Challenges to the bio(geo)chemist

Marine gases

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Gases are produced and consumed by numerous biological processes in the oceans and some volatiles are readily exchanged with the atmosphere where they can affect our climate. They can be waste products but they are also fundamental as nutrient and energy sources and terminal electron acceptors in respiration, and can facilitate communication and interactions via diffusive signalling. Here we will introduce some major gases and mention a few more enigmatic volatiles before focussing on the bio(geo)chemistry of dimethyl sulfide (DMS), ethene and isoprene, three important gases that are currently at the forefront of our research.

Key words: *dimethyl sulfide (DMS), ethene, infochemistry, isoprene, marine gas, volatile organic compound (VOC)*

Major gases

Oxygen (O_2) is probably the most-recognized example. Since half of global photosynthetic productivity occurs in the ocean, every second breath we take is provided by phototrophic organisms including oxygenic bacteria and algae. Because photosynthesis utilizes carbon dioxide (CO_2), the oceans are also vital for the removal of this greenhouse gas from the atmosphere and effectively dampen man-made warming. In the vast central gyres of the world's oceans, such as the Sargasso Sea, the inorganic macronutrients ammonium (NH_4^+) and nitrate (NO_3^-) are so sparse that these areas are often referred to as the 'ocean deserts'. This is where growth is fuelled by certain cyanobacteria that can utilize atmospheric nitrogen gas (N_2) to produce 'fixed' nitrogen as ammonium that is vital for the biosynthesis of proteins and nucleic acids within the entire marine food web. The removal of N_2 from the atmosphere in this process is balanced by the return of nitrogen gas via the denitrification of nitrate and the release of nitrous oxide (N_2O), a powerful greenhouse gas with a warming potential nearly 300 times greater than that of CO_2. Further warming potential is released from the oceans in the form of methane (CH_4) that can be produced from the breakdown of organic matter in anaerobic environments such as fish guts or organic-rich sediments. Luckily, a consortium of bacteria is also responsible for removing about 80% of oceanic methane[1] so that it never enters the atmosphere where its global warming potential is 25 times higher than CO_2.

Marine gases close the biogeochemical cycling of many elements

Next to some of the major gases introduced above, the oceans also release a suite of 'trace' gases that may be less abundant, but can be intricately linked with human health. A combination of agriculture and erosion can rapidly remove trace elements from soil that are washed downriver and accumulate in the sea. One example is iodine, an essential micronutrient for the biosynthesis of the animal hormone thyroxine. The uptake of iodine by seaweeds and phytoplankton, and subsequent biogenic release of iodine-containing volatile organic compounds (VOCs) such as iodomethane (CH_3I) is vital for the return of this important trace nutrient, via the atmosphere and wet or dry deposition to our soils and hence into our diet. In the past, iodine deficiency in areas remote from the sea such as the Alps caused endemic goitre – a health risk that was effectively removed with the introduction of synthetic fertilizers in agriculture and iodinated salt in food preparation.

Other gases can be rather damaging. The episodic 'eruptions' of toxic hydrogen sulfide (H_2S) in the upwelling region along the coast of Namibia are well documented and were known to the colonial settlers as 'The Black Painter' that arrives through the air and gave their precious silverware a black coating with metal sulfides. This gas results from the anoxic degradation of organic material that is massively produced in the nutrient-rich surface waters and then trapped in marine sediments. Fish kills often occur alongside these events and provide a feast for birds, whereas lobsters flee to land from toxic waters and are a welcome addition to the dinner tables of local residents.

Numerous other marine gases are currently being studied, often with reference to their warming potential in the atmosphere and their role in climate regulation. In the following sections, we highlight three gases that are the focus of our current investigations and provide opportunities for deepening our understanding of gases in the biochemistry, ecology and physiology of marine organisms.

