

Cryptic Cross-Linkages Among Biogeochemical Cycles: Novel Insights from Reactive Intermediates

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The biogeochemical cycling of major and minor elements in the ocean has direct bearing on the health of the planet and its inhabitants. Reactive intermediates, of both chemical and biological origin, are emerging as important players in these biogeochemical cycles. Due to their rapid production and consumption, these reactive intermediates are short-lived and typically in low concentration. Involvement of these “invisible” species in biogeochemistry may therefore be hidden, or cryptic, with no obvious lingering chemical signature. Here, we highlight reactive intermediates of the oxygen, manganese, and sulfur cycles and how these intermediates are involved in cryptic cross-linkages between marine biogeochemical cycles of global importance.

KEYWORDS: reactive intermediates, biogeochemistry, manganese, sulfur, superoxide, hydrogen peroxide, disproportionation

INTRODUCTION TO REACTIVE INTERMEDIATES

Modern global biogeochemical element cycles are ultimately controlled by the light-driven reduction of CO₂ to form organic matter and O₂ (oxygenic photosynthesis) and the subsequent oxidation of this organic matter coupled to the reduction of O₂, i.e. respiration. The rest is detail; but it's the detail that yields the fascinating diversity of chemistry and life on our planet's surface. Recent chemical and microbiological advances in monitoring the production and consumption of so-called reactive intermediates and the enzymes responsible for their formation have begun to highlight the importance of these short-lived species in microbially mediated processes. These intermediates encompass numerous elements (e.g. O, N, S, Mn) and phases (gas, liquid, mineral) and are involved in myriad microbial activities, ranging from respiration to cell signaling.

Enzymatic reactions often involve the formation of transitory intermediate compounds. Reactive intermediates can also arise from purely chemical (abiotic) reactions: for instance, by the reaction of an end-product with other compounds, either in solution or in the solid-phase. As many of the globally important reduction–oxidation (redox)

processes and cycles may extend over several oxidation states, a large variety of intermediates may form. Many of these intermediates are (bio)chemically reactive; they exist for extremely short periods of time (seconds to days), especially when measured against the geological timescales that are associated with the cycling of many of the major bioactive compounds (e.g. O₂, CO₂, SO₄²⁻, Mn and Fe oxides, N₂ etc.). Low concentrations of dissolved reactive intermediates reflect steady-state conditions where rates of production and consumption are tightly coupled. Perturbations, for example due to bottlenecks at a specific enzyme in

a microbial pathway, or externally due to rapid changes in temperature or some physical disturbance of the environment, may lead to sudden excursions in concentration of the intermediate, but these will shortly return to some low metastable steady-state value.

Although short-lived and in low concentrations, many of these intermediate compounds exist long enough to interact with the surrounding geochemical and biological environment, often in confounding and surprising ways. And they may even be essential to the overall efficiency of other element cycles, despite a lack of evidence that they are, or were ever, present. Due to their rapid production and consumption, the cycling of these “invisible” species may, therefore, be hidden, or cryptic, with no obvious lingering chemical signature. Indeed, despite their relatively short lifetimes, cryptic processes involving reactive intermediates can have a disproportionately large influence on the cycling of several elements (such as the micronutrient iron and contaminant chromium), on the degradation of organic carbon, and on the activity of microbial populations.

Here, we provide a brief glimpse into the biogeochemistry and geomicrobiology of selected intermediates (of O, Mn and S), taking examples from processes ranging from oxidizing to highly reducing sulfidic environments. While there is a growing appreciation for cryptic cycles in lacustrine and terrestrial systems, our current knowledge of reactive intermediates has emerged primarily from investigations in marine sediments and waters. Thus, for our purposes, marine systems will serve as the focus.

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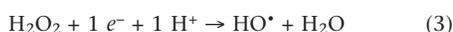
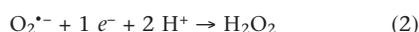
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REACTIVE INTERMEDIATES IN THE OXYGEN, MANGANESE, AND SULFUR CYCLES

Reactive oxygen species: friend or foe?

