# Overall summary

In addition to the traditional circulation of carbon via multicarbon compounds in the biosphere ($CO_2$ → green plants → animals → $CO_2$; death and decay → non-living organic compounds → $CO_2$) a large quantity of carbon also circulates through methane and reduced $C_1$ compounds. This has only been fully appreciated in recent years since accurate data for the methane content of the atmosphere have been available. Cycles of the type $CO_2$ → methanogenic bacteria → $CH_4$ → methylotrophic bacteria → $CO_2$; death and decay → non-living organic matter → $CO_2$ and analogous systems involving methanol and carbon monoxide exist as what we call the methane cycle. The abundant methane in this cycle is produced by anaerobic bacteria called *methanogenic bacteria*. Some seven genera of methanogens exist: they are thought to be very ancient forms of life, and the genera are taxonomically greatly different. These bacteria live in three main types of habitat: in anaerobic sediments in lakes, paddy fields, tundra or anaerobic sludge digesters; in the rumen of cattle, sheep and similar mammals; and in geothermal springs. In these ecosystems we have a mixed bacterial population that breaks down organic compounds anaerobically to methane and carbon dioxide. Whether other organic compounds are also formed depends on the ecosystem. Thus, in the rumen volatile fatty acids are formed that are used as an energy source by the ruminant, and the gas produced thus contains less methane and more carbon dioxide. In other ecosystems, the final products are often merely methane and $CO_2$ in a ratio of $2:1$.

The methane is formed by the eight-electron reduction of $CO_2$ by hydrogen gas, catalysed by the methanogenic bacteria. The standard free energy change for this reaction, $-136 \, \text{kJ} \, \text{mol}^{-1}$, is sufficient for the methanogen to obtain energy for growth. The hydrogen and $CO_2$ are produced by other bacteria in the ecosystem, and the removal of hydrogen by the methanogens has a marked effect on both the growth and fermentation products of the other bacteria. This is called inter-species hydrogen transfer. Methane is formed by the reduction of bound $C_1$ units, probably as thiol-esters of coenzyme $M(HSCH_2CH_2SO_3^-)$. The reduction process involves several novel coenzymes and results in ATP formation in intracellular membranes. Many details of the biochemistry of this process are not yet understood. The pathway of carbon assimilation into cell material involves the conversion of two molecules of $CO_2$ into acetyl-coenzyme A by an unknown route not involving acetate. Acetyl-coenzyme A is then carboxylated successively to $C_3$ and $C_4$ derivatives from which the cell constituents are formed. The methanogens are distinguished from the eubacteria by a series of unusual cell components (Table 2.2). Their cell walls are different and their metabolism involves the use of novel coenzymes, which are

significantly different from those in eubacteria—they contain deazaflavins, nickel–porphyrin compounds and novel pteridines—as well as the more usual flavins and cytochromes.

The methane formed by methanogenic bacteria can be used as a growth substrate by certain groups of aerobic bacteria. In addition to methane, a wide range of other reduced $C_1$ compounds such as methanol, carbon monoxide, formate, methylated amines and methylated sulphur compounds can often also support growth of these *methylotrophic bacteria*. Methylotrophs recycle the methane formed by methanogens by converting it to cell material (biomass) and $CO_2$. (There is also a significant chemical transformation of methane to methanol, formaldehyde and carbon monoxide in the upper atmosphere. Some of this material comes back to earth and is available to methylotrophic bacteria). Methylotrophs can be obligate, in which case they can only grow on reduced $C_1$ compounds, or facultative. Facultative methylotrophs are either heterotrophic methylotrophs, which as an alternative to $C_1$ compounds can also use multicarbon compounds, or autotrophic methylotrophs. The latter, as an alternative to methylotrophic growth, can also grow photosynthetically or chemoautotrophically and fix $CO_2$. Three pathways of carbon assimilation are known in methylotrophic bacteria. The ribulose bisphosphate cycle or Calvin cycle of $CO_2$ fixation operates in autotrophic methylotrophy. Heterotrophic methylotrophs use either the hexulose phosphate cycle of formaldehyde fixation, or the serine pathway of carbon assimilation. In the latter carbon is assimilated both as formaldehyde and as $CO_2$.

Energy is obtained in methylotrophic bacteria by the oxidation of the $C_1$ substrate to $CO_2$. Generally speaking, the tricarboxylic acid cycle does not fulfil any oxidative role, and indeed probably does not function in obligate methylotrophs. Sometimes the $C_1$ oxidation pathway is a linear sequence: Methane $\rightarrow$ Methanol $\rightarrow$ Formaldehyde $\rightarrow$ Formate $\rightarrow CO_2$ or $(CH_3)_nN \rightarrow$ Formaldehyde $\rightarrow$ etc., but with some organisms using the hexulose phosphate cycle there are also present in the cells enzymes that make up a dissimilatory hexulose phosphate cycle that oxidizes formaldehyde to $CO_2$ via sugar phosphates rather than via formate. How significant this cycle is has not yet been established for certain. Methane is converted to methanol by two kinds of hydroxylation system which are non-specific and can hydroxylate various other compounds. Methanol is oxidized in bacteria by methanol dehydrogenase, a novel enzyme with a quinone prosthetic group called methoxatin or pyrrolo-quinoline quinone. This enzyme transfers electrons from methanol to feed into the electron transport chain at the level of cytochrome $c$. This means that only one molecule of ATP can be formed per molecule of methanol oxidized to formaldehyde. Methanol dehydrogenase lies on the periplasmic surface of the cell membrane. Cytochrome $c$ plays an essential role in methylotrophic growth, but it is not yet certain that this is merely because it accepts electrons from the prosthetic group of methanol dehydrogenase. Methylated amines are oxidized via alternative pathways involving either mono-oxygenases or dehydrogenases. Possession of the latter enables these amines to be used as carbon source during denitrifying growth in the total absence of oxygen. At least three mechanisms for the oxidation of methylamine have been described.

