THE MICROBIOLOGY OF CRYOPEDOGENIC SOILS OF THE SUBARCTIC WITH PARTICULAR REFERENCE TO THE CHURCHILL REGION

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A Thesis Submitted to
The Faculty of Graduate Studies and Research
University of Manitoba
In Partial Fulfillment of the
Requirements for the Degree
Doctor of Philosophy

1968

ABSTRACT

Soil materials taken from sampling sites in the Churchill and Inuvik areas were examined in an attempt to determine the role of various micro-organisms contributing to cryopedogenesis. Examination of the samples taken during September, 1964 and 1965 included a critical study of the physico-chemical, microbiological and general biological characteristics. The findings suggest that soil development in the area is typical of that proceeding in other cryopedogenic regions in North America. High C:N ratios, low levels of mineral nitrogen and the uneven thickness of partly decomposed organic-matter layers were the chief characteristics of the soils developing under the influence of native vegetation, wind erosion, moisture levels and permafrost. The active layer, in terms of root penetration and microbial action, is shallower than in the more temperate soils. Upper layers of the soil are mostly acidic in nature.

These soils, further, are characterized as harbouring considerable population of bacteria, fungi and actinomycetes which, in general, tend to decrease abruptly (in a numberical sense) with depth. Soils are biologically active and are comparable to temperate soils in terms of their potential biological activity as determined by respiration intensity,

mineralization of organic nitrogen and other enzymic activities. Soils not differing appreciably in their physico-chemical make-up showed a direct relationship between their general biological activity and organic matter content. A weak positive correlation was also established between organic-matter content and total plate-counts.

Microbial population in the areas consists mainly of cold-adapted native species similar in many respects to those forms prevalent in more temperate regions. Among the actinomycetes and fungi, the total number of genera encountered was low but, where present, these constituted a significant proportion of the total microbial population. The extent of cold-adapted populations noted in the soils was quite similar to that reported for Alaskan tundra and Antarctic soils. The extent of the cold-adaptation phenomenon among the microbial populations from the Old Beach and Inuvik soils tends to support the geological concept that these soils are older than the Goose Creek and Twin Lake formations. Evidence is presented that the respiration intensity in these soils is largely governed by the rate of enzymic hydrolysis of the organic-matter. This, in turn, is partially a function of the nature and type of microbial

agents involved in the process. Fungi, bacteria and actinomycetes all seemed to participate on the basis of evidence provided by functional group analyses. This finding is contrary to the general held belief that such activity under arctic conditions is mainly the result of action of fungi.

The process of organic-nitrogen mineralization was slow and, in acidic soils, stopped at the ammonia level. Detailed studies on the physiology of the nitrification process in these soils indicates the diverse nature of microbial agents involved and provides evidence that the major limiting factor in nitrification is the acidic nature of the soils. Ammonium-oxidizing strains, in general, were found to be much more sensitive to abnormal environmental conditions (low pH and temperature) than the nitrite-oxidizing population.

A large number of quite different microbial isolates were found to be capable of growing under nitrogen-fixing conditions. Although they were mainly bacterial in type, some nitrogen-fixing species of fungi, yeasts and actinomycetes were noted. Counts of nitrogen-fixers ranged from several tens of thousands to several millions per gram of soil. The significance of these findings in terms of cryopedogenesis is discussed.

ACKNOWLEDGEMENT

The writer is most grateful to Dr. N.E.R. Campbell, his advisor, for his invaluable aid and continual encouragement during the course of this investigation. His interest and stimulating discussions contributed much to this work.

Appreciation is also extended to Dr. H. Lees, Chairman and Head of the Department of Microbiology for advice and for the provision of facilities whereby this study was possible. The stimulating atmosphere for graduate research provided by all members of the Department is gratefully acknowledged. As well, the writer acknowledges with thanks the financial support provided by the University of Manitoba in the form of a University studentship. Warm appreciation is extended to Miss U.P. Dasani for her willing assistance in the preparation of the manuscript and to Mrs. Barbara Cleugh for her cheerful contributions in typing.

An acknowledgement for this thesis would be incomplete without extending the writer's appreciation to personnel of the Rocket Research Facility at Fort Churchill and to Mr. Eugene Hermanson and others in the Department of Public Works, Fort Churchill who gave willingly of their time and advice in the field trips.

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GENERAL INTRODUCTION

Although the presence of viable micro-organisms in soils of the polar and subpolar regions has been fairly well established and some evidence has accumulated to suggest that they are adapted in a varying degree to the prevailing low temperature conditions, virtually nothing is known about their activities in their natural environment. Accordingly, their direct role in the evolution of cryopedogenic soils is poorly understood.

This lack of information has been due in some extent to the limited numbers of investigators interested in these regions. Of greater significance, perhaps, is that the majority of those who have investigated subarctic and arctic microbiology were concerned with purely qualitative determinations and gave little attention to questions of temperature adaptation, physiological role and other activities of the microorganisms in terms of their natural environment.

In the past few years, however, a greater interest has developed and published reports mostly in the field of microbial ecology of cold soils are starting to appear. The bulk of information on tundra soils, thus far available, was derived from purely pedological studies and in these reports every little information is included concerning the role of micro-organisms. Although such information has

accumulated in terms of soil evolution under temperate and tropical climates, little is known concerning the contributions by microorganisms in soils developing under the rigours of arctic and subarctic climates.

This investigation was designed to evaluate the ecological significance of micro-organisms existing in the cold soils and to determine their contributions, if any, in cryopedogenesis. For purposes of clarity the thesis has been divided into three main parts: Part I deals with the general physico-chemical and microbial characteristics of the soils; Part II reports on studies of the physiology of nitrification, and Part III deals with nitrogen fixation. Part I is preceeded by a general review of the literature and a separate introduction is given to each Part.

GENERAL DESCRIPTION OF THE AREAS UNDER STUDY, SAMPLING SITES AND SOILS

This present investigation is restricted to an area within a radius of 60 miles from the mouth of the Churchill River and, to a much lesser extent, to a narrow section of the delta of the Mackenzie River near Inuvik, N.W.T. As such, it is not proposed that the findings to be presented subsequently are necessarily typical or representative of the entire subarctic region of Canada. One might reasonably expect, however, that since roughly the same topography and micro relief conditions pertain, and further, that environmental conditions in respect to temperature, annual precipitation, vegetative cover, wind force and the like are similar, that observations made in one area of the subarctic will not differ markedly from those derived from another distant yet similar region.

The Churchill Region

Physiography[1]

The Hudson Bay coastal plain is a wide, flat plain of late quaternary marine clays extending from Cape Jones on the east side of James Bay, around the south side and up the

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[1]This description is based largely on the report of Brown - Beckel *et al* (1954).

west coast of Hudson Bay to the Arctic Ocean. The greatest width of the plain is approximately 150 miles.

Over the Palaeozoic limestone, south and south-west of the Bay, the flat surface is broken only by a few hills of rock or drift and by post-glacial stream valleys. The unconsolidated materials composing the coastal plain are made up of four more or less distinct aggregate classes as follows:

1. glacial drift of a dense blue clay carrying a few pebbles;
2. marine clays and silt commonly carrying marine shells;
3. stratified sands; and
4. sands and gravels forming beach ridges, terraces and eskers.

Inland from the mouth of the Churchill River, the terrain is more typical of the region: monotonous expanses of organic terrain, pockmarked by innumerable lakes and ponds with the monotony relieved occasionally by gravel ridges. The town of Churchill located at the mouth of the Churchill River lies within the northern subarctic area (Latitude 50° 45'N and Longitude 94° 5' W) in terms of mean temperature and precipitation. Soils in the vicinity represent tundra and forest transition zone types.

Climate

Churchill weather is transitional between arctic and subarctic. Average temperature for the warmest month, July, is 53.7°F, which places it just within the subarctic boundary (Brown Beckel et al, 1954). Mean annual air and ground temperatures of 19°F and 27.5° to 28.9°F respectively have been recorded for the Churchill region by Brown (1960). Distribution of permafrost is continuous according to Pihlainen (1962). Yearly rainfall averages a little over 10 inches which when combined with an average snowfall of 56.9 inches gives a yearly precipitation of nearly 16 inches. Wind plays an important part in this climate; its effect on vegetation, for example, being most noticeable close to the coast.

Vegetation

Vegetation in this area is largely governed by climate and drainage characteristics of the soils. Dominant plant species include white and black spruce, larch, bearberry, labrador tea, dwarf birch, rye grass, lichens, mosses, arctic avens and so forth.

Sampling Sites and Soils

Soil samples were taken from three different sites in the Churchill region. Within each site one could find

examples of well and poorly drained soils, variations in vegetative cover and thickness of organic matter deposit, variations in depth to the permafrost level and so forth. Sample depths generally were shallow because of sampling difficulties encountered in water-logged areas and where abundant loose gravel and stones prevented the emplacement of sampling tubes. It should be pointed out that the 'active' zone in these soils seldom is deeper than 6 inches since, even in mid-summer, soil temperatures at this depth seldom exceed 2° to 3°C as a consequence of proximity of the permafrost. Most of the investigations reported herein are based on soil samples collected to a depth of from 4 to 9 inches. Where possible, soil cores up to a depth of 12 inches were collected for soil morphology comparisons.

Churchill Site #1 (Twin lakes Hill)

This site is located in a transition region between the tundra-like terrain of the coastal zone and the boreal forest zone which properly begins further to the south. The hill is about 15 miles south and slightly east of the town of Churchill and has an elevation of some 160 feet above the surrounding terrain. Good internal drainage is characteristic of this hill soil. Occasional surface and sub-surface drainage channels are made obvious by the lush growth of

sedge, moss and willow. At the foot of the sharp to moderately sloped terrace faces, there are poorly drained areas marked by the presence of a thick mat of spongy moss and heath which displays an exceedingly uneven surface contour. In spring and summer, the ground thaws to a depth of 12 inches on the lower and middle slopes and to a depth too great to measure at the top of the hill. Black and white spruce (Picea mariana and P. glauca) and larch (Larix laricina) predominate on the hill. The southern slope of the hill is covered by a thick mat of reindeer moss (Cladonia rangiferina and C. alpestris). Labrador tea and spruce make up the other dominant plants at the southern end of the hill.

Sample Number	Description of sampling sub-sites
TL-2, TL-8, TL-11	Soils developing under a mixed coniferous and deciduous vegetation and can best be described as being of a sand type. Samples were taken at the peak hill elevation of about 120 feet and from a well drained soil.
TL-3, TL-10	Samples taken at the base of the hill under a thick vegetation of mosses and lichens. Soils were morphologically similar to TL-1 and TL-8, except richer in organic matter and comparatively wet.

Site #2 (Old Beach)

The Old Beach sampling area lies approximately 60 air miles south west of the mouth of the Churchill River.

Soils of the beach are generally of the sandy type, carrying a varying depth of peaty organic matter and supporting a thin vegetative cover. The major vegetation, where it develops at all, is coniferous (mostly black spruce and tamarack). Poor drainage and high water table level are characteristic of the site. In the low area between the dunes where the water table is close to the surface, a relatively heavy growth of sedge and dwarf birch is common.

Sample Number	Description of sampling sub-sites
OB-1, OB-7	Collected from an area devoid of vegetation; sands with or without organic matter.
OB-4, OB-9	Poorly drained soils developed under deciduous shrubs. Surface soils similar to those of Twin Lakes Hill in morphology but wet due to high water table and poor drainage.
OB-5, OB-6	Represents peat deposits near the shore of a small lake. No vegetation over the deposit.

Site #3 (Goose Creek)

The Goose Creek area is about 10 miles below the mouth of the Churchill River. The soil structure is mainly clay and silt originating, in part, from alluvial deposition. The vegetative cover is composed mainly of grasses and low shrubs, i.e., buffaloberry, bearberry, and so forth.

Sample Number	Description of sampling sub-sites
GC-12, GC-13	Waterlogged to poorly drained soils; vegetation mainly grasses and shrubs clays and silt intermixed with partly decomposed plant residues.
GC-15, GC-16, GC-17	High organic-matter content, consisting mainly of black amorphous humic material, vegetation mainly shrubs.

The Inuvik Region

Physiography[1]

The Mackenzie delta is approximately 50 miles wide and 100 miles long. The delta is a low flat area interlaced and dissected by several large channels and numerous smaller meandering channels and is spotted with hundreds of stagnant ponds and lakes. Most of the area north of 68° 45' is treeless and so is wholly in the arctic. Inuvik is located on the east channel of the Mackenzie River about half way to the arctic coast from the head of the delta and about 30 miles south of Reindeer Depot, at 68° 21'N; 133° 41'W. The area represents tundra-boreal forest transition. The lower valley and the delta of the Mackenzie are built of alluvial materials and are forested with spruce and willow. The oldest known rocks in the region are possibly Proterozoic but more probable Cambrian to Silurain and are exposed near Inuvik with flanking younger Palaeozoic and Cretaceous rocks on both sides;

[1]This description is based largely on the reports of MacKay (1963) and Day and Rice (1964).

Mackay (1963). South of Inuvik are Devonian limestones. Bedrock hills rise 300 to 400 feet south of Inuvik as far as Campbell Lake.

The locality is under continuous permafrost according to Brown (1960) and in late August the depth of thaw ranges from 18 to 30 inches for clayey mineral soils with peaty cover to about 51 inches for gravelly soils with peaty cover; Day and Rice (1964). All the soil material appears to consist of unconsolidated to poorly consolidated sands, silts and gravels. The area has a rough microtopography and peat deposits are apparent in the depressions.

Climate

Although the region lies over 200 miles within the Arctic Circle, portions of the Mackenzie delta are considered to have a subarctic climate in terms of mean temperature for the warmest month, July (Aklavik, 56.4°F) and on the basis of tundra-forest delineation (Inuvik being some 30 miles south of the present tree line). Winter temperatures in the Inuvik-Aklavik areas tend to be slightly higher than those for the Fort Good Hope area to the south. This also applies to extreme temperatures which range from a low of -62°F at Aklavik to -79°F at Fort Good Hope; Mackay (1963). Continuous areal distribution of permafrost and mean annual

distribution of permafrost and mean annual air and ground temperatures of 16°F and 25.9°F respectively have been recorded in the region as reported by Brown (1960). Annual precipitation in the form of rain is low; around 10 inches at Aklavik. For the three consecutive years of 1949 to 1952, snow depths at Aklavik were below five inches; Mackay (1963).

Vegetation

The dominant plant species is white spruce. Also present are black spruce, willow and alder with scattered strands of tamarack. In the lower valley the vegetation is mainly deciduous and, along the river banks, growth of horse-tail, sedges and -rasses appear as soon as the Spring river levels subside.

Sampling Sites and Soils

The area examined at Inuvik includes the gently undulating river terrace and moderately sloping upland plain to the east.

Sample Number	Description of sampling sub-sites
Iv-X, Iv-Z	Samples were taken about 15 feet above mean Mackenzie River level near the town of Inuvik. These are alluvial clays and silts for the most part and are developing under a mixed vegetation cover, mainly deciduous.
Iv-Y	About 7 to 10 feet above mean river level. No vegetation cover; soils are characteristically deep reddish brown in color.

REVIEW OF LITERATURE

Although the volume of research literature dealing with microbial contributions to soil development in temperate and tropical regions has become very large over the last half-century, the amount of similar information pertaining to soils that develop under arctic and subarctic conditions is still very small. To some extent this disparity has been due to the relatively high cost of transportation and subsistence for research workers in the terrestial polar and subpolar regions. More importantly, perhaps, has been the general realization that such areas are not likely to be of significant agricultural importance for some time to come; thus, the impetus that stimulated soil microbiological research in the more temperate and inhabited zones has been lacking. Whatever the reasons are for this lack of research interest, the polar and subpolar regions still remain as some of the least known and understood areas of the world.

In high arctic and antarctic soils where extreme cold, low relative humidity, generally light precipitation and dessicating winds prevail for most of the year, the entrance or at least the meaningful activity of the 'biotic' element is lacking. Here, one can assume that soil-building processes are proceeding mainly, if not entirely, under the stimuli of what can be called "simple weathering".

In the subpolar regions, however, particularly in the Northern Hemisphere there are transitional zones where climatic conditions permit the entrance of the biotic element to a significant extent in terms of soil development. Although the climatic environment is still rigorous and although the periods of biotic activity are restricted to the short summer interval, contributions by microorganisms to soil development are positive and easily detected.

Answers to questions concerning arctic and antarctic microbiology began to emerge some 60 years ago with the aquatic studies of Gran (1901) and Hesse (1914) which established on a qualitative basis the presence of nitrifying and denitrifying bacteria in the Arctic Sea and from waters off the coasts of Norway, Iceland and Spitzbergen. As well, in 1914 there appeared a comprehensive monograph by Isachenko which dealt with the microflora of the mud and waters of the Barents Sea. This author reported the presence of aerobic and anaerobic nitrogen fixers, ammonium and nitrite oxidizers and denitrifying bacteria. His publication may be considered as one of the first cornerstones upon which Russian research interest in the microbiology of arctic and subarctic soils has developed. Indeed, over the next several decades the principal contributions to our knowledge of arctic soil microbiology have come from the Soviet Union

with the exception of relatively small but significant contributions by Danish and Scandanavian scientists working in Greenland and the northern Scandanavian countries (Bartel, 1922; Russel, 1940; Favre, 1959).

Following the publication of Isachenko's monograph, the next major Soviet contribution appeared in 1932 when Kazanski reported on a qualitative survey of soils from Nova Zemlya which showed the presence of aerobic and anaerobic cellulose decomposers, nitrifiers, denitrifiers, nodule bacteria and anaerobic asymbiotic nitrogen fixers. Further qualitative studies were reported by Bukevich (1932) on the low optimum growth temperatures of polar soil organisms; by Isachenko and Simokova (1934) dealing with similarities in species from the arctic with those from more temperate regions, and by Levinskaya and Mamicheva (1936) working on quantitative estimates of soil micro-organisms in soils of the Murmansk area of the Soviet arctic. The latter investigators reported seasonal changes in numbers of soil organisms with winter counts as low as 1×10^5 organisms per gram.

Kriss (1940) reported on qualitative studies of soils of the Kolyuchin and Wrangel Islands wherein physiological groups similar to those reported earlier by Kazanski (1932) were found, but included evidence for the presence of

Azotobacter species.

More recently, extensive investigations in the Soviet subarctic have been carried out by Rybalkina (1957) and by Sushkina (1960). In the latter report based on soils of the Zemlya Franz Joseph area, Sushkina noted that the temperature ranges for optimal growth of soil micro-organisms from this region were rather narrow, from 15°C to 20°C; and, as such, represented much narrower ranges than those reported for soil organisms from the Moscow district, i.e., from 20°C to 34°C. Sushkina further reported that the numbers of colonies appearing when the incubation temperature was 3°C represented usually less than 10 percent of those which developed at temperatures in the 15°C to 20°C range. It is interesting to note that Sushkina was unable to isolate any filamentous fungi from the Zemlya Franz Joseph soils although bacterial counts ranged up to 1.4×10^5 per gram and actinomycetes were present to the extent of 4×10^4 per gram of soil.

In the interests of brevity, only a few of the many Soviet papers have been included in this review; for a more comprehensive listing, the Arctic Bibliography is recommended as the most authoritative index of Soviet contributions to arctic soil microbiology.

Of more pertinent interest to North Americans were

the investigations of Setchell and Collins (1908) and Howe (1927) on the algal flora of Hudson Bay and adjacent areas. Their reports indicate the dominence of Nostoc species which now are recognized as photosynthetic nitrogen fixers.

Further interest in the microflora of the subarctic Hudson Bay area was not apparently forthcoming until 1942 when James and Sutherland reported the presence of micro-organisms comprising physiologically different groups from soils taken in the proximity of Churchill, Manitoba. Their studies were limited mainly to the qualitative and vertical distribution of soil micro-organisms. Although their results were not conclusive for clay soils, they encountered viable micro-organisms in gravel deposits to a depth of nine feet (normally well below the permafrost line). They postulated that this could have resulted from percolation of surface waters during the summer retreat of the permafrost. In soils above the permafrost layer James and Sutherland were able to identify anaerobic bacteria and cellulose decomposers.

The few earlier reports on the microfloral populations of subarctic Canada were concerned with isolations of specific groups of micro-organisms and little attention was given to quantitative evaluations or other considerations. Indeed, the only comprehensive quantitative study thus far carried out in this region of Canada was by Ivarson (1965).

This investigator reported on three different soil profiles from the Mackenzie valley and concluded that arctic soils supported a rather large microfloral population. The highest viable counts of bacteria, actinomycetes and molds were 1.7×10^6, 2.3×10^6 and 0.4×10^6 per gram of soil respectively. As well, he examined microbial temperature relationships and found that the maximum number of colonies developing on plates occurred for a given medium where 25°C was selected as the incubation temperature. The ratios of colony numbers developing at 25°C as opposed to 4°C were approximately 3:1 for bacteria, 4:1 for actinomycetes and 2.5:1 for filamentous fungi. From these results he concluded that the actinomycetes were more sensitive to low temperatures than either the bacteria or fungi.

A number of largely qualitative studies have been carried out on soils in the vicinity of Point Barrow, Alaska and in the western arctic. These include estimates of psychrophiles, mesophiles and thermophiles by McBee and McBee (1956) and by Boyd (1958). The presence of thermophilic species found deep in the permafrost layer in some instances was quite unexpected. Where found in surface soils, their presence has been attributed to contamination by man and animals in the recent past. The presence of viable thermophiles at depths of several meters into the

permafrost (well below the annual summer retreat) is more difficult to account for. Either these cells have remained viable in a resting stage since the last warm period in the arctic (circa. 900 to 1100 A.D.) or have entered by percolation of contaminated surface waters through fissures during the summer months. The question of whether thermophiles should be considered as part of the 'normal' flora remains unanswered. Brockman and Boyd (1963) have also reported the presence, qualitatively, of members of the Myxobacteria in Alaskan and Canadian arctic soils.

In terms of arctic soil fungi, the review by Singer (1954) is considered the most comprehensive thus far. He concluded that all four Classes of fungi occur in the arctic and subarctic, probably in approximately the same proportions as they do in soils of warmer climates. The number of different species, however, tends to be smaller and members of the Ascomycetes and Basidiomycetes predominate. In part, this opinion was confirmed by Cooke and Fournelle (1960) and by Ivarson (1965).

In recent years, considerable interest has also developed in antarctic soil microbiology. The areas most extensively studied include Ross Island and McMurdo Sound (Boyd and Boyd, 1963), Taylor and Wright valleys (Boyd, Staley and Boyd, 1966) and Little America (Darling and Siple, 1941).

Boyd and Boyd on Ross Island found viable counts ranging as high as 9.8×10^7 for bacteria and 6.4×10^4 for fungi per gram of soil while in the non-glaciated, a humic soils of the Wright and Taylor valleys microbial populations were much lower; in fact, a number of the soil samples collected were essentially sterile. Straka and Stokes (1960) working near one of the American research stations in Antarctica found psychrophile to mesophile population ratios (organisms growing at 0°C and 30°C incubation respectively) to be as high as 1:2000. They considered this high proportion of mesophiles to psychrophiles to reflect the adaptation of introduced (as opposed to native) mesophilic organisms to a life at below normal temperatures. A similar finding on rations of psychrophiles to mesophiles was reported by Boyd, Staley and Boyd (1966) for soils of Ross Island and the Taylor and Wright dry valleys.

Enrichment studies on the Ross Island soil samples showed, to a varying degree, the presence of iron oxidizers, photosynthetic nonsulfur organisms, heterotrophic nitrogen fixers, ammonium oxidizers and sulfate reducing bacteria. At McMurdo Sound, evidence of ammonification, weak nitrification and nitrate reduction in soils was obtained. Although an Azotobacter species was present in some of the soil samples no nitrogen fixation could be demonstrated "in situ". Earlier, Darling and Siple (1941) had concluded

that in soils of Little America the indigenous species represent low temperature adaptation forms of species commonly found in soils of more temperate climates. In soils of the Australian antarctic, Bunt and Rovira (1955) found that most bacterial isolates grew optimally over a temperature range of 10°C to 25°C but a much higher proportion grew optimally at 25°C than at 10°C.

Where soils as a "whole system" have been examined, as by Bunt and Rovira (1955) in Antarctica and by Douglas and Tedrow (1959) in Alaska, soil respiration and CO_2 evolution were shown to be slow. It is interesting, however, that respiration intensities at 25°C are relatively higher for antarctic soils than for sub-tropical soils incubated at the same temperature. On this evidence, Bunt and Rovira conclude that low temperature adaptation has occurred among the cold-soil organisms enabling them to function better under their environmental temperatures than would otherwise be supposed.

PART I

GENERAL PHYSICO-CHEMICAL AND MICROBIAL CHARACTERISTICS OF SOIL

INTRODUCTION

Micro-organisms, it is generally agreed, assume a dynamic role in soil development except in a situation (temporarily, at least) where the soil is frozen for extended periods of time or where soils are more or less completely dry. This role, however, is continually changing both in a qualitative and in a quantitative sense as fluctuations occur in the soil environment. Since physical and chemical factors play a large part in determining the environmental nature of the soil and these factors are subject to change, one can not say that the soil environment is uniform. Rather, it is an intricate, interwoven biological system composed of a myriad of micro-environments (Stevenson, 1964).

It was considered essential, therefore, to provide information on the physico-chemical nature of these soils developing under extremely cold-climatic conditions before any evaluation was made of the microbial statics of the soil.

Soil Characteristics

Though several reports are available on the characteristics of soils from the tundra and subarctic regions of Canada (Day and Rice, 1964; Beschel, 1961; McMillan, 1960; Wright et al, 1959; Leahey, 1954, 1957, 1947; Lajoie, 1954),

little is known about the nature of soil development in the Churchill region of Northern Manitoba, the exception being a report available by Brown Beckel et al (1954) on the major terrain types.

Studies on the characteristics of permafrost soils in the Mackenzie Valley (N.W.T.) by Day and Rice (1964) and in Alaskan Tundra by Hill and Tedrow (1961), Douglas and Tedrow (1960), Tedrow and Cantlon (1958), and Tedrow et al (1958) concluded that under well-drained conditions a remarkable degree of similarity exists in the morphological features of these soils throughout the cryopedogenic region in North America. Across a distance of 35° of latitude, there appears to be no single characteristic or combination of characteristics that will permit a distinction, above the series level, between the well-drained cold soils of the arctic and those in mountains at mid latitudes (Retzer, 1965). Retzer further suggested that well-drained cold soils represent a zonal type of development; and because the time for development under the unique climatic controls has been short, the surface layer (A_1) is the most altered. Day and Rice (1964) showed that soil that had developed under the influence of poor drainage had an A_h horizon high in organic matter and he classified it as a subarctic peaty carbonated Rego Humic Gleysol (in the Canadian System, 1963).

The present investigation, though confined and restricted to a relatively few analyses, covers a range of morphologically different types of soils. No attempts are made to classify or map the soils.

Microbiological Characteristics

Results of the studies thus far conducted on the soils of polar regions have suggested the occurrence of bacteria to be ubiquitous. Some earlier as well as more recent reports (Isachenko and Simakova, 1934; Darling and Siple, 1941; McBee and McBee, 1956; Boyd, 1958; Flint and Stout, 1960; and Straka and Stokes, 1960) commonly agree that the bacteria in arctic soils are, in reality, variants of species quite common in temperate zones. As such, they represent merely adaptation to low temperature conditions. Further, data reported by Sushkina (1960) and Straka and Stokes (1960) dealing with soil samples of Soviet Arctic and Alaskan areas respectively suggest that soil bacteria are adapted in a varying degree to low temperatures.

However, the significance (in a statistical sense) of micro-organisms that are able to grow at low temperatures in the natural environment is not completely clear since the amount of data on the number of cold-adapted organisms in the natural habitat is limited. The microbial population of subarctic and arctic soils of Canadian North has been

investigated only to a limited extent (James and Sutherland, 1942; Brockman and Boyd, 1963; Ivarson, 1965) and these reports tend to cover, largely, points of purely taxonomical interest. In the present investigation, attempts are made to give a comprehensive characterization to the microbial population in terms of their distribution, abundance, ecological significance and functions in the quite different and diverse groups of soils of the Churchill and Inuvik regions. Fungi and actinomycetes, which have been largely neglected by the majority of the investigators in Arctic Microbiology have received considerable attention.

Biological Activities

It is most difficult to evaluate, from numbers alone, the ecological significance of micro-organisms in a given habitat. Although the plate-count is still widely used for this purpose, it does not necessarily reflect the biological state of the organisms as they exist in the soil. Although studies of the chemical changes brought about by isolated soil organisms (examined in pure culture) are of obvious importance in the understanding of the chemical potentialities, this method does not provide for an evaluation of the biological processes as they occur in the complex soil system (Quastel, 1965). In the present investigation, therefore, the data on microbial populations are supplemented

with several examples of biological activities occurring in the soils. These examples include measures of respiration intensity, enzymic activity, ammonification, nitrification, and denitrification all based on 'whole' soil systems.

MATERIALS AND METHODS

Soil Sampling

Soils were sampled principally according to the recommendations of Johnson et al (1959). Before sampling, fresh leaf litter not mixed with the soils was removed. Initially, core samples were taken using 3 inch steel sampling tubes from which the soil core was subsequently removed by a hydrolic piston device.

Composite samples used later in the investigation were taken from depths ranging to 4 to 8 inches below the fresh leaf litter, mixed and stored temporarily in polyethylene bags. The depth of sample in this case depended on the soil type, vegetation cover and proximity of the permafrost to the surface.

Preparation of samples for analysis

Preparation (a)

The day of arrival at the laboratory samples were spread separately over Saran paper in a constant temperature chamber at 13 $\pm$ 0.5°C. Living roots and germinating seedlings, if any, were removed and samples were left over-night for partial drying. Samples were then evenly mixed and sieved through a 2 mm sieve, the portion retained in the sieve was weighed and corrections were made in this

respect when calculating the analytical results. Precautions were taken to minimize contaminations from external sources.

Soil passed through the sieve was remixed and distributed aseptically in small quantities (100 to 500 g) in air-tight polyethylene bags. Unless otherwise stated, all the microbiological analyses were made on this preparation without any further storage. Where necessary, samples were stored at -20°C. Each small quantity thus represented a subsample of the soil in question and individual samples could then be withdrawn from frozen-storage for the various analyses, without disturbing the remainder. Samples once thawed were not refrozen, but were used for experimental purposes and then discarded.

Samples other than the composite ones received similar treatments.

Preparation (b)

Small subsamples from preparation (a) of each soil were redried at room temperature for several days and stored in polyethylene bags at room temperature until required.

Preparation (c)

Small portions (approx. 20 g) of preparation (b) of the different soils were separately ground by a mechanical grinder to a particle size small enough to pass through a 100 mesh sieve. These ground samples were stored in polyethylene bags at room temperature.

Soil Physical and Chemical Determinations

Moisture, pH and hygroscopic moisture

Moisture contents of the fresh samples were determined soon after the arrival of the soils in the laboratory. This was done by drying a known wet-weight sample at 105°C in a simica crucible for 12 hours; moisture percentage, before and after drying, was determined by weight loss. Hygroscopic moisture was similarly determined on air-dried samples of the soils. Values of pH for the fresh samples (preparation (a)) were determined with a radiometer (Type PHM 22r) on the basis of 1:2.5 aquous soil suspension after holding for 30 minutes at room temperature. Soil samples equivalent to 10 g oven-dry weight were used for this purpose and radiometer readings were taken repeatedly until a constant value was reached in each case.

Total Nitrogen (organic and mineral)

Total organic nitrogen was determined by the semi-microkjeldahl procedure of Bremner (1960), modified as follows: One gram of soil (preparation (b)) in a micro-kjeldahl flask was digested for three hours using the digestion mixture of Bremner (6 ml H_2SO_4; 2.0 g K_2SO_4; 0.2 g $CuSO_4$ and 20 mg Se powder). After digestion, flasks and contents were cooled to room temperature and the digest

in each case was taken up with 20 ml distilled water in a 50 ml volumetric flask. To ensure complete transfer, a further 15 ml of distilled water was carefully added to each flask 5.0 ml at a time, rinsing the inner surface of the flask neck. All the washings were combined with the digest and the collected volume was made up to a total of 50 ml. After thorough mixing, 5.0 ml of a 20% sodium hydroxide solution was added. Distilled ammonia was received in 125 ml Erlenmeyer flasks each containing 3.0 ml of a boric acid-indicator mixture*. A total of 20 ml of distillate was collected in each case and titrated against 0.01 N potassium biiodate [$KH(IO_3)_2$] solution delivered by a micro-burette. A blank containing all the reagents except soil was treated in the same way as the samples. Milligrams of nitrogen per sample were calculated according to the following equation:

mg N in 5.0 ml digest = 0.14 (A-B),

where, A = ml 0.01 N biiodate to titrate the sample,
B = ml 0.01 N biiodate to titrate the blank.

---

*2% Boric acid and Indicator: 10 g Boric acid + 5 ml of mixed indicator made to 500 ml.

Mixed indicator: 0.2% methyl red in 95% ethanol
0.2% methylene blue in 95% ethanol

Mixed in a ratio of 2:1.

Results were calculated by taking into consideration the dilution factor and the moisture content of the soils. All determinations were conducted in duplicate and the average values were presented as the percent nitrogen content on an absolute dry weight basis.

Mineral Nitrogen

Ammonium, nitrite and nitrate nitrogen were determined in 1 N KCl extracts (Bremner and Shaw, 1958) of the soils (Preparation (a)) as follows:

To 10 g of soil in a 250 ml flask was added 100 ml 1 N KCl solution. Flasks were corked and shaken on a mechanical wrist shaker for two hours at room temperature. Finally, the contents of each flask were filtered separately through Whatman #44 filter papers over a Buchner funnel under mild suction.

Ammonium was determined following the formation of indophenol blue (Crowther and Large, 1956) using the reagents*

---

*Sodium phenate (12.5% w/v) was prepared by mixing the following solutions (a) and (b) in 20 ml amounts each and making up to a final volume of 100 ml with distilled water.

(a) 62.5 g phenol (crystalline) dissolved in smallest amount of ethanol was added to 2 ml methanol and 18.5 ml acetone and finally diluted to 100 ml with ethanol. The solution was stored in refrigerator.

(b) Sodium hydroxide (27% w/v) solution in distilled water. Hypochlorite solution: NaOCl diluted with distilled water to yield a final concentration of 0.9% active chlorine.

and procedure described by Hoffman and Teicher (1961). The procedure was adapted to these determinations as follows:

Aliquots of filtrate were brought to a volume of 1.0 ml with 1 N KCl solution and to this were added 0.4 ml phenate solution and 0.3 ml of hypochlorite solution. After 20 minutes incubation at room temperature, the volume was made up to 5.0 ml by adding 3.3 ml of distilled water. A control reagent blank was similarly prepared except that soil extract was replaced by 1.0 ml 1 N KCl. After shaking, aliquots were removed from the reaction vessels and transferred to glass cuvettes (1.0 cm light path) for optical density measurement by a Unicam SP 700 recording spectrophotometer. Optical density was measured at 580 mμ in comparison with the reagent blank. A standard curve was prepared following the above procedure using known concentrations of nitrogen in the form of ammonium sulphate. This standard curve, Fig. 1, (coordinate as optical density, abscissa as μg-N) is linear up to 10 μg-N. Under the test conditions an O.D. value of 0.427 corresponds to 10 μg ammonical nitrogen.

Nitrite was determined by the sulfanilic acid procedure of Bratton et al (1939). The reagents consist of 0.02% N-(1-naphthyl) - ethylene diamine dihydrochloride in distilled water and 1.0% sulfanilic acid in 20% HCl. Aliquots of sample (1 to 5 ml) were brought to a volume of 6.0 ml with distilled water in Klett tubes and to this were

Fig. 1. Calibration curve for ammonia nitrogen.

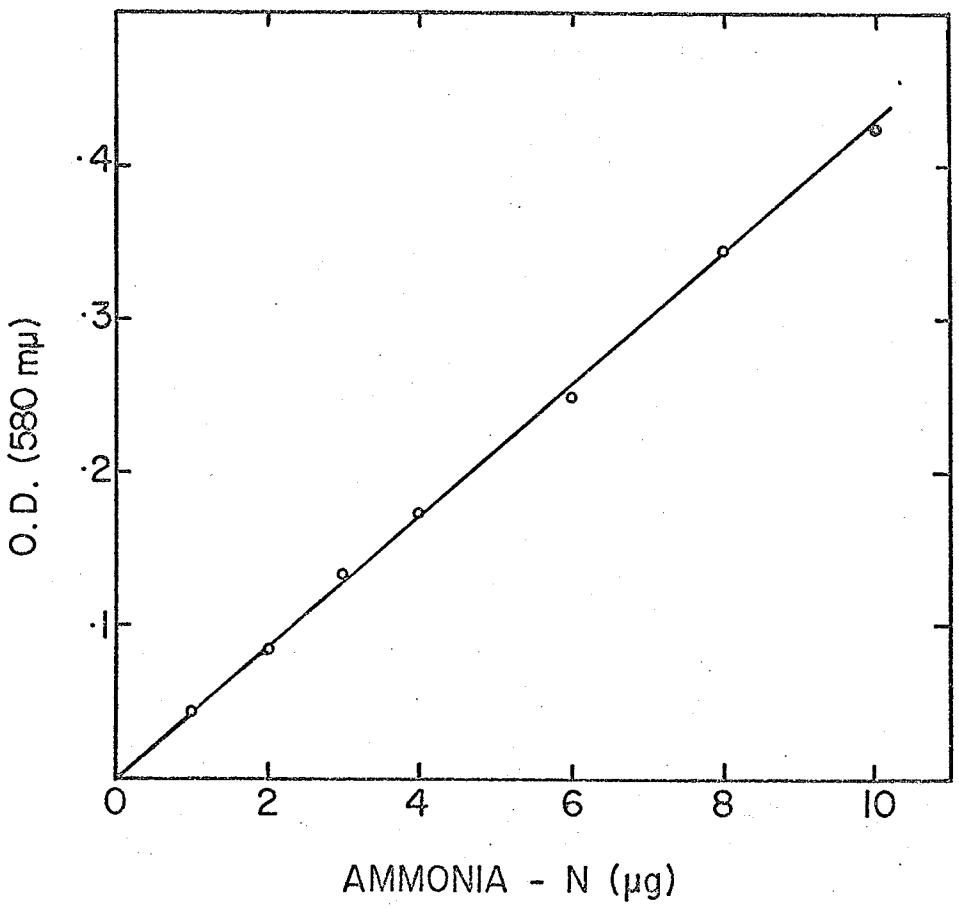
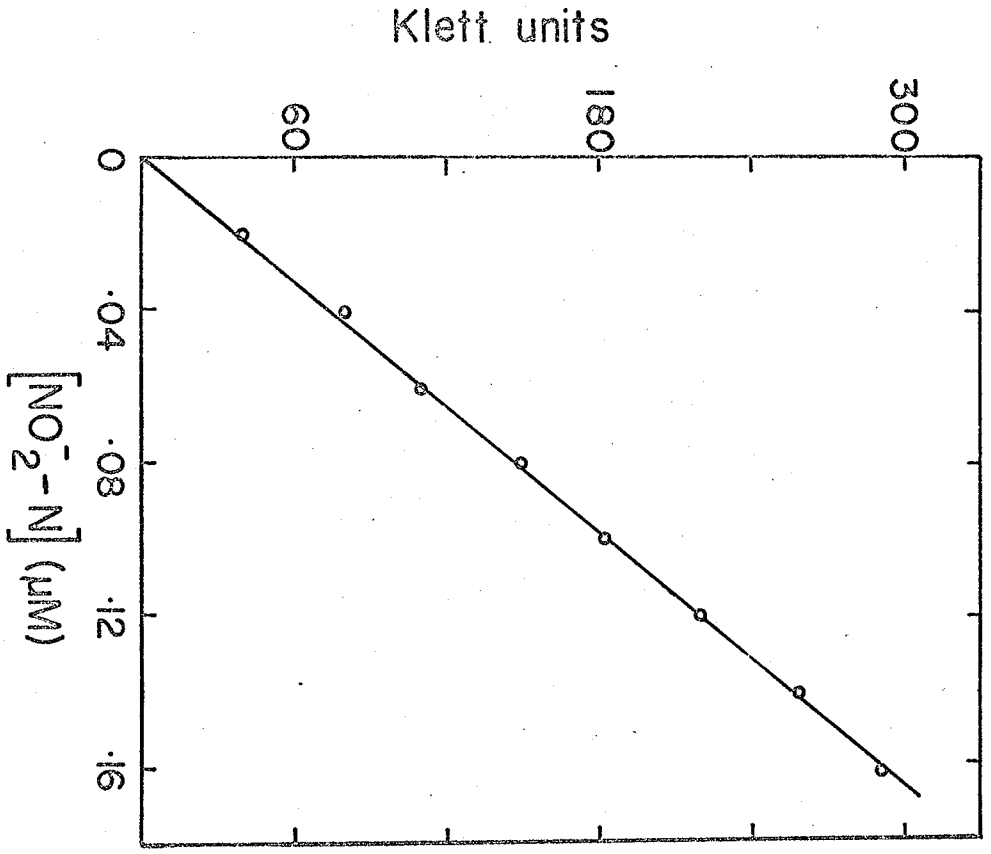
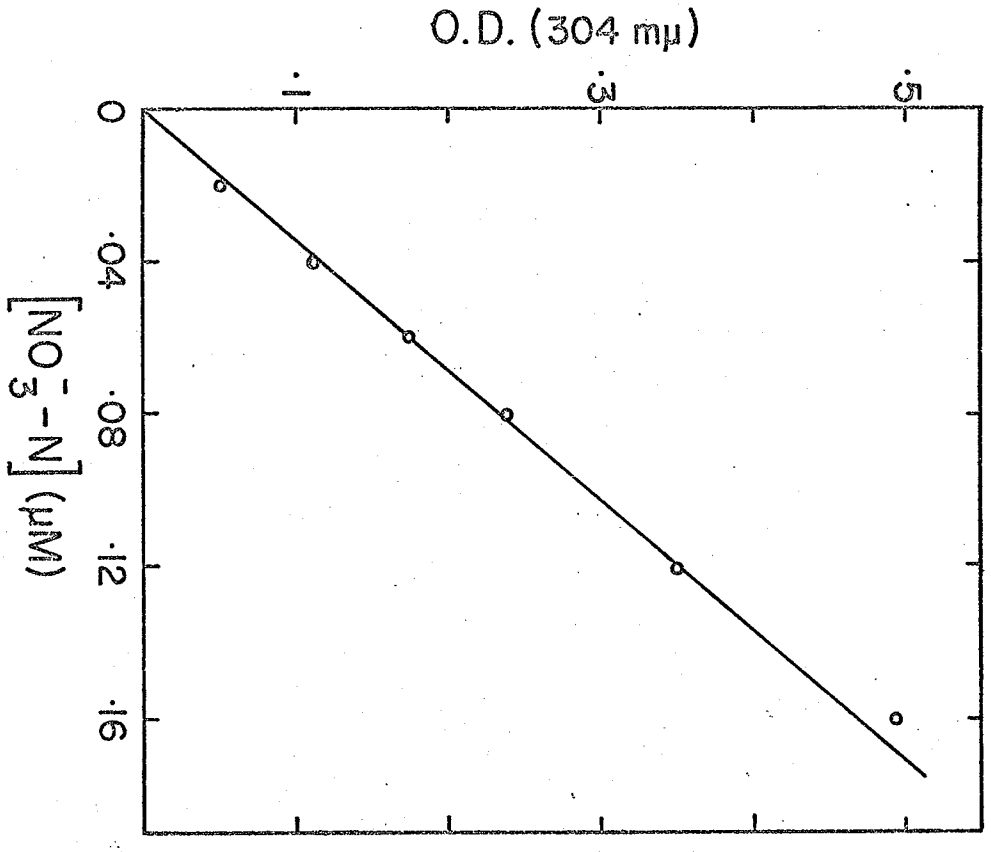

O.D. (580 mμ)
·1
·2
·3
·4
0
2
4
6
8
10
AMMONIA - N (μg)

Fig. 2. Calibration curve for nitrite-nitrogen.

Fig. 3. Calibration curve for nitrate-nitrogen.