Reactive oxygen species (ROS) are most simply defined as short-lived oxygen-containing molecules with half-lives ($t_{1/2}$) in aquatic systems that range from nanoseconds to a few hours. The most common forms of ROS in natural systems are hydrogen peroxide (H_2O_2), superoxide ($\text{O}_2^{\bullet-}/\text{HO}_2^{\bullet}$), hydroxyl radical (HO^{\bullet}), singlet oxygen ($^1\text{O}_2$), and carbonate radical ($\text{CO}_3^{\bullet-}$). These ROS are commonly found at picomolar to micromolar levels in environmental systems, where they are generated by a number of (photo) chemical and biological processes. The formation of ROS within aqueous systems occurs via sequential one-electron transfer reactions (Fridovich 1998). For instance, the ROS superoxide, hydrogen peroxide, and hydroxyl are the intermediates of the sequential one-electron reduction of molecular oxygen (O_2) to water (H_2O):



Reactive oxygen species are, therefore, inevitable by-products of oxygen redox chemistry. The oxygen cycle is restored through the oxidation and ultimate splitting of water by photosynthetic organisms forming molecular oxygen.

In fact, all aerobic organisms (including you!) form ROS inside their cells (intracellular) as a by-product of respiration or photosynthesis. Oxidative stress involves the accumulation of these ROS to toxic levels within the cell, where the ROS alter the redox state of critical enzymes or destroy essential biomolecules, such as membranes and proteins. Since ROS other than H_2O_2 have limited ability to passively diffuse or be actively transported across biological membranes, organisms must deal with these toxic molecules internally. Thus, to minimize cellular damage, and evade death, levels of intracellular ROS are highly regulated through the production of molecules and enzymes that specifically target and destroy oxygen radicals (termed antioxidants).

Nevertheless, ROS, particularly superoxide, is also produced extracellularly (outside the cell) by a broad range of microorganisms, including fungi, phytoplankton, and bacteria (Rose 2012). In eukaryotes, this extracellular production is known to mediate a number of essential physiological processes, including cell development, signaling, and nutrient acquisition. On the flip side, superoxide is produced as a means of microbial warfare. It is used, for instance, as a virulence factor by some pathogenic organisms. In fact, excessive extracellular superoxide production by the toxic phytoplankton *Chattonella* has been implicated in massive fish kills associated with red tides. Despite the widespread ability of organisms to produce extracellular superoxide, the full repertoire of reasons for this process remains unknown. What is clear is that microorganisms have a finely tuned redox environment around the cell where a regulated balance between ROS production and decay is required in order to sustain healthy ROS levels.

Most investigations of ROS distributions and production rates within the environment have focused on hydrogen peroxide and/or superoxide within marine systems. Concentrations of these ROS are typically highest in sunlit surface waters. Although the historical perception has been that photochemical reactions represent the

dominant source of these ROS, photosynthetic organisms (algae, cyanobacteria, diatoms) are now considered important, if not dominant, contributors within sunlit systems (Rose 2012). Levels of hydrogen peroxide and superoxide in surface waters, however, only inconsistently show a light dependence, and production in the dark (both at night and below the photic zone) has been widely observed (Diaz et al. 2013). This dark production is attributed, in part, to O_2 reacting with reduced labile redox-active compounds, such as sulfide and organic compounds. Dark production has also recently been attributed to superoxide produced by aerobic bacteria, which include a broad spectrum of taxonomic groups from various ecological niches (Diaz et al. 2013). Thus, biogenic ROS are unquestionably important reactive intermediates in environmental systems, including the dark biosphere—the 95% of our global habitat not touched by light.