Marine gases are integral to the functioning and health of marine eco-systems and connect across biological processes at various scales (Image: Glynn Gorick)

DMS and the 'smell of the sea'

Although often not recognized by its name, dimethyl sulfide [DMS; $(CH_3)_2S$] is a key marine gas that is known by virtually all human beings that ever ventured to the seashore as the 'smell of the sea'. It was only accepted as the missing link in the biogeochemical cycling of sulfur between the ocean and land after James Lovelock, the 'father of the Gaia Hypothesis', returned with the first oceanographic measurements of this gas from research cruises to the south of England and along a south-north transect in the Atlantic in 1972[2]. This established DMS as the natural sulfur compound which provides a vehicle for the transfer of sulfur from the sea through the air to the land surface originally assigned by biogeochemists to H_2S. Lovelock was further involved in linking the oceanic release of DMS and the formation of clouds in the remote marine atmosphere that we now refer to as the 'CLAW hypothesis' after the initials of the authors of a seminal publication, Charlson, Lovelock, Andreae and Warren, in 1987[3]. Since then, much research focused on the sources and relevance of atmospheric DMS, the conversion of DMS into sulfate aerosols, and their role in Earth's albedo and climate.

Despite its importance for sulfur biogeochemistry and climate, surprisingly little is known about 'why' and 'how' the estimated 54.5 Tg of DMS that transfer annually from the oceans to the atmosphere are produced. Research on the biochemistry of DMS was gathering new momentum with the publication of the first molecular genetic evidence on its production in bacteria[4]. Since then, two metabolic pathways for the production of DMS from the secondary metabolite dimethylsulfoniopropionate (DMSP) have been identified in various bacteria and a fungus, including the

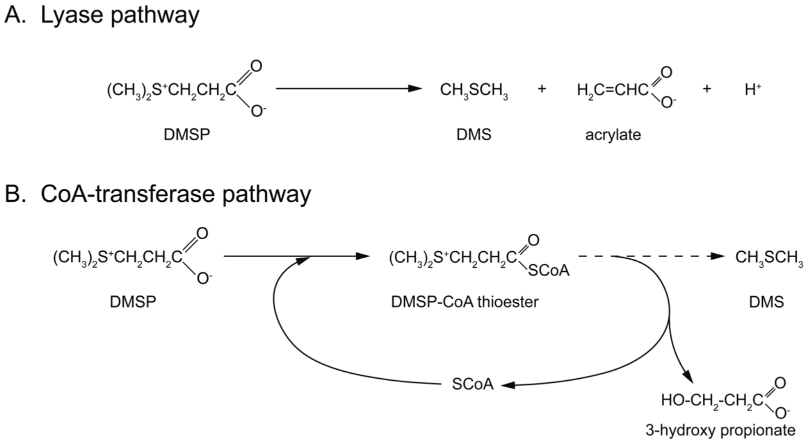

Figure 1. Pathways for DMSP-dependent DMS production. (a) 'Lyase pathway' described in fungi and bacteria, and suggested for algae; (b) 'Co-A transferase pathway' in bacteria (modified from Todd et al.[4])

original 'lyase' pathway that cleaves DMSP to equimolar concentrations of DMS, acrylate and a proton (Figure 1).

Discovering and describing the proteins involved in making DMSP and DMS in eukaryotic algae is the next challenge for the marine biochemist. Initial experiments show direct production of DMS in seaweeds and phytoplankton, but, so far, hard biochemical evidence for the existence of this metabolic pathway is lacking. There is also the possibility that DMS can be produced enzymatically or abiotically from naturally occurring non-DMSP dimethylsulfonium compounds including gonyauline, *cis*-2-(dimethylsulfonio)cyclopropanecarboxylate, and gonyol, 3S-5-dimethylsulfonio-3-hydroxylpentanoate, two secondary metabolites in *Gonyaulax polyedra* and other phytoplankton dinoflagellates. However, the abundance of such alternative DMS precursors and their contribution to the global production of DMS is currently unknown.

Marine gases as infochemicals?

