

The Institute of Biology's
Studies in Biology no. 123

The Biochemistry of Pollution

J. H. Ottaway



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General Preface to the Series

Because it is no longer possible for one textbook to cover the whole field of biology while remaining sufficiently up to date, the Institute of Biology has sponsored this series so that teachers and students can learn about significant developments. The enthusiastic acceptance of 'Studies in Biology' shows that the books are providing authoritative views of biological topics.

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Readers' comments will be welcomed by the Education Officer of the Institute.

1980

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Preface

We have all become aware of environmental issues in the last two decades. Pollutants are chemicals; their persistence in or disappearance from the environment, and their biological consequences, can be explained fully only in biochemical terms. There are thousands of possible pollutants – the European Economic Community alone has a 'black list' of some 1500 chemicals – and it has been necessary to be very selective, or the book would have become a mere catalogue of unrelated reactions. Some of the more important xenobiotics are metabolized by pathways that have only recently been elucidated, and which are not fully described in most textbooks. Special attention has been given to these pathways. Otherwise it has been necessary, for reasons of space, to assume that the reader has access to a standard biochemical text.

A chapter on radiation hazards has been included, because this is a live environmental issue, and one on which it is difficult to find plain technical information. The book as a whole is designed to provide sufficient factual information at the biochemical level for the reader to make up his own mind on the issues involved. The author has tried to lay his own opinions strictly aside – with what success, the reader must judge.

Edinburgh, 1980

J. H. O.

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1 Fresh-water Pollution

1.1 Introduction

Pollution of fresh water is one of the most serious environmental problems for the world as a whole. Unless circulation from the deep oceans is inadequate (see Chapter 2), heavy marine pollution does not extend more than a few miles offshore. The situation is very different for fresh waters, and it is difficult to see how many of the problems can, with present technology, be alleviated. We will restrict ourselves to surface waters, which may be divided into running streams, and lakes. Even in highly-populated hard-rock areas, such as the Scottish Highlands, surface water always contains nutrients in sufficient quantities to support many kinds of life. The further a body of water (running or still) lies within a highly-populated area, the higher the concentration of nutrients that it will contain. It is not necessarily more active biologically.

Even in the absence of accidental pollution, there is not enough unused water in many temperate and tropical countries for the needs of the population. About 0.06% of the total fresh water in the world at any one time is in lakes and rivers – about 3×10^{15} litres. With a world population of 3000 million people, in theory everyone has a million litres to call his own. In practice, most of this water is not within reach of the major centres of population. In the U.S.A., the total stream flow is only 21000 litres day⁻¹ per person, and the *actual* amount withdrawn from streams is less than 4000 litres day⁻¹ per person. Even the larger volume is barely capable of dissolving enough oxygen to oxidize the nitrogenous compounds which the population of the U.S.A. excretes each day, leaving nothing over for the non-nitrogenous components, or the waste products of animals or of industry. As far as possible, then, these oxidations must not be allowed to occur in lakes and streams.

Industrial processes require vast quantities of water, often for cooling, but sometimes to dilute waste products to an acceptable concentration before returning them to rivers. The very large volumes sometimes quoted for industry's requirements must be assessed with caution – the water does not simply disappear, as ultimately most of it returns to source, and the water need not be drinkable. Nevertheless, effluent water from industrial plants is always warmer than the intake, and almost always more impure. The temperature differential is often the most important factor, as explained below. Power stations, particularly nuclear plants, are especially bad sources of heat pollution (see Chapter 5).

A serious source of industrial pollution is the leaching of sulphides (particularly pyrite, FeS₂) from mine workings or dumps. Dissolved O₂ converts this to FeSO₄ and then to ferric sulphate, which is hydrolysed.

Ferric hydroxide ($\text{Fe}(\text{OH})_3$) is precipitated in the stream bed, and dilute H_2SO_4 flows off. The consequent high acidity and the loss of O_2 makes the water biologically sterile, so it is both useless as a diluent, and unfit to drink. In heavily polluted waters downstream the sulphate may be reduced by anaerobic bacteria to sulphide (see p. 12), which is both toxic and unpleasant. Salts, especially potassium chloride from salt mines, may also cause problems.

