

**A REVIEW OF MICROBIOLOGICAL  
PROCESSES RELEVANT TO THE  
EFFECTS OF ACIDIC PRECIPITATION**

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**A REVIEW OF  
MICROBIOLOGICAL PROCESSES RELEVANT TO THE  
EFFECTS OF ACIDIC PRECIPITATION**

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## 1. INTRODUCTION

The current attention focused on the increasingly widespread problems of acidic precipitation and long-range transport of air pollutants has brought with it a deluge of papers describing the effects on fish, vegetation and other macrobiotic components of the ecosystem. Little information, however, is available on the impact of microbiological activities in ecosystems affected by "acid rain". Microbes themselves are sources of significant quantities of gaseous pollutants. They contribute large amounts of both organic and inorganic gases and volatile compounds; they also remove pollutants from soil, water and the atmosphere through a variety of scavenging processes, often transforming the contaminants into more or less toxic forms. In aquatic and terrestrial ecosystems, acidic precipitation can affect the growth and survival rates of microorganisms; it may alter morphology or adversely affect various microbial interrelations (12).

The purpose of this report is to review some of the potential effects of acidic precipitation on the microbiota, and also to discuss contributions to this problem by microorganisms. Since all life in our biosphere is ultimately dependent on microbial activities, the dearth of supportive data on microbial processes related to acid rain is a serious shortcoming (13). Although not directly applicable, some information can be gleaned from studies on environments simulating the effects of acidic precipitation (e.g. acid mine waters, coal ash effluents and *in vitro* experiments). Another indirect source of information is the literature on the major groups of microorganisms involved: that is, those responsible for the cycling of sulphur, nitrogen and carbon, primarily in aquatic and terrestrial ecosystems. For the purpose of clarity, as there is much overlap, this review will be organized into three main sections on the three cycles followed by a section on cycle interactions. The discussion of the reactions and the bacteria involved in the three cycles is not intended as an exhaustive review of the latest

research in these areas as this would not be within the scope of this report. It is intended only as a guide to focus on bacterial and chemical processes which may be involved in environments affected by acid rain.

## 2. SULPHUR CYCLE

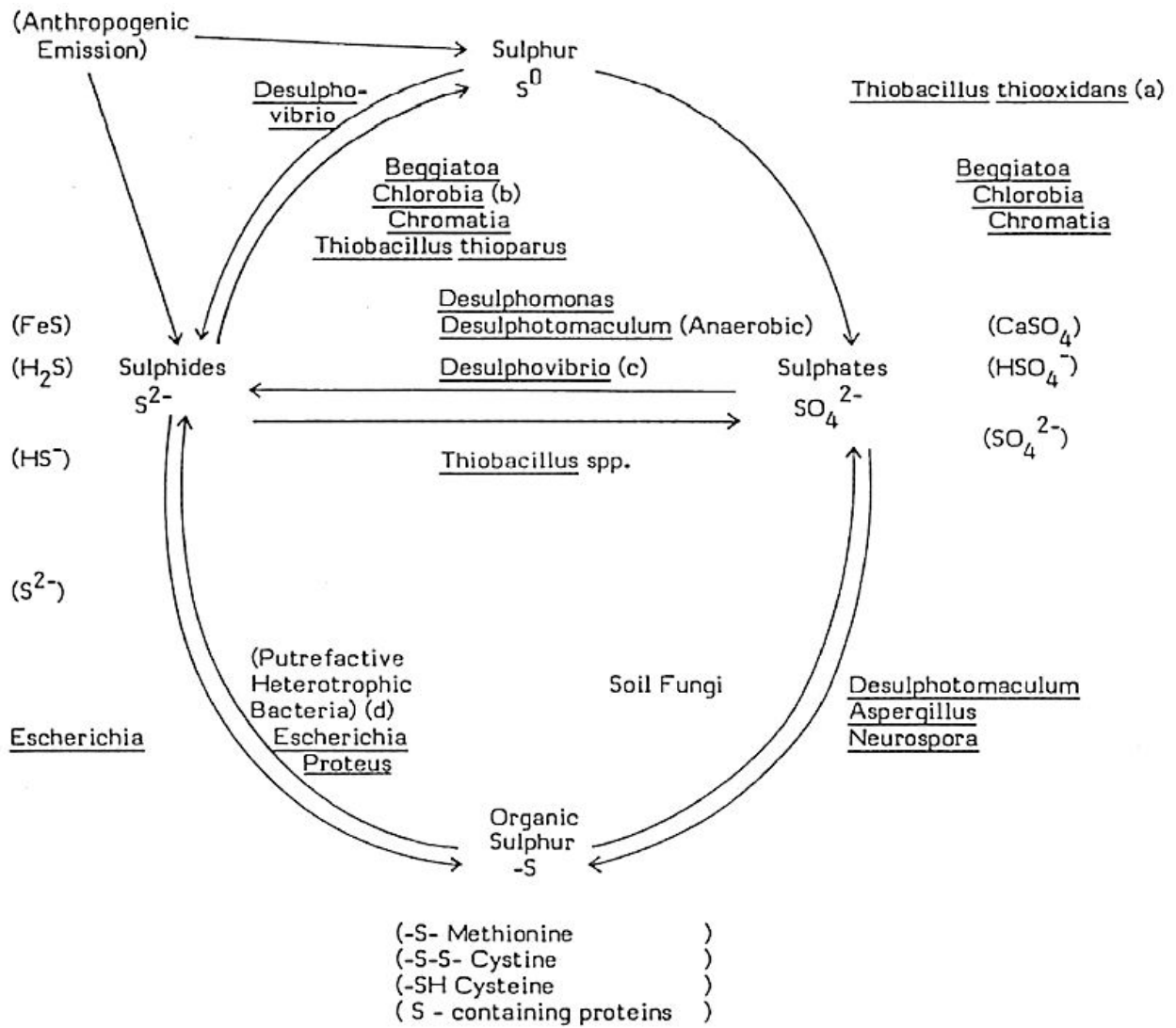
### 2.1 Microbial Transformations of Sulphur Compounds

Microorganisms are involved in a wide variety of sulphur transformations in the environment, including reductions, oxidations, incorporation into organic matter, mineralizations, and formation of gaseous and volatile products (184). These reactions are shown in Figure 1 which has been adapted from several sources (69, 121, 180, 184), and is intended as a guide throughout this section.

Nriagu and Hem (121) have stated that the influence of sulphur and its derivatives on the pH of the aquatic environment is profoundly influenced by biological activity, since this determines the rates and pathways of sulphur transformations. These transformations can broadly be classified as reduction or oxidation processes. The role of microbially mediated reactions that may be involved in an environment affected by acidic precipitation will be discussed under these two headings.

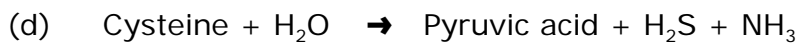
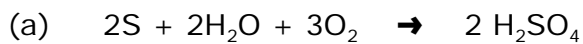
#### 2.1.1 Sulphate Reduction

The sulphate-reducing bacteria were originally described by Beijerinck (20) but little information about them is to be found in standard microbiological textbooks. Much of the literature on these and the other sulphur and nitrogen cycle bacteria is found in soil microbiology studies which are often 30 to 40 years old. Sulphate-reducers are usually strict anaerobes which grow more slowly than the more common water or soil organisms, but have a marked capacity for survival in terrestrial and aquatic environments. This group of bacteria contributes to both the biological sulphur cycle (Figure 1) and to the geochemical sulphur cycle (94) which describes the translocation of sulphur in various chemical forms. This may be mediated by the burning of fossil fuels, dissolving of soluble sulphur compounds in rain, and the retention or exclusion in soils and vegetation by ion exchange. The biological reduction of sulphur compounds constitutes the greatest natural source of atmospheric sulphur (47).



**Figure 1.** Microbiological Sulphur Cycle in Nature.

Letters after bacterial names refer to chemical reactions given below (121, 125).



Sulphate-reducing bacteria are widely distributed. They can tolerate and metabolize at pH values ranging from 4.2 to 9.5 under a variety of osmotic conditions (130, 180). They generate hydrogen sulphide ( $H_2S$ ) in quantities sufficient to support growth of the sulphide- and sulphur-oxidizing bacteria. In so doing,  $H_2$  is scavenged and this process may be responsible for the incompatibility of sulphate-reducing and methane-producing bacteria (see p. 29). During periods of active sulphate reduction, the surrounding environment tends to become alkaline, unless acid formation is taking place simultaneously, or unless the sulphide is trapped as insoluble heavy metal derivatives. The sulphide ion thus formed suppresses the multiplication of aerobic microorganisms and is toxic to many of them. Re-oxidation of  $H_2S$  by sulphur oxidizers prolongs the dominance of sulphate reducers in a self-perpetuating "sulphuretum" until sulphate supplies become limited and methane-producing bacteria predominate (130).

#### 2.1.1.1 $H_2S$ Production

Much of the atmospheric sulphur is emitted in the form of  $H_2S$  produced either by the anaerobic microbial reduction of sulphates as described above, or by anthropogenic emissions. Hydrogen sulphide evolution can also occur by bacterial decomposition of sulphur-containing amino acids (see Figure 1). Approximately  $9.8 \times 10^7$  tons of  $H_2S$  per year are produced in this way (136). The  $H_2S$  can easily be oxidized to sulphur dioxide ( $SO_2$ ) which, when dissolved in fog or cloud droplets, becomes sulphurous acid ( $H_2SO_3$ ). This is rapidly oxidized by dissolved oxygen to sulphuric acid ( $H_2SO_4$ ) which is the predominant source of hydrogen ions in acidic precipitation.

On a global scale, approximately one-third (136) to one-half (94) of the atmospheric sulphur is of anthropogenic origin. In a regional or seasonal distribution, bacterially produced sulphur may exceed industrial emissions. Isotopic studies near Salt Lake City, Utah, showed that atmospheric sulphur oxides formed from  $H_2S$  released by anaerobic sulphate reducers may dominate over industrial sulphur

emissions (67). These authors suggested that a better understanding of the role of sulphate reducers would be essential to rational environmental sulphur management. In an early discussion on the "economic activities" of sulphate-reducers, Postgate (128) gave examples of gross atmospheric H<sub>2</sub>S pollution, massive fish kills, bird mortalities and metal corrosion. He stated that because of their ubiquity and their generation of large quantities of H<sub>2</sub>S, sulphate-reducing bacteria were responsible for "a variety of impressive industrial, economic and ecological effects." These effects are comprehensively described in several review articles (128, 129, 130).

