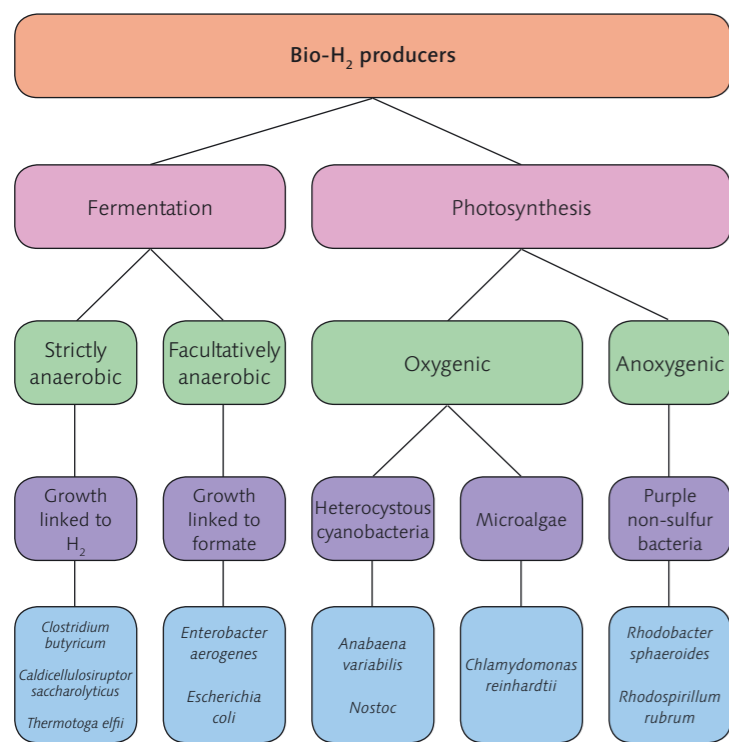


Life's a gas ... and it's hydrogen



▲ Biohydrogenic micro-organisms.

► H₂ is the only fuel with sufficiently high energy content for space exploration and its single combustion product is water, hence it is environmentally 'clean'.
Comstock Images / Jupiter Images

Hydrogen (H₂) contains around three times more potential energy by weight than petrol, making it the highest energy-content fuel available, a property exploited in space exploration. Perhaps unsurprisingly, a multitude of micro-organisms have developed the ability to derive energy from H₂, but this is not the focus of this short article. Paradoxically, there are special and yet prevalent circumstances under which micro-organisms have no better way of gaining energy than to release H₂ into their environment. The study of these phenomena began early in the last century, but biohydrogen (biologically produced H₂) remained merely an academic curiosity before the fuel crises of the 1980s. The rising profile of energy issues in the public consciousness and in political agendas, combined with scientific advances and the expansion of interdisciplinary research, have contributed to a fresh revival and new developments in biohydrogen technologies.

Biohydrogen production by microbes

The capacity for biohydrogen (bio-H₂) production is associated with the activity of either of two very common enzymes (hydrogenase and nitrogenase), but the shortlist of candidates targeted for focused study represents relatively few classes, including the fermentative bacteria and photosynthetic micro-organisms such as cyanobacteria, microalgae and purple bacteria. The ways by which these produce H₂ are summarized in the diagram on the left.

The mechanisms of bio-H₂ production within these groups are diverse, but some generalizations can be made. First, bio-H₂ production is strictly an anaerobic phenomenon because both hydrogenase and nitrogenase enzymes are destroyed by oxygen. Second, the circumstances under which it occurs always challenge the cell in some way, be it to dispose of excess reducing power, to dispatch a toxic substance or to cope with the absence of an important nutrient.

For example, in anaerobic fermentation H₂ is produced from oxidizable carbohydrates like sugars, and the generation of ATP is inextricably linked to the release of reducing power, which must be deposited onto a suitable acceptor for the fermentation to proceed. In the cases of strictly anaerobic bacteria, hydrogenase enzymes can function to 'dump' the excess reducing power onto H⁺, forming H₂. Therefore the fermentation is so dependent upon H₂ production that feedback inhibition caused by the produced H₂ stalls growth if H₂ is not allowed to escape. Facultative bacteria carry out a similar reaction, but in this case H₂ is produced primarily via the decomposition of formic acid, a mildly toxic fermentation product, hence the connection between growth and H₂ production is indirect.

Seeing the light

In contrast to the dark world of fermentation, photosynthetic micro-organisms have tapped into the Earth's most abundant

The ability of certain microbes to generate hydrogen gas has many exciting potential applications according to **Mark Redwood** and **Lynne Macaskie**. One new development uses biodegradable wastes that would normally go into landfill to make biofuel.