Up to a point, river water may be re-used. Estimates have shown that much of the water running from London's taps has been taken from the Thames, purified, and returned to the river at least four times previously. On each occasion the water that is returned, although technically unobjectionable, adds a quota of nutrients, particularly nitrate, so this re-use cannot go on indefinitely without dilution by 'virgin' water.

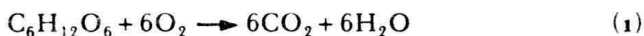
Dilution of polluted effluent by purer water is a simple thing to specify, but the purity characteristics of the diluent water need to be discussed in some detail. Water flowing from the drains or sewage works of a town can almost always become biologically and biochemically more pure, if time is allowed for microbial action to go to completion. This requires a large volume of water so that the concentration of pollutants remains low, a relatively neutral pH, absence of obvious biochemical poisons such as cyanide, phenols, or copper salts, and abundance of dissolved O_2 . In practice, the minimal diluent flow is five times the flow of effluent, if regeneration within a reasonable distance downstream is to be achieved.

Of all these requirements, the need for O_2 is the greatest. Since the solubility of gases decreases with rising temperature while the rate of biochemical activity often doubles for every 10°C rise in temperature, one may see why the temperature of water entering rivers from industrial plant is so important. Thus a large volume of effluent, even pure distilled water, coming from a power station at, say 40°C , would be disastrous. The effect of increased temperature on river life was shown in England in 1976, when prolonged drought and intense sunlight raised the temperature of many rivers to 25°C , resulting in the death of most fish. Along the S.E. coastline of the U.S.A., the summer heat and thermal pollution from industry regularly raise the temperature of rivers to $33\text{--}35^\circ\text{C}$. All living things die except a few thermophilic bacteria, which are useless for regeneration purposes.

Why is the oxygen so vital? To see this, we must consider how human effluent is dealt with in Western countries. A well-nourished adult excretes about 8 g of combined nitrogen per day. Of this about 6 g is in urea, $\text{NH}_2\text{CO.NH}_2$, 1 g in uric acid and 1 g, mostly as bacterial protein, in faeces. The latter two can fairly readily be broken down to derivatives that may be re-assimilated by growing bacteria. Urea is quickly hydrolysed by the enzyme *urease* to CO_2 and ammonia, which is toxic to most higher animals, probably because it reduces the activity of the Citric Acid Cycle in the brain. The limit of toxicity for ammonia is low and often surprisingly sharp. Trout, for instance, will die after 24 h in water containing

2 parts per million of NH_4^+ but will survive at a concentration of 1 ppm. Thus the ammonia problem may be solved by dilution (for example when untreated sewage is pumped into the sea), otherwise it must be converted into something less toxic, preferably by biological means. Fortunately there are bacteria which will convert ammonia to the far less toxic nitrite and nitrate. This solution is the one commonly adopted in inland cities of the Western world. The nitrate is very soluble and is removed from the vicinity by streams and rivers.

Conversion of NH_4^+ to NO_3^- is obviously an oxidation. In addition, human beings excrete about 100–150 g of solids each day, much of it as undigested plant polysaccharides (roughage). Microorganisms present in almost any natural water will slowly hydrolyse these polysaccharides to monosaccharides, and if an oxidizing agent is available, will convert the latter to CO_2 and H_2O . This uses a lot of O_2 :

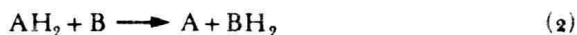


Oxidation of ammonia and carbohydrate are the major chemical processes requiring dissolved O_2 in surface waters. If the oxidations can be carried out, using forced aeration, in an effluent treatment plant, this O_2 demand can be avoided. Unfortunately, treatment plants are seldom completely efficient, and the problem is made much worse if industrial effluents also contain oxidizable organic material. Among the worst offenders are industries that use polysaccharides as raw material, for example sugar refineries, paper or cellulose pulp producers and other users of wood products. These can release very large quantities of soluble carbohydrate into local rivers, to compete with the human effluent for the O_2 brought downstream. What happens if this O_2 supply is inadequate?

In order to answer this question, we need to look at some fundamental biochemistry.