When attempting to unravel the biochemical pathways for DMS production, it may also be useful to address the ecology and physiology of DMS and other gases to gain improved insight into their biological function in nature. Nitric oxide (NO) is a well known messenger gas that serves as an 'infochemical' in cell–cell communication in many organisms. It is possible that some of the marine gases described here could also be involved in conveying information between different cells or from one organism to another. Several mechanisms govern the chemical communication between planktonic predators and their prey, including chemical attraction via membrane-bound receptors for cell-surface prey recognition, motility in response to dissolved infochemicals and antipredator proteins that inhibit phagocytosis[5]. Numerous aquatic infochemicals have been recognized 'in principle', but their structural identity and biochemical activity is in many cases unknown[6]. DMS provides a notable exception to this, since several studies suggest that it provides directional information about the presence of prey. The oceans are a structured, but very patchy, environment, and organisms have evolved various behavioural and sensory mechanisms to exploit areas of high biological productivity with abundant prey. For example, African penguins (*Spheniscus demersus*) and storm petrels (e.g. *Oceanites oceanicus*) use DMS as a directional olfactory cue in their search for food such as krill (*Euphausia superba*) that is often associated with high DMS production when grazing on DMSP-containing phytoplankton. DMS triggers search

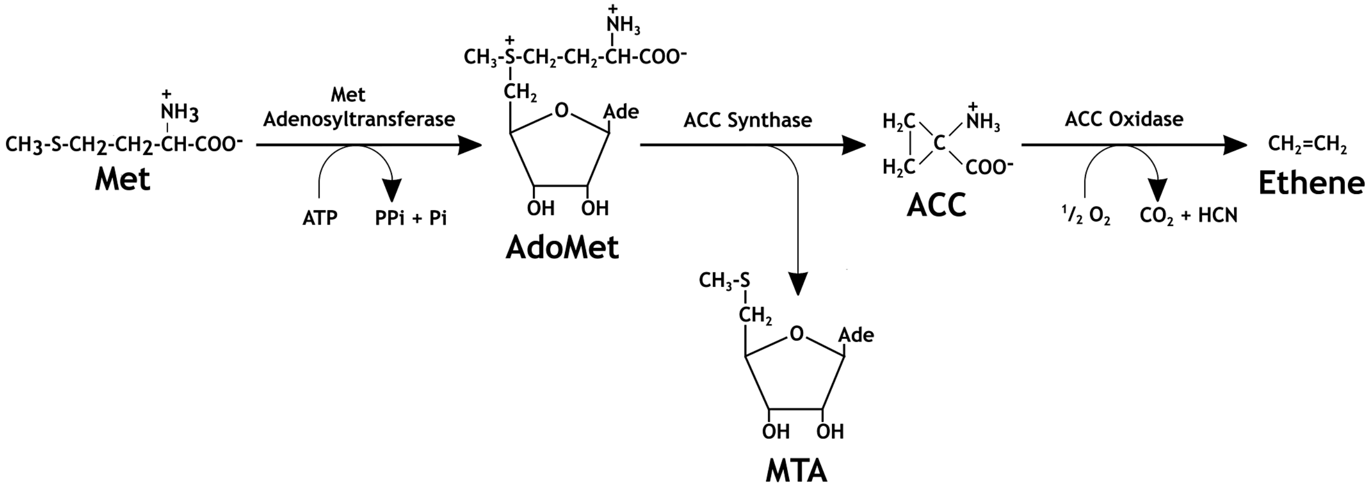

Figure 2. Biosynthesis of ethene via the 1-aminocyclopropane-1-carboxylic acid (ACC) pathway in terrestrial plants and algae. Abbreviations: Met, methionine; AdoMet, *S*-adenosyl-L-methionine; Ade, adenosine; Acc, 1-aminocyclopropane-1-carboxylic-acid; MTA, 5′-methylthioadenosine (modified after Plettner et al.[11]).

behaviour in copepods[7] and, recently, DMS was shown to attract unicellular planktonic organisms[8]. Considering that DMS may provide information on the presence and location of prey is fascinating and provides a new angle to research the ecology of DMS.

The examples above provide a first glimpse into the potential for gases to mediate trophic interactions between one predator and its prey. In addition, gases can also have a role in mediating interactions between several trophic levels. Numerous examples exist on the role of gases in mediating tritrophic or multitrophic interactions between terrestrial plants, herbivores and carnivores. Once a plant is under attack by a herbivore, gases released into the environment can attract carnivores that assist the plant in reducing the grazing pressure. DMS may be involved in such multitrophic interactions in the sea[5] and it is not surprising that algae with a high potential for DMS production are generally very successful in establishing large populations.