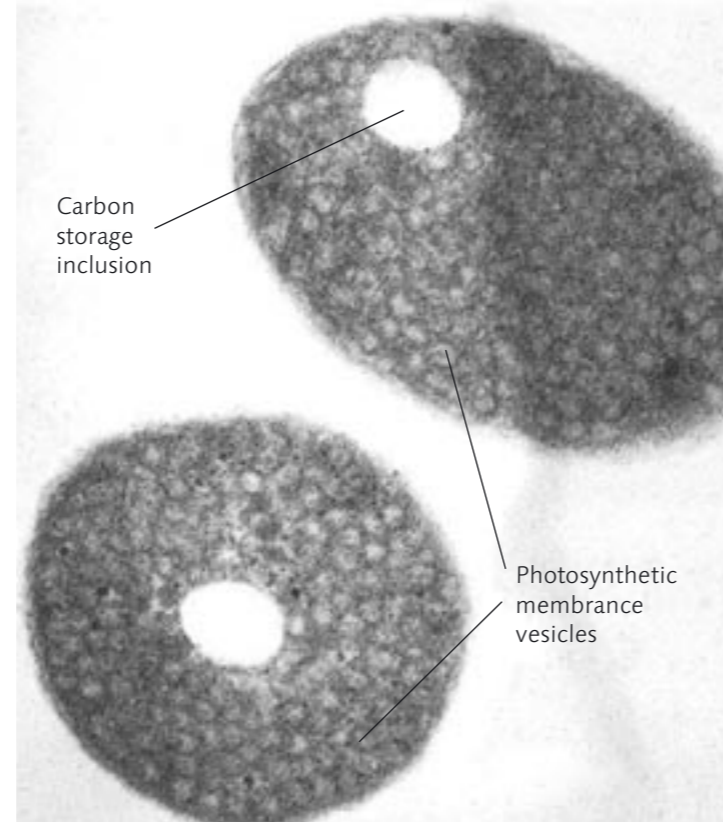


▲ H₂ production by heterocystous cyanobacteria occurs due to the exchange of nutrients between specialized cell-types; heterocysts and vegetative cells. *John Walsh / Science Photo Library*

energy source: sunlight. When photosynthesis is in full swing energy is plentiful and H₂ production results from the need to overcome different barriers. Access to light energy enables photosynthetic micro-organisms to live by endothermic chemical reactions, which could not support life in darkness. For example, anoxygenic photosynthetic bacteria (APB) are able to derive carbon for growth from relatively inaccessible substrates, including organic acids, such as those formed in the process of dark fermentation. This metabolism releases reducing power from the substrates, which must be disposed of so that more substrate can be processed (hence the term photofermentation). APB solve this problem by producing highly reduced storage material (e.g. poly-β-hydroxybutyrate) and, when they are limited for their nitrogen supply, by fixing atmospheric nitrogen when readily available nitrogen sources are scarce. This is where nitrogenase makes a dramatic entrance. This enzyme



▲ Fermentative bacteria consume sugary substrates to produce hydrogen and smelly organic acids requiring disposal. *Courtesy Geoff Gadd*



▲ Purple bacteria. Sections of *Rhodospirillum rubrum* cells showing inclusions of carbon-storage polymer (poly-β-hydroxybutyrate: the clear bodies) and photosynthetic membrane vesicles. *Lynne Macaskie*

functions to split the N₂ molecule to form 'ready nitrogen' (NH₃), a reaction requiring an enormous activation energy to break the N≡N triple bond, one of the strongest bonds found in nature. Power comes at the expense of selectivity and here H₂ is formed as a wasteful byproduct. However, the purple bacteria can be fooled into running nitrogenase even though N₂ is absent, so that only H₂ and not NH₃ is produced.

Different branches of photosynthetic micro-organisms (including cyanobacteria and microalgae) carry out oxygenic photosynthesis, so-named because it generates oxygen. H₂ production by oxygenic micro-organisms relies on separating the production of H₂ and O₂ either in space or in time. The simplest way of doing this is termed 'indirect photolysis' as it involves the photosynthetic generation of carbohydrate by day, followed by its decomposition by night when the photosynthetic supply of oxygen ceases, allowing H₂ to be generated by anaerobic fermentation. Conversely, according to 'direct photolysis', the reducing power generated by photosynthesis is dissipated by hydrogenase enzymes, such that the complex pathway can be approximated to water-splitting: $H_2O \rightarrow H_2 + \frac{1}{2}O_2$. Nitrogen-fixing cyanobacteria form chains of connected cells (filaments). Like the purple bacteria, the cyanobacteria use nitrogenase to access 'ready nitrogen', but due to the abundance of damaging oxygen, it is necessary to protect nitrogenase in a specialized anaerobic cell called a heterocyst. Nitrogenase can function only in the heterocyst because the oxygen-producing part of the photosynthetic machinery is absent, but the crippled photosystem is unable to produce enough energy for carbohydrate production, so it is dependent upon its vegetative neighbours to provide carbohydrate in exchange for 'ready nitrogen'.