1.2 Terminal electron acceptors

The standard metabolic pathways for glucose breakdown and for the Citric Acid Cycle need not be discussed in detail here; essentially they release free energy locked in the organic molecules which are degraded, and transfer it to *adenosine triphosphate* (ATP), which can be used as a source of free energy by many processes in all cells (e.g. growth and cell division, osmotic regulation). The energy transfers accompany a succession of oxidations ending in the oxidized end-products H_2O and CO_2 . All oxidations imply a concomitant reduction; the chemical which is reduced in many oxidations in all cells is the coenzyme *nicotinamide adenine dinucleotide* (NAD). The general oxidation–reduction process may be written:

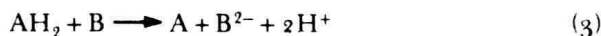


where AH_2 is to be oxidized (dehydrogenated), and B is the oxidant. In the case of cells we have the more particular equation:



where AH_2 is some oxidizable nutrient or intermediate. The amount of NAD within any cell is very small and finite: it must be continuously re-oxidized, ultimately by an oxidant from the external environment, the reduced product of which can return to that environment. The most familiar oxidant is O_2 , with H_2O as the reduced product, but in the present context several other compounds may be of importance.

Oxidation by transfer of hydrogen atoms (as in equations 2 and 2a) may be theoretically treated as a transfer of electrons and protons, thus:



This enables us to connect this type of oxidation with one involving only electrons, as in



Oxidants and reductants may then be compared quantitatively with one another in terms of their ability to gain and lose electrons. A table of *standard redox potentials* may be drawn up, based on a comparison with the tendency of hydrogen molecules to lose electrons in standard conditions of acidity (pH):



The redox potential of this system in 1 N acid (that is, when the pH is equal to zero) is taken to be 0.0 volts. However, even in acid waters the hydrogen ion concentration is never as great as 1 Normal, and inside cells $[H^+]$ is usually near that in pure water, namely $10^{-7}M$, which is equivalent to pH 7. Thus biochemists use a secondary standard of redox potential, E'_0 , at pH 7. The tendency of hydrogen atoms to lose electrons at this pH can be expressed as a voltage of -0.42 V, while the tendency of O_2 to accept electrons is denoted by a redox potential of $+0.81$ V. Lest any readers think that these 'tendencies' are very abstract affairs, many microorganisms, including the sulphate-reducing bacteria referred to later in this chapter, possess an enzyme called *hydrogenase*, whose function is precisely to catalyse the reversible reaction expressed in equation 5.

The free energy (chemical potential) made available when electrons or hydrogen atoms are exchanged between two redox pairs may be precisely calculated (for details see MORRIS, 1972). This is useful, because it enables the energy released in oxidations to be compared with that needed for energy-requiring (endergonic) reactions, and in particular with the requirement for the synthesis of ATP. This is coupled with redox processes

in various complex ways. Such computations provide useful guides to biological possibilities. For example, the (standard) free energy change required for the synthesis of one mole of ATP is 33 kilojoules (kJ). The free energy released by the oxidation of one mole of NADH_2 by O_2 is

$$2 \times 96.5 \times (0.82 - (-0.32)) = 220 \text{ kJ}$$

(2×96.5 (Faraday's constant) because *two* electrons are involved); the numbers inside the brackets are the standard redox potentials of the oxidant (O_2) and reductant (NADH_2) respectively.

Thus in principle, the oxidation by O_2 of one molecule of any natural substrate whose redox potential is roughly that of NAD/NADH_2 , could support the synthesis of at least six molecules of ATP. The experimentally determined ratio is three.

Table 1 Biological energy yield of various inorganic oxidants.

	E'_0 (mV)	$^*\Delta G_0/2e$ (kJ)
$\text{HCO}_3^- + 9\text{H}^+ + 8e^- \rightarrow \text{CH}_4 + 3\text{H}_2\text{O}$	-230	-8.4
$\text{SO}_4^{2-} + 8\text{H}^+ + 8e^- \rightarrow \text{S}^{2-} + 4\text{H}_2\text{O}$	-200	-9.6
$\text{NO}_3^- + 2\text{H}^+ + 2e^- \rightarrow \text{NO}_2^- + \text{H}_2\text{O}$	+420	-162
$\text{NO}_3^- + 6\text{H}^+ + 5e^- \rightarrow \frac{1}{2}\text{N}_2 + 3\text{H}_2\text{O}$	+750	-344
cf $\frac{1}{2}\text{O}_2 + 2\text{H}^+ + 2e^- \rightarrow \text{H}_2\text{O}$	+820	-236

* The figures in this column refer to the useful chemical energy (ΔG_0) that can be obtained when 1 mole of the oxidant is reduced under specific conditions.

