were observed on gelatin agar at 20° C than at 5° C, indicating that many of these organisms are apparently not psychophilic strains.

Results of the liquid enrichments for sulphate-reducing bacteria show that these organisms were present in the mud but not in the water samples from lakes 2, 4 and 10. Wet-film and Gram-stained preparations from the positive enrichments showed the predominance of Gram-

TABLE I. INCIDENCE OF PROTEOLYTIC BACTERIA IN THREE SIGNY ISLAND LAKES*

| *Sample* | | *Incubation temperature* 5° C | 20° C |
|---|---|---|---|
| Lake 2 | water | $1{\cdot}3\times10^3$ | $2{\cdot}4\times10^4$ |
| | mud | $21\times10^3$ | $37\times10^3$ |
| Lake 4 | water | 76 | 465 |
| | mud | $17{\cdot}7\times10^3$ | $31{\cdot}3\times10^3$ |
| Lake 10 | water | $4{\cdot}3\times10^3$ | $9{\cdot}4\times10^3$ |
| | mud | $13{\cdot}1\times10^3$ | $12{\cdot}4\times10^4$ |

* Bacterial count/ml. of water or 1 g. wet weight mud.

negative, actively motile, single rod-shaped bacteria. Older cultures show marked pleomorphism. These features together with the extremely active formation of $H_2S$ indicate the presence of *Desulfovibrio* sp. No Gram-positive, spore-forming sulphate-reducing bacteria of the genus *Desulfotomaculum* were observed in any of the positive enrichment cultures examined.

Enrichment cultures for purple and green photosynthetic sulphur bacteria have so far failed to yield any of these organisms from the mud and water samples from lakes 2, 4 and 10. The enrichments have been repeated using higher and lower light intensities but without success.

Bacterial growth was observed in all the aciduric and non-aciduric *Thiobacillus* medium enrichments inoculated with water and mud from the three lakes. Growth initially appeared as a thin pellicle on the surface of the liquid enrichments whilst in older cultures the whole medium became turbid. Serial ten-fold dilutions from the positive enrichments were plated out on to the enrichments media solidified by the addition of Ionagar No. 2. Small colourless colonies developed which later became white on ageing. Examination of slide preparations from single colonies showed the presence of small, regularly shaped, single Gram-negative rods which were actively motile. 20 colonies were sub-cultured from the plates into the liquid enrichment medium and the disappearance of thiosulphate determined by titration with 0·1 N iodine using starch as the indicator (Vogel, 1961). Thiosulphate slowly disappeared from the medium as bacterial growth occurred in the inoculated enrichments but not from the uninoculated controls. These features positively support the presence of *Thiobacillus* sp. in the three lakes.

Data presented in Table II show the incidence of bacteria capable of degrading organic material with the release of hydrogen sulphide ($H_2S$). Relatively few bacteria present in the water samples were capable of releasing $H_2S$ from organic material compared with the activity of the bacteria present in the mud. Greater counts of $H_2S$-producing organisms were observed at 20° C than at 5° C, suggesting that many of these organisms were not psychrophilic. No attempt has been made to classify these isolates, although slide preparations show that they are predominantly Gram-negative rods.

TABLE II. INCIDENCE OF $H_2S$-PRODUCING BACTERIA IN THREE SIGNY ISLAND LAKES*

| *Sample* | | *Incubation temperature* 5° C | 20° C |
|---|---|---|---|
| Lake 2 | water | 279 | $1{\cdot}1 \times 10^3$ |
| | mud | $16 \times 10^3$ | $23{\cdot}9 \times 10^3$ |
| Lake 4 | water | 0 | 131 |
| | mud | 370 | $1{\cdot}5 \times 10^3$ |
| Lake 10 | water | 765 | $2{\cdot}7 \times 10^3$ |
| | mud | $19 \times 10^3$ | $29 \times 10^3$ |

* Bacterial count/ml. of water or 1 g. wet weight mud.