Manganese(III): The Overlooked Oxidation State

Manganese (Mn), as one of the most abundant redox-active transition metals in the Earth's crust and has been an essential bioelement throughout the history of life on Earth. It is used for a variety of enzymatic reactions and biological functions in Bacteria, Archaea and Eukarya because of its ability to cycle between 3 primary oxidation states, Mn(II), Mn(III) and Mn(IV). Well-known for its role in oxygen-producing photosynthesis, Mn is also essential as a key metal cofactor for many other cellular functions and is used as an intracellular antioxidant. In fact, Mn is an essential cofactor in Mn-containing superoxide dismutase (Mn-SOD), a critical enzyme required for mitigating oxidative stress in most oxygen-based life.

Both the oxidative and reductive parts of the Mn cycle are largely driven by microbial activities, either through direct catalysis or indirectly through the production of chemical oxidants (e.g. superoxide) or reductants (e.g. sulfide). The intermediate Mn(III) results from a variety of one-electron transfer reactions during either Mn(II) oxidation or Mn(IV) oxide reduction, irrespective of whether this process is directly catalyzed by enzymes or occurs indirectly. The Mn(III) produced is available to react or form complexes with other chemicals in the environment. Even in cases where a single enzyme is capable of oxidizing Mn(II) to Mn(IV), this occurs in one-electron steps with Mn(III) still formed as a labile intermediate.

Historically, Mn(III) has generally been regarded as environmentally unimportant because it is extremely unstable in solution and quickly undergoes chemical disproportionation, i.e. the reaction in which a compound of intermediate oxidation state decomposes into both its more oxidized and reduced forms. In this case, Mn(III) disproportionates to Mn(II) and Mn(IV). Despite the assumption that the natural occurrence of soluble Mn(III) in aqueous systems is rare, substantial levels of soluble Mn(III) complexes have more recently been observed in suboxic marine waters where O_2 and H_2S were undetectable (Trouwborst et al. 2006; Schnetger and Dellwig 2012). Furthermore, soluble Mn(III) complexes have now been measured in sedimentary systems where Mn(III) accounted for up to 90% of the total dissolved Mn pool within hemipelagic sediments (Madison et al. 2013). The composition, reactivity, and fate of the detected and quantified Mn(III) complexes are not yet known but will likely vary as a function of the chemical and microbial framework.

Sulfur Intermediates: Lubricating the Biogeochemical Element Cycles

Cycling between the globally important pools of oxidized and reduced sulfur in the forms of sulfate (SO_4^{2-}) and the mineral pyrite (FeS_2) helps to balance the redox state of the ocean and atmosphere. The overall driver in the sulfur cycle is microbially mediated sulfate reduction to sulfide (H_2S). In the absence of oxygen, sulfate-reducing bacteria use the oxidizing power contained in dissolved sulfate to respire detrital organic matter to CO_2 . In near-surface sediments, a vigorous, and bewildering, series of abiotic and microbial oxidation reactions operate. Only a small fraction of the sulfide produced via sulfate reduction becomes permanently buried as pyrite, and a wide variety of inorganic sulfur compounds are produced over the six intermediate oxidation states between sulfate and sulfide (Fig. 1).

bacteria closely related to known sulfate-reducing bacteria can harness the small amount of energy available in the disproportionation of sulfur intermediates—thiosulfate or elemental S (S^0)—simultaneously producing the higher and lower oxidation state compounds, sulfate and hydrogen sulfide. Bacterial disproportionation, which can be thought of as an inorganic S fermentation process, is considered to be a key process in driving the oxidative side of the sulfur cycle, especially in sedimentary environments where elemental sulfur may form.

What makes sulfur in its elemental oxidation state particularly fascinating is its ability to concatenate: that is, to form chains and rings of continuous S atoms, such as the eight-membered S ring, which is the most common form of elemental sulfur. In its common form, solid-phase elemental sulfur is rather unreactive. Yet, a diverse range of bacteria actually store intracellular elemental sulfur

