

FIG. 3 Re-Os isochron plot of Udachnaya eclogites; error bars are smaller than plot symbols. $\lambda^{187}\text{Re} = 1.64 \times 10^{-11} \text{ yr}^{-1}$, from ref. 29. Samples UV1/79 and UV25/84 are omitted from the plot for reasons stated in the text. See Table 1 for definitions of λ and γOs .

Oxygen isotope characteristics of some diamond-bearing eclogites from Udachnaya are consistent with their protoliths having experienced low-temperature hydrothermal alteration, possibly as oceanic crust⁵. It is also possible that some eclogites crystallized as high-pressure magmatic products in the mantle with no crustal influence^{6,9}. Although the oxygen isotope characteristics of some of the samples studied here are closer to typical mantle values, they show significant variation ($\delta^{18}\text{O} = 4.8\text{--}7.0\text{‰}$)²³ and do not preclude an origin as oceanic crust. Indeed, some Udachnaya eclogites have trace-element concentrations²⁴, including Re and Os, similar to those of mid-ocean-ridge basalts.

The processes involved in generating the elemental and isotopic systematics of the Udachnaya (and other) bi-mineralic eclogites are multi-stage and complex²⁴, rendering it difficult to constrain ages using isotope systems where parent and daughter are large-ion lithophile elements. Indications of Mid- to Late-Archaean ages from Re-Os and Pb-Pb isotope systematics do not favour an origin for these rocks as vestiges of the Earth's primary differentiation in the Hadean (>4 Gyr). Strong arguments have been made for subducted basaltic protoliths for some of these rocks^{6,19}. Although the Re-Os isotope systematics do not provide definitive evidence for a subducted origin, the data do not preclude it, and the very high jadeite contents of the pyroxenes argue against a simple magmatic origin¹⁹. If subduction played a role in the genesis of these rocks then the Re-Os isotope data indicate that it took place in the Archaean.

The crustal basement in the Siberian craton does not appear to be significantly older than the age range seen in the mantle lithosphere defined by Re-Os isotopes¹². This general correspondence between lithospheric mantle age and basement age is similar to that inferred for the Kaapvaal craton^{25,26}, and suggests that either major crust-building and mantle differentiation events are linked by a genetic process, or that survival of voluminous continental crust in the Archaean required the protection of rigid lithospheric mantle 'keels'. □

Received 15 September 1994; accepted 14 March 1995.

- McCulloch, M. T. in *Kimberlites II* (ed. Ross, N.) 864-876 (Geol. Soc. Australia, 1989).
- Snyder, G. A. et al. *Earth planet. Sci. Lett.* **118**, 91-100 (1993).
- Jagoutz, E., Dawson, J. B., Hoernes, S., Spettel, B. & Wanke, H. *Lunar planet. Sci.* **XV**, 395-396 (1984).
- Neal, C. R. et al. *Earth planet. Sci. Lett.* **99**, 362-379 (1990).
- Jacob, D., Jagoutz, E., Lowry, D., Matthey, D. & Kudrjavtseva, G. *Geochim. cosmochim. Acta* **59**, 5191-5207 (1995).
- O'Hara, M. J. & Yoder, H. S. *Scott J. Geol.* **3**, 67-117 (1967).
- Hatton, C. J. thesis, (Univ. Cape Town, 1978).
- Smyth, J. R., Caporuscio, F. A. & McCormick, T. C. *Earth planet. Sci. Lett.* **93**, 133-141 (1989).
- Caporuscio, F. A. & Smyth, J. R. *Contr. Miner. Petrol.* **105**, 550-561 (1990).