## DISCUSSION

From the data presented it is clear that representatives of all the bacterial genera, with the exception of N-fixing *Clostridium* sp. and purple and green photosynthetic bacteria, necessary for performing the specifilized reactions of the nitrogen and sulphur cycles, are present in the three fresh-water lakes studied. Further, the results show that several of these specialized bacterial groups are physiologically active at 5° C, a temperature commonly attained in these lakes following disappearance of the ice cover. Thus, the *Azotobacter* sp. isolated fix appreciable amounts of nitrogen at 5° C as measured by the acetylene-reduction method (Stewart and others, 1967). This finding is of some interest since *in vitro* studies show that nitrogenase is cold-labile (personal communication from W. D. P. Stewart). Similarly, denitrification as

measured by $N_2$ gas formation occurred after 3–5 days incubation at 5° C. Nitrification, the conversion of $NH_4$-N to $NO_3$-N however appears to be an extremely slow process at 5° C and only a little more rapid at 20° C. Painter (1970), in a review on inorganic nitrogen metabolism, indicated that nitrifying bacteria grow extremely slowly even under good conditions, whilst data by Buswell and others (1950) suggest that at a temperature of 5° C or less *Nitrosomonas* sp. would not grow.

Two groups of bacteria in lakes 2, 4 and 10 are able to produce hydrogen sulphide ($H_2S$). Thus $H_2S$ is produced from inorganic sulphates by *Desulfovibrio* sp. and from proteinaceous material by heterotrophic bacteria. It must be stressed that the counts obtained for the heterotrophic $H_2S$-producing bacteria are not absolute figures, since growth will inevitably have occurred from the time the samples were taken to when they were examined. As is to be expected, the main activity is in the sediment of each lake where anaerobic conditions which favour $H_2S$ production prevail. Lake 4, which receives little organic material, has a low count of $H_2S$-producing bacteria, whereas lakes 2 and 20, which receive drainage from a gull colony and a seal wallow, respectively, have much higher counts. Heywood (1968) suggested that $H_2S$ produced in the Signy Island lakes was converted under anaerobic conditions, in the presence of $Fe^{++}$ ions, to ferrous sulphide. Presumably the process does not go to completion and thus, under anaerobic conditions and in the presence of radiant energy, purple and green sulphur photosynthetic bacteria could use $H_2S$ as an electron donor in photosynthesis. However, these bacteria are absent from the samples so far examined. The most probable explanation for their absence is that the lakes are shallow (Heywood, 1967) and this factor, coupled with wind turbulence and oxygen evolved as a product of algal photosynthesis, prevents the surface mud from becoming sufficiently anaerobic for these organisms to develop.

The presence of the facultative anaerobe *Thiobacillus denitrificans* in mud samples from lakes 2, 4 and 10 is of some importance because it provides a link between the nitrogen and sulphur cycles. The organism is able to oxidize reduced inorganic sulphur compounds under anaerobic conditions using $NO_3$-N as the terminal electron acceptor. It would be of interest to determine the significance of this organism in the cycling of nitrogen and sulphur compounds in the three lakes, in light of the findings by Mann and others (1972) that the addition of sulphur to soil increased the rate of denitrification by *Thiobacillus denitrificans*. Under aerobic conditions, such as those occurring after disappearance of the ice cover, aciduric and non-aciduric *Thiobacillus* sp. are probably actively involved in the oxidation of reduced sulphur compounds. Although growth of *Thiobacillus* sp. was observed in liquid enrichments (pH 4), it is unlikely that in the natural environment they are exposed to such acid conditions because Heywood (1968) reported that the pH of the lakes was 7.

Further studies are now necessary to determine whether the organisms involved in the cycling of nitrogen and sulphur compounds in the Signy Island lakes are specifically adapted to function at low temperatures, i.e. psychrophilic species, or whether they are mesophilic strains which are operating at greatly reduced efficiencies.

Parallel with these studies, field data to make possible the determination of the rates of these transformations in the lakes are also required.

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