as a bacterial sulfur “battery” for energy-yielding reduction and oxidation reactions. In the presence of dissolved sulfide, elemental sulfur reacts within minutes to form polysulfides—reactive, unprotonated chains of elemental sulfur atoms ranging from 2 to 9 atoms, with negative charges distributed at their sulfidic ends (see Fig. 1). Individual polysulfides (such as S_4^{2-} , S_5^{2-} , and S_6^{2-}) can reach micromolar concentrations in sulfidic marine environments (Kamyshny and Ferdelman 2010). Such polysulfides are thought to be involved in a number of geochemically important reactions, including reactions that “cross-link” organic compounds in petroleum-bearing formations and in the globally important reaction to form pyrite (Rickard and Luther 2007). Polysulfides also arise during sulfide oxidation reactions. For example, a surface-attached disulfide has

been observed during the reaction of dissolved sulfide with iron oxides; thus, disulfide plays a critical role in the formation of pyrite (Wan et al. 2014). Recent experiments also show that hydrodisulfide (HS_2^-) serves as a substrate for microbial disproportionation even under sulfidic conditions (Milucka et al. 2012).

CROSS-LINKAGES AMONG KEY REACTIVE INTERMEDIATES AND ELEMENTAL CYCLES

Reactive intermediates are the essential, yet often invisible, links between elemental cycles (Fig. 2). The impact of these coupled cycles is pervasive and influences a plethora of environmental processes, including, but not limited to, carbon degradation, contaminant transport, photosynthetic activity, and microbial metabolism. At the root of many of these linkages are intermediates of the O, Mn, Fe, N, and S cycles, a subset of which are discussed below.

The Broad Reach of Reactive Oxygen Species

Reactive oxygen species (ROS) are key players in the degradation of carbon, the bioaccessibility of metal micronutrients, and the cycling of other metals and toxins. For instance, superoxide and hydrogen peroxide are capable of oxidizing and/or reducing a number of metals, including copper (Cu), iodine (I), and mercury (Hg). Importantly, superoxide also has the ability to reduce Fe(III) to Fe(II). Because Fe is an essential nutrient that limits photosynthesis in vast regions of the ocean, superoxide-mediated reduction and the subsequent release of Fe from strong Fe(III)-ligands has been suggested as an important process controlling biological activity in the surface ocean (Rose

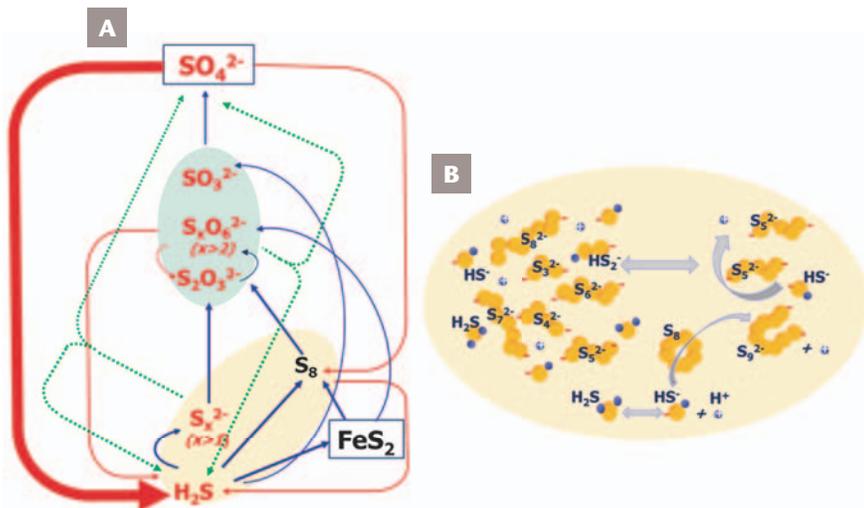


FIGURE 1 (A) Microbial sulfate (SO_4^{2-}) reduction to sulfide (H_2S ; thick red line) is the main driver in the sulfur cycle and the link between globally important sulfate and sulfide (as FeS_2) pools. Key reactive S compounds of intermediate oxidation state include compounds within the polysulfide-sulfur system (S_n^{2-} - S_8 ; yellow shading) and sulfur oxyanions ($\text{S}_x\text{O}_y^{2-}$; green shading), and whose concentrations are regulated by reduction (red), oxidation (blue) and disproportionation (stippled green) reactions. Red and black type indicate dissolved and solid phase S species respectively. (B) An expanded version of the polysulfide-sulfur system (yellow shading in Fig. 1A) illustrates the reaction between dissolved hydrogen sulfide (H_2S and HS^-) and elemental sulfur rings (S_8) and the subsequent formation of a metastable equilibrium between dissolved hydrogen sulfide, polysulfides (S_n^{2-}) and elemental sulfur.