- Nutman, A. P., Chernyshev, I. V., Baadsgaard, H. & Smelov, A. P. *Precambrian Res.* **54**, 195-201 (1992).
- Turchenko, S. I. in *Precambrian Geology of the USSR* (eds Rundqvist, D. V. & Mitrofanov, F. P.) 247-264 (Elsevier, Amsterdam, 1993).
- Pearson, D. G. et al. *Geochim. cosmochim. Acta* **59**, 959-977 (1995).
- Sobolev, N. V. et al. (abstr.) *5th Int. Kimberlite Conf. Araxa 391* (CPRM, 1991).
- Walker, R. J., Shirey, S. B. & Stecher, O. *Earth planet. Sci. Lett.* **87**, 1-12 (1988).
- Walker, R. J., Shirey, S. B., Hanson, G. N., Rajamani, V. & Horan, M. F. *Geochim. cosmochim. Acta* **53**, 3005-3013 (1989).
- Martin, C. E. *Geochim. cosmochim. Acta* **55**, 1421-1434 (1991).
- Reisburg, L. et al. *Earth planet. Sci. Lett.* **120**, 149-167 (1993).
- Hauri, E. & Hart, S. R. *Earth planet. Sci. Lett.* **114**, 253-271 (1993).
- Ireland, T. R., Rudnick, R. L. & Spetsius, Z. *Earth planet. Sci. Lett.* **128**, 199-213 (1995).
- Richardson, S. H. *Nature* **322**, 623-626 (1986).
- Smith, C. B. et al. *Geochim. cosmochim. Acta* **55**, 2579-2590 (1991).
- Taylor, L. A. & Neal, C. R. *J. Geol.* **97**, 551-567 (1989).
- Snyder, G. A. et al. *Am. Mineral.* (in the press).
- Jerde, E. A., Taylor, L. A., Crozaz, G., Sobolev, N. V. & Sobolev, V. S. *Contr. Miner. Petrol.* **114**, 189-192 (1993).
- Richardson, S. H., Gurney, J. J., Ertank, A. J. & Harris, J. W. *Nature* **310**, 198-202 (1984).
- Pearson, D. G., Carlson, R. W., Shirey, S. B., Boyd, F. R. & Nixon, P. H. *Earth planet. Sci. Lett.* (in the press).
- Walker, R. J. et al. *Geochim. cosmochim. Acta* **58**, 4179-4197 (1994).
- Martin, C. E., Esser, B. K. & Turekian, K. K. *Aust. J. Earth Sci.* **38**, 569-576 (1991).
- Lindner, M., Leich, D. A., Russ, G. P., Bazan, J. M. & Borg, R. J. *Geochim. cosmochim. Acta* **53**, 1597-1606 (1989).
- Shirey, S. B. & Walker, R. J. (abstr.) *Eos* **75**, 355-356 (1994).

ACKNOWLEDGEMENTS. We thank F. R. Boyd for encouragement and guidance. The manuscript benefited greatly from reviews by F. R. Boyd, S. Galer, N. Rogers and C. Hawkesworth.

Concentration and transport of nitrate by the mat-forming sulphur bacterium *Thioploca*

H. Fossing*, V. A. Gallardo†, B. B. Jørgensen*, M. Hüttel*, L. P. Nielsen‡, H. Schulz*, D. E. Canfield*, S. Forster*, R. N. Glud*, J. K. Gundersen*, J. Küver*, N. B. Ramsing‡, A. Teske*, B. Thamdrup* & O. Ulloa§

* Max Planck Institute for Marine Microbiology, Fahrenheitstrasse 1, D-28359 Bremen, Germany

† Centro EULA-Chile, Universidad de Concepción, Casilla 156-C, Concepción, Chile

‡ Department of Microbial Ecology, Institute of Biology, Aarhus University, Ny Munkegade Build. 540, DK-8000 Aarhus C, Denmark

§ Department of Geophysics, Niels Bohr Institute, University of Copenhagen, Haraldsgade 6, DK-2200 Copenhagen N, Denmark

MARINE species of *Thioploca* occur over 3,000 km along the continental shelf off Southern Peru and North and Central Chile¹⁻⁴. These filamentous bacteria live in bundles surrounded by a common sheath and form thick mats on the sea floor under the oxygen-minimum zone in the upwelling region, at between 40 and 280 m water depth. The metabolism of this marine bacterium^{5,6} remained a mystery until long after its discovery^{1,7}. We report here that *Thioploca* cells are able to concentrate nitrate to up to 500 mM in a liquid vacuole that occupies >80% of the cell volume. Gliding filaments transport this nitrate 5-10 cm down into the sediment and reduce it, with concomitant oxidation of hydrogen sulphide, thereby coupling the nitrogen and sulphur cycles in the sediment.

Thioploca mats, sediment and water chemistry were studied along a shelf transect off the Chilean coast during late summer, March 1994 (Fig. 1). In the densest *Thioploca* mat, at 87 m water depth, the wet weight of sheathed *Thioploca* reached nearly 1 kg m⁻², of which the total bacterial biomass accounted for 120 g m⁻² (wet weight). Single filaments up to 7 cm in length were able to glide into the sediment in sheaths that were 10-15 cm long and 1.5 mm diameter (Fig. 2a, b). Most of the sheaths were horizontally oriented at the sediment surface where they formed yellowish mats up to 2 cm thick, with sheaths

LETTERS TO NATURE

stretching vertically deep into the sediment (Fig. 3). Up to 100 *Thioploca* filaments, sometimes of two different widths, were observed to share one sheath. Phylogenetic characterization through 16S ribosomal RNA sequencing confirmed that there were two species of *Thioploca*, *T. chileae* (15–20 µm wide) and *T. araucae* (30–40 µm wide), previously described on the basis of filament diameter⁸.

