ALBERTA OIL SANDS ENVIRONMENTAL RESEARCH PROGRAM

A Preliminary Study of Chemical and Microbial Characteristics of the Athabasca River in the Athabasca Oil Sands Area of Northeastern Alberta

WS 2.3

March 1979

Sponsored jointly by

Alberta ENVIRONMENT

Environment Canada

Environnement Canada

15th Floor, Oxbridge Place
9820 - 106 Street
Edmonton, Alberta, Canada
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ALBERTA OIL SANDS ENVIRONMENTAL RESEARCH PROGRAM

RESEARCH REPORTS

These research reports describe the results of investigations funded under the Alberta Oil Sands Environmental Research Program, which was established by agreement between the Governments of Alberta and Canada in February 1975 (amended September 1977). This 10-year program is designed to direct and co-ordinate research projects concerned with the environmental effects of development of the Athabasca Oil Sands in Alberta.

A list of research reports published to date is included at the end of this report.

Enquiries pertaining to the Canada-Alberta Agreement or other reports in the series should be directed to:

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A Preliminary Study of Chemical and Microbial Characteristics in the Athabasca River in the Athabasca Oil Sands Area of Northeastern Alberta

Project WS 2.3

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The Hon. J. W. (Jack) Cookson
Minister of the Environment
222 Legislative Building
Edmonton, Alberta

and

The Hon. John Fraser
Minister of the Environment
Environment Canada
Ottawa, Ontario

Sirs:

Enclosed is the report "A Preliminary Study of Chemical and Microbial Characteristics of the Athabasca River in the Athabasca Oil Sands Area of Northeastern Alberta".

This report was prepared for the Alberta Oil Sands Environmental Research Program, through its Hydrology Technical Research Committee (now the Water System), under the Canada-Alberta Agreement of February 1975 (amended September 1977).

Respectfully,

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A PRELIMINARY STUDY OF CHEMICAL AND MICROBIAL CHARACTERISTICS OF THE ATHABASCA RIVER IN THE ATHABASCA OIL SANDS AREA OF NORTHEASTERN ALBERTA

DESCRIPTIVE SUMMARY

BACKGROUND

The Athabasca River is central to the surface water system in the Alberta Oil Sands Environmental Research Program study area and thus the maintenance of its quality is of paramount importance. A thrust of AOSERP sponsored research is aimed at developing a biological model of the Athabasca River to enable the prediction of its response to oil sands development impacts. One component of this model is the microbiological activity of the river and the capacity of the river to assimilate organic compounds and the present project proposed to address this component.

Objectives for this project were:

1. To produce a thorough and definitive review of the literature on the assimilative capacity of river systems due to microbial degradation of organic compounds and on the methods for the measurements of nutrient uptake by microbial populations.
2. To determine total numbers and define physiological groups of microbes upstream from the Athabasca Oil Sands deposit and to assess the effects of passage through this formation on these parameters on a seasonal basis.
3. To determine the rates of nutrient uptake by physiological groups of microbes in the Athabasca River system on a seasonal basis.

Subsequent to completion of this research it was anticipated that a biological model concerning nutrient fluxes could be used to anticipate the fate of organic

compounds arising from natural and man-induced loadings into the Athabasca River.

ASSESSMENT

Field work for this project was initiated in the fall of 1977. After fall and winter field surveys were completed, further field work was suspended by AOSERP to insure compatability of the project design with the design of other projects looking at chemical and physical processes in the Athabasca River. Thus, some of the seasonal aspects of the project objectives could not be fulfilled by the researchers and the development of techniques of organic analysis was cut short. This report has been reviewed by scientists at the University of Alberta and the University of Calgary and AOSERP is satisfied that the authors have responded to the reviewers' suggestions. However, the conclusions of the report do not necessarily reflect the views of Alberta Environment or Fisheries and Environment Canada and the mention of trade names for commercial products does not constitute an endorsement or recommendation for use. The Alberta Oil Sands Environmental Research Program is pleased with the efforts put forth by the researchers in this project and accepts this report "A Preliminary Study of Chemical and Microbial Characteristics of the Athabasca River in the Athabasca Oil Sands Area of Northeastern Alberta" as an important and valid document suitable for wide distribution.

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A PRELIMINARY STUDY OF CHEMICAL AND MICROBIAL CHARACTERISTICS OF THE ATHABASCA RIVER IN THE ATHABASCA OIL SANDS AREA OF NORTHEASTERN ALBERTA

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Project WS 2.3

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ABSTRACT

A literature review and evaluation of methods used to determine rates of bacterial uptake (heterotrophic assimilation) of organic substrates in fresh water systems was undertaken. The uptake of a "universal" substrate - radiolabelled glutamic acid - was determined in order to assess the effects of organic compounds from both natural (oil sands) and industrial (oil extraction and refining) sources on the rate of heterotrophic assimilation in the Athabasca River. Only partial results are available due to an unanticipated termination of this project for reasons beyond our control.

A mobile field laboratory was used during fall (1977) and winter (1978) surveys to determine ^{14}C-glutamic acid uptake rates. The total planktonic heterotrophic potential (V_{max}) was similar at various river sites and showed no increase downstream from the current mining area. V_{max} values ranged from 0.16 to 0.86 µg/l/h for six river sites. This compares to a V_{max} of 3.9 to 4.6 µg/l/h for refinery process wastewater. During the winter survey, a preliminary enumeration of specific physiological groups such as sulphur oxidizers, sulphide producers, hydrocarbon oxidizers (^{14}C-hexadecane), and organic acid oxidizers (benzoic acid) indicated that these populations may increase 10-fold or more at a site just below the area of mining activity.

In order to determine the actual uptake rate (v) for glutamic acid, extensive efforts were devoted to developing a suitable methodology for the determination of natural levels of this substrate. Levels of 0.5 to 3 µg/l of glutamic acid were determined and thus an actual uptake of from 0.02 to 0.05 µg/l/h was calculated for Athabasca River water samples during the winter survey. In addition, a description of extraction techniques and gas chromatography methods for the analysis of other organic compounds in both water and sediment samples is provided. The premature termination of this project precluded final identification or quantification of these compounds.

On the basis of this preliminary investigation, it was concluded that oil sands mining wastes have no significant stimulatory or toxic effect on the uptake of glutamic acid by the planktonic bacterial populations of the Athabasca River.

ACKNOWLEDGEMENTS

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In addition, sincere thanks to Bob Salahub and Aram Yousif (Chemical & Geological Laboratories Ltd.) for their technical support, as well as Carol Henderson for her work in preparing this report.

And finally, thanks to F.S. Liu (University of Alberta) for her chemical analysis.

This research project WS 2.3 was funded by the Alberta Oil Sands Environmental Research Program, a joint Alberta-Canada research program established to fund, direct, and co-ordinate environmental research in the Athabasca Oil Sands Area of northeastern Alberta.

1. GENERAL INTRODUCTION

The uptake of organic compounds by fresh water microorganisms has not been extensively studied. Yet the the importance of this phenomena of "heterotrophic assimilation" specifically as it relates to the ecology of the Athabasca River and both natural and (perhaps) industrial (oil sands mining) inputs of organic compounds cannot be dismissed.

Because of the relative lack of attention given by aquatic biologists to this subject area, a significant portion of this paper (Section 2) has been devoted to the review of techniques used to measure the bacterially-mediated uptake of organic substrates in fresh water systems. The technique of measuring the uptake of radiolabelled organic compounds has been selected for use in this study and has thus been explained in some detail in our review.

The original intention of this study was to utilize ^{14}C-glutamic acid as a "universal substrate" to compare heterotrophic uptake rates at various sites; then to proceed with uptake studies of specific organic molecules either found naturally in the river or associated with wastewater effluents. However, the premature termination of our contract, for reasons beyond our control, allowed only the former objective to be partially met during two surveys carried out in October 1977 and January 1978. It is hoped that this work can provide a basis for future studies.

At the onset of this study, it became apparent that a significant effort was required to establish reliable methodologies for the analysis of organic compounds in both sediment and water - especially with respect to glutamic acid. A special report concerning the extraction and analysis of glutamic acid is contained in Section 7.2. We have also attempted to provide detailed descriptions throughout this report of the methodologies used for quantification of other organic compounds so that although incomplete, they can provide a useful reference for similar studies.

The sites for this study were located in order to provide information about the effects of both natural and industrial inputs of organic compounds into the Athabasca River throughout the oil sands area. In order to assess the effects of natural

inputs, a site (1A) was chosen upstream from the oil sands area at the town of Athabasca, approximately 334 kilometres upstream from Great Canadian Oil Sands Ltd. (G.C.O.S.). The other sites were located within the Alberta Oil Sands Environmental Research Program (AOSERP) study area (Figure 1) at points already established by a previous microbiological study (Costerton and Geesey in review), and to avoid any confusion we have used the same site numbers. These sites are listed in Table 1.

Thus sites 1A, 2, and 4 are located upstream from the area of effluent discharge from G.C.O.S. Ltd. The two principal wastewaters are from the tailing pond (dike drainage water from the oil extraction process) and process waters (upgrading pond wastewater from the oil refining process). Sites 5A, 7, and 10 are located downstream from the G.C.O.S. plant site.

The objectives of this study were as follows:

1. To monitor and compare the potential uptake of a "universal" substrate (glutamic acid) by aquatic bacterial populations at six river sites and from two different wastewaters (dike drainage and the upgrading pond effluent) in order to assess any effects - toxic or stimulatory - of compounds released into the Athabasca River from natural or industrial sources in the oil sands area.
2. To measure the level of glutamic acid at these sites so that an actual measurement of uptake of this compound can be determined.
3. To monitor total bacterial populations and certain physiological groups (sulphur-oxidizers, sulphide producers, hydrocarbon oxidizers, and organic acid oxidizers) in river water, river sediments, and wastewaters.
4. To provide some information regarding chemical compounds in both the river water and sediment so that further studies can determine the most suitable substrates for use in bacterial uptake experiments.

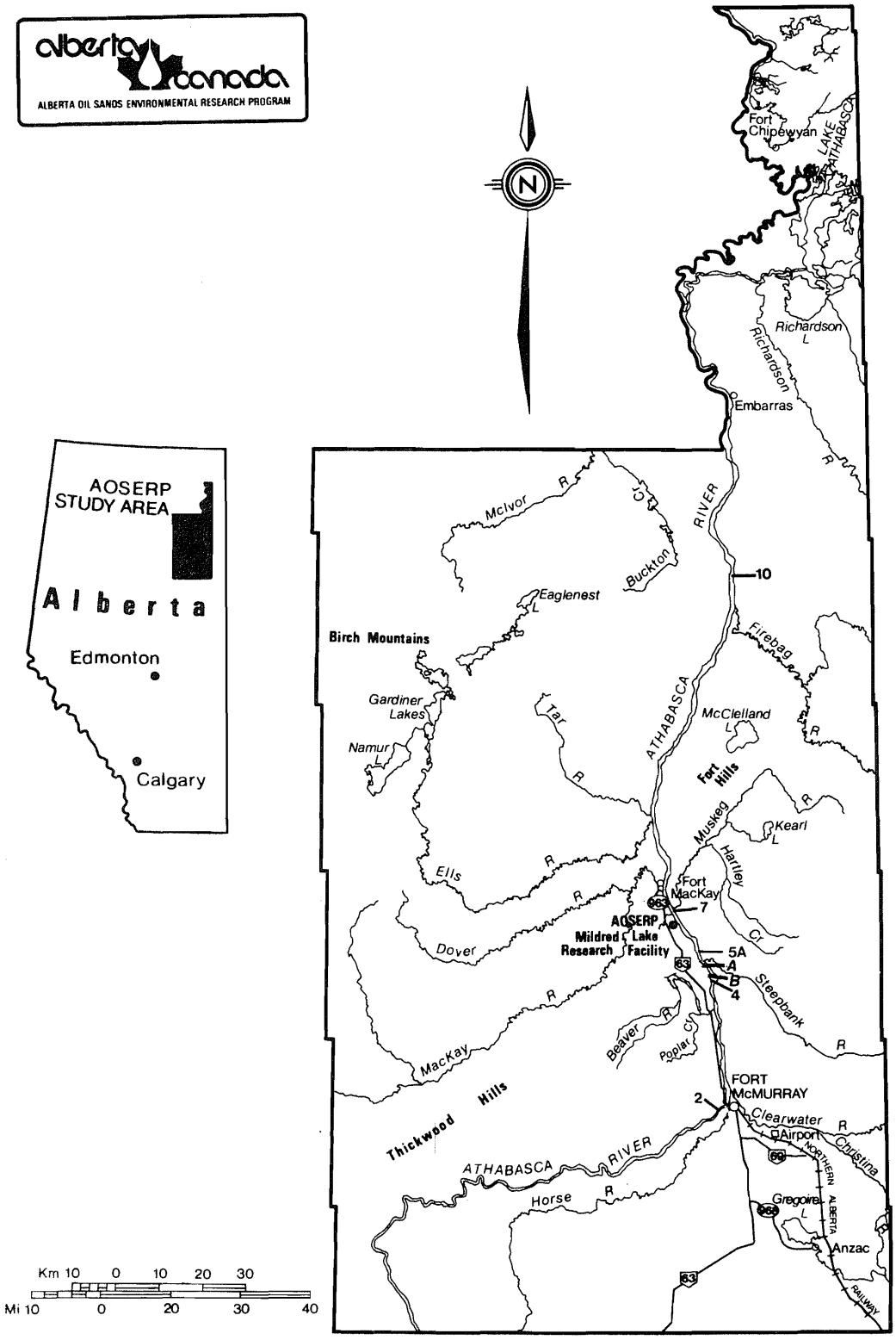

Figure 1. Sampling sites within the AOSERP study area.

Table 1. Brief description of sample sites.

Sample Location		Approximate Distance from GCOS in kilometres[a]
1A.	Upstream from the town of Athabasca	+ 334
2.	Athabasca River 1 km upstream from Fort McMurray	+ 37
4.	Athabasca River at upstream of Tar Island 5 km above GCOS	+ 5
5A.	At AOSERP dock 3 km downstream from GCOS	- 3
7.	Athabasca River 300 m upstream from Muskeg River	- 18
10.	Athabasca River at township 102	- 122
A.	GCOS upgrading pond wastewater	<1.0
B.	GCOS dike drainage water from tailings pond	<1.0

[a]Symbols: + = upstream
- + downstream

5. To determine if measurements of bacterial biomass, such as direct counting using epifluorescence microscopy, or assays for LPS (lipopolysaccharide), can be used to estimate heterotrophic activity and thus the ability of planktonic bacteria to assimilate waste organic compounds.

2. A LITERATURE REVIEW OF HETEROTROPHIC ASSIMILATION IN FRESH WATER SYSTEMS

2.1 INTRODUCTION

The study of freshwater bacteria has characteristically suffered from a lack of suitable methods that would allow valid interpretations of events that occur in situ. This deficiency has been caused in part by a long tradition of laboratory based research. In addition, there are severe difficulties in studying chemical transformations of very dilute mixtures (natural waters) by unseen organisms that react swiftly to sampling-induced changes.

The characteristics of aquatic bacteria in natural systems undoubtedly differs dramatically from those studied in the laboratory. Over forty years ago, Henrici (1932) noted that generally aquatic bacteria were attached to surfaces, not floating free in "test tubes" of nutrient broth. Other workers (as summarized by Schmidt 1973) have stressed the importance of this type of contradiction in evaluating the usefulness of, for example, plate counts in determining bacteria populations. In the environment many species of bacteria react with a wide variety of substrates, while laboratory studies have usually dealt with a very few substrates in pure cultures. The net result is that very little information (Hobbie 1971; Hynes 1970) is available to describe the uptake of organic substrates by aquatic bacteria.

In fresh water systems, this biological uptake or assimilation of organic compounds is mediated largely by bacterial cells (Sepers 1977), and the process is called "heterotrophic" assimilation.

The term "primary productivity" has historically referred to the unique and exclusive photosynthetic process of transforming energy from the sun into plant tissue. However, in aquatic systems, bacterial heterotrophic production is also a unique and virtually exclusive method of enabling dissolved organic compounds to enter the food chain. Thus it too may be described as a primary productive process (Sorokin 1965). And, in at least some fresh water systems (e.g., woodland streams which receive a great deal of organic compounds), heterotrophic production may be of greater consequence to the total aquatic community than is photosynthetic production (Anderson and Dokulil 1977; Sorokin 1965).

Thus in environmental studies, the uptake of organic molecules by bacteria is of great significance. In a more specific context, this process must also be understood in order to determine the biodegradation and assimilation of specific molecules that enter water systems due to industrial activity.

2.2 METHODS FOR THE MEASUREMENT OF BACTERIAL ACTIVITY

Bacterial cells are highly sensitive to environmental changes and can respond to these changes within a very short time interval. It is likely therefore, that most existing methods of measuring bacterial activity cannot be "extrapolataed back to the natural undisturbed situation" (Hobbie 1973).

An excellent review of existing techniques has been published by Sorokin and Kadota (1972) as part of the International Biological Program. The authors stress that the study of heterotrophic assimilation is of great importance for two reasons: "microorganisms are capable of attacking substrates that cannot be utilized by animals, and they produce particulate food material from dissolved organic materials and therefore represent an important link in the natural food chain".

In the laboratory, the measurement of oxygen consumption has been a useful tool to determine metabolic activity. In the field, the procedure utilizes "dark bottles" suspended in the water or incubated at in situ temperatures. Bacterial uptake of oxygen was distinguished from other organisms by size separation (filtration) or by the use of antibiotics in control bottles. However, bacterial cells cannot be completely separated by filtration and since antibiotics do not inhibit all bacterial activity (Hall and Hyatt 1974), either method has severe deficiencies.

In addition, the long incubation periods (usually 24-48 hours) necessitated by low substrate levels create the possibility of significant bacterial proliferation and adaptation to their new environment. If substrate is added, Hobbie (1973) estimates that uptake rates begin to increase after only one hour of incubation.

Stevenson (1959) modified a technique of measuring enzyme activity (Lenhard 1956, cited by Stevenson 1959) and produced a utilizable assay of

dehydrogenase activity "as a method of measuring total (anaerobic and aerobic) microbial activity in soil". This method depends on the reduction of tetrazolium salts by dehydrogenase enzymes to a coloured product which can be measured photometrically. Thus a measurement of the activity of the electron transport cycle is possible. This technique has been used with some success in soils (Ross 1970; Casida et al. 1964) and more latterly, in marine and fresh water sediment (Lenhard et al. 1962; Weiser and Zech 1976).

However, since only incomplete information is available on the operation of the electron transport pathway, it may be hazardous to extrapolate from the potential activity measured by the dehydrogenase assay to activity in situ. The technique essentially "uncouples" the process of energy generation from energy accumulation and this must drastically alter the cells internal "environment". Also, the activity of non-bacterial dehydrogenases is measured and the results may not reflect activity due solely to heterotrophic mechanisms.

Assays for specific enzyme activity are available for a few substrates only, e.g., glucose (Cavari and Phelps 1977). In most cases, however, suitable assays have not been developed for the wide variety of substrates found in the environment.

Anaerobic processes, such as these occurring largely in sediments, can also be estimated by measurement of the amount of gas evolved; however, this cannot be quantitative since the reduction of organic compounds may not be fully completed to gaseous products. The long periods of incubation necessary (Sorokin and Kadota 1972) also limits use of this technique in analyzing specific in situ rates of uptake.

Zeikus and Brock (1971) have used a technique of comparing bacterial growth on glass slides which act as a kind of artificial substrate. This was effective in comparing the growth rates among different locations, but cannot be used to measure in situ rates of the total bacterial community. A related technique is the use of dialysis tubing (Baskett and Lulves 1974) which allows at least some nutrients to pass into an inoculated chamber where growth can be studied. Either method creates severe selective pressures (e.g., favoring adherent growth) on both the

types and numbers of bacteria; and since from 1 to 4 days are required for changes in growth to be observed, large alterations in population are possible. Thus these experimental systems cannot reasonably be used to estimate in situ growth rates or assimilation rates.

Because of the lack of precise methods to measure nutrient uptake, past workers have often relied upon measurements of cell numbers (e.g., plate counts) or certain cell constituents (e.g., ATP) to at least estimate the activity of heterotrophic bacteria. It is not within the scope of this work to discuss these methodologies in detail; however, even as a precise measure of bacterial numbers, many of them have serious deficiencies.

Plate counts have been critically evaluated by many workers (King and White 1976; Francisco et al. 1973) and generally conceded to be unsatisfactory. The fact that many cells occur in clumps and only an unknown (but usually small) percentage grow on any one nutrient agar medium, renders this technique virtually useless.

The measurement of ATP (cellular energy supply) provided much stimulus for research. However Qureshi and Patel (1976) have recently reviewed this technique and its many remaining difficulties. Some notable problems are that ATP levels vary during different stages of growth and under different environmental conditions. Organisms other than bacteria may contribute to ATP levels and thus these values would no longer relate solely to heterotrophic activity.

Recently, more promising methods of calculating total bacterial biomass or numbers have been developed. There are presently three important and potentially useful techniques: direct cell counts using epifluorescence microscopy; an assay for muramic acid which is found in the cell walls of bacteria and blue-green algae (King and White 1976); and an assay for LPS (lipopolysaccharide) which is a constitute of all gram-negative bacterial cell walls (Watson et al. 1976). The latter two methods are very recent developments and thus a critical evaluation of their usefulness, in both theoretical and practical terms, cannot be made.

Fluorescent counting has a much longer history however, and recent developments (epifluorescent microscopes, nucleopore filters, etc.) have made it a much improved technology (Francisco et al. 1973; Jones 1974; Daley and Hobbie 1975). Because of the use of epifluorescent counting in the present study, a section of the annotated bibliography (Section 7.8) has been included which deals primarily with this technique.

Besides physical (gas evolution, oxygen consumption) and biological (enzyme activity,growth) measurements of heterotrophic activity might be measured by monitoring changes in the various organic substrates. This has frequently been done in laboratory research and also in environmental work but, in the latter case, only as an estimate of "biodegradation potential" (Walker and Colwell 1975a; Westlake et al. 1974). In either case, substrate levels are very much higher than normally found in natural waters and existing chemical techniques have been adequate.

More sensitive chemical techniques would enable the determination of specific uptake rates for individual compounds whereas most biological techniques assess only total levels of activity. The application of sophisticated techniques such as computerized mass spectrometry may allow changes in the concentration (at natural levels) of various molecules to be monitored over sufficiently short incubation periods so that specific or total uptake rates could be measured.

The natural concentrations of many common substrates, such as amino acids, glucose, etc., have been reported in the range between 1 to 20 ng/l (Hobbie 1973; Wright and Hobbie 1966; Crawford et al.1974) for many waters. The present study has shown that it is possible to measure glutamic acid in the nanograms per litre and thus in the future it may be possible to monitor the decreasing concentrations of this or other substrates from freshly incubated water samples.

The disappearance of a molecule does not, however, necessarily imply its assimilation by bacteria. The phenomena of co-oxidation whereby a substrate is oxidized but not used as a source of carbon or energy occurs widely in microbial metabolism (Horvath 1972). Also, many molecules are more complex than

simple carbohydrates or amino acids and may be only partially utilized by bacteria.

At present, the necessary increase in sensitivity of chemical techniques is obtained by employing radiolabelled substrates. The use of ^{14}C-labelled bicarbonate to measure algal productivity provided the the necessary expertise to allow later use of ^{14}C-labelled organic compounds in the study of bacterial assimilation.

Wright (1971) has provided an excellent summary of the difficulties encountered utilizing this technique and more recently (Wright 1978) has provided an update and critical evaluation of various theoretical aspects. These two papers have provided the basis for our study of bacterial assimilation in the Athabasca River. Other relevant papers are described in the annotated bibliography (Section 7.8).

Wright (1978) points out that "no method measures bacterial production and mineralization in total". Much of the work done to-date has utilized relatively simple ^{14}C-labelled organic compounds such as, glucose (Harrison et al. 1970), amino acids (Robinson and Hendzel 1973), and acetate (Hall et al. 1972). However, in a recent (1977) American Society of Testing Materials (A.S.T.M.) symposium, Dr. Wright indicated that glutamate may be a preferred substrate since it can be utilized as a source of energy (respiration), carbon (assimilation), and nitrogen, by many bacterial species. Burnison and Morita (1973) reported that in tests utilizing sixteen amino acids, glutamic acid had one of the highest values for rate of uptake and also was respired to a greater extent than most others.

By adding different quantities of substrate, one is able to measure the response of the transport enzymes (uptake) of the bacterial cells by measuring after a suitable interval the amount of radioactivity in the respired carbon dioxide and cellular material. This application of radiolabelled carbon uptake studies has been described as the "Transport Kinetic" approach and was first utilized by Parsons and Strickland (1962). It utilizes the traditional Michaelis-Menton equation relating bacterial growth to substrate concentration:

$$\frac{S}{v} = \frac{1}{V_{max}} S + \frac{K_m}{V_{max}}$$

where K_m is the Michaelis constant, S is the substrate concentration, v is the velocity of uptake at S, and V_{max} is the theoretical maximum velocity of uptake by the existing bacterial population when S is at saturation levels (of the transport enzymes).

With respect to radiolabelled substrates, Hobbie (1973) has shown that this relationship can be described as:

$$\frac{Cut}{c} = \frac{K + S}{V_{max}} + \frac{A}{V_{max}}$$

where c is the counts per minute (cpm) of radioactive carbon taken up, Cu is the total cpm added, t is the incubation time, S is the total substrate, while A is the added substrate. This equation is particularly useful since the values for Cut/c when plotted against A show a straight line relationship providing, of course, that Michaelis-Menton kinetics do apply. The The slope of the plotted line is $1/V_{max}$ and the Y intercept is t_n, the turnover time or the theoretical time required for the natural concentration of substrate (S_n) to be fully utilized. A model graph is shown in Figure 2 to demonstrate this relationship.

V_{max} and t_n thus become important parameters which describe the heterotrophic potential of a given sample of water. Hobbie (1971) has described how well these parameters relate to the trophic status of various Swedish lakes, ranging by four orders of magnitude when applied to waters taken from polluted ponds and from oligotrophic lakes.

In some cases, especially in oligotropic ocean samples (Vaccaro 1969) uptake responses have not followed Michaelis-Menton kinetics and interpretation of the data has not been possible. This may be due to low bacterial populations or to the necessity of existing bacterial cells to adapt their enzymes to an unfamiliar substrate. At the other extreme, poor data (in terms of transport kinetics) found when high substrate levels exist, has often been related to diffusion kinetics caused by the increasing importance of algal uptake as substrate levels become high (Wright and Hobbie 1966).

The uptake of labelled substrates in sediments has posed more difficult problems due to its more heterogeneous nature and the difficulties in

dealing with an undisturbed sample. Harrison et al. (1970) and many others have shown that heterotrophic activity is often very high in sediments, and Hall and Hyatt (1974) have provided a review of existing techniques. If only mineralization (respired carbon dioxide) is measured, then relatively undisturbed samples can be incubated and the carbon dioxide collected; however, if assimilated carbon is measured, then the sediment must be diluted so that filtered samples (bacterial cells) can be obtained (Boylen and and Brock 1973).

Wright (1978) also points out that the data are analyzed "as if it (the bacterial population) were functionally uniform in its response to substrate concentration". He notes that there is no clear explanation as to why the uptake of mixed populations follows Michaelis-Menton kinetics (which were derived from studies of pure cultures). It is possible that many species have a similar affinity for the relatively simple organic compounds used to-date, or that a few dominant species are responsible for much of the uptake. Clearly, too little is known about the activity of natural populations to resolve this question.

The great advantage of the technique is that, for the first time, the fate of specific organic molecules can be traced at natural (low) levels in aquatic systems. By using "universal" substrates like glutamic acid, a comparison of the heterotrophic activity of many diverse environments can be made although what fraction this represents of the total uptake is is not yet known.

The work with common substrates like amino acids should develop our ability to investigate in a similar fashion more complex molecules that may be introduced into aquatic systems by natural or industrial activities. The work by Geesey et al. (1976) to adapt this technique using a mobile field laboratory has further increased its usefulness as a valid description of natural rates of nutrient uptake.

^{14}C-hydrocarbons have also been utilized to measure the "biodegradation potential" of various compounds (Walker and Colwell 1975b). In this method, relatively large quantities of labelled hydrocarbons are incubated for a period of days before a determination of the amount assimilated or respired is made.

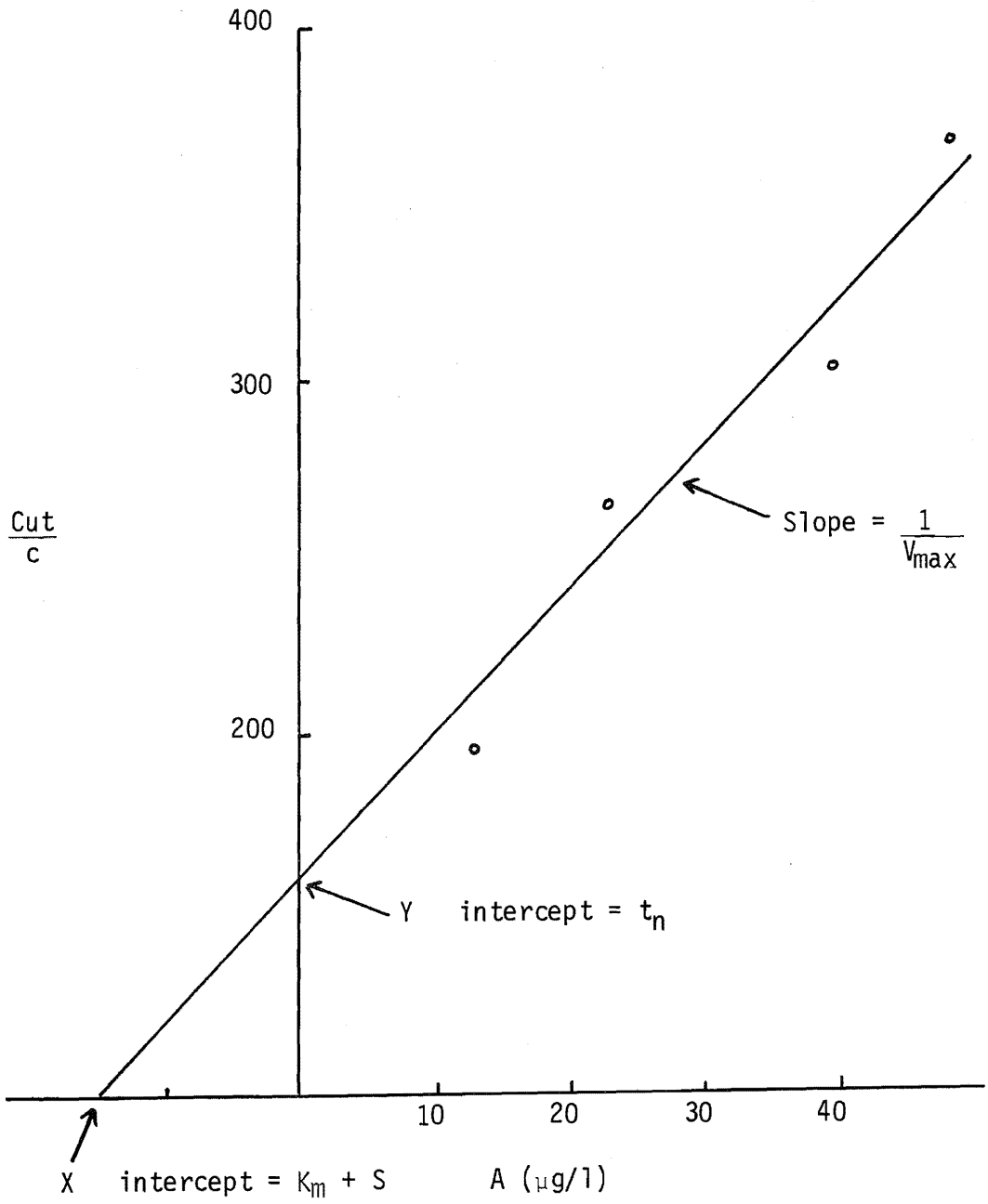

Figure 2. A theoretical plot of the added substrate concentration (A) versus the uptake into bacterial cells (Cut/c).

In areas where oil pollution is a recurring process and thus a population of hydrocarbon oxidizing bacteria is part of the "normal" environment, shorter incubation times are possible and a V_{max} can be calculated.

Specific uptake rates for individual cells, or the percentage of cells in a population that can assimilate a certain substrate, may be determined by a fairly recent adaptation of radiolabelled uptake studies called "autoradiography". In this technique, the uptake of tritiated compounds can be measured by analyzing the amount of exposed silver grains on a photographic plate.

This method has been used by Stanley and Staley (1977) to measure the uptake of tritiated acetate by cells in a pulp mill waste lagoon. The results indicated that different species were active at very different rates. They note that "a comparison of several different cell types indicate a minimum uptake activity range of 20-fold".

While this technique has not yet been as well developed as the "total community" uptake methods, it undoubtedly has an important place in future studies. Ramsay (1973) for example, has used this technique to determine the portion of metabolizing bacteria in leaves and it seems possible that in future work, methods could be devised to quantitatively study uptake by sediment bacteria using autoradiography.

Other techniques utilizing radiolabelled isotopes include the work of King et al. (1976) in studying the rate of uptake of ^{14}C and ^{32}P into various microbial lipids as a measure of bacterial activity in decomposing leaves. In contrast, microcalorimetry (Forest 1972) has been used in laboratory cultures as well as natural systems such as rumen fluids, sewage, and soil; but has yet to be applied successfully to less active systems such as natural waters. At present, however, most of the knowledge about heterotrophic activity has been derived using the ^{14}C-carbon uptake techniques initiated by Parsons and Strickland (1962).

2.3 CURRENT INFORMATION ON THE ASSIMILATIVE CAPACITY OF FRESH WATER SYSTEMS

Because of the inadequacies of many traditional methods little detailed information is available concerning nutrient uptake experiments that realistically reflect in situ heterotrophic activity. The data that does exist has been developed in the last 15 years and has been produced by the utilization of the techniques of ^{14}C-carbon uptake. Much of the work has been done on lake and marine samples, but very little on rivers. Thus while our objective is to provide a background of knowledge regarding river systems, a great deal of information has been, of necessity, taken from work dealing with lakes.

As discussed previously, this uptake is primarily bacterial. It has been known for sometime that the uptake of certain organic substrates by algae can occur; however Wright and Hobbie (1966) have shown that this is always less than 10% of bacterial uptake of the low substrate concentrations normally found in natural waters.

Much work has been done by Wright and Hobbie (1966) in Lake Erken, Sweden; which has a climate not too dissimilar from central Alberta. It is a "naturally eutrophic" lake with a dissolved organic content of approximately 50 mg/l which also is similar to Athabasca River values of between 10 to 30 mg/l (Seidner in prep). Table 2 summarizes values obtained for the uptake of glucose and acetate; natural substrate concentrations (S_n) were in the range of 1 to 20 µg/l.

A more recent study by Geesey et al. (1976) of an oligotrophic alpine stream in Alberta, has detected V_{max} for planktonic bacteria of approximately .003 µg/l/h in October (using glutamic acid and arginine), a 100-fold reduction compared with eutrophic Lake Erken. The problem of comparing different substrates is a very difficult one and it is to be hoped that the increasing use of a standard substrate such as glutamic acid will enable more valid comparisons in the future.

In West Blue Lake, Manitoba, Robinson and Hendzel (1973) found that the V_{max} for planktonic samples was in the range of .003 to .046 µg/l/h for nine organic acid substrates. A comparison with plate counts specific for each substrate showed a correlation (r) of .98 with the respective V_{max} values

Table 2. Values for V_{max} (µg/l/h) and tn (h^{-1}) for planktonic bacteria in Lake Erken, Sweden.[a]

Substrate	Oct.		Nov.		Dec.		Jan.	
	V_{max}	t_n	V_{max}	t_n	V_{max}	t_n	V_{max}	t_n
^{14}C-glucose	.18	17	.045	70	.026	100	.023	70
^{14}C-acetate	.19	37	.041	200	.054	270	.019	430

[a]Summarized from Wright and Hobbie (1966)

indicating a good relationship between V_{max} and bacterial numbers. However, with respect to total bacterial numbers as measured by direct counts, Wright (unpubl.) has evidence showing that V_{max} varies much more than do cell counts. Even for specific populations Wright (1978) claims that the findings of Robinson and Hendzel (1973) are the exception rather than the rule; and thus that, "V_{max} is probably a legitimate indicator of in situ heterotrophic activity towards a given substrate but cannot be used to indicate biomass".

In addition to measuring heterotrophic activity, this technique has been used by Ward and Brock (1975) to measure the biodegradation potential of hydrocarbons in Lake Mendota, Wisconsin. They were able to conclude that the potential to degrade hydrocarbons was evident but was limited by "seasonal and regional variations in temperature and nutrient content" and that "despite the constant presence of oil-degrading bacteria, such lakes do not provide optimal conditions for hydrocarbon metabolism". However, in a study of a gasoline spill in an Arctic freshwater lake, Horowitz and Atlas (1977) used ^{14}C-hexadecane to show that the "indigenious micro-organisms could extensively convert hydrocarbons to carbon dioxide, but also noted "that the use of fertilizers (nutrients) would be beneficial".

In the Red Deer River, Alberta, Baker et al. (1977) found a V_{max} for glutamic acid of 0.61 µg/l/h during the summer. However, values for sediment bacterial were about 108 µg/l/h. This dramatic increase is reflected to an even greater extent by total counts, 5.6×10^4 cells/ml in the planktonic sample and 1.4×10^8 cells/ml in the sediment. The values for the sediment V_{max} in this study were stated to be "preliminary" since much work is needed in adopting suitable techniques for incubating and analyzing sediment samples. It does, however, emphasize the tremendous activity of sediment bacteria.

In rivers of the Fraser Valley, B.C., Albright and Wentworth (1973, cited by Sepers 1977) found planktonic V_{max} values in the range of .004 to .7 µg/l/h using glucose. This wide range in values is not unusual. In Sweden, Hobbie (1971) has found that V_{max} can differ by four orders of magnitude and

this suggests that it can be a sensitive indicator of heterotrophic activity.

Boling et al. (1975) state that evidence "overwhelmingly supports the generalization that running-water habitats in the temperate zone are dependent on their major energy supply upon reduced carbon compounds". The peak flow of the Athabasca River, through the study area is approximately 100,000 cfs (Loeppky and Spitzer 1977) or 2.7×10^6 l/s. If uptake rates (V_{max}) for glutamic acid in Red Deer River are comparable (.6 µg/l/h), then this would translate into an assimilation rate of 1.6 g/h for the amount of water flowing by a specific point each second.

This theoretical value completely omits the significant capabilities of sediment bacteria to assimilate organic compounds. However, a valid interpretation of this value is very much complicated by the physical and chemical environment and the V_{max} cannot therefore be used as an absolute value. Again, most work has been done with lake samples.

In Marion Lake, B.C., Hall et al. (1972) found V_{max} values for sediments ranging between 4 and 17 µg/g/h (acetate). He also compared winter and summer values for three substrates (acetate, glycine, and glucose) and these data are reported in Table 3. In the context of summer-winter variations, Boylen et al. (1973) showed that values for lake sediment V_{max} decreased greatly during winter months (Wisconsin, U.S.) and that true psychrophiles did not develop.

Harrison et al. (1970) reported a V_{max} (glucose) of .17 µg/ml/h for sediment in Upper Klamath Lake, Oregon. By relating uptake to one mil of sediment rather than per gram, comparisons are more readily made with planktonic values which in this case were very much lower (2.6×10^{-5} µg/ml/h). Harrison notes that "by comparison, the top square centimetre of sediment was capable of mineralizing 24 times as much glucose as the overlying square centimetre of water based on a mean depth of 2.7 metres". This comparison assumes, possibly incorrectly, that the existing methodology produces a realistic value for V_{max} in sediments.

Unfortunately there has been no work to evaluate the importance of the physical structure of

Table 3. Values for V_{max} (μg/g/h) for sediment bacteria in Marion Lake, B.C.[a]

Substrate	Summer V_{max}	Winter V_{max}
^{14}C-glucose	3.8	2
^{14}C-glycine	3.8	0.6
^{14}C-acetate	70	3.6

[a]Summarized from Hall *et al.* (1972)

sediments on bacterial activity. For example, the variation in the sand-silt-clay composition may greatly affect V_{max} values. Stotzky (1973) has reported that clay can greatly affect soil microbial activity both as an inhibitory or stimulatory factor. There is no reason to suspect that sediment bacteria would not also be affected and that a sample taken a few metres (or less) from another might as a result have a completely altered V_{max}.

In summary, it has been through the utilization of ^{14}C-carbon uptake techniques that much of the limited knowledge regarding heterotrophic rates has been achieved. Reliable methods of determining both assimilation and respiration for planktonic bacteria have been developed while sediment samples still pose considerable difficulties. In the future it will be important to be able to confirm if the uptake of radiolabelled "universal" substrates such as glutamic acid accurately reflects the total heterotrophic activity of a system and; in addition, to be able to study the assimilation of more complex molecules that enter fresh water systems.

3. MATERIALS AND METHODS

3.1 BACTERIAL POPULATIONS AND HETEROTROPHIC POTENTIAL

Water samples were collected using sterile sampling bottles from six locations on the Athabasca River. The temperature at each site was determined at the time of sampling and activity measurements were done within 30 min of collection at in situ temperatures utilizing a modified camper as a laboratory. The number of heterotrophic bacteria in the water column was determined by epifluorescence microscopy as described by Zimmerman and Meyer-Reil (1974). Samples to be counted were fixed in the field with 0.5% glutaraldehyde and transported to Chemical & Geological Laboratories Ltd. for staining.

Sediment samples were obtained using a large (50 cc) plastic syringe with the end cut open. Two 5ml samples were drawn up the syringe at each site and dispensed into a 10 ml solution of glutaraldehyde (5%) buffered at pH 8.0 with .067 M cacodylate acid. In the laboratory,the preserved samples were blended in a waring blender and diluted using cacodylate buffer at a pH of 8.0, then filtered and counted as with planktonic samples.

The heterotrophic activity determination was based upon the uptake and mineralization of uniformly labelled ^{14}C-glutamic acid (sp. act. greater than 235 μC/μM, New England Nuclear) by the planktonic microflora. Kinetic data which demonstrated a saturation function were analyzed by Michaelis-Menton enzyme kinetics as described by Wright and Hobbie (1966) and Crawford and Hobbie (1969). Assimilation respired carbon dioxide and total uptake were determined by the modified Lineweaver-Burke equation:

$$\frac{Cut}{c} = \frac{K_t + S_n}{V_{max}} + \frac{A}{V_{max}}$$

where:

C = 2.22 x 10^6 disintegrations/minute (DPM)
u = microcuries of substrate added
t = incubation time in hours
c = number of experimentally determined DPM's taken up
K_t = the uptake constant

S_n = natural substrate level of glutamic acid at the time of sampling

A = amount of experimentally added substrate (µg/l)

V_{max} = maximum velocity of uptake

A computerized plot of Cut/c versus A allows one to calculate V_{max} since the slope of the line is $1/V_{max}$, and to estimate the turnover time, $Kt + S_n/V_{max}$, which intercepts the Y axis.

Uptake of the radioactive labelled metabolite was measured over a range of concentrations (0.16 to 32 µg/l). Incubation times were selected so that not more than 5% of the substrate at the lowest concentration was removed. Ten mil water samples were pipetted into sterile 50 ml serum vials fitted with a rubber stopper. Triplicate assays were done for assimilation and mineralization determinations at each substrate concentration. Acid killed controls for each concentration were prepared by injecting 0.2 ml of 6N hydrochloric acid into the vials prior to the addition of labelled substrate. Controls and reaction flasks were incubated in the dark for 1 hour in a polypropylene glycol cooled laboratory shaker (100 rpm) at in situ temperature. After one hour the assimilated uptake was terminated by filtration through a 0.2 µm Nucleopore filter, followed by a 10 ml wash with sterile stream water. Mineralization was measured by acidifying the reaction flask to about pH 1.5 and collecting the evolved ^{14}C-carbon dioxide for 1.5 h in the center well filter wick soaked with phenetheylamine (0.2 ml). The Nucleopore filters were air dried for 5 min and placed into scintillation vials containing 10 ml of a toluene-based cocktail consisting of 2,5-diphenyloxazole (0.4%) and 1,4-bis-(5-phenyloxazole-2)-benzene (0.1%). Filter wicks were placed in vials containing this cocktail immediately following the carbon dioxide trapping incubation period.

Radioactivity was counted on a Packard Tri Carb liquid scintillation counter and counts were corrected for background activity, quenching, non-specific absorption to acid killed controls, and machine efficiency. Data was analyzed by computer facilities available at the University of Calgary, Alberta.

Physiological groups of bacteria were enumerated using standard MPN (Most Probable Number) techniques. One millilitre of a serial dilution of the sample was inoculated into test tubes of minimal media within 30 min of collection. For organic acid oxidizers, benzoic acid was the sole carbon substrate (Rodina 1972). Media for sulphate-reducing bacteria was prepared according to Torien et al. (1968) and Marna and Williams (1970); and for sulphur-oxidizers, according to Laishley et al. (1978).

Hydrocarbon utilizers were enumerated according to the techniques of Atlas (1978). To each serially inoculated 60 ml serum bottle 50 µl of 1-^{14}C-hexadecane spiked diesel oil (0.4 µc/ml) was added and the bottles closed with rubber serum tops. Samples were inoculated for six weeks and acidified (0.2 ml conc. hydrochloric acid) to stop the reaction. Radioactive ^{14}C-carbon dioxide was collected from each vessel and those bottles having twice the radioactive ^{14}C-carbon dioxide of the control were counted as positive. The numerical characteristic obtained by this method was converted to a number of hydrocarbon utilizers per millilitre by use of MPN tables (Rodina 1972).

3.2 ORGANIC CHEMISTRY OF WATER AND SEDIMENTS

At the six river sites sediment samples were collected with a small shovel (at a water depth of approximately 0.3 metres) and frozen within one hour during the October survey. Samples were a composite of five sub-samples taken within 10 m of each other. Air-dried 100 g samples were extracted with benzene and these extracts were delivered to Xenotox Services Ltd. for analysis. General analytical procedure was as follows. Each evaporated extract was dissolved in an ether/methylene chloride mixture (14:11 v/v; 100 ml) and extracted for a) weak acids (phenols), b) strong acids (carboxylic acids), c) bases and d) neutral compounds as shown in Figure 3. The extracted neutral materials and organic acids (as their n-butyl esters) were examined by gas chromatography (GC); the the extracted Bases and Phenols were not further examined at this time (reserved). The conditions for GC separation of the acid and neutral compounds are shown in Section 7.1.

Four water samples from the January survey (approximately 800 ml each) were analyzed. Three of them (from sites 1A, 5A and 10) had been acidified

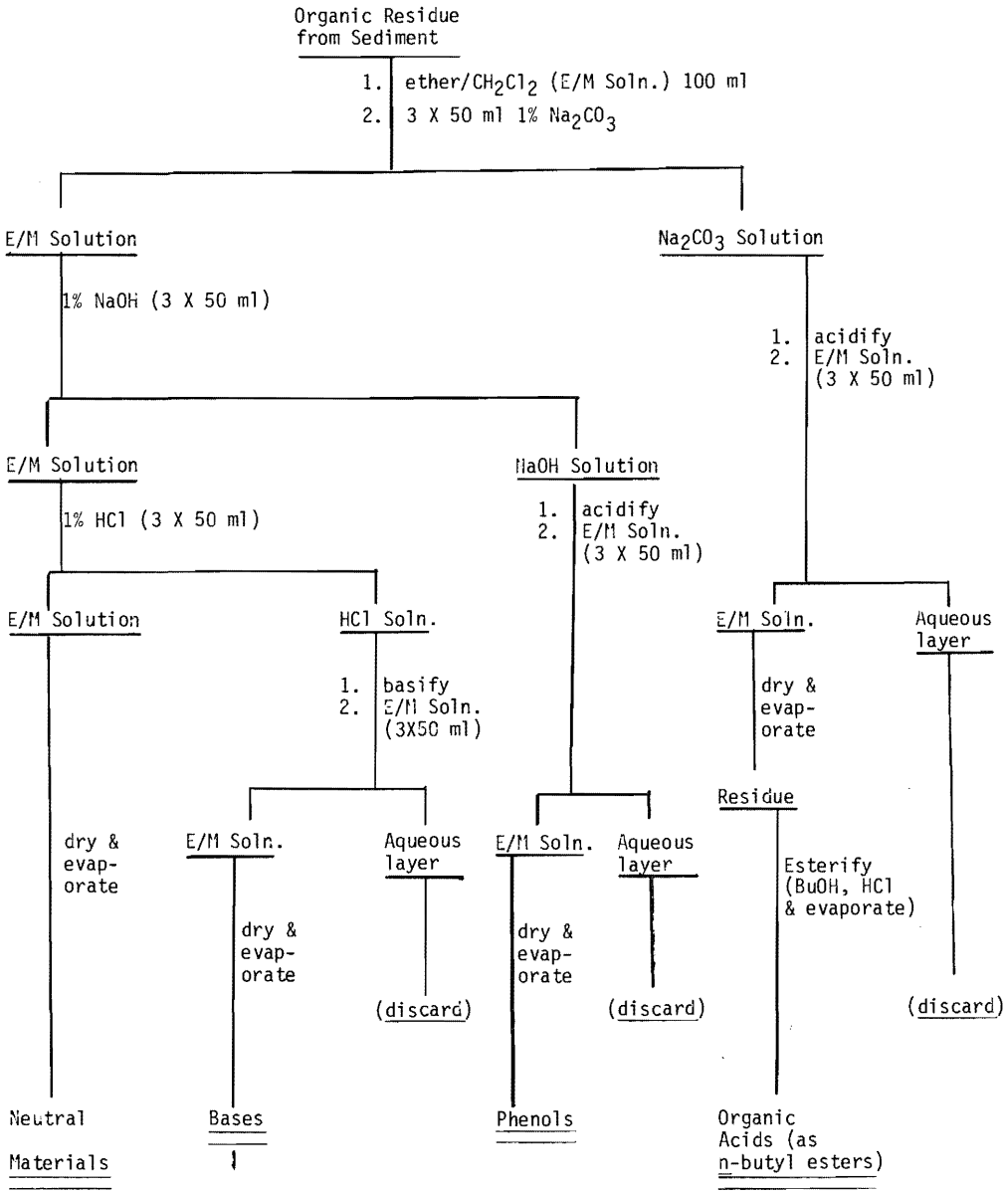

Figure 3. Separation of components in sediment extracts.

approximately to pH 2 immediately after collection to prevent bacterial action. The fourth sample (from site 4) had been frozen immediately after collection and was supplied in the frozen state. All samples were stored in thoroughly rinsed (distilled water) one litre glass bottles with plastic caps.

Each river water sample (500 ml) was concentrated to 25 ml in a rotary evaporator at 40^{o}C under reduced pressure. (The flask used and the evaporator were thoroughly cleaned with concentrated sulphuric acid, double distilled water and boiling methanol before use). The concentrate was basified (sodium bicarbonate) and extracted with diethyl ether/methylene chloride (14:11 v/v; 3 x 20 ml) and, by using essentially the scheme depicted in Figure 3, neutral organic compounds, phenols and organic acids were separated from water samples 1A, 5A and 10. In addition to these three classes of compounds, organic bases were separated from water sample 4. However, only the neutral and organic acid fractions were investigated further.

The water concentrates remaining after extraction with ether/methylene chloride contained non-extractable materials, including glutamic acid. They were reserved for further treatment.

Extracts containing the neutral compounds were examined in the same way as the corresponding extracts obtained from sediments (see before). GC conditions were identical (see Section 7.1) except that a helium flow rate of 50 ml/m was used. In addition, a non-distilled sample of ether was inadvertently used to prepare the ether/methylene chloride solvent system. Normally the solvent blank trace contains no GC-detectable compounds; in the present study the solvent blank trace contained one large peak and three small peaks which must be ignored in the GC traces of the neutral compounds in river water samples 1A, 5A and 10. Extracts containing the acidic compounds (as their n-butyl esters) were likewise examined.

3.3 GLUTAMIC ACID CONTENT OF RIVER WATER

Heterotroph potential studies employing ^{14}C-glutamic acid provided data on the V_{max} or potential activity of the river micro-organisms to metabolize this compound. However, if the natural substrate concentration (S_n) is known, then the actual uptake

rate (v) can be determined according to the following equation (Wright and Hobbie 1965):

$$v = \frac{c\,(S_n + A)}{Cut}$$

where c is the radioactivity of the filtered organisms (count/min), C the count/min. from one microcurie of carbon 14, t the incubation time (h), u the number of microcuries added, and A is the added substrate.

In order to identify glutamic acid levels that were anticipated to be in the nanogram per litre or microgram per litre range, Dr. Coutts undertook an evaluation of existing methods and an extensive deciption of the method used for this project. This report is contained in Section 7.2.

4. RESULTS

4.1 BACTERIAL POPULATIONS AND HETEROTROPHIC POTENTIAL

A summary of values for V_{max} and t_n for the six river sites and two wastewaters is provided in Table 4. Fewer values were obtained from the January survey due to wide discrepancies in ^{14}C-carbon dioxide production data. This was attributed to cracks in the rubber caps of the serum bottles perhaps caused by the extreme cold, outside temperatures were between -30°C and -40°C.

The data includes values for planktonic samples only since there was insufficient time to undertake studies of sediment uptake during the fall survey, and bottom ice prevented suitable sampling during January. Preliminary experiments at a few locations using diluted sediments indicated that glutamate was taken up in the October sediments although the data did not fit Michaelis-Menton kinetics. Uptake from sediments collected in January appeared to be minimal.

Values for V_{max} of between 0.1 and 1.0 µg/l/h were obtained for most river sites with no evidence of a decreasing rate of assimilation during winter. These results are comparable to those of Baker et al. (1977) who found a V_{max} (glutamic) of 0.61 µg/l/hr in water samples of the Red Deer River; but are approximately 100-fold greater than values for an alpine stream (Geesey et al. 1976).

The wastewater effluent from the upgrading pond had a V_{max} approximately 10-fold greater than did river samples. However, the V_{max} for dike drainage water was similar to river samples and appeared to decrease greatly during winter.

The wide fluctuations in mean percent carbon dioxide respired may indicate differences in environment (e.g., substrates) but also may indicate experimental errors perhaps due to leakages of carbon dioxide from the serum bottles during incubation. Raw data for all V_{max} and t_n values listed in Table 4 are provided in Section 7.3.

Tables 5 and 6 summarize the results of total cell counts for both planktonic and sediment samples. The October planktonic values were quite

Table 4. A summary of heterotrophic potential experiments using ^{14}C-glutamic acid for the months of October and January.

Site No.	Temperature (°C)		V_{max} (µg/l/h)		t_n hours		Mean % CO_2 respired		Correlation Value (r)	
	Oct.	Jan.	Oct.	Jan.	Oct.	Jan.	Oct.	Jan.	Oct.	Jan.
1A	5	-	0.86	-	30.1	-	61.4	-	-	-
2	6	-	0.59	-	13.2	-	15.5	-	.95	-
4	3.5	1	0.62	2.2	13.6	41	17.4	67	.90	.91
5A	3.5	1	-	0.17	-	124	-	39	.54	.96
7	4	1	0.27	0.75	2.2	13	36.7	20	.93	.97
10	4	-	0.16	-	14.2	-	40.6	-	.92	-
A	14	5	3.9	4.6	0.54	2.0	18.9	9.6	.99	.97
B	14	15	0.35	.01	10.5	-	48.4	-	.72	.89

Table 5. Planktonic cell counts using epifluorescence microscopy.

Site No.	Total Cell Counts (cells/ml)	
	Oct.	Jan.
1A	9.7×10^4	-
2	4.5×10^5	1.0×10^4
4	2.4×10^5	3.0×10^5
5A	6.0×10^5	2.0×10^6
7	5.4×10^5	9.2×10^4
10	6.3×10^5	6.1×10^4
A	6.2×10^5	6.1×10^6
B	2.7×10^6	6.6×10^4

Table 6. Sediment cell counts using epifluorescence microscopy.

Site No.	Total Cell Counts (cells/ml) Oct.	Jan.[a]
1A	5.5×10^5	
2	3.9×10^7	
4	4.1×10^6	
5A	1.6×10^8	
7	-	
10	3.8×10^6	

[a] A great deal of difficulty was experienced in obtaining sediment samples due to a layer of ice covering the river bottom. A few samples were obtained using very crude procedures and results indicated almost no cells or at least less than 10^4 cells/ml.

consistent among sites at about 10^5 cells/ml; however, values in January were very irregular (Table 5). Sediment counts were also irregular although this was not unexpected due to its more heterogeneous nature (Table 6).

During the January survey, most probable number (MPN) tests indicate the presence of only a very few (10 to 100 cells/ml) hydrocarbon oxidizing bacteria in water samples, but much larger numbers in sediment (Table 7). This result is supported by chemical analysis which showed some hydrocarbons like hexadecane in all sediment samples (Table 8) but none detected in the river water. The fact that modest populations (100 to 10,000 cells/ml) of hydrocarbon-oxidizing sediment bacteria were found in January, when the sediment was covered with ice, indicates the probability of much larger populations during ice-free periods.

The remaining data regarding physiological groups are also summarized in Table 7. It should be noted that these data are the result of only one survey and should be regarded as preliminary. The difficulties in obtaining sediment samples during the January survey should also be noted.

With respect to water samples, the populations of hydrocarbon oxidizing bacteria were larger in the upgrading pond effluent which, of course, contains relatively large amounts of oil. It is interesting to note that the toxic effects of dike drainage water on fish (Hrudey 1975) does not seem to affect bacterial activity as demonstrated by these data as well as V_{max} values and direct counts.

4.2 CHEMICAL ANALYSIS

The objectives of this initial report were to establish suitable chemical techniques and to identify some major compounds; and since these data are based on only one sample at each site, results are presented in most instances as qualitative data only.

4.2.1 Analysis of Sediments

Air-dried samples were analyzed for total organic content and benzene-extractable organic material; and these data are summarized in Table 9. The benzene-extractables appear to be present at higher

Table 7. Preliminary estimates of specific populations of various physiological groups from sediment[a] and water samples using most probable number technique. Samples were collected during the January 1978 survey.

Site No.	Physiological Group (cells/ml) hydrocarbon oxidizers (^{14}C-hexadecane)	organic acid oxidizers (benzoic acid)	sulphur oxidizers	sulphide producers
1A - planktonic	$1.2 \times 10^{2}_{3}$	$1.4 \times 10^{3}_{5}$	3	-
- sediment	2.4×10^{3}	1.4×10^{5}	0	2.0×10^{4}
2 - planktonic	$2.1 \times 10^{1}_{2}$	$1.4 \times 10^{3}_{3}$	$2.5 \times 10^{1}_{3}$	-
- sediment	1.1×10^{2}	1.4×10^{3}	6.5×10^{3}	-
4 - planktonic	$1.1 \times 10^{2}_{3}$	$1.4 \times 10^{3}_{4}$	$1.5 \times 10^{2}_{4}$	-
- sediment	2.4×10^{3}	1.4×10^{4}	1.4×10^{4}	1.4×10^{4}
5A - planktonic	-	$1.4 \times 10^{4}_{5}$	$1.4 \times 10^{3}_{5}$	-
- sediment	2.4×10^{4}	1.4×10^{5}	1.4×10^{5}	1.4×10^{5}
7 - planktonic	$2.1 \times 10^{1}_{2}$	1.4×10^{3}	1.4×10^{3}	-
- sediment	2.0×10^{2}	-	-	-
10 - planktonic	2.5×10^{1}	$1.4 \times 10^{3}_{4}$	1.1×10^{2}	-
- sediment	-	1.4×10^{4}	4	4.0×10^{3}
A - planktonic	2.4×10^{3}	1.4×10^{5}	1.0×10^{3}	-
B - planktonic	6.4×10^{2}	1.4×10^{4}	1.0×10^{3}	-

[a] See Table 6.

Table 8. Alkanes extracted from sediments at Site 1A.

Identity	Peak T_r [a]	ΔT_r [b]
(? n-$C_{21}H_{44}$)	23.53	2.21
(? n-$C_{20}H_{42}$)	21.32	2.22
(? n-$C_{19}H_{40}$)	(19.10)	2.29
n-$C_{18}H_{38}$	16.81	2.45
(? n-$C_{17}H_{36}$)	14.36	2.53
n-$C_{16}H_{34}$)	11.83	2.41
n-$C_{15}H_{32}$	9.42	2.36
n-$C_{14}H_{30}$	7.06	2.38
(? n-$C_{13}H_{28}$)	(4.68)	

[a] Peak T_r = Retention time (in minutes) of GC peak;

[b] ΔT_r = Difference in retention times (in minutes).

Table 9. Organic material in sediment (October survey).

Site No.	Total Organic Material (% by weight)	Benzene-extractable Organic Material (mg/kg)
1A	1.4	175
2	3.4	775
4	3.4	752
5A	2.4	545
7	1.7	352
10	1.0	875

levels within the oil sands area (Sites 2, 4, 5A, 7) or downstream (Site 10) than they are in upstream sediments (Site 1A). These values do not appear to be influenced by the mining operations at Great Canadian Oil Sands Ltd. (Sites 4, 5A, and 7). Visually, the benzene extract from Site 1A appeared clear while all the others appeared as varying degrees of brown colour.

All extracts (1A, 2, 4, 5A, 7 and 10) contained numerous neutral compounds. Not surprisingly, all contained varying amounts of higher molecular weight alkanes. n-Tetradecane (C14), n-pentadecane (C15), n-hexadecane (C16) and n-octadecane (C18) were positively identified by GC (gas chromatography), MS (mass spectrometry), and by comparison with authentic reference compounds as components of each extract, but other n-alkanes are undoubtedly present. For example, the trace of extract of sediment 1A contained nine peaks, of which four are positively, and five are tentatively identified (Table 8) by their GC retention times.

Other neutral compounds are also present in each extract. An examination of each GC trace (Section 7.4) and the accompanying data reveals that all contain compounds with Tr (retention time of GC peak) values of approximately 26.7 and 31.6 min, in apreciable quantities (Table 10). They have not been identified. Their Tr values, however, correspond to what would be expected for the n-alkanes, n-C22 and and n-C24 respectively, but this requires confirmation. The peak of T4 19.4 was identified as di-n-butyl phthalate (DBP) by comparing its GC behaviour with that of an authentic sample of DBP.

The acid components were examined as their n-butyl esters. Examination of the GC traces of the organic acids in the sediments (Section 7.5) showed that they contain numerous compounds, four of which were present in appreciable quantities (Table 11), i.e., those with Tr values of approximately 19.4, 25.0, 25.9, 31.6 min. The first of these (Tr = 19.4 min) was confirmed, by reference to an authentic samples, to be n-butyl phthalate, thus indicating the presence of phthalic acid in the sediment extract. The source of this phthalic acid is not known. It may be a true component of the sediment but it could also have been introduced during the analytical procedure. The other components remain unidentified.

Table 10. Approximate percentage age composition of long retaining time neutral components of sediments.

Site No.	% Composition Approximate Retention Times (min) 19.4	26.7	27.4	29.6	31.6
1A	11.5	49.3	-	-	9.4
2	9.1	64.3	-	1.7	5.5
4	-	10.0	5.6	18.3	21.1
5A	8.4	43.6	-	7.4	6.5
7	5.5	41.0	-	21.9	20.5
10	1.7	18.1	19.5	10.9	15.9

Table 11. Approximate percentage age content of acidic compounds (as n-butyl esters) in sediments.

Site No.	% Composition Approximate Retention Times (min) 19.4	25.0	25.9	26.7	28.3	29.6	31.6
1A	22.0	6.9	30.8	-	3.8	3.9	25.7
2	29.2	0.9	34.3	-	2.3	3.4	29.6
4	32.7	32.4	-	-	2.8	5.6	26.5
5A	31.8	4.0	17.2	-	2.5	-	38.3
7	55.9	17.7	11.1	-	15.4	-	-
10	31.5	5.6	13.4	4.5	5.9	-	36.1

4.2.2 Analysis of Water

As observed with the sediment extracts, the extracts of neutral compounds in river water at sites 1A, 5A, and 10 give rise to many peaks on the GC (Section 7.6). Two peaks in each trace must be ignored (i.e., peaks Tr 6.34 and 26.38), since they were present in the solvent system. The major peaks present in these extracts are summarized in Table 12. None has yet been identified, but it is very likely that most are phthalate esters, which are common constituents of water samples, including distilled water. It is noticeable that the hydrocarbons so abundant in the sediment extracts are present in very low concentrations in these water extracts.

The procedure of acidifying water samples prior to analysis, to prevent bacterial action is open to question in view of the results obtained when water sample 4 (preserved by freezing) was examined. The results obtained in the latter case were quite different from those depicted in Table 12 (see GC trace in Section 7.6). The difference in the composition of water sample 4 compared with 1A, 5A, and 10 might reflect a genuine difference in the nature of the chemical components. The frozen sample may have undergone microbiologically-induced changes when thawed in the laboratory. On the other hand, the acidifying process may have been responsible for these differences. This problem should be addressed if future samples are to be preserved by acidification.

In comparison with the GC traces of neutral components in water samples 1A, 5A and 10, the water sample from Site 4 contained numerous peaks, one of which (Tr 26.75) was present in large quantities. Other components were present in significant amounts (Tr 7.22, 16.91, 19.28 and 31.45). Three of these compounds (Tr 19.28, 26.75 and 31.45) are also present in sediment extracts (see Table 8). The sources of these compounds are not known; they were not introduced during the assay process. One of them (Tr 19.28) is probably di-n-butyl phthalate. It, and the other major components can be identified by GC/MS should this project be revived.

Numerous acidic compounds were present in small quantities (Table 13) and some (converted Tr 19.4, 25.0, 25.9, 26.7, 29.6 and 31.6) were also present in sediment extracts. GC traces are located

Table 12. Approximate percentage age composition of major neutral components extracted from water samples 1A, 5A, and 10.

T_r	Converted T_r[a]	% Composition of Sample		
		1A	5A	10
6.34[b]	9.1	42.1	55.1	61.6
8.19	11.3	5.6	6.1	4.3
11.45	14.7	2.0	3.7	3.5
14.18	17.3	9.7	8.8	7.1
23.86	26.7	2.3	2.0	1.8
26.38[b]	28.9	6.5	6.1	9.6
28.85	31.6	22.6	4.8	5.5

[a] T_r values are at a He flow rate of 50 ml/min. Converted T_r values are at 30 ml/min to enable direct comparison with results obtained from sediment studies.

[b] These two peaks are also present in the solvent blank and should be ignored. The shorter retention peak (T_r 6.34) is butylated hydroxytoluene (BHT), a preservative commonly added to diethyl ether which is not present in distilled ether samples.

Table 13. Relative proportions of acidic components (as n-butyl esters) in Athabasca River water samples - qualitative data.

T_r	Converted T_{ra}	% Composition of Sample		
		1A	5A	10
11.49	14.8	2.3	1.7	0.3
16.23	19.4	15.1	13.0	18.4
18.67	21.5	8.2	4.2	0.5
20.40	23.3	3.4	-	2.2
22.19	25.0	8.2	2.5	4.4
23.02	25.9	8.9	16.5	20.3
23.85	26.7	14.5	7.2	1.8
26.41	29.6	18.8	14.1	8.6
28.87	31.6	16.8	34.7	26.4
30.76	33.7	1.5	5.6	6.2

[a]See footnote to Table 12.

in Section 7.7. Two compounds are apparently present in all extracts, i.e., Tr 19.4 and 31.6, He flow rate 30 ml/min. The former is known to be di-n-butyl phthalate, the latter was not identified. It is conceivable that both are spurious compounds, present, for example, in the caps of the bottles which were used to transport the samples. This should be investigated further.

Values for total organic carbon (TOC) in the river are reported in Table 14 and appear to increase slightly at Sites 2 and 4. It is interesting to note that while TOC values for the wastewater effluent (Site A) are comparable to values found in the river, V_{max} values for this site (Table 4) are of an order of magnitude higher. This suggests that those components in the wastewater effluent are more susceptible to bacterial degradation than are the organic compounds found in the river water. Dike drainage water, on the other hand, has a much higher TOC concentration but bacterial activity as measured by V_{max} (glutamic acid) appears to be similar or even less than river water samples, suggesting the presence of compounds more resistant to bacterial attack.

4.2.3 Glutamic Acid Analysis and Uptake

Using the method described in Section 7.2, the concentration of glutamic acid is given for five river sites (Table 15). Since the natural substrate concentration is known, the actual velocity of uptake can be calculated according to the equation (Wright and Hobbie 1966):

$$v = \frac{c\,(S_n + A)}{Cut},$$

when $A = 0$ and $t_n = Cut/c$

$$v = \frac{S_n}{t_n}$$

where:

v = velocity of uptake at the natural concentration of substrate
S_n = natural substrate concentration
t_n = the time for complete utilization or turnover of the substrate at its natural concentration

Table 14. Total organic carbon of water samples.

Site No.	Total Organic Carbon (TOC) (mg/l)	
	Oct.	Jan.
1A	9	11
2	12	9
4	14	10
5A	14	9
7	13	9
10	18	9
A	19	12
B	158	50

Table 15. Preliminary values for the glutamic acid content and v (glutamic acid) of water samples, January survey.

River Water Sample	Glutamic Acid (μg/l)	v (μg/l/h)
1A	0.856	-[a]
4	1.786	.04
5A	2.730	.02
7	0.660	.05
10 (3 determinations)	0.738, 0.755, 0.746 (av. 0.747)	-[a]

[a] a value for t_n was not available.

Thus by utilizing the values for t_n (Table 4), the value for v for sites 4, 5A, and 7 can be calculated (Table 15). These values must be regarded as tentative since they are based on only one sampling per site. As a percentage of V_{max} (the potential rate of uptake) the values for v are 1.8% at Site 4, 11.7% at Site 5A, and 6.6% at Site 7. This would indicate that at the existing populations of microorganisms, there is a substantial potential for the increased assimilation of at least this one organic substrate.

5. DISCUSSION AND SUMMARY

The use of ^{14}C-carbon (glutamic acid) uptake experiments, utilizing a mobile field laboratory, has provided information enabling a comparison to be made regarding in situ planktonic heterotrophic activity (V_{max}) at various sites in the Athabasca River. Data from the October 1977 survey (Table 4) indicates that values obtained for V_{max} are comparable to the value obtained by Baker et al. (1977) in the Red Deer River (.61 µg/l/h) and tended to decrease from Site 1A, located above the oil sands area, through to Site 10, located well below the mining area.

This trend, if valid, was unexpected since organic compounds from mining effluents as well as the natural oil sands deposits do enter the river. Our data (Table 14) indicated an increase in total organic carbon (TOC) of 33% as the river water enters the oil sands area and a further increase of 17% as it enters the area of mining activity. Strosher and Peake (1978) also showed an increase of 13% in TOC as the river passes this latter area.

Unfortunately, the limited nature of this study precludes a broader discussion of the validity or implications of such a trend toward decreasing values for heterotrophic uptake. It seems improbable that the components (unknown) of dike drainage waters toxic to fish (Hrudey 1975) are responsible for any decrease in V_{max} values especially when the value for V_{max} of dike drainage water (.35 µg/l/h) was comparable to values found in the river itself.

The approximate 10-fold increase in V_{max} values for the upgrading pond wastewater compared to river water almost certainly reflects the presence of more readily degradable organic compounds. The work of Strosher and Peake (1978) indicated that almost 50% of the TOC (36 mg/l) in this effluent "likely exists as dissolved or dispersed liquid hydrocarbons and other compounds of low polarity which are readily extractable with benzene". In contrast only 24% of the river TOC (13 mg/l) was benzene extractable. It should be noted, however, that the validity of making direct comparisons between V_{max} values of river water and wastewater is open to some question since the latter represents a significantly different environment and there is no guarantee that V_{max} remains a constant percentage of total organic uptake in these different situations.

The winter values for river water V_{max} do not indicate a great decrease in assimilatory capacity and this is not unexpected since temperatures (Table 4) and even substrate (TOC) levels (Table 14) remain fairly constant throughout the year. During the winter months, a thick layer of bottom ice indicated that, whatever the number or activity of bacterial cells, assimilation of substrates from the aqueous phase of the river by sediment bacteria must be minimal or non-existant. This layer of bottom ice appeared to extend some distance from the shore although no attempt was made to determine if it covered the entire width of the river.

The importance of sediment bacteria in contributing to heterotrophic uptake has been outlined in Section 2.3. Our data (Table 6) showed erratic but generally high levels of bacteria in the sediments in comparison to planktonic bacteria. Lock and Wallace (in review) also found evidence of high levels of epilithic bacteria in tributaries to the Athabasca River and also found indirect evidence of high rates of heterotrophic activity within sediments. However, the lack of concrete data for heterotrophic uptake in sediments precludes any estimae for the total heterotrophic capacity of the Athabasca River.

In Section 2.3 (Table 2), we noted the values for V_{max} determined for a eutrophic lake in Sweden, Lake Erken (Wright and Hobbie 1966). The value for v or actual uptake using acetate was calculated to be .04 µg/l/h; and for glucose, less than .026 µg/l/h. These values are comparable to an average value for v (glutamic acid) of .037 µg/l/h found in three samples from the Athabasca River (Table 15).

We note that the values for v are in the order of 2 to 12% less than the value for V_{max} at these three sites. Thus it would appear that the existing populations of microorganisms have the potential to assimilate substantially greater amounts of organic material. However, it should be noted that this small value for v relative to Vmax is typical of similar values found by other workers (Wright and Hobbie 1966; Crawford et al. 1974); and it therefore cannot be assumed that the Athabasca River is uniquely capable of assimilating additional inputs of organic compounds.

In comparison to the value for v of .0007 µg/l/h (glutamic acid) determined from an oligotrophic alpine stream in October (Geesey et al. 1976), these values from the Athabasca River are higher by approximately a factor of 100. This may indicate an expected increase in heterotrophic activity when comparing an oligotrophic mountain stream to a woodland river. However, TOC values increase by only factors of between 2 to 6 and this would indicate that the organic substrates in the Athabasca River may be more susceptible to bacterial degradation.

As mentioned, results obtained during the fall survey showed a trend of decreasing values for planktonic V_{max} from Site 1A to Site 10 (downstream). The fact that bacterial numbers tended to increase slightly (Table 5) along this same direction does, however, indicate that cell numbers cannot be used as an estimate of heterotrophic activity. This conforms to the evidence of Hobbie and Wright (1965) that the physiological condition or activity of cells cannot be determined by epifluorescence microscopy. Also, Daley (1975) has noted that "recent indirect evidence suggest that most if not all native bacteria are viable, but that many are metabolically dormant".

Thus any attempt to utilize direct counts or, for example, an assay for LPS as an indicator of periods when the inputs of wastes to the river could be increased due to increased bacterial activity seem unlikely to succeed. In any case, that particular assay technique (LPS) appears to be more complex and expensive than anticipated and we did not proceed with that phase of the work.

The estimates of specific groups (Table 7) of bacteria, although based on only one sampling in January 1978, did show a tendency towards increased populations (hydrocarbon oxidizers, organic acid oxidizers, and sulphur bacteria) in both sediments and water just downstream from the GCOS plant (Site 5A). In their study, Strosher and Peake (1978) did show that organic sulphur compounds, organic acids, and hydrocarbons were indeed significant components of the upgrading pond wastewater; thus it appears that these specific populations of bacteria have responded to an increase in the concentration of certain compounds. At Site 7, 15 kilometres further downstream, the populations of these particular physiological groups of bacteria returns to more "normal" levels.

The presence in river sediments of appreciable quantities of hydrocarbons (Table 8) known to be metabolized by bacteria (Zobell 1969) indicates that these substrates may be a significant portion of the total heterotrophic uptake of the Athabasca River. Indeed, the existence of hydrocarbon oxidizing bacteria in the sediments (Table 7) even during the conditions of bottom ice in January supports this assumption. It seems likely therefore that any spill of refined oils would be easily assimilated by these pre-adapted populations of bacteria.

There is, however, no information available regarding the assimilation of specific organic compounds released routinely into the Athabasca River due to mining activity. Strosher and Peake (1976; 1978) have identified certain groups of compounds present in various waste streams; but no attempts have been made to identify the components separated by GC analysis. We believe that many of these organic constituents can be identified and quantitated utilizing the methodologies presented in this study.

This would then form the basis for an assessment of the significance of various compounds, due either because of their toxicological effects or because of the quantities involved. Assimilation studies, using one of the techniques outlined here for glutamic acid and hexadecane, could then assess the persistence of the compounds in river water and sediments.

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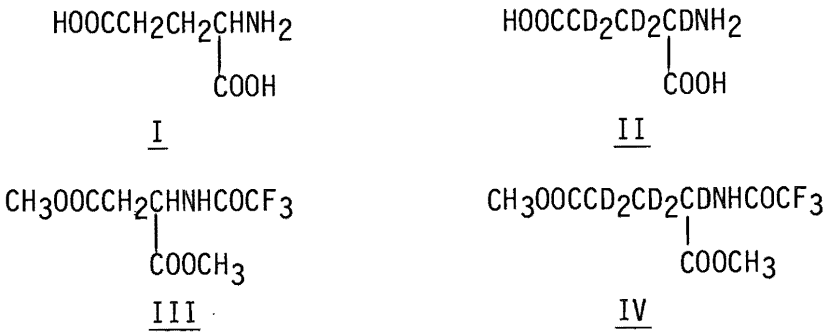
7. APPENDICES

7.1 CONDITIONS FOR THE GC SEPARATION OF SEDIMENT EXTRACTS

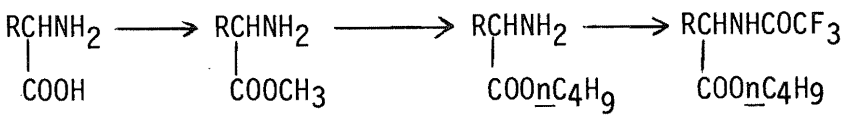
1. Column: 51 cm x 2.5 mm i.d. stainless steel containing 10% UC-W98 on 80-100 chromosorb W.
2. Mode: Dual column.
3. Column temperature: Programmed at 110^{o} for 2 min, then program at 4^{o}/min to 230^{o} and hold. Total time of run normally 35 min.
4. Carrier gas; flow rate: Helium; normally 35 ml/min. In some analyses, approximately 50 ml/min was used. Retention times (Tr) of compounds emerging from the column at the latter flow rate are easily converted to Tr values at a He flow of 30 ml/min.
5. Reference GC Traces. A GC trace of the solvent system used (ether/methylene chloride) was recorded on the day of the run (solvent blank). A GC trace of a mixture of authentic hydrocarbons n-tetradecane, n-pentadecane, n-hexadecane, and n-octadecane, identified on traces as C14, C15, C16, and C18 respectively (reference solution) was regularly recorded.

7.2 GLUTAMIC ACID CONTENT OF RIVER WATER

Natural concentrations of many organic compounds in environmental water samples are generally less than 20 µg/l (Hobbie 1973; Wright and Hobbie 1966; Crawford et al. 1974) and in some instances (Telang et al. 1976) are lower than 1 µg/l. Telang et al. (1976), for example, showed that levels of individual amino acids in the Marmot Creek drainage basin were around 350 ng/l. It was expected, therefore, that the levels of glutamic acid (I) in the River Athabasca samples could be less than 1 µg/l and that a specific sensitive and reproducible method of analysis for glutamic acid, capable of repetitive application should be developed. Initially an attempt was made to use the method reported by Telang et al. (1976) which was a modification of a procedure described by Gehrke et al. (1968), Roach and Gehrke (1969a) and Roach and Gehrke (1969b), for the analysis of protein hydrolysates. Telang et al. (1976) concentrated each water sample (10 ml - 5 ml) and passed the concentrate down a cation exchange column. Amino acids were retained on the column and were subsequently eluted with ammonium hydroxide solution. The eluate was evaporated to dryness, and the residue was then converted e.g., esterified then treated with trifluoroacetic anhydride (Scheme 1). Quantitative analysis of the final products (II) was achieved using a gas chromatographic (GC) method.



Glutamic Acid and its Converted Forms



Scheme 1

When this method of analysis was used on Athabasca River water, however, it proved impossible to separate completely, by GC means, other converted water components which also eluted from the cation exchange column. An equally sensitive but more specific method of analyzing nanogram quantities of glutamic acid was required. Numerous preliminary experiments were then conducted to see whether the glutamic acid in the water samples could be separated from other components prior to GC analysis.

Activated charcoal has been used successfully as an analytical sorbent for the purification of water for domestic use (Eichelberger and Lichtenberg 1971). A water sample (500 ml) was passed down a column (10 cm x 1 cm) of thoroughly washed (methanol, acetone, then distilled water) and reactivated (heating at 180^{0}C for four hours) powdered activated charcoal in the hope that most organic materials would be adsorbed onto the column while the glutamic acid, being very polar, would pass through the column. After much experimentation, it was concluded that most of the glutamic acid was also retained in the column. Since it proved impossible to elute only the glutamic acid from the column, this attempt to separate glutamic acid from other organic materials in the water sample had to be abandoned.

The next attempt to separate glutamic acid from other organic compounds was to use a column (10 cm x 1 cm) packed with neutral resin XAD-2. This material is used (Stolman and Pranitis 1977; Technical Bulletin No. 10) to isolate drugs and metabolites from urine and blood samples. Again it was hoped that interfering organic compounds present in the water sample would be adsorbed onto the XAD-2 resin while the glutamic acid would not be adsorbed and would pass down the column with the water. At first the method looked promising since glutamic acid was detected in the residue remaining after the water emerging from the column was evaporated, but subsequent studies showed that almost 99% of the glutamic acid has remained on the XAD-2 column. This method to separate glutamic acid from other water-soluble organic components was also abandoned. It was then decided to use the method employed by Telang et al. (1976) to separate glutamic acid from other components in the water sample, but to replace their GC method of quantitatively analyzing the amino acid with a mass spectrometric method.

Mass spectrometric methods of analysis, which permit the isolation, identification and quantification of subnanogram amounts of drugs and metabolites, are now routinely used in biochemistry and medicine (Millard 1977; Lehmann and Schulten 1978). Since pentadeuterated glutamic acid (L-glutamic-2,3,3,4,4,-d_5 acid, II) was readily available commercially (Merck and Co., Rahway, New Jersey), it seemed reasonable to expect that trace quantities of natural glutamic acid in aqueous solution could be quantitatively analyzed by mass spectometry. The method proved to be successful and, after much experimentation, the procedure which is now described in detail, was adopted.

7.2.1 Experimental Details

Combined GC/MS was performed on a Hewlett Packard 5710A gas chromatograph coupled to a Hewlett Packard 5981A mass spectrometer with a Newlett Packard 5934A data system. The mass spectrometer was operated at 70eV.

Freshly prepared demineralized double-distilled water, obtained from a Corning LD-2a demineralizer coupled to a Corning AG-11 distillation apparatus, was used to prepare all aqueous solutions and to wash glassware.

All glassware was soaked overnight in chromic acid and then thoroughly washed with double-distilled water and methanol prior to use.

Stock solutions of glutamic acid and d^5-glutamic acid were prepared by dissolving 10.0 ml of each amino acid in separate litre volumes of double-distilled water. Suitable dilutions of these stock solutions were made immediately prior to when the diluted solutions were required. Both stock solutions retained their potency over a three-month period whereas very dilute solutions (10-1000 ng/l) slowly lost potency on standing.

A Pipetman pipet (Gilson France, Villiers le Bel, France), 200 µl maximum capacity, was used to pipet the 50 µl and 100 µl samples employed in the study. The pipet delivered reproducible volumes.

7.2.2 Conversion of Glutamic Acid (I) and its Pentadeuterated Derivative (II)

A solution containing 10 mg of glutamic acid in double distilled water (1 litre) was prepared. One mil of this solution (=10 μg) was evaporated to dryness in a round-bottom flask on a rotary evaporator (water temp. 80°C). Methanolic hydrogen chloride (1.4 N, 10 ml) was added to the flask which was sealed with Parafilm M (American Can Co., Neenah, Wisconsin) and heated for 30 min at 60°C in a water bath. The solution was then concentrated, transferred in small portions to a Reactivial (0.6 ml size) (Pierce Chemical Co., Rockford, Illinois) and evaporated to dryness. The residue was dissolved in methylene chloride (0.2 ml) and trifluoroacetylated by adding trifluoroacetic anahydride (0.4 ml) and heating for 30 min in the sealed Reativial at 120° C. The solution was then evaporated to dryness under nitrogen and dissolved in methylene chloride (0.2 ml). This solution (3 μl) of III was examined by gas chromatography/mass spectrometry (GC/MS).

In a manner similar to that described above, 1 ml (= 10 μg) of a solution of L-glutamic-2,3,3,4,4-d_5 acid in double-distilled water was evaporated and the residue converted with methanolic hydrogen chloride and trifluoroacetic anhydride. The final solution in methylene chloride contained the pentadeuterated derivative (IV); a portion (3 μl) was examined by GC/MS.

7.2.3 GC/MS Examination of Converted Glutamic Acids

Derivatized glutamic acid (III) and d_5-glutamic acid (IV) were chromatographed on a glass column (2.12 m x 4 mm i.d.) packed with 3% PC-3210 (Pierce Chemical Co., Rockford, Illinois) on acid-washed silane-treated Chromosorb 750 (80-100 mesh) (Johns-Manville, Denver, Colorado); temperatures: oven 140°C, injection port 250°C, detector 300° C; He flow rate at 60 ml/min. Under these conditions, compounds III and IV both had retention times of 1.7 min.

Mass spectra were recorded of each GC peak. The spectra of III and IV are illustrated (Figure 4). A fragmentation pathway consistent with the formation of the major fragments in the spectra of III and IV is illustrated in Figure 5.

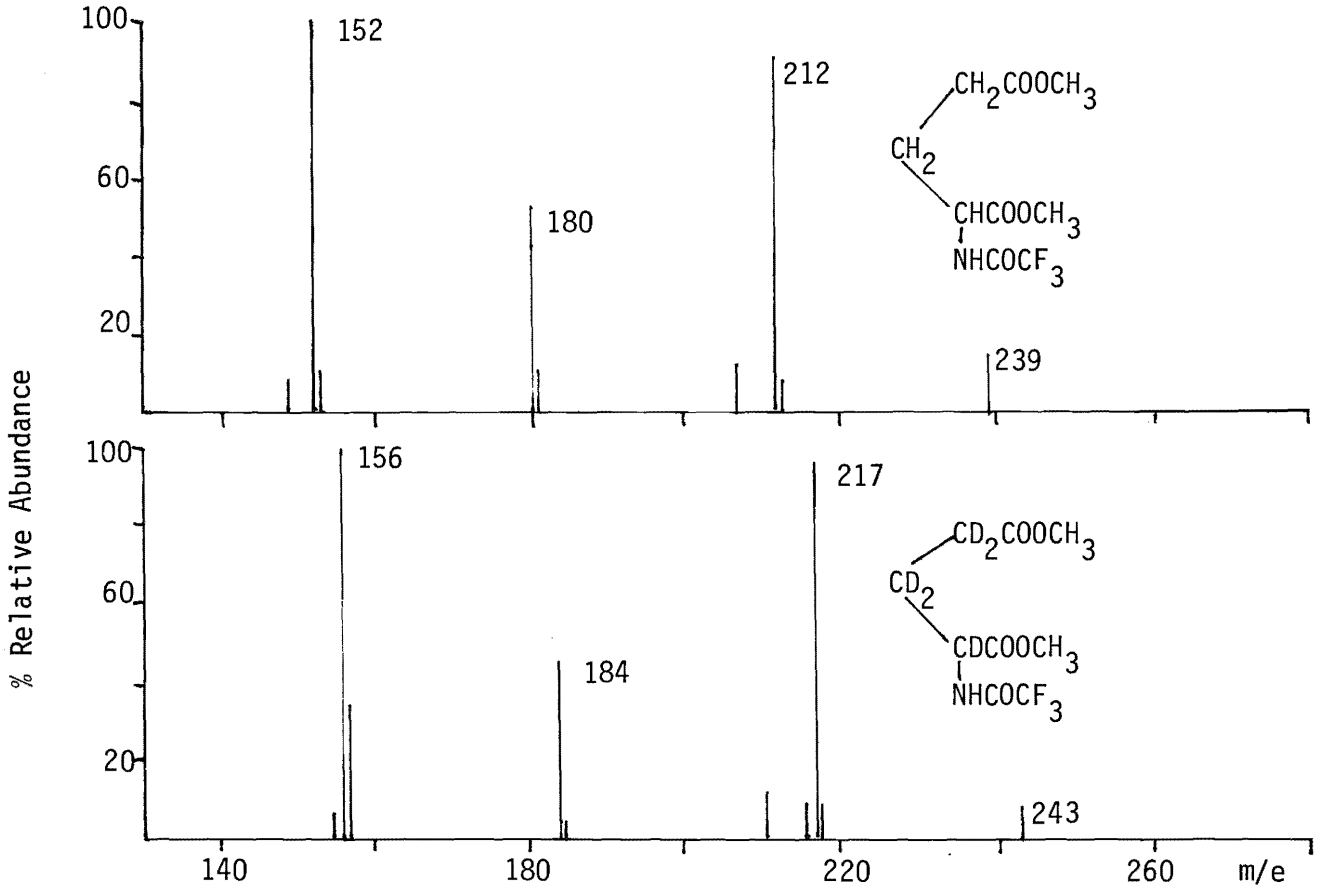


Figure 4. Mass spectra of dimethyl (N-trifluoroacetyl)glutamate (III) and dimethyl (N-trifluoroacetyl)glutamate-2,3,3,4,4-d_5.

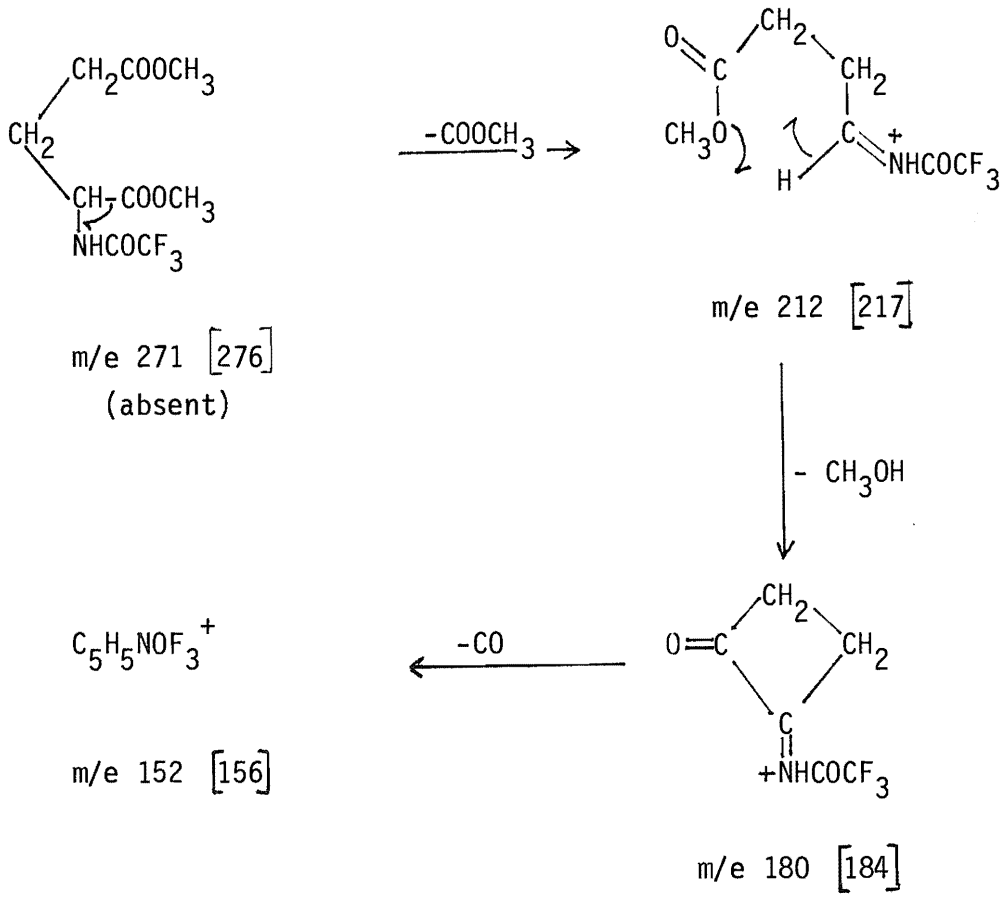


Figure 5. MS fragmentation pattern for dimethyl (N-trifluoroacetyl) glutamate (III). The m/e values of the equivalent ions in the spectrum of d_5-deuterated compound (IV) are in square brackets.

For the quantitative mass spectrometric study, the ions m/e 212 and m/e 217 in the spectra of derivatized glutamic acid (III) and d_5-glutamic acid (IV) respectively, were selected for single ion monitoring.

Separate solutions of 0, 50, 100, 200, 500, 750, 1000, 1500 and 2000 ng of glutamic acid in 25 ml freshly distilled water were prepared and to each was added 600 ng of d_5-glutamic acid (in 50 µl D_2O). Each solution was acidified to pH 4 by the addition of a few drops of dilute hydrochloric acid then slowly (1 ml/min) passed down an Amberlite IR-120 (Mallinckrodt Chemical Works, St. Louis, Missouri) strong acid ion-exchange column (6 cm x 1 cm) followed by 25 ml of distilled water. Eluates were discarded. The column, which retained the glutamic acid and d_5-glutamic acid, was eluted with 7N ammonium hydroxide solution (10 ml) followed by distilled water (5 ml). The combined eluate (15 ml) was evaporated to dryness on a rotary evaporator and the residue was reacted with methanolic hydrogen chloride, followed by trifluoroacetic anhydride as described in Section 7.2.2. A plot of ion current ratio m/e 212/m/e 217 versus nanograms of glutamic acid added was constructed. It was linear (Figure 6). A linear regression program was used in a PDP-8/L digital computer (Digital Equipment Corp., Maynard, Massachusetts) to provide an unweighted linear least squares fit to the data in Figure 6. The calculated slope of the line (m) was 1.514×10^{-3}, and the intercept (c) = 0.038 (correlation coefficient 0.9984). To confirm the accuracy of these figures, two separate solutions containing 382.5 ng and 1275.0 ng of glutamic acid in 100 µl of distilled water were prepared and assayed for their glutamic acid content by the method just described. Calculated values of glutamic acid content were 384.4 ng and 1282.4 ng respectively.

7.2.4 Calculation of the Glutamic Acid Content of an Athabasca River Water Sample

The example which follows is typical. A portion (960 ml) of the water sample adjusted to pH 4 with dilute hydrochloric acid was concentrated on a rotary evaporator at 50°C to about 25 ml. Pentadeuterated glutamic acid (d_5-glutamic acid, 600 ng in 50 µl D_2O) was added and the mixture was slowly passed down an Amberlite IR-120 ion-exchange column (6 cm x 1 cm). Subsequent treatment was identical to

that described in Section 7.2.3. An ion current trace of ions m/e 212 and m/e 217 was recorded (Figure 7) and the ratio m/e 212/m/e 217 (i.e., 6421/5721 = 1.1224) determined. A reagent blank was also determined by repeating the above procedure, except that the 960 ml river water sample was replaced by 25 ml of distilled water. The ion current ratio m/e 212/m/e 217 of the blank was 0.045, in good agreement with the computer-calculated value (0.038). The quantity of glutamic acid, (in nanograms) in the 960 ml sample of river water (x) was calculated from the equation $y = mx + c$, where $y = 1.1224$, $m = 1.514 \times 10^{-3}$ and $c = 0.038$ (see Figure 6). Thus, 960 ml of water sample contained $1.1224-0.039/1.514 \times 10^{-3} = 716.25$ ng glutamic acid, equivalent to 746.1 ng/l. Repeat determinations on different volumes of the same water sample gave values of 738.3 and 755.4 ng/l.

Using the method just described, results were obtained for the five water samples investigated. These results show that small quantities of glutamic acid (0 to 2000 ng) can be readily removed from water sample by concentrating the acidified water sample to a small volume (approximately 25 ml) and passing the concentrate down a strong cation exchange column. The glutamic acid retained on the column is quantitatively eluted with strong ammonia solution. If the residue remaining after evaporation of the column eluate is methylated then trifluoroacetylated, the resulting derivative III is suitable for quantitative analysis by mass spectrometry using the single ion monitoring technique. Although the ions m/e 212 and m/e 217 (see Figures 4 and 5) were selected for single ion monitoring in the present study, other abundant ions in the mass spectra of III and its pentadeuterated derivative (IV), e.g., m/e 180 and m/e 184, m/e 152 and m/e 156, may also be suitable for quantitative mass spectrometry if additional components in a water sample interfered in the assay process employing ions m/e 212 and m/e 217.

The quantitative mass spectrometric method used in the present study is reproducible provided certain precautions are taken. All solutions of glutamic acid and d_5-glutamic acid should be freshly prepared in freshly double-distilled water. The strengths of solutions containing 2000 ng or less per litre of these acids slowly fall on storage due presumably to their adsorption onto glass surfaces. For the same reason, river water samples should be assayed

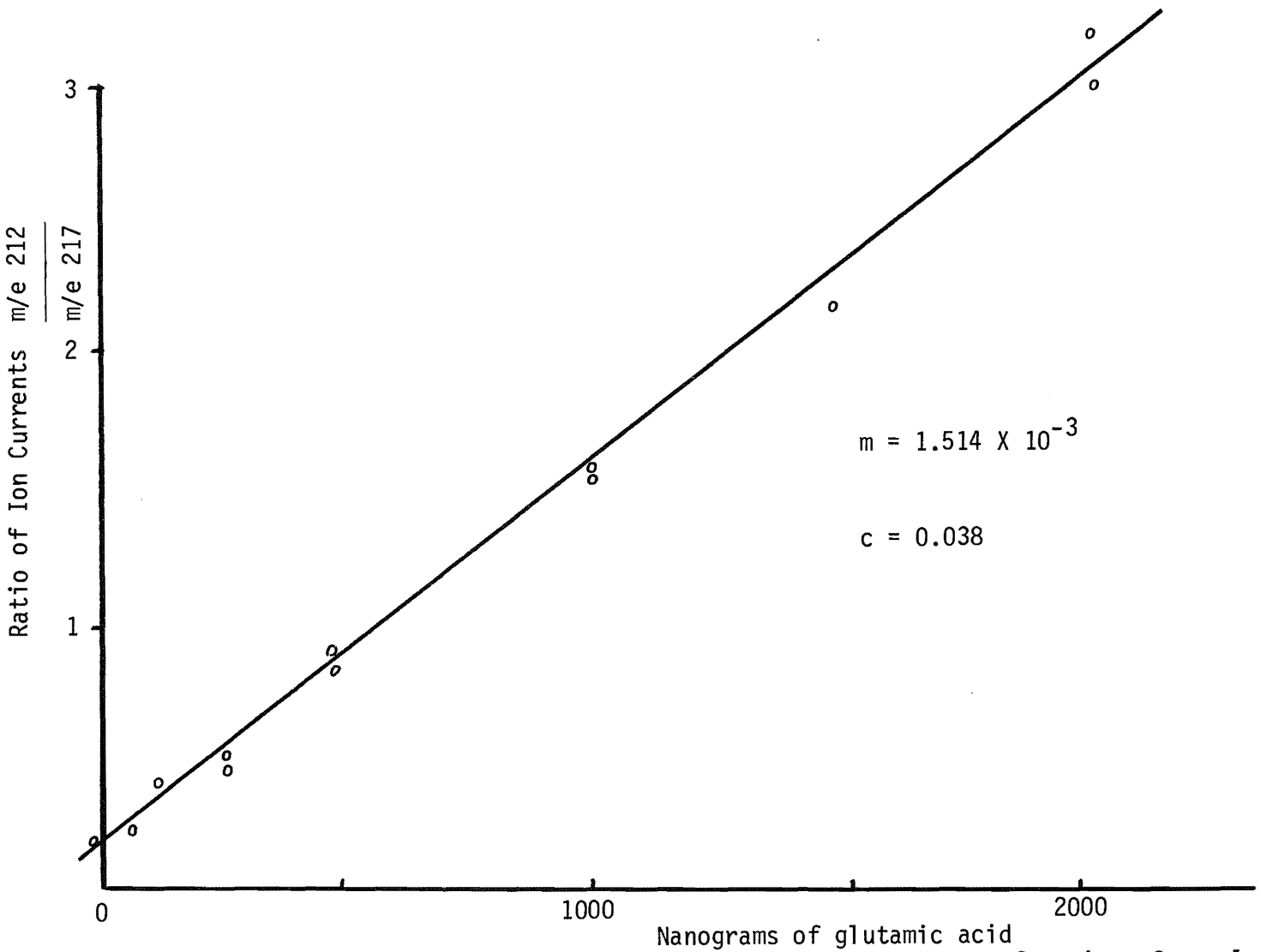


Figure 6. Calibration curve: Ratio of single ion current responses as a function of sample size. Line indicates the best fit by linear regression.

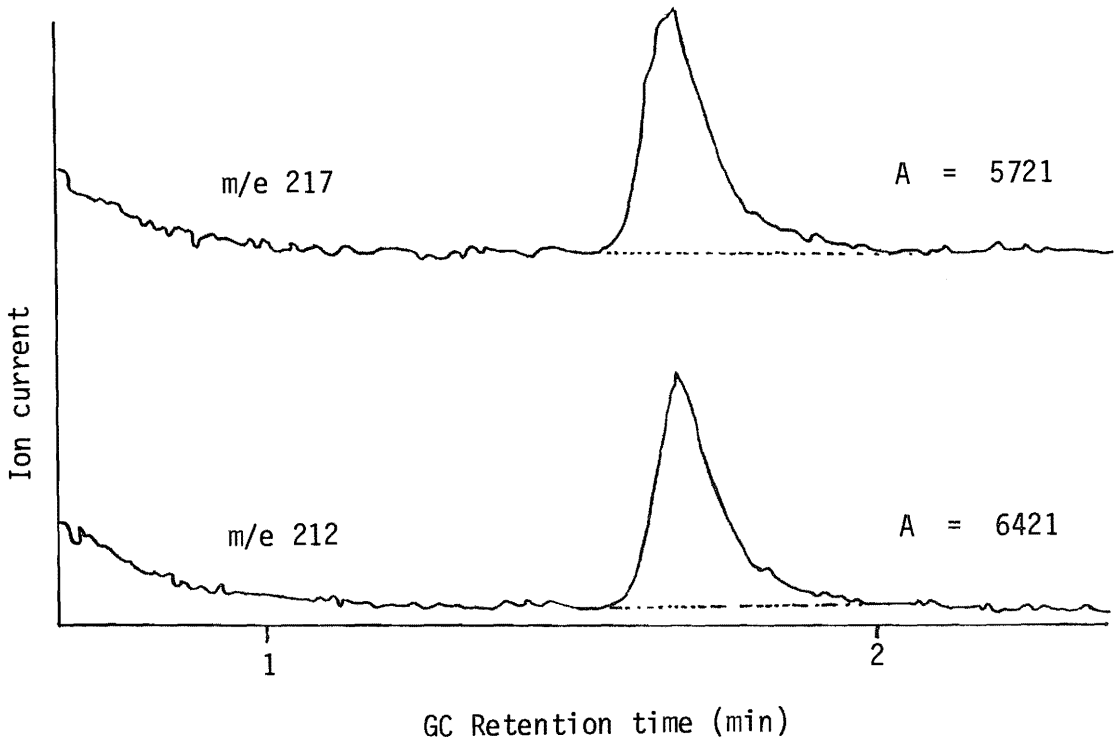


Figure 7. Selected ion chromatogram. Computer plots of the ion currents of two selected ions (m/e 212 and m/e 217). A = Area of ion current peak.

for glutamic acid content immediately after collection or should be frozen during storage. Since mass spectrometer detector responses vary to a small extent whenever the instrument is tuned, fresh calibration curves must be constructed prior to a series of analyses.

This new method of analysis of trace amounts of glutamic acid in water samples was still not ideal because the method required a tedious concentration of the water sample to a small volume (25 ml). Experiments have been carried out to see if this concentration step could be omitted from the analytical procedure. A larger Amberlite IR-120 ion-exchange column (10 cm x 1.2 cm) was used and a portion (750 ml) of river water sample 10 was passed down this column at a rate of 20 ml/min, followed by 25 ml of distilled water. The column was then eluted with 7N ammonium hydroxide (25 ml) followed by distilled water (25 ml). The combined eluate (50 ml) was evaporated to dryness on a rotatory evaporator and the residue was converted as described in Section 7.2.2. The glutamic acid content of the converted residue was calculated from the equation $y = mx + c$ as described before, and found to be 752.1 ng/l, in excellent agreement with the value obtained (746.6 ng/l) when the water sample was initially concentrated. When water sample 7 was similarly analyzed without initial concentration, the glutamic acid content (712.4 ng/l) was in reasonable agreement with the value obtained (659.9 ng/l) when sample was initially concentrated. Further studies on this topic are required.

7.2.5 Conclusion

A sensitive, specific and reproducible method of determining the glutamic acid content of environmental water samples has been developed. Both 'free' (i.e., unbound) glutamic acid and 'bound' (i.e., glutamic acid in conjugated form) glutamic acid in a water sample could be estimated by this method by a) passing the water sample immediately after collection down the Amberlite IR-120 column to remove the 'free' glutamic acid, then b) hydrolyzing the eluate from the column by heating it under reflux after the addition of hydrochloric acid (which would release glutamic acid from its 'bound' form), and passing the resulting solution down another Amberlite IR-120 column.

The method could also be applied to the analysis of other amino acids in the water sample.

7.3 RAW DATA FROM HETEROTROPHIC POTENTIAL ANALYSIS OF ^{14}C-GLUTAMIC ACID.

Table 16 summarizes the data collected from 7 sites in October 1977 and 3 sites in January 1978. For a explanation of the theory and a definition of terms, refer to Section 2.2.

Table 16. Raw data from heterotrophic potential analysis of ^{14}C-glutamic acid[a].

Site	A (µg/l)	U (MICROCURIE)	MEAN DISINTEGRATIONS/MIN RESPIRED	ASSIMILATED	(CORRECTED) TOTAL UPTAKE	PERCENT RESPIRED	Cut/c (HOURS)
1A (OCT.)	.800	.0126	15.7	50.7	66.4	23.6	422.0
DILUTION = 1/10	3.200	.0505	263.8	129.5	393.4	67.1	284.8
INCUBATION = 2 H	4.800	.0757	206.9	203.0	409.9	50.5	410.0
	8.000	.1262	417.1	271.3	688.5	60.6	406.8
	16.000	.2523	791.6	385.5	1177.0	67.3	476.0
2 (OCT.	.800	.0126	37.9	195.7	233.6	16.2	119.9
DILUTION = 1/10	1.600	.0252	30.8	356.9	387.7	7.9	144.5
INCUBATION = 1 H	3.200	.0505	95.3	389.3	484.6	19.7	231.2
	4.800	.0757	279.9	612.3	892.3	31.4	188.4
	8.000	.1262	59.5	869.7	929.2	6.4	301.5
	16.000	.2523	166.4	1294.1	1460.5	11.4	383.6
4 (OCT.)	.800	.