Substances having a function similar to that of O_2 are called *terminal electron acceptors*. Oxygen itself is used by almost all terrestrial and most marine animals, by plants and by many microorganisms. The release of O_2 during photosynthesis ensures that the vast quantities present in the atmosphere do not sensibly diminish. However, in principle, any electron acceptor which is kinetically active (in practice, one for which an enzymic catalyst has evolved), and which has a standard redox potential more positive than zero volts, can support the synthesis of at least one molecule of ATP per two electrons transferred from NADH_2 .

Many microorganisms have evolved pathways based on the electron transport chain shown in Fig. 1-1. Dozens of terminal electron acceptors are known, many of them organic aldehydes or ketones, which can be reduced to alcohols. These need not concern us here. Three inorganic acceptors are of importance: CO_2 (HCO_3^-), SO_4^{2-} and NO_3^- . They may be reduced as shown in Table 1. The negative sign implies that energy is released when the reaction goes in the direction of the arrow. Because some of the reactions are more complex than others, they have been

released to the atmosphere every year by biological fermentations (see p. 21).

The *sulphate-reducing bacteria* will be considered in a later section, because of the peculiarly noxious nature of the end-product, hydrogen sulphide.

From Table 1, we see that about as much free energy is gained, per two electrons transferred to nitrate, as would be obtained by the more familiar reduction of O_2 . Many microorganisms use NO_3^- as a terminal electron acceptor in the absence of O_2 . Frequently O_2 represses the synthesis of the enzyme *nitrate reductase*, so that nitrate is only used in completely anaerobic conditions, but it is then readily reduced. The immediate product is the toxic nitrite, which may diffuse from the cells. Several species of microorganisms will use nitrite itself as an oxidant, some taking the process, by way of NO and N_2O , as far as N_2 . (This is *dis-similatory* nitrate metabolism.) Syntrophic nitrogen-fixing anaerobes may use the N_2 . Other organisms use nitrate in the way plants do; they reduce it, in a controlled fashion, by way of nitrite, to NH_4^+ , which is used for intracellular synthesis of amino acids, and hence proteins and nucleic acids (*assimilatory* nitrate metabolism).

Thus an anaerobic body of water may be far from sterile. It can support a large variety of microorganisms using terminal electron acceptors other than O_2 . However, the biological activity is limited; no oxygen-requiring animal can live there. More important, it will not support plant life, including phytoplankton, because photosynthesizing organisms generally require O_2 for respiration in order to maintain cell integrity during the hours of darkness. As anaerobic water cannot be re-oxygenated by plants, it must depend on diffusion of O_2 through the air-water interface, which may be very slow. If the organic content of the water is high, even in the absence of anaerobiosis aerobic bacteria will compete for the incoming O_2 with photosynthesizing organisms, and limit the growth rate of the latter.

1.3 Polluted fresh-water streams

A simplified description of a stream which does not receive enough well-oxygenated diluent is that it has a high content of solids, which settle on the bottom as an anaerobic mud, from which bubbles of H_2S and CH_4 may rise. The water will be free from almost all animal and plant life, but a few specialized worms may survive in the sediment. Nitrate arriving in the stream from treated sewage will be immediately reduced; the state of the water will be far worse if untreated excreta are allowed to enter it. Not only is such a stream obnoxious and useless as a source of drinking water, but conditions are favourable for the growth of pathogens such as cholera and typhoid organisms. For this reason officials in many large cities of Europe began, in the mid-nineteenth century, to prohibit the dumping of

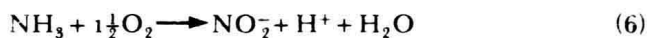
untreated sewage into rivers, and to develop sophisticated methods of sewage treatment. The 'activated sludge' method was invented in Manchester. The social and financial gain from this development was enormous, and it can still reap benefits today. If such methods of water treatment could be adopted throughout the Middle East they would, for example, eliminate the crippling economic burden of trachoma blindness.