The coastal upwelling zone off central Chile has a very high primary production, 9.6 g carbon m⁻² day⁻¹ at the time of our study, among the highest observed in marine environments. Mineralization of the settling organic matter resulted in extensive

oxygen depletion of the water column from the *Thioploca* mat and up to 60 m above the sea floor, with temporal O₂ variation between 0 and 5 µM (Fig. 1). Seasonal oxygen variations are however, pronounced and strongly influence the mat distribution and biomass (V.A.G., unpublished result). During spring-summer, dense *Thioploca* mats inhabit the sea floor below about 40 m water depth. But the shallower mats break up during winter as bottom-water oxygen increases and nitrate becomes depleted so dense *Thioploca* mats are observed only at depths below 60 m.

Fine-scale *in situ* measurement of O₂ with microelectrode mounted on a benthic lander showed no oxygen even in the

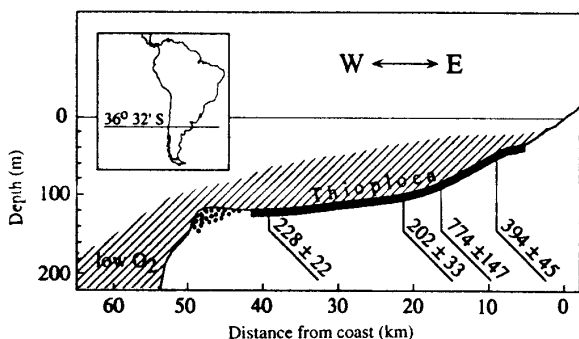


FIG. 1 The continental shelf off Chile at the Bay of Concepción (36°32' S), studied during late summer 1994 (6 March–1 April). The *Thioploca* mat (black) underlies the oxygen minimum zone (<5 µM O₂ equivalent to <0.1 ml O₂ litre⁻¹ or <2% air saturation; shaded) in the upwelling region from a water depth of 40 m down to a phosphorus reef on the shelf edge at 120 m. Mat abundance is shown for four stations as wet weight of *Thioploca* filaments plus sheaths (g m⁻² ± S.E., n = 3). Undisturbed mat samples of 28 cm² were taken by a multipoint corer. The mat was gently sieved out of the sediment through a 0.5-mm mesh sieve and infauna and debris were removed before the mat was weighed. From RV *Vidal Gormaz*, the transect was surveyed in a joint research project between the Max Planck Institute for Marine Microbiology, Bremen, Germany, and the Centro-EULA, University of Concepción, Chile.

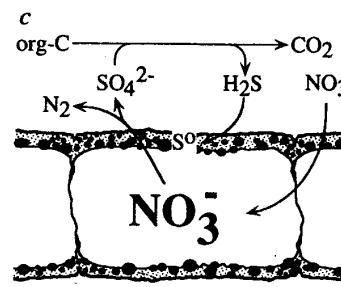


FIG. 2 a, *Thioploca* filaments observed inside sheaths of various thickness and length. The photo was taken after the sediment had gently been washed away from the mat (bar inside white frame represent 1 mm). b, Three multicellular *Thioploca araucae* filaments sharing common sheath, visible around the filaments as a fibrillar shadow (~100 µm thickness). Each cell is about 40 µm wide and 60 µm long and filled with light-refracting sulphur globules embedded in the cytoplasm and surrounding a large vacuole (cannot be seen; bar represent 40 µm). c, Schematic drawing of a *Thioploca* cell with elemental sulphur globules (grey) embedded in the cytoplasm (dotted) and the large central liquid vacuole (white) in which nitrate is concentrated up to 500 ml (bar, 40 µm). Nitrate reduction and carbon oxidation are coupled through the sulphur cycle. Hydrogen sulphide is produced by sulphate-reducing bacteria oxidizing organic carbon compounds. *Thioploca* oxidizes hydrogen sulphide using nitrate with concurrent storage of elemental sulphur in the cytoplasm. We suggest the following stoichiometry: $2\text{NO}_3^- + 5\text{HS}^- + 7\text{H}^+ \rightarrow \text{N}_2 + 5\text{S} + 6\text{H}_2\text{O}$, followed by oxidation of elemental sulphur to sulphate: $6\text{NO}_3^- + 5\text{S} + 2\text{H}_2\text{O} \rightarrow 3\text{N}_2 + 5\text{SO}_4^{2-} + 4\text{H}^+$.