The search for other 'multipurpose' gases: ethene and isoprene

Research into terrestrial plants may help to identify further suitable infochemicals that could assist in deciphering chemical communication in the sea. One such example is the gaseous plant hormone ethene (ethylene; $CH_2{=}CH_2$) that is important for overall plant development since it can regulate the timing of fruit ripening and the production of protective plant pigments[9]. It has even been shown to provide a 'language' for the communication between individual neighbours: plants can react to relatively low concentrations of ethene produced in their vicinity and invest more resources into growth to outcompete their neighbours for access to light[10]. Our own research shows that seaweeds use the same metabolic pathway for ethene production as terrestrial plants[11] (Figure 2), but what is the purpose of this gas in seaweeds? Could the development of reproductive cells be regulated by ethene or does it assist with adjusting to the correct level of protective pigments to fine-tune the photosynthetic machinery? Ethene can easily diffuse across biological membranes and could facilitate cell–cell or even plant–plant communication. In the latter case, one may think that the surrounding water would easily remove infochemical gradients by dilution; however, the diffusivity of gases in water is orders of magnitudes lower in comparison with the atmosphere. If ethene is a suitable infochemical for terrestrial plants, it is likely that it works for marine plants as well.

Isoprene [2-methyl-1,3-butadiene; $CH_2{=}C(CH_3)CH{=}CH_2$] is produced by many terrestrial plants