Control is important in methylotrophic metabolism, because the largely separate assimilatory and dissimilatory pathways have clear-cut branch points, where the two pathways diverge. Control of enzyme activity is essential to divert metabolites into the correct pathway according to the physiological needs and energy status of the cell. Because many of the enzymes of methylotrophic metabolism play no other role in the cell, control at the level of enzyme formation is also necessary, so that during the heterotrophic or autotrophic growth of facultative methylotrophs, the 'methylotrophic enzymes' can be repressed.

The only eukaryotes studied that are capable of methylotrophic growth are a relatively small number of yeast species, particularly members of the genera *Candida*, *Hansenula*, *Pichia* and *Torulopsis*. Both the assimilatory and dissimilatory pathways show significant differences from bacteria. Dissimilation of methanol involves its oxidation to formaldehyde by an oxidase, which does not generate energy. The formaldehyde is oxidized to $CO_2$ by extramitochondrial enzymes that generate NADH, which on reoxidation produces less ATP than intramitochondrial NADH. Assimilation proceeds via the dihydroxyacetone pathway, a newly characterized route in which the key enzyme is a special type of transketolase.

The two major applications at the industrial level of micro-organisms described in this book are the use of methanogenic bacteria in the anaerobic digestion of domestic, sanitary, industrial and agricultural waste, and the use of methylotrophic bacteria for single-cell protein production. (Single-cell protein is protein from microbial sources used as food for vertebrates.)

Anaerobic digestion is widely used in the disposal of sewage and of waste from food-processing factories. Applications have also been suggested for the disposal of cattle, poultry or piggery wastes without spreading obnoxious smells. The digestion process turns animal excrement into a relatively odourless sludge (which can be used as a fertilizer) with the generation of biogas (67% methane, 33% $CO_2$), which can be used for heating purposes or for generating electricity. It is likely that in the future most large farms will become energy-sufficient by the use of biogas.

Methanol has now become an important source of single-cell protein. Yeasts can be used for this, but are not as efficient as bacteria in terms of energy yield. Methanol has several advantages as a carbon source over methane, the major one being its water solubility. ICI Ltd are now producing 40 000 tonnes per year of broken, dried bacterial cells of the hexulose phosphate cycle bacterium *Methylophilus methylotrophus*. The product, called Pruteen, is used mainly as a substitute for soyabean- and fish-meal in poultry feeds.

There are also several possible applications that are not yet beyond the laboratory stage, including biotransformations using methylotrophic bacteria with particular reference to methane mono-oxygenase, the use of immobilized cells and enzymes and the possibilities of improving the industrial efficiency of micro-organisms and their adaptation to new uses, by genetic manipulation.

# Further Reading

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# Glossary

This list does not include terms that are defined in the text.

**Atractyloside.** A toxic glycoside isolated from thistles, which inhibits the transfer of adenyl nucleotides through the mitochondrial membrane.

**Biosphere.** That component of the earth's surface which comprises living organisms and their products.

**Chromophore.** The chemical grouping in a molecule responsible for its light-absorbing properties and hence its colour.

*N,N'*-**Dicyclohexylcarbodiimide** $C_{13}H_{22}N_2$. Very toxic reactive chemical inhibiting ATP synthesis in mitochondria.

**Ferredoxin.** A family of nonhaem iron–sulphur proteins functional as electron carriers found mainly but not exclusively in anaerobic bacteria. $E_0'$ about $-430\,mV$.

**Food chain.** Relationship between living organisms A, B, C, in which A eats B, which in turn has eaten C etc. Sometimes the components of a food chain may be products of living organisms, such as milk, or dead bodies, etc.

**Muramic acid.** 2-Amino-3-*O*-(lactyl)-2-deoxy-D-glucose $C_9H_{17}NO_7$, one of the two amino sugars making up the carbohydrate backbone of murein (see below).

**Murein.** The major component of the eubacterial cell wall consisting of a polysaccharide backbone of alternating *N*-acetylmuramic acid and *N*-acetylglucosamine cross-linked by peptide chains containing D- and L-amino acids attached to the muramic acid. Also called peptidoglycan.

**Nigericin.** Macrotetralide antibiotic that allows cations to be conducted through membranes.

**Pectin.** Polymer found in the roots, stems and fruits of plants, molecular weight 20 000 to 400 000, mainly polygalacturonic acid with up to 60% of the carboxyl groups esterified with methanol.

**Pentosan.** Polymer of pentoses, particularly of xylose. Xylans are the major component of the *hemicellulose* fraction of the plant cell wall. Hemicelluloses are non-cellulose, non-lignin cell wall components.

**Peroxisome.** Intracellular organelle of eukaryotes with single membrane boundary containing oxidases such as urate, glucose or D-amino acid oxidase, and catalase. Also called microbody.

**Silage.** Green grass and vegetable crops stored damp and anaerobically in a pit. Primary fermentation (see Chapter 2) of starch and cellulose occurs, giving a product rich in fatty acids.

**Squalene.** Isoprenoid hydrocarbon $C_{30}H_{50}$ found in many natural oils. Biosynthetic precursor of sterols.

# Index