Ivanov (81) has suggested that "... the problem of the anaerobic microbiological formation of ... hydrogen sulphide and dimethylsulphides has acquired great practical importance in connection with the contamination of the atmosphere and the resulting ultraacid atmospheric precipitation". Ivanov used labeled sulphate to directly determine the rate of sulphate reduction in water and sediments. The results indicated that a large amount of the H<sub>2</sub>S in the freshwater lake studies was microbiologically produced in the water column. The H<sub>2</sub>S formed here and in the sediments was removed from the sulphur cycle as pyrite and organically bound sulphur in the bottom sediments; however, in shallow waters, the H<sub>2</sub>S could directly enter the atmosphere.

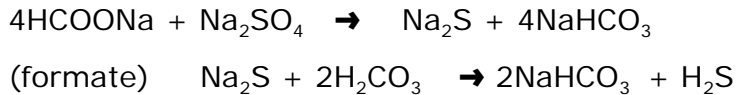
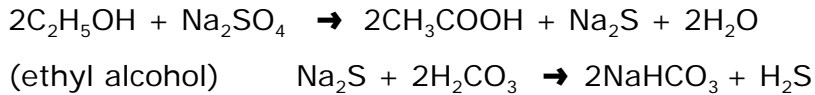
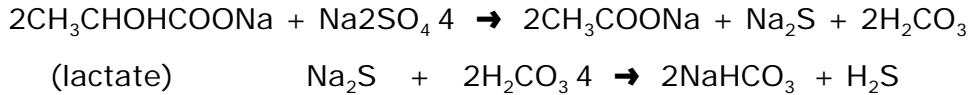
There has been some controversy in the literature over the contribution of biogenically produced sulphur to the global sulphur cycle. Nriagu and Coker (120) quoted several sources (74, 94, 106) as having suggested that the sulphur contribution from lacustrine sediments was substantial, and might even be the main source of the "missing" volatile sulphur compound in the sulphur cycle (54, 75, 94). Nriagu and Coker measured the sulphur emission from Lake Ontario sediments, and found the contribution was insignificant compared to the total atmospheric sulphur emitted annually within the lake basin. This publication aroused a strong protest from D. R. Hitchcock who disagreed with the data interpretation, followed by an equally strong rebuttal from Nriagu and Coker, both comments in a later volume of *Limnology and*

Oceanography (March, 1978).

#### 2.1.1.2 Buffering activity

Sulphate reducers therefore contribute to the problem of acid precipitation by generating  $H_2S$ , one of the precursors of acid rain; they may also have a role in counteracting the effects of acid rain. In his most recent review on sulphate-reducing bacteria, Postgate (130) briefly refers to their potential for purification of sulphate-rich waters. Demonstrating this potential, Schindler *et al.* (143) experimentally acidified a lake with sulphuric acid over a three-year period in order to simulate the effects of acidic precipitation. They found that sulphate reduction increased as the sulphate concentration in the lake increased, and that the resistance to acidification was higher than expected. It was suggested that this buffering effect was caused by the generation of dissolved inorganic carbon (stored as bicarbonate) by sulphate reducers under anoxic conditions.

In another demonstration of this capability for producing alkalinity, Abd-el-Malek and Rizk (1) studied the effect of initial pH on sulphate reduction, and observed activity (production of ferrous sulphide) at pH's ranging from 3.5 to 8. The final pH in all the cultures showing active sulphate reduction was between 8.6 and 8.8, regardless of the initial pH. The developed alkalinity was greatest when the initial pH of the medium was 5 or 6. These findings may be compared with those of Domka and Szulczynski (51) who stated in their study that the optimal pH range for sulphate reduction was 6.8 to 7.0, and that the final pH from a variety of carbon sources did not exceed 8.5. As in Schindler's work (143), this was due mainly to bicarbonate production as expressed in the following pairs of equations for *Desulphovibrio desulphuricans* (1):



In a complementary paper on laboratory experiments with sulphate reduction in soils (2), Abd-el-Malek and Rizk found that the presence of organic matter greatly enhanced sulphate reduction. A linear relationship was observed between the amount of sulphate reduced and increases in titratable alkalinity and insoluble carbonates. These *in vitro* observations were confirmed in a third paper by these authors (3) in field experiments near Cairo. From the results, they postulated a symbiotic relationship between *Desulphovibrio* spp. and decomposer bacteria in the swamps. The organic acids produced by anaerobic decomposition would probably hinder microbial activity unless neutralized. The resultant alkalinity from the activity of sulphate reducers, speculated Abd-el-Malek and Rizk, kept the pH near neutrality, which was favourable for both types of organisms.

Further support for this role of sulphate-reducing bacteria was put forth by Wong (179) who showed that it was possible to obtain via the reduction of sulphates, not only sulphur, but also lime, of which calcium carbonate is the main component. This compound has been used in the treatment and reclamation of lakes acidified by rain. Using a combination of calcium carbonate and calcium hydroxide, Scheider *et al.* (141) neutralized acidic lakes and studied the effects on a variety of biological parameters. Levels of sulphate-reducers increased in both the water column and sediments after liming.

It has been suggested (113) that the process of microbial sulphate reduction might be applied to raise the pH of acid mine drainage, or to reclaim calcium sulphate sludges. Tuttle *et al.* (163) investigated the feasibility of this approach in a stream receiving acid mine drainage. A mixed culture system was developed, using sawdust as a carbon source, which could reduce sulphates at a pH of 2.8. Sulphate reducers isolated from the mixture, however, were unable to grow below pH 5.0. In this case, the sawdust may have provided a micro-environment of higher pH. A general discussion of pH buffering by sulphate reduction is given by Goldhaber and Kaplan (64).

#### 2.1.1.3 Determination of rate of sulphate reduction

Determining populations of sulphate-reducers in acidified waters provides little information on rates of sulphate reduction. In the few studies on direct rate measurements in a natural environment, labeling techniques have usually been used. Ivanov (80), Sorokin (151) and Lien *et al.* (103) incubated sediment from various sources labeled with <sup>35</sup>S-sulphate, and obtained rates ranging from 0.0098 to 19 mg H<sub>2</sub>S per day (184). In later experiments by Ivanov (81), sulphur-labeled samples of mud and water were incubated under natural conditions at the same horizons from which the samples had been collected. The chemical composition of the samples was measured as well as the isotopic composition of sulphur compounds and the distribution of sulphate reducers. The rate of sulphate reduction was calculated from the labeled sulphur distribution in reduced sulphur compounds at the end of the experiment, compared to the initial content of sulphate sulphur.

Another approach to determining the activity of sulphate-reducing bacteria can be found in stable isotope studies. In the work by Grey and Jensen (67), discussed previously, sulphur isotope measurements were used to compare the bacteriogenic production of sulphur compounds to contributions from industrial sources.

Indirect calculations of sulphate reduction rates have been made by determining the accumulation of metal sulphides, assuming complete precipitation of the  $H_2S$  formed (90). Such measurements, however, may lead to underestimation of the actual reduction rate (86). Also, the chemical methods for measuring concentrations of sulphates or sulphides are not accurate enough to monitor the small changes occurring during short-term incubation experiments. Direct measurements using radiotracer techniques are now the preferred approach in such investigations (85).

In addition to the effects of pH (51), the ratios N/S and C/S also influence the rate of sulphate reduction. These effects have been studied extensively by Domka and Gasiorek (48, 49, 50) in fermenter experiments under controlled conditions. These workers found that the highest "reduction degree" was obtained at a N/S ratio of 0.33 and an optimal C/S ratio of 1.84. Therefore, in order for microbial reduction of sulphates to have any substantial buffering effect, an adequate amount of utilizable nitrogen and carbon is necessary.

### 2.1.2 Oxidation of Sulphur Compounds

Much of the sulphide produced by the respiratory metabolism of sulphate reducers is precipitated with metal ions, but some is oxidized back to sulphate. This can occur as a spontaneous chemical reaction or be catalyzed by chemoautotrophic or photoautotrophic sulphur bacteria. In an investigation into possible causes for the acidification of headwater streams in the New Jersey Pine Barrens (83), biological oxidation of sulphides to form  $H_2SO_4$  in the cedar bogs was considered a possibility, although acidic precipitation was considered a more likely cause. The oxidation of sulphur compounds can be mediated by sulphur-oxidizing bacteria, heterotrophic bacteria and fungi, the latter being able to utilize sulphide, thiosulphate and more oxidized forms of inorganic sulphur.

Sulphur oxidizers are usually divided into two groups: the colourless aerobic

bacteria such as *Thiobacillus* spp, which lack photosynthetic pigments, and the coloured, phototrophic bacteria such as Chromatiaceae, which derive their energy from photosynthesis in anaerobic environments.

The most comprehensively studied colourless sulphur bacteria belong to the genus *Thiobacillus*. Among the chemicals oxidized by various thiobacilli are H<sub>2</sub>S, elemental sulphur, thiosulphate and polythionates. Non-acidophilic thiobacilli are present in soils, muds, fresh and salt waters. The bacteria which are more important to ecosystems acidified by precipitation are the acidophilic species *T. thiooxidans* and *T. ferrooxidans*.

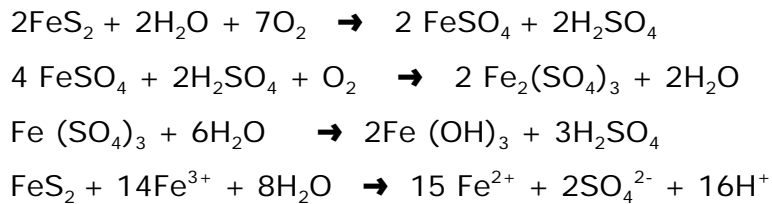
#### 2.1.2.1 The thiobacilli and other colourless sulphur bacteria

Economically, the most important characteristic of *Thiobacillus* spp. is their ability to oxidize elementary sulphur to sulphate. It has been reported that, with bicarbonate as carbon source, *T. thiooxidans* can produce up to 10 percent sulphuric acid, and can survive at a pH of 0.6 (36). This property can be useful in agriculture; however, it can also be very destructive. The sulphuric acid produced by these organisms has been responsible for corrosion of concrete (36), corrosion of stone (127) and deterioration of rubber (156). Parker and Prisk (123) have provided a detailed description of *Thiobacillus* spp. isolated from corroded concrete and have outlined the modes of oxidation - the end product in each case was sulphuric acid. The papers cited above are 25 to 35 years old and show that the contribution to acidic wet and dry deposition by bacteria has long been a problem.