Even the water of a river polluted as heavily as that described in the previous paragraph would become pure many miles downstream, if left to itself. All too often, however, conurbations downstream of the original source of pollution add further burdens before the first has been removed, so that the river remains sterile until it is finally diluted by the ocean (cf. ELKINGTON, 1977).

The aim of pollution control is to prevent the development of such sterility, or to reverse it if it has been allowed to occur. Our expectations must not, however, be too idealistic. They may indeed be fulfilled, as with the Thames and London. Other cities have not had such success.

1.4 The principles of sewage treatment

The fundamental biochemistry of sewage treatment is that oxygen is used to oxidize ammonia, first to nitrite and then to nitrate.



These are both exergonic processes, carried out by bacteria which derive energy for making ATP from the oxidations. Reaction 6 is catalysed by *Nitrosomonas*; the nitrite is excreted and used as substrate for reaction 7 by *Nitrobacter*. The skill in the process lies in keeping a continuous fermentation of these organisms going as they are both autotrophs, whose growth is inhibited by high concentrations of organic substrates, and also obligate aerobes. Thus there has to be a pre-treatment period, during which solids are removed by sedimentation or centrifugation, while heterotrophic organisms oxidize the organic molecules. During the conversion to nitrate, there is usually forced aeration, to make sure that O_2 is always present in excess.

The ideal sequence is one in which NH_3 disappears completely, to be replaced by nitrite, which in turn is replaced by nitrate as shown in Fig. 1-2. However, there are many things which can go wrong; for example, foaming caused by household detergents can reduce the efficiency of aeration. Thus water treatment engineers are usually happy if the effluent leaves the treatment plant at a point somewhere on the right-hand side of Fig. 1-2, i.e. when most of the ammonia has been converted to nitrate or nitrite.

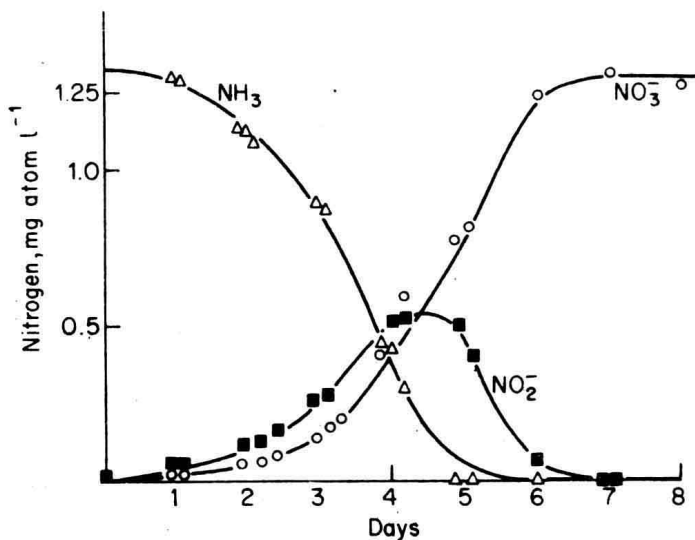


Fig. 1-2 Interrelationship between ammonia, nitrite and nitrate. The symbols show the concentrations of NH_3 (Δ), NO_2^- (\blacksquare) and NO_3^- (\circ) in water drawn from the Thames estuary in 1960, and incubated for eight days at 30°C in the presence of O_2 . The lines are the predicted concentrations of the three chemicals, based on the presence in the original water sample of low concentrations of *Nitrosomonas* and *Nitrobacter*, and on the known growth rates of these two organisms. (From KNOWLES *et al.* (1965). *J. Gen. Microbiol.*, 38, 263.)

1.5 The burden of sewage effluent on rivers

It is desirable to quantify the work that the river has to do before its waters become potable, and this may be done in several ways. A simple test is that of the Biological Oxygen Demand (BOD), which measures the amount of O_2 consumed by a sample of river water in a given time (usually five days). This test does not work very well with heavily polluted water, because there may be a long lag period before conditions are suitable for the growth of *Nitrosomonas* and *Nitrobacter*. A more sophisticated test is to measure the O_2 consumed by the sample over a short period of time under optimal conditions, and to assume that consumption will subsequently fall off exponentially with a characteristic decay constant (see Fig. 1-3). This gives the Ultimate Oxygen Demand (UOD).