These intermediate oxidation-state S compounds are typically present in low micromolar concentrations that, under the right conditions, can be reduced or oxidized by microbes. Any given sulfur atom may actually undergo several cycles of oxidation and reduction among these intermediate states before returning to the sulfate pool. For instance, within oxidized marine surface sediments, tetrathionate ($\text{S}_4\text{O}_6^{2-}$) may form during microbially mediated oxidation of reduced sulfide or pyrite. Although tetrathionate is a common oxidation product during sulfide oxidation, it is rarely detected. Rather, the capacity of other, as yet unidentified microorganisms, to completely reduce it within hours to thiosulfate ($\text{S}_2\text{O}_3^{2-}$) appears to be widespread throughout surface oxidizing environments, thereby keeping tetrathionate concentrations well below micromolar concentrations (Zopfi et al. 2004). Bacterial sulfur disproportionation, a process discovered in the early 1990s (Thamdrup et al. 1993), further complicates this internal (re)cycling of sulfur. In particular, some

elemental sulfur and polysulfides. The (re)generation and bacterial disproportionation of these sulfur intermediates allows for enough sulfate to be produced to sustain bacterial sulfate reduction below the sulfate–methane transition zone.

Microbes may facilitate the reaction between hydrogen sulfide and metal oxides to produce the reactive sulfur intermediates that drive this deep cryptic sulfur cycle. For example, intermediate sulfur species are used in electron shuttling in bacterially driven reduction of Fe(III) oxides (Flynn et al. 2014; Lohmeyer et al. 2014). Sulfur compounds with an intermediate oxidation state may also be produced during anaerobic oxidation of methane. In an enrichment of methane-oxidizing archaea and sulfate-reducing bacteria elemental sulfur and polysulfides accumulate. Milucka et al. (2012) have suggested that the observed elemental S is a product of a novel pathway for sulfate reduction performed by the Archaea to produce elemental S, thereby providing an alternate source of S intermediates for cryptic S cycling. Thus, the interplay between carbon respiration, methane oxidation, iron oxides, and the deep cryptic sulfur cycle may be even more complex and challenging than previously imagined.

TRACKING ELUSIVE REACTIVE INTERMEDIATES

Measuring reactive intermediates is intrinsically difficult due to their characteristically low abundance and short lifetimes. Some compounds (e.g. polysulfides), which may exist in a dynamic metastable steady state, immediately respond to even minor perturbations in their surrounding chemical environment. For the particularly unstable or short-lived species (e.g. superoxide), direct measurement of in situ concentrations is not feasible and, as such, there are many environments where their presence and environmental relevance has not yet been explored. To detect and quantify these species, a number of conventional and novel techniques are utilized that encompass a wide range of approaches, including targeted chemical assays, electrochemical methods, high-resolution microscopies, and both bulk and spatially resolved spectroscopies. An exhaustive review of these options is beyond the scope of this article, and instead we provide a few examples of some of the more recent technological or methodological advances made in measuring reactive intermediates in aqueous and sedimentary systems.

The detection and quantification of aqueous- and particle-associated reactive intermediates is, as mentioned, hindered by their short lifetime and the difficulty of preserving these reactive phases without significant modification. The options are limited, but directly making measurements in situ is one approach. In particular, cyclic voltammetry has provided great insight into the speciation and distribution of sulfur (e.g. nanocrystalline elemental S) (Boyd and Druschel 2013) and of Mn intermediates (Trouwborst et al. 2006) in various environmental and microbial systems. Furthermore, measurements that are made at the tip of a microsensor can provide microscale resolution of these intermediates in situ in active microbial cultures or along spatial gradients in complicated matrices.