top 100 μm of the mat-covered sediment. Organic carbon in the sediment was therefore mineralized entirely through anaerobic processes. Carbon oxidation rates were up to $50 \text{ mmol C m}^{-2} \text{ day}^{-1}$ in the upper 10 cm of the sediment, principally as a result of sulphate reduction. In spite of the production of $25 \text{ mmol H}_2\text{S m}^{-2} \text{ day}^{-1}$, less than $2 \mu\text{M H}_2\text{S}$ was detected in the pore water within this sediment layer. Therefore, the sulphide must have been rapidly reoxidized within the sediment, a surprising result considering the complete absence of oxygen in the sediment. Analysis of nitrate within the cells of *Thioploca*, however, revealed a potential of these bacteria to oxidize part of the sulphide with NO_3^- .

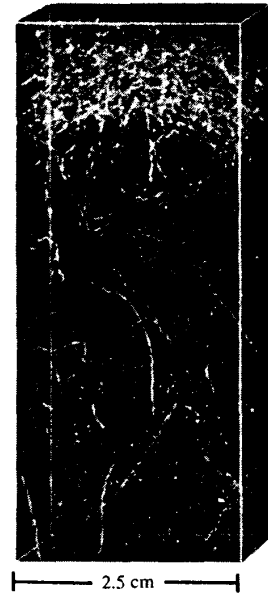
The nitrate concentration in the sea water overlying the *Thioploca* mats was about $25 \mu\text{M}$; in the sediment the concentration decreased to zero at 2–3 cm depth. In contrast, extremely high concentrations of up to 500 mM NO_3^- were measured in clean *Thioploca* filaments collected from the top 6 cm at the same site. *Thioploca* was obviously able to concentrate NO_3^- inside the cell to up to 20,000 times the ambient seawater concentration. To our knowledge, such an extreme nitrate accumulation, comparable to the seawater chloride concentration of 550 mM , has not been observed in any other prokaryote.

A peculiar behaviour and adaptation to this nitrate accumulation by *Thioploca* was demonstrated by addition of nitrate to water overlying *Thioploca* mats in an aquarium with flowing water (flume) under *in situ* conditions. *Thioploca* filaments partly emerged from their sheaths embedded in the sediment and stretched several centimetres up into the sea water after NO_3^- addition. They had the appearance of white hair, swaying in the anoxic or hypoxic water to provide optimal conditions for nitrate uptake.

The large central liquid vacuole, which serves as a store for nitrate, occupies $>80\%$ of the cell volume and has been described as an extracytoplasmic reservoir formed by an inflated membrane intrusion⁷. This vacuole is surrounded by numerous sulphur globules embedded in the cytoplasm (Fig. 2b and c). Sulphur globules are also seen in *Beggiatoa*, another mat-forming, filamentous, colourless sulphur bacterium capable of H_2S oxidation with oxygen or nitrate^{10,11}. As shown by 16S rRNA sequencing, *Beggiatoa* is phylogenetically the closest relative to *Thioploca*, sharing many morphological similarities even in its ultrastructure¹².

Beggiatoa and other sulphide-oxidizing sulphur bacteria such as *Thiothrix* spp. and *Thiovulum* spp.^{13,14} depend on the coexistence of their electron donor (H_2S) and acceptor (O_2 or NO_3^-) in order to maintain their energy metabolism and, consequently, they often live in steep, opposed diffusion gradients^{13,15}. Our observations suggest that *Thioploca* is also a sulphide-oxidizing denitrifier. However, by their unique ability to store both nitrate and sulphur in their cells, *Thioploca* become independent of coexisting substrates. By continuous vertical migration between the nitrate-containing sea water and sulphide-producing sediment layers, *Thioploca* can therefore also thrive in sediments at depths where nitrate is lacking. In fact, *Thioploca*

FIG. 3 A computer-generated schematic of a 1.5-cm thick *Thioploca* mat of mainly horizontally oriented sheaths, showing the sheaths stretching more than 6 cm into the sediment. *Thioploca* filaments were observed to move up to 1 cm h^{-1} inside their sheaths and were thus able to transport NO_3^- much faster than diffusion (for example, *Thioploca* filaments may move down to 6 cm within 6 h, whereas diffusion would take $\sim 10 \text{ d}$). Immediately after sampling, the sediment-mat core was frozen in a nitrogen/isopentane slush. The top end of the core liner was tightly closed to allow only a downward expansion of the sediment in order to prevent major changes of the *Thioploca* mat due to swelling. Vertical sections of $100 \mu\text{m}$ thickness were removed by a freeze microtome and after each cut a photo was taken of the remaining core surface in polarized light to enhance the contrast of the sulphur globules. A series of 101 photos were scanned, digitized, and used to construct a 3D image (NIH Image Program) of a 1-cm-thick vertical sediment block. Thus the discontinuity of *Thioploca* filaments at depth is due to their penetration out of the block. The white strands therefore show the orientation of the sulphur-containing filaments, mostly within sheaths.



may outcompete other sulphur bacteria by keeping H_2S and NO_3^- separated.