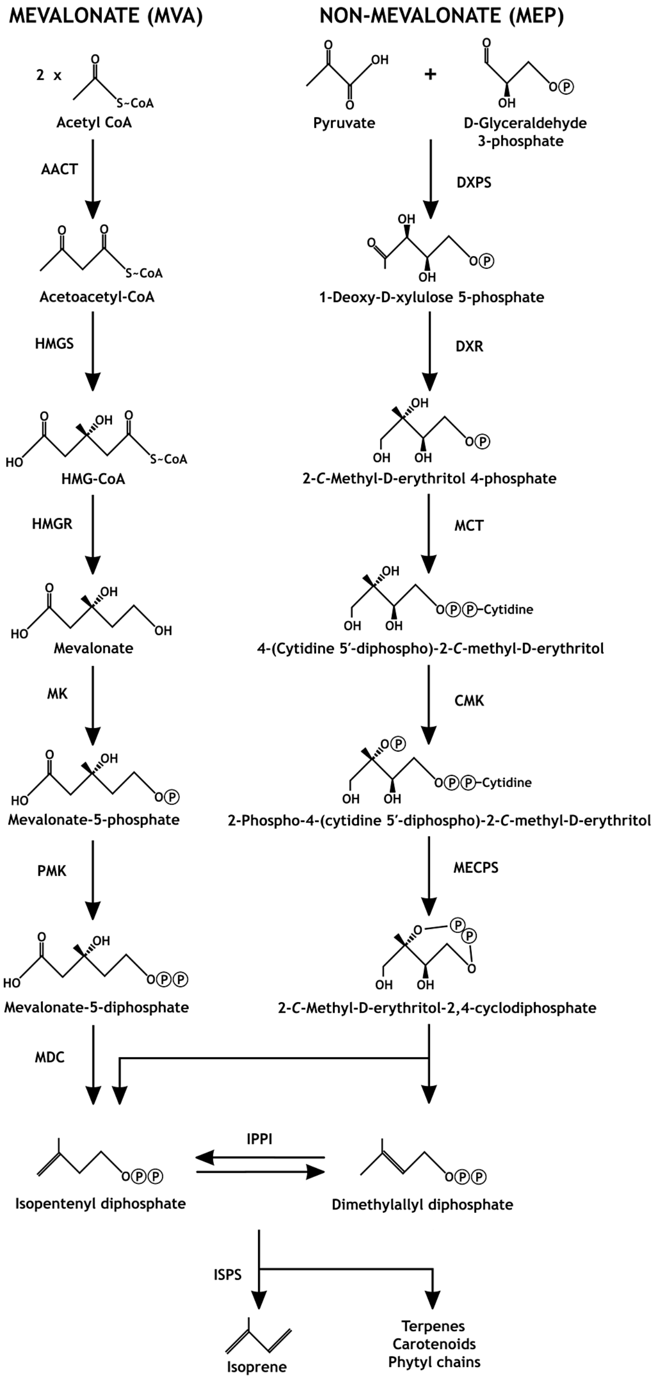

Figure 3. Biosynthesis of isoprenoids via the mevalonate (MVA) and non-mevalonate or methylerythritol phosphate (MEP) pathways of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) production. Enzymes shown are: AACT, acetoacetyl-CoA thiolase; HMGS, 3-hydroxy-3-methylglutaryl-CoA synthase; HMGR, 3-hydroxy-3-methylglutaryl-CoA reductase; MK, mevalonate kinase; PMK, phosphomevalonate kinase; MDC, mevalonate-5-diphosphate decarboxylase; DXPS, 1-deoxyxylulose-5-phosphate synthase; DXR, 1-deoxyxylulose 5-phosphate reductoisomerase; MCT, 2-*C*-methylerythritol 4-phosphate cytidyltransferase; CMK, 4-(cytidine 5′-diphospho)-2-*C*-methylerythritol kinase; MECPS, 2-*C*-methylerythritol 2,4-cyclodiphosphate synthase; IPPI, isopentenyl diphosphate isomerase; ISPS, isoprene synthase (modified from Lange et al.[22]).

and nearly all marine algae, and is a highly reactive, volatile and abundant hydrocarbon (its atmospheric emission rate is a close second to methane). Its impact on climate is complex; for example, isoprene can lead to increases in tropospheric ozone, and, by mopping up hydroxyl radicals, it can extend the lifetime of less reactive gases, such as methane, in the atmosphere[12]; both methane and ozone are potent greenhouse gases!

Nearly all of our knowledge about isoprene's biological function comes from studying terrestrial plants, especially tree species that liberate the vast majority of isoprene. Vickers et al.[13] propose an overarching biological mechanism whereby isoprene protects against reactive oxygen species that result from diverse abiotic stresses. Membrane stabilization by isoprene is also proposed to guard against thermal shock[14], and, additionally, isoprene can be liberated by plants to attract 'bodyguards' that prey on herbivorous insects to provide multitrophic feedbacks between plant, herbivore and carnivore as already outlined for DMS above[15]. Isoprene production in the marine environment is variable, and there is a correlation with photosynthesis[16]. However, it remains to be determined whether isoprene serves as a signalling molecule and the extent to which it protects against thermal, light or other stresses in marine algae.

The cocktail of organic gases generated by marine algae provides food for diverse heterotrophic bacteria, and more is often consumed than reaches the atmosphere. We have found that most marine isoprene-degrading bacteria also utilize *n*-alkanes, a major component of crude oil, as a carbon and energy source[17]. It will therefore be interesting to learn the extent to which algae-derived isoprene serves to sustain viable populations of oil-degrading microbes in the absence of oil, thereby enabling rapid growth in the event of a spill. Algal isoprene also helps to maintain a community of bacteria that can co-metabolize chlorinated VOCs, such as trichloroethene ($CHCl{=}CCl_2$), from algal and industrial sources[18]. Such microbes are therefore likely to contribute to remediation of polluted estuaries and more generally influence the flux of reactive halogens to the atmosphere.

Two routes are known for the biosynthesis of terpenes and higher isoprenoids that are synthesized from isoprene units: the mevalonate (MVA) and mevalonate-independent or methylerythritol phosphate (MEP) pathways (Figure 3). In plants, the MEP pathway is located in the chloroplast, whereas the MVA pathway is in the cytosol. For eukaryotic algae, the picture is less clear, with euglenoid algae (phylum Euglenozoa) possessing the MVA pathway, green algae (phylum Chlorophyta) only having the MEP pathway, whereas golden algae (class Chrysophyceae) have both[19]. To date, there is no information about isoprene biosynthetic routes in important marine phototrophs such as diatoms, and sketchy details about pathways in cyanobacteria. Many bacteria, in addition to cyanobacteria, produce isoprene, and although most employ the MEP biosynthetic route, there are exceptions.