For solid-phase intermediates, synchrotron-based techniques have provided an attractive and powerful approach because the X-rays are highly intense and are tunable such that each element can be individually interrogated with high sensitivity within complex, even hydrated, matrices (see Templeton and Knowles 2009 for examples).

New capabilities in coupling X-ray microscopy and absorption spectroscopy have allowed for unprecedented progress in high-resolution mapping of short-lived elemental and mineral species within microbial and natural samples. Recent advances in confocal laser Raman spectroscopy have helped identify and map reactive intermediates at the single cell level. Over the past decade, these approaches have provided a greatly improved understanding of sulfur metabolism and storage in microorganisms: for instance, by revealing the importance of polysulfides as short-lived intermediates in the metabolism of sulfur (Fig. 3) (Berg et al. 2014).

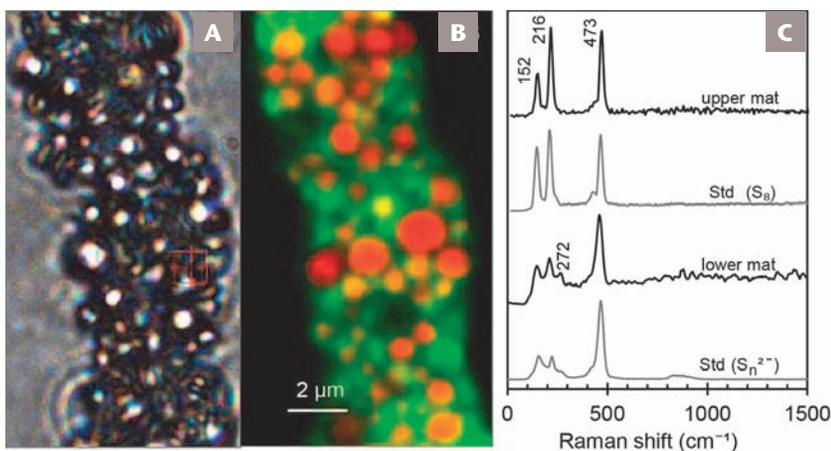


FIGURE 3 Confocal Raman spectroscopy has tremendous utility for mapping intermediate sulfur species at the cellular level. (A) An optical image of the sulfur-oxidizing bacterium *Beggiatoa*, cultured from a microbial mat. (B) Corresponding Raman map of the bacterium in 3A showing the sulfur peak (yellow/red) and autofluorescence of the cell (green). (C) Representative point spectra of the sulfur inclusions in the bacterium as compared to standards of polysulfide (Std S_n^{2-}) and cyclooctasulfur (Std S_8). IMAGES FROM BERG ET AL. (2014) WITH PERMISSION OF THE AMERICAN SOCIETY FOR MICROBIOLOGY.

In addition, isotopic approaches are particularly well suited to revealing the dynamics of reactive intermediates. The isotopic signatures of natural abundance N and S oxyanions, including key information in the O isotopic composition, have been used to confirm rapid recycling and turnover of intermediate nitrite and sulfite species in a variety of systems (e.g. Casciotti and McIlvin 2007). Labeling with both stable and radioactive isotopes has also yielded key information on the fate of reactive intermediates. There now exists the possibility to image and quantify cellular uptake of isotopically labeled atoms (e.g. ^{34}S or ^{15}N) that have been assimilated into microbial cells using nanoscale secondary ion mass spectrometry (nanoSIMS) and to link the uptake directly with the identity of a given individual cell (Musat et al. 2008).

Major advances, however, are not restricted to the development of sophisticated instrumentation. In fact, recent discoveries of the widespread presence of Mn(III) in aqueous and sedimentary systems is attributed to the development of a novel spectrophotometric kinetic method that uses a chemical probe selective to Mn (Madison et al. 2013). This past decade has seen a surge of superoxide measurements within natural and microbial systems that were all made possible through recent developments of new, highly selective, and sensitive chemical probes (see review in Burns et al. 2012).