Perhaps the best documented activity of *T. thiooxidans* and *T. ferrooxidans* is their participation in the oxidation of iron and sulphur compounds, contributing to the problem of acid mine drainage (107). These acidic waters pollute streams and rivers, and are corrosive. Studies on acid mine drainage waters cannot be directly extrapolated to predict bacterial effects in water affected by acidic precipitation. The

uncontaminated waters of coal or gold mines often have higher alkalinity and hardness than the soft waters acidified by low pH precipitation (36, 72), and usually have low oxygen concentrations and high concentrations of heavy metals. For these reasons and because there are many excellent reviews on the subject (9, 18, 107, 159), the role of thiobacilli in acid mine waters will only be briefly discussed.

Whenever water comes in contact with sulphides, often in the form of pyrites, in mine tailings or in metal ore, acid mine drainage is produced. Barton (18) has given the qualitative mechanism for the formation of acid water when water and air come in contact with iron disulphide as associated with a coal seam:



One of the variables which can accelerate this process is the presence of iron-oxidizing *T. ferrooxidans*. These bacteria are common in the acidic waters associated with deposits of metal sulphides and sulphide-bearing coals. The second chemical reaction listed above proceeds slowly at a pH of less than 4.5; however, *T. ferrooxidans*, if present, can accelerate this process by a factor of over  $10^6$  at pH values less than 3.5 (18). *Thiobacillus thiooxidans* also contributes to the detrimental effects of acid mine drainage by oxidizing sulphur compounds to sulphuric acid. These bacteria have a pH optimum in the range of pH 1 to 5 and are indigenous to acidic aquatic environments containing metal sulphides and oxygen. Both *T. thiooxidans* and *T. ferrooxidans*, therefore, enhance the acidifying influence of mine drainage on the surrounding terrestrial environment and affected streams. The effects of this level of acidity on bacteria will be discussed in a later section (see 6.4).

Another colourless sulphur bacterium is *Sulfolobus acidocaldarius* which is a

thermophilic, acidophilic autotroph capable of oxidizing elemental sulphur. It also oxidizes  $\text{H}_2\text{S}$  and can grow heterotrophically (3). *Sulfolobus* has been shown to be responsible for the oxidation of elemental sulphur to sulphuric acid in acid hot springs (115), and can grow under such conditions to a level of  $10^8$  cells/ml.

*Beggiatoa* and *Thiothrix* are both sulphur-oxidizing bacteria which accumulate intracellular sulphur granules, via the oxidation of  $\text{H}_2\text{S}$  in the case of *Beggiatoa*. *Beggiatoa* may have an indirect role in alleviating adverse effects of acidic precipitation on plants, by reducing the  $\text{H}_2\text{S}$  levels near the roots, thereby reducing toxicity (88).

#### 2.1.2.2 Heterotrophic sulphur oxidizers

A wide variety of heterotrophic bacteria are also able to oxidize sulphur compounds. These bacteria include members of the genera *Arthrobacter*, *Bacillus*, *Micrococcus*, *Pseudomonas*, *Mycobacterium*, certain actinomycetes, and the fungi *Saccharomyces* and *Debaryomyces* (184). The heterotrophic sulphur oxidizers have been studied much less extensively than their autotrophic counterparts. A slight pH increase may be associated with the oxidation of thiosulphate to tetrathionate. In the Black Sea, the oxidation of thiosulphate may be due completely to the presence of these heterotrophs, since no thiobacilli have been isolated (184). Sulphur oxidizers can be isolated from rivers, soils and salt waters (152, 164).

#### 2.1.2.3 Coloured photosynthetic bacteria

This group of sulphur oxidizers includes the green sulphur bacteria (*Chlorobacteriaceae*) and the purple sulphur bacteria (*Chromatiaceae*). The former are generally found in areas with high concentrations of  $\text{H}_2\text{S}$  (4 to 8 mM), while the *Chromatiaceae* are usually present at intermediate (0.8 to 4 mM)  $\text{H}_2\text{S}$  concentrations. As illustrated in Figure 1, the green sulphur bacteria oxidize  $\text{H}_2\text{S}$  to  $\text{SO}_4^{2-}$  via the intermediate of elemental, extracellular sulphur. The purple sulphur bacteria oxidize

H<sub>2</sub>S, forming intracellular sulphur globules, which are then oxidized to sulphate (184). Pfennig (126) discusses the sulphur relations of these photosynthetic bacteria more completely.

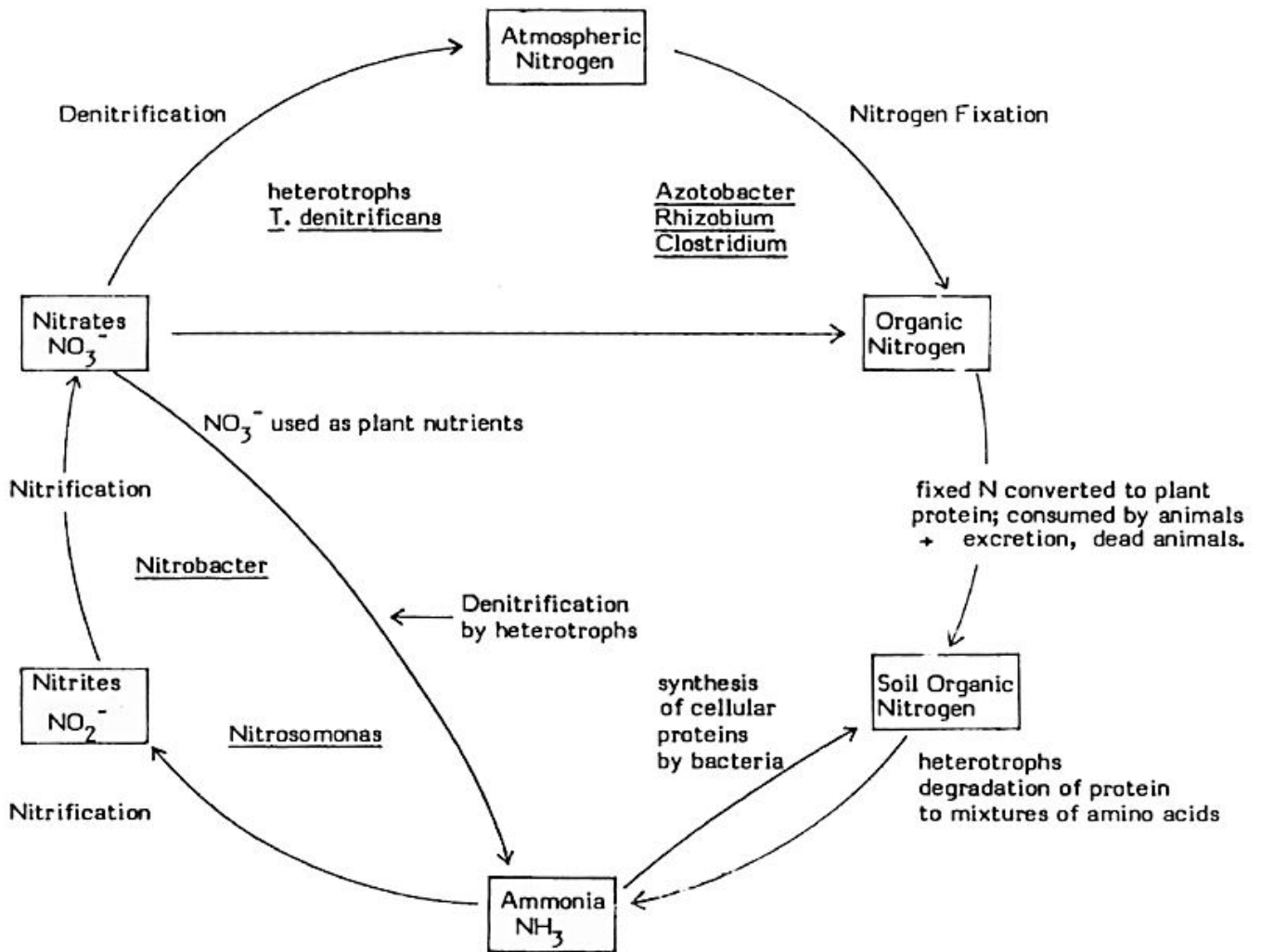
An important role is played by the photosynthetic sulphur bacteria, in conjunction with sulphate reducers, in the formation of sulphur deposits. The H<sub>2</sub>S produced by the sulphate reducers is photosynthetically oxidized to elemental sulphur. In fact, the purple sulphur bacteria, which have a pH range of 4.8 to 10.5 (180), grow so closely together with the sulphate reducers, that only a small amount of sulphur is necessary for growth, since it is cycled back and forth many times.