Isoprene synthase (ISPS) is the key enzyme that catalyses the elimination of pyrophosphate from dimethylallyl diphosphate to produce isoprene (Figure 3). The structure of ISPS and the underlying genetics have been worked out in poplar (genus *Populus*)[20], and there has been some genetic characterization in the bacterium *Bacillus subtilis*, but major gaps in our knowledge for marine organisms remain. This is surprising, given the ecological and climatic importance of isoprene; but may change with the realization of BioIsoprene's industrial value.

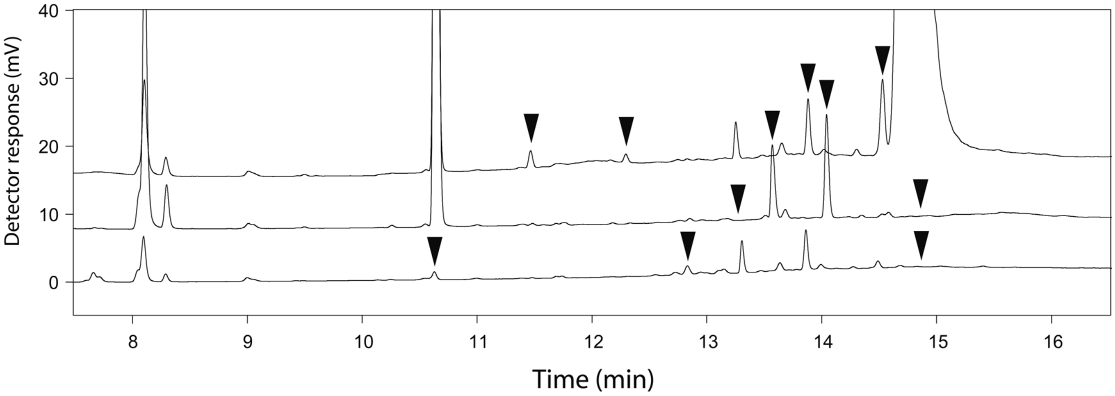

Figure 4. Gas chromatogram of volatile organic compounds in the phytoplankton species *Dunaliella tertiolecta* (bottom), *Emiliania huxleyi (middle) and Cylindrotheca* sp. (top). Arrowheads indicate major differences in trace gas signatures. Note that chromatograms are offset along the *y*-axis for clarity.

Biosynthesis of isoprene: a route to replace oil-derived products?

Isoprene biosynthesis is starting to replace petroleum-derived chemical synthesis of isoprene, for example in the production of 'green tyres' manufactured from poly-isoprene. Genencor and Goodyear are currently collaborating in a multi-million dollar venture using recombinant *Escherichia coli* with plant MVA pathways and isoprene synthase genes[21]. Isoprene also has potential as a biofuel, with several advantages over ethanol. Consequently, interest is growing in the potential application of novel genetically tractable isoprene-synthesizing algae.

Marine gases and our future

Our brief overview of some selected marine gases illustrates the complexity of their biochemistry and suggests possible directions for future research. Many other gases are being produced by biological processes in the oceans for reasons that are currently poorly understood. The high diversity in their biosynthesis results in specific 'signatures' that can be associated with individual groups of organisms or particular metabolic processes (Figure 4). Hence, it may be unrealistic to describe the infochemistry of an isolated gas if the biological activity stems from the synergism of a complex mixture of infochemicals. Some of the marine gases are climate relevant since they affect atmospheric chemistry and the formation of clouds. Future environmental change could have significant consequences for the production of marine gases and their transfer from the ocean to the overlying atmosphere. We know little about how climate change will affect gas release; with DMS, for example, an increase in gas transfer could result in more clouds and added cooling, whereas a decrease may constitute a harmful positive feedback where CO_2-induced warming will diminish the oceans' capacity to produce clouds with acceleration of a general warming trend. If we can harvest marine resources for the sustainable production of hydrocarbons such as BioIsoprene, we may be able to decrease our reliance on fossil fuels and lessen the ecological footprint our species puts on this planet. ■

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