The emergence of the “omics” revolution (see Dick and Lam 2015 this issue) has provided valuable insight and continued

promise in the unveiling of cryptic biogeochemical cycles and their underpinning reactive intermediates. Specifically, in systems where these reactive species are too elusive for detection, the identification and quantification of genetic markers involved in either their formation or destruction provides evidence of their presence. For instance, the detection of enzymes involved in the reduction of sulfate and the concomitant oxidation of reduced and intermediate sulfur species (such as thiosulfate) revealed a cryptic sulfur cycle in suboxic surface ocean waters (Canfield et al. 2010). This was a key discovery, especially when considering that the defining chemical indicators (e.g. sulfide) could not be measured due to their rapid production and decay.

INTO THE FUTURE: PEERING INTO THE BIOGEOCHEMICAL MICROSCOPE

Over the coming decades, the scientific community will continue to be called upon to develop new strategies and methodologies to overcome the challenges associated with investigating low abundance, short-lived, but vitally important reactive intermediates in the natural environment. There is a specific need not only to detect and quantify these short-lived species but also to quantify their corresponding rapid production and decay rates so that they

can be integrated into biogeochemical models. Advances in our technological and methodological approaches will no doubt lead to discoveries of new reactive intermediates that are central to a myriad of biogeochemical cycles and will reveal the broader environmental relevance for intermediates already known. The difficulty of this challenge is particularly amplified when considering the need to measure short-lived chemical species in systems that are not easily accessible, the deep sea being just one.

Despite, or perhaps because of, the challenge of investigating these elusive species, it is an exciting time to be disentangling cryptic biogeochemical cycles and to witness the emergence of reactive intermediates as key players in biogeochemistry.