These measurements and assumptions apply only to the place from which the sample was actually taken. Clearly, industrial effluent downstream from the sewage works, whither it carries large amounts of fermentable material, or too much waste heat, may undo the best efforts of civic engineers; thus pollution of large rivers has to be considered along their entire length.

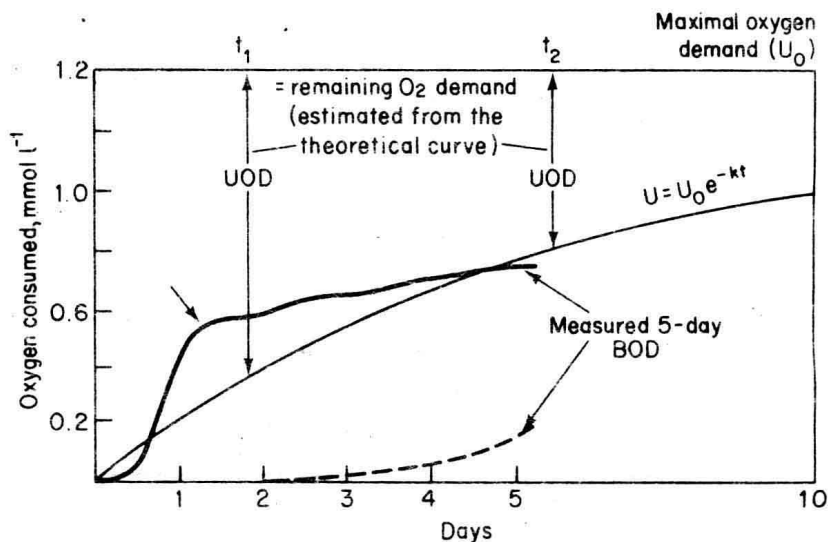
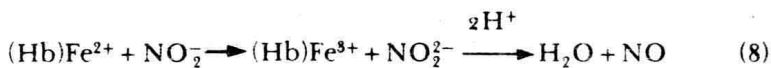


Fig. 1-3 Biological Oxygen Demand (BOD) and Ultimate Oxygen Demand (UOD). The graph shows two possible curves for oxygen uptake against time in a highly polluted waste water. — shows the uptake expected if the water contains a readily degradable substrate; at the point indicated by the arrow all the substrate will have been taken up by bacteria, and the slow oxygen utilization thereafter is due to the endogenous respiration of the cells formed in the growth phase. ---- shows oxygen uptake in water containing a slowly degradable organic substrate, or effluent from a treatment plant in which the NH_3 has only partially been converted to NO_3^- . Note that the 5-day BOD test might grossly underestimate the true oxygen requirement. — shows how UOD may be estimated (for example, at t_1 and t_2) from a theoretical curve. This method can only be used if the oxygen demand at infinite time (U_0) can be estimated by some means.

1.6 Nitrate and nitrite

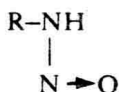
The best modern sewage works may reduce the nitrogen output of their effluent by as much as 40% by using denitrifying bacteria to convert NO_3^- to N_2 (Table 1). It is, nevertheless, inevitable that the mineral content downstream of a centre of population is higher than that upstream. Sodium, potassium, phosphate and inorganic nitrogen compounds are all nutrients which may cause troublesome overgrowth (eutrophication) in rivers, lakes or coastal seas. However, nitrate and nitrite pose a unique problem in addition to their role as nutrients.

Nitrite is toxic to man and many animals, and is likely to be produced in an anaerobic environment by many organisms, according to the equations of Table 1. The most widely known poisonous effect of nitrite is its ability to cause methaemoglobinaemia. Nitrite oxidizes the ferrous iron in the haemoglobin of the body's red blood cells to ferric iron:



$(\text{Hb})\text{Fe}^{3+}$ represents *methaemoglobin*, which is unable to transport oxygen in the blood. The victim suffers from anaemia which can be fatal. Infants are most at risk from this, partly because their haemoglobin is more easily oxidized, and partly because nitrate reduction can occur readily in the anaerobic, only faintly acid conditions of a baby's stomach. The secretion of hydrogen chloride by the stomach develops very slowly during the first six months of life.