### 3. NITROGEN CYCLE

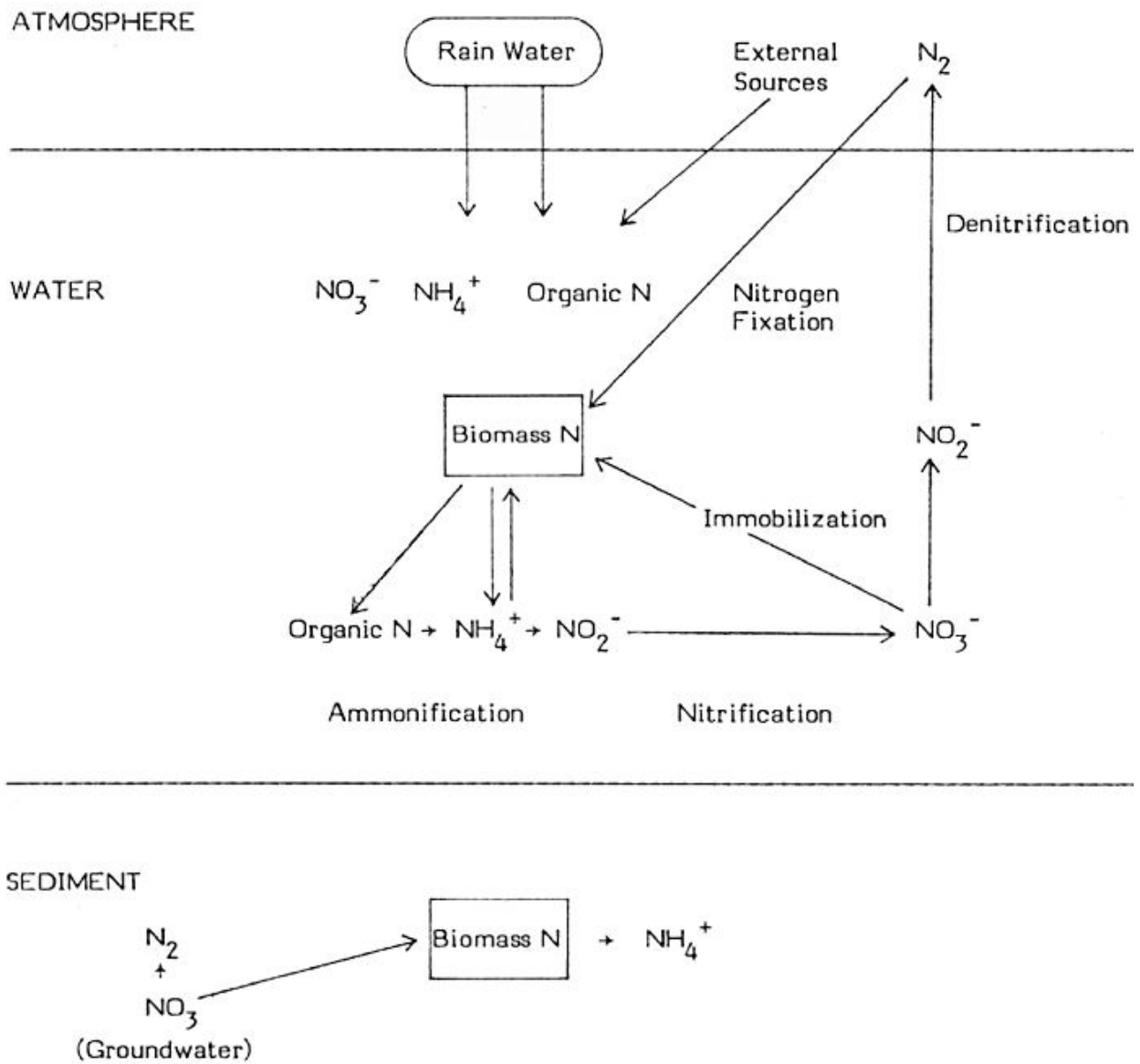
#### 3.1 Microbial Transformations of Nitrogen Compounds

The nitrogen transformations essential to all life are largely the result of microbial activity which supplies the protein nitrogen for plants and animals. In fact soils could become completely barren without nitrogen fixation by bacteria and blue-green algae (33). Microbial influence on the nitrogen cycle is basically the same, whether in fresh or salt waters, in soils or in estuarine environments (180). The microbial nitrogen cycle is illustrated in Figure 2 showing the three main reactions involving bacteria: nitrification, denitrification and nitrogen fixation (36, 125, 180). Another reference diagram is given in Figure 3, taken from Keeney (93), showing the nitrogen cycle in sediments and water systems. By referring to Figures 2 and 3 as guides throughout the following discussion and in Section 3.2, the importance of microbial nitrogen transformations to the problem of acid rain will be made more clear.

Many methods have been used for studying the nitrogen cycle bacteria. Nitrifiers in the ocean have been estimated using ammonium enrichment techniques (41); however, such methods may seriously underestimate the active population (170). The more direct approach of fluorescent-antibody techniques has been used by Schmidt and co-workers to study *Rhizobium* (144), and more recently by Ward and Perry (170) in their study on the marine ammonium-oxidizing *Nitrosococcus oceanus*. More detail on the nitrogen cycle in sediments and waters, and on the study of microbial processes involved may be found in the review article by Keeney (93). In addition to denitrification and mineralization processes, microorganisms exert indirect effects such as pH change, alteration of the oxidation-reduction status, consumption of oxygen, gas production and formation of soluble exchangeable cations. Some of these effects and the bacterial transformations responsible - nitrification, denitrification and nitrogen fixation - are described in the next three subsections, following which is an overview (Section 3.2) of how these processes can directly affect acidic precipitation.



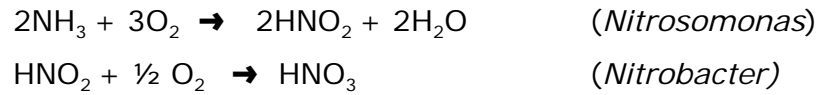
**Figure 2.** Microbiological Nitrogen Cycle in Nature.



**Figure 3.** The Nitrogen Cycle in Sediments and Water (93).

### 3.1.1 Nitrification

The oxidation of ammonia to nitrate is termed nitrification and is carried out in two stages:



Other genera are capable of performing these reactions, but only *Nitrosomonas* and *Nitrobacter* have been studied in detail. Both these organisms are obligate autotrophs and are strictly aerobic. Nitrification in soils is carried out almost completely by these bacteria, although Belser and Schmidt (21) have recently put forward a strong case for the importance of *Nitrospira* and *Nitrosolobus* in the oxidation of ammonia in terrestrial environments. In aquatic environments, nitrifiers are not present in significant numbers in surface waters but are active in sediments. Nitrate concentrations are thus highest near the bottom and are available immediately to the plant community (180).

Various heterotrophic microorganisms, including bacteria, algae and fungi, are also able to produce  $\text{NO}_2^-$ -N and  $\text{NO}_3^-$ -N from  $\text{NO}_4^+$ -N (6). The rate of nitrification by these organisms however, is almost negligible compared to that of the autotrophic bacteria (93), and has been the subject of little research.

Much progress has been made in mathematical modeling of nitrogen transformations in soil since the 1965 classic column studies by Macura and Kunc (108). In 1980, Macura and Stotzky (109) studied the effects on nitrification of the two clay minerals montmorillonite and kaolinite. They found that clay minerals appeared to inhibit as well as stimulate nitrification in soil. Of more relevance to the present paper is the discussion that followed on the effect of pH on nitrification. Weber and Gainey (173) had previously shown that nitrification occurred in acidic soils and that  $\text{NO}_3^-$ -N accumulated until a pH of approximately 4 was reached. This was confirmed by Macura and Stotzky who found that nitrification continued as the pH of soils and

perfusates fell to almost 4. Both the rate of nitrification and the pH increased with increasing amounts of montmorillonite or  $\text{CaCO}_3$  added (98), presumably at least partially due to the higher buffering capacity in soils thus treated.

These recent studies contradict somewhat the earlier reports indicating that maximum rates of reaction for both *Nitrosomonas* and *Nitrobacter* occurred in a pH range of 7 to 9 (29, 53), although nitrite oxidizers appeared to have a lower pH optimum in soil than did ammonium oxidizers (114, 173). Experiments on the effect of pH and ammonia on the rate of nitrification in surface waters were published by Kholdebarin and Oertli in 1977 (95) using river water. These authors determined a pH optimum of 8.5 for oxidation of nitrite, which is considerably higher than reported values for soil conditions and much higher than the lower pH limit of 4 observed by other workers (108). Kholdebarin and Oertli also suggested that the process of nitrite oxidation would be stimulated by  $\text{NH}_4^+$ -N.

### 3.1.2 Denitrification

The process of transforming nitrates to gaseous nitrogen or nitrous oxide is called denitrification. This transformation results in a net loss of nitrogen from the soil and may be mediated by both autotrophs (e.g. *Thiobacillus denitrificans*) and heterotrophs (e.g. *Micrococcus denitrificans*, *Serratia*, *Bacillus* and *Pseudomonas*) (93, 125). *Thiobacillus denitrificans* is widely distributed in water, mud and soil, and is a facultative anaerobe which oxidizes sulphur and thiosulphate in order to obtain the energy for nitrate reduction (36). There have been many reported isolations of denitrifying *Pseudomonas* from water, but often activity could only be demonstrated in the laboratory. Venkataraman and Sreenivasan (167) isolated 20 strains of *Pseudomonas* which required 1000 mg/L peptone for denitrification unlikely in the ocean, but more frequent in plankton swarms and marine sediments. In an earlier study, Waksman *et al.* (169) could isolate no complete denitrifiers in the sea and only one strain in the sediment, although organisms reducing nitrates to nitrites were common in the sediment.

Keeney (93) has stated that denitrification will occur in any microbial microenvironment that is anaerobic. The rate is related to the content of organic matter which acts as H donor for electron transport and which consumes oxygen in providing energy for bacterial growth. Nitrification and denitrification can occur simultaneously in the same system. Patnaik (124), using  $^{15}\text{NH}_4^+\text{-N}$ , observed nitrification in the oxidized surface layers of paddy soils and subsequent denitrification of  $\text{NO}_3^-\text{-N}$  in the reduced subsurface zone.

Recently, a kinetic model has been developed (19), which describes autotrophic denitrification using elemental sulphur. This model, confirmed by experimental results, predicts that the unit rate of denitrification is proportional to the ratio of sulphur concentration/biomass concentration at low values of this ratio and when nitrate is present in excess. At high values of the sulphur to biomass ratio, the unit rate approaches a maximum as predicted by the model. Batchelor and Lawrence, the developers of this system, proposed the use of *T. denitrificans* for the removal of nitrogen from wastewaters. This organism was suggested because it could oxidize a wide variety of reduced sulphur compounds ( $\text{H}_2\text{S}$ ,  $\text{S}_2\text{O}_3^-$ ,  $\text{S}_4\text{O}_6^{2-}$ ,  $\text{SO}_3^{2-}$ ), while reducing nitrate or nitrite to elemental nitrogen.

The possibility of using denitrification to remove nitrogen from wastewater was examined a year later by Blaszczyk *et al.* (26), who studied the influence of several carbon substrates on the efficiency of bacterial denitrification under high concentrations of  $\text{NO}_2^-$ . These workers could not obtain total denitrification of  $\text{NO}_2^- \text{-N}$  in chemostatic continuous cultures. They suggested a three-step system for cleaning nitrogen waste including nitrification, denitrification and removal of bacteria.

In a study on gas production from aquatic sediments, van Kessel (166) determined that the sequence of different nitrogenous compounds detected during denitrification was  $\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2$ , confirming work by previous investigators (37, 42, 43). In one sediment, nitrate was the limiting factor, while in the other sediment, available organic matter was the limiting factor to denitrification. Both

*T. denitrificans* and aerobic heterotrophic bacteria were suggested as using nitrate for terminal electron acceptor under anaerobic conditions, although no bacterial studies were done.