Klett units
60
180
300
0
·04
·08
·12
·16
[NO2-N] (μM)


O.D. (304 mμ)
·1
·3
·5
0
·04
·08
·12
·16
[NO3-N] (μM)

added 0.5 ml of the sulfanilic acid solution followed by 0.5 ml of N-(1-naphthyl) - ethylene diamine diHCl solution. Contents of the Klett tubes were then mixed and, precisely after 20 minutes incubation at room temperature, the volume was made up to 10.0 ml in each case. Color intensity was then measured at 540 mμ in a Klett-Summerson colorimeter. A standard curve was prepared using known concentrations of KNO_2, Fig. 2. Under the experimental conditions used, 1.0 mμ mole nitrite corresponds to 1.82 Klett units.

Nitrate nitrogen was determined spectrophotometrically by the method of Montgomery and Dymock (1962). Nitrite present in the sample was removed by reacting the sample with sulfamic acid-treated papers. The reagents consisted of 80.5 - 83.3% (W/W) sulphuric acid (455 ml 98% H_2SO_4 mixed with 17 ml deionized water) and 100 ml 2,6-dimethyl phenol solution (0.244% in glacial acetic acid) and 50 ml aquous ammonium chloride solution (24% W/V). Sulfamic acid papers were prepared by soaking Whatman #5 filter paper discs (16 equal segments cut from 5.5 cm filter disc) in a 50% aquous solution of sulfamic acid. The pieces were allowed to air-dry and were stored in a stoppered bottle.

To 1.6 ml of the H_2SO_4 (cooled to 0 to 10°C) in pyrex tubes were added 0.1 ml of the sample and 0.1 ml of 2,6-dimethyl phenol reagent. After five minutes, 3.0 ml of

deionized water was added and the mixture was incubated at room temperature for a further 15 minutes. Then, the optical density was measured at 304 mμ by a Unicam SP 700 recording spectrophotometer using silica cuvettes (1.0 cm light path). If nitrite was present in the sample, it was removed prior to nitrate determination by adding to the tube discs of sulfamic acid-treated paper (one disc per 0.1 mg of nitrite-nitrogen). After a five minute reaction time, samples were withdrawn for nitrate determination. A standard curve was prepared using known concentrations of KNO_3, Fig. 3.

Organic matter, easily hydrolysable carbohydrates and humic acids.

Organic matter was determined on soil preparation 'c' of each sample collected by the wet-combustion procedure of Walkley (1947). Total organic carbon was calculated by dividing the observed organic matter value by the conventional carbon to organic matter factor of 1.724.

For the determination of carbohydrates, soil hydrolyzates were prepared using air-dried soils (preparation 'c') by the procedure of Brink et al (1960). Hydrolysis was performed at 85°C for 24 hours using 10 ml 3N H_2SO_4 per gram of soil in 250 ml Erlenmeyer flasks. After hydrolysis, contents of the flasks were filtered through fritted filter discs and the residue thereon was washed with hot water (10 ml per gram

Fig. 4. Calibration curve for hexose sugars.

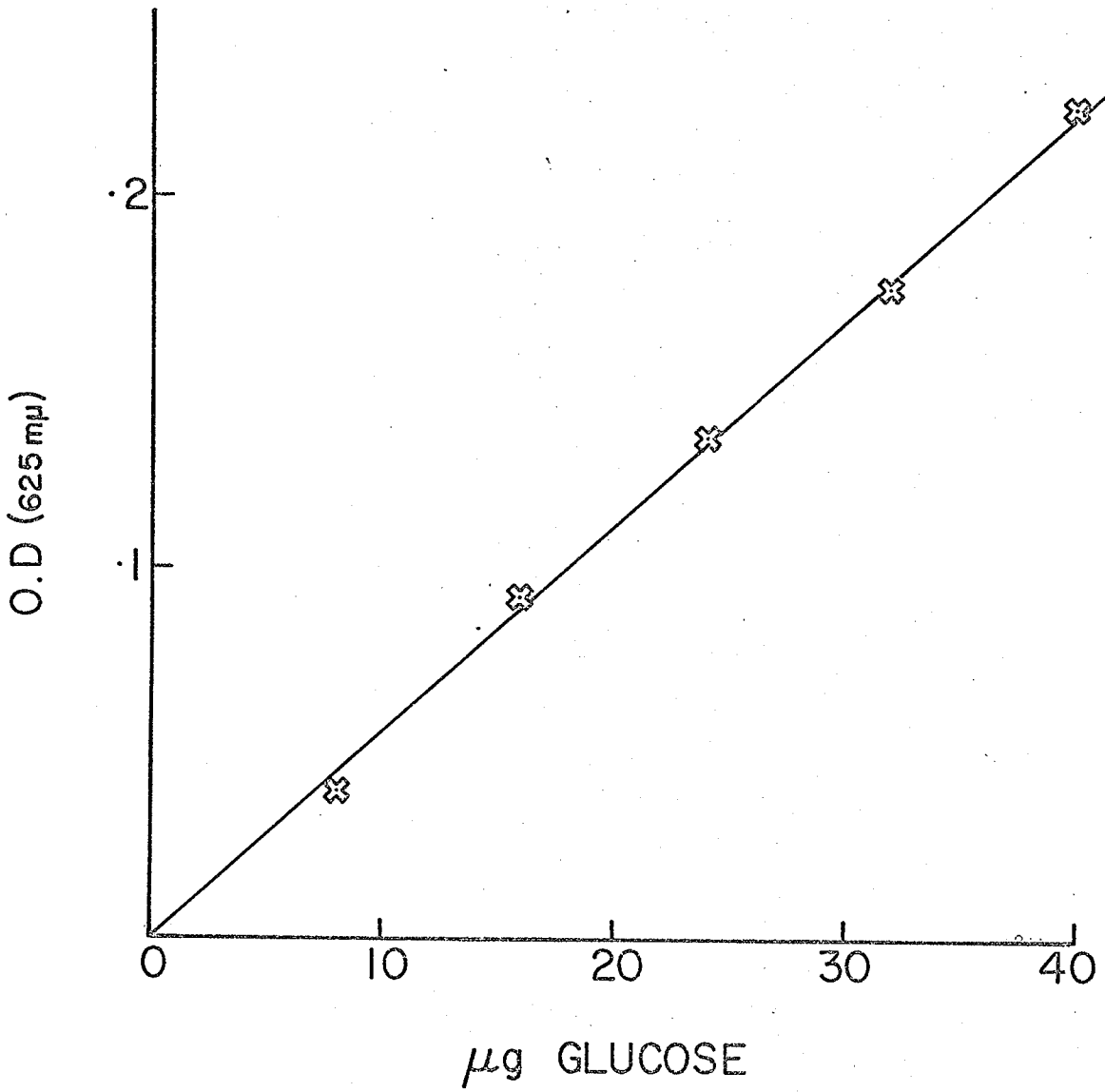

O.D (625 mμ)
·2
·1
0
10
20
30
40
μg GLUCOSE

Fig. 5. Calibration curve for pentose sugars.

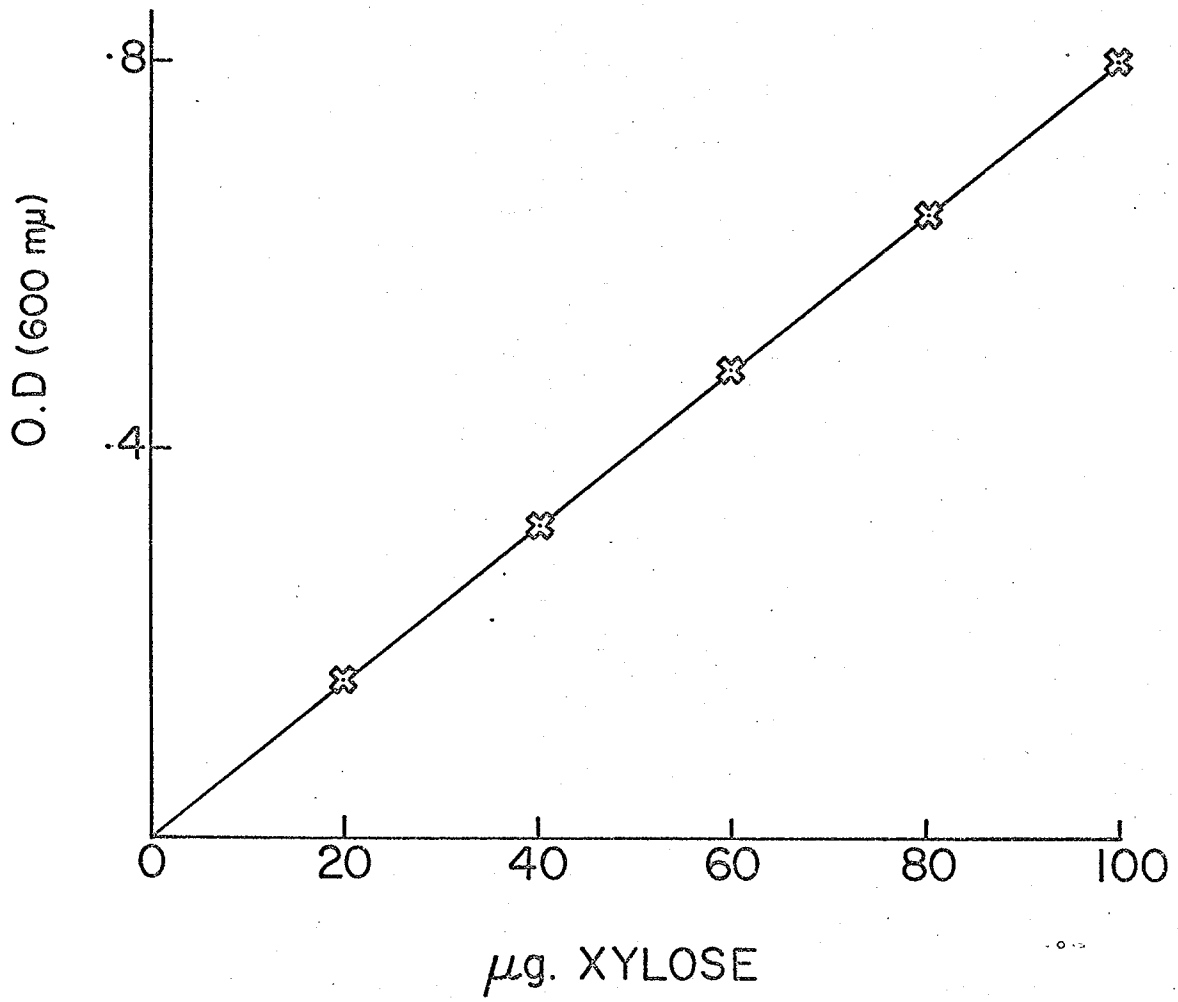
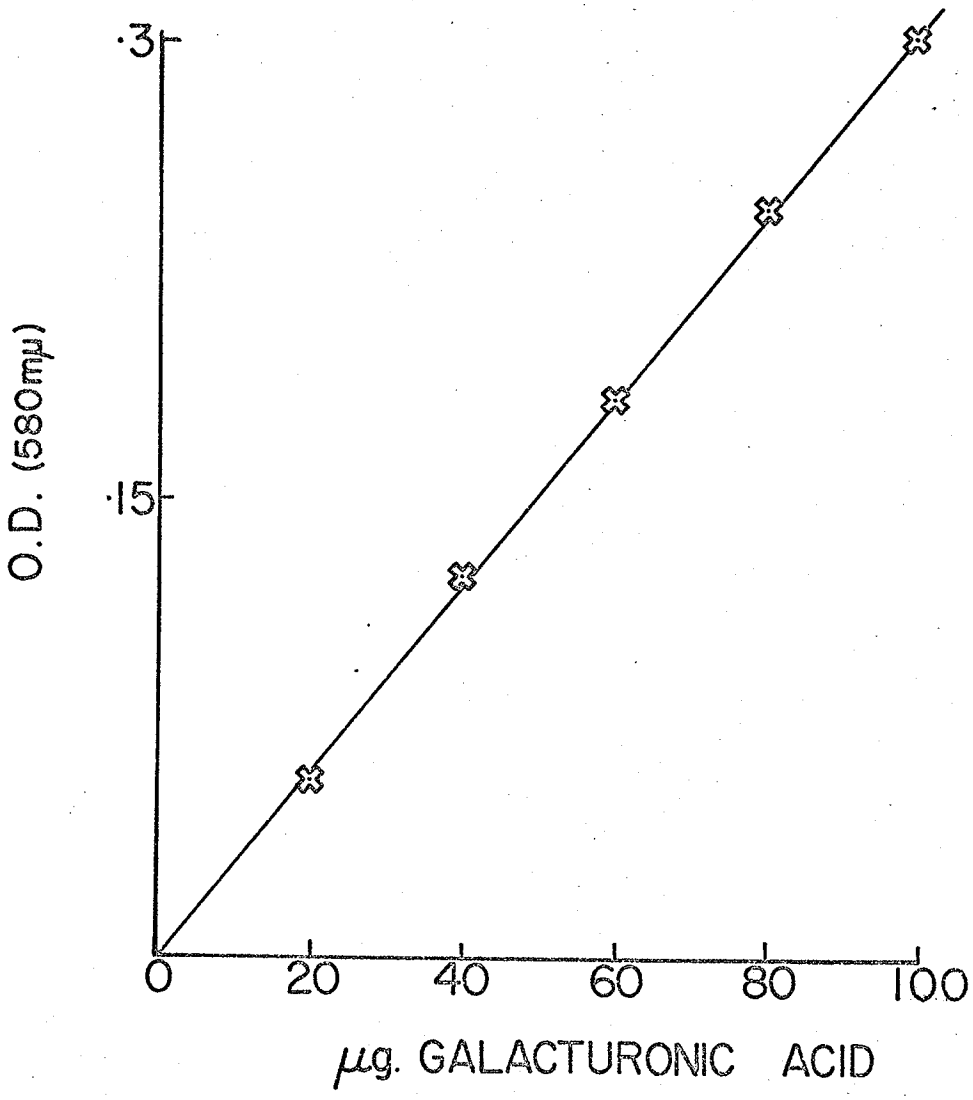

.8
.4
O.D (600 mμ)
0
20
40
60
80
100
μg. XYLOSE

Fig. 6. Calibration curve for uronic acids.


O.D. (580mμ)
.3
.15
0
20
40
60
80
100
μg. GALACTURONIC ACID

of soil). Filtrates were cooled to room temperature and then treated with $CaCO_3$ to raise the pH to 6.8. Following pH adjustment, samples were centrifuged (1085 X g for 10 minutes) in nalgene centrifuge bottles. Clear supernatants were brought up to 200 ml in volumetric flasks with distilled water. Appropriate dilutions of this hydrolyzate were used for carbohydrate analyses.

Total hexose sugars were determined by the anthrone method of Brink et al (1959), pentose sugars by orcinal reagent as reported by Thomas et al (1961) and uronic acids by carbazole reagent, Lynch et al (1957). For each group of sugars, representative monosaccharides, e.g., glucose for hexoses, xylose for pentoses and galacturonic acid for uronic acids were used for preparing standard curve data, Figs. 4, 5 and 6.

Humic acids (humic + fulvic acids) were determined in the sodium pyrophosphate extract of the soil according to the method of Aleksandrova (1960).

Cation-exchange capacity and exchange acidity.

The procedures of Mehlich (1948) and Peech et al (1947) as outlined by Chapman and Pratt (1961) was used for determinations of cation-exchange capacity and for total exchange acidity estimations using 10 g of air-dried soil (preparation 'c').

Total exchangeable metallic cations and exchange status

Total exchangeable metallic cations was determined by the equilibrium pH method of Brown (1943) as outlined by Jackson (1958). The soils used were preparation 'c'. The meq. exchangeable metallic cations per 100 g of soil was calculated by the formula of Brown (Jackson, 1958). Exchange status (Percentage base saturation) was calculated as follows:

$$\% \text{ Base saturation} = \frac{S}{T} \times 100$$

where S = Total exchangeable metallic cations as meq per 100 g of soil and

T = Cation-exchange capacity as meq per 100 g of soil.

Determinations of General Biological Activities

To assess what might be called the general biological activities of intact or whole soils, a series of determinations were carried out which included a measure of soil respiration, ammonification, nitrification, denitrification, ureolysis and, finally, assays for the presence of various carboyhydrases in the soils.

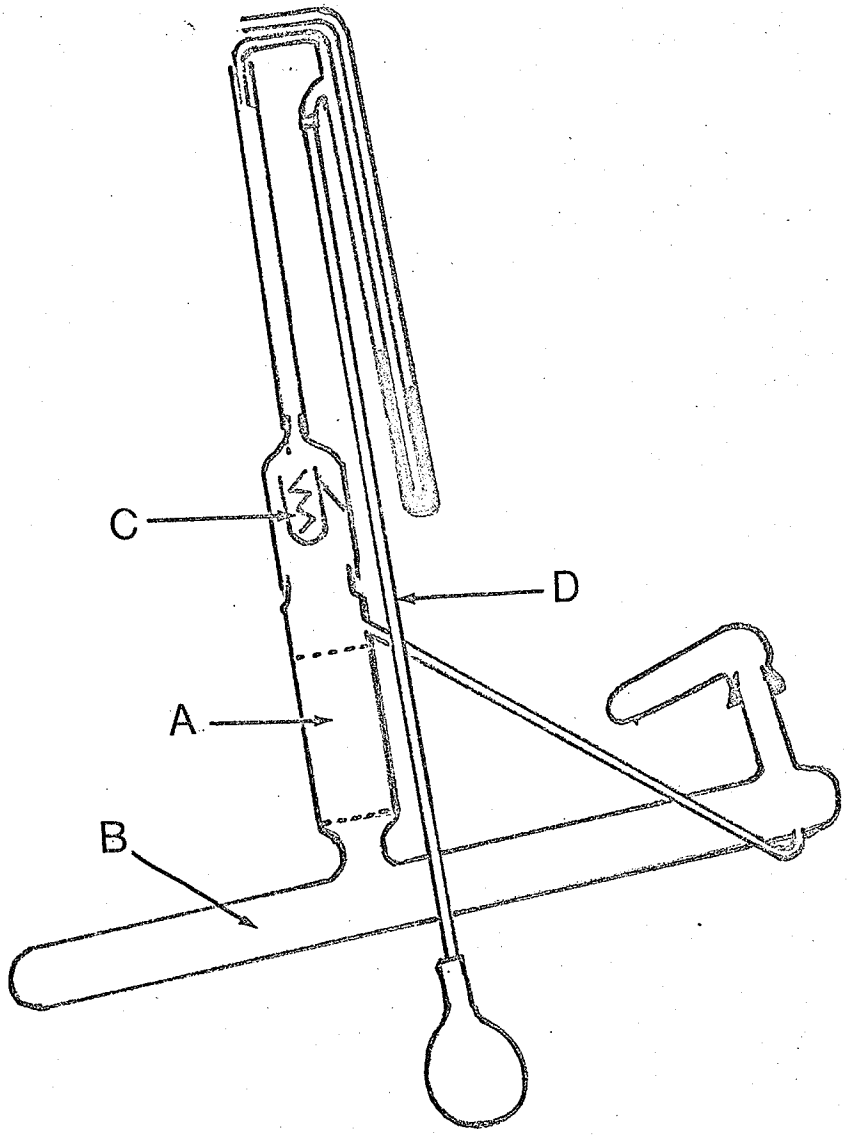
Soil respiration

Soil respiration as an index of the total mineralization capacity of the soils was estimated under a closed percolation system. The rocking percolator according to the design of Greenwood and Lees (1956) was constructed in the Science Technical Laboratory under the supervision of Mr. Gordon Trider. A photograph of the assembled equipment appears in Fig. 7 as a schematic diagram.

Endogenous respiration in the soil samples was measured in comparison with a sterile sand column control. This control column received the same treatment accorded to the soil columns under study. The set-up procedure may be briefly described as follows:

Soils and sterile sands (equivalent to 10.0 or 20 g oven-dried weight) were loosely packed to give uniform columns in the vertical chamber, marked A, in Fig. 7. To the horizontal reservoir, marked B, was added 50.0 ml of sterile water; the apparatus was reassembled and percolation of the fresh column was carried out for 15 minutes. After moisture stabilization had occurred in the soil column, a further amount of sterile water was added to bring the reservoir volume up to 50.0 ml. At this time, 2.0 ml of 40% sodium hydroxide was introduced into the plastic cups, marked C, which held fluted filter paper strips.

Fig. 7. A schematic diagram of Rocking Percolator. A single unit of the assembly.


C
D
A
B

This portion of the assembly constituted the trap for evolved CO_2.

The percolator was reassembled and following an equilibrium percolation interval of 30 minutes, readings of O_2 uptake, as measured by water displacement in the calibrated tube, marked D, were taken every hour for a period of five hours.

Ammonification and nitrification

Endogenous ammonification and nitrification capacities of the soils were determined on the bases of three replicates of each soil incubated separately at 15 $\pm$ 0.5°C over a period of 15 days. Soil sample replicates equivalent to 10.0 g oven-dried weight of soil were uniformly spread over the bottom of 250 ml conical flasks without packing. A pre-calculated volume of sterile distilled water was carefully added to each flask to bring the soil sample in question to 60% of its moisture holding capacity. Flasks were plugged with cotton and each then placed in a 2 litre beaker containing 50.0 ml of tap water. Beakers were tightly covered with aluminum foil and incubated in a constant temperature (15°C) chamber for 15 days. Initial levels of mineral nitrogen (NH_4^+, NO_2^- and NO_3^-) were determined on triplicate 10.0 g samples of each soil after adjustment to 60% of their respective moisture holding capacities. These determinations were carried out by

procedures as previously described for the general soil analyses and constituted the zero-time controls for the experiment.

Denitirfication

The procedure of Bremner and Shaw (1958) based on the loss of combined nitrogen on incubation of soils under water logged conditions was used as a measure of denitirifcation. Six Kjeldahl flasks (300 ml) were used for each soil sample tested; to each was added 5.0 g equivalent oven-dried weight of soil, 5.0 mg KNO_3 and 12.0 ml distilled water. Three flasks of each soil set were analyzed at zero time for total nitrogen content by the procedure outlined below while the remaining three soil flasks per sample were incubated at 15°C for 15 days and then analyzed for total nitrogen.

Total nitrogen determinations at zero time and at the end of the incubation interval were carried out by adding 10.0 ml of 5% (w/v) $KMnO_4$ and 20.0 ml of 50% H_2SO_4 (v/v) to each replicate flask slowly with constant shaking. After 5 minutes each replicate flask received two drops (0.1 ml) of octyl alcohol and 5.0 g of reduced iron[1] (to pass 100 mesh sieve). A 25 ml Erlenmeyer flask was

[1]Available from B.D.H. Laboratory, Chemical Division, England.

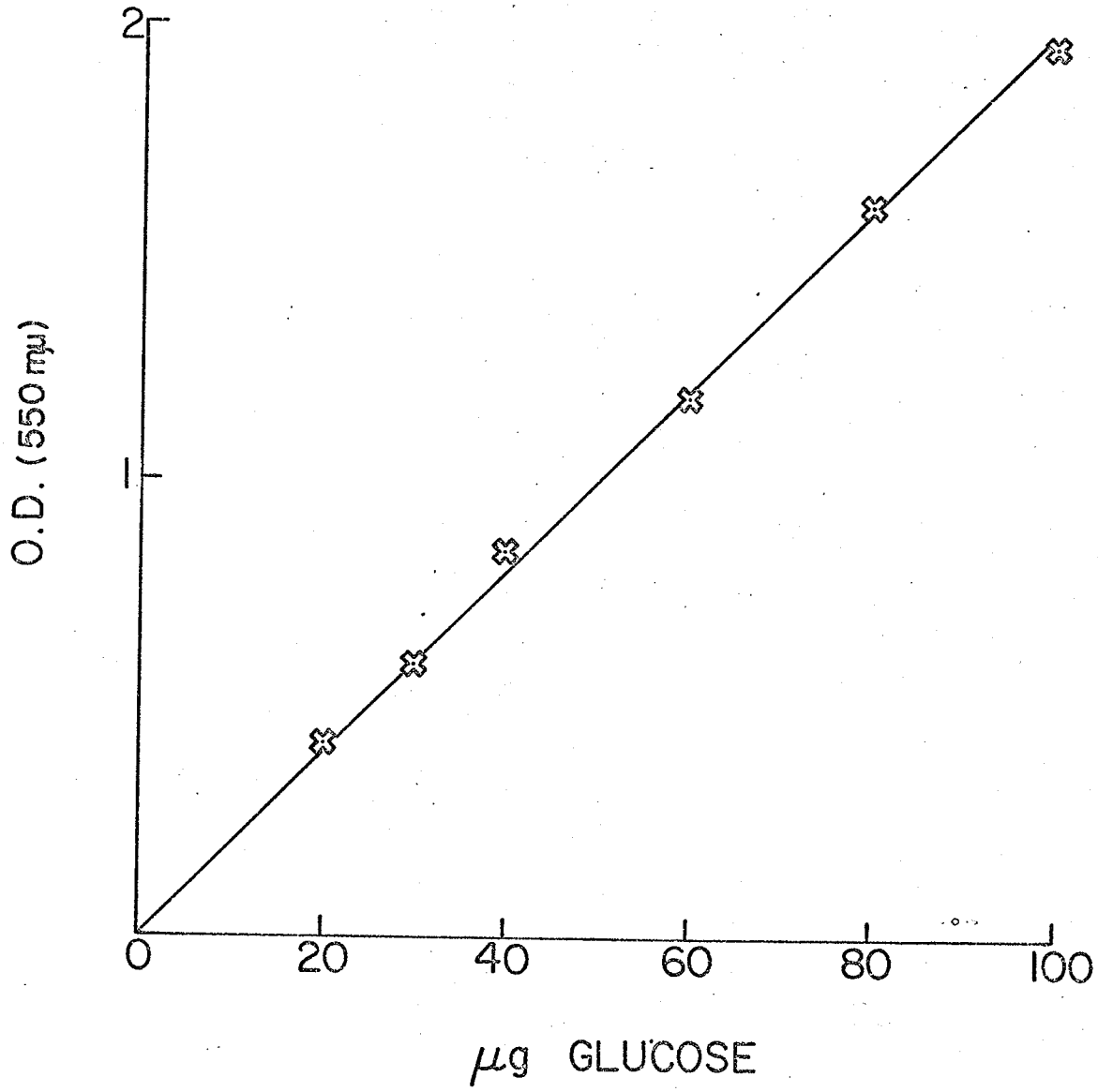
inverted over the neck of each Kjeldahl flask (as a cap) and flasks were reshaken until the initial strong effervescence had subsided. These flask assemblies were then transferred to a Kjeldahl digestion rack (Erlenmeyer caps still in place) and heated gently for 45 minutes. Flasks and contents were then cooled, caps removed, and to each flask were added 10.0 g K_2SO_4, 1 g $CuSO_4$ $5H_2O$, 0.1 g of Se and 40.0 ml concentrated H_2SO_4. Flasks and contents were then reheated until a yellowish-green color developed therein, then boiled vigorously for five hours. Following digestion, flask contents were carefully transferred to volumetric flasks and each brought up to a final volume of 100 ml with distilled water.

Ammonium-nitrogen was determined on aliquots of these diluted samples by the microkjeldahl procedure described previously in the section on soil nitrogen analysis. Corrections in the amount of added alkali were made on the bases of the dilution factors for sulphuric acid used in the digestion mixture.

Ureolysis

Ureolytic activity of the soils was estimated after inhibiting the microbial growth by toluenization. The assay procedure was similar to that proposed by Hoffman and

Fig. 8. Calibration curve for glucose.


2
1
O.D. (550 mμ)
0
20
40
60
80
100
μg GLUCOSE

Teicher (1961) except that toluenization in each case was carried out according to the method of Ross (1965).

Samples equivalent to 10.0 g oven-dried weight of soil were dispensed to 100 ml volumetric flasks and then mixed with 3.0 ml of toluene. Immediately after a 15 minute incubation interval at room temperature, each flask received 10.0 ml of urea solution (10% w/v) and 20.0 ml 1 M citrate buffer, pH 6.7 (1 M citrate in 1 M KOH). Flasks were stoppered tightly after mixing and incubated at 37°C for three hours in a constant temperature water bath. After incubation, contents were adjusted to a final volume of 100 ml with warm distilled water (38°C). During volume adjustment contents of the flasks were shaken to ensure uniform mixing. Ammonia as the end product of ureolysis was determined on soil-free filtrates (What #12 filter paper) by the procedure described earlier under physico-chemical analyses methods.

Extracellular carbohydrases

Sucrase, amylase, cellulase and xylanase activities in the soils under study were determined by procedures similar to that used for urease determinations again after the soils had been treated with toluene to stop microbial growth. End products (as reducing sugars in all cases) as determined by the modified dinitrosalicylate reagent[1] (Reese,

[1]Dinitrosalicylate Reagent: 3,5-Dinitrosalicylic acid, (1.0%); Phenol (crystalline), (0.2%); Sodium sulfite, (0.05%); Sodium hydroxide, (1.0%); Rochelle salt, (20%).

1964) gave evidence of the activity of the various enzyme systems.

To the duplicate tubes, each containing 3.0 ml of dinitrosalicylate reagent, was added 1 ml of clear filtrate and the tubes were then heated for 10 minutes in a boiling water bath. After cooling to room temperature, the total volume in each tube was made to 8.0 ml and O.D. was determined immediately. This is to be noted that soil extracts did not interfere with the determination of reducing sugars. Figure 8 shows a typical standard curve obtained by using known concentrations of glucose.

In all the cases except xylanase, glucose was used as the standard reducing sugar and the O.D. was recorded at 550 mμ. For xylanase, xylose replaced glucose for preparing standard curve and the O.D. was recorded at 575 mμ.

Sucrase

Activity was measured by a method based upon that of Hoffman and Seegerer (1951) as amended by Ross (1965). To 10.0 g toluenized soil in 150 ml conical flasks were added 20.0 ml 0.5 M acetate-phosphate buffer, pH 5.5 (0.5 M acetate in 0.5 M Na_2HPO_4) and 20.0 ml of 5% (w/v) sucrose solution. Contents were mixed and incubated in a constant temperature water bath at 37°C. After 24 hours of reaction, flask contents were filtered through fluted filter paper (Whatman #12) and aliquots therefrom were used for reducing sugar determinations.

Amylase

The reaction mixture was similar to that used for sucrase except that 20.0 ml (2%) soluble starch was used as substrate and a reaction period of 24 hours was selected. Reaction mixture was incubated at 28°C.

Cellulase

Again the reaction mixture was similar except 20.0 ml (1%) Walceth cellulose[1] was used as substrate and reaction period was extended to 48 hours. Reaction mixture was incubated at 28°C.

Xylanase

Reaction mixture temperature of incubation and period of reaction were as for cellulase except xylan (1%) was used as substrate and in the reducing sugar assay the O.D. was determined at 575 m.

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[1]Walceth Cellulose: Phosphoric acid treated cellulose prepared in the laboratory by the method of Walceth (1952). Purified cotton linters (Type A 1000 PSF, Hercules Powder Company, Delaware, U.S.A.) were ground in a grinding mill to pass through a 100 mesh sieve. The ground linters thus obtained were treated with cold (0-2°C) 85% phosphoric acid (20 ml per gram of air-dried cellulose) for 2 hours. After 4 washings (repeated cycles of suspension and filtration) with ice-cold water, the cellulose was resuspended in a 1% sodium carbonate solution (1 liter per 20 g cellulose) for overnight and washing was then continued with distilled water at room temperature till the pH of the suspension was the same as that of distilled water. The treated cellulose was finally dispersed in distilled water and concentration was determined by drying replicate aliquots over phosphorus pentaoxide invacuo.

General Microbiological Methods

All microbiological determinations were made on fresh soil samples after removal of living plant roots, stems and leaves. If necessary, soils were stored at 4°C in air-tight polyethylene bags but in no case did the storage period extend over two weeks. Unless otherwise stated, for all viable count determinations of micro-organisms, 10 g of fresh soil were aseptically added to 90 ml of sterile saline water (0.85% NaCl) in 250 ml flasks and dispersed by shaking on a mechanical wrist-shaker. To aid homogenization of the samples, a few sterile glass beads (3 mm diameter) were added to each flask. After 10 minutes shaking, homogenized samples were serially transferred (diluted) in sterile saline water and plated on various media by spreading 0.1 ml aliquots of appropriate dilutions on the surface of the plated agar media with sterile bent glass rods. All operations involved in the preparation of soil suspensions, dilutions and platings were performed in a constant temperature chamber at 13 $\pm$ 0.5°C. All media were prepared with distilled water and sterilized at 121°C for 15 minutes.

Viable counts and isolation of micro-organisms

Bacteria

For the enumeration of total heterotrophic bacteria, four different media were used and plates were incubated at

3.5 $\pm$ .5 and 15°C. Colonies developing on the media were randomly picked and purified. Stock cultures of the isolates were maintained by once-a-week transfer on fresh agar slants and grown at 15°C.

Medium A — Medium A prepared from the salt solutions of Cook and Lochead (1959) contained K_2HPO_4, 0.1 g; KNO_3, 0.5 g; $MgSO_4.7H_2O$, 0.2 g; $CaCl_2$, 0.1 g; NaCl, 0.1 g and $FeCl_3.6H_2O$, 0.01 g in 750 ml of water and 250 ml of soil extract prepared by the method of Lochead and Burton (1957). The medium was adjusted to pH 7.0 with 5N NaOH and solidified with 15 g Bacto Agar (Difco).

Medium B — One liter of medium B, essentially the salts medium of Stanier, Doudoroff and Adelberg (1957) contained K_2HPO_4, 1.0 g; $MgSO_4.7H_2O$, 0.2 g; $FeSO_4.7H_2O$, 0.05 g; $CaCl_2$, 0.02 g; $MnCl_2.9H_2O$, 0.002 g; Yeast Extract (Difco), 1.0 g; glucose, 0.1 g and Bacto-Agar (Difco) 15 g. Glucose was autoclaved separately and added aseptically.

Medium Sc — Stock Culture Agar (Difco), a dehydrated medium was rehydrated according to the directions prescribed.

Medium T — Thornton's Standardized Medium (Thornton, 1922) contained in one liter, K_2HPO_4, 1.0 g; $MgSO_4.7H_20$, 0.2 g; $CaCl_2$, 0.1 g; NaCl, 0.1 g; $FeCl_3$, trace; KNO_3, 0.5 g; asparagine, 0.5 g; mannitol, 1.0 g and Bacto Agar (Difco) 15 g. This was supplemented with 0.25 g yeast extract (Difco) and the pH was adjusted to 7.2 with HCl or NaOH solutions as required.

Total Gram negative bacteria

Plates of Medium B containing crystal violet (1:500,000 final concentration were incubated after inoculation at 15°C for two weeks.

Filamentous fungi

Peptone-Dextrose Agar (Difco) plus Rose Bengal Streptomycin of Martin and Johnson (Martin, 1950; Johnson, 1957) was used for isolation and enumeration of fungi. One liter of this medium contained: K_2HPO_4, 1.0 g; $MgSO_4.7H_20$, 0.5 g; peptone, 5.0 g; dextrose, 10 g; Bacto Agar (Difco), 20 g; streptomycin, 30 mg and Rose Bengal, 10 ml (1:300 dilution). All the components except streptomycin and Rose Bengal were dissolved in distilled water and heated slowly while stirring until the solution started to boil. After removal from the

heating source, Rose Bengal (1:300 dilution) was added at the rate of one ml per 100 ml of the medium. After autoclaving and before plates were poured, streptomycin was added to the medium at 40°C. Inoculated plates were incubated at 15°C for three weeks. Randomly picked isolates were checked for purity by morphological comparisons and pure cultures were maintained on cornmeal agar (Difco) slants by two-week transfers and incubated at 15°C.

Actinomycetes

The starch-casein medium of Kuster and Williams (1964), to which was added 50 µg/ml actidione, 5.0 µg/ml Polymyxin B sulfate, 1.0 µg/ml sodium penicillin and 50 µg/ml Nystalin, was used for the selective isolation and enumeration of actinomycetes. One liter of the starch-casein medium contained: starch, 10 g; casein (vitamin free, Difco), 0.3 g; KNO_3, 2.0 g; NaCl, 2.0 g; K_2HPO_4, 2.0 g; $MgSO_4.7H_20$, 0.05 g; $CaCO_3$, 0.02 g; $FeSO_4.7H_20$, 0.01 g and Bacto Agar (Difco) 18 g. All the components were dissolved by heating, stirred and autoclaved. Antibiotics were added to the autoclaved and cooled (45°C) medium must before plating. Inoculated plates were incubated at 15°C for 3 weeks. Colonies were randomly picked and purified. Pure cultures were maintained on the same medium devoid of antibiotics.

Enumeration of cellulolytic bacteria, actinomycetes and fungi

The basal medium of Eggins and Pugh (1961) with 0.5% Walceth cellulose (see Determination of General Biological Activities) was used for the enumeration of cellulolytic fungi. Colonies, producing a clear halo (cellulose hydrolysis) were counted after 15 days incubation at 15°C. The medium contained in one liter of glass-distilled water $(NH_4)_2SO_4$, 0.5 g; L-asparagine, 0.5 g; KH_2PO_4, 1.0 g; KCl, 0.5 g; $MgSO_4.7H_20$, 0.2 g; $CaCl_2$, 0.1 g; yeast extract (Difco), 0.5 g; Walceth cellulose, 0.5 g and agar 20 g. The pH was adjusted to 6-6.2. The medium was autoclaved at 121°C for 20 minutes.

Total cellulolytic bacteria and actinomycetes were counted using the medium and method of Emerson and Weiser (1963). The medium contained in one liter of glass-distilled water, K_2HPO_4, 1 g; $MgSO_4.7H_20$, 1.0 g; Na_2CO_3, 1 g; $(NH_4)_2SO_4$, 2 g; and agar 15 g.

Plates were first inoculated with soil dilution and then a 0.2% water solution of sodium carboxymethyl cellulose (High viscocity type 70; Hercules Powder Company, Welmington, Del., U.S.A.) previously autoclaved and cooled was poured over the plates to form a thin layer. After 15 days incubation at 15°C, colonies surrounded by depressed areas were counted.

The Influence of Temperature on the Number of Colonies Appearing

Bacteria

Development of bacterial colonies at different incubation temperatures was studied by spreading 0.1 ml diluted soil suspensions on plates of Medium B.

Fungi

The influence of different incubation temperatures on numbers of fungal colonies appearing was examined essentially in the same way as for bacterial colonies except that a peptone-dextrose-rose bengal-streptomycin medium was used.

Studies on the Temperature Range of Growth of Different Isolates

Bacterial isolates

The temperature range of growth of isolated bacterial strains was evaluated by transferring a loopful of broth culture of each isolate (36 hour old cultures in medium B, grown at 15°C) to a series of tubes each containing 7.5 ml of liquid medium B. Inoculated tubes were incubated in duplicate at 3.5°, 15°, 20°, 28° and 37°C. Subsequent growth was measured by optical density determinations (Klett-Summerson) at 540 mμ wave-length.

Fungal isolates

Growth at different temperatures (3.5°, 15°, 22°, 28° and 37°C) was determined by measuring the increase in colony diameter by isolates grown on Sabouraud's dextrose agar (Difco) plates. This was done as follows: freshly prepared agar plates were spot-inoculated with mycelial fragments or spores from the slant cultures and incubated at 15°C for from 3 to 5 days. One centimetre discs were then aseptically cut from the fungal growth areas on each plate by means of a sterile cork-borer. The discs were transferred aseptically to fresh medium plates, one disc per plate, and positioned more or less centrally. Plates so prepared were incubated in duplicate at the different incubation temperatures and increases (mm) in colony diameters were recorded at twelve hour intervals.

Actinomycetes

The temperature range of growth of actinomycetes was determined by preparing uniform slightly turbid cell suspensions of each isolate in sterile saline water and streaking a loopful of each suspension on separate casein-starch medium plates. The inoculated plates, in duplicate, were incubated at 3.5°, 15°, 22°, 28°, 37° and 45°C. A record of the amount of growth as estimated by increases in colony diameters was made at regular intervals.

Classification of the Microbial Isolates

Bacteria

Taxonomic grouping of bacterial isolates was based mainly on the gram reaction, shape and size of the cells. These determinations were made according to the procedures described in Manual of Microbiological Methods (1957). After 36-48 hrs incubation at 15°C on medium B, the organisms were gram-stained by the Burke and Kopeloff-Beerman modification. Shape and size determinations were based on the gram-stained preparations.

Fungi[1]

Identification of the majority of the fungal isolates was carried out according to the Manual of Soil Fungi (Gilman, 1957). Media used for colony characteristics and sporulations were Czapek's agar (Waksman, 1952) and bacto-cornmeal agar (Difco). Oidiodendron spp. were identified on the basis of developmental morphology of the conidia according to Barron (1962); medium used for colony characteristics and sporulation was bacto-malt agar (Difco). Wardomyces spp. were identified on the basis of the colonial morphology and the sporulating structures developing on bacto-malt agar

[1]Identification of fungi in most of the cases was up to generic level.

(Difco) according to Hennebert (1962). Chrysosporium spp. were identified according to the key proposed by Carmichael (1962); media used were bacto-malt agar and bacto-cornmeal agar (Difco).

Actinomycetes

Genera of actinomycetes were identified mainly on the basis of colony characteristics, production and nature of aerial mycelium, spores and sporophores. Colony characteristics were studied on Czapek's agar (Waksman, 1952) and nutrient-agar (B.B.L.). Production of aerial mycelium, spores and sporpphores were studied by a simple modification of Vernon's slide culture technique (Vernon, 1931), suggested by Poonawalla (1955). Generic identifications are based on Bergey's Manual of Determinative Bacteriology (1957).

Biochemical and Physiological Tests Carried Out on the Isolates

Tests for urease production, nitrate reduction and gelatin liquifications by bacterial isolates were carried out according to the procedures described in the Manual of Microbiological Methods (1957).

Fungal isolates were tested for their ability to hydrolyse cellulose (Walceth-cellulose), Pectin (B.D.H.),

and laminarin (Seaweed Res. Inst., Inuvesk, Great Britain) by growing them in the liquid medium of Eggins and Pugh, containing 0.5% of the respective compounds as the sole carbon sources and analysing the culture-filtrates for extra cellular enzymes. For growing the fungi, 15 ml media were taken in 125 ml conical flasks and, after sterilization (121°C for 15 minutes), were inoculated with mixed mycelial and spore suspension obtained from 7 days growth on corn-meal agar. After 15 days incubation at 15°C, the contents of the flasks were filtered through sintered glass and the filtrates were analysed for respective enzymic activity. Methods were essentially the same as described for cellulose and xylanase of soils. Reaction mixtures contained: 0.5 ml of substrate (0.5%), 0.1 ml culture filtrate and 0.4 ml acetate buffer (0.2 M, pH 5.0). After reaction times of 0.0, 30, 60 and 120 minutes, mixtures were assayed for reducing sugar by the dinitrosalicylate reagent.

Cellulolytic activity of bacteria and actinomycetes was determined by the same procedures as described for fungi except that the pH of the growth medium was adjusted to 7-7.2 by dilute NaOH solution and the buffer used was K_2HPO_4- KH_2PO_4, pH 7.0.

RESULTS AND DISCUSSION

Soils: Morphology, Physico-Chemical and Microbial Characteristics Varying with Depth.

Initial studies on the morphology, physico-chemical and microbial characteristics of the Churchill soils were based on cored samples taken, on the average, to a depth of 12 inches. One to two samples each consisting of duplicate cores were taken during the Fall of 1964 from three of the sampling sites selected on the bases of general soil type, drainage characteristics and vegetative cover. General observations recorded for each pooled sample are given in Table I and include a brief morphological description, estimates of total organic matter and total nitrogen as well as the reaction (pH) of subsamples taken at different depths within each core. In addition, the Table includes data on the vertical distribution of microorganisms as measured by the total plate count. Counts of bacteria and actinomycetes were grouped together as were counts for filamentous fungi and yeasts.

From a general study of the soil cores it is quite apparent that the root zone is limited to a very shallow depth (7 to 8 inches) or even less. This is not surprising since the low temperature (permafrost) doubtless retard deep root penetration (Richards et al, 1952). The tendency for

Table I

Some morphological, physico-chemical and microbial characteristics of Churchill soils.

Sample	Depth Inches	Morphological Description
TL	0-2	Partly decomposed organic matter intermixed with sand; penetrated by roots; dark brown; friable.
	2-5	Partly decomposed organic matter, intermixed with sand and gravel; penetrated by roots; yellowish brown; friable; strongly effervescent.
	5-8	Sands and pebbles, single grained intermixed with humified olive brown mineral soil; high concentration of roots; strongly effervescent.
	8-12	Fine sand, silt and gravels; mederately plastic; no roots; dirty chalky white; strongly effervescent.
GC	0-3	Partly decomposed organic matter, well rooted; discontinuous with woody and fibrous plant remains; black.

Table I. Cont'd.

Sample	Depth Inches	Morphological Description
GC	3-5	Partly decomposed organic matter, fine roots, sticky; yellowish brown.
	5-8	Clayey; pebbles with brown surface coating; sticky, olive brown; strongly effervescent.
OB	0-1	Brownish-black organic matter deposit; partly decomposed, fluffy.
	1-4	Yellowish brown to reddish brown, partly decomposed organic matter.
	4-8	Olivaceous brown, partly humified soil; sands and pebbles dominate; loose and friable; highly effervescent.

*O.M. = Organic matter (Organic carbon x 1.72)
Bact. = Bacteria
Actino. = Actinomycete

‡Counts less than one thousand per gram soil.

Table I. Cont'd.

Particles >2 mm	pH	O.M.*	Total N %	C/N Ratio	Microbial counts (x 10^6)/g soil	
					Bact. & Actino.	Fungi
0.0	5.95	10.44	0.31	19.70	4.10	0.90
25.0	7.30	2.13	0.07	16.74	4.50	0.42
45.0	7.70	1.25	0.04	k8,99	2.45	0.01
35.0	7.85	0.96	0.03	18.57	0.04	∓
0.0	5.50	50.16	1.62	18.00	1.50	1.74

Table I. Cont'd.

Particles >2 mm	pH	O.M.*	Total N %	C/N Ratio	Microbial counts (X 10^6)/g soil	
					Bact. & Actino.	Fungi
0.0	6.50	33.28	1.32	14.55	2.80	1.03
65.0	7.80	5.24	0.22	14.00	1.40	0.04
00	4.70	77.0	2.30	19.40	0.10	2.80
0.0	3.80	80.67	2.95	15.90	0.40	1.30
57.0	7.45	0.08	0.03	15.20	8.00	0.05

root system to spread laterally is particularly evident for the larger trees such as black spruce and Tamarack and is responsible for the relatively poor anchor effect which often results in uprooting during the frequent wind storms prevalent in the region (Campbell, 1968).

As well, pH values tend to increase with depth while the C:N ratio remains broad throughout the length of the core. The tendency for an increase in alkalinity with depth is due to the nature of parent material which in all the three areas is limestone. Low pH values of the top layers of the soils could probably be explained on the basis of decomposition of carbonates with calcium being removed (Wright et al (1959). The broad C:N ratio is also quite characteristic of Cryopedogenic soils (Retzer, 1965) and probably reflects the over-all slow rate of organic matter decomposition.

As might be expected, the total numbers of micro-organisms tended to decrease with depth but at a much greater rate than is usually observed in more temperate soils. Very likely this results from the drastic permafrost effect. Though not reported in Table 1, temperature measurements made during mid summer at a depth of four to six inches in Old Beach and Twin Lake soil, for example, varied from 3.4 to 4.0°C.

Surface accumulation of organic matter, on the other hand, reflects the nature of the vegetative cover and, in a quantitative sense, is apparently quite strongly influenced by the wind erosion so characteristic of the region. In the more exposed areas where wind-force effects are most evident, leaf litter, branches of trees and the like on falling tend to be swept into low depressions or sheltered areas leaving the higher ground mostly bare. This results in a particularly uneven deposition of plant materials.

In the Goose Creek area the alluvial soil is of a clay type with an organic matter overburden that ranges from thin to quite thick (1 to 6 inches). Some intermixing of the organic matter to a relatively shallow depth was also noted in this area. This sampling area is subject to rather wide fluctuations in water table largely due to high water level in Goose Creek during the spring run-off. At times the sampling area is even flooded.

Twin Lakes soils give the impression of being very primitive (largely composed of sands and gravel) and yet support a good vegetative cover. At higher elevations in the Twin Lakes area, soils show a 2 to 4 inch deep cover of thick dark brown amorphous humus overlying a sand-gravel substrate. Interspersed with the gravelly sands are fragments

of weathered limestone. As the elevation decreases down-slope, the soil picture is similar except that thicker deposits of organic matter have accumulated on the surface. The prevailing winds are mostly from the north and it is not surprising that organic matter should accumulate to a greater extent on the sheltered southern slopes.

The old Marine Beach soil area presents a very diversified picture. Soils sampled near the crest of the beach where vegetative cover is scanty to absent are made up of a 4 to 6 inch sand and silt deposit over increasingly larger aggregates. Short distances away on the down-slope of the beach drainage is poor and peat development is not uncommon. Below the peat accumulations, the sandy-gravel substrate characteristic of the area is quite evident. It should be pointed out that the insulatory properties of the peat retard the permafrost retreat during summer and consequently soil temperatures at the peat-sand interface are either below freezing, or at best, close to freezing throughout the year.

While it is difficult to generalize on the nature of the tundra soils, one fact emerged quite clearly from the examination of cored samples: i.e., the active biotic element is invariably restricted to a layer which seldom exceeds 8 inches in depth regardless of the soil area under consideration. For this reason, subsequent soil sampling and investigations were confined to what should be called 'surface sampling'.

Physico-chemical and Biological Characteristics of Surface Soils

Physico-chemical characteristics

Analyses presented in Table II on the composite surface samples of soil collected during September 1965 clearly show that Twin Lakes Hill and Old Beach soils tend to be acidic in nature and poorly base-saturated. In contrast, Goose Creek and Inuvik soils are neutral to slightly alkaline and represent reasonably well developed soils. Goose Creek samples taken from water-logged areas, however, have an acidic layer of varying depth at the surface, (Table I). This is most evident where peat deposits have accumulated.

Carbon and nitrogen analyses show the C/N ratio to be broad in all the soils examined with the highest C/N ratio values evident in water-logged soils of the Old Beach formation. A similar range of C/N ratios (13 to 26) in cryopedogenic soils from the Alaskan area have been reported by a number of American investigators (Brown and Tedrow, 1959); Douglas and Tedrow, 1959; Drew and Tedrow, 1957; Retzer, 1956; Tedrow and Hill, 1955; Ugolini et al, 1963 and Retzer, 1965). Humic acids constitute, generally, a rather low proportion of total organic matter in most of the soils examined. The entry of organic materials into

Table II

Some physico-chemical characteristics of the upper layers of Churchill and Inuvik soils.

Soil Sample	Depth Inches	Moisture %	Field Capacity	pH	Exchange Acidity	Cation Exchange Capacity	Base Saturation
TL-2	0-4	36.0	136.0	5.50	14.0	34.30	38.40
TL-3	0-4	30.4	55.0	5.65	7.0	23.38	18.80
OB-4	0-4	30.0	53.0	4.60	38.0	30.80	17.85
OB-5	0-4	78.0	350.0	4.90	118.0	136.30	10.00
GC-12	0-5	52.8	70.0	7.60	-	35.60	107.00
Iv-X	0-5	45.0	98.0	8.10	-	50.50	-
Iv-Z	0-5	50.0	100.0	7.35	-	-	-

Table II. Cont'd.

Organic Matter %	Humic Acid (%OM)	Organic Nitrogen %	Mineral nitrogen (ppm) NH_4^+	NO_2^-	NO_3^-	C/N Ratio
10.44	70.3	0.32	17.0	0.00	3.30	18.5
12.22	52.0	0.41	8.0	1.03	4.16	17.4
27.00	31.0	0.55	17.0	1.10	1.00	28.7
95.00	20.0	1.73	15.0	1.36	0.80	31.0
8.40	44.0	0.29	10.0	1.20	6.10	14.0
15.00	34.0	0.50	2.0	0.45	9.00	17.4
21.00	30.0	0.54	3.0	0.47	2.00	23.5

Table III

Easily hydrolysable polysaccharides in Churchill surface soils expressed as a percent of organic matter.

Soil Sample	Pentoses	Hexoses	Uronic acids	Total
TL-2	1.940	4.951	1.11	8.00
TL-3	2.133	3.600	2.20	7.93
OB-4	1.481	8.160	2.52	12.16
OB-5	4.166	12.604	2.78	18.50

soils seems almost to occur at a rate faster than it can be decomposed.

Although mineral nitrogen levels were low in all the samples taken, water-logged soils tend to show higher ammonia levels than are evident in well-aerated soils. No significant accumulation of nitrite or nitrate was observed in any of the samples. Old Beach soils yielded nitrate levels ranging from 0.8 to 1.0 ppm while Goose Creek, Inuvik and Twin Lakes soils gave values ranging from 2 to 11 ppm. Judging by the amount of nitrate-nitrogen present, the process of nitrification seems to be not very active in the Old Beach acid soils; however, the ammonification process seems to operate. In Twin Lakes, Goose Creek and Inuvik soils, both ammonification and nitrification seem to occur; the former being more active than the latter in Twin Lakes' soils.

The presence of easily hydrolysable polysaccharides measured as pentoses, hexoses and uronic acids in considerable amounts, Table III, suggests a good potential source of energy materials available for soil metabolic activities.

Biological characteristic

Data on biological activities including respiration, ammonification, nitrification, denitrification and specific enzymic activities are detailed in Table IV.

Table IV

Some biochemical activities of Churchill and Inuvik soils.

Soil Sample	Respiration Intensity (µl O_2/hr/g)	Ammonifying and Nitrifying capacity					
		prior to incubation			after 15 days incubation		
		NITROGEN (ppm)					
		NH_4^-	NO_2^-	NO_3^-	NH_4^+	NO_2^-	NO_3^-
TL-2	18.88	12.40	0.47	6.30	5.30	1.00	13.20
TL-3	20.00	8.30	0.20	6.00	8.00	0.19	13.14
OB-4	13.05	52.50	0.40	0.81	67.50	0.20	0.93
OB-5	50.00	8.00	1.36	3.00	36.00	0.00	0.00
GC-12	25.67	12.00	0.75	20.28	7.00	0.30	35.49
Iv-18	-	6.20	3.70	7.00	7.20	1.70	43.75

Table IV. Cont'd.

Denitrifying capacity (% loss of N after 15 days incubation)	Urease[1] (units/ 10 g soil)	Saccharase[2] (units/ 10 g soil)
0.00	10.97	2.15
2.63	14.52	0.81
0.91	11.00	1.33
0.00	17.28	2.77
6.60	4.00	0.58

Data not available

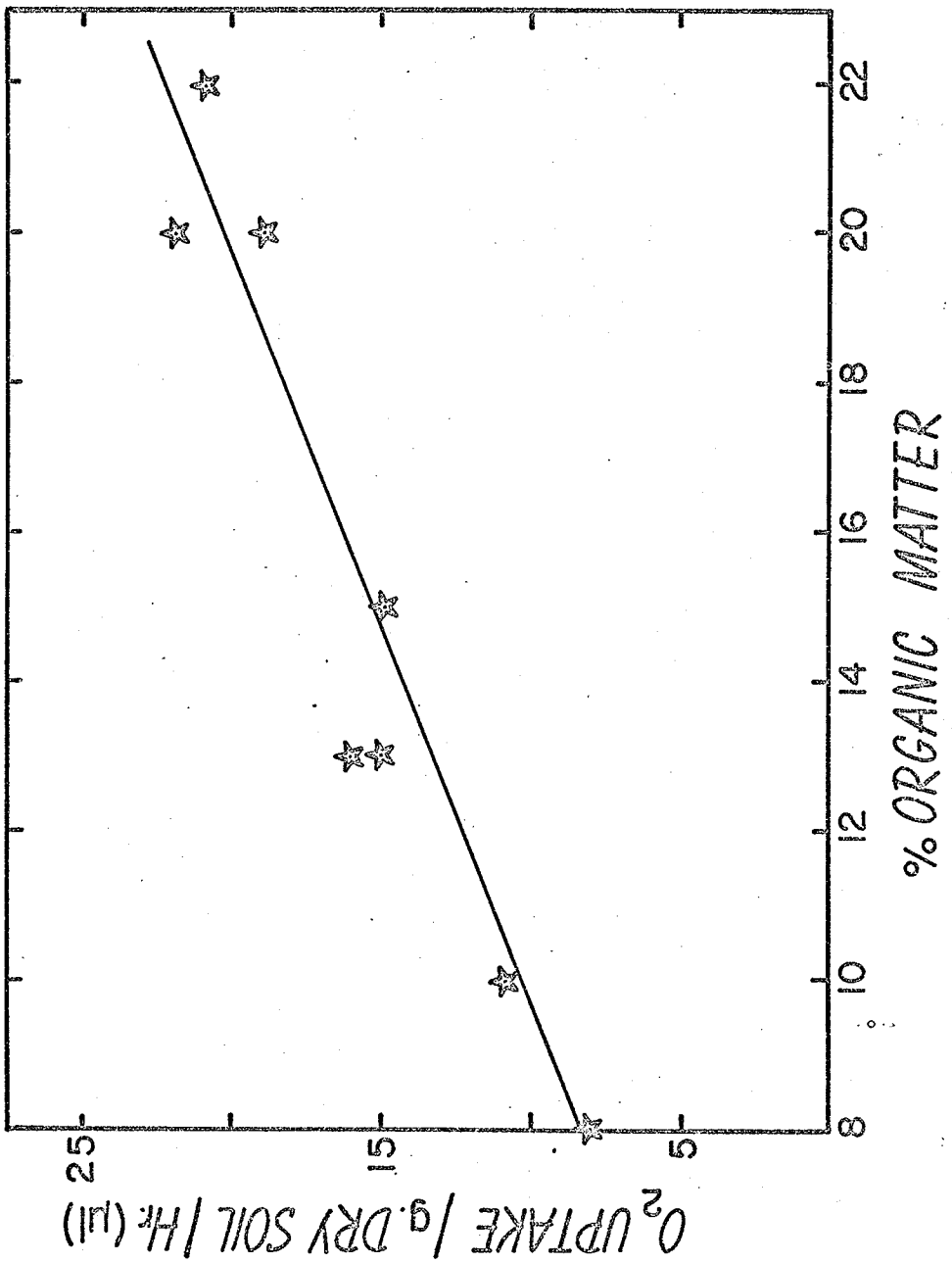
[1]Urease units = (urease number X 0.32). The urease number 1 corresponds to the amount of enzyme which, under the conditions described, hydrolyses 1 mg of nitrogen as NH_3.

[2]One unit represents the amount of enzyme which catalyses the hydrolysis of sucrose to give 1 μM of reducing sugars per minute at 37°C.

Soil Respiration

Respiratory activity of surface soils was examined under the closed percolation system as described in Materials and Methods. Endogenous respiration activities were measured using fresh 20 g equivalents of air-dried soils except in the case of the OB-5 soil where only 10 g equivalent was permissible because of the high volume/weight ratio. The Goose Creek soil which was neutral and low in organic-matter content respired at a much faster rate than the Twin Lakes' soils. When one compares the respiration intensity observed between OB-4 and GC-12, it would seem that relative organic matter concentrations in these soils has no influence. A similar lack of relationship in terms of organic-matter content is obvious when one compares the OB-4 soils (O.M. content, 27%) with Twin Lakes soil (O.M. contents, 10.44, 12.22). In all other comparisons, respiration intensity in these soils did seem to be related directly to the respective organic-matter concentrations. These considerations then lead to the assumption that a comparison of respiration intensity between soils can only be made properly when factors other than organic-matter content are taken into account. These factors would include differences in pH, aerobiosis and general make-up of the soils in a physical

Fig. 9. Relationship between the organic matter content and respiration intensity in Twin Lake soils.
Eight random surface samples were analysed for organic matter content and the respiration intensity was determined by the rocking percolator technique.
Respiration rate calculated on the basis of four hours percolation at 25°C.
Correlation coefficient (r) = 0.913.


O_2 UPTAKE /g. DRY SOIL / Hr. (µl)
25
15
5
8
10
12
14
16
18
20
22
% ORGANIC MATTER

and chemical sense, as has been suggested by Douglas and Tedrow (1959). These investigators reported that respiration intensity varied widely under the influences of temperature and moisture with respect to the type of soil.

The data on eight Twin Lakes samples which differed to a considerable extent in their organic matter content (8 to 22%), though otherwise similar, yielded a highly significant correlation ($r = 0.913$) between the organic matter content and the soil respiration intensity (Fig. 9) However, it was not possible to observe if there was a close relationship of this type among the soils of Goose Creek and Old Beach because of the few numbers of samples available. Bunt and Rovira (1955) and Ross (1965) observed a similar highly significant correlation between organic matter content and the respiration intensity in soils not differing appreciably in their physico-chemical characteristics.

Ammonification, nitrification and denitrification

Evidence for the mineralization of organic nitrogen (ammonification and nitrification) and for subsequent losses of mineralized nitrogen through denitrification in some of these soils is also given in Table IV. In general, the acidic soils (OB-4, OB-5) from the Old Beach sampling areas showed little, if any, nitrification although the accumulation

of ammonium nitrogen suggests an active ammonification process is underway. Traces of nitrite and nitrate nitrogen present in OB-5 soil prior to incubation could not be detected after the 15 day incubation period. Twin Lake, Goose Creek and Inuvik soils gave strong evidence of both ammonification and nitrification and, of these, nitrification seemed to be dominant although no significant indigenous accumulation of nitrite was observed in any of the soils examined.

Denitrification (percent loss of added plus indigenous nitrogen) was not observed experimentally in TL-2 and OB-4 soils. TL-3 soils, however, showed an experimental denitrification loss of 2.63% while the maximum loss of 6.6% was encountered in the GC-12 soil.

Accumulation of ammonia coupled with the absence of detectable amounts of nitrate in the acidic Old Beach soils could be accounted for in two ways. Either nitrification was depressed by the acidic nature of the soils or, nitrate, if formed, was utilized at an accelerated rate by the indigenous microflora. Alexander (1961) has suggested that acidic soils do, in fact, discourage nitrification (thus implying little contribution by fungal heterotrophic nitrifiers). Ammonification, however, according to Harmsen and Van Schreven (1955) does proceed well in acidic peaty soils. The rather low level of ammonium-nitrogen detected in these acidic soils might be

accounted for because under the conditions of low pH and high C/N ratios there is a rapid immobilization of ammonium-nitrogen. Jensen (1929), in proposing this concept, explained it on the basis of the enhanced activities of fungi which require greater amounts of nitrogen than do the bacteria when assimilating a given amount of organic-carbon. Results to be reported in Part II of this thesis show that the population of indigenous nitrifiers is, indeed, low in the OB soils and that pH does limit the process of nitrification. Although Twin Lake soils have pH and C/N values that would permit active nitrification, very little nitrate formation was detected under experimental conditions. Here again, immobilization of the product could be responsible for the low values obtained. Goose Creek and Inuvik soils which are mainly alluvial slightly alkaline clays gave more positive evidence for the accumulation of nitrate.

Failure to demonstrate denitrification in the Old Beach soils is not surprising since these soils show little nitrification potential. Nitrification may be considered an 'obligatory process' as suggested by Hiltbold and Adams (1960) that must occur before any biological or non-biological denitrification takes place. Moreover, the preference for neutral to alkaline reactions by the

denitrifying organisms as reported by Jensen (1934), Nomnik (1956), Bremner and Shaw (1958), Valera and Alexander (1961) and Swaby (1962) probably accounts for the failure of denitrifiers to develop in the acidic peats of the Old Beach area. The well-drained, aerated soils of the TL-2 area would also tend to discourage the development and activities of anaerobic denitrifiers while the TL-3 samples taken from a down-slope vegetation-covered area where conditions are comparatively wet might be expected to display reducing conditions (anaerobiosis) that were more favourable to denitrifiers. Maximum denitrification losses reported from GC-12 soils are to be expected in view of the alkaline condition and heavy alluvial texture of the soil material. Here, the water table is close to the surface and the seasonal flooding during spring run-off would provide, temporarily at least, a wet anaerobic environment that would encourage the activity of denitrifiers. The duration of the anaerobic environment, however, is assumed to be brief and the return to relatively aerobic conditions is evidenced by the nitrification activities displayed by these soils.

Urease and saccharase activities

Table IV also presents evidence for urease and saccharase activities in the soils examined. Activity of

both enzymes as a measure of their concentration in the soils increased with increasing organic matter content (see Table II). Similar relationships have been reported for urease in soils by Chin and Kroontje (1963), McGarity and Meyers (1967) and for saccharase by Hofmann and Braunlich (1955), Balicka and Trzebinska (1956), Alexandrova (1959) and Galstyan (1961). Both the saccharolytic and ureolytic activities in these soils are comparable to those observed in soils from temperate regions, (Ross, 1965; Bergmeyer, 1963). The significance of these enzymes in soils is in dispute. Kuprevish (1951) and Mashtakov (1954) consider that enzymic activity of this type serves as a good indication of the total biological activity and fertility of the soil. Drobnik (1956) and Quastel (1965) however, are of the opinion that these enzymic activities can not serve as criteria of total biological activity and fertility but merely as indicators of specific biochemical processes in the soils in question.

Microbial Characterization

Choice of medium and incubation intervals

Over the past fifty years or so a rather considerable number of general and selective media have been developed for qualitative and quantitative estimates of soil microbial populations. Of these, only a few have gained wide acceptance and no single medium thus far developed will adequately support all soil bacteria, actinomycetes, yeasts and fungi. Thus, the choice of any one medium for a given situation is arbitrary and, at best, represents a compromise.

In selecting media for enumeration, isolation and characterization of subarctic soil microorganisms, consideration was given to the relatively low incubation temperatures to be provided in an attempt to simulate 'normal' subarctic soil temperature conditions. With incubation temperatures ranging from 4°C to 15°C, times of incubation required for the development of representative populations are prolonged; in some cases, for as long as four weeks.

Four of the commonly used media for soil bacteria enumeration were selected for use in a preliminary study based on three different subarctic soils collected from the Fort Churchill region in November 1964. The purpose of this investigation was to select, if possible, one medium which would yield maximum counts for the shortest incubation period.

Table V

Plate counts as influenced by the types of medium, temperature and duration of incubation.

Soil Sample	Medium*	Numbers of bacteria (x 10^6)/g soil counted after the incubation periods of							
		7 days		14 days		21 days		28 days	
		4°C	15°C	4°C	15°C	4°C	15°C	4°C	15°C
G	B	33.0	37.5	37.0	49.0	47.5	50.0	47.5	50.0
	A	2.5	13.5	5.2	17.5	6.7	18.5	6.9	18.5
	T	3.5	9.0	4.5	17.9	6.2	19.0	10.0	19.0
	Sc	5.0	46.0	8.5	46.5	9.0	46.7	9.0	46.70
TL	B	2.4	2.2	3.1	4.0	3.3	4.0	3.4	4.0
	A	1.8	2.3	2.8	3.2	2.8	3.2	2.8	3.2
	T	1.0	1.1	1.5	3.2	2.2	3.2	2.3	3.2
	Sc	1.5	2.8	2.7	3.1	2.8	3.1	2.8	3.1
F	B	0.2	2.2	2.0	3.0	2.2	3.0	2.2	3.0
	A	1.0	1.2	1.2	3.0	2.2	3.0	2.2	3.0
	T	0.5	1.2	0.5	1.5	1.7	2.5	1.8	2.5
	Sc	0.5	0.5	2.2	3.0	3.0	3.0	3.0	3.0

*Media designation (B, A, etc.) as given in Materials and Methods.

Spread plates were prepared and used as described in Materials and Methods: care was exercised during all pipetting operations to reduce to a minimum the adsorption of bacteria on the inner surfaces of pipettes, according to the recommendations of Wieringa (1958). These results are presented in Table V.

As noted in Table V, considerable variations in total counts were observed between media as well as between incubation temperatures for each soil sample examined. These variations, moreover, tended to increase markedly as the time of incubation was prolonged. It may also be noted that although considerable variation in total counts occurred between the three soils examined, medium B gave consistently higher counts of representative bacterial flora even for the shorter incubation intervals.

Variations in plate counts are not unexpected and often the ultimate choice of medium to be used will depend, in a large measure, on the type of soil to be examined (Jensen, 1968). Further, as reported by Prakasam and Dondero (1967) and others, considerable variation in total plate counts can be expected from a single soil when different media compositions are employed.

On the basis of this preliminary experiment medium B was chosen for all subsequent plating requirements as far as bacteria were concerned. Where different media are used for particular purposes in succeeding parts of this investigation they will be mentioned specifically.

Table VI

The influence of different incubation temperatures on plate-counts of micro-organisms in soils of the Churchill and Inuvik areas.

Soil Sample	Bacteria (x 10^6)/g soil						Fungi (x 10^5)/g soil				
	3.5°C	15°C	22°C	28°C	37°C	48°C	3.5°C	15°C	22°C	28°C	37°C
TL-2	1.02	1.83	1.80	0.99	0.29	0.05	2.00	3.57	3.56	0.31	0.15
TL-3	3.00	7.00	7.00	3.10	1.00	1.00	3.33	3.33	3.37	2.50	0.15
OB-4	0.40	0.60	0.60	0.16	0.12	0.00	1.70	2.64	2.54	2.14	0.42
OB-5	1.35	1.50	1.45	0.24	0.10	0.00	3.00	3.30	3.26	0.80	0.13
GC-12	3.75	7.52	7.50	6.10	0.90	0.18	3.90	4.00	4.01	1.85	0.90
Iv-X	42.80	44.20	39.00	6.00	5.00	0.08	- Data not available -				

No fungal growth was evident at an incubation temperature of 48°C.

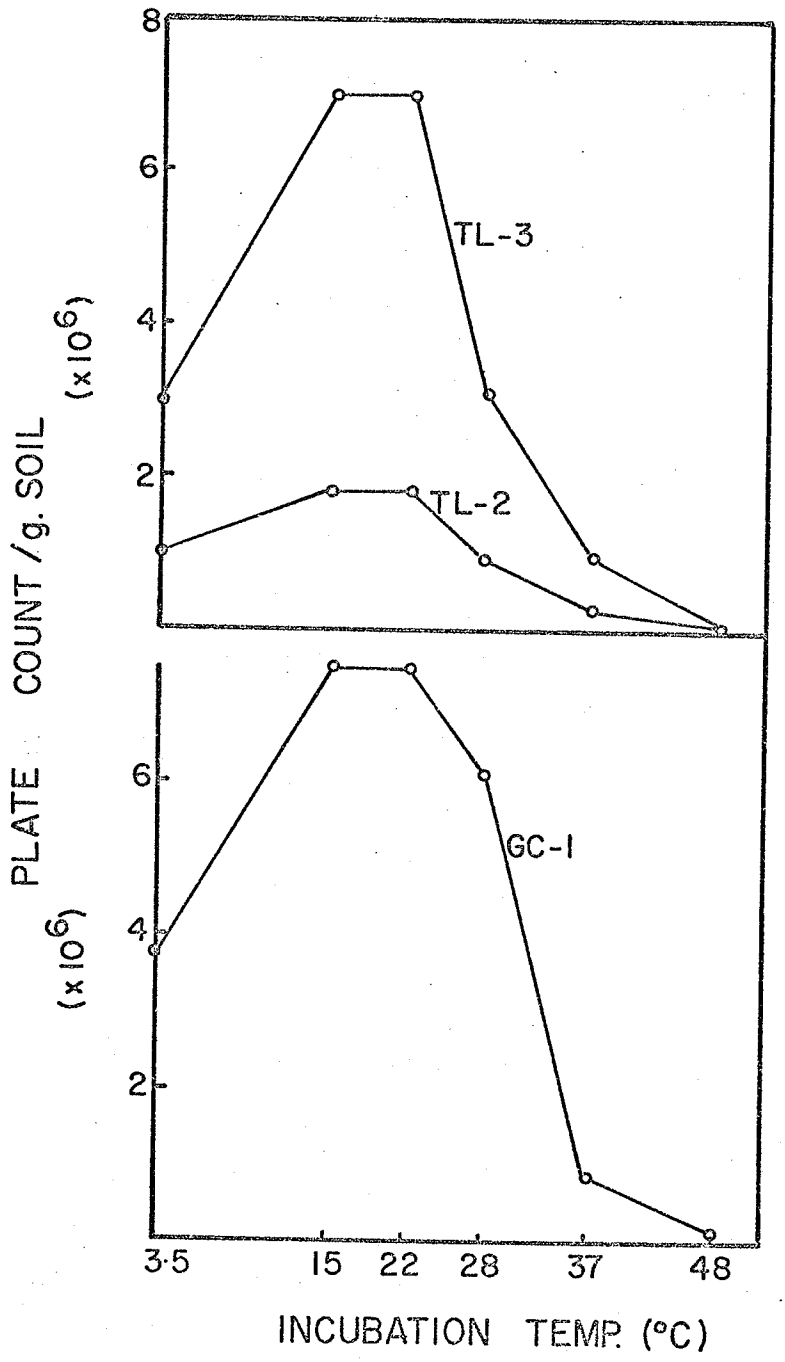
The Influence of Temperature on the Development of Microorganisms

In the interests of establishing the incubation temperature optima for subarctic microorganisms in Hudson Bay and Inuvik soils, and of establishing the range of temperatures over which they would develop, an experimental series was carried out. This consisted of a series of replicate spread-plates prepared from dilute soil suspensions using medium B for the bacterial flora and Rose Bengal streptomycin agar for fungal enumeration. Incubation temperatures ranged from 3.5 $\pm$ .5°C to 48°C and incubation times accordingly varied (inversely as the temperature) from a few days to several weeks.

Estimated numbers of bacteria and fungi per gram of soil were based on the number of colonies appearing on the plates. These data are given in Table VI. From these results it appears that the optimal temperature range for growth of bacteria from soils of all sample sites lies between 15°C and 22°C. In the case of Twin Lakes and Goose Creek soils, the numbers of colonies developing above and below the optimal temperature range decreased sharply. A much less noticeable decline in numbers of developing colonies on either side of the optimal range is evident for the Old Beach and Inuvik soils.

In terms of the numbers of fungal colonies developing during incubation the effect of temperature is less evident.

Fig. 10. Influence of temperature on plate counts of bacteria in Twin Lake, Goose Creek, Old Beach and Inuvik soils.


8
6
4
2
TL-3
TL-2
PLATE COUNT /g. SOIL (x10^6)
6
4
2
GC-1
(x10^6)
3·5
15
22
28
37
48
INCUBATION TEMP. (°C)

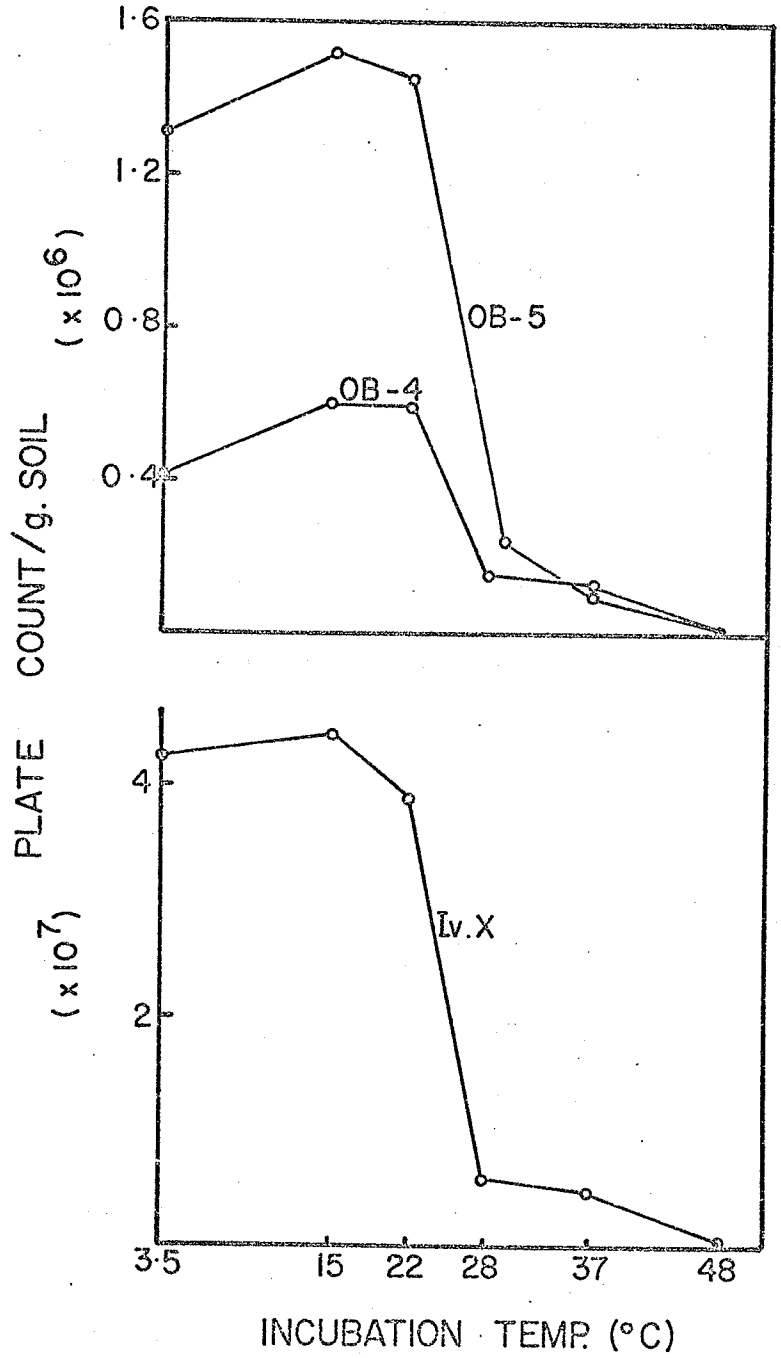

1·6
1·2
0·8
0·4
(x10⁶)
PLATE COUNT/g. SOIL
OB-5
OB-4
4
2
(x10⁷)
Iv.X
3·5
15
22
28
37
48
INCUBATION TEMP. (°C)

Fig. 11. Influence of temperature on plate counts of fungi in Old Beach and Twin Lake soils.

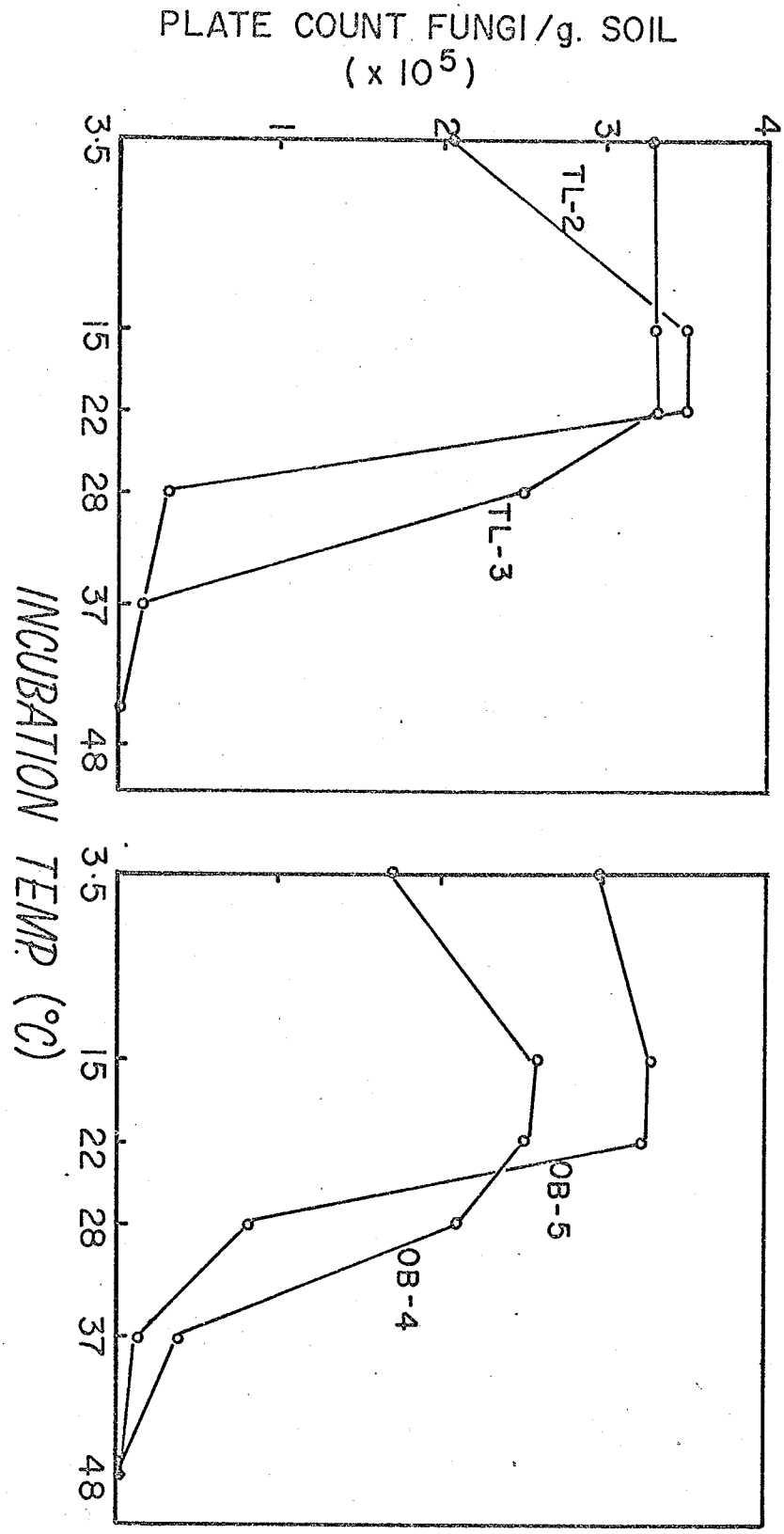


PLATE COUNT FUNGI/g. SOIL
(x 10^5)
INCUBATION TEMP. (°C)
3·5
15
22
28
37
48
1
2
3
4
TL-2
TL-3
OB-5
OB-4

The number of fungal colonies remained fairly constant over the incubation range from 3.5 to 22°C. These data for bacteria and fungi temperature responses are presented graphically in Figs. 10 and 11.

A further analysis on the plate-count data obtained from 3.5° $\pm$ .5°C and 15°C incubations for soils from different localities are presented in Table VII. The data show that bacterial counts at 3.5° $\pm$ .5°C (expressed as the % of total bacterial counts at 15°C) average about 50% for the Goose Creek and Twin Lake areas and about 80% for the Old Beach and Inuvik areas.

Completely random analyses of variance and t-tests for individual and multiple comparisons (Table VIII) carried out according to Steel and Torrie (1960) further suggest that degrees of bacterial adaptation to low temperature conditions are similar for the population in the Twin Lake and Goose Creek soils; as well, statistical evaluation indicates that these sampling sites differ from the Old Beach and Inuvik areas where soils are very similar to each other in terms of harbouring a cold-adapted bacterial population.

In general, results obtained from Churchill and Inuvik soils are not too different from those reported for Soviet Arctic soils by Sushkina (1960) with some notable exceptions. The major difference in the microbial population in our soils as compared to soils from the Soviet Arctic was noted

Table VII

Counts of cold-adapted bacteria (3.5° ± 0.5°C) as % of total bacterial counts at 15°C incubation.

Soil Sample		% bacteria counted at 3.5 ± 0.5°C	Soil Sample		% bacteria counted at 3.5 ± 0.5°C
OB-1		81	GC-12		54
OB-4		67	GC-13		52
OB-5		90	GC-14		45
OB-6		90	GC-15		49
	Average	80.75	GC-16		45
				Average	49.0
TL-2		56	Iv-X[1]		80
TL-3		45	Iv-X		76
TL-8		50	Iv-Y		72
TL-10		53	Iv-Z		84
TL-11		51		Average	78.0
	Average	51.0			

[1]Composite sample (see Table VI)

Table VIII

Analysis of variance* on data from Table VII

Source of Variations	Degree of freedom	Sum of Squares	Mean of Squares	F.
Total	17	4364.94		
Locations	3	3860.19	1286.73	35.69 (Highly significant)
Error	14	504.72	36.05	

t. tests for individual comparisons

1. OB vs Iv
   t = 0.97 (not significant), t. .05, 6 d.f.= 2.45 — Old Beach does not differ from Inuvik.

2. TL vs GC
   t = 0.74 (not significant), t. .05, 8 d.f.= 2.31 — Twin Lake does not differ from Goose Creek

t. test for multiple comparisons

OB + Iv vs TL + GC
t = 10.31 (significant at P >0.01) Old Beach and Inuvik differ from Twin Lake and Goose Creek.

* The autor gratefully acknowledges Dr. R.J. Baker, Canadian Department of Agriculture, Winnipeg, Manitoba for the analysis of the data.

in the degree of adaptation to low temperature. In terms of low temperatures the 3°C counts amounted to 4.4 to 12.3% of the optimum in the Soviet soils; where as in soils from the Churchill and Inuvik areas, low temperature counts (3.5° ± .5°C incubation) amounted 50% and 80% respectively. The optimal temperature range, however, was the same, i.e., in the range of 15°-22°C.

Rather similar observations on soils from Antarctica and Mackenzie Delta were reported by Boyd and Boyd (1963) and Ivarson (1965) respectively. These authors noted that on the average counts of colonies incubated at 2°-4°C constituted some 60-70% of those counts obtained when the incubation temperatures were 22°-25°C. The extremes in the ratios of low temperature/optimum temperature counts varied much more widely in Antarctic soils than had been reported by Ivarson (1965) for soils of the Mackenzie Delta. A similar narrow variation in ratios is reported here for soils from the Churchill and Inuvik areas.

Boyd, Staley and Boyd (1966) suggested that extremely low counts obtained at low temperature incubation in Antarctic soils were largely the result of 'de novo' introduction resulting from relatively recent contamination by man and animals. The constancy of low temperature counts/total bacterial counts in all our soils, on the other hand, would tend to imply that here the microbial population is native rather than newly introduced and displays more obvious low

temperature adaptation characteristics.

The higher percentages of low-temperature-adapted organisms noted in the Old Beach and Inuvik soils suggest that these populations have had a longer period of time to achieve the adaptation. Thus, one might conclude that the Old Beach and Inuvik soils are significantly older than the Goose Creek and Twin Lake formations. Geological evidence tends to support this conclusion (Campbell, 1968).

Growth Temperature Responses by Isolates from Churchill Soils

Bacteria

To examine further the growth temperature requirements of subarctic soil bacteria, one hundred isolates randomly picked from medium B plates (incubated at 15°C) of the previous experiment were transferred in replicate to tubes of liquid medium B. These isolates, by groups of one hundred, were incubated at different temperatures as described in Material and Methods. Results of this study are presented in Fig.s 12a and 12b where percentages of strains growing optimally at the different temperatures are presented in Fig. 12a, while percentages of strains able to grow (even minimally) at the different incubation temperatures are given in Fig. 12b.

Almost all of the strains examined in this part of the investigation grew at incubation temperatures of 15° to 28°C.

Fig. 12. Growth-temperature responses by bacterial isolates from Churchill soils.

(a) Percent bacterial isolates growing optimally at different temperatures.

(b) Percent bacterial isolates capable of growing at different temperatures.

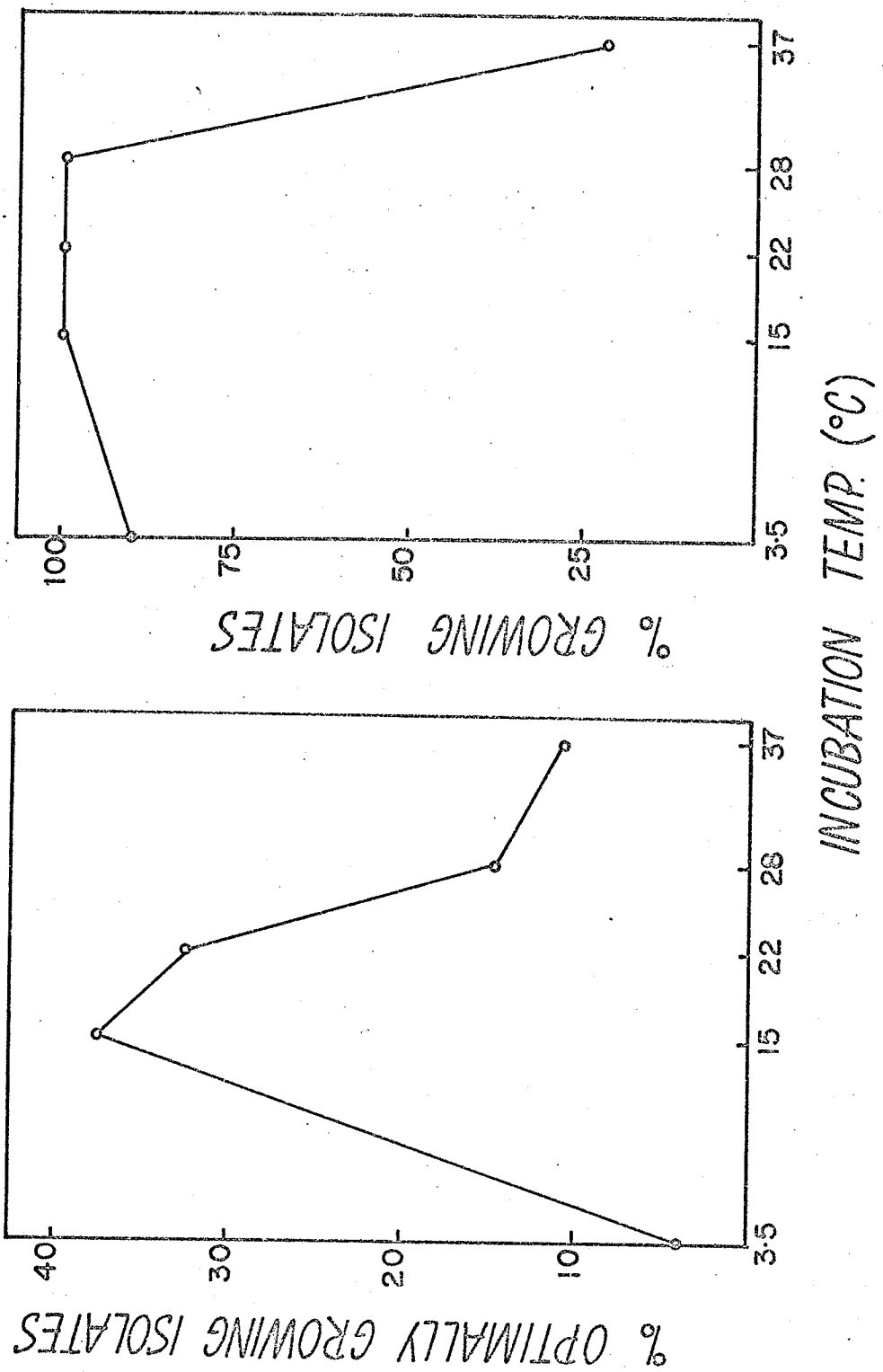

% GROWING ISOLATES
100
75
50
25
3·5
15
22
28
37
% OPTIMALLY GROWING ISOLATES
40
30
20
10
3·5
15
22
28
37
INCUBATION TEMP. (°C)

Table IX

Optimum growth temperatures of morphologically distinct groups of bacteria isolated from Churchill soils.

No. of isolates	Gram stain and morphology	No. of isolates growing optimally at				
		3.5°C	15°C	22°C	28°C	37°C
71	gram(-) rods	4	28	24	10	5
14	gram(+) rods	0	6	4	2	2
13	gram(+) cocci	0	3	5	3	2
2	gram(-) filaments	0	0	0	0	2

At and above 37°C a marked decrease in number of strains able to grow was noted while almost 90 percent of the isolates gave growth at 3.5 $\pm$ .5°C.

In terms of optimum temperature for growth, however, it is apparent from the plot given in Fig. 12a that 15° to 22°C proved to be best suited for the majority of the isolates; i.e., 37 percent and 32.5 percent of isolates grew optimally at 15°C and 22°C respectively. Some 15 percent of the strains displayed an optimum temperature for growth at 28°C, thus the sharply decreasing trend above 22°C further emphasizes the cold environment adaptation phenomenon of bacteria isolated from subarctic soils. Here it should be noted that 18 of the 100 isolates grew from 3.5 $\pm$ .5°C to 37°C while 71 of the strains had a more restricted range from 3.5 $\pm$ 5 to 28°C.

The distribution of Gram characteristic and simple morphology of the isolates in terms of the optimum temperature ranges is given in Table IX. These data show that although the majority of isolates studied were Gm^{-ve} rods which grew optimally between 15° and 22°C, there were 10/71 strains with an optimum of 28°C and 5/71 showing an optimum temperature of 37°C. In studies of psychrophilic organisms by Ingraham and Stokes (1959) and by Stokes (1963), variations of this kind in spread of optimal temperatures have been reported and are not considered unusual. Gm^{+ve} rods and cocci gave similar

spreads in temperature optima while the two Gm^{-ve} filamentous isolates, possibly Myxobacteria, showed growth optima of 37°C. $Gram^{-ve}$ bacteria constituted the largest proportion of the total isolates and displayed varying temperature optima ranging from 3.5 ± 0.5°C to 37°C with the greatest numbers showing an optimum temperature range between 15°C and 22°C. This latter range is quite characteristic of psychrophilic organisms as noted by Ingraham and Stokes (1959) and Stokes (1963).

Fungi

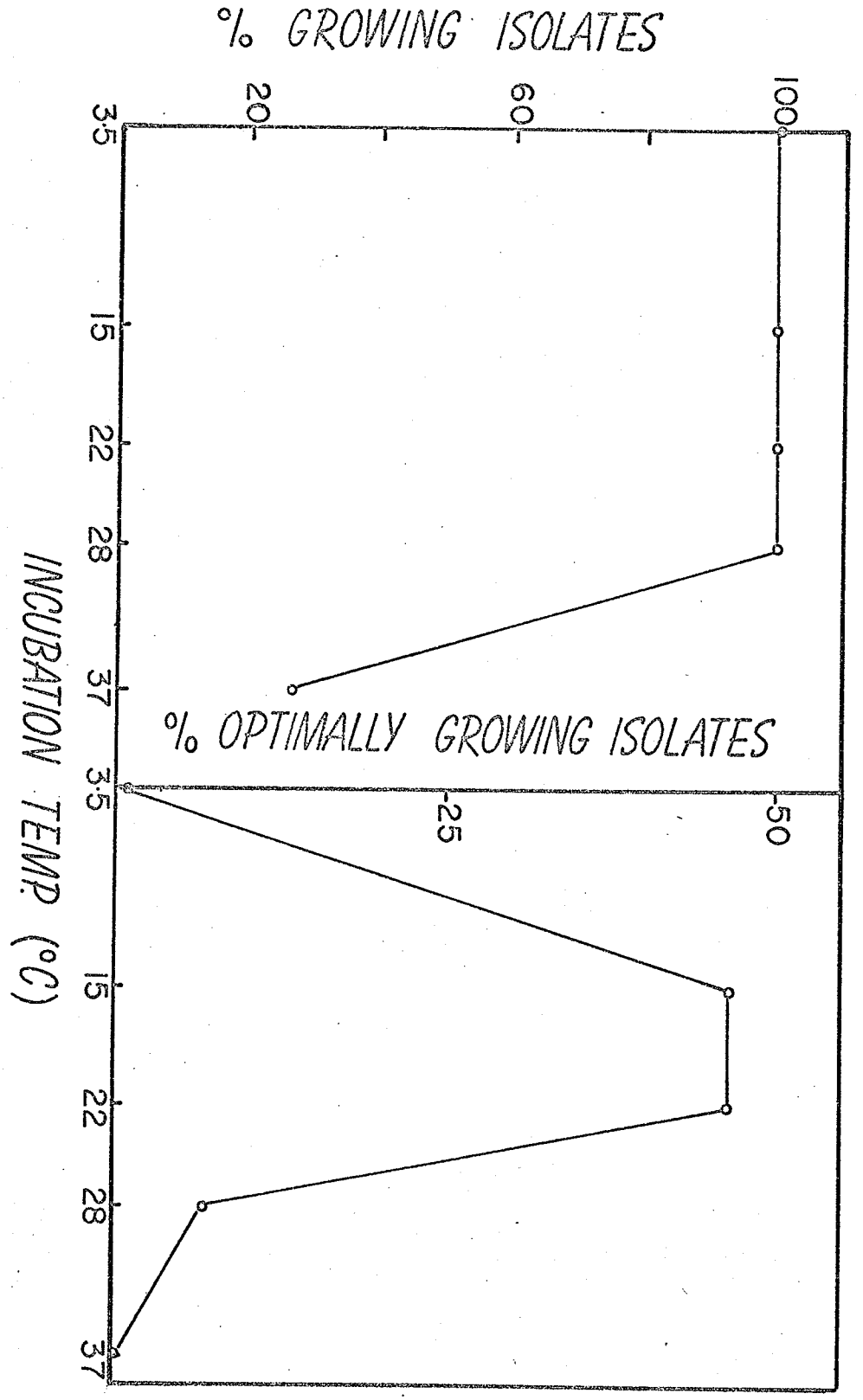
Thirty fungal strains isolated from Hudson Bay soils were examined for their growth temperature requirements on malt extract agar (Difco). Results are presented in Fig. 13. All strains grew from 3.5° to 28°C, Fig. 13a, and although some strains grew at 37°C, growth was poor. The optimum temperature range for growth of the majority of the fungal isolates was from 15° to 22°C, Fig. 13b.

In general, these findings agree with those of Ivarson (1965) on fungi from Mackenzie Valley Delta and differ little from the results obtained for more temperate soils (Timonin, 1939) where fungi seem to be less adapted to low temperature growth. Relatively little is known about the psychrophilic habit among fungi but according to Gunderson (1962) these fungi can be classified as psychrophilic strains as they are able to grow at temperature below 5°C.

Fig. 13. Growth-temperature responses by fungal isolates from Churchill soils.

(a) Percent bacterial isolates capable of growing at different temperatures.

(b) Percent bacterial isolates growing optimally at different temperatures.


% GROWING ISOLATES
20
60
100
3.5
15
22
28
37
INCUBATION TEMP. (°C)
% OPTIMALLY GROWING ISOLATES
25
50
3.5
15
22
28
37

Quantitative Studies on Subarctic Soil Microflora

Having selected appropriate media and incubation temperatures on the bases of the experiments just reported, viable counts per gram of soil (oven-dried weight) of bacteria (excluding streptomycetes) were determined for each of the composite and individual soil samples under study according to the techniques outlined in Materials and Methods. The results of this survey are presented in Table X.

Variations in soil reaction (pH) as reported in Physico-chemical Considerations ranged from moderately acidic to slightly alkaline. Indeed, within a single sampling area, pH variations occur to a considerable degree. Despite this, bacteria constituted the predominant group in all the soil samples. In the acidic Old Beach soils, although bacteria predominate, the difference between total bacterial counts and total fungal counts is much less than that noted for Goose Creek and Inuvik soils. Again, despite higher acidity, Old Beach soils gave higher counts of actinomycetes than did the less acidic Twin Lakes soils. Considerably larger numbers of actinomycetes were encountered in Goose Creek soils than in any of the other soils examined.

In Goose Creek surface soils which range from slightly acidic to alkaline, numbers of fungi per gram tend to increase, as might be expected, with increasing acidity. In contrast, however, fungal populations were highest in the two Inuvik soils which were slightly to moderately alkaline. Thus, pH

Table X

Distribution of microorganisms in relation to some physico-chemical characteristics of surface soils from the Churchill and Inuvik areas.

Soil Sample	Depth Inches	Moisture %	pH	Organic Matter %	Total N %	C/N ratio	Microorganisms X 10^6/g soil			
							Total Bacteria	Gm neg.* Bacteria	Fungi	Actinos.
OB-1	0-5	25.0	6.50	19.20	0.443	25.2	0.27	-	0.04	-
OB-4	0-4	30.0	4.60	22.94	0.547	24.3	0.54	15.65	0.22	-
OB-5	0-4	78.0	4.95	84.67	1.730	28.45	1.44	41.66	0.20	0.26
OB-6	0-8	75.0	6.05	72.60	1.930	21.80	5.20	56.00	0.36	0.42
OB-7	0-4	30.0	5.25	9.50	0.255	21.60	0.13	35.00	-	0.04
OB-9	0-4	43.0	4.50	27.00	0.60	26.10	0.70	21.11	0.25	0.07
TL-2	0-4	32.0	5.00	9.50	0.30	18.40	2.22	51.80	0.33	0.06
TL-3	0-4	38.0	5.60	12.24	0.47	15.12	8.15	73.70	0.38	0.06
TL-8	0-5	40.0	6.40	8.35	0.26	19.00	2.20	80.00	0.28	0.10
TL-10	0-5	30.3	6.80	11.35	0.33	20.00	7.17	83.00	0.20	0.06
TL-11	0-4.5	40.0	6.67	8.32	0.26	18.98	6.80	74.26	0.22	-

Table X. Cont'd.

Soil Sample	Depth Inches	Moisture %	pH	Organic Matter %	Total N %	C/N ratio	Microorganisms X 10^6/g soil			
							Total Bacteria	Gm neg.* Bacteria	Fungi	Actinos.
GC-12	0-4-5	60.0	6.30	45.40	1.620	16.50	51.00	60.00	0.35	0.40
GC-13	0-5	52.8	7.60	6.70	0.289	13.50	7.06	90.0	0.11	2.64
GC-14	0-4	75.0	6.00	68.00	2.90	13.79	18.96	78.0	1.56	3.50
GC-15	0-4	65.0	5.80	70.00	2.238	18.32	4.74	80.0	1.85	1.42
GC-16	0-4	52.5	5.80	27.52	1.160	13.80	4.50	-	-	-
GC-17	0-4	40.0	5.60	30.10	1.710	10.23	1.60	-	-	-
Iv-X	0-5	42.0	7.35	17.95	0.540	19.30	32.00	-	2.40	-
Iv-Y	0-5	35.0	8.15	12.71	0.500	14.78	17.60	-	0.04	-
Iv-Z	0-5	45.0	8.10	20.64	0.548	22.0	37.00	-	0.34	-

*Percent of total bacteria at 15°C

- Data not determined

does not seem to be a deciding factor in determining the size of fungal populations in subarctic soils.

Goose Creek soils gave high numbers of actinomycetes; in fact, these micro-organisms constituted a rather large proportion of the Goose Creek microflora despite the fact that their (actinomycete) distribution was irregular and seemingly unrelated to either pH or organic matter content. Examination of Inuvik soils which did not include actinomycete determinations gave high bacterial and fungal counts.

The finding that bacteria usually occur in much larger numbers than other groups of organisms in acidic soils was noted by previous investigators including Waksman and Purves (1932), Jensen (1934), Timonin (1935), Ivarson and Katznelson (1960) and Holding et al (1965) for soils of temperate regions. This also appears true for acidic soils of the subarctic. Jensen (1930) had observed that the frequency of occurrence of actinomycetes was related in soil to pH, i.e., they favoured slightly alkaline soils; this observation was not confirmed in this study.

In view of the differences in topography, soil physical characteristics, soil pH, vegetative cover, wind erosion effects and moisture contents, any comparisons of the microbial populations either qualitatively or quantitatively are probably not very meaningful. As has been established, such variations occur not only between sample sites but also to some extent

are evident within a single sample area. As well, variations in kind and numbers of micro-organisms occur in terms of vertical distribution within a single sample. Superimposed upon these naturally occurring variations are the inherent errors of estimation in the plate-count techniques where a single medium (for practical purposes) was used for both acidic and near neutral soils. Other investigators have encountered the same limitations in plate count techniques. Corke and Chase (1964) for example found significantly different counts of actinomycetes when using acidic and near neutral media for enumeration in soils of differing reaction.

Total viable counts of bacteria, fungi and actinomycetes did not show significant relationship with the organic matter content of the soils examined. Statistical analyses of the data for organic matter versus total counts of micro-organisms gave correlation coefficients on the bases of five samples for each soil of 0.65 and 0.32 for Twin Lake and Old Beach soils respectively. Bunt and Rovira (1955), for example, in comparison studies of sub antarctic soils found maximum counts of bacteria in a beach sand almost devoid of organic matter.

The Occurrence of Some Major Groups of Micro-organisms in Hudson Bay Soils

Bacteria

Evidence based on 'direct' counts of Gm^{-ve} bacteria developing on selective agar plates (containing Crystal Violet) indicated that these organisms constituted the major population group in Goose Creek and Twin Lake soils. The more acidic soils of the Old Beach area contained fewer Gm^{-ve} bacteria, ranging from 15 to 56 percent of the total viable count, as compared to Twin Lakes and Goose Creek soils where Gm^{-ve} bacteria formed 80 to 90 percent of the total bacterial population (see Table X).

The dominance of Gm^{-ve} bacteria was also noted in observations made during examination of the random isolates (Table XIa). Here, the media used were not selective for Gm^{-ve} bacteria but none the less the majority of cultures examined from Goose Creek and Twin Lake soils were of this type. The more acidic Old Beach soils, again, gave evidence of fewer Gm^{-ve} organisms. Gm^{+ve} bacteria were more frequently encountered in Old Beach and Twin Lake soils than in the Goose Creek mineral or peaty soils. Cocci (mostly Micrococcus spp.) made up approximately 44 percent of the total isolates in OB-1 samples but were not isolated either from Goose Creek or OB peaty soils. Coccoid rods (mostly Gm variable to Gm^{-ve})

Table XI

Occurrence of some major groups of micro-organisms in Churchill soils.

A. BACTERIA

Soil Sample	Total No. of Isolates	Gram Stain			Morphology			
		Percent of Total Isolates						
		(-)	(+)	(±)	Rods	coccoid rods	Fila-ments	cocci
OB-1	18	33.3	55.1	11.2	44.4	11.2	0.0	44.4
OB-4 & 5	21	61.9	33.3	4.8	95.0	4.8	0.0	0.0
TL-2 & 3	20	65.0	30.0	5.0	75.0	5.0	10.0	10.0
GC-12	18	66.6	16.6	16.6	66.6	22.2	5.5	5.5
GC-15 & 16	24	100.0	0.0	0.0	66.6	25.0	8.3	0.0

Table XI. Cont'd.

B. FUNGI

Soil Sample	Dominant Species	Associated Species
OB-1	Penicillium purpurrescens Sopp, Penicillium sp.(1), Mortierella sp.(1)	Oidiodendron sp., Penicillium chrysogenum Thom, Chryso-sporium sp., Pachy basidium sp.
OB-4	Penicillium sp.(1), Oideodendron sp., Chrysosporium sp., Mortierella sp.	Penicillium chrysogenum Thom
OB-5	Penicillium chrysogenum Thom, Trichoderma album Preuss, Trichoderma glaucum Abbott	Penicillium sp.(2)
TL-2 & 3	Chrysosporium sp., Gliocladium atrum Gilman and Abbott, Chrysosporium pannorum, Hughes, Cephalosporium acremonium Corda, Trichoderma album Preuss, T. glaucum Abbot	Penicillium restrictum, Gilman and Abbot, Cephalosporium humicola, Oudemans, Pullularia sp., Pachybasidium, Epicoccum sp.*, Mucor sp.
GC-12	Epicoccum sp., Pullularia sp., Gliocladium atrum, Gilman and Abbott, Cephalosporium spp.	Penicillium spp., Chrysosporium sp.
GC-15 & 16	Penicillium spp., Trichoderma glaucum, Abbott, Trichoderma album, Preuss	Wardomyces sp., Cladosporium sp.

*Epicoccum sp. was identified by Dr. P.K. Isaac, Dept. of Botany, University of Manitoba, Winnipeg.

Table XI. Cont'd.

C. ACTINOMYCETES

Soil Sample	Dominant Species	Associated Species
OB-1	Streptomyces sp.(1)* Streptomyces sp.(2)	
TL-2 & 3	Streptomyces sp.(2), Nocardia(1) Nocardia(2), Nocardia(3)	Actinomycetes were restricted to a few 'type species'
GC-12	Streptomyces sp.(1), Streptomyces sp.(2)	
GC-15 & 16	Streptomyces sp.(1), Streptomyces sp.(2)	

*(1, 2, 3) - morphologically distinct colonies appearing on the plates.

formed significant numbers in all the soils examined while filamentous forms (mainly Cytophaga spp.) were isolated only from Goose Creek and Twin Lake soils.

Variation in frequency of occurrence of gm^{-ve} bacteria did not seem to be related to soil factors except, possibly, the small proportion found in acid soils of the Old Beach formation. However, it did seemingly bear a relationship to the rhizosphere effect as reported by Holding (1960) and Rouatt and Katznelson (1961) in temperate soils. High percentages of these organisms were encountered in soils of the Goose Creek area (good vegetation) while the percentages decreased in Tsin Lake and Old Beach soils where vegetation was moderate to sparse. The isolation of gm^{+ve} cocci from TL-2, TL-3, OB-1 and GC-12 soils but not from OB-4, OB-5, GC-15 and GC-16 soils suggests that these bacteria could be regarded as insignificant in terms of numbers in acid-peat soils. Again, similar observations have been made in this respect by Stout (1958) and Jensen (1963) dealing with temperate soils.

Unfortunately, information on the distribution of cytophaga species is not available. Culture isolates from the first series of samples failed to survive in storage.

Fungi

Among the fungi isolated during the course of this study only relatively few genera were regularly encountered.

These dominant fungal groups are noted in Table XIb.

The OB-5 soils, for example, was characterized by the dominance of Trichoderma and Penicillium species. In OB-1 and OB-3 soils, the dominant fungal groups were Penicillium, Mortierella, Chrysosporium and Oidiodendron.

Twin Lakes -2 and -3 soils contained large numbers of fungi whose dominant members were Chrysosporium, Gliocaldium, Cephalosporium and Trichoderma. The Goose Creek mineral soil (GC-12) yielded Epicoccum, Pullularia and Gliocladium as the principal fungal genera present. In the peaty Goose Creek soils (GC-15 and GC-16) the principal fungi were Penicillium and Trichoderma.

The number of distinct genera present in these soils appear to be low; only a total of 12 genera were identified in all samples from the three areas studied. Most of the genera isolated from soils were found to be common to fungal genera encountered in soils of Mackenzie Delta (Ivarson, 1965). Aspergillus and Fusarium which are known to be abundant in the soils of temperate regions (Gilman, 1957) were absent. However, all the fungal species encountered in these soils seem to be common to those recorded for the temperate regions of Canada (Gilman, 1957; Hennebert, 1962; Morrall, 1968). Penicillium spp. were found in all the soil samples in some cases as the dominant species, in other cases they were associated with other dominent groups

Species of Trichoderma, Cephalosporium, Epicoccum, Pullularia, Mortierella, Gliocaldium, Oidiodendron and Chrysosporium were found to be rather restricted to certain soil types. Occurrence of relatively few genera in OB-5 and Goose Creek peat soils (GC-15 and GC-16) could possibly be explained on the basis of antagonistic action of the dominant Trichoderma and Penicillium spp. as has been suggested by Welvaert and Veldeman (1955).

Actinomycetes

Visual and microscopic examination of colonies developing on agar plates suggested that the actinomycete population consisted of only two generic groups, Streptomyces and Nocardia. Within the Streptomyces group two morphologically distinct members were found in almost all the soils examined, Table XIc. The culture designated as Streptomyces-1 was dominant in the less acidic soils. All three species of Nocardia were found only in Twin Lake soils.

Physiological characteristics of the microbial flora

Physiological activities of some isolated strains of bacteria and fungi are detailed in Tables XIIa and b. On the bases of a few selected reactions mainly involving nitrogen transformations it was found that Hudson Bay soils do harbour metabolically active groups of micro-organisms adapted

Table XII

Some physiological activities of isolates from Churchill soils.

A. Bacteria

Gram Reaction & Morphology	No. of isolates	% Isolates reducing Nitrate to Nitrite	Urea to Ammonia	gelatin liquification
Gram(-) rods	32	50.0	34.3	9.4
Gram(+) rods	7	28.5	14.0	0.0
Gram(+) cocci	8	62.5	50.0	0.0
Gram($\pm$) coccoid rods	4	50.0	33.0	0.0
Gram(-) coccoid rods	11	27.0	18.0	20.0

Table XII. Cont'd.

Biochemical Activities of some fungal isolates from Churchill soils.

B. Fungi

Fungal isolate	Source of Isolation	Hydrolytic activity toward			
		Cellulose	Xylan	Pectin	Laminarin
Trichoderma album	Twin Lake	+	+	n.d.*	+
Trichoderma glaucum	Old Beach	+	+	+	+
Chrysosporium pannorum	Twin Lake	+	+	+	+
Chrysosporium sp. (1)	Twin Lake	very poor	+ n.d.	+	+
Chrysosporium sp. (2)	Old Beach	(-)	n.d.	+	(-)
Penicillium chrysogenum	Old Beach	(-)	n.d.	+	(-)
Penicillium restrictum	Twin Lake	+	+	+	+
Penicillium sp. (1)	Old Beach	(-)	+	+	+
Penicillium sp. (2)	Old Beach	(-)	+	+	+
Penicillium sp. (3)	Old Beach	+	n.d.	n.d.	n.d.
Epicoccum sp.	Goose Creek	+	+	+	+
Cephalosporium acremonium	Twin Lake	+	n.d.	n.d.	n.d.

Table XII. Cont'd.

Fungal isolate	Source of isolation	Hydrolytic activity toward			
		Cellulose	Xylan	Pectin	Laminarin
Gliocladium atrum	Twin Lake	+	n.d.	n.d.	+
Oidiodendron sp.	Old Beach	+	+	(-)	+
Wardomyces sp.	Goose Creek	+	+	+	+
Mucor sp.	Twin Lake	+	n.d.	n.d.	n.d.

*not determined

to carry out the various reactions at low temperatures equivalent to those pertaining in the area studied. Since only a limited number of strains were tested no generalization can be made concerning specific contributions by any morphologically distinct group of bacteria.

Fungal isolates from Hudson Bay soils when tested for their hydrolytic potential towards complex carbohydrates displayed significant activities. A majority of the dominant species were found to degrade cellulose, xylan and pectin. Laminarin, a β-D-1,3 glucan (Whistler and Smart, 1953) now known to be of wide occurrence in algae, fungi and higher plants, was found to be hydrolyzed by a majority of the isolates.

Streptomyces and Nocardia species were all found to be cellulose decomposers when tested by appropriate techniques at an incubation temperature of 15°C.

Decomposition of Organic Matter in Churchill Soils

One of the main features of Churchill soils noted was the accumulation therein of relatively large quantities of organic matter, the decomposition of which would actively contribute to the soil forming process. Although there is ample information available on the microbiology of organic-matter decomposition with particular references to the microbial agents involved, little is known in this regard about the arctic or subarctic soils. In his recent paper on arctic mycology, Savile (1963) pointed out that the bacterial action on organic-matter decomposition under arctic environments seems to be ineffective. He also suggested that fungal agents would probably be the chief operative agents in this process. Since soils in the area are generally acidic, one might reasonably expect fungal decomposition activities would likely be dominant. To evaluate the respective contribution in organic matter decomposition by fungi and bacteria, a series of experiments were conducted. The planning of these experiments was based principally on two quite different approaches involving the breakdown of added polysaccharides. These experiments included:

1) Functional group analysis of micro-organisms involved in cellulose decomposition and 2), estimation of the enzymic hydrolysis of some added organic matter constituents

(cellulose, xylan and starch). This latter approach was based on the assumption that decomposition of soil organic matter proceeds firstly by enzymic hydrolysis to simple compounds. These hydrolytic products then can be absorbed or metabolized by micro-organisms.

In this sense the rate of breakdown of organic-matter constituents will be determined mainly by the rate at which extracellular enzymic hydrolysis proceeds. The principal polysaccharide component used for this investigation was cellulose. However, xylan and starch have also been used in some of the experiments.

Micro-organisms involved in organic matter (cellulose) decomposition

Cellulolytic fungi, bacteria and actinomycetes were counted on selective agar media as described under Materials and Methods. Results are presented in Table XIII.

Characteristics of extracellular enzymes of some microbial isolates

In vitro studies on cellulase and partly on xylanase, produced by fungal isolates from different soils, Table XIV, shows that fungal cellulolytic and xylan hydrolysing enzymes are most active in the pH range of 4.0 to 5.0, while cellulolytic enzymes from bacteria and actinomycetes are active

Table XIII

Estimation of cellulolytic micro-organisms and associated activities in Churchill soils.

Soil Sample	Cellulolytic micro-organisms		Ratio
	Total ($\times 10^6$)/ g soil	Fungi (%) of total	Cellulase activity at pH 4-5 / Cellulase activity at pH 6-7
OB-9	0.90	44.0	0.90
OB-5	2.88	35.0	0.50
TL-2	2.00	37.5	0.80
TL-3	2.80	45.0	1.20
TL-10	1.68	17.0	0.38
GC-12	1.40	30.0	0.82

Table XIV

Some characteristics of extracellular enzymes (cellulase, xylanase) of certain soil isolates.

Fungal or bacterial isolate	Source of isolation	Temperature-Growth Characteristics		Cellulase activity		Xylanase activity	
		optimum temp(°C)	Temp. range of growth(°C)	optimum pH[1]	optimum temp(°C)[2]	optimum pH	optimum temp(°C)
Mucor sp.	TL-2	22	3-28	4.0	37	d.n.a.*	d.n.a.
Epicoccum sp.	GC-12	15	3-28	5.0	50	d.n.a.	d.n.a.
Chrysosporium pannorum	TL-3	22	3-37	4.5	50	3.4	40
Penicillium sp.	OB-1	22	3-37	5.0	50	5.0	50
Trichoderma glaucum	TL-3	15	3-28	4.0	50	4-5	40
Penicillium sp.	OB-5	22	3-37	4.0	50	d.n.a.	d.n.a.
Penicillium sp.	OB-4	22	3-37	-	-	4.5	50
Cladosporium sp.	GC-15	22	3-28	4.0	50	d.n.a.	d.n.a.
Trichoderma album	TL-2	15	3-28	4.0	50	5.0	40
Gliocladium sp.	TL-2	15	3-37	5-6	37	d.n.a.	d.n.a.
Oidiodendron sp.	OB-1	22	3-28	4.0	50	4-5	40

Table XIV. Cont'd.

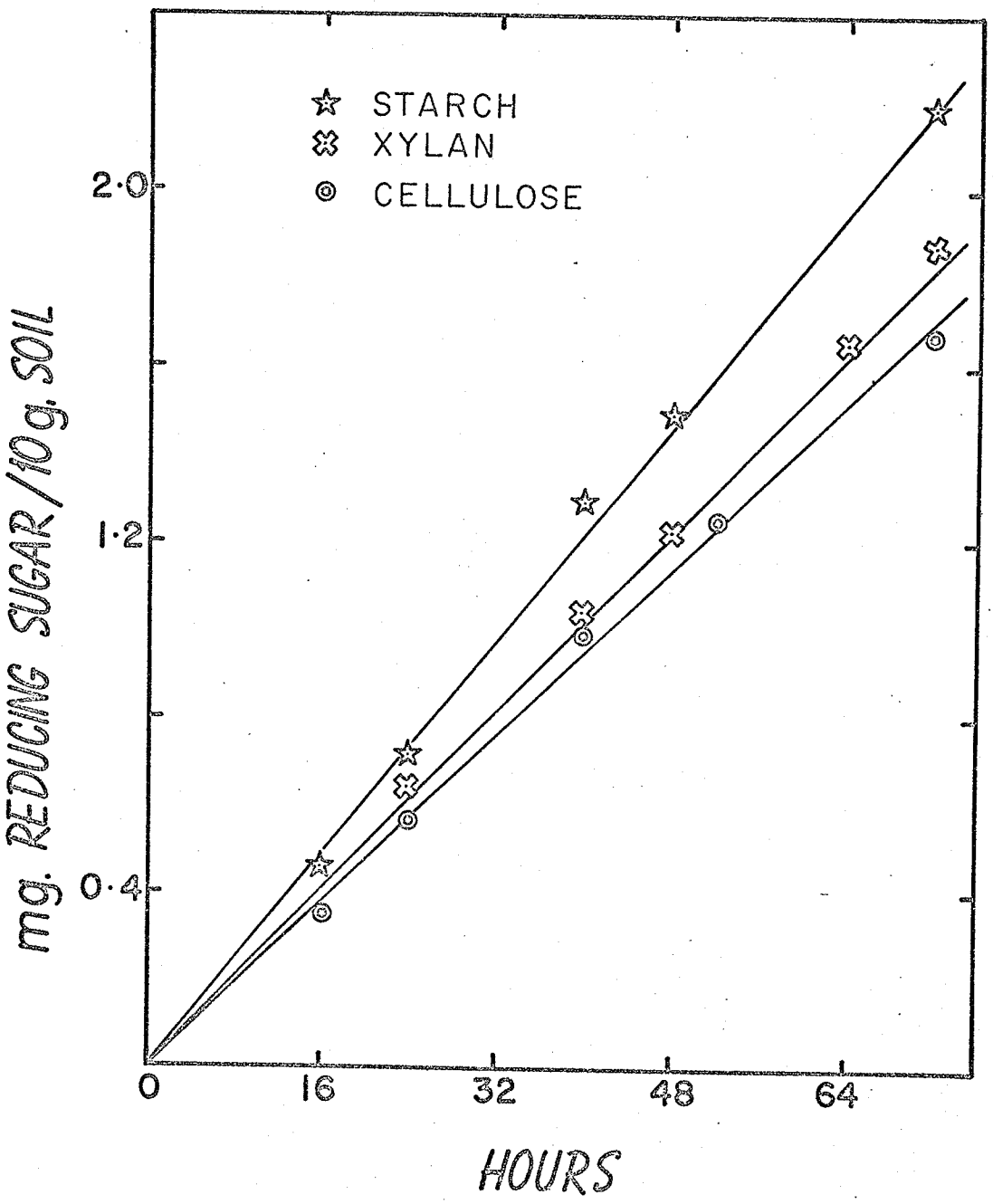
Fungal or bacterial isolate	Source of isolation	Temperature-Growth Characteristics		Cellulase activity		Xylanase activity	
		optimum temp(°C)	Temp. range of growth(°C)	optimum pH	optimum temp(°C)	optimum pH	optimum temp(°C)
Penicillium sp	OB-5	22	3-37	5.0	50	d.n.a.	d.n.a.
Cephalosporium sp.	TL-3	22	3-37	d.n.a.	d.n.a.	d.n.a.	d.n.a.
Streptomyces sp.(1)	TL-2	28	3-37	7.0			
Nocardia sp.(2)	TL-3	28	3-37	7.0	Optimum temperature for cellulase, and data for xylanase not available for Streptomycetes and Bacteria.		
Bacterial isolate (1)	OB-5	22	3-28	7.0			
Bacterial isolate (2)	TL-2	22	3-28	7.0			

*d.n.a. = Data not available

[1]Enzymic activities at different pH values were determined, using 0.2 M acetate buffers (pH 3.5-5.0), 0.2 M phosphate, KH_2PO_4-K_2HPO_4, buffer (pH 6-8). Reaction was carried out at 30°C for 1 hour.

[2]Activities at different temperatures (15°, 30°, 40°, 50°, 60° and 70°C) were followed at optimum pH values. Reaction time in each case was 1 hour.

Fig. 14. Time-course hydrolysis of cellulose, xylan and starch in TL-10 soils.


STARCH
XYLAN
CELLULOSE
2·0
1·2
0·4
0
16
32
48
64
mg. REDUCING SUGAR / 10 g. SOIL
HOURS

in the pH range from 6.0 to 7.0. Additional data from the literature (Mandel and Reese, 1965) on cellulolytic enzymes of different organisms suggests that fungal cellulases have optimum pH values similar to those determined in this study and, moreover, the pH optima they reported for bacterial and actinomyces cellulolytic enzymes are in close agreement with those reported above.

Analysis of cellulolytic and some other polysaccharide-hydrolysing enzymes of soils

Time-course hydrolysis of cellulose, xylan and starch

A preliminary observation that soil treated with toluene (bacteriostatic and plasmolytic agent) catalyzed the hydrolysis of added polysaccharides, e.g., cellulose, xylan and starch, suggested the presence of extracullular enzymes and/or metabolyzing (but nondividing) micro-organisms. Production of reducing sugars as a measure of hydrolysis end-products was linear for 72 hours incubation at 28°C, Fig. 14.

Effect of pH on the hydrolysis of added polysaccharides

The effect of pH on the hydrolysis of cellulose and starch was observed in the pH range from 4.0 to 8.0 and on xylan from 4.0 to 7.0. These results are presented in Fig. 15a, b and c.

Fig. 15. Effect of pH on the hydrolysis of added polysaccharides by different soils.

(a) Cellulose

(b) Starch

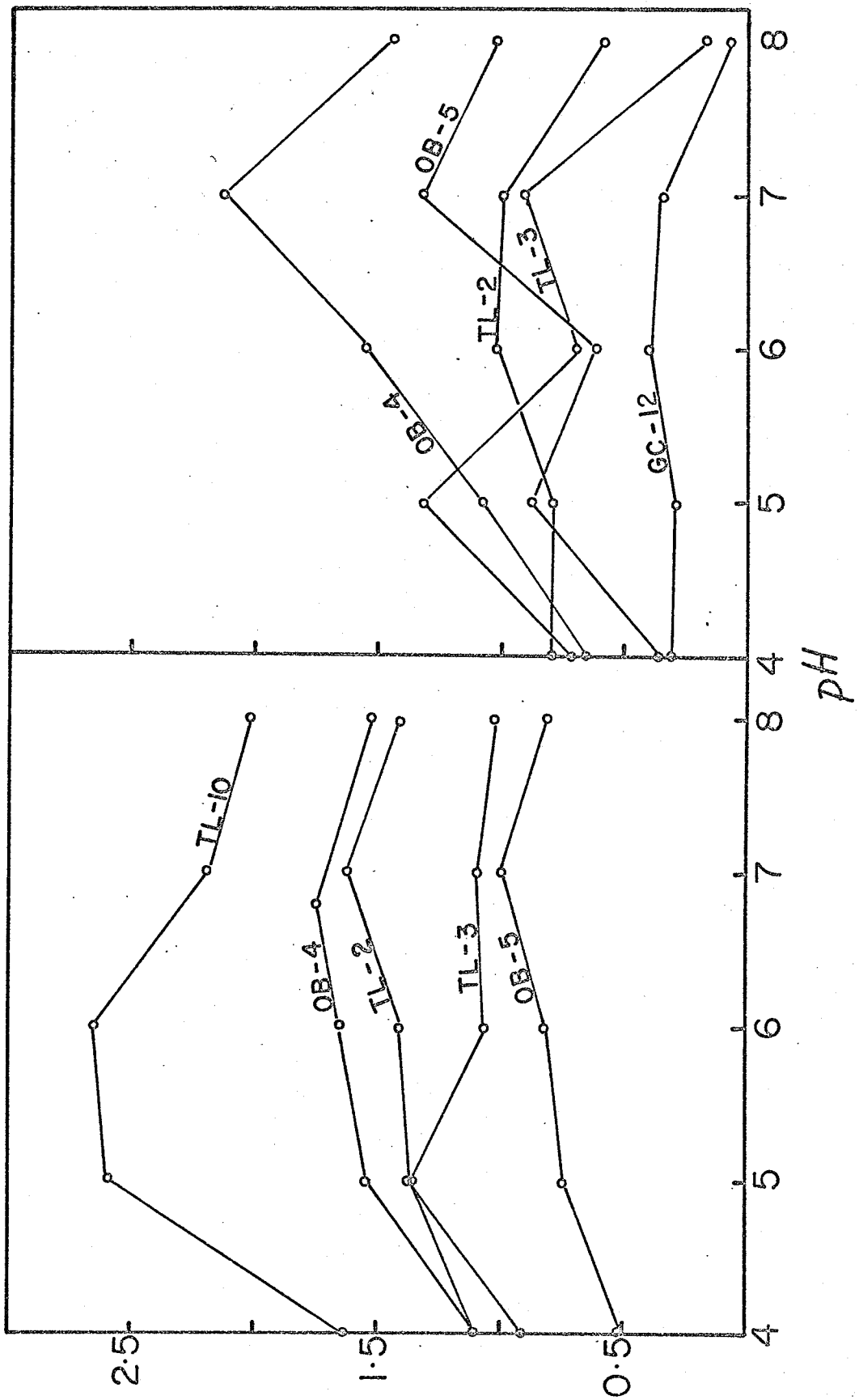
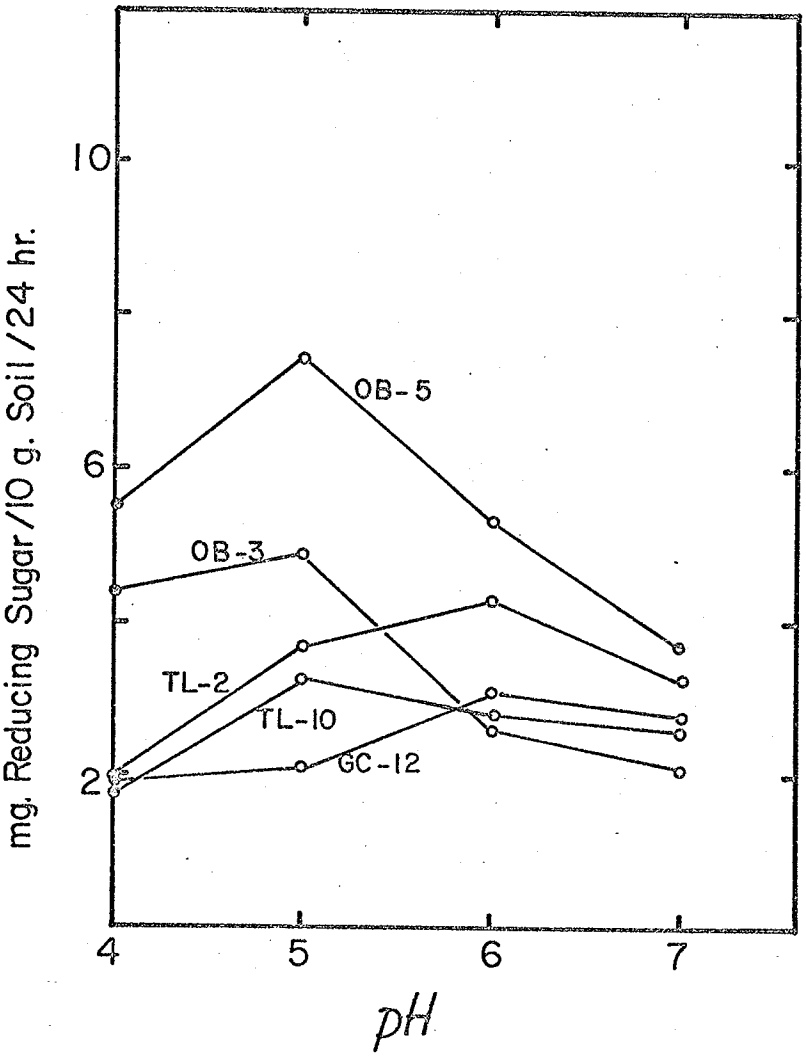

mg. Reducing Sugar/10 mg. Soil/24 hr.
2.5
1.5
0.5
4
5
6
7
8
pH
TL-10
OB-4
TL-2
TL-3
OB-5
OB-4
TL-2
TL-3
OB-5
GC-12

Fig. 15. Effect of pH on the hydrolysis added polysaccharides by different soils (C) Xylan.


mg. Reducing Sugar/10 g. Soil/24 hr.
10
6
2
OB-5
OB-3
TL-2
TL-10
GC-12
4
5
6
7
pH

A variable response to pH was observed in all the soils examined. The OB-5 soil gave two distinct peaks for hydrolysis of cellulose in the acid and neutral pH ranges respectively. With starch, however, the same soil showed a single broad peak for hydrolysis activity that increased in intensity towards the alkaline side. In the case of added xylan, a single peak was obtained which indicated the pH optimum to be in the acid range. OB-4 soil gave pH optima curves very similar to OB-5 for starch hydrolysis but, with cellulose as a substrate, a single peak in the neutral range was noted. TL-3 soil gave similar responses to pH with xylan and starch, having peaks on the acid side; however, with cellulose two distinct peaks at pH 5.0 and pH 7.0 again were noted. TL-3 soil gave almost identical responses with all three substrates. GC-12 soils, on the other hand, showed pH optima for both cellulase and xylanase to be in the neutral to alkaline range.

Cellulolytic micro-organisms and activities in soils.

The intensity of cellulose hydrolysis at acid pH (4.0 to 5.0) and at neutral pH (6.0 to 7.0) as determined for the different soils are also detailed in Table XIII, along with the number and types of micro-organisms present. As evident from the Table, the observed decrease in the ratio of activities (enzymic hydrolysis) at acid to neutral pH

paralleled the percentage decrease in the occurrence of cellulolytic fungi. The development of two peaks for cellulose hydrolysis, one on the acid side and the other on the neutral side, could be accounted for by the nature of the enzymes derived from different groups of micro-organisms. Thus, it would seem that both fungi and bacteria (including actinomycetes) are active in acid and in neutral subarctic soils and that pH is only one of a number of factors that determines the type and intensity of polysaccharide hydrolysis.

These data are further supported by the respiration intensity of some of the soils at different pH values[1]. Respiration rates calculated from the cumulative oxygen uptake values after four hours percolation at different pH levels are presented in Fig. 16. A marked decrease in the O_2 uptake by the Goose Creek (GC-12) sample was noted as pH values were lowered toward the acid side. TL-2 soil, on the other hand, gave two distinct respiration peaks; one which developed as pH values were adjusted toward the acidic side and the other which developed toward the alkaline side. OB-4 soil showed two respiration peaks similar to those displayed by the TL-2 soil although peak intensities were much lower.

---

[1] pH of the soils was adjusted to desired values by percolating 0.2 M phosphate buffers (KH_2PO_4 - K_2HPO_4) through the water saturated soils and sand columns. pH values recorded are based on the final values of soil:buffer suspension.

Fig. 16. Influence of pH on the respiration intensity of GC-12, TL-2 and OB-4 soils. Respiration intensity was measured at 25°C.

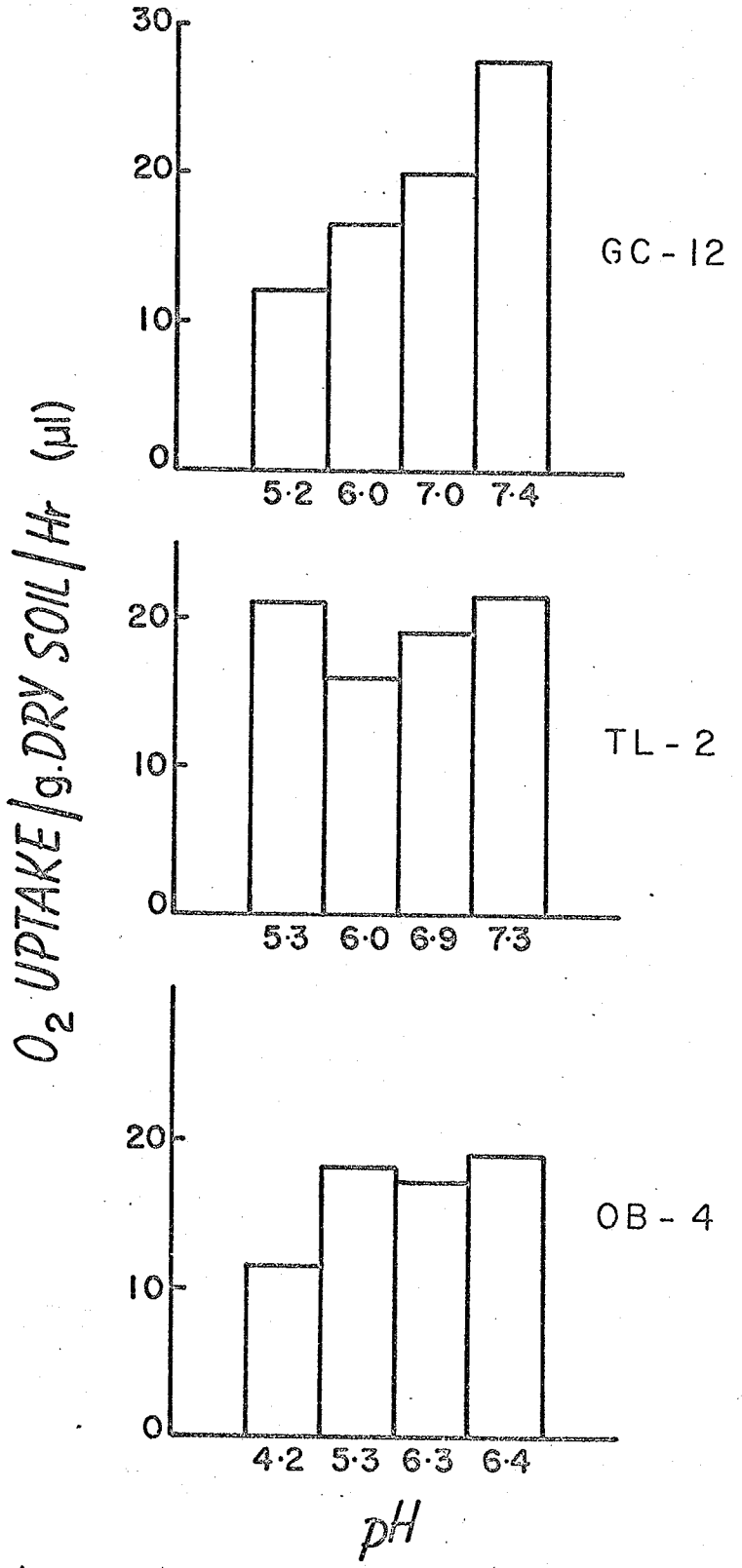

30
20
10
0
5·2 6·0 7·0 7·4
GC - 12
20
10
0
5·3 6·0 6·9 7·3
TL - 2
20
10
0
4·2 5·3 6·3 6·4
OB - 4
O2 UPTAKE/g.DRY SOIL/Hr (µl)
pH

Table XV

Oxygen uptake and cellulolytic activities of Churchill soils at acid (4-5) and neutral (6-7) pH values.

Soil Sample	Oxygen uptake (l)/hr/g soil		Cellulolytic activities (mg. red. sugar)/10 g soil/48 hrs	
	at acid pH (a)	at neutral pH (b)	at acid pH (c)	at neutral pH (d)
OB-9	17.00	20.00	0.79	0.87
OB-4	18.00	19.00	0.87	1.80
OB-5	50.00	45.00	2.77	4.70
TL-2	18.00	21.00	0.80	1.00
TL-3	17.00	21.00	1.01	0.80
TL-10	16.00	-	0.80	2.10
GC-12	25.00	19.60	0.31	0.38

Correlation coefficients between: (a) and (c) = 0.90 for 6 d.f.
(a) and (d) = 0.56 for 6 d.f.
(b) and (d) = 0.97 for 5 d.f.

Enzymic activity in comparison with oxygen uptake in the soils

Results obtained on cellulolytic activity in the different soils, Table XV, suggests a significant correlation between oxygen uptake and cellulolytic activity at the lower pH ranges. When cellulolytic activity was measured at neutral pH in soils correlation with oxygen uptake was low. If the soil reactions, on the other hand, were amended by treatment to give slightly acid to neutral reactions (pH 6.0 to 7.0), 0_2 uptake correlation with cellulolytic activity gave a highly significant value of 0.97.

Short time-course respiration studies (up to four hours) are considered by Drobnik (1960) only to reflect enzyme systems originally present in the soils and would exclude, therefore, any respiratory contributions due to increasing population numbers (reporduction). Stevenson and Katznelson (1958) and Hoffman (1952) have proposed that such short-time respiration studies give a measure of the biological potency of soils. If one accepts the generally held belief that soil pH influences not only numbers of micro-organisms present, but also exerts a strong selective effect on the micro-organism types extant (bacteria/ fungi) then the respiratory activity peak noted in TL-2 in the acid pH range could reflect the activities of fungi present. Similarly the peak noted in the alkaline range could be

attributed to bacterial respiration mainly. This interpretation is analagous to that reported by Hoffman (1962) in his measurements of saccharase activities in soils. He noted that neutral soils showed higher saccharase activity at neutral pH values while soils that were inherently acidic displayed higher saccharase activities in the acid pH range. Strong activity at or near neutral pH values was assumed to be due to bacterial saccharases while high activities at acid pH values were probably of fungal origin.

Considerations of the data on microbial counts, the suitability of the isolates to low temperature conditions, and their apparent hydrolytic potential toward polysaccharides combined with the evidence of extracellular enzymic activity all suggest that both fungi and bacteria (including actinomycetes) are involved in organic matter decomposition in these soils. The experimental evidence in this investigation supports the statement of Greenwood (1968) that the decomposition of organic-matter in soils is limited by the rate of enzymic hydrolysis, and further, that decomposition can be measured by aerobic respiration. In contrast to the views of Alexander (1961) that fungi are the principal active agents in organic-matter decomposition in acid habitats (pH 5.5 and below), evidence suggests that in these acidic soils (pH less than 5.5) fungi, bacteria and actinomycetes all seem to be active. This could possibly be explained on the basis of their

adaptation to a low pH soil environment. The actual participation of bacteria and actinomycetes was not examined experimentally in pure culture in this investigation and only indirect evidence is available to support this assumption.

SUMMARY

Studies have been made on the physico-chemical, microbiological and the general biological characteristics of 7 composite and 20 individual surface soil samples from 4 sampling sites within the Canadian sub-arctic. Additional data on cored samples from some of the sampling sites have been provided with respect to general morphology, physico-chemical and microbiological characteristics of soils varying with depth. The pH of these soils generally tended to increase with depth and the C:N ratio remained broad throughout the length of the core. Active layer in terms of root penetration and intensity of microbial population was shallow. Surface soils were characterized by high organic matter content, low degree of humification, and low levels of ammonia and nitrate.

General biological activities of the soils were quite comparable to more temperate soils and, in general, biological activity was related with the organic matter content of the soils. Mineralization of organic nitrogen was evident in all the soils but in acidic ones it stopped at the ammonia level. Denitrification was also evident in some soil samples examined.

It was concluded that these soils could support relatively large numbers of bacteria, actinomycetes and fungi. Degree of cold-adaptation among microbial populations varied from site to site and region to region. In general, the extent of cold adapted populations noted in these soils was quite similar to that reported for Alaskan tundra and Antarctic soils. The majority of the isolates from Churchill region showed optimum growth-temperature in the range of 15° to 22°C and significant proportions were capable of growing at 3.5° $\pm$ .5°C.

Evidence was produced that both in the acidic and neutral-alkaline soils organic-matter decomposition proceeded via the action of bacterial and fungal groups. This finding is contrary to the generally held belief that such activity under arctic conditions is mainly the result of action of fungi.

PART II

PHYSIOLOGY OF NITRIFICATION

INTRODUCTION

Nitrification is defined as the biological oxidation of ammonia to nitrite and the subsequent conversion of nitrite to nitrate according to the stoichiometric equations:

(1) $2NH^{+}_{4} + 3O_2 - - - - - 2\ NO^{-}_{2} + 4H^{+}$

(2) $2NO^{-}_{2} + O_2 - - - - - 2NO^{-}_{3}$

Though several nitrifying genera, i.e., Nitrosogloea, Nitrosococcus, Nitrospira (ammonia oxidizing) and Nitrocystis (nitrite oxidizing) have been isolated from soils (Alexander, 1965), these oxidations are believed to be carried out principally by members of the genera Nitrosomonas and Nitrobacter. In view of the well documented ability of certain heterotrophs to produce nitrite (Doxtader and Alexander, 1966; Eylar and Schmidt, 1959; Hirsch, et al, 1961) and the reported ability of certain fungi to produce nitrite and nitrate (Marshall and Alexander, 1962; Schmidt, 1954) it is possible that at least a portion of the nitrification taking place in soils could be due to organisms other than Nitrosomonas and Nitrobacter.

Interpretation of the data from the organic-nitrogen mineralization involving nitrification of ammonium-nitrogen or of physiological studies of these processes with added ammonium-nitrogen is difficult because of the heterogeneity

and complex nature of the soils in which nitrifying bacteria operate. Dissimilar nitrification capacities that have been observed in soils have been attributed to a number of environmental factors. These include pH (Kelly, 1916; Harper and Boatman, 1926; Martin, et al, 1943; Meiklejohn, 1954; Broadbent, et al, 1957; Aleem, et al, 1957; Munk, 1958 and Dijkshoorn, 1960); oxygen supply (Parker and Larson, 1962); moisture regime (Justice and Smith, 1962; and Parker and Larson, 1962). The effect of temperature on nitrification has been examined by Anderson and Purvis (1955); Calder (1957); Stojanovic and Broadbent (1957); Tyler et al (1959); Sabey et al (1959); Anderson (1960); Linke (1961); Justice and Smith (1962) and Mahendrappa et al (1966). The influence of organic matter concentration has been evaluated by Frederick (1957); CO_2 effects were examined by Clark (1962) and the relationship of nitrification to the cation exchange capacity of the soil was investigated by Lees and Quastel in 1946. Although the above-noted factors have been extensively studied, attempts to characterize the nitrification process in terms of optimum values for any individual factor have been largely unsuccessful. For instance, the ability of a strain of nitrifying bacteria isolated from an acid soil (pH 5.6 to 6.3) to grow well in an alkaline medium (pH 7.1 to 7.2) led Meiklejohn (1954) to conclude that an optimum H^+

ion concentration can not be sharply defined for nitrifying bacteria. In fact, some strains of these bacteria isolated from acid soils are more tolerant to high H^+ ion concentrations than those from neutral and alkaline soils (Gaarder and Hagem, 1922-23). Recorded data relative to nitrification in acid media are extremely variable and hence controversial. Despite evidence (Olsen, 1923, and Weber, 1959) indicating that nitrification proceeds in soils of pH 4.0 or even lower, the general concept still prevails that the rate of nitrification falls off rapidly at pH values below 6.0 and becomes negligible in soils with a pH below 5.0 (Harper and Boatman, 1926; Broadbent, et al, 1957; Munk, 1958 and Dijkshoorn, 1960).

An examination of the literature on the influence of temperature on nitrification likewise leads to the conclusion that there is no general optimum temperature although values between 30° to 35°C (Alexander, 1961) and around 25°C (Quastel and Scholefield, 1951) have been proposed. Several studies on the temperature effect in temperate soils have shown that the process, though slow, proceeds at temperatures as low as 2°C (Frederick, 1956 and Gerretsen, 1946). In general, however, nitrification has been shown to be adversely effected by soil temperatures below 5°C (Parker, 1957; Broadbent, et al, 1957; Fischer and Parks, 1958; Sakai, 1959; Sabey et al, 1959 and Anderson, 1960) and above 40°C by Sakai, 1959 and by Linke, 1961.

From the point of view of the microbiologist interested in cryopedogenic soils, it is unfortunate that the nitrification process and the physiology of these important organisms have been studied almost exclusively in temperate and tropical soils. Though the nitrifiers have been reported to occur in arctic and antarctic soils (Winogradsky, 1904; Kazanski, 1932; Jensen, 1951 and Boyd and Boyd, 1963), detailed information on the physiology of nitrification under cryopedogenic conditions is unavailable.

In preliminary studies on Hudson Bay and Inuvik soils, it was noted that, in some of the soils examined, mineralization stopped at the ammonia stage. This was particularly evident in the case of acidic soils although it also occurred to a variable extent in the other soils examined. These findings led to a detailed study of the nature of nitrification in subarctic soils. The principal objectives, then, were to examine the adaptability of 'native' nitrifiers, if present, in terms of their temperature and pH response; and finally, to attempt an evaluation of the nitrification process as it may contribute to the development of soils under subarctic environmental conditions.

MATERIALS AND METHODS

Soils

The soils used in this part of the investigation were structural aggregates (passed through a 2.0 mm sieve) of each of the soil types and are similar to those described and used for Biological Analyses, Part I.

Determinations of Soil Moisture, Water-holding Capacity and pH

Unless otherwise stated, procedures used were as described in Part I.

Determination of Ammonia, Nitrite and Nitrate Nitrogen

As described in Part I.

Calcium Carbonate-Treated Soils

Calcium carbonate was added, 1 g/20 g soil sample, and mixed before packing.

Preparation of Inoculum of Nitrifiers

The inoculum of nitrifiers was prepared just before inoculating the soil columns. This was done as follows: TL-10 soil that had been percolated with 0.01 M ammonium sulfate solution at 15°C for 15 days was allowed to drain overnight while still in the column. Ten grams well-drained soil was dispersed by shaking in 100 ml sterile distilled water and the suspension thus prepared

was used as the enrichment inoculum.

Inoculation of Soil Columns with Nitrifiers

Five ml of the soil suspension (enrichment inoculum) was percolated with distilled water (45 ml) through soil columns. After 3 days percolation, the percolates were discarded and the columns were subsequently used for nitrification activity.

Rocking Percolator Technique for Nitrification

The rocking percolator used was the manometric type (Greenwood and Lees, 1956). Description of the apparatus has been detailed previously in Part I of this thesis. For nitrification studies, oxygen uptake was not recorded and the individual percolates each served as a single unit. Soil columns were packed as described for soil respiration studies, Part I.

After packing the soil columns, percolators were run for two days with distilled water (50 ml each) until the percolates were quite clear and free from soil particles. Usually, these water percolates were then discarded and the columns (now water-saturated) were used for nitrification experiments. In this procedure, a new percolate solution consisting of 50 ml of $(NH_4)_2SO_4$ or KNO_2 at appropriate concentrations was introduced into the reservoirs below the columns. The percolators were then run at 15°C and

at prescribed intervals samples of each percolate in the series were removed for determination of pH and for nitrite and nitrate analyses.

Flask Incubation Technique for Nitrification

The method used was that described by Frederick (1956). Unless stated otherwise, soil samples equivalent to 10 g of oven-dried soil were placed in 250 ml Erlenmeyer flasks to which ammonium sulphate or potassium nitrite (anhydrous, analytical grade) was added at the rate of 33 mg and 21.25 mg respectively in the form of aquous solutions. Solutions were added in these cases to bring the soils to 60 percent of their moisture-holding capacity. After addition of the solutions the flasks were each placed in 2 litre beakers containing 15 to 20 ml of tap water. The tops of the beakers were covered with aluminum foil and the beaker-flask assemblies were incubated at constant temperatures for 15 days. After incubation the flasks were removed from the beakers and to each was added 100 ml of 1 N KCl solution (10 ml per 1 g soil); then, the flasks were shaken for 30 minutes on a wrist shaker. After shaking, contents of each flask were filtered through Whatman #44 filter paper and the resultant clear filtrates were stored for subsequent nitrogen analyses.

Soil Column Enrichment Experiments for Ammonia- and Nitrite Oxidizers

Columns of bacteria-saturated soils (Lees and Quastel, 1946) were prepared as follows:
Moist soils, equivalent to 20 g oven-dried weight, were packed in the percolators and washed with 50 ml distilled water by percolating at 15°C for two days. After discarding the wash-water the soil columns were percolated with increasing concentrations (0.005, 0.01, and 0.02 M) ammonium sulphate or potassium nitrite for a period of 30 days. Each concentration was retained for 10 days. After 30 days of percolation, columns were washed several times with distilled water until only barely detectable traces of ammonia, nitrite or nitrate remained in the respective wash waters. Columns were allowed to drain.

Isolation of Ammonium-Oxidizers in Liquid Medium

Enrichment cultures for ammonia oxidizers were started by adding 1.0 g of the appropriate enriched soil to 100 ml of the Nitrosomonas medium of Meiklejohn (Barkworth

and Bateson, 1964). This medium contained in 1 litre, NaCl, 0.3 g; $MgSO_4 17H_2O$, 0.14 g; $FeSO_4.7H_2O$, 0.03 g; KH_2PO_4, 10 ml (M/10 aquous soln); $CaCO_3$, 10 g; $(NH_4)_2SO_4$, 0.66 g; A-Z trace element solution[1], 1.0 ml and distilled water to volume.

Ammonium sulphate was sterilized separately and added to the rest of the medium after autoclaving and cooling (15 psi for 15 min.). Following incubation at 15°C for 10 days on a reciprocal shaker (140 rpm) contents of the flask were centrifuged at 4°C (11000 x g for 15 min.). The resulting pellet was resuspended in 50 ml sterile distilled water and 10 ml of this suspension was used to inoculate 100 ml of fresh medium devoid of calcium carbonate. This enrichment process was repeated three times over a period of three weeks following which the culture was centrifuged again and the pellet used to inoculate 200 ml of fresh medium. This medium now contained half the amount of calcium carbonate that had been present in the original medium. For this transfer, the incubation interval was reduced to 5 to 7 days. After two further transfers to carbonate-free medium, the culture was collected by

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[1] A-Z trace element solution contained in 1 litre, $MnSO_4.7H_2O$, 0.554 g; H_3BO_3, 0.63 g; $CuSO_4.5H_2O$, 0.33 g; $ZnSO_4.7H_2O$, 0.343 g; $Co(NO_3)_2.6H_2O$, 0.356 g.

centrifugation and inoculated to 200 ml of fresh medium with carbonate. Following this final incubation, the culture was washed twice with sterile distilled water and the pellet now consisting of calcium carbonate and bacteria was resuspended in 150 ml of distilled water and stored at 4°C for subsequent metabolic studies and as inoculum for growth characteristics in liquid medium. No attempt was made to eliminate possible contaminant heterotrophs although their proliferation under the prevailing cultural conditions would be unlikely. The final cell preparation thus obtained failed to show oxidation of nitrite to nitrate suggesting either the absence of nitrite oxidizers in the original soil enrichment or their loss during the serial transfer process.

Growth of Ammonium-Oxidizers in Liquid Medium

After inoculation with 5 ml enrichment preparation to 50 ml of sterile autotrophic medium dispensed in 250 ml Erlenmeyer flasks, incubation followed at constant temperatures on mechanical shakers (140 rpm). At regular intervals, samples were removed from each flask and analyzed for nitrite as a measure of culture growth.

Isolation and Growth of Nitrite-Oxidizers in Liquid Medium

The composition of the medium and procedure used was essentially the same as described for ammonium-oxidizers

except that 0.5 g KNO_2 replaced the ammonium sulfate in the medium. The isolation was followed by enrichment technique in liquid medium (50 ml medium in 250 ml Erlenmeyer flask) using enriched soil (nitrite percolated) equivalent to 2 to 3 g wet-weight per flask as the initial inoculum. After 10 days incubation (shaken at 140 rpm) at 15°C, 5.0 ml aliquots of the growing culture were transferred to flasks of fresh medium (again 50 ml) with further incubation for another 10 days. Finally, the contents of each flask were centrifuged (11, 700 x g for 15 min) at 4°C and the resultant pellet then resuspended in 50 ml of fresh carbonate-free medium. This suspension was used as inoculum for the growth studies of the organisms.

Five ml aliquots of this suspension were added to 50 ml lots of fresh medium dispensed in 250 ml Erlenmeyer flasks. These were incubated on reciprocal shakers (140 rpm) at temperatures of 4, 15, 28 and 35°C for 13 days. Growth was measured on the basis of nitrate accumulation in the culture medium.

Assay of Ammonium Oxidation by Washed Cell-suspensions

The oxidation of ammonium was observed by measuring the appearance of nitrite in open Warburg flasks (Campbell and Aleem, 1965) shaken at different temperatures. Each Warburg flask contained 4.5 ml of cell suspension in a

buffer solution (0.01 M KH_2PO_4 + 0.005 M $Na_2B_4O_7$); the pH was adjusted to 7.7 by the addition of 0.1 M HCl or NaOH. Cell suspensions prepared as described under Materials and Methods were preadjusted to pH 7.7 with 0.1 N HCl as described by Hoffman and Lees (1953), centrifuged at 11,700 x g at 4°C for 15 minutes and the pellet was dispersed in buffer. After 10 minutes shaking at each selected temperature, 0.5 ml of 0.1 M ammonium sulphate solution was added to the Warburg vessels containing the cell suspensions. Samples were withdrawn at regular intervals for the determination of nitrite produced by the organisms.

RESULTS AND DISCUSSION

Influence of Ammonium-Nitrogen Concentrations and Soil Sample Size on Nitrification

In a preliminary investigation on nitrification of ammonium sulphate (0.01 M, 50 ml) by TL-10 soil (20 g) it was found that about 65 to 75 percent of ammonium-nitrogen was transformed to nitrite and nitrate nitrogen after 15 days percolation at 15°C. In a further experiment with different concentrations of ammonium sulphate in solution (0.005 M, 0.01 M, 0.02 M and 0.05 M) where the pH values of the concentrations had been preadjusted to approximate the pH of the soil in question, a relatively short lag phase for nitrification was observed in all the soil columns. The lengths of these lag phases increased with increasing concentrations of ammonium sulphate, Fig. 17a. With concentrations varying from 0.005 M to 0.02 M ammonium sulphate, the lag phases for oxidation to nitrite were similar, i.e., six days; however, the lag phases of oxidation from nitrite to nitrate increased directly with increasing concentrations of ammonium sulphate, Fig. 17 b. Lag phases of 5, 5.5 and 7 days were observed for 0.005, 0.01 and 0.02 M ammonium sulphate concentrations respectively. Soil columns receiving 0.05 M ammonium sulphate did not yield any appreciable amount of nitrite or nitrate in their percolates even after 15 days.

Fig. 17a. Influence of different concentrations of ammonium sulfate on nitrification. 50 ml ammonium sulfate solutions (0.005, 0.01, 0.02 and 0.05 M) percolated through 20 g TL-10 soil at 15°C for 15 days. The cumulative value of nitrite- and nitrate-nitrogen was used as an index of nitrification.

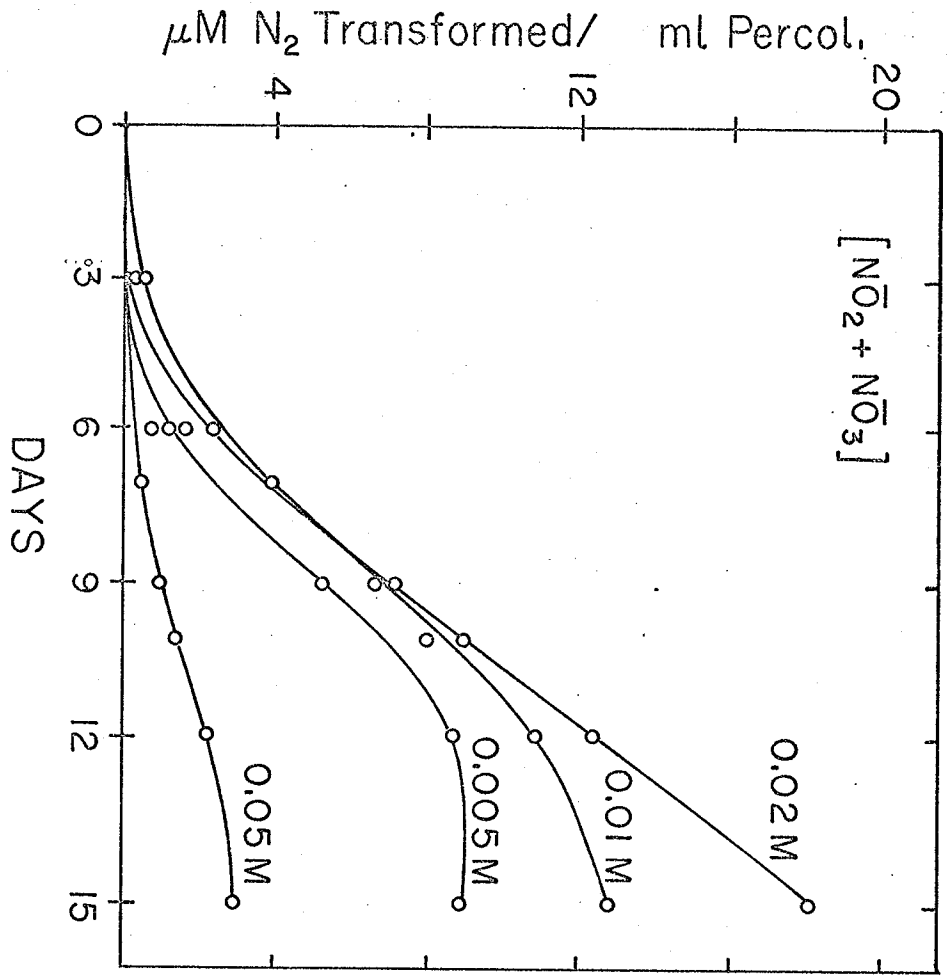
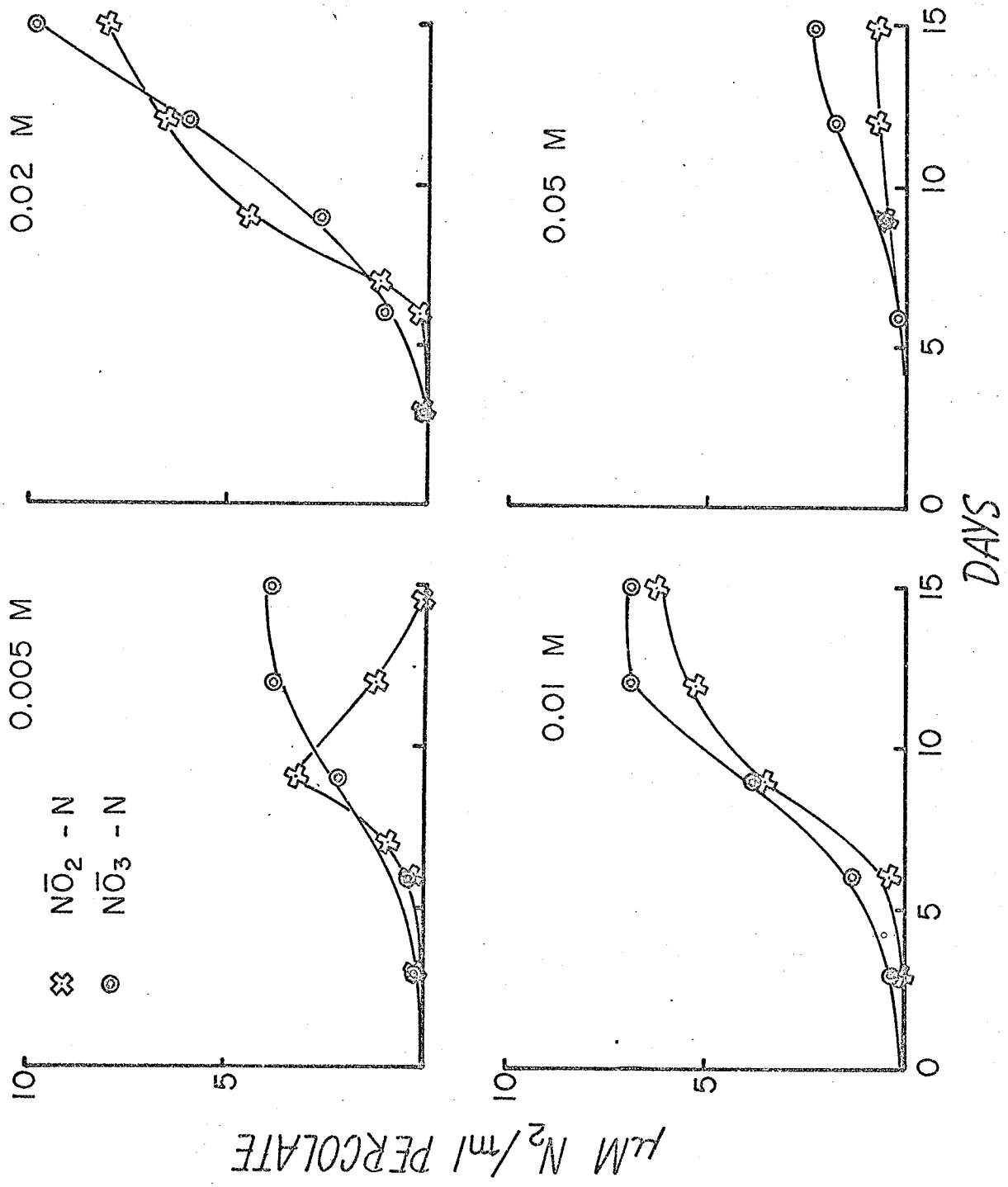

μM N2 Transformed/ ml Percol.
4
12
20
0
3
6
9
12
15
DAYS
[NO2 + NO3]
0.05 M
0.005 M
0.01 M
0.02 M

Fig. 17b. Replots of Fig. 17a, individual variation in nitrite- and nitrate-nitrogen with time.


0.02 M
0.05 M
0.005 M
0.01 M
NO_2 - N
NO_3 - N
DAYS
μM N_2/ml PERCOLATE

Data on the nitrogen balances in the resevoirs after 15 days percolation at the various concentrations of ammonium sulphate showed that nitrification was most effective with 0.005 and 0.01 M solutions. In these, 90% and 70% of the added nitrogen was oxidized to nitrite and nitrate respectively.

The initial increase in lag phase of nitrite oxidation (with increasing concentration of ammonium sulphate) is not surprising since the nitrification process has been identified as a surface phenomenon, Lees, and Quastel (1946): i.e., only ammonium adsorbed on the surface of soil particles by the base-exchange phenomenon is oxidized in the nitrification process; the remaining unadsorbed (free) ammonium has been shown to exert a selective inhibition on the nitrite oxidizers, Smith (1964), Nommik and Nilsson (1963); Stojanovic and Alexander (1958); Broadbent et al (1957). In other words, the greater the amount of free ammonium in the percolate (as substrate concentration rises) the greater the degree of nitrite oxidizer inhibition.

An additional experiment was carried out to demonstrate the inhibitory effect by free ammonium on the nitrite oxidizing organisms present in these soils. In this experiment an ammonium sulphate solution (0.01 M, 50 ml) was percolated for two weeks through soil columns of 5, 10, 20 and 30 g weight respectively. The duration of the

Table XVI

Influence of soil-size on the nitrification of 0.01 M ammonium sulfate (50 ml) percolated through the columns for two weeks at 15°C.

Soil Weight g	Column Height cm	Concentration of mineral-nitrogen (μM/ml) in the percolate										pH, initial and final
		2 days					14 days					
		NH^+	NO_2^-	NO_3^-	$\frac{NO_2^-}{NO_3^-}$	$\frac{\Sigma NO_2^-}{+NO_3^-}$	NH^+	NO_2^-	NO_3^-	$\frac{NO_2^-}{NO_3^-}$	$\frac{\Sigma NO_2^-}{+NO_3^-}$	
5	2.00	2.60	0.29	0.05	5.50	0.34	1.43	3.97	0.84	4.70	4.81	6.8-6.9
10	3.58	2.15	0.45	0.13	3.40	0.58	1.04	6.38	4.26	1.50	10.64	6.6-6.3
20	6.75	1.30	0.95	0.32	3.01	1.26	0.53	8.45	5.90	1.43	14.35	6.8-6.6
30	8.95	1.17	0.99	0.45	2.23	1.44	0.43	7.98	6.55	1.21	14.53	6.8*

* pH value remained constant

percolation period was selected on the basis that the maximum phase of nitrification is usually reached between the first and second week of percolation. Analytical details of these percolates are presented in Table XVI. These results show that the extent of nitrification (formation of nitrite-nitrogen and nitrate-nitrogen) depended on the weight of the soil. Toward the end of the percolation interval, the 30 g soil column appeared to become water-logged and both nitrite- and nitrate-nitrogen production slowed down noticeably. The concentration of free ammonium in the percolate (which varies inversely by adsorption with the weight of the soil) did not have any effect on nitrite formation; however, the observed increase in nitrate formation paralleled the decreasing free ammonium concentration in the percolates. Thus, the nitrite:nitrate ratios decreased with an increase in soil column weight. In other words, columns of greater soil height (weight) adsorbed any excess of ammonium ions thus promoting the nitrification of ammonium to nitrite and, at the same time, reducing the toxicity effect of free ammonium in the percolate toward the nitrite-oxidizers.

Patterns of Nitrification in Different Soils (influence of pH and initial population of nitrifiers)

In the two previous experiments it was observed that an increase in soil column height (weight of soil per column)

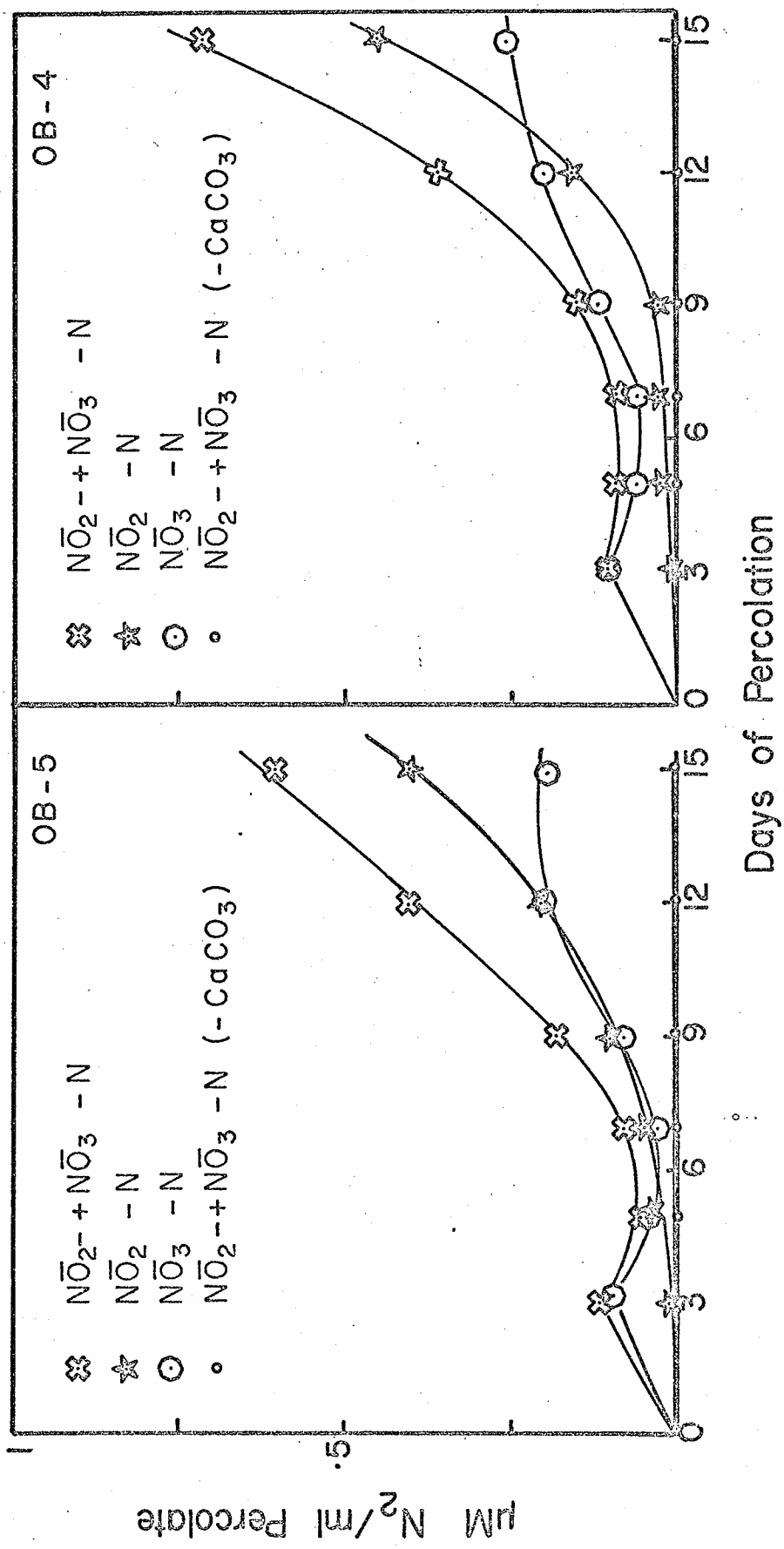
resulted in water-logging conditions toward the end of the percolation interval. Presumably this was due to the amorphous nature and water-retention capacities of the organic-matter present to a varying degree in the soils examined. Further, any excess of free ammonium was found to extend the lag phase of nitrite oxidizers. For these reasons, all subsequent percolation experiments were based on 20 g oven-dried weight of soil in each column and ammonium sulphate concentrations were limited to 0.01 M in 50 ml of percolate solution.

Old Beach Soils (OB-1, OB-4 and OB-5)

In a preliminary investigation, acidic soils from the Old Beach sampling areas did not produce nitrite- or nitrate-nitrogen even after 15 days percolation with ammonium sulphate solution. According to Alexander (1961) the pH range as displayed by acidic soils is generally too low for nitrification to be active. Moreover, in our experiments pH determined on the percolates showed a marked decline with time. In the OB-4 soil percolate, for example, the pH dropped to 3.9 from an initial value of 4.7 while in the OB-5 percolate the pH dropped from 4.8 to 4.2. Ammonium-nitrogen determinations made at the end of the percolation period showed a 103% and 118% recovery over the zero-time

Fig. 18. Nitrification of ammonium-nitrogen to nitrite and nitrate in OB-5 soil (with and without $CaCO_3$ treatment). 50 ml of ammonium sulfate (0.01 M) solution percolated through 20 g soil at 15°C. Initial pH of untreated soil was 4.7. Initial pH of $CaCO_3$ treated soil was 7.65.

Fig. 19. Nitrification of ammonium-nitrogen to nitrite and nitrate in OB-4 soil (with and without $CaCO_3$ treatment). 50 ml of ammonium sulfate (0.01 M) solution percolated through 20 g soil at 15°C. Initial pH of untreated soil was 4.7. Initial pH of $CaCO_3$ treated soil was 7.5.


OB-4
OB-5
$NO_2^- + NO_3^-$ - N
NO_2^- - N
NO_3^- - N
$NO_2^- + NO_3^-$ - N (-$CaCO_3$)
0
3
6
9
12
15
Days of Percolation
μM N_2/ml Percolate

controls[1] in these soils respectively. These findings suggested that there likely was excess mineralization of native organic matter which stopped at the ammonia stage; further mineralization being inhibited by the low pH of the soil. These observations led to further investigations on the nitrifying population status of these soils.

As reported in Fig. 18 and 19, treatment of these soils with $CaCO_3$ prior to percolation provided the pH shifts (to pH 7.5-7.65) which in turn permitted nitrate and nitrate formation to proceed. However, even under these conditions nitrate formation did not attain a maximum rate phase during the percolation interval. On the contrary, a slow decline in the rate of nitrate formation was observed. In the case of both soils pH values of the percolates remained almost unchanged. The comparatively long lag periods (about 10 days) observed in nitrate formation also suggested that either ammonia inhibited nitrite formation (at the concentration used) or that the initial population of nitrifiers in the soils was quite low. The decline in nitrate formation toward the end of the percolation period and the over-all slow rate thereof could be attributed to

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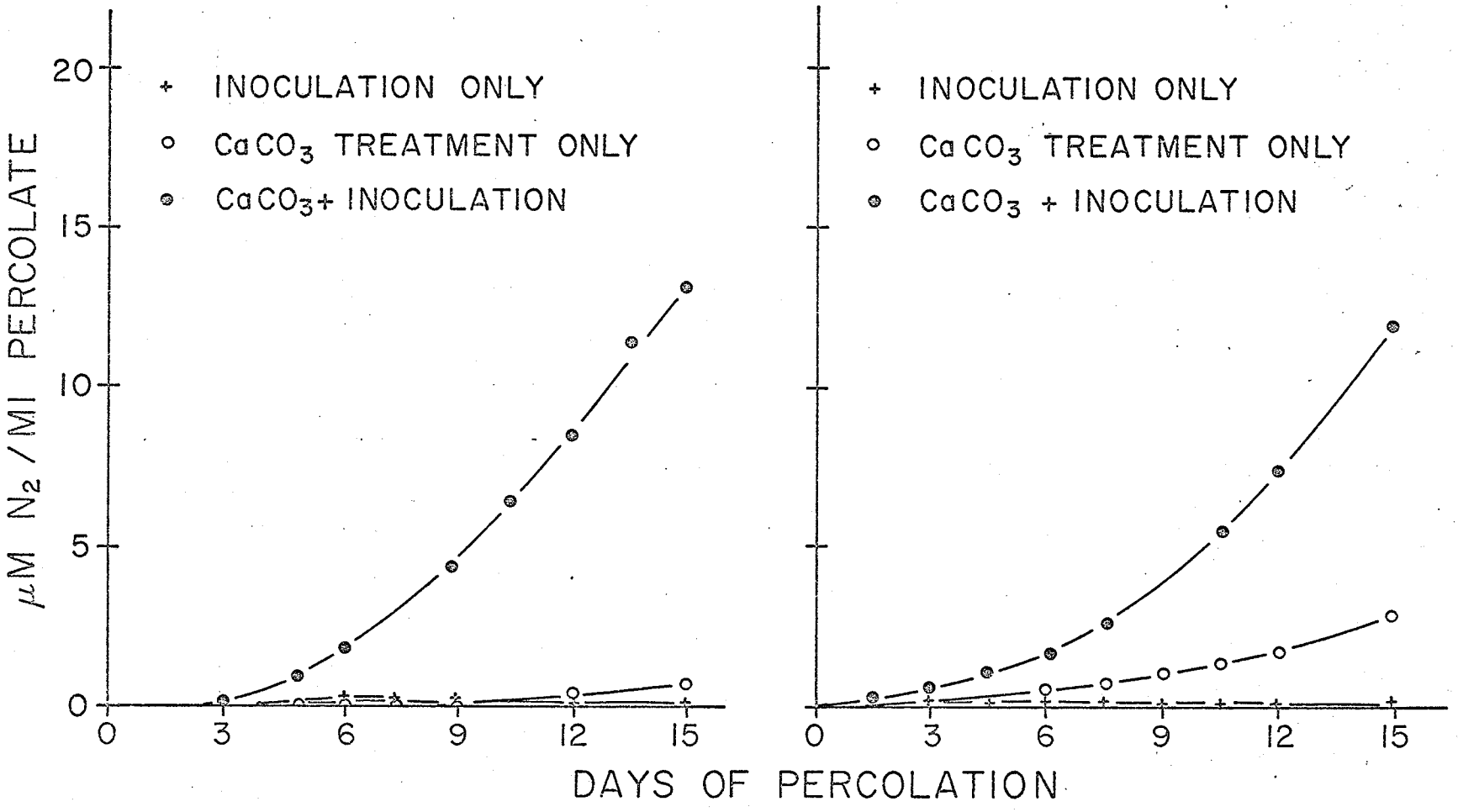
[1]The term 'zero-time control' as used here may be misleading since the actual concentration of ammonium sulphate in the percolate dropped because of adsorption by the soil in the columns. The zero-time control value, therefore, was determined after five hours percolation through the columns and represents the value of unadsorbed ammonium in the percolate.

Fig. 20a. Influence of pH and initial population of nitrifiers on nitrification of ammonium-nitrogen in OB-4 soil. 50 ml ammonium sulfate (0.01 M) solution percolated through 20 g soil columns at 15°C. Treatments as described in text. Sums of nitrite- and nitrate-nitrogen were taken as an index of ammonium oxidizing activity.

Fig. 20b. Influence of pH and initial population of nitrifiers on nitrification of nitrite to nitrate. 50 ml potassium nitrite (0.01 M) solution percolated through 20 g soil columns at 15°C. Treatments as described on text.


20
15
10
5
0
μM N2 / MI PERCOLATE
+ INOCULATION ONLY
o CaCO3 TREATMENT ONLY
CaCO3+ INOCULATION
0
3
6
9
12
15
DAYS OF PERCOLATION
+ INOCULATION ONLY
o CaCO3 TREATMENT ONLY
CaCO3 + INOCULATION
0
3
6
9
12
15

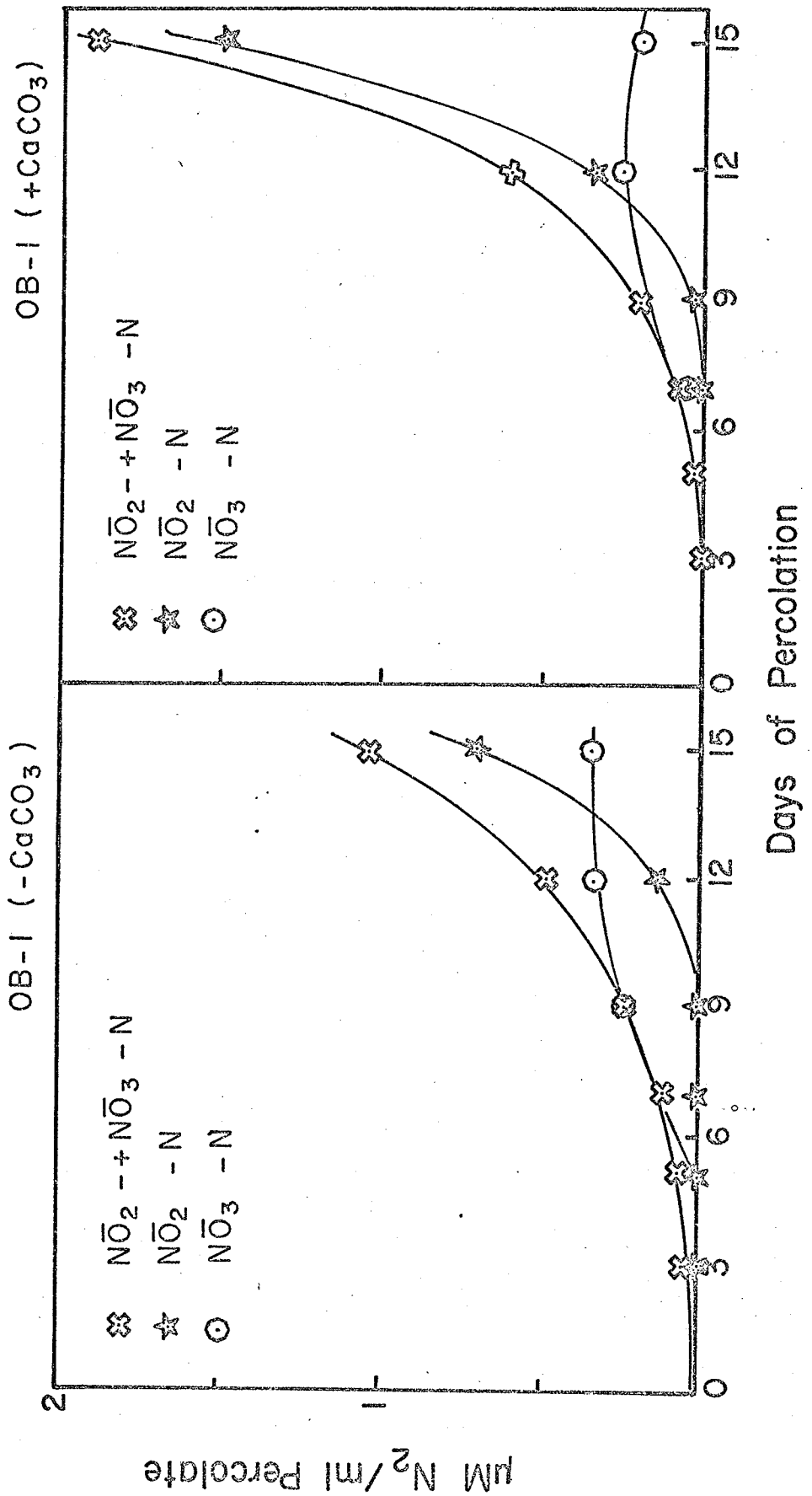
several factors: (a) the adjusted high pH and the possible low population level of nitrite-oxidizers; (b) the preferential rapid immobilization of nitrate formed, or (c) the presence of some toxic materials in the soils.

To examine these assumptions, fresh OB-4 soil columns with and without carbonate treatment were inoculated with nitrifier-enriched suspensions and then received ammonium sulphate or potassium nitrite solutions (0.01 M concentrations). These were percolated for 15 days. The results are presented in Fig. 20a and b. Both in the ammonium sulphate 'a' and potassium nitrite 'b' soil columns ($CaCO_3$ treated and enrichment inoculated) the lag phases were reduced to about six days and nitrification rates were increased. Simple enrichment inoculation to non-carbonate treated soils did not yield any appreciable amount of nitrite or nitrate in the percolates while on the average the pH dropped to 3.9 from the initial value of pH 5.0 after 15 days. Carbonate treatment maintained the pH at an almost constant value (initial) during the percolation period. Uninoculated but carbonate treated soils (also reported previously) showed slow nitrification of ammonia and of nitrite after long lag periods.

These results, then, indicate that the failure of nitrification in this soil was due mainly to the initial

Fig. 21a. Nitrification of ammonium-nitrogen to nitrite and nitrate in OB-1 soil (initial pH 6.5). 50 ml ammonium sulfate (0.01 M) solution percolated through 20 g soil at 15°C.

Fig. 21b. Nitrification of ammonium-nitrogen to nitrite and nitrate in OB-1 soil treated with $CaCO_3$ (pH 7.7). 50 ml ammonium sulfate (0.01 M) solution percolated through 20 g soil at 15°C.

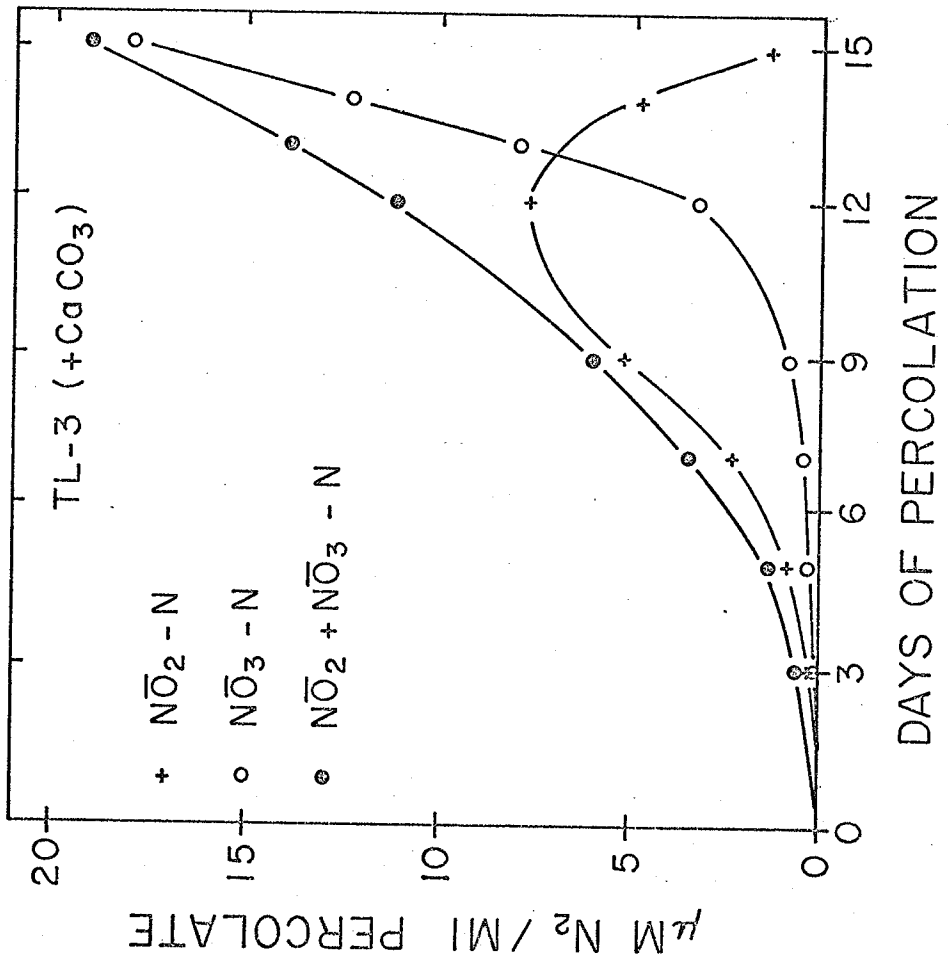

OB-1 (+CaCO3)
OB-1 (-CaCO3)
NO2- + NO3 - N
NO2 - N
NO3 - N
Days of Percolation
μM N2/ml Percolate

low pH and likely to a low initial population of nitrifiers. As has been suggested by Sabey et al (1959) for temperate soils, the delay or lag period is a characteristic of initial low population levels and as well, perhaps, a reflection of unfavorable growth conditions for the nitrifiers. The comparatively slow rates of nitrification both of ammonium and of nitrite is this soil as compared to TL-3 soil receiving similar treatments (see Fig. 22) could probably be attributed to soil factors, i.e., toxicity, immobilization, etc., which govern the activities of microorganisms.

In OB-1 soil (initial pH 6.5) the addition of calcium carbonate to give a final value of pH 7.7 did not result in any change in the nitrification pattern, Fig. 21a and b. In both cases, at pH 6.5 and at pH 7.7, nitrite accumulation was observed while nitrate formation again slowed down toward the end of the percolation interval.

Nitrite accumulation in all three soils of this series after carbonate treatment and in OB-1 soil without carbonate treatment, thus, cannot be assigned to a high pH effect as has been suggested by Broadbent et al (1957) for temperate soils. The enrichment inoculation experiment with OB-4 soil gives evidence that the nitrite oxidation has followed a normal pattern. The possible high free ammonium concentration and the shorter incubation interval

Fig. 22. Nitrification of ammonium-nitrogen to nitrite and nitrate in TL-3 soil treated with $CaCO_3$ (pH, 7.5-7.6). 50 ml ammonium sulfate (0.01 M) solution percolated through 20 g soil at 15°C.


TL-3 (+CaCO3)
NO2 - N
NO3 - N
NO2 + NO3 - N
μM N2 / Ml PERCOLATE
DAYS OF PERCOLATION
0
5
10
15
20
3
6
9
12
15

used in this case could account for the observed low nitrate accumulation, Sabey et al (1959). Nitrite concentrations in the percolater were too low to inhibit the activities of nitrite-oxidizers according to the report of Lees and Simpson (1957).

Twin Lakes Soil (TL-3)

In TL-3 soil (initial pH 5.5) no nitrification of ammonium sulphate took place during the percolation period. After 15 days, however, the pH of the percolate had dropped to pH 4.1. The addition of calcium carbonate prior to percolation, on the other hand, stimulated both nitrite and nitrate formation as shown in Fig. 22 . The relatively long lag period observed in nitrite oxidation could probably be accounted for by the high free ammonia concentration in the percolate and the low population level of nitrifiers. As is evident in Fig. 23a, and b, the enrichment inoculations remarkably decreased the lag periods both in ammonium and nitrite oxidations. Furthermore, even nitrate formation was enhanced in the ammonia percolated soils,(Fig. 23c). Thus, it seems clear that the high pH (7.5-7.6) pertaining after carbonate treatment was not the sole factor in causing the delay in nitrite oxidation. The relatively high concentrations of ammonia were apparently utilized more efficiently by the combined native and enrichment populations than by the native

Fig. 23a. Influence of pH and initial population of nitrifiers (ammonium-oxidizers) on nitrification of ammonium-nitrogen to nitrite in TL-3 soil. 50 ml of ammonium sulfate (0.01 M) solution percolated through 20 g soil columns at 15°C. Treatments as described in the text. Sum of nitrite- and nitrate-nitrogen was taken as an index of ammonium oxidation.

Fig. 23b. Influence of pH and initial population of nitrifiers on nitrification of nitrite to nitrate in TL-3 soil. 50 ml of potassium nitrite (0.01 M) percolated through 20 g soil columns at 15°C. Treatments as described in the text.

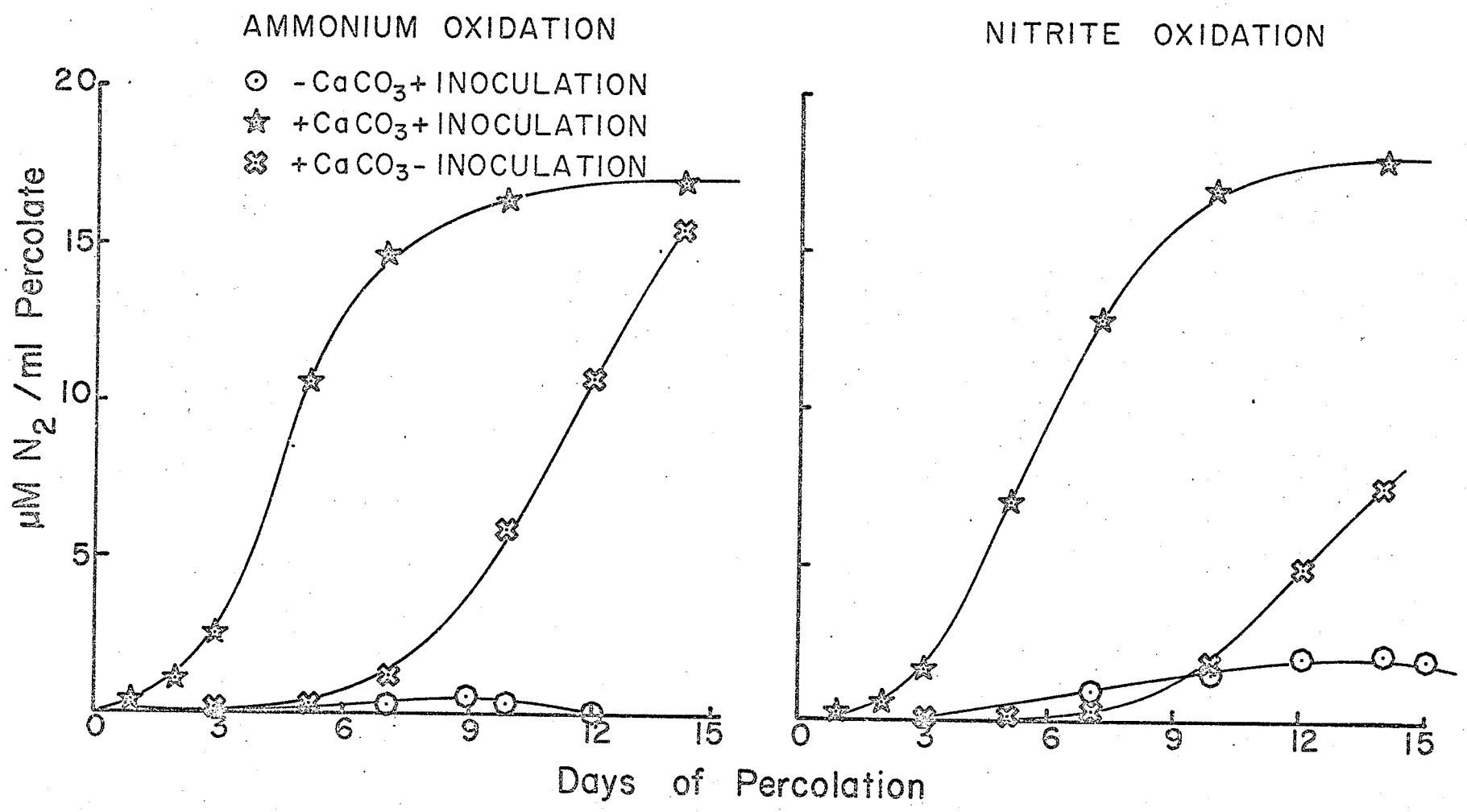
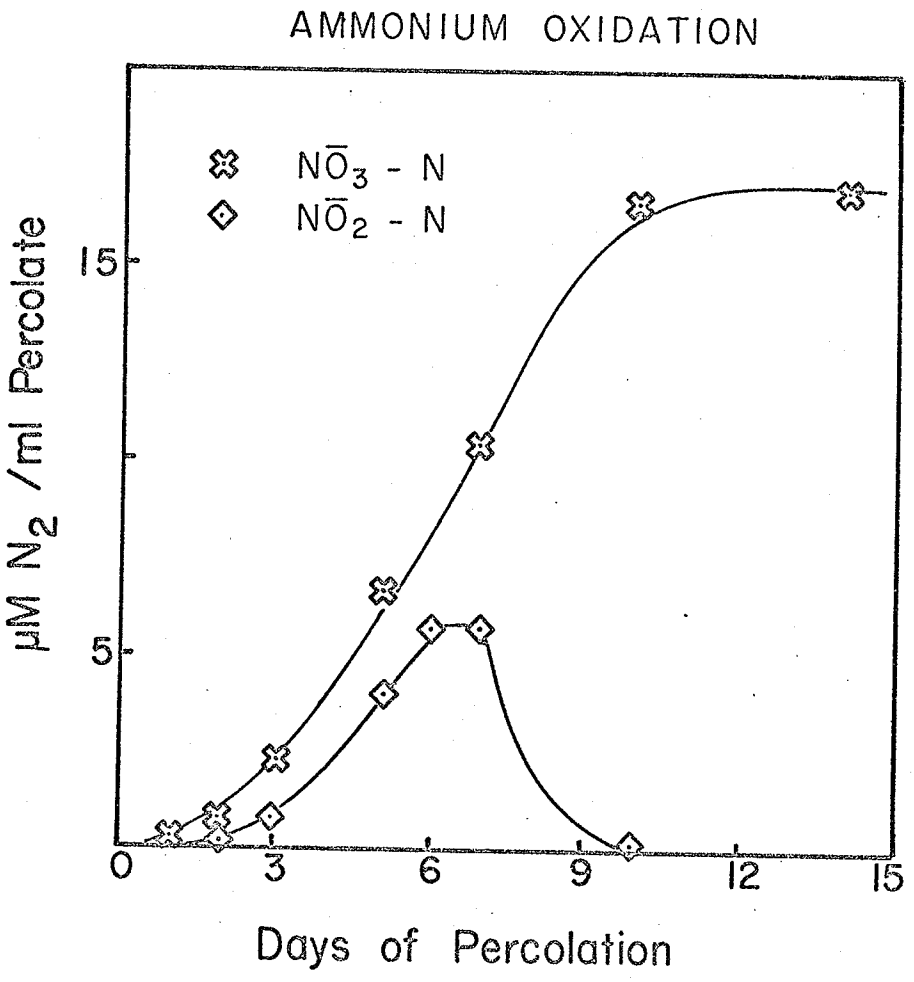

AMMONIUM OXIDATION
NITRITE OXIDATION
⊙ -CaCO3+ INOCULATION
☆ +CaCO3+ INOCULATION
✲ +CaCO3- INOCULATION
μM N2 /ml Percolate
20
15
10
5
0
3
6
9
12
15
Days of Percolation

Fig. 23c. Replot of Fig. 23a, individual variation in nitrite- and nitrate-nitrogen.


AMMONIUM OXIDATION
NO_3 - N
NO_2 - N
μM N_2 /ml Percolate
15
5
0
3
6
9
12
15
Days of Percolation

nitrifier population alone.

Simple enrichment inoculation (without carbonate treatment) did not enhance nitrification. Nitrite percolation columns showed some appreciable amount of nitrate formed but at the later stages this activity slowed down probably because of the decline in pH. On the other hand, ammonium oxidation was not very evident. The small quantity of nitrate detected during ammonium percolation did not exceed the level to be expected from oxidation of the nitrite carried over in the enrichment inoculation. This may be taken as evidence that nitrite-oxidizing organisms were less sensitive to low pH levels than were the ammonium-oxidizers.

Goose Creek Soil (GC-12)

GC-12 soil (initial pH 7.7) behaved in a quite similar way to carbonate-treated TL-3 soils in that no appreciable difference in lag times of ammonium- and nitrite- oxidizing activities was evident, Fig. 24 . Here, nitrite did accumulate in early stages of percolation but began to disappear rapidly toward the end of the percolation interval. According to Morrill and Dawson (1967), this sort of nitrification pattern could be attributed to the effect of the initial high pH of the soil. As well, the high initial activity of ammonium-oxidizers (leading to a temporary accumulation of

Fig. 24. Nitrification of ammonium-nitrogen to nitrite and nitrate in GC-12 soil (pH, 7.7). 50 ml of ammonium sulfate (0.01 M) solution percolated through 20 g soil at 15°C.

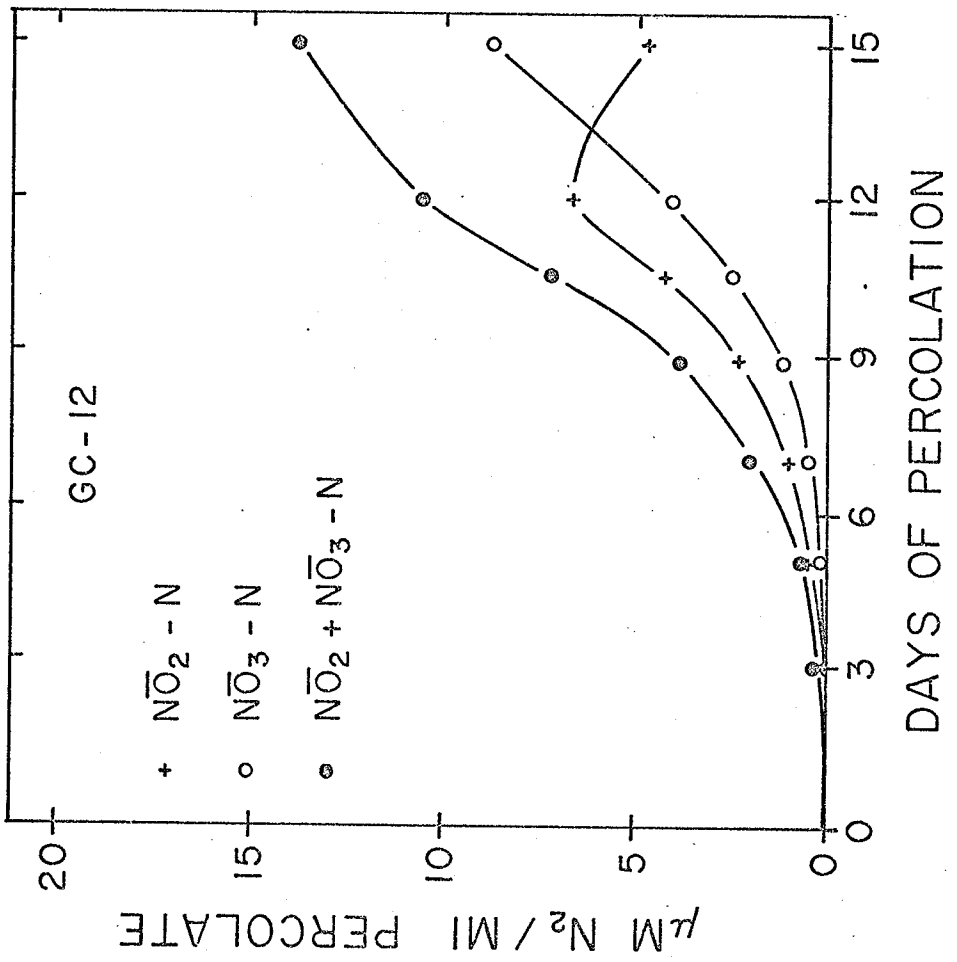
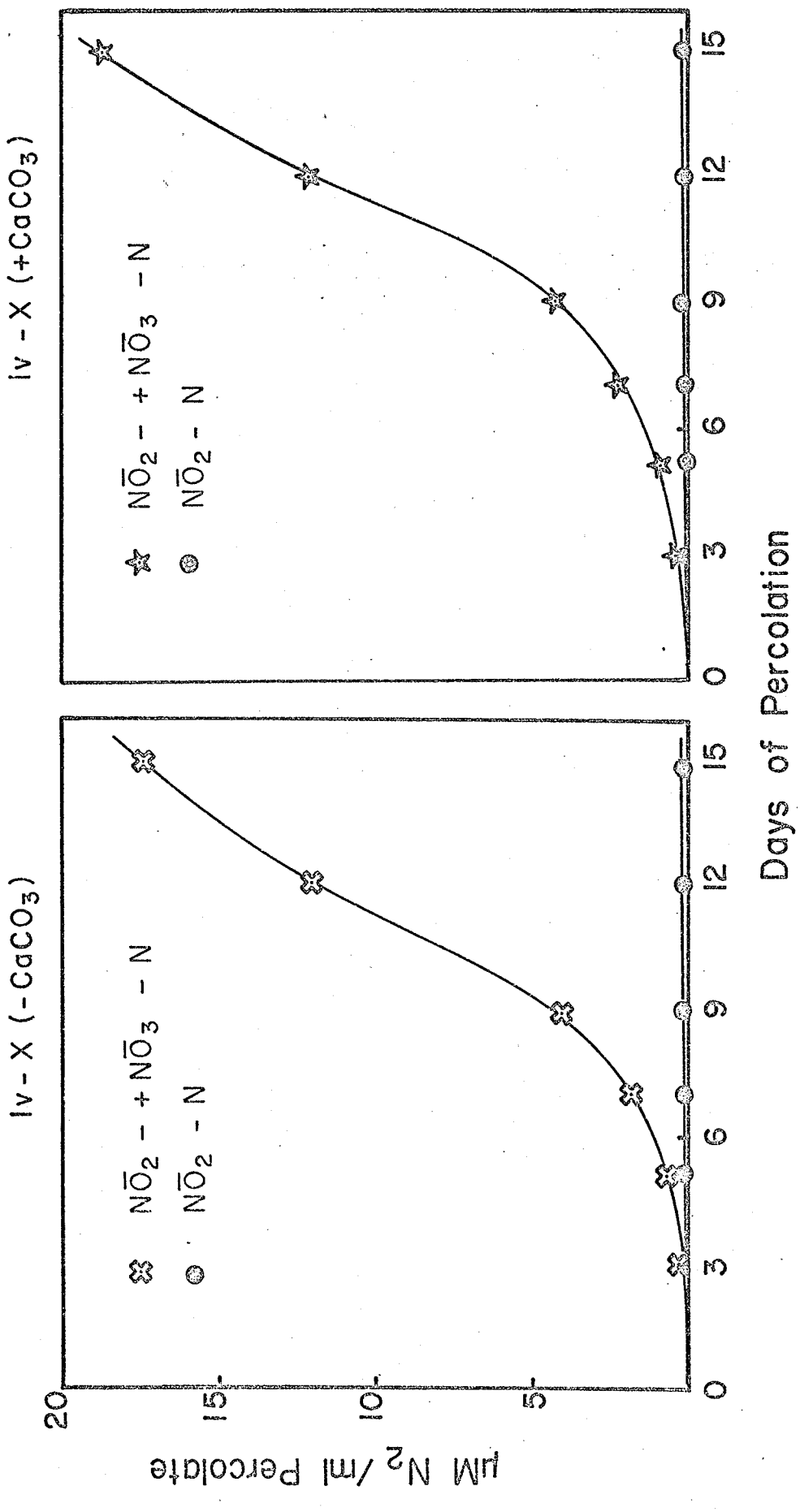

GC-12
$N\bar{O}_2$ - N
$N\bar{O}_3$ - N
$N\bar{O}_2 + N\bar{O}_3$ - N
μM N_2 / Ml PERCOLATE
DAYS OF PERCOLATION
0
5
10
15
20
0
3
6
9
12
15

Fig. 25a. Nitrification of ammonium-nitrogen to nitrite and nitrate in Iv-X soil. 50 ml of ammonium sulfate (0.01 M) solution percolated through 20 g soil (pH, 7.8) at 15°C.

Fig. 25b. Nitrification of ammonium-nitrogen to nitrite and nitrate in Iv-X soil treated with $CaCO_3$ (pH, 7.8). 50 ml of ammonium sulfate (0.01 M) solution percolated through 20 g soil at 15°C.


Iv - X (-CaCO3)
Iv - X (+CaCO3)
NO2 - + NO3 - N
NO2 - N
μM N2 /ml Percolate
Days of Percolation
0
3
6
9
12
15
5
10
15
20

nitrite) would probably account for the longer observed lag in proliferation of nitrite oxidizers. Ultimately, however, the rapid post-lag proliferation of these organisms and their relatively high rate of nitrite oxidation would account for the marked reduction in nitrite levels noted in later stages of the percolation.

Inuvic Soil (Iv-X)

Iv-X soil (initial pH 7.8) showed similar nitrification pattern with and without $CaCO_3$ treatment (Fig. 25a and b). In this case accumulation of nitrite to detectable levels did not occur. Nitrate formation, on the other hand, was prompt and by 15 days almost all the ammonium-nitrogen added had been converted to NO_3^- - N. Carbonate treatment did not appreciably alter the pH of this soil. The lower activity of ammonium oxidizers and the very short lag noted for nitrite-oxidation would probably account for the non-appearance of nitrite.

Of all the soils examined in this investigation, the Inuvik and Goose Creek soils in general, gave by far the highest levels of nitrification. The favourable pH and alluvial nature of the soils account for the establishment of the nitrifying population. High river levels in the spring could quite conceivably provide the vehicle by which nitrifying population from further south might be introduced.

Another reason for the high nitrifying potential of the Goose Creek and Inuvic soils may be due to the rather high cation-exchange capacity of these soils as compared to some other soils from Twin Lake and Old Beach areas. As noted by Lees and Quastel (1946) for temperate soils, this phenomenon would tend to encourage nitrification provided the appropriate population and the substrates were present.

Twin Lake soils which are fairly well-drained, in comparison to Old Beach soils, seemed to have better nitrification potential, probably because of their higher pH reactions and low C/N ratios.

In the Old Beach soils, the failure of nitrification is apparently not due to the absence of appropriate species. Slow nitrification activity after calcium carbonate treatment suggests that, although viable nitrifiers are present (albeit in quite small numbers), a major curtailment of their activity arises from the acidic nature of the soils. When one considers the apparent limitation of the nitrification activity in Old Beach acid soils 'in situ', another important factor concerns the question of soil aeration. Downslope from the Old Beach crest, the poor drainage combined with the tendency for peat deposition would provide for conditions tending toward the anaerobic. Thus, although the potential for nitrification is present in soils of this region, any meaningful nitrification will not occur until

nitrite) would probably account for the longer observed lag in proliferation of nitrite oxidizers. Ultimately, however, the rapid post-lag proliferation of these organisms and their relatively high rate of nitrite oxidation would account for the marked reduction in nitrite levels noted in later stages of the percolation.

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drainage conditions improve and soils become more aerobic and less acidic. At the present rate of permafrost-retreat observed in this latitude such an event may be a very long time in coming.

Effect of Temperature on Nitrification

The effect of variations in temperature on the nitrification of added ammonium- or nitrite nitrogen was studied in soils by the flask-incubation technique as described in Materials and Methods. Soils were incubated under moist conditions at constant temperatures of 4°, 15°, 22°, 28°and 35°C. Nitrification in each case was measured by analyzing at intervals for the production of (a) nitrite plus nitrate, and (b) for nitrate nitrogen. Each reported nitrification value was based on the average of three replicate samples taken at each time interval.

Time Course of Nitrification for Ammonium and Nitrite Nitrogen

The temperature influence on nitrification of ammonium nitrogen in TL-10 soils is presented in Fig. 26a. As noted in the data, nitrification was not evident at 28°C or at 35°C but otherwise was quite evident at temperatures as low as 4°C.

Evidence for the failure of nitrification at the higher temperatures (28°and 35°C) led to a further study of the production of nitrate from nitrite to determine whether the

Fig. 26a. Time-course of nitrification of ammonium-nitrogen at different temperatures. Ten grams of TL-10 soil was added to 33 mg of ammonium sulfate (in solution form) and incubated at constant temperatures. Sum of nitrite- and nitrate-nitrogen was taken as an index of nitrification.

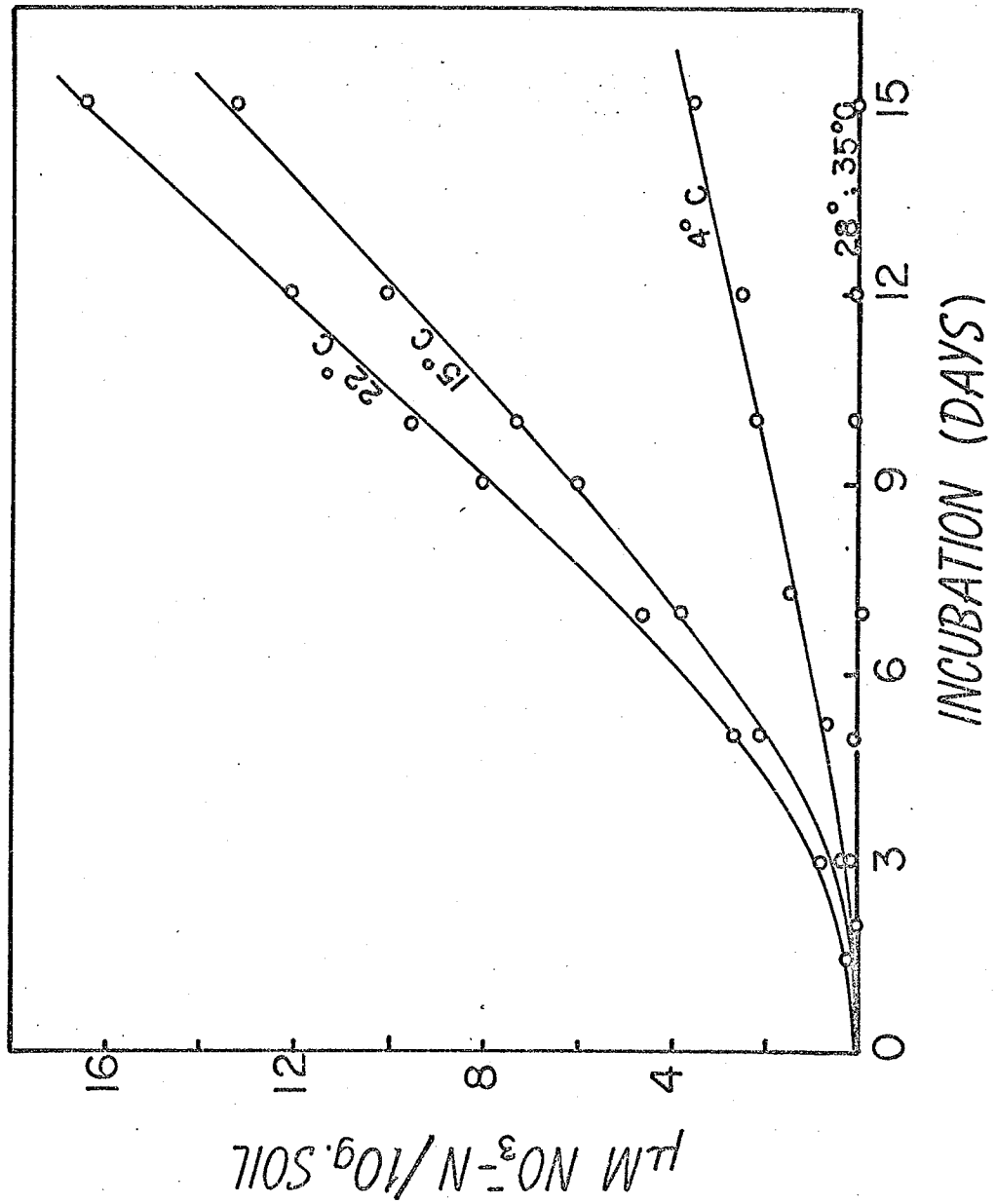
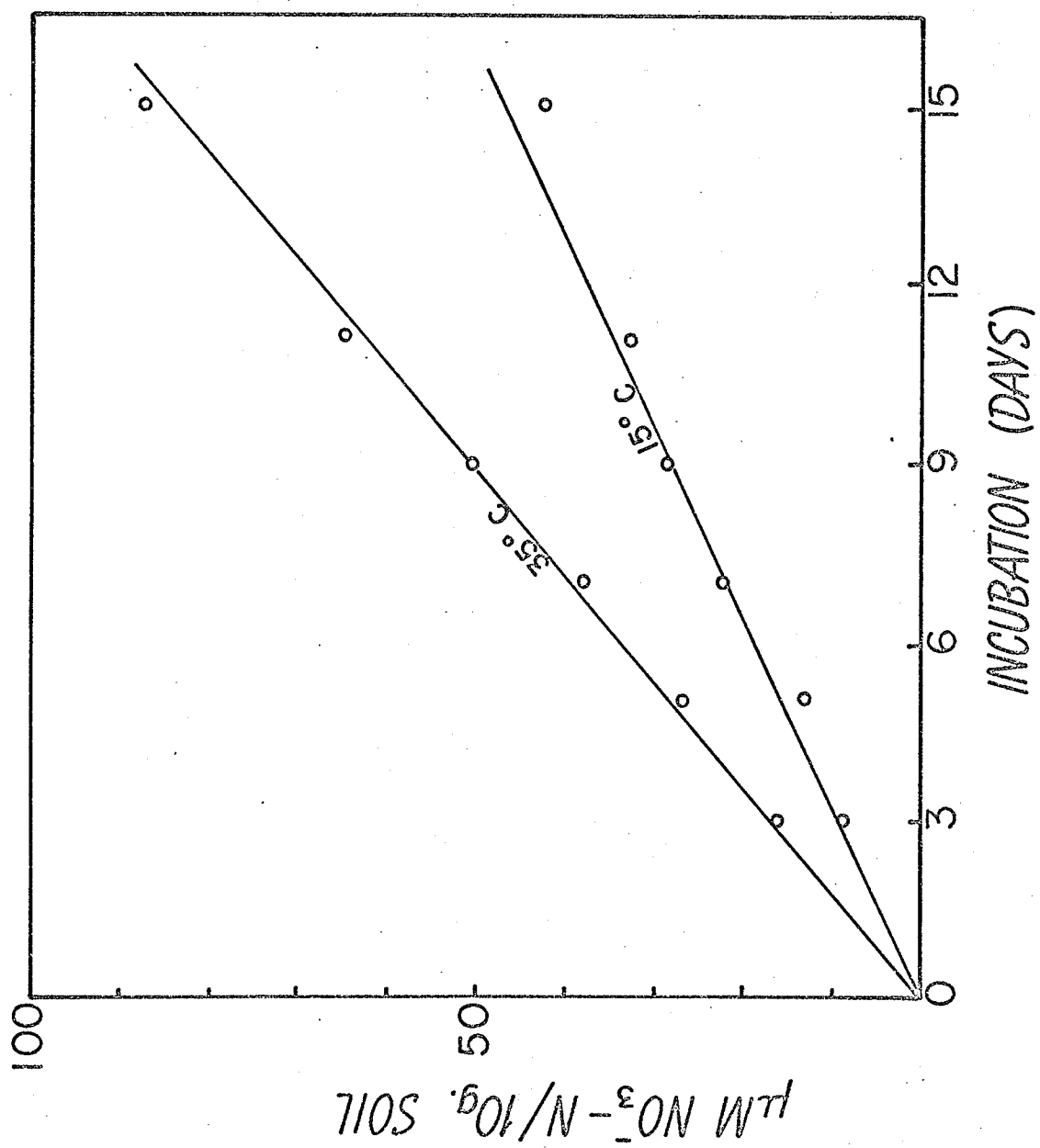

μM NO3-N/10g. SOIL
0
4
8
12
16
INCUBATION (DAYS)
0
3
6
9
12
15
4°C
15°C
22°C
28°; 35°C

Fig. 26b. Time-course of nitrification of nitrite to nitrate at 15° and 35°C. Ten grams of TL-10 soil was added to 21.25 mg of KNO_2 (in solution form) and incubated at constant temperatures.


μM NO3- -N/10g. SOIL
100
50
0
3
6
9
12
15
INCUBATION (DAYS)
35° C
15° C

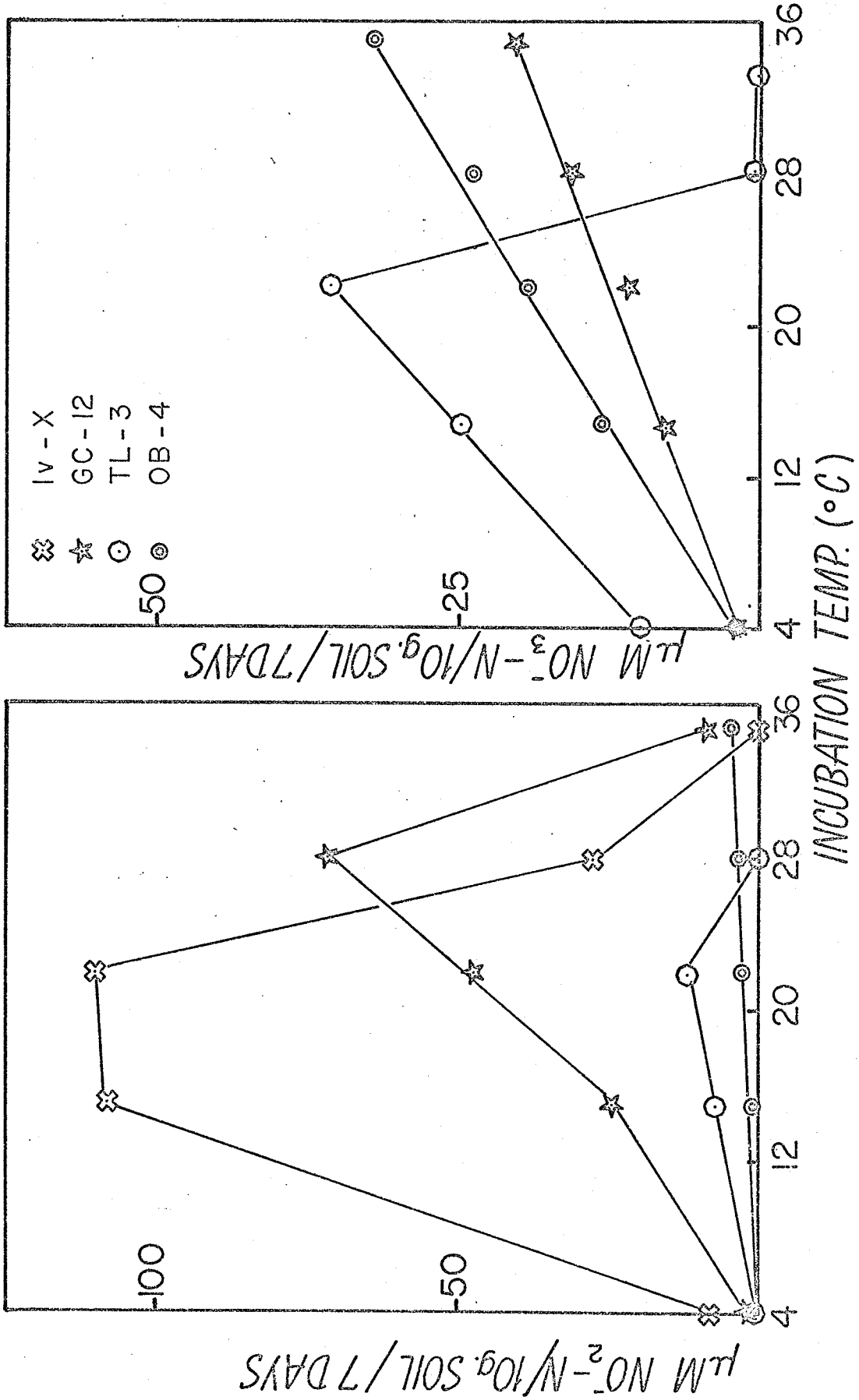
lack of nitrification was due to a failure of the first major oxidative step ($NH_4^+ - NO_2^-$) or to the second phase of the over-all reaction ($NO_2^- - NO_3^-$). Evidence presented in Fig. 26b shows clearly that nitrite oxidation to nitrate does in fact proceed at all the temperatures under consideration and further that the intensity of nitrite oxidation increased with increasing incubation temperature.

Nitrification of ammonium-nitrogen at incubation temperatures of 4°, 15° and 22°C but not at 28° and 35°C suggests that these ammonium oxidizing strains are either psychrophiles or at least are cold-adapted. The good nitrite-oxidizing activity, however, over a wide range of temperature from 4° to 35°C suggests that this group favours somewhat higher temperatures but is functionally adapted to low temperature conditions. The ability of the nitrite oxidizers to grow over a wider and higher temperature range as compared to the ammonium-oxidizers suggests that in these soils a state of unbalance between the two major nitrifying groups might occur during the colder (in terms of soil temperature) periods of the summer.

As noted previously in TL-10 soil, over-all nitrification of ammonium-nitrogen at different incubation temperatures was influenced by the considerable differences in temperature tolerance of the two groups of nitrifiers. To determine

Fig. 27a. Influence of temperature on nitrification of ammonium-nitrogen to nitrite in different soils. Sum of nitrite- and nitrate-nitrogen was used as an index of ammonium-oxidizing activity.

Fig. 27b. Influence of temperature on nitrification of nitrite to nitrate in different soils.


μM NO₂⁻-N/10g. SOIL/7 DAYS
100
50
4
12
20
28
36
INCUBATION TEMP. (°C)
μM NO₃⁻-N/10g. SOIL/7DAYS
50
25
lv-X
GC-12
TL-3
OB-4

whether such differences were evident in other soil samples of the area and also in soils from different sampling areas within the subarctic range, nitrification of ammonium- and nitrite-nitrogen was followed at the selected incubation temperatures. Results of these experiments are graphically presented in Fig. 27a and b. Here, nitrification rates (slopes of the linear portions of the maximum rate phase) are compared in terms of incubation temperatures. In TL-3 soil, nitrification of ammonium and nitrite was very sensitive to the higher incubation temperatures and no nitrification occurred either at 28°C or at 35°C. The maximum rates of nitrification (both of ammonium and nitrite) were displayed at 22°C. In comparison, GC-12 soil gave a maximum rate of ammonium nitrification at 28°C with significant activity as low as 4°C and as high as 35°C. In this soil, rates of nitrite-nitrification, on the other hand, were linear over the temperature range from 4°C to 35°C with the maximum being reached at the upper temperature limit.

In the OB-1 soil, both ammonium- and nitrite - nitrification proceeded most rapidly at the higher temperature (35°C). Although the rate of nitrite-nitrification was not measured separately, the optimum temperature for maximum rate of ammonium-nitrification was observed at 15° to 22°C.

These findings on the influence of temperature in all the soils examined indicated that over-all nitrification

increased with increasing temperature; however, the optimum temperature for these reactions varied with the soils. In all the soils studied nitrification was evident at 4°C. A similar observation has been made in many temperate soils by Anderson and Purvis (1955), Justice and Smith (1962), Sabey et al (1959) and Anderson and Boswell (1964) to name a few. The most interesting observation on nitrification activity in these soils was the difference in the temperature tolerance of ammonium- and nitrite-oxidizing organisms. In some soils the failure of nitrification of ammonium to nitrite and nitrate at higher temperatures due to the failure of the first step in the process, $NH_4^+ - NO_2^-$. Application of nitrite to these soils gave nitrate formation. In general, ammonium-oxidizing strains were found to be much more sensitive to temperature than the nitrite-oxidizers.

Nitrification of ammonium at low incubation temperature gave a slow but constant rate of nitrate accumulation in most of the soils and no evidence of proliferation of the cells was observed. The only exception to this was in the GC-12 soil where a proliferation curve was evident during the 15-day incubation period. It is not clear whether ammonium-oxidizing strains in the other Churchill soils are capable of growing at low temperatures (4°C). According to reports by Sabey et al (1959) and Anderson and Boswell (1964) nitrification in temperate soils is slow at low temperatures and

requires an incubation period of 8 to 12 weeks. These authors also proposed that nitrification at low temperatures is highly influenced by the texture of the soil and the initial population level of nitrifiers. A long term incubation experiment in subarctic soils, though not included in this investigation, might cast some light on the fate of nitrification at low temperatures and provide some more accurate measure of the role of nitrification in the prevailing environment.

The variations in soil type and in initial nitrifier population levels and, moreover, the differential response to temperature noted among the organisms examined would indicate that no reasonable comparison can be made between soils in terms of nitrification potential. Accordingly, no general rule can be formulated to establish suitable incubation temperatures for the evaluation of nitrification activities in these soils.

Temperature Relationships of Nitrifiers in Liquid Media

As reported previously, TL-3 soil failed to show significant nitrification of ammonium sulfate at temperatures above 22°C while at 4°C the rate of nitrification was limited because of the substrate (NO_2^- - N) unavailability to nitrite - oxidizing strains. This finding suggested that either the ammonium-oxidizing strains were not acclimatized

Fig. 28. Growth-curves of ammonium-oxidizing strains (TL-3 soil) at different incubation temperatures. Conditions as described under Materials and Methods.

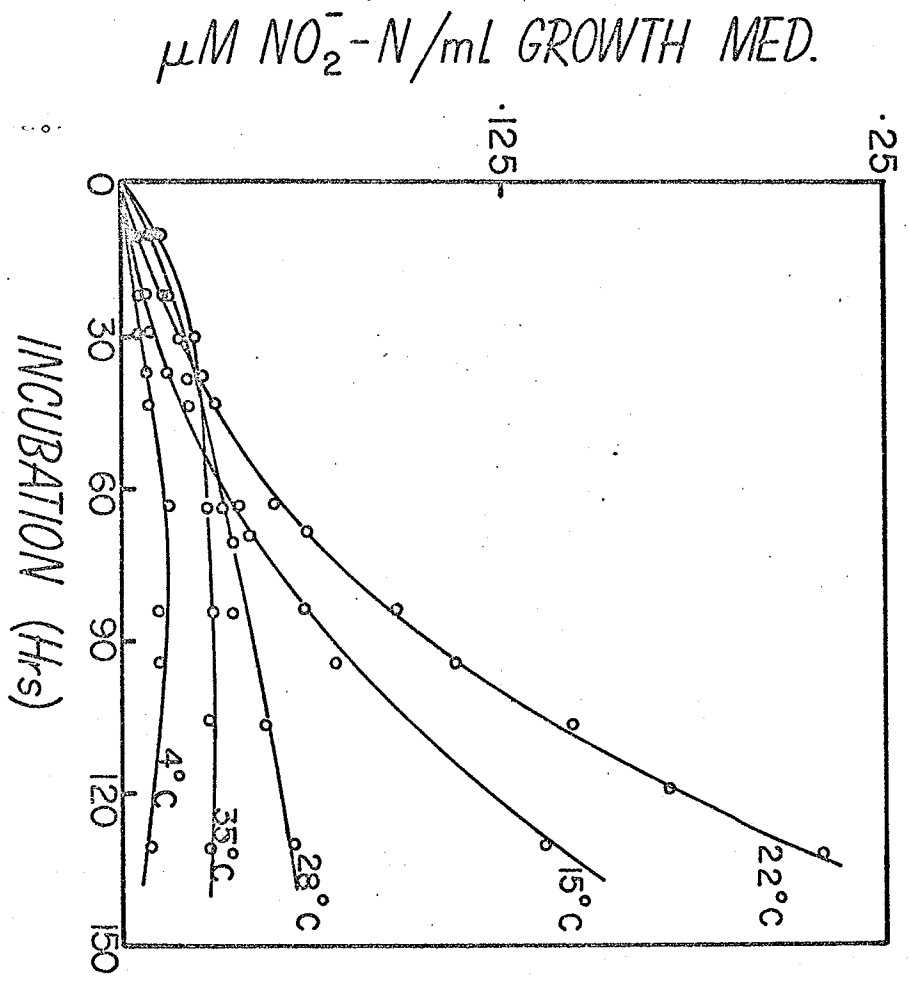
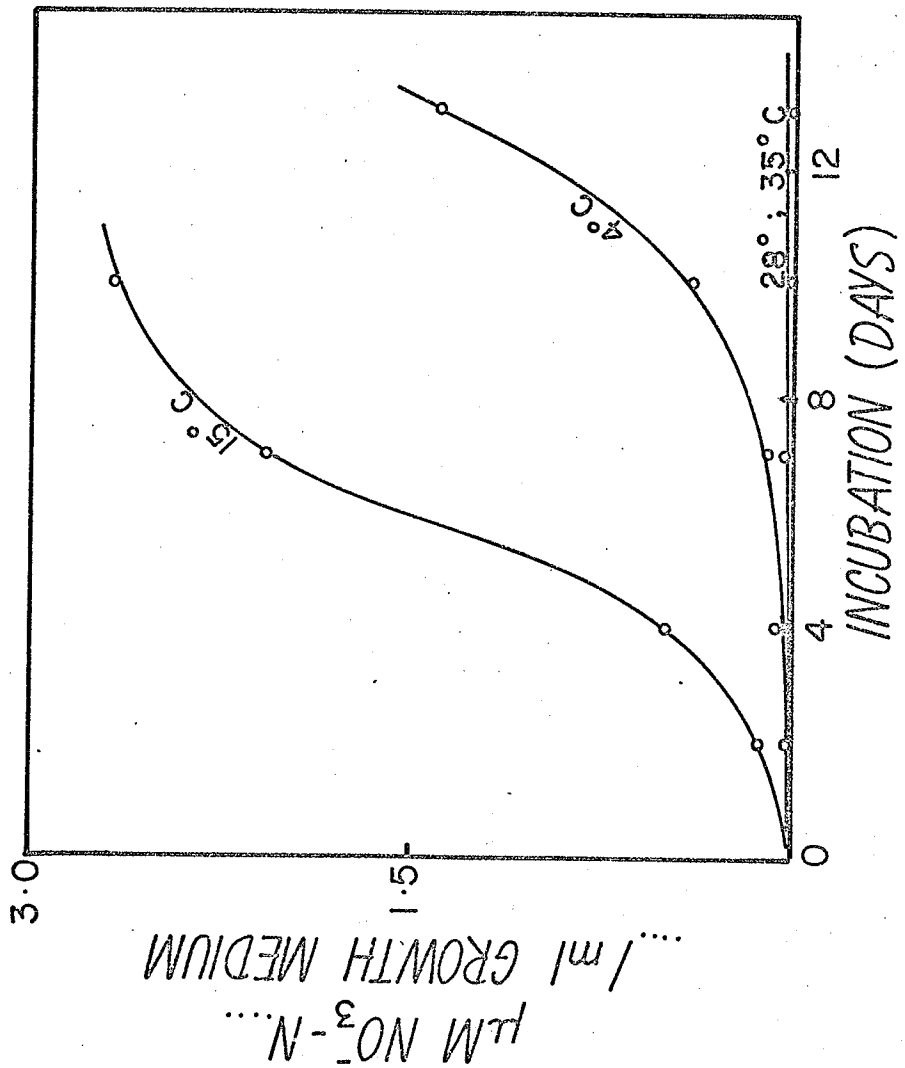

μM NO2- - N/ml GROWTH MED.
.125
.25
INCUBATION (Hrs)
0
30
60
90
120
150
4°C
35°C
28°C
15°C
22°C

Fig. 29. Growth-curves of nitrite-oxidizing strains (TL-3 soil) at different incubation temperatures. Conditions as described under Materials and Methods.


μM NO3-N
.../ml GROWTH MEDIUM
3.0
1.5
0
4
8
12
INCUBATION (DAYS)
28°, 35°C
4°C
15°C

to low temperature conditions or that the soil characteristics and the initial low population restricted their proliferation. This led to further studies on the growth-activity of ammonium-oxidizing strains isolated from TL-3 soil. For comparison, nitrite-oxidizing strains were also cultivated in an artifical medium.

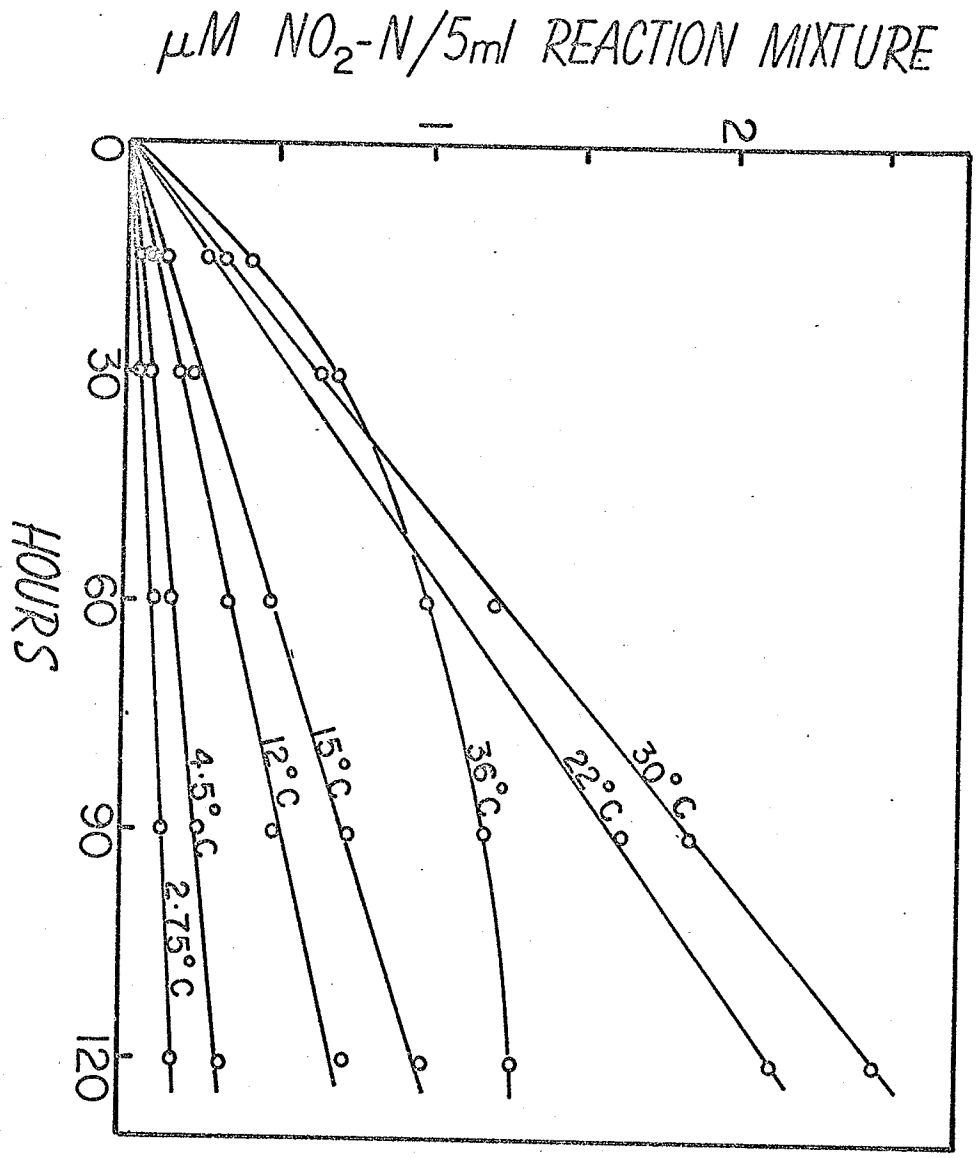
Growth of ammonium-oxidizing strains

Fig. 28 illustrates typical growth curves at different incubation temperatures. No growth was evident at 4°, 28° and 35°C. Slight accumulation of nitrite at these temperatures represents only the gradual accumulation of end-product by a non-proliferating population. The characteristic proliferation curves are exemplified by the plots for 15° and 22°C. Repeated experiments conducted by increasing the size of initial inoculum or by extending the incubation period to 21 days failed to yield evidence of any significant growth at 4°C. This finding emphasizes the fact that ammonium-oxidizers are, in this case at least, poorly adapted to growth at low temperatures but display a marked growth response in the temperature range from 15° to 22°C that normally prevails in these subarctic surface soils during the summer period of activity.

Growth of nitrite-oxidizing strains

Figure 29 illustrates typical growth curves of nitrite-oxidizing strains at various incubation temperatures.

Fig. 30. Oxidation of ammonium by washed cell-suspension of ammonium-oxidizers at different temperatures. Reaction mixture and analytical details as described under Materials and Methods.


μM NO2-N/5ml REACTION MIXTURE
1
2
0
30
60
90
120
HOURS
4.5°C
2.75°C
12°C
15°C
36°C
22°C
30°C

From these results it is evident that the nitrite-oxidizing strains from the TL-3 soil were not able to grow at 28° or at 35°C, but grew profusely at 4° and 15°C. No incubation was followed at 22°C.

Oxidation of ammonium by washed cell-suspension of ammonium oxidizers

Data presented in Figure 30 show that the rate of oxidation was linear with time at all the temperatures except 36°C where a rate decrease was observed after 30 minutes.

Effect of heat treatment of the cells on their ammonium-oxidizing activity

To examine the thermal lability characteristics of the ammonium-oxidizing activity, a cell suspension adjusted to pH 7.7 was distributed into 10 ml lots and heated at various constant temperatures for a period of one hour. Immediately after heat treatment culture tubes were cooled rapidly to 4°C in an ice bath. Residual ammonium-oxidizing activity was assayed as previously described. The results are presented in Table XVII.

Table XVII

The effect of heat treatment for one hour at different temperatures on the ammonium-oxidizing activity.

Treatment Temperature	% remaining of oxidation activity
15°C	100
22°C	100
28°C	98
36°C	88
50°C	0

Results obtained on temperature relationships of nitrifiers in artificial media compliment the previous observations in whole soil in showing that the nitrite-oxidizing strains in TL-3 soil are acclimatized to low temperature conditions. A temperature range of 28-37°C, generally observed as the optimum range for most of the mesophilic strains, failed to support any significant growth of either the ammonium- or nitrite-oxidizing strains. It is rather surprising that the ammonium-oxidizers from the same soil were poorly adapted to low temperature conditions. Their growth response to a restricted temperature range suggests that under low temperature conditions the activity displayed by these organisms (in terms of ammonium-oxidation

by resting cells) would probably never permit nitrite accumulation.

It was also found that soil characteristics were apparently not a factor in governing the proliferation of ammonium-oxidizers both at low and high temperatures. Failure to grow at the low temperature even after increasing the inoculum size or extending the incubation period further suggests that incapability of proliferation at this temperature could be attributable to the nature of the strains themselves.

SUMMARY

Nitrification in nine soil samples from the Twin Lake Hill, Old Beach, Goose Creek and Inuvik areas was studied in the laboratory by the rocking percolator and flask incubation techniques. All the samples though varying to a great degree in their physico-chemical characteristics showed a nitrification potential. The major limiting factor to nitrification appeared to be the pH level of the soil. For example, though the viable organisms were present in acidic soils of the Old Beach and Twin Lake areas, nitrification activity was not displayed until the soils were amended with calcium carbonate to raise the pH. Nitrification capacity of the soils depended largely on the initial existing nitrifying population and on the physical and chemical nature of the soils themselves.

A marked variation in the degree of temperature acclimatization was observed among the nitrifying strains from soil samples of the same region as well as from soils of the different regions. Temperature variations (both optimum and effective range) were even more pronounced between the ammonium and nitrite-oxidizing strains in the same sample. On the whole, ammonium-oxidizing strains were found to be more sensitive to both low and high temperatures

within the experimental range than the nitrite-oxidizers. The varying nature and extent of temperature adaptations among the strains of a single group (ammonium- or nitrite-oxidizers) as well as variations between the two groups suggest that future investigations of this kind should be based on a critical analysis of nitrification in which the ammonium, and nitrite oxidations would be considered as independent processes in terms of their temperature characteristics.

PART III

NITROGEN FIXATION

INTRODUCTION

Over a half-century has passed since the first recognition of nitrogen fixation by free-living organisms was noted for a member of the genus Clostridium by Winogradsky in 1890 followed somewhat later by the contributions of Beijerinck in the case of the genus Azotobacter. Since then, the majority of reports have dealt with these classical nitrogen fixers and, for the most part, studies have been restricted to strains isolated from temperate soils (Jensen, 1940; Meiklejohn, 1956; Becking, 1961; Chang and Knowles, 1965 and others). A more restricted number of reports have been based on strains isolated from tropical soils (Becking, 1961; Meiklejohn, 1962; Moore, 1963 and Domergues, 1963).

Whether or not asymbiotic fixation plays a truly significant role in soil nitrogen economy is still open to question. Results of applied azotobacter cultures (in terms of soil treatments) have been reported as beneficial by some Soviet investigators; these results have not been confirmed in similar soil treatments by English and American workers. The relative importance of naturally-occurring azotobacter and clostridium soil populations as agents in nitrogen fixation is difficult to assess since azotobacter is not always found in agricultural soils and only fragmentary data are available thus far on the distribution of the probably more promising clostridial group (Chang and Knowles, 1965).

Until comparatively recently, members of the genus Azotobacter have been accepted as the principle asymbiotic organisms operating under aerobic conditions. The fixation efficiency of this group has received considerable attention and, in general, quite low values for fixed nitrogen on an acre-foot basis has been reported. In all probability, these findings have been responsible for the general opinion that asymbiotic aerobic fixation is only of minor benefit to the soil nitrogen economy.

Within the last few years, however, as exemplified by the report of Chang and Knowles (1965) among others, a growing realization has emerged that the capacity for asymbiotic fixation (aerobic and anaerobic) is more widely distributed among soil microorganisms than hitherto had been believed. A more recent survey of the literature suggests that a wide group of organisms including bacteria, actinomycetes, molds, yeasts and blue-green algae possess the capacity to fix atmospheric nitrogen in culture as noted in the following list of genera:

1. Pseudomonas spp. (Paul and Newton, 1961; Procter and Wilson, 1958; Anderson, 1955; Voets and Debacher, 1955).
2. Aerobacter spp. (Tribe Klebsielleae, Mahl et al, 1965; Jensen, 1956; Hamilton and Wilson, 1955; Hamilton et al, 1953).
3. Mycobacterium spp. (Coty, 1967).
4. Methanobacterium sp. (Pine and Barker, 1954).

5. Achromobacter spp. (Jensen, 1958; Proctor and Wilson, 1958).

6. Bacillus polymyxa (Hino and Wilson, 1958).

7. Rhodotorula and Saccharomyces spp. (Metcalfe and Chayen, 1954).

8. Pullularia sp. (Brown and Metcalfe, 1957).

9. Derxia spp. (Jensen et al, 1960; Roy, 1962).

10. Nocardia spp. (Metcalfe and Brown, 1957).

11. Methane oxidizers (Pine and Barker, 1954; Davis et al, 1964).

12. Blue-green algae (Reviewed by Singh, 1961).

There is, however, no positive evidence to substantiate the role of these nitrogen fixers (except the blue-green algae, Singh, 1961) in the soil nitrogen economy. In part this is due to the lack of information on the numerical or ecological distribution of these genera in soils. More important, perhaps, are the practical difficulties inherent in designing appropriate experimental systems to evaluate contributions by these organisms under field conditions. The recent introduction of the acetylene reduction assay method by Hardy (1968) which measures nitrogenase activity in soils may provide a partial solution to some of these difficulties.

A bibliography of Arctic Microbiology also gives evidence of attempts to demonstrate asymbiotic nitrogen

fixation by soil and marine cultures isolated from polar and adjacent areas. Some of the earlier attempts to demonstrate the presence of Azotobacter species in these habitats were unsuccessful (Bartel, 1916-1918 in Greenland; and Burgvits, 1929; Levinskaya and Mammicheva, 1936 in the Soviet Kola Peninsula). Isachenko, (1914) on studies of micro-organisms in the water and mud of the Barent's Sea reported the presence of anaerobic and aerobic nitrogen fixers while Kazanski (1932) detected the presence of aerobic asymbiotic nitrogen fixers in soils of the Nova Zemlya and Murmansk areas of the Soviet Union. Later, attempts in Greenland by Russel (1940) and in the Kola Peninsula by Roizin and Ezrukha (1958) did show the presence of Azotobacter spp. in the respective soils.

An extensive ecological survey of microorganisms in Alaskan tundra soils by Boyd and Boyd (1962, 1963) confirmed the presence of azotobacter but no actual nitrogen fixation by the strains was quantitatively demonstrated either in culture or "in situ" (Boyd et al, 1966).

The concept of nitrogen fixation as being more widespread among soil microorganisms than previously considered was strengthened by the investigations of Jensen et al (1960) in which the existence of facultative nitrogen fixers was reported. Proctor and Wilson (1958), earlier had demonstrated the adaptive nature of nitrogen fixing ability in certain strains of Pseudomonas while Nemeth (1959) reported fixation

by an unknown red-pigmented bacterial species.

The critical evaluation of newly-isolated species showing some degree of fixation potential has been hampered by the use of methodologies not well suited to the task. Often, where optimal conditions for nitrogen fixation have not been worked out, investigators tend to rely on estimates based almost solely on growth of these cultures in or on media devoid of combined nitrogen (As described in review by Virtanen and Miettinen, 1963). In this procedure an increase in total nitrogen content of the medium (inoculated and incubated) usually determined by Kjeldahl procedures is frequently used. Unfortunately, such a method is not well suited for critical estimates of nitrogen fixed by these organisms since, for one reason or another, their fixation is only marginal. The use of $^{15}N_2$ as a tracer was introduced by the Wisconsin group (Burris and Miller, 1941; Burris et al, 1943) and still remains as one of the more sensitive methods for detecting fixation. Its great disadvantage, however, lies in the cost involved which makes the assay prohibitively expensive for large scale screening surveys. Manometric techniques proposed by Mortensen (1964) have considerably improved the reliability of nitrogen fixation estimates for work done at the cell-free level.

The present investigation was aimed primarily to apply an ultra sensitive assay method using $^{13}N_2$ (Campbell et al, 1967) for screening cultures obtained through the use

of nitrogenous and nitrogen-free media selection from soils of the subarctic. Major emphases have been placed on the several parameters that influence the ecological distribution of nitrogen fixers in the diverse soils of the region under the extremes of the climatic environment.

METHODS AND MATERIALS

A. Plate Count Estimation of Nitrogen Fixers on Selective Media

I. Azotobacter and total heterotrophic nitrogen fixers

Numbers of Azotobacter were determined on a nitrogen-free medium described by Clark (1965). The medium contained in one liter of glass distilled water, K_2HPO_4, 0.5 g; $MgSO_4.7H_2O$, 0.2 g; $CaSO_4$, 0.1 g; $FeSO_4$, 0.02 g; $MnSO_4$, 0.02 g; $MoO_3.H_2O$, 0.01 g; KI, 0.01 g; sucrose, 10 g; $CaCO_3$, 3.0 g and agar (Noble), 15 g. The medium was autoclaved at 121°C for 20 minutes. The method of preparing soil suspensions, serial dilutions, pipetting and spread plating were the same as described for total bacterial counts, Part I. After incubation at different temperatures (3.5, 15, 28 and 37°C) for varying periods of time as determined by the temperature effect on growth, Azotobacter, distinguishable as cream colored mucilagenous colonies larger than one millimeter in diameter (usually 2 to 3 mm), were counted. Colonies other than the azotobacter type appearing on the medium were also counted and considered as either contaminants (growing at the expense of soluble nitrogen secreted by Azotobacter) or other slow-growing nitrogen fixers.

II. Clostridium and Total anaerobic nitrogen fixers

The method of counting clostridial types and the medium used was as described by Ross (1958). The medium contained in one liter of tap water, K_2HPO_4, 1.0 g; $MgSO_4.7H_20$, 1.0 g; $FeCl_3.6H_20$, 10 mg; glucose, 10 g; $ZnSO_4.7H_20$, 1.0 mg; $Na_2MoO_4.2H$ 0, 0.5 mg; soil extract (Lochead and Burton, 1957), 10 ml and agar (Noble), 15 g. All the ingredients except glucose were dissolved in 900 ml tap water and autoclaved at 121°C for 15 minutes. Glucose was autoclaved separately after dissolving in 100 ml of water, cooled and added aseptically to the rest of the medium (cooled). Plating procedures were similar to those used for azotobacter and total aerobic nitrogen fixers. Plates were incubated at 15°C under an atmosphere of nitrogen and carbon dioxide (95:5) for two weeks after which time the total number of colonies appearing on the plates were counted. After enumeration, plates were flooded with Gram's iodine solution and a further count was made (after excess iodine was poured off) of the dark brownish-blue stained colonies representative of the clostridial type.

III. Members of the genera Pullularia and Chromobacter

Members of both the genera were counted on modified medium B agar (similar to that used for total bacterial

counts, Part I, except that combined nitrogenous ingredients were omitted). The choice of this medium was based on the prior experience that members of these genera had been more frequently encountered on this medium than on any other medium used for enumeration. After two weeks incubation at 15°C, colonies that were dark brown to black, raised, 1 to 2 mm in diameter represented Pullularia; violet colored colonies of varying sizes were considered to be representative of the genus Chromobacter. It should be noted that this medium was not selective (except in a N-free sense) and counts were based solely on the appearance of characteristic colony color. No other colorfully-pigmented bacterial nitrogen fixers were observed.

B. Isolation of Nitrogen Fixers Developing on Nitrogenous Media

Soils collected during the Fall of 1964 were examined for their bacterial populations on medium B as described in Part I of this thesis. As part of the characterization studies, colonies developing on the plates incubated at 15°C were randomly picked and purified by successive transfers to plates of the same medium in order to yield monomorphic types. Pure cultures thus obtained were then challenged with Burk's nitrogen-free medium[1] and incubated

[1]Burk's nitrogen-free medium contained in one liter of distilled water: sucrose, 20 g; K_2HPO_4, 0.16 g; $MgSO_4.7H_2O$, 0.2 g; $CaSO_4.2H_2O$, 0.05 g; NaCl, 0.2 g; Na_2MoO_4, 0.001 g; $FeSO_4.7H_2O$, 0.003 g and agar (Noble), 15 g. Final pH, 7.2.

at 15°C under an ammonium-free atmosphere[1]. After three successive transfers on nitrogen-free medium, cultures that grew well were transferred again to nitrogen-free Burk's agar slants. These cultures were routinely maintained by retransfer every two weeks.

C. Cultivation of Nitrogen Fixing Isolates in Liquid Medium

For liquid medium propagation, unless otherwise stated, all bacterial isolates were grown in Winogradsky's solution as modified by Hino and Wilson (1958). This medium was prepared by mixing equal portions of the following autoclaved solutions:

Solution A. In 500 ml distilled water, sucrose, 20 g; $MgSO_4.7H_2O$, 500 mg; NaCl, 10 mg; $CaCl_2.2H_2O$, 55 mg; $FeSO_4.7H_2O$, 15 mg; $Na_2MoO_4.2H_2O$, 5 mg.

Solution B. In 500 ml of distilled water, para-amino benzoic acid, 10 µg; biotin, 5µµg; K_2HPO_4 - KH_2PO_4 buffer (0.1 M, pH 7.7).

The inoculum was prepared by growing the cells in the medium at 15°C.

As reported by Campbell et al (1967) Pullularia and Rhodotorula species were cultured in a medium of the following composition: In one liter of glass-distilled water, KH_2PO_4, 875 mg; K_2HPO_4, 125 mg; $MgSO_4.7H_2O$, 500 mg;

[1]Burk's medium plates were incubated in a closed chamber through which sulfuric-acid-washed moist air was passed. Estimates of ammonium present were obtained by a modified Conway diffusion technique[2] and results were routinely negative.

[2]Mortensen, L.E. (1961) Anal. Biochem. 2, 216.

NaCl, 100 mg; $CaCl_2$, 100 mg; $FeSO_4.7H_20$, 15 mg; biotin, 20 µg; and $Na_2MoO_4.2H_20$, 5 mg. The pH was adjusted to give a final value of 5.0. Subsequently, the medium of Hino and Wilson as described for bacterial isolates was found to be equally suitable and in all further studies this medium replaced the medium of Campbell et al (1967).

Characteristics of Nitrogen Fixing Isolates

Morphological characterisitics

Gram and flagella stains were prepared on each of the isolates according to the procedures described in Manual of Microbiological Methods (1957). After 36 hours incubation at 15°C on nitrogen-free medium, the organisms were Gram-stained by the Burke and Kopeloff-Beerman modifications. Motility was determined first by examination of wet mounts under oil immersion microscopy followed by confirmatory flagella staining using Bailey's procedure as modified by Fisher and Conn. Cell size was estimated, in each case, on the basis of the Gram stained preparations.

Cultural characteristics

All the bacterial isolates that demonstrated a nitrogen fixing capacity were cultured on the following

listed media for studies of growth, pigmentation and colony characteristics.

1. Nutrient Agar (BBL).
2. Ashby's nitrogen-free medium.[1]
3. Ashby's medium + nitrate or ammonium nitrogen.[2]
4. Ashby's medium + asparagine (0.5%).

Other media as used for cultural studies are specifically detailed in Results and Discussion.

Environmental characteristics

The ability of isolates to grow in nitrogen-free liquid medium at 3.0, 15, 22, 28 and 37°C incubation temperatures was determined after seven days incubation. Growth was evaluated by turbidity measurements.

The effect of pH on the growth of the isolates was determined in the liquid medium of Hino and Wilson. The initial pH of the medium was adjusted, in each case, by changing the composition of portion 'b' of the medium as follows:

for pH 5.0, 6.0, 7.0 and 8.0	add 0.1 M KH_2PO_4 - K_2HPO_4 buffer
for pH 9.0	add 0.1 M KH_2PO_4 - KOH buffer
for pH 4.0 to 4.5	add 0.1 M KH_2PO_4 titrated with dilute HCl (10% V/V) if necessary.

[1]Ashby's nitrogen-free medium contained in one liter of distilled water: mannitol, 20 g; K_2HPO_4, 0.2 g; $MgSO_4$, 0.2 g; NaCl, 0.2 g; K_2SO_4, 0.1 g, and $CaCO_3$, 5.0 g. Medium was autoclaved at 121°C for 15 minutes.

[2]Potassium nitrate or ammonium sulfate at 0.1% concentration.

The medium was dispensed 25 ml at a time in 125 ml Erlenmeyer flasks and inoculated. Incubation was carried out at 15°C on a rotary shaker.

Physiological characteristics

The ability of the isolates to hydrolyze starch was tested on nutrient agar and Burk's agar plates supplemented with 0.2% soluble starch. After seven days incubation at 15°C, the plate surfaces were flooded with Gram's iodine solution and a clear halo surrounding the colony was accepted as evidence of starch hydrolysis by that isolate.

Gelatin liquification was determined, in each case, by stab inoculation in a 12% gelatin in distilled water. Stab cultures were incubated at 15°C for three weeks.

Test for the reduction of nitrate to nitrite was carried out according to the procedure described in the Manual of Microbiological Methods (1957). Urease production was examined in a urease medium (BBL) after seven to ten days incubation at 15°C.

Cultural responses in milk were noted using litmus milk (BBL) according to the procedure outlined in Manual of Microbiological Methods (1957). Utilization by the isolates of different carbon sources, and tests for concomitant acid production were carried out using nitrogen-free medium

of Hino and Wilson with bromcresol purple (0.04%) as acid-base indicator.

Measurements of Growth and Fixation

Estimation of growth

Growth, estimated as the increase in turbidity, was measured with a Klett-Summerson photoelectric colorimeter at 540 mμ.

Assay for nitrogen-fixation by resting cell-suspensions

Isolates able to grow on nitrogen-free media were confirmed for their nitrogen-fixing potential using the $^{13}N_2$ radio-tracer technique of Campbell et al (1967). Fresh cultures of isolates growing on nitrogen-free solid media were harvested by washing the surface growth with phosphate buffer (0.3 M, KH_2PO_4 - K_2HPO_4, pH 7.0) and centrifugation (10,000 X g, for 15 minutes at 4°C). Pellets were further washed twice using the same buffer and finally resuspended in liquid nitrogen free media (bacteria and actinomycetes in the medium of Hino and Wilson and Rhodotorula and Pullularia in the medium of Campbell et al). The energy source, usually glucose sterilized in solution by filtration, was added to yield a final concentration of

10 mg/ml. The cultures were then returned to incubation conditions at 15°C for 2-4 hrs before exposure to $^{13}N_2$ in the assay.

Each experimental series of eight flasks normally included one 'positive' control (usually Azotobacter vinelandii[1]), one 'negative' control (usually Escherichia coli), and two isolates whose growth performance on nitrogen-free media suggested a fixation capacity. For each culture tested, two flasks were required: one to contain a heavy suspension of fresh cells, the other containing an equivalent amount of boiled cells as a control. Cells were exposed to the $^{13}N_2$ under the continuous flow of the gas-mixture (0_2 at 20 ml/min, $^{14}N_2$ + CO_2 (95:5), at 50 ml/min and $^{13}N_2$ carrier gas (helium) at 200 ml/min) for a maximum of 30 minutes; during the exposure period the flasks were shaken continuously to ensure maximal gas exchange. At the conclusion of the exposure period the $^{13}N_2$ supply was shut off and the culture flasks were flushed (while shaken vigorously) for 10 minutes with a 95:5 nitrogen-carbondioxide mixture at a flow rate of 0.5 l/minute to remove any unfixed $^{13}N_2$. After the flushing, the cultures were poured from the culture flasks into aluminum foil dishes for counting the radioactivity resulting from fixed $^{13}N_2$. Radioactivity, 0.51 Mev gamma pulse (positron

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[1]Courtesy of Dr. P.W. Wilson, Univ. of Wisconsin, Madison, Wis., U.S.A.

decay, ^{13}N to ^{13}C), was counted by an external sample scintillation-detector coupled to a single-channel pulse height analyzer. Generally, counts on each sample of exposed cells were taken for 60 seconds.

Assay for nitrogen-fixation in growing cultures

Increase in total nitrogen content of the growing culture was the estimate of nitrogen fixed; the method used was a conventional microkjeldahl technique. The method of distillation, apparatus used and subsequent titration were the same as described for the total organic-nitrogen determination of soils in Part I of this thesis except that the digestion mixture was changed. Digestion was performed as follows: to Kjeldahl flasks containing 1-2 ml of cell-samples were added one Henger granule and 10 mg of digestion mixture ($CuSO_4.5H_20$ - K_2S0_4, mixed in a ratio of 3:1). Finally 2.0 ml of concentrate H_2S0_4 (reagent grade) was added to each sample flask. Samples were digested until clear.

Estimation of protein in whole cells

Protein in whole cells was calculated on the basis of the microkjeldahl-nitrogen content.

Protein = Kjeldahl Nitrogen X 6.25.

RESULTS AND DISCUSSION

Plate Count Estimation of Nitrogen-Fixers:

Results of the plate-count estimation of non-symbiotic nitrogen-fixers (15°C incubation) are detailed in Table XVIII. These data show that nitrogen-fixers were abundant in these soils. Total numbers in these soils ranged from a few hundreds of thousands to nearly two million per gram. These figures are more or less comparable to the estimates made on Canadian temperate soils (Chang and Knowles, 1965). The lowest numbers were detected in the acidic soil of Twin Lake (TL-3) and the soil of Old Beach areas.

Numbers of azotobacter-like organisms varied from soil to soil but always formed a significant proportion of the total nitrogen-fixing population. Though relati-ely fewer in numbers, azotobacters were none the less encountered in the acidic soils. Many earlier as well as more recent observations (Gonick and Reuszer, 1949; Jensen, 1940, 1950; Sushkina, 1949) have indicated that Azotobacter spp. rarely occur in temperate soils with a pH level below 6.0. However, there is some indication that in the soils of subarctic and arctic regions, Azotobacter spp. are widely distributed. Although no quantitative data are available Azotobacter has been detected in the acid soils (pH 4.1 to 6.4) of Alaskan arctic (Boyd and Boyd, 1962).

Table XVIII

Plate-count estimates of total aerobic and anaerobic nitrogen-fixing bacteria, developing at an incubation temperature of 15°C.

Soil Sample	Numbers of bacteria (x 10^3)/g oven-dry soil			
	Aerobic		Anaerobic	
	Total	Azotobacter	Total	Clostridium
TL-3	230	46.1	85.2	0.0
TL-10	1430	1072.5	85.7	0.0
OB-1	200	160.0	69.0	0.0
OB-5	800	192.0	160.0	0.0
GC-12	1600	1120.0	200.0	0.0
Iv-X	1250	797.5	330.0	92.0
Iv-Z	1671	901.8	500.0	83.0

Table XIX

Distribution of *Chromobacter* (G-15) and *Pullularia* (Ao-7) in Churchill soils.

Soil Sample	Numbers (x 10^3)/g oven-dry soil	
	Chromobacter	Pullularia
TL-2	10.00	5.70
TL-3	23.00	17.00
OB-1	16.70	10.00
OB-5	0.00	0.00
GC-1	110.00	64.00

Anaerobic nitrogen-fixers constituted a significant proportion of the total nitrogen-fixing population in these soils. Clostridium, however, was only encountered in the Inuvic soils.

The distribution of Chromobacter and Pullularia species is given in Table XIX . Both types were obtained in numbers ranging from several thousands to several tens of thousands per gram in soils from the Twin Lake and Goose Creek areas. Both Chromobacter and Pullularia were absent in OB-5 soil. Whether their absence was attributable to the low soil pH prevailing or to some other causes was not established.

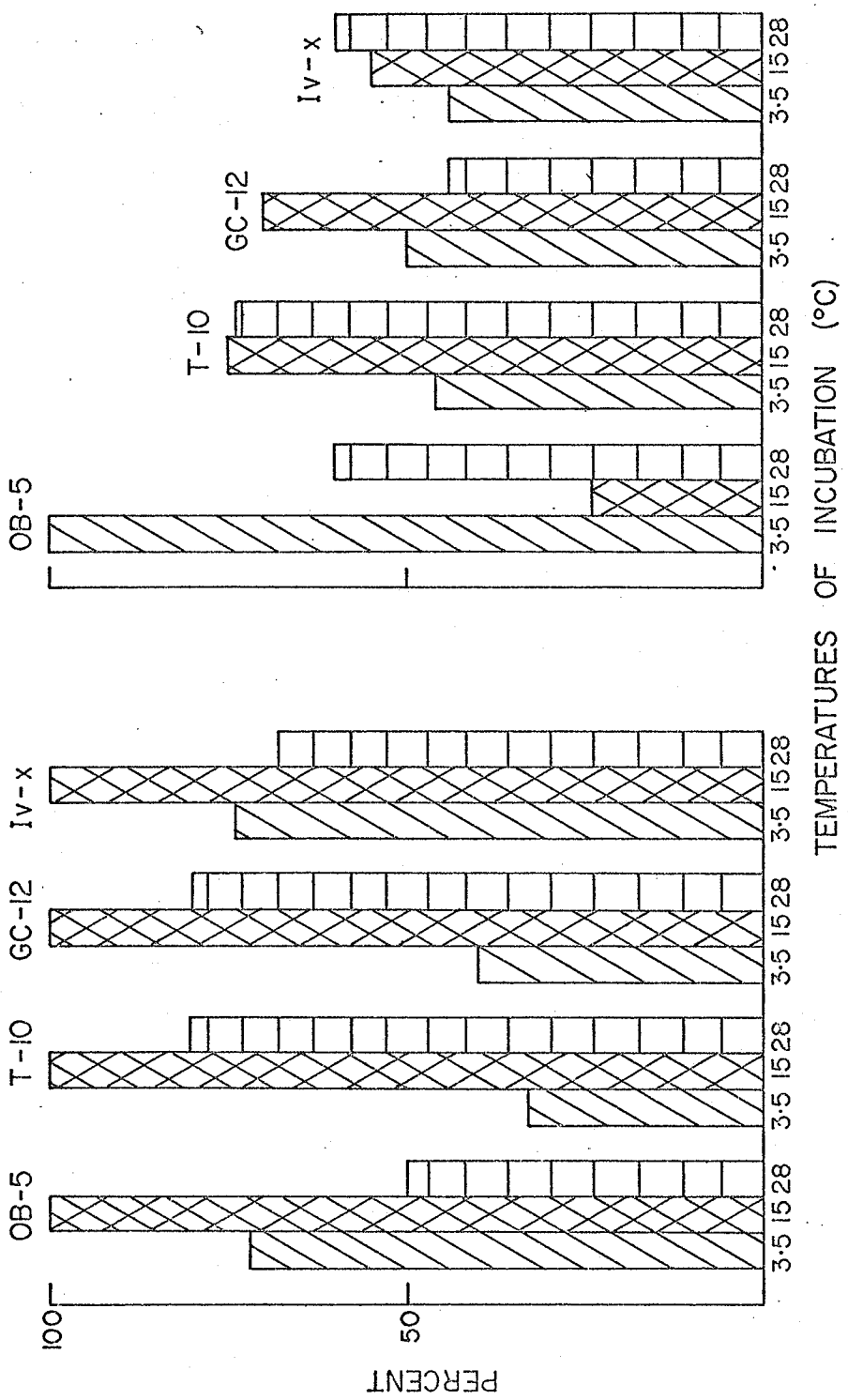
Influence of Incubation Temperatures on Plate-Counts of Total Aerobic Non-Symbiotic Nitrogen-Fixers

Data for total nitrogen-fixers and Azotobacter spp. at 3.5, 15 and 28°C expressed as percentages of total counts at 15°C incubation, are histogrammed in Fig. 31 (a and b). Here, plate counts following a 15°C temperature incubation are assumed to constitute, for comparison purposes, 100% growth of nitrogen-fixers. Data for the 37°C incubation temperature were not recorded because only a few colonies appeared on the plates. The data show that bacteria capable of growing at 3.5°C constituted 27 to 76% of the total nitrogen-fixing counts at 15°C. The most interesting

Fig. 31. Influence of incubation temperatures on plate-counts of total aerobic non-symbiotic nitrogen-fixers in different soils.

(a) Percentage of bacterial counts at different temperatures as compared to counts at 15°C.

(b) Azotobacter counts at different temperatures, as percent of total count of nitrogen-fixers.


PERCENT
100
50
OB-5
T-10
GC-12
Iv-x
3·5 15 28
TEMPERATURES OF INCUBATION (°C)

observation in this experiment was that the increase in the counts at 3.5°C for certain soils (OB-5 and Iv-X) was invariably accompanied by a decrease in counts at 28°C. The converse was also true. The highest percentage of nitrogen-fixing bacteria was counted at 3.5°C in Old Beach and Inuvic soils.

Azotobacter counts obtained at different incubation temperatures showed much variation; the degree of variation depended to a great extent on the soil in question. In Twin Lake and Goose Creek soils, maximum numbers were encountered with incubation temperature of 15°C. In Old Beach soil maximum numbers were evident at 3.5°C incubation; the next highest numbers were evident at 28°C incubation. However, in Inuvic soil, the counts increased with increasing temperatures of incubation within the experimental range up to 28° but not at 37°C. These variations noted in the appearance of Azotobacter spp. might be analogous to the observation of Ivarson (1965) on fungal counts in arctic soil. He demonstrated that a particular group will dominate at incubation temperature best suited for their growth and will, thereby, supress or minimize the appearance of other groups less suited to that temperature.

The observed difference between soils in the percentages of azotobacter populations (able to grow at a 3.5°C incubation temperature) is very likely due to the degree of acclimatization to low temperature. Conditions which, according to Mishustin

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(1947), depends on the time period elapsed since the introduction of the species into the particular area. According to this concept Azotobacter introduction into the Old Beach and Inuvic soils could be assumed to be an earlier event historically. However, there are no data to support this concept other than that the Old Beach soil dates back to immediate post-glacial emergence. Thus, soils sampled closer to the coast are of more recent origin.

Isolation of Nitrogen-Fixers from Colonies Developing on Nitrogenous Medium

The frequency of occurrence of nitrogen-fixers based on the screening of random isolates for their nitrogen-fixing potential and the isolates selected for further studies are detailed in Table XX . On the whole about 41% of the isolates showed a nitrogen-fixing potential as indicated by their continued growth on nitrogen-free media. All the three soils and a subsurface water sample (mud water) contained a wide variety of nitrogen-fixing species.

Table XX

Isolation of nitrogen-fixers from colonies developing on nitrogenous Medium B.

Sample	Isolates tested (number)	No. of isolates capable of growing on N_2-free medium	Isolates, selected
Old Beach soil	20	9	A-1, A-6, A-11, A-13
Goose Greek soil	17	7	G-11, G-12, G-15, G-16, G-17 G-22
Twin Lake soil	20	8	T-2, T-4, T-6, T-1,
Twin Lake mud water	20	4	Ao-6, Ao-12, Ao-7, Aol-11
Total	67	28	18

Morphological Characteristics of Isolates

The results pertaining to morphological characteristics of the isolates from nitrogenous and non-nitrogenous media are listed in Table XXI. Out of 34 isolates, 29 were gram^{-ve} coccoid rods and of these 12 displayed motility by peritrichous flagellations. Of the other 17 isolates, one showed lophotrichous flagellation; two gave evidence of amphitrichous flagellation; two were monotrichous and six isolates were non-motile. Motility in the remaining six isolates was not determined either due to technical difficulties or to failure of the cultures to survive in storage. Two gram^{+ve} isolates (T-1 and G-12) were identified as Nocardia and Streptomyces species respectively, based on study of their morphological and cultural characteristics (see Part I, Materials and Methods). Isolate T-1 showed short, irregularly curved and sparsely branched mycelia after 18 hours slide-culture incubation at 25°C. After 36 hours, however, these mycelia began fragmenting to yield the short curved rods characteristic of Nocardia species. Isolate G-12 produced aerial mycelia which were unevenly gram-stained. Slide-culture propagation (25°C incubation) gave long, profusely branched vegetative mycelia, well developed sporulating aerial mycelia slightly curved at the tips and small round spores (0.3-0.4 μ).

Table XXI

Morphological characteristics of some nitrogen-fixing isolates

Isolate	Method of Isolation	Gram-stain and Morphology	Motility and Flagellation
A-1	Non-selective*	Gram(-) rods, 0.6 x 3-4 μ, single or in clumps	Motile, peritrichous
A-6	"	Gram(-) rods, 0.6 x 2-3 μ, single	Motile, Not determined
A-11	"	Gram(-) rods, 0.6 x 1.2-2 μ, single two distinct granules, one at each end	Motile, peritrichous
A-13	"	Gram(-) rods, 0.6 x 1.2-2 μ, single two distinct granules one at each end	Motile, peritrichous
G-11	"	Gram(±) coccoids, 0.8 x 1.0 μ, single	Motile, lophotricous (at one end)
G-12	"	Gram(+), produces aerial mycelium (Streptomyces sp.)	
G-15 [1]	"	Gram(-) rods, 0.7 x 1.5-2 μ, single	not determined
G-16	"	Gram(-) rods, 0.5 x 3-6 μ, single	non-motile
G-17	"	Gram(-) rods, 0.6 x 4 μ, single or in clumps	Motile, peritrichous
G-22	"	Gram(-) rods, 0.6 x 2.5-4 μ, single, granulated	Motile, peritrichous

Table XXI. Cont'd.

Isolate	Method of Isolation	Gram-stain and Morphology	Motility and Flagellation
T-1	Non-selective	Gram(+) rods, fragmenting hyphae (*Nocardia*)	Non-motile
T-2	"	Gram(-) coccoid rods, 1.5 x 2.0	Non-motile
T-4	"	Gram(-) rods, 1.3 x 1.8 , single	
T-6	"	Gram(-) rods, 0.5 x 1.5 , single	Motile, amphitrichous
Ao-6	"	Gram(-) rods, 0.7 x 2-4 , single	Motile, peritrichous
Ao-7‡	"	*Pullularia* sp.	
Ao-1-11	"	Gram(-) rods, 1-1.3 x 2-2.5 , single	Motile, lophotrichous (at one end)
Ao-12	"	Gram(-) coccoid rods, 0.8 x 1.2 , single	Motile, peritrichous
Ao-15‡	"	*Rhodotorula* sp.	
Iv-X-1	Selective	Gram(-) rods, 0.5 x 2-3.5 , single	Motile, peritrichous
Iv-X-2	"	Gram(-) rods, 0.5 x 2 , single or in in pairs or triplets	Motile, monotrichous
Iv-X-3	"	Gram(-) rods, 0.65 x 4-5 , single	Non-motile
Iv-Z-1	"	Gram(-) rods, 0.65 x 4-5 , single	Non-motile

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Table XXI. Cont'd.

Isolate	Method of Isolation	Gram-stain and Morphology	Motility and Flagellation
TL-3-2	Selective	Gram(-) rods, 0.5 x 2-3.5 μ, single	Motile, peritrichous
TL-3-3	"	Gram(-) coccoid rods, 1.3 x 2.0 μ, single	non-motile

*Non-selective = nitrogenous medium
Selective = N_2-free medium

‡Isolates (Ao7 and Ao15) were identified by Dr. J.F.T. Spencer, Prairie Regional Laboratory, National Research Council, Saskatoon, Saskatchewan, Canada.

[1]Identified and studied as Chromobacter sp. by J. Vincent (M.Sc. theses, 1966, University of Manitoba).

All the identified cultures (T-1, G-12, G-15, Ao=7 and Ao-15) and A6, Ao-6, G-16 and Ao-12 were, unless otherwise stated, not futher considered in later studies.

Cultural Characteristics of Isolates

The growth, colony forms and color of isolates on nutrient agar and Ashby's nitrogen-free agar medium are given in Table XXII. All the isolates listed in the Table were capable of growth on nutrient agar, but growth was very scanty in majority of cases. In comparison, the isolates (e.g., A-1, G-17, T2, T-4, T-6, GC-1, TL-10-3 and TL-3-1) showing insignificant growth on nutrient agar, grew more profusely on the nitrogen-free agar medium. All the isolates grew on Ashby's medium supplemented with ammonia-, nitrate- and asparagine-nitrogen. Pigmentation (color) was most pronounced on Ashby's medium with combined nitrogen sources.

Physiological Characteristics of some Isolates

Tables XXIII and XXIV list the physiological characteristics of the isolates on nitrogenous and non-nitrogenous media. On nitrogenous media, as described under Material and Methods, none of the isolates hydrolysed starch. Gelatin liquification, ureolysis, litmus milk reduction and nitrate reduction, however, were noted for several isolates.

Table XXII

Colony form and colour of some nitrogen-fixing isolates[1]

Isolate	Colonies on nutrient agar	Colonies on Ashby's medium (-N)	Colour on Ashby's Medium (+N) supplied as		
			Ammonia	Nitrate	Asparagine
A-1	chalk white, raised, circular, minute colonies	chalk white, raised, circular, minute	yellow, diffusible	yellow, diffusible	yellow, diffusible
A-11	chalk white, raised, circular, large	white, raised, circular, large	light brown, diffusible	no colour	light brown, diffusible
A-13	chalk white, raised, circular, large	white, raised, circular, large	light brown, diffusible	no colour	light brown, diffusible
G-11	slightly yellow, opaque, raised, circular, large	turbid white, raised, circular, minute	n.d.*	n.d.	n.d.
G-17	chalk white, shiny, raised, circular, minute	chalk white, raised, circular, minute	yellow, diffusible	yellow, diffusible	yellow, diffusible
G-22	translucent, with slight brownish center, circular, raised, minute	chalk white, slimy, circular raised, large	slightly yellow	slightly yellow	slightly yellow

Table XXII. Cont'd.

Isolate	Colonies on nutrient agar	Colonies on Ashby's medium (-N)	Colour on Ashby's Medium (+N) supplied as		
			Ammonia	Nitrate	Asparagine
T-2	deep yellow, circular, raised, minute	colourless, slimy, shiny, slightly raised, large	yellow	yellow	yellow
T-4	chalk white, circular, raised, minute	chalk white, circular, raised, large	white	white	faint yellow-brown,
T-6	deep yellow to orange, shiny, circular, raised, minute	slightly yellow, shiny, circular, raised, large	faint yellow	faint yellow	faint yellow
AO1-11	translucent, slightly raised, circular to hemis-pherical, large	colourless, shiny with blue tinge, slimy, circular, raised, large	n.d.	n.d.	n.d.
Iv-X-1	slightly brown, opaque, with translucent margin, circular, raised, large	colourless, slimy, irregular, generally hemis-pherical, flat, large	yellow brown	yellow brown	yellow brown

Table XXII. Cont'd.

Isolate	Colonies on nutrient agar	Colonies on Ashby's medium (-N)	Colour on Ashby's Medium (+N) supplied as		
			Ammonia	Nitrate	Asparagine
Iv-X-2	deep yellow to orange, raised, circular, minute	slightly yellow, raised circular, large	n.d.	n.d.	n.d.
Iv-X-3	slightly brown with blue tinge, opaque, circular, raised, minute	colourless, translucent, circular, raised, large	white	white	dirty white
Iv-Z-1	slightly brown with blue tinge, opaque, circular, raised, minute	colourless, translucent, circular, raised, large	white	white	dirty white
Iv-Z-2	chalk white, raised circular, very	chalk white, raised, circular, minute	yellow, diffusible	yellow, diffusible	yellow diffusible
Iv-Z-3	slightly brown, with opaque center, circular, raised	colourless, shiny, flat, hemispherical	orange yellow	light orange yellow	deep yellow
GC-1	slightly brown with blue tinge, circular, raised, minute	white, opaque to translucent, circular, raised, large	white	white	dirty white

Table XXII. Cont'd.

Isolate	Colonies on nutrient agar	Colonies on Ashby's medium (-N)	Colour on Ashby's Medium (+N) supplied as		
			Ammonia	Nitrate	Asparagine
GC-2	slightly brown with blue tinge, circular, raised, minute	white, opaque to translucent, circular, raised, large	white	white	dirty white
GC-3	translucent, slightly brown in center, circular, raised, minute	chalk white, slimy, raised, circular, large	slightly yellow	slightly yellow	slightly yellow
TL-10-1	dirty white, shiny, circular, raised, large	colourless, shiny with blue tinge, raised, circular	slightly yellow	slightly yellow	slightly yellow
TL-10-2	deep yellow to orange, raised, circular, large	slightly yellow, circular to hemis-pherical, large	yellow	yellow	yellow
TL-10-3	chalk white, raised, circular, minute	chalk white, raised, circular, very mucoid	white	white	faint yellow

Table XXII. Cont'd.

Isolate	Colonies on nutrient agar	Colonies on Ashby's medium (-N)	Colour on Ashby's Medium (+N) supplied as		
			Ammonia	Nitrate	Asparagine
TL-3-1	very poor growth	shiny white, circular, raised, large	white	white	faint yellow
TL-3-2	dirty brown, translucent, circular to hemispherical, flat large	colourless, shiny	yellow	yellow	deep yellow
TL-3-3	deep yellow, circular, raised, minute	colourless, very slimy, shiny, slightly raised to flat	yellow	yellow	yellow

[1]Incubation in each case was at 15°C for 5 days.

*n.d. = not determined

Table XXIII

Physiological characteristics of some nitrogen-fixing isolates.

Characteristics	Isolates Positive
Gelatin liquification	T-2, T-6, GC-2, GC-3, G-22, TL-10-1, TL-10-2, AO1-11, Iv-Z-1, Iv-X-3, GC-1, TL-3-1.
Starch Hydrolysis	none
Ureolysis	A-1, A-11, A-13, G-17, Iv-Z-2
Litmus-milk reduction	T-6, T-2, G-22, GC-3, TL-3-1, TL-3-2, TL-10-2, TL-10-1, AO1-11, Iv-X-1, Iv-Z-3
Nitrate reduction	T-6, T-4, T-2, G-22, GC-3, TL-3-2, TL-10-3, G-17, A-1, Iv-Z-2, TL-10-2, Iv-X-1, TL-3-1, GC-2, Iv-X-3, Iv-2-1

Table XXIV

Utilization of different carbon sources and concomitant acid production by isolates growing under nitrogen fixation conditions.*

Carbon source		Isolation showing positive response
Arabinose	(1)	All
	(2)	All except, A-11, A-13, Iv-X-2 and TL-3-1
Mannose	(1)	All
	(2)	All except, A-11, A-13, Iv-X-2 and TL-3-1
Xylose	(1)	All
	(2)	All except, A-11, A-13, T-4, TL-3-2, TL-10-1 and TL-10-3
Fructose	(1)	All
	(2)	All except, T-4, TL-10-3, Iv-X-1, Iv-Z-3, G-17 A-1, Iv-Z-2, TL-3-2, TL-10-1, Aol-11, Iv-X-3, Iv-Z-1, and G-11

Table XXIV. Cont'd.

Carbon source		Isolation showing positive response
Lactose	(1)	All exeept T4
	(2)	All except T4, A-11, A-13, G-17, A-1, Iv-Z-2, TL-10-1
Raffinose	(1)	All except T4, Aol-11
	(2)	All except G-22, Iv-X-1, Iv-Z-2, T4, TL-10-3, Aol-11, GC-1, GC-2, GC-3
Amylose	(1)	All except G-22, GC-1, GC-3, Iv-X-1, Iv-X-3, Iv-Z-1, Iv-Z-2, Iv-Z-3, T4, TL-10-3, A-11, A-13, TL-3-1, G-11, G-17
	(2)	Same as above
Glycerol	(1)	All except T-2, T-4, TL-3-3
	(2)	All except T-2, T-4, T-6, Iv-X-1, Iv-Z-3, TL-3-2, TL-3-3, TL-10-2, TL-10-3, Aol-11

Table XXIV. Cont'd.

Carbon source		Isolation showing positive response
Mannitol	(1)	All except T-6, TL-10-2, Iv-Z-2
	(2)	All except GC-1, GC-3, G-22, T-2, T-4, T-6, Iv-X-1, Iv-Z-2, Iv-Z-3, TL-10-1, TL-10-2, TL-10-3, TL-3-2, TL-3-3
Inositol	(1)	All
	(2)	All except GC-1, GC-3, G-22, Iv-X-1, Iv-Z-2, Iv-Z-3, T-4, TL-3-2, TL-10-3, A-11, A-13, A-1, AO1-11, G-17

(1) = Utilization

(2) = Acid production

*Culture tubes each containing 5 ml of nitrogen-free medium with test-carbon source were inoculated with 0.1 ml of cell suspension and were incubated at 15°C for 15 days.

As presented in Table XXIV all the isolates described under 'Cultural Characteristics' grew under nitrogen-fixing condition on arabinose, mannose, dextrose, xylose, fructose and inositol. Lactose, raffinose, amylose, glycerol and mannitol were found to be not suitable as carbon and energy sources for several of the isolates. Production of acidity was also noted for some of the cultures when grown in media supplemented with the different carbon sources.

Environmental Characteristics of Nitrogen-Fixing Isolates

Effect of temperature

Table XXV shows the temperature response by the isolates in liquid medium containing 15 µg N/ml in the form of ammonium sulfate. Most of the isolates were able to grow in the temperature range of 3.5° $\pm$ .5° to 28°C with optima ranging from 15° to 22°C. Certain isolates, however, preferred higher temperatures for optimum growth. A few of the isolates were able to grow over a wide temperature range of 3.5° $\pm$ .5° to 37°C. In this study of incubation temperature response by the isolates, growth was measured turbidimetrically rather than on the basis of an increase in total nitrogen. Thus, it is not certain that growth at 3.5°C would proceed in the absence of combined nitrogen in

Table XXV

Temperature optima and ranges for growth of some isolates in nitrogen-free liquid medium.[1]

Isolate	Optimum temp. (°C)	Temperature range of growth (°C)
A-1, G-17, Iv-Z-2, AO1-11, GC-1, T-6, TL-10-2	15	3.5-28
A-11, A-13, T-4, TL-10-3, TL-3-1, Iv-X-2, Iv-X-3, Iv-Z-1, GC-2	22	3.5-28
G-22, GC-3	22	15-28
Iv-X-1, Iv-Z-2	28	3.5-37
T-2, TL-3-3	28	15-37
G-22, GC-3, TL-10-1	15	3.5-37
Rhodotorula	28	15-28
Pullularia[2]	28	15-28
Nocardia sp.	22	3.5-28

[1]Medium was supplemented with 15 µg N/ml in the form of ammonium sulfate.

[2]Growth measured in medium containing no combined nitrogen.

the liquid medium.

Unfortunately, for reasons of time, it was not possible to examine the growth curves of all the isolates at different incubation temperatures. However, for certain isolates (A-1, G-22 and Pullularia sp.) growth curves are presented in Figs. 32, 33 and 34. A-1 and G-22 both failed to grow at 37°C (data not recorded in the Figures), A-1, however, grew well at 4°C (3.5 $\pm$ .5°C). At 28°C, both these isolates grew quite well at 4°C (3.5 $\pm$ .5°C). At 28°C, both these isolates grew quite well for 2 days after which growth rates declined and by the 6th day a decrease in turbidity was noted. This suggests that the added substrate-nitrogen became exhausted and the populations stopped growing. As also noted in these Figures, growth at 15°C continued after the substrate-nitrogen in the medium became exhausted and the populations stopped growing. As also noted in these figures, growth at 15°C continued after the substrate-nitrogen in the medium became exhausted. This apparent anamoly in the temperature-growth response can not be explained on the basis of this present study. The possibility that at 15°C the rate of substrate-nitrogen depletion was slow and that the exhaustion of added combined-nitrogen did not occur within 10 days was examined.

Fig. 32. Temperature-growth responses by the nitrogen-fixing isolate, A-1. 50 ml of Hino and Wilson's medium containing 15 ppm combined-N (in the form of ammonium sulfate) in 250 ml sidearm flask was inoculated with 72 hours old culture grown in the similar medium at 15°C.

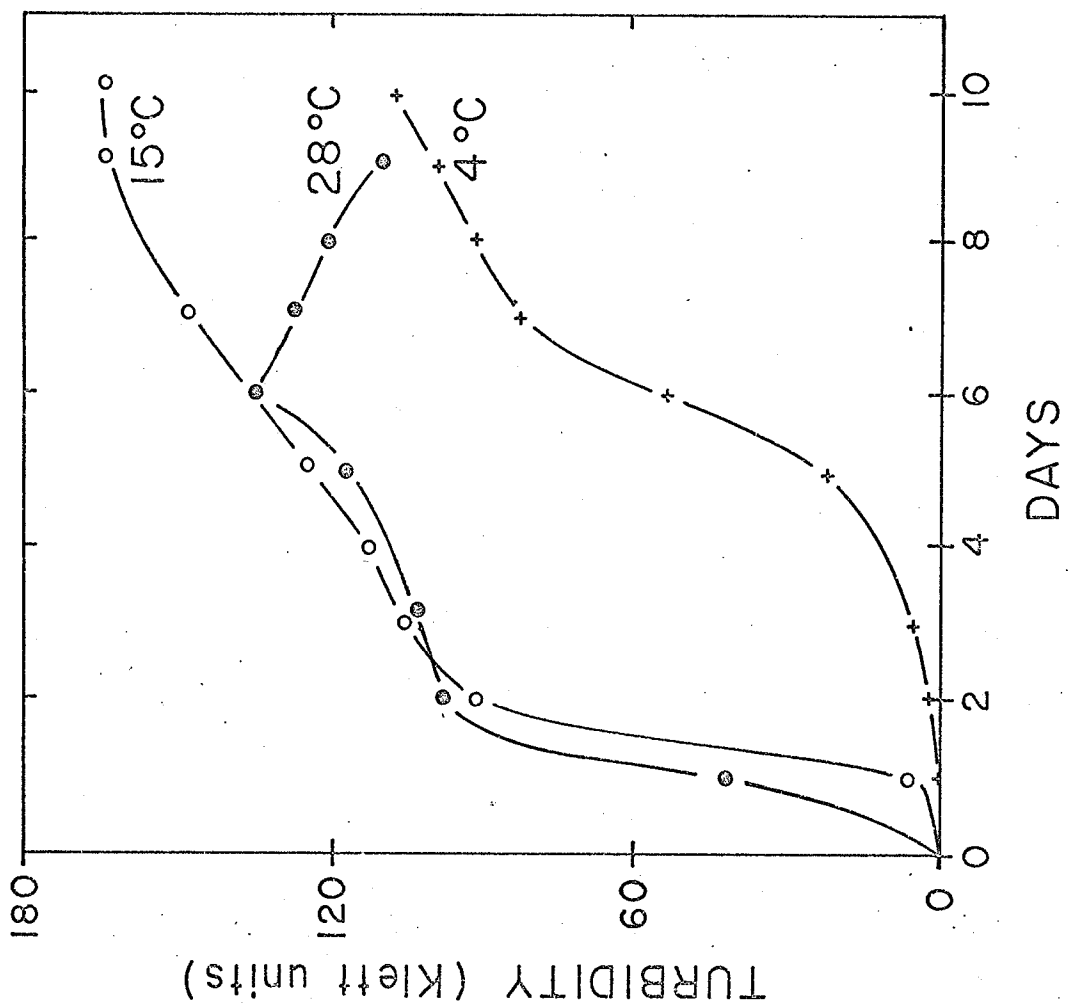

15°C
28°C
4°C
TURBIDITY (Klett units)
180
120
60
0
0
2
4
6
8
10
DAYS

Fig. 33. Temperature growth response by the nitrogen-fixing isolate G-22. Conditions as described for Fig. 32.

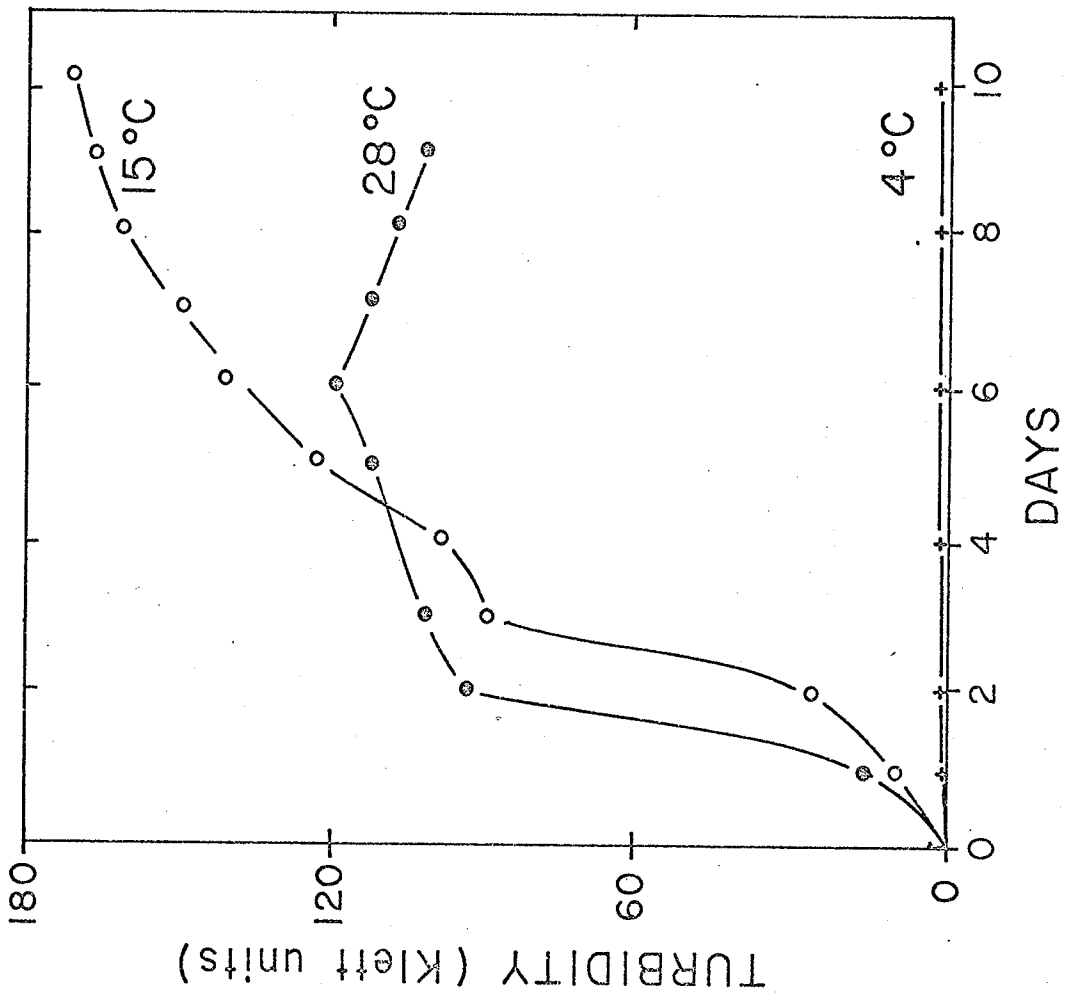
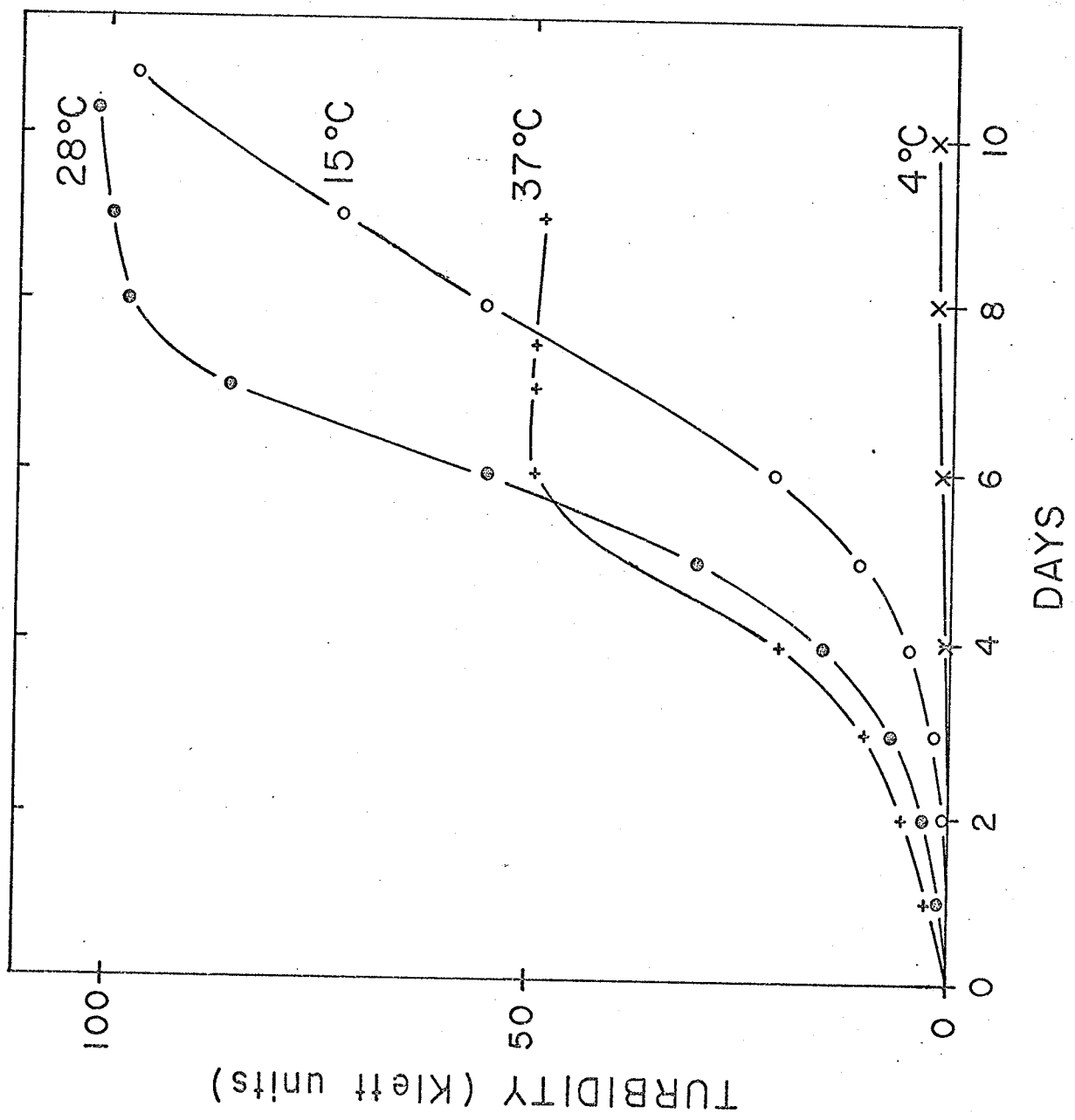

TURBIDITY (Klett units)
180
120
60
0
0
2
4
6
8
10
DAYS
15°C
28°C
4°C

Fig. 34. Temperature-growth response by the nitrogen-fixing Pullularia sp (Ao-7). Complete nitrogen-free medium of Campbell et al. Other conditions as described for Fig. 32.


28°C
15°C
37°C
4°C
TURBIDITY (Klett units)
100
50
0
0
2
4
6
8
10
DAYS

Auxiliary data (Table XXVI) on Kjeldahl analyses of these isolates gave evidence that the 15°C inoculated culture did, in fact, fix nitrogen. Further work is needed to verify these observations and to examine the mechanism which permits nitrogen fixation and growth at 15°C, but not at 28°C.

Table XXVI

Nitrogen fixation by A-1 and G-22 growing at 15° and 28°C.

Isolate	Increase in nitrogen content*/ml culture	
	15°C	28°
A-1	57.0	7.0
G-22	27.0	8.0

*Total nitrogen content/ml on 10 days harvest - initial nitrogen present/ml.

Effect of pH

Table XXVII shows the optimum pH and pH ranges for active growth of these nitrogen-fixers in liquid media. The optima ranged from 6.0 to 7.3 for the majority of the isolates studied. However, some isolates, e.g., GC-1, TL-3-1 and Rhodotorula sp. (Ao-15) showed pH optimum values close to 5.0. The interesting observation generally

Table XXVII

pH optima in N-fixation and permissible pH ranges for growth (fixation) for some isolates in a liquid N-free medium.

Isolate	Optimum pH	pH range of active growth[1]
A-11, A-13	6.0	5.0-8.0
TL-10-1	6.0	4.0-9.0
TL-3-2	6.0	4.0-8.0
Iv-X-1, Iv-Z-3	7.0	4.0-8.0
GC-1	5.0	3.5-8.0
Iv-X-3, Iv-Z-1, GC-2	7.3	6.0-9.0
TL-3-1	5.5	3.5-8.0
Rhodotorula sp.	5.0	4.0-7.5
Pullularia sp.	7.0	4.0-7.5

[1]Growth was measured by the increase in nitrogen content of growing culture (15°C incubation for 10 days).

noted was that a majority of the isolates were able to grow over a wide range of pH. For certain selected isolates the growth response (fixation) at different pH level is given in Table XXVIII. From data presented in this Table, it is obvious that the optimum pH for maximum fixation under the cultural conditions varies considerably among the isolates.

Table XXVIII

Nitrogen-fixation by Ao-15, TL-3-1 and TL-10-1 isolates at different pH

Isolate	Initial pH of the medium	Incubation (days)	µg N/ml culture medium
Ao-15 (*Pullularia* sp.)	4.0	14	50
	5.0		57
	6.0		60
	7.0		74
	7.5		70
TL-3-1	5.1	20	180
	5.6		425
	6.2		250
	7.0		120
	8.0		75
TL-10-1	5.1	20	75
	5.6		100
	6.2		115
	7.0		125
	8.0		90

Table XXIX

Nitrogen fixation activity of the isolates[1]

Isolate	Isotopic $^{13}N_2$ Experiments* (cpm/mg protein)	Total nitrogen experiments	
		Incubation (days)	Increase in total nitrogen as µg Kjeldahl-N/ml culture medium
A-1	13,000	7	40.0
A-6	7,000	7	10.0
A-11	13,000	7	21.0
A-13	12,000	7	25.0
G-11	7,500	7	22.0
G-12	3,000	21	10.0
G-15	16,000	10	17.0
G-16	n.d.	15	15.0
G-17	14,000	7	39.0
G-22	8,000	7	27.0
T-1	5,500	21	23.0
T-4	3,000	10	14.0
T-6	3,000	15	8.0
AO-6	5,600	5	13.0
AO-7	3,000	14	30.0
AO1-11	9,000	10	32.0

n.d. = not determined

[1]Cultures incubated aerobically at 15°C.

Table XXIX. Cont'd.

Isolate	Isotopic $^{13}N_2$ Experiments* (cpm/mg protein)	Total nitrogen experiments	
		Incubation (days)	Increase in total nitrogen as µg Kjeldahl-N/ml culture medium
AO-12	18,300	7	17.0
AO-15	5,700	10	21.0
Iv-X-1	6,000	10	18.0
Iv-X-2	2,000		
Iv-X-3	n.d.	10	25.0
Iv-Z-1	9,000	10	22.0
Iv-Z-2	1,000	10	12.0
Iv-Z-3	7,500	7	27.0
GC-1	12,000	10	37.0
GC-2	8,000	10	35.0
GC-3	n.d.	7	25.0
TL-10-1	3,000	10	11.0
TL-10-2	n.d.	10	12.0
TL-10-3	5,900	10	15.0
TL-3-1	6,700	20	49.0
TL-3-2	n.d.	20	33.0
TL-3-3	n.d.	20	14.0

*Specific activity determined on 30 min exposure time. Preparation of cell-suspension, exposure to $^{13}N_2$ etc. as detailed under Materials and Methods.

Nitrogen-Fixation Activity of the Isolates

Nitrogen-fixation activity of the isolates in growing culture and by resting cell suspension as assessed by micro-Kjeldahl analysis and by $^{13}N_2$ incorporation are detailed in Table XXIX. All the isolate tested showed some degree of nitrogen-fixing potential. $^{13}N_2$ incorporation studies confirm in a semi-quantitative as well as qualitative sense the nitrogen-fixing properties of the isolates.

Taxonomy and Ecological Significance of the Nitrogen-Fixing Isolates

The results obtained in this investigation concerning the morphological, cultural, physiological and nitrogen-fixation characteristics of the isolates gives evidence of a diverse group of nitrogen-fixing organisms present in these soils. To precisely identify each of the isolates on the basis of the various physiological and biochemical tests required is quite outside the scope of this investigation. To be done properly, such identification would require, in all likelihood, a period of several years.

To summarize the nature of the isolates the following descriptive Key is presented:

I. Bacteria. The nitrogen-fixing bacterial isolates are grouped into two main categories based on their cell-size and flagellation characteristics.

A. Small rods to coccoidal cells (0.5-1.3 x 1.2-2.5 μ)

(a) Motile with peritrichous flagella, A-11, A-13, Ao-12, Iv-Z-1

(b) Motile with lophotrichous flagella, G-11, Ao1-11

(c) Motile with monotrichous flagella, Iv-X-2

(d) Motile with amphitrichous flagella, T-6, TL-10-2

(e) Non-motile, G-16, T-2, TL-3-1, TL-3-3

(f) Isolates of uncertain motility and/or flagellation, A-6, T-4, TL-10-3.

B. Large rods to coccoidal cells (0.5-1.3 x 3-5 μ).

(g) Motile with peritrichous flagella, A-1, G-17, G-22, Ao-6, Iv-Z-2, Iv-Z-3, TL-10-1, TL-3-2, GC-3

(h) Motile with monotrichous flagella, GC-1

(i) Non-motile, Iv-X-3, Iv-Z-1, GC-2

II. Fungi and Yeasts

(j) Pullularia sp. (Ao-7)

(k) Rhodotorula sp. (Ao-15)

III. Actinomycetes

(l) Streptomyces sp. (G-12)

(m) Nocardia sp. (T-1)

Some bacterial isolates, having morphological cultural, environmental and physiological characteristics in common are grouped as follows:

(1) A-1, G-17, Iv-Z-2
(2) G-22, GC-3
(3) A-11, A-13
(4) T-2, TL-3-3
(5) T-6, TL-10-2
(6) Iv-Z-3, Iv-X-1
(7) T-4, TL-10-3
(8) Tv-X-3, Iv-Z-1, GC-2

According to Baillie et al (1962) the nitrogen-fixing bacterial isolates of the larger cell-sizes with peritrichic flagellation can be assigned to the Azotobacter group (A. chroococcum, A. agilis and A. vinelandii). Bacterial isolates comparatively smaller in size but showing peritrichic flagellation (A-11, A-13, Ao-12, Iv-Z-1) can be assigned to Azomonas agilis. Bacterial isolates of varying cell sizes showing polar flagellations could be assigned to Azomonas insigne and Azomonas macrocytogenes (Baillie et al 1962) or to Pseudomonas spp. (Paul and Newton, 1961; Anderson, 1955; Voets and Debacher, 1955).

Isolate TL-3-1 can be classified as a Beijerinckia sp. based on its non-motility, morphology (oval with two distict granules, one at each end), gum production in the medium and its tendency to grow at lower pH.

The presence of diverse group of nitrogen-fixing micro-organisms comprising bacteria, actinomycetes and molds in these soils and their repeated isolation and occurrence

thus suggests that the nitrogen-fixation process,in all likelihood,is occurring under the sub-arctic environment. Ability of the is-lates to grow under low temperatures and over a wide pH range combined with the enormous supplies of organic matter available (as the non-limiting energy substrate) in these soils would imply that naturally-existing conditions are favourable for activity of this kind. This assumption becomes even more reasonable when one considers the relatively low available nitrogen content prevailing generally in these soils. Thus, it would seem that nitrogen-fixation conditions are almost ideal in this environment.

It is arguable that what occurs in a test tube on a chemically defined medium is often quite different from what occurs in soil, but this work has the virtue of showing that the potential for nitrogen-fixation in the environment exists. However, no final answers as to the real contribution to the nitrogen-economy of these soils can be found until quantitative studies are carried out "in situ". The use of the acetylene reduction as an assay of nitrogenase activity may make the type of study more feasible than it hitherto has been. A strong suspicion exists in the mind of the principal investigator that a considerable measure of fixed nitrogen in these soils may be entering by way of photosynthetic nitrogen-fixation by members of the

Schizophyceae. Again, this possibility should be explored at the earliest opportunity by either the acetylene reduction assay or by the $^{15}N_2$ incorporation techniques.

The occurrence of Beijerinckia sp. in these soils suggests that the distribution of the group is more widespread that has hitherto been believed and is not confined only to the tropics as suggested by Becking (1961). Boyd and Boyd (1962) have also detected this group in some soils of Antarctica. In the present investigation, however, it has been confirmed that the Beijerinckia sp. is capable of growing and fixing nitrogen under low temperature conditions.

SUMMARY

Although the soils examined represented quite diverse soil-types, the presence of considerable number of nitrogen-fixing organisms, - both aerobic and anaerobic - was encountered in each sample examined. In terms of quantitative determinations of total nitrogen-fixing organisms (aerobic and anaerobic), plate counts ranged from several tens of thousands per gram to as high as several millions per gram of soils. Clostrial types were found to be restricted to Inuvik soils only. Among the aerobic bacteria isolated, were members of the genera Azotobacter, Beijerinckia, Chromobacter and the Azomonas-Pseudomonas group in addition to others not identified; as well, fungi as represented by Pullularia, yeast as represented by Rhodotorula and actinomycetes (Nocardia and Streptomyces species) were also encountered. The majority of the isolates were shown to be either cold-adapted or psychrophiles.

The wide diversity of types of nitrogen-fixing organisms encountered further supports the evidence by other recent workers that the potential for nitrogen-fixation among micro-organisms is more wide spread than was originally believed.

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*Not read in the original.