In oxygen-deficient oceanic waters, carbon oxidation may be coupled directly to nitrate reduction. In the upwelling waters off the Pacific coast of South America, denitrification rates of $25 \times 10^{12} \text{ g N year}^{-1}$ ($3.9 \text{ mmol NO}_3^- \text{ m}^{-2} \text{ day}^{-1}$) have been estimated, comprising up to 25% of the global denitrification in marine water masses^{16,17}. The efficient nitrate uptake and vertical transport by *Thioploca* extends the denitrification zone into the sediment. Carbon oxidation and nitrate reduction are, however, here linked by the sulphur cycle as an intermediate electron carrier (Fig. 2c). Nitrate uptake by a *Thioploca* mat was measured *in situ* as $2.1 \text{ mmol m}^{-2} \text{ day}^{-1}$, which can oxidize up to 20% of the H_2S produced. The *in situ* uptake, however, could be increased by one order of magnitude when seawater nitrate was changed from 20 to $60 \mu\text{M}$. This demonstrates the efficiency by which *Thioploca* filaments are able to take up nitrate. In marine sediments, NO_3^- is normally taken up by diffusion and bio-irrigation when sea water is pumped into tubes and burrows. On the South American Pacific shelf, however, nitrate is transported into the sediment by a previously unknown but unique mechanism, the migration of nitrate-filled *Thioploca* bacteria. □

Received 4 January; accepted 6 March 1995.

- Gallardo, V. A. *Nature* **286**, 331–332 (1977).
- Rosenberg, R. et al. *J. mar. Res.* **41**, 263–279 (1983).
- Henrichs, S. M. & Farrington, J. W. *Limnol. Oceanogr.* **29**, 1–19 (1984).
- McCaffrey, M. A., Farrington, J. W. & Repeta, D. J. *Org. Geochem.* **14**, 61–68 (1989).
- Morita, R. Y., Iturriaga, R. & Gallardo, V. A. *Kiel. Meeresforsch. Sonderb.* **5**, 384–389 (1981).
- Maier, S. & Gallardo, V. A. *Arch. Microbiol.* **139**, 218–220 (1984).
- Gallardo, V. A. *Gayana Zool.* **10**, 3–15 (1963).
- Maier, S. & Gallardo, V. A. *Int. J. Syst. Bact.* **34**, 414–418 (1984).
- Maier, S., Völker, H., Beese, M. & Gallardo, V. A. *Can. J. Microbiol.* **36**, 438–448 (1990).
- Nelson, D. C., Revsbech, N. P. & Jørgensen, B. B. *Appl. Environ. Microbiol.* **52**, 161–168 (1986).
- Sweerts, J. P. R. A. et al. *Nature* **344**, 762–763 (1990).
- Maier, S. & Murray, R. G. E. *Can. J. Microbiol.* **11**, 645–656 (1965).

- Jørgensen, B. B. & Revsbech, N. P. *Appl. Environ. Microbiol.* **45**, 1261–1270 (1983).
- Larkin, J. M. & Strohl, W. R. A. *Rev. Microbiol.* **37**, 341–367 (1983).
- Nelson, D. C., Jørgensen, B. B. & Revsbech, N. P. *Appl. Environ. Microbiol.* **52**, 225–233 (1986).
- Codispoti, L. A. & Christensen, J. P. *Mar. Chem.* **16**, 277–300 (1985).
- Codispoti, L. A. et al. *Science* **233**, 1200–1202 (1986).

ACKNOWLEDGEMENTS. We thank the following participants in the *Thioploca* Cruise, who have all contributed to the research: B. M. Bebout, U. Berninger, Y. Cohen, T. G. Ferdelman, D. Ganzhorn, Y. Helman, G. Herz, M. F. Isaksen, S. Klöser, A. Krack, C. Lee, K. Neumann, S. Pantoja, N. Peter Revsbech and M. Salamanca. We thank Captain J. Boto and his crew and technicians from the Hydrographic and Oceanographic Service of the Chilean Navy for their help, cooperation and enthusiasm onboard *RV Vidal Gormaz* during *Thioploca* Cruise 1994. The study was supported by the Max Planck Society, the University of Concepción, and the FONDECYT 1994 Project.