The methods applied to the study of denitrification in sediments and water systems have included labeling studies with  $^{15}\text{N}$  (32) and  $^{13}\text{N}$  (61), gas chromatographic measurements of  $\text{N}_2\text{O}$  reduction (60) and acetylene inhibition (149). Determination of nitrate reductase has been used in both marine systems (122) and in fresh water sediments (84) to study denitrification. Referral to these papers will provide information on the effects of nitrate concentration, temperature, oxygen tension and electrode potential ( $E_h$ ) on denitrification. The role of this process related to acidic precipitation will be discussed in Section 3.2.

### 3.1.3 Nitrogen Fixation

The conversion of molecular nitrogen into nitrogenous compounds is termed nitrogen fixation and may involve symbiotic or non-symbiotic microorganisms. Symbiotic nitrogen fixation is usually carried out by bacteria belonging to the genus *Rhizobium* which live in roots of leguminous plants. The fixation process is intimately associated with the metabolism of both the host plant and the microorganisms and is difficult to demonstrate in a cell-free system (125).

Non-symbiotic nitrogen fixation is accomplished by bacteria which live independently in the soil. Heterotrophic bacteria include *Azotobacter*, *Beijerinckia*, *Pseudomonas*, *Spirillum*, *Clostridium* and *Achromobacter*. Some of the autotrophic bacteria capable of nitrogen fixation are *Methanobacterium*, *Desulphovibrio*, photosynthetic *Rhodospirillum*, and the green and purple sulphur bacteria *Chromatium* and *Chlorobium*. The fungi *Rhodotorula* and *Pullularia* also have been found to fix nitrogen (125). Nitrogen fixed non-symbiotically is incorporated into cellular components. The actual role of the sulphate reducers and coloured sulphur bacteria in fixing nitrogen under marine conditions is not well understood, although their ability

to do so was recorded 30 years ago (148).

Another heterotrophic bacterium capable of nitrogen-fixing ability is *Klebsiella* sp. Knowles *et al.* (96) in their study on nitrogen fixation by pulp and paper mill effluents, isolated 129 strains of *Klebsiella* from pulp mills, lakes, rivers, and drainage and sewage systems. Of these, 32% were able to fix nitrogen by acetylene ( $C_2H_2$ ) reduction. Nitrogen-fixing strains of *Klebsiella pneumoniae* have also been isolated from animal gut contents (23), soil (105), plants (57) and decaying wood (145). Other nitrogen-fixing members of the *Enterobacteriaceae* have been isolated from animals (23), soil (105) and corn (131).

While blue-green algae are generally considered the predominant nitrogen fixers in surface waters (100), bacteria may be more important in soils. Chen and co-workers (42), in their study of lake sediments, found 40 to 70% of added  $^{15}NO_3^-$ -N in the  $NH_4^+$ -N fractions of sediments following treatment of samples with  $^{15}NO_3^-$ -N in sealed systems. Chen *et al.* hypothesized that some of the  $^{15}N_2$  formed from denitrification was being fixed by bacteria.

The significance of nitrogen fixation to the nitrogen budget in aquatic systems is poorly understood. In tropical climates, nitrogen fixation may occur year-round (31). Kuznetsov (100) reported that this process was responsible for the major portion of nitrogen in a reservoir studied. Nitrogen fixation rates in lake and estuary sediments are generally low and probably of little significance to the nitrogen economy in waters (31).

### 3.2 Specific Contributions of Microbial Nitrogen Transformations to Acidic Precipitation

Data from New York State and parts of New England have indicated that 30 to 40% of the acidity in acidic precipitation is due to the presence of nitric acid, primarily from atmospheric nitrogen oxides ( $NO_x$ ) (63). A high proportion of  $NO_x$  are attributed to automobile or other mobile sources and to fertilizers, but the following papers

suggest that microorganisms play a significant role in their contribution to acidity in aquatic and terrestrial environments.

The most abundant atmospheric nitrogen compound is nitrous oxide ( $\text{N}_2\text{O}$ ) which is emitted primarily from soil (5). It has been estimated that as much as  $5.92 \times 10^8$  tons/year of  $\text{N}_2\text{O}$  are evolved from soil as a result of anaerobic bacterial denitrification (137). The emission of  $\text{N}_2\text{O}$  is promoted by increased moisture content in soils, and under acid conditions,  $\text{N}_2\text{O}$  is the main product of denitrification (10, 174). Other bacterial sources of  $\text{N}_2\text{O}$  include *Nitrosomonas europaea* which oxidizes  $\text{NH}_4^+$  to  $\text{N}_2\text{O}$ , and the fungi *Aspergillus flavus* and *Penicillium atrovirens* which both emit  $\text{N}_2\text{O}$  due to nitrite reduction (12). Certain heterotrophic bacteria active in denitrification, such as *Bacillus subtilis*, *Enterobacter aerogenes* and *Escherichia coli*, reduce nitrate thereby releasing  $\text{N}_2\text{O}$  (181).

Large quantities of atmospheric  $\text{NO}_x$  are also of biotic origin. In 1970, Robinson and Robbins (137) calculated that, on a global scale, biological production of  $\text{NO}_x$  ( $768 \times 10^6$  tons/year) exceeded industrial emissions ( $53 \times 10^6$  tons/year) by a factor of 15. Anaerobic bacterial nitrate reduction is the main source of  $\text{NO}_x$  in soil. The  $\text{NO}_2^-$  produced is converted to nitrous acid ( $\text{HNO}_2$ ) which may decompose to form nitric oxide ( $\text{NO}$ ) and nitrogen dioxide ( $\text{NO}_2$ ) (118). Greater quantities of  $\text{NO}_x$  are evolved from acidic soils (89). Another primary bacterial source of atmospheric  $\text{NO}_2$  may be photosynthesizing microorganisms in top soil layers (111). Reuss (134) has stated that the oxidation of ammonium to nitrate in rain may result in a  $\text{H}^+$  production of an order of magnitude similar to the direct input of  $\text{H}^+$  in acidic precipitation.

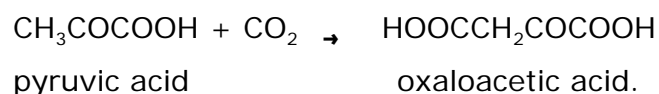
One of the minor activities of nitrifiers is also relevant to the problem of acid rain. Kauffmann (92) attributed the corrosion of stone monuments partly to the oxidation of atmospheric ammonia to nitrous and nitric acids by these organisms. The actual corrosion was caused by the leaching out of calcium nitrite and nitrate (36).

Atmospheric ammonia produced by bacteria has also been shown to aggravate surface corrosion under acidic conditions (97). When sulphur dioxide is absorbed on moist particles or dissolved in droplets of water, it is oxidized to sulphuric acid. This sulphuric acid can then be neutralized by the ammonia produced by bacteria to form  $(\text{NH}_4)_2\text{SO}_4$  and different forms of acid ammonium sulphates (e.g.  $\text{NH}_4\text{HSO}_4$  and  $(\text{NH}_4)_3\text{H}(\text{SO}_4)_2$ ). When deposited on surfaces such as painted metals, these gaseous and particulate sulphur compounds cause corrosion.

#### 4. CARBON CYCLE

As with the sulphur and nitrogen cycles, carbon cycle bacteria are involved in a complex series of chemical changes which ultimately support plant and animal life. Especially important are their roles in nutrient cycling and decomposition of detritus. Unlike the other groups of bacteria, however, microorganisms active in carbon transformations do not appear to directly contribute to the problem of acidic precipitation. The synthesizing and degradative microbial processes involved in carbon cycling will be described in this section. The direct effects of acidic precipitation on these bacteria and organic matter decomposition will be discussed in more detail in a later section (6.3).

The microbial carbon cycle in fresh water lakes is shown diagrammatically in Figure 4 (69, 99). Synthesizing reactions in this cycle include photosynthesis and the utilization of carbon dioxide (CO<sub>2</sub>) to form carbohydrates. Many heterotrophic bacteria are capable of "fixing" CO<sub>2</sub> into an organic compound already in existence. An example of this is the transformation of pyruvic acid into oxaloacetic acid (125):



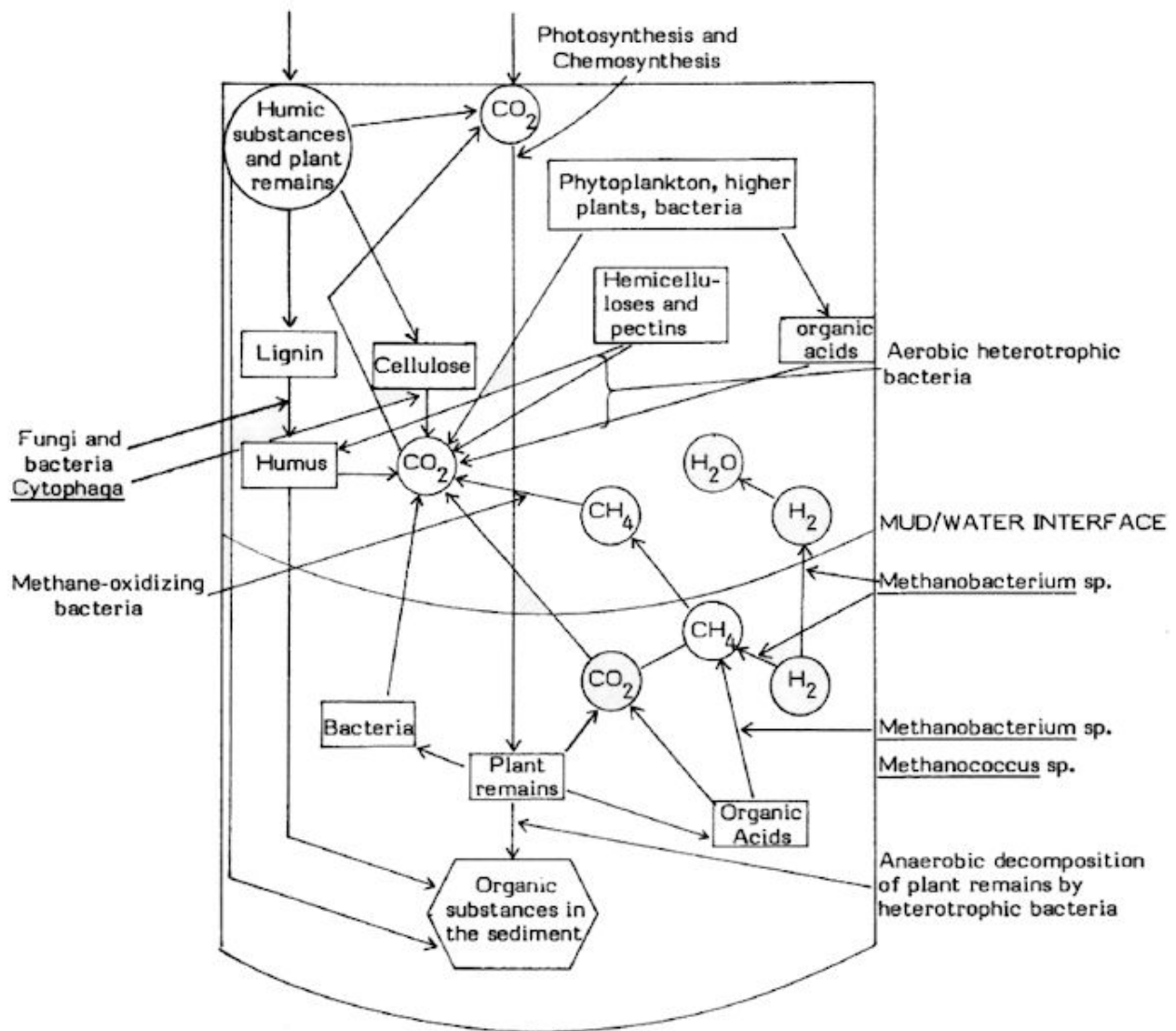
Microorganisms are able to synthesize a great variety of carbohydrates from simple compounds.