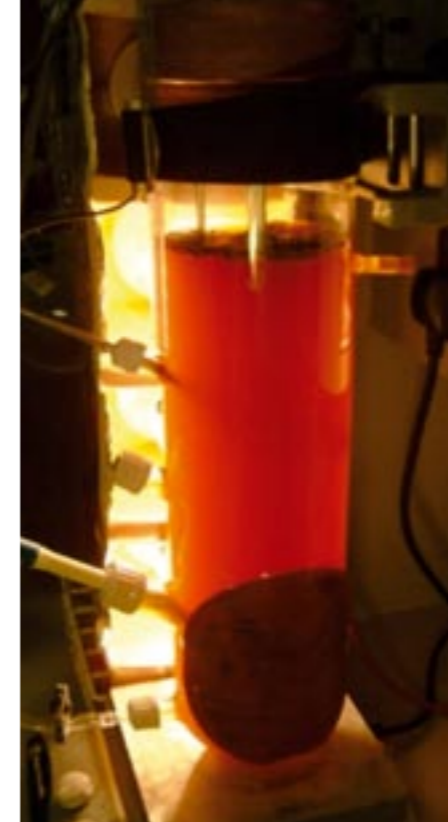
This situation has been recreated artificially using sulfur-deprived microalgae, which cannot maintain the oxygenic part of the photosynthetic apparatus during a shortage of sulfur.



▲ Made for each other? Dark (left) and light (right) bio-H₂ reactors co-operate to make bio-H₂ with high efficiency. The fan (arrowed) is powered by a fuel cell which generates electric power from bio-H₂. See movie at <http://bst.portlandpress.com/bst/033/bst0330076add.htm>

Light and dark: a new way to help save the planet

Such biochemical phenomena provide endless fascination for scientists, but increasing attention is becoming focused on applying this knowledge to address some of mankind's worsening problems. Recent work at the University of Birmingham focuses on combining dark fermentation and photofermentation to generate H₂ from sugary feedstocks. These two bioreactions fit together as the organic acid products of dark fermentation represent the ideal substrates for purple bacteria. When assembled in the laboratory, the bioprocess represents an everyday process occurring in nature where the two types of bacteria co-exist, but in the bioprocess the two bioreactors are optimized to provide the ideal conditions for H₂ production by the two different mechanisms. The maximum quantity of H₂ that could be potentially recovered from sugary feedstocks is 12 mol H₂ per mol hexose unit, but this kind of efficiency cannot be approached by a single organism. The dual bioreactor process can approach this maximum by producing up to 4 mol H₂ in the dark reactor and up to 8 mol H₂ in the photobioreactor. A significant challenge for the development of this process to a productive scale is to design a kind of photobioreactor that is cheap to construct and capable of capturing



light from a large area and transmitting it into the photosynthetic culture. A second issue is connecting the process with a reliable supply of sugary feedstock.

Immense quantities of suitable substrates can be found in biodegradable wastes, which if dumped into landfill would generate landfill gases, including methane, a greenhouse gas 25 times more potent than CO₂. For example, a third of all household food is wasted in the UK, totalling 7 million tonnes a year. However, this represents only a fraction of the actual food-linked waste as the UK food industry generates at least a further 6 million tonnes of biodegradable waste annually. With a more advanced pre-treatment, bio-H₂ can even be produced from the cellulosic residues from food-crop cultivation (e.g. corn stalks and husks), which represent tens of millions of tonnes annually in the UK. Diverting these wastes from landfill into bio-H₂ production addresses both climate change and energy security.

In a final twist, the hydrogenase in the leftover bacterial cells can be used to scavenge precious metals from spent automotive catalysts to make the catalytic ingredients of the fuel cell that converts H₂ into electricity. Hence nothing is wasted and an important new application can be found for today's waste mountain in tomorrow's non-fossil fuel transport and energy.

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Further reading

- Baxter-Plant, V.S., Mabbett, A.N. & Macaskie, L.E. (2002). Bacteria, their precious metal armour and a new weapon against waste. *Microbiol Today* 29, 80–81.
- Kapdan, I.K. & Kargi, F. (2006). Bio-hydrogen production from waste materials. *Enzyme Microb Technol* 38, 569–582.
- Macaskie, L.E. & others (2005). Applications of bacterial hydrogenases in waste decontamination, manufacture of novel bionanocatalysts and in sustainable energy. *Biochem Soc Trans* 33, 76–79.
- Redwood, M.D., Paterson-Beedle, M. & Macaskie, L.E. (2008). Integrating dark and light biohydrogen production strategies: towards the hydrogen economy. *Rev Environ Sci Technol* (in press).
- Waste and Resources Action Programme (April 2008). *The Food We Waste*. <http://wrap.s3.amazonaws.com/the-food-we-waste.pdf>