Supporting information

(A) Experimental conditions of microcosm batch experiments

D. toluolica was grown anaerobically with toluene as sole source of carbon and energy, batch-wise, in a bicarbonate-buffered mineral salt medium for marine sulfate-reducing bacteria containing the following stock ingredients (in g l^{-1}): NaCl, 21; $MgCl_2$, 3; KH_2PO_4, 0.5; NH_4Cl, 0.4; KCl, 0.4; and $CaCl_2$, 0.1. The basal medium (prepared in batches of one liter) was completed by adding the following amounts of stock solutions: 30 ml $NaHCO_3$ (84 g l^{-1}, CO_2-saturated), 1 ml SL-10 trace element solution chelated with EDTA (5.2 mg l^{-1} stock solution), 5 ml vitamin solution (containing, in mg l-1: 4-aminobenzoate, 8; D(+)-biotine, 2; nicotinic acid, 20; Ca-D(+)-pantothenate, 10; pyridoxamine hydrochloride, 30; thiamine dichloride, 20), and 1 ml selenite-tungstate solution (containing, in mg l^{-1}: NaOH, 500; Na_2SeO_3 x 5 H_2O, 3; Na_2WO_4 x 2 H_2O, 4). The sulfate concentration of the basal medium was adjusted to ca. 20 mM by adding the appropriate amount of Na_2SO_4. Finally, the medium was reduced with a few grains (15-20 mg) of sodium dithionite. The pH was adjusted to 7 with HCl (1 M) or NaOH (1 M). To avoid toxic effects, toluene was not added directly to the medium, but dissolved (1-2 % [v/v]) in 2,2,4,4,6,8,8-heptamethylnonane (HMN, Fluka, Germany), which served as an inert hydrophobic phase continuously providing toluene for the culture. Usually, the medium was spiked with 30 ml l^{-1} HMN-toluene mixture. The toluene-metabolizing freshwater enrichment culture Zz 5-7 was cultivated in the same manner as described, except that less NaCl and $MgCl_2$ were added (0.5 g l^{-1} NaCl, 0.5 g l^{-1} $MgCl_2$). Also, a smaller amount of Na_2SO_4 was supplied to set the initial sulfate concentrations of the medium to ca. 12 mM.

Besides the experiments with Na_2SO_4 as the sulfate source, a second set of experiments was conducted using ferrous iron as sulfide scavenger. In those experiments, sulfate was supplied as $FeSO_4$ x 7 H_2O from a 1 M stock solution, to reach final ferrous iron and sulfate concentrations of ca. 17 mM for *D. toluolica* cultures and ca. 12 mM for the enrichment

culture Zz 5-7. No Na_2SO_4 was added into the culture media within these experiments. For the $FeSO_4$ experiments, three different basal media were prepared using waters of different oxygen isotopic composition (-35.5 ‰, -9.0 ‰, +4.5 ‰ VSMOW).

All mentioned solutions were sterilized by filtration or autoclaving and flushed with N_2 (to remove oxygen) before use. The media were subsequently prepared in an anoxic glove-box (gas atmosphere: 95 % nitrogen, 5 % hydrogen; Coy Laboratory Products Inc., USA) to ensure anoxic conditions. For cultivation, 118 ml serum flasks were filled with the respective media and inoculated with 10 % of *D. toluolica* or Zz 5-7, each grown to late exponential phase. The serum flaks were filled almost completely with culture solution and HMN-toluene (remaining gas phase: 1 ml), and closed with teflon-coated butyl rubber stoppers (ESWE Analysentechnik Gera GmbH, Germany). For growth, cultures were incubated statically at 28 °C in the dark. For maintenance, cultures were grown on toluene, stored at 4 °C, and transferred every 8 weeks as described above.

(B) Preparation of sulfate and sulfide for isotope measurement

For sulfur isotope analyses, dissolved sulfide was precipitated as ZnS by adding 10 ml 0.2M $Zn(CH_3COO)_2*2H_2O$ solution (oxygen free, pH 12 adjusted with 25 % NH_3 solution) per 100 ml of sample. Any precipitated ZnS and FeS formed during batch experiments using $FeSO_4$ as sulfate source was collected by filtration through 0.45µm membrane filters. Until further handling, the wet filters with the sulfide precipitate were kept under an oxygen free atmosphere to prevent oxidation, particularly of FeS. After transferring the filters to a distillation apparatus, they were reacted with 6 M hydrochloric acid under permanent nitrogen gas flow. ZnS and FeS was simultaneously liberated as H_2S, again precipitated as ZnS by bubbling the gas flow through a 3% Zn-acetate/NH_3 solution, and subsequently converted to Ag_2S by addition of 0.1 M $AgNO_3$ solution. To recover dissolved sulfate for isotope analyses, the filtrate remaining after removing precipitated sulfide was acidified to a pH of 3 with 0.1M

HCl to remove dissolved inorganic carbon. Sulfate was then precipitated as $BaSO_4$ by adding $BaCl_2$ solution at 70°C. After 12 hours, $BaSO_4$ was collected on an ash-free filter (Blue Ribbon 589[3], Schleicher and Schuell, Germany) that was completely removed by baking the sample at 800°C for two hours. The baking procedure also removed any sample-related organic residue that may otherwise interfere with the oxygen isotopic composition of the sulfate. By preparing and analyzing dissolved sulfate reference materials, the baking procedure was proven not to affect the sulfur and oxygen isotopic composition of prepared sulfate.

For normalizing the $\delta^{34}S$ data, the IAEA-distributed reference materials NBS 127 ($BaSO_4$) and IAEA-S1 (Ag_2S) were used. The assigned values were +20.3 ‰ (CDT) for NBS 127 and -0.3 ‰ (CDT) for IAEA-S1.

The normalization of oxygen isotope data of sulfate was carried out using the reference material NBS 127 with an assigned $\delta^{18}O$ value of +9.3 ‰ (V-SMOW).