Plant and animal tissues in the soil contain carbon as a major constituent of organic compounds. By their degradative processes, microorganisms release this carbon to be used again by plant life. Organic carbon-containing compounds in plants are cellulose, lignin, pectin, starch and sugars. In animals, glycogen, lipids and proteins all contain carbon which is ultimately released via bacterial action as carbon dioxide or methane. The heterotrophic bacteria are as important chemically and biologically as the autotrophs to the degradation and translocation of organic matter (69, 180), and

can perform many transformations that cannot be mediated by larger organisms. In natural waters, the uptake of dissolved organic compounds was shown to be primarily a bacterial process, when compared to the uptake characteristics of phytoplankton and invertebrates (146).

One of the most important groups of bacteria involved in the carbon cycle is comprised of the methanogens or methane-producing bacteria. The biology of these bacteria has been extensively reviewed, particularly by Barker (17), Wolfe (177) and, most recently, by Zeikus (182), and will only briefly be described here. The bacterial formation of methane ( $\text{CH}_4$ ) is ubiquitous in most anaerobic environments (see Section 5). The association of this process with anaerobic decomposition of organic matter in the intestinal tract of animals, and in sediments and muds of various aquatic habitats has been studied for more than a century (182). As a group, the methanogens are morphologically diverse and include four genera: *Methanospirillum*, *Methanobacterium*, *Methanococcus* and *Methanosarcina*. All methanogenic bacteria are able to use hydrogen as the sole energy source for methanogenesis and for cell carbon synthesis. Substrates include  $\text{H}_2$ - $\text{CO}_2$ , formate, methanol and acetate. Several species are capable of synthesizing all cellular carbon from  $\text{CO}_2$  utilizing the energy from hydrogen oxidation.

The ecological aspects of this group of bacteria are of more relevance to the problem of acidic precipitation. Hydrogen-oxidizing methanogens have been shown to predominate in mud, water, sediment and flooded soils of marine and freshwater environments (183). Relatively little, however, has been known until recently about the environmental factors influencing methanogenesis or the *in situ* microbial activities responsible for methane formation in aquatic sediments. Methanogenesis has been shown to be inhibited by the addition of sulphate, nitrate, nitrite and acetylene to sediments (28, 39, 110). The effects of sulphate, in particular, on methanogenesis have been well reviewed (182). It appears that the interrelationships between the



**Figure 4.** The Carbon Cycle in a Fresh Water Lake(69, 99).

Methanogens and sulphate reducers are dependent on sulphate concentration. In sulphate-depleted freshwater sediments, synergistic relationships may develop instead of the competition active under high sulphate concentrations. From calibrated watershed studies in the Experimental Lakes Area, Schindler (142) suggested that decomposition processes were usually dominated by methanogenic bacteria. When sulphate concentrations increased due to acidic precipitation, the methanogenic bacteria were apparently suppressed and supplanted by sulphate reducers. Schindler reported that the consequences of this shift, while potentially serious, were not yet well understood. The interactions between methanogens and sulphate reducers are discussed further in Section 5.

## 5. CYCLE INTERACTIONS

The sulphur, nitrogen and carbon cycles interact with each other under various conditions, and their oxidizing and reducing processes exist in a symbiotic relationship. In any closed aqueous system containing organic material, redox reactions occur in a prescribed sequence starting with reduction of  $O_2$ . The chemical series of reactions follows the decreasing  $E_n$  level, and is accompanied by a parallel succession of microorganisms (e.g. aerobic heterotrophs, denitrifiers, sulphate reducers and methanogens) (153). Hutton and ZoBell (78), have shown that autotrophic methane-oxidizing bacteria from marine sediments were able to oxidize ammonia to nitrite and that the nitrite formed was related quantitatively to the methane oxidized. *Thiobacillus denitrificans*, as described previously, uses the energy of denitrification to oxidize sulphur anaerobically. Andersen (7) has demonstrated the importance of the denitrification process for the rate of degradation of organic matter in the sediment, when nitrate concentration was over 2 mg/L.

In anaerobic microbial ecosystems containing sulphate, methanogenesis is inhibited by sulphate-reducing bacteria which compete for the hydrogen or acetate required by methanogens (4, 34). In marine sediments, other studies have suggested that, since methane concentrations increased with depth, while sulphate reduction rates and concentrations decreased, methanogenesis was limited by sulphate reduction (24, 133). In a recent study on the relation of methanogenesis and sulphate reduction in sediments, Mountfort and co-workers (116) found that sulphate-reducing bacteria were usually the important agents in carbon and electron flow where high sulphate concentrations were present. Where low sulphate concentrations occurred, however, methanogenic bacteria appeared to have a more important role in the dispersal of carbon and electrons, and may have been benefiting from the activity of the sulphate reducers.

Cappenberg *et al.* (40) investigated the influence of hydrogen-producing, sulphate limited *Desulphovibrio desulphuricans* on the fermentation of hydrogen by

*Methanobacterium formicicum*. In continuous culture experiments, the hydrogen produced by *Desulphovibrio* was used by *Methanobacterium* suggesting a type of symbiotic relationship between the two. Also, the acetate-fermenting *Methanobacterium* was able to utilize the acetate produced by *Desulphovibrio*.

The observation that the denitrification intermediates, NO and N<sub>2</sub>O, accumulated near the sulphide-rich deeper layers of coastal marine sediments, prompted a study by Sorensen *et al.* (150). These authors found that the sulphide, not the low redox potential, was responsible for partial inhibition of NO reduction and strongly inhibited N<sub>2</sub>O reduction in denitrifying *Pseudomonas fluorescens*. This resulted in an increase in the proportion of N<sub>2</sub>O and NO at the expense of N<sub>2</sub>. In laboratory experiments in soil, Tam and Knowles (155) had previously reported that the presence of sodium sulphide inhibited N<sub>2</sub>O reduction by *Pseudomonas aeruginosa*.

In a study by van Kessel (166) on the effect of nitrate on gas production from sediments, nitrate was shown to suppress the formation of methane, possibly by raising the redox potential of the sediment. Ward and Frea (171), studying the distribution patterns of four species of methanogens in lake sediments, found that their distribution was related to the type and concentration of carbon source, and also to the activities of the heterotrophic and sulphate-reducing bacteria.

## 6. DIRECT EFFECTS RELATED TO ACIDIC PRECIPITATION

In this final section, the effects of acidity on microorganisms in a variety of environments will be discussed. In some cases the direct effects of acid precipitation have been studied; in other investigations the results have been obtained under conditions which may simulate the effects of acid rain. These papers will be reviewed according to the habitat involved.

### 6.1. Atmospheric Effects

Although there have been many papers on the influence of atmospheric pollutants on vegetation and animals, there is a conspicuous lack of research on the effects on microorganisms. This may be due to their small size, the variety of metabolic states affected and the numerous microbial habitats. The effects of air pollution, primarily based on laboratory observations, have been extensively reviewed by Babich and Stotzky (12, 13), and only the sulphur and nitrogen compounds implicated in acidic precipitation will be considered here.

In evaluating the effects of various atmospheric compounds on bacteria, the anionic solubility products must also be considered. Before a gaseous pollutant has an effect on a microorganism it must first pass through the aqueous films surrounding the cell. The effects, therefore, are probably the results of the pollutant's molecular or ionic solubility products. Babich and Stotzky (14) applied this rationale in their study on the effects of bisulphite ( $\text{HSO}_3^-$ ) and sulphite ( $\text{SO}_3^{2-}$ ), the anionic solubility products of sulphur dioxide. Bisulphite was found to be more inhibitory to bacteria, fungi and coliphages than was  $\text{SO}_3^{2-}$ . The lower the pH, the greater the toxicity of both compounds.

Some of the criteria which may be considered in studying the effects of acidity are: lethality; growth inhibition, retardation or stimulation; pigmentation alteration; spore production or germination; enzyme inhibition, stimulation or induction; net

respiratory or photosynthetic capabilities; and mutagenicity. Babich and Stotzky (12) have reviewed some of the major difficulties in studying the effects of gaseous and volatile compounds. Problems which are often encountered include choice of concentration (relative to that likely in the environment), and maintenance of constant relative humidity, temperature, flow rates and pH.

A reciprocal relationship between pollutant concentration (C) and length of exposure time (T) has often been noted. The toxicity of SO<sub>2</sub> to fungal spores was observed to be a function of C x T (45), and reciprocal C x T relationships were also recorded for several species of fungi exposed to SO<sub>2</sub>, NH<sub>3</sub> and H<sub>2</sub>S at concentrations of 1 to 10<sup>3</sup> ppm for 1 to 960 minutes (112).

The pH of the environment often determines toxicity of a compound. Decreases in pH increase the toxicity of SO<sub>2</sub>, and it is probable that H<sub>2</sub>SO<sub>3</sub> is the most toxic form of the HSO<sub>3</sub><sup>-</sup> ion; in fact, H<sub>2</sub>SO<sub>3</sub> was shown to be more inhibitory to bacteria, yeasts and fungi than was sulphuric acid (12). At pH 7, sulphite was found generally to be nontoxic (46). Sulphur dioxide was also shown to be toxic to a variety of plant and animal viruses at concentrations of 0.6 to 1.5% (132).

The effects of N<sub>2</sub>O and NO were shown to be generally non-bactericidal (58, 147). The maximal bactericidal activity of HNO<sub>2</sub> was found at pH 4.5 to 5.5 while HNO<sub>3</sub> was generally nontoxic in this pH range (147). Nitrogen dioxide, however, at concentrations as low as 0.18 and 0.36%, was lethal to bacteria such as *Pseudomonas fluorescens* and *Clostridium* sp. on filter paper discs. Following the treatment, the pH was 4.6 after exposure to 0.18% NO<sub>2</sub>, and 4.3 for the 0.36% NO<sub>2</sub> condition. The bactericidal effect was probably due to a combination of pH reduction and NO<sub>2</sub> formation. Irradiated atmospheres containing 0.5 ppm NO<sub>2</sub> were found toxic to *Serratia marcescens* (82). In laboratory studies on the effects of artificial smog, the growth rate of *E. coli* was decreased after exposure to gaseous mixtures of NO<sub>x</sub> and hydrocarbons (55), and NO<sub>2</sub> and butene (56).

Other major factors determining the toxicity of an atmospheric pollutant to microorganisms are the concentration, relative humidity and length of exposure (12). The bactericidal activity of  $\text{NO}_2$  against airborne bacteria was found to be dependent upon relative humidity in experiments with *Rhizobium meliloti* (178). When the bacteria were exposed to atmospheres containing 0.15  $\mu\text{L/L}$   $\text{NO}_2$  or 20  $\mu\text{L/L}$   $\text{SO}_2$ , greater toxicity was observed at 95% relative humidity than at 50%. Conversely, exposure to  $\text{SO}_2$  was more toxic to aerosols of *S. marcescens* held at 60% relative humidity than at 100% (104).

Light intensity also influences the toxicity of atmospheric pollutants. A level of 3.6 ppm  $\text{SO}_2$  was more toxic to Venezuelan equine encephalomyelitis virus at 30% relative humidity compared to 60% (22). High light intensity increased this lethality, while at 30% relative humidity, low light intensities had a protective effect on the virus particles.

Atmospheric  $\text{SO}_2$  may affect the reproductive potential of microorganisms. Toxicity to fungal spores was observed to vary with relative humidity and exposure time in experiments with *Botrytis* and *Alternaria* (44, 45). At sublethal concentrations, however,  $\text{SO}_2$  has been found to stimulate spore germination in *Diplocarpon rosae* (140). In other laboratory studies, an increase in pigmentation of *S. marcescens* occurred after exposure to irradiated mixtures of  $\text{NO}_2$  (0.5 ppm) and 1-hexane (2 ppm) (82). Exposure to 50 ppm  $\text{SO}_2$  for 2 hours in aqueous solutions was found to delay pigmentation in *S. marcescens* and *Sarcina lutea* (12).

The stimulatory effect of low concentrations of atmospheric pollutants has been observed in  $\text{SO}_2$  polluted areas, where several genera of fungi proliferated under these conditions (71). In such field observations, however, it is difficult to establish that the dominant atmospheric pollutant is responsible for the observed effects. In laboratory experiments, direct effects have been observed at the cellular level. Both  $\text{HSO}_3^-$  and  $\text{HNO}_2$  have been demonstrated to be mutagenic for viruses (102, 135), lambda coliphages (117) and various bacteria (65, 76, 91). Other effects of atmospheric

sulphur and nitrogen compounds have been observed in studies on enzyme activity and bioluminescence, both of which are reviewed by Babich and Stotzky (12).

Air pollutants may also have the ability to increase or decrease the resistance of the host organism to infection. Artificially acidified rain water at pH 3.5 increased the susceptibility of kidney bean plants to infection by *Pseudomonas phaseolicola*; however, exposure to 60 ppm SO<sub>2</sub> in solution almost eliminated infectivity of potato leaflets by *Phytophthora infestans* (71).

The preceding examples have shown that atmospheric SO<sub>2</sub> and NO<sub>x</sub>, precursors of acid rain, affect all aspects of microbial ecology as well as microbial interactions with other biological systems.

## 6.2 Terrestrial Habitats

More information is available on the effects of soil acidity on microorganisms, compared to atmospheric or aquatic effects. Two major causes of soil acidification are industrial pollutant emissions and acid mine run-off water. It has been found that the addition of elemental sulphur causes a rapid decrease in soil pH which may be attributed to the production of sulphuric acid by sulphur-oxidizing bacteria. This reduces heterotrophic bacterial populations and eventually reduces nutrient cycling and decomposition of organic matter (175). Recently Bryant *et al.* (35) studied the long- and short-term effects of soil acidification on the microbial ecosystem in several Alberta field soils. Effects were assessed by measuring total heterotrophic bacteria and soil respiration as CO<sub>2</sub> evolution. In both cases, bacterial numbers decreased significantly with decreased pH, and did not recover while the soil remained acidic. These workers concluded that nutrient cycling would be ultimately affected and that the effects could seriously disturb other major biological transformation cycles. When the physiological groups of affected bacteria were studied, the starch- and cellulose-degrading organisms showed no activity after short-term acid exposure, while the glucose and urea metabolizing organisms were still present, though less active, after long-term

exposure.

It was reported by Tamm (154) that acidification inhibited nitrification in a forest soil. He suggested, in a hypothetical model, that increases in acid deposition would result in decreased microbial activity, followed by decreased immobilization of nitrogen, leading eventually to reduced tree production. Tamm reported initial increases in ammonification after acidification of forest soils, and suggested that microbial growth may be more inhibited than the decomposition rate. In studies on microbial activity and biomass in a Norwegian forest soil treated with artificially acidified rain, Baath *et al.* (11) also concluded that decreased microbial activity could be a long-term effect of acidified rain, although the pH in their experiments was adjusted to as low as 2.0. Bacterial cell size was found to be smaller in the most acidic treatment areas.

Bancroft and co-workers (16) studied the effects of bisulphite and nitrite in an acid forest soil at pH 4.0 to 4.2. They reviewed other published reports that the antimicrobial effects of SO<sub>2</sub>, NO<sub>2</sub> or their anionic solubility products increased as pH decreased (15, 176). A level of 1.0 µg NO<sub>2</sub><sup>-</sup>-N/g soil inhibited the rate of oxygen utilization and carbon dioxide evolution temporarily. Heterotrophic populations were sensitive to low concentrations of nitrite and therefore presumably to NO<sub>2</sub>. Bancroft *et al.* (16) confirmed the pH dependency of nitrite toxicity but suggested that the actual soil concentration would be a function of NO<sub>x</sub> in the atmosphere, soil pH and the rate of nitrite-nitrous acid utilization by microorganisms. The authors concluded that the observed suppression of oxygen consumption and organic matter breakdown, in addition to the published reports of toxicity to nitrification (62, 101), indicated a need for further evaluation of the potential ecological impact of NO<sub>x</sub> on soil microorganisms. These findings disagree somewhat with earlier studies where nitrification was observed occurring readily in acid soil below the pH normally considered essential for autotrophs (38).

A paper on the effects of simulated acid rain on the respiration and growth of soil microorganisms was presented by Bewley in 1981 (R.J.F. Bewley and G. Stotzky, Abst.

Ann. Meet. Amer. Soc. Microbiol., 1981, Q60, p. 210). The pH of soils was adjusted to values ranging from pH 5.4 to 0.8 and CO<sub>2</sub> evolution was monitored. At pH 2.0, respiration was completely inhibited; above this, at pH 2.5, an extension in the lag phase was observed. In soil replica plating studies with several species of fungi, growth was markedly reduced or completely inhibited in soils acidified to less than pH 3.5. Growth was enhanced, however, by the addition of montmorillonite.

### 6.3 Decomposition Processes and Aquatic Habitats

One of the most frequently reported effects of acidic precipitation is the decreased rate of decomposition in acidified waters (73). In small oligotrophic lakes, an important source of energy for bottom invertebrates is the input of organic litter from allochthonous sources. Detritus from both allochthonous and autochthonous sources can either be biologically transformed in the water column, or it can sink to the sediments where it may be transformed or accumulate. Microbial activity is primarily responsible for the transformations involved in decomposition. Bacterial consumption and mineralization of both particulate and dissolved detritus organic matter also allow a cycling of carbon which influences the structure and functioning of the system, providing basic stability (72). In deep, open water, bacteria rapidly assimilate dissolved labile organic substances and convert them into biomass which is then available to higher trophic levels. This results in conservation of energy, and the bacterial and fungal communities transform other particulate detritus into usable forms.

In laboratory studies in 1973 (25), it was demonstrated that, as pH was lowered, numbers of bacteria and protozoans decreased, populations of fungi increased, and rates of decomposition and nitrification were reduced. It was also shown that decomposer organisms adapted to the lower pH by extending their lag period by several hours, although the exponential phase remained the same down to a pH value of 3.5.

Allochthonous litter in bottom sediments is usually decomposed by microbial processes and animal grazing. In field studies, an increase in accumulation of bottom sediments in acidified lakes has been reported (66), and was presumed due to a corresponding decrease in rate of decomposition by bacteria (30). Hendrey (72) has also reported that pH decrease due to acidic precipitation caused reduced microbial activity and therefore resulted in decreased rate of decomposition. As decomposition processes slow, coarse organic debris accumulates and nutrient recycling is impeded. This may interfere with nutrient supplies for plants and may also cause a reduction in the microbial biomass available to higher trophic levels. This in turn affects the invertebrates which prefer conditioned (colonized by bacteria) detritus (27).

More specific laboratory studies were conducted by Traaen and Laake in 1980 (162) who measured decomposition of homogenized birch litter and glucose/glutamate using oxygen uptake. When the pH was decreased from 7.0 to 3.5, litter decomposition dropped from 50-80% to 30-50% and a shift from bacterial to fungal dominance was observed. These authors also conducted leaf litter weight loss experiments in lakes and brooks in southern Norway, and in the laboratory. Significantly higher weight loss was observed at higher pH's than under acidified conditions (pH 4 to 5) after 12 months. Also, as pH decreased from 6.1 to 4.0, bacterial diversity was reduced, community respiration decreased, and there was a possible inhibition of nitrification. Traaen and Laake suggested that the overall effect may be an increased accumulation of organic matter and nutrient depletion in lakes.

In earlier experiments with homogenized leaf litter, Traaen (160) did not observe any adaptation to lower pH over a period of three weeks. Oxygen consumption was reduced by 50% when the pH was decreased from 7.0 to 5.2. When wilted birch leaves were incubated in flowing brook water at three pH levels, a significantly higher degree of decomposition was observed at pH 6 than at pH 4.5 to 5.2. The rate of decomposition was lowest in brook water adjusted to pH 4.0 (161).

Further evidence for this effect of acidification comes from lake reclamation studies where the practice of liming to raise the pH also increased the population of aerobic heterotrophic bacteria (157, 158) and accelerated the decomposition of organic matter (8, 141). An interesting study of the effects of liming and simulated acidic precipitation on pine litter decomposition was carried out by Ishac and Hovland in 1976 (79). Decomposition rates increased with temperature and incubation period. At pH 3, pine needles from limed plots showed less weight loss than needles from unlimed plots. Only fungal isolates were identified, but the composition of fungal flora did not differ according to treatment. *Trichoderma harzianum* was more tolerant to acidic conditions than the other cellulose decomposers.

The effects of decreasing pH on methane oxidizers (see Figure 4) have also been investigated, in a small eutrophic Canadian Shield lake (138). The pH was not found to be an important controlling factor; in fact the methane-oxidizing organisms appeared to tolerate acidic conditions and were active at pH 4.

At the cellular level, bacteria may adapt to a lower pH by maintaining the proper ion balance and internal pH (159), or, in the case of *Staphylococcus aureus*, may form a membrane phospholipid containing a basic amino acid (77). Vorbeck and Martinelli (168) observed an increasing amount of lysine in the membranes of *Streptococcus faecalis* with decreasing pH of the medium. In a chemostatic culture of *Bacillus megaterium*, cells accumulated lipids, phospholipids and acid-insoluble polyphosphates; more oxygen was utilized and more CO<sub>2</sub> was produced at a pH of 4.6 to 4.9 (139). Cell numbers decreased and the ATP pool was reduced. Many of the effects noted were nonspecific and also occurred under very alkaline conditions (pH 9.6). In a much earlier paper on the effects of pH on *E. coli* and *Micrococcus lysodeikticus*, Gale and Epps (59) found that a change in the external pH (e.g. to 4.5) was followed by an alteration in the cell enzyme content. An attempt was made in this way to counter the external change while maintaining essential metabolic activities at a constant level.

A more direct study of aquatic effects was made in Finland (119), where the numbers of acidophilic (pH 4.5 to 4.8) thiosulphate-oxidizing and iron-oxidizing thiobacilli were monitored in a river affected by seasonal acid run-off. The most important group of microorganisms involved in the oxidation of sulphur compounds to  $H_2SO_4$  was comprised of thiobacilli which were found to be indigenous to acidic sulphur-oxidizing soils. Passive transport of both bacteria and acidity from the surrounding soil into the river was observed; however, lack of evidence of biological activity suggested a die-off effect which may have been temperature dependent.

#### 6.4 Acidic Conditions Simulating Acidic Precipitation Effects

The problem of acid mine drainage and the role of thiobacilli were introduced in Section 2.1.2.1. The effects of this level of acidity can be compared to some extent to the effects of acid rain.

Thompson and Wilson (159) studied the effects of low pH on *Escherichia coli* under simulated acid mine water conditions. The *E. coli* survived the initial shock of low pH, continued to metabolize slowly and were able to grow actively again when the conditions improved. The reduction in pH caused loss of nuclear material, phosphates and amino acids; and clumping as a means of self protection was observed.

In a study by Tuttle *et al.* (163) on the sulphur cycle in acid mine water, low numbers of heterotrophic bacteria, particularly anaerobes, were observed at a pH of 3.1. Iron- and sulphur-oxidizing bacteria were present, but no sulphate reducers were isolated. Gram positive bacteria (*Bacillus* spp.) were present, although in an earlier paper Tuttle *et al.* (165) had reported that Gram positive bacteria of neutral streams were extremely susceptible to acid mine water. After enrichment with organic nutrients from wood dust, the acidic waters (pH 3.4) supported high numbers of aerobic heterotrophs including many yeasts and fungi, and *Pseudomonas* spp. Also, a 200-fold increase in the population of anaerobic bacteria was noted after the enrichment. Although these pH levels are lower than those generally observed in ecosystems

affected by acidic precipitation, the effects of the organic content on the redox potential may be relevant to both situations.

In a review on the impact of acid mine drainage on recreation and stream ecology (9), large and varied populations of bacteria, fungi and yeasts were reported characteristic of streams acidified with mine drainage. Genera of bacteria isolated from acid mine waters included *Bacillus*, *Micrococcus*, *Sarcina*, *Escherichia*, *Thiobacillus*, and *Ferrobacillus* (87, 172). Evidence of balanced microbiological systems in acid mine drainage water as low as pH 2.5 has been reported (52). In this study, organisms belonging to the *Ferrobacillus* / *Thiobacillus* group were present associated with yeasts.

In general, yeasts and filamentous fungi predominate in acid mine water. Populations of bacteria decrease initially when subjected to the low pH, then stabilize and may adapt to some extent by increasing the pH in a microhabitat around the surviving cells. This theory and many other reports of bacterial isolations from acid mine drainage waters are extensively reviewed by Thompson and Wilson (159).

In another habitat which may simulate the effects of acid rain, a coal ash basin was monitored over a period of two years for total heterotrophic bacteria (68). A pH drop of 6.5 to 4.6 was observed in this time period. Total culturable bacteria and numbers of colony types decreased by 30 to 44%; however, the percentage of chromogenic (pigmented) bacteria increased by 51%. The overall population structure also changed: in the first year the predominating genera were *Bacillus*, *Sarcina*, *Achromobacter*, *Flavobacterium* and *Pseudomonas*; in the second year the order of predominating bacteria changed to *Pseudomonas*, *Flavobacterium*, *Chromobacterium*, *Bacillus* and *Brevibacterium*.

A recent study was conducted on the changes in populations of various groups of bacteria in an artificial coal spoil (70). Heterotrophic bacteria dominated first, followed by sulphur-oxidizing *Thiobacillus* sp. and finally, where acidity was greatest, *T. ferrooxidans*. Maximum numbers of *T. ferrooxidans* ( $> 10^7$  cells/g) and lowest pH (2.6) both occurred at the summit of the coal spoil.

## 7. SUMMARY

In this report, the major groups of bacteria which may be implicated in or influenced by acidic precipitation have been reviewed. Processes which are potentially relevant to the effects of acid rain have been described, and studies on the microbial effects directly related to this problem have been discussed.

Microbially mediated transformations of sulphur, nitrogen and carbon compounds are essential to all forms of life. Under certain conditions, these reactions are contributing to the acidity in our environment. The magnitude of this contribution is poorly documented and probably underestimated. Acidic precipitation is also affecting many of the essential microbial processes and much more intensive research is warranted in this field.

Topics for future research should include laboratory and field studies on:

- (a) the effects of acidic precipitation on levels of sulphur, nitrogen and carbon cycle bacteria and fungi;
- (b) the effects of acidic precipitation on the activities of sulphur, nitrogen and carbon cycle microorganisms, by radiotracer techniques or fluorescent emission methods;
- (c) the taxonomy of the bacteria most sensitive to pH decreases;
- (d) identification of the microorganisms associated with the decreased decomposition rates reported observed in acid-stressed lakes;
- (e) the contribution of microbially mediated processes such as sulphur oxidation to acidification in various environments; and
- (f) the counter effects of microbially mediated processes such as sulphate reduction on acidification of aquatic ecosystems.

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## 9. ADDITIONAL REFERENCES OF INTEREST

Reynolds, D.M. Laishley, E.J. and Costerton, J.W. 1981. Physiological and ultrastructural characterization of a new acidophilic *Thiobacillus* species (*T. kabobis*). Can. J. Microbiol. 59 : 151.

A new acidophilic species of *Thiobacillus* was isolated from acidic soil near a natural gas processing plant's sulphur stockpile. The organism grew in a pH range of 1.8 to 6.0 with an optimum of pH 3.0. It was able to oxidize  $S_2O_3^{2-}$  to  $S_4O_6^{2-}$  during growth with a corresponding pH increase of 3.6 to 4.1. During the stationary phase, the isolate, named *T. kabobis*, produced  $SO_4^{2-}$  followed by a concurrent drop in pH. The culture was also able to oxidize  $S^0$  to  $H_2SO_4$  during growth with a rapid drop in pH from 3.7 to 2.2.

Muller, M.M., Sundman, V. and Skujins, J. 1980. Denitrification in low pH spodosols and peats determined with the acetylene inhibition method. Appl. Environ. Microbiol. 40 : 235.

Potential denitrification rates were determined for predominantly acid (pH 3.6) soils in southern Finland by the acetylene inhibition method. A highly significant correlation was observed between denitrification rate and soil pH. Denitrification, therefore, can occur in acid soils though at a considerably lower rate than for soils of pH > 5.

Apel, W.A. Dugan, P.R., Filppi, J.A. and Rheins, M.S. 1976. Detection of *Thiobacillus ferrooxidans* in acid mine environments by indirect fluorescent antibody staining. Appl. Environ. Microbiol. 32 : 159.

Acidophilic thiobacilli, the predominant bacteria in acid coal mine drainage, and their reactions were reviewed in the introduction to this paper. The development of an indirect fluorescent antibody stain specific for *T. ferrooxidans* was described. The technique was applied to detect the presence of this organism, and to evaluate its role in acid production in coal refuse.

Lowendorf, H.S., Baya, A.M. and Alexander, M. 1981. *Rhizobium* survival in acid soils. Abstr. Annu. Meet. Am. Soc. Microbiol. N 71, p. 185.

Three species of *Rhizobium* were tested for their ability to survive in acid and neutral soils. In sterile soil at pH 4.4, numbers of *R. meliloti* and *R. phaseoli* fell rapidly while the cowpea *Rhizobium* population did not change. At a near neutral pH, population densities of all three strains remained at inoculum level.

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Biogenic sources were described as contributing a significant fraction of atmospheric sulphur compounds. An emission flux measurement technique for determining fluxes of these compounds was applied to estimate biogenic sulphur contributions. Results were compared to global sulphur measurements.

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Even small additions of H<sub>2</sub>SO<sub>4</sub> or sulphur were described as affecting soil biological processes, especially nitrogen turnover.

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