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REFERENCES

- Berg JS, Schwedt A, Kreuzmann A-C, Kuypers MMM, Milucka J (2014) Polysulfides as intermediates in the oxidation of sulfide to sulfate by *Beggiatoa* spp. *Applied and Environmental Microbiology* 80: 629-636
- Boyd ES, Druschel GK (2013) Involvement of intermediate sulfur species in biological reduction of elemental sulfur under acidic, hydrothermal conditions. *Applied and Environmental Microbiology* 79: 2061-2068
- Burns JM and 11 coauthors (2012) Methods for reactive oxygen species (ROS) detection in aqueous environments. *Aquatic Sciences* 74: 683-734
- Canfield DE and 7 authors (2010) A cryptic sulfur cycle in oxygen-minimum-zone waters off the Chilean Coast. *Science* 330: 1375-1378
- Casciotti KL, McIlvin MR (2007) Isotopic analyses of nitrate and nitrite from reference mixtures and application to Eastern Tropical North Pacific waters. *Marine Chemistry* 107: 184-201
- Diaz JM and 5 coauthors (2013) Widespread production of extracellular superoxide by heterotrophic bacteria. *Science* 340: 1223-1226
- Flynn TM, O'Loughlin EJ, Mishra B, DiChristina TJ, Kemner KM (2014) Sulfur-Mediated Electron Shuttling During Bacterial Iron Reduction. *Science* 344: 1039-1042
- Fridovich I (1998) Oxygen toxicity: A radical explanation. *Journal of Experimental Biology* 201: 1203-1209
- Hansard SP, Easter HD, Voelker BM (2011) Rapid reaction of nanomolar Mn(II) with superoxide radical in seawater and simulated freshwater. *Environmental Science and Technology* 45: 2811-2817
- Hansel CM, Zeiner CA, Santelli CM, Webb SM (2012) Mn(II) oxidation by an ascomycete fungus is linked to superoxide production during asexual reproduction. *Proceedings of the National Academy of Sciences of the United States of America* 109: 12621-12625
- Holmkvist L, Ferdelman TG, Jørgensen BB (2011) A cryptic sulfur cycle driven by iron in the methane zone of marine sediment (Aarhus Bay, Denmark). *Geochimica et Cosmochimica Acta* 75: 3581-3599
- Kamyshny A Jr, Ferdelman TG (2010) Dynamics of zero-valent sulfur species including polysulfides at seep sites on intertidal sand flats (Wadden Sea, North Sea). *Marine Chemistry* 121: 17-26
- Learman DR, Voelker BM, Vazquez-Rodriguez AI, Hansel CM (2011) Formation of manganese oxides by bacterially generated superoxide. *Nature Geoscience* 4: 95-98
- Learman DR, Voelker BM, Madden AS, Hansel CM (2013) Constraints on superoxide mediated formation of manganese oxides. *Frontiers in Microbiology* 4: doi: 10.3389/fmicb.2013.00262
- Lohmayer R, Kappler A, Lösekann-Behrens T, Planer-Friedrich B (2014) Sulfur species as redox partners and electron shuttles for ferrihydrite reduction by *Sulfurospirillum deleyianum*. *Applied and Environmental Microbiology* 80: 3141-3149
- Luther GW III, Sundby B, Lewis BL, Friedel PJ, Silverberg N (1997) Interactions of manganese with the nitrogen cycle: Alternative pathways to dinitrogen. *Geochimica et Cosmochimica Acta* 61: 4043-4052
- Madison AS, Tebo BM, Mucci A, Sundby B, Luther GW III (2013) Abundant porewater Mn(III) is a major component of the sedimentary redox system. *Science* 341: 875-878
- Milucka J and 9 coauthors (2012) Zero-valent sulphur is a key intermediate in marine methane oxidation. *Nature* 491: 541-546
- Musat N and 9 coauthors (2008) A single-cell view on the ecophysiology of anaerobic phototrophic bacteria. *Proceedings of the National Academy of Sciences of the United States of America* 105: 17861-17866
- Rickard D, Luther GW III (2007) Chemistry of iron sulfides. *Chemical Reviews* 107: 514-562
- Rose AL (2012) The influence of extracellular superoxide on iron redox chemistry and bioavailability to aquatic microorganisms. *Frontiers in Microbiology* 3: doi: 10.3389/fmicb.2012.00124
- Schnetger B, Dellwig O (2012) Dissolved reactive manganese at pelagic redox-clines (part I): A method for determination based on field experiments. *Journal of Marine Systems* 90: 23-30
- Templeton A, Knowles E (2009) Microbial transformations of minerals and metals: Recent advances in geomicrobiology derived from synchrotron-based X-ray spectroscopy and X-ray microscopy. *Annual Review of Earth and Planetary Sciences* 37: 367-391
- Thamdrup B, Finster K, Hansen JW, Bak F (1993) Bacterial disproportionation of elemental sulfur coupled to chemical reduction of iron or manganese. *Applied and Environmental Microbiology* 59: 101-108
- Trouwborst RE, Clement BG, Tebo BM, Glazer BT, Luther GW III (2006) Soluble Mn(III) in suboxic zones. *Science* 313: 1955-1957
- Wan M, Shchukarev A, Lohmayer R, Planer-Friedrich B, Peiffer S (2014) Occurrence of surface polysulfides during the interaction between ferric (hydr)oxides and aqueous sulfide. *Environmental Science and Technology* 48: 5076-5084
- Wuttig K, Heller MI, Croot PL (2013) Pathways of superoxide (O₂⁻) decay in the Eastern Tropical North Atlantic. *Environmental Science and Technology* 47: 10249-10256
- Yakushev EV and 5 coauthors (2007) Analysis of the water column oxic/anoxic interface in the Black and Baltic seas with a numerical model. *Marine Chemistry* 107: 388-10
- Zopfi J, Ferdelman TG, Fossing H (2004) Distribution and fate of sulfur intermediates—sulfite, tetrathionate, thiosulfate, and elemental sulfur—in marine sediments. In: Amend JP, Edwards KJ, Lyons TW (eds.) *Sulfur Biogeochemistry—Past and Present*. Geological Society of America Special Paper 379: 97-116