Adults are much less at risk from nitrite-induced methaemoglobinaemia, but there is also the danger of nitrite reacting with certain aromatic amines, such as food additives, in the gut to form compounds called *nitrosamines*. These contain the grouping



Nitrosamines are carcinogenic, and there is good evidence to connect them with the development of cancer of the stomach lining.

Thus even the inflow of completely treated sewage into river water places a biological time-bomb in the environment. If the river becomes anaerobic anywhere in its subsequent course, nitrate will be partially reduced to nitrite in the stream. In addition, nitrate itself is potentially dangerous to babies because their intestinal flora can reduce it to nitrite. High nitrate concentrations are more likely to occur in the summer months, when diluent inflow is low. In the great English drought of 1976, the nitrate content of the tap water in several cities rose above the safe limits for babies, and supplies of pure water from springs were stockpiled for issue. It is fairly certain that within the next ten years, the issue of nitrate-free water for making-up infant feeds will be routine in the London area. It will not be easy to find water on this scale without distillation, because nitrate, like other inorganic ions, can percolate down to the underground aquifers from which pure water has hitherto been drawn. This nitrate often comes from excessive use of chemical fertilizer.

1.7 Other aspects of sewage disposal

Attention has been concentrated on the problem caused by human nitrogenous excreta, although pollution of surface water by ammonia, nitrate and phosphate, derived from leaching of excess fertilizer applied to agricultural land, can also be a serious problem. In some areas it is estimated that up to half the nitrogen content of a stream may come from fertilizer run-off.

It is nevertheless right to think of people as the more fundamental problem, because over-use of fertilizers can be cured by education or

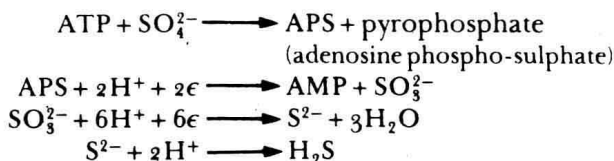
rising costs. It is interesting that the population centres with the worst pollution problem, such as Calcutta, are those where farmers can least afford to be wasteful with fertilizer. The only solution to the sewage problem is for there to be fewer people. In effect, the use of rivers as a depository for human and industrial effluent, even with enlightened biological treatment, creates a problem that cannot be cured, quite apart from the continuous desperate search for increasing amounts of oxygenated diluent water. Civil engineers have hardly begun to think about the problem (see HANLON, 1977). For example, biological recycling, (e.g. by using fishponds) is not a solution on the urban scale. Fishponds are shallow ponds – so that the water at the bottom does not become de-oxygenated – whose high rate of nitrogen input from sewage supports a vigorous growth of aquatic plants and hence of fish which are netted at intervals. BONEY (1975) gives a more detailed discussion. The technique makes sound biochemical sense, but leads to dangers of endemic disease, especially by parasites.

There are other serious chronic freshwater pollutants besides nitrogen. Chief among them is phosphate, which comes both from breakdown of metaphosphate water softeners of the 'Calgon' type and from leaching of agricultural fertilizer.

1.8 Sulphate and sulphide

Sulphate-reducing bacteria are exacting anaerobes, with several odd, perhaps primitive, properties. Their wide distribution, and tolerance of extreme environments, makes it important to discuss them briefly.

The overall energy yield of SO_4^{2-} reduction has been shown in Table 1, but in fact the activation of the rather stable sulphate ion and its reduction to SO_3^{2-} is energy-requiring, so that these organisms might be thought of as sulphite-reducing. This reminds us first that they will use many sulpho-ions other than sulphate as terminal electron acceptors, and second, that sulphite, used in the purification of wood pulp for paper-making, is an important pollutant of fresh-water environments. The overall reduction may be written



The electrons and protons needed for these reductions usually come from one of two sources; one is molecular hydrogen (see p. 4). As Table 1 shows, this does not provide much useful energy. The more common source of reducing power is the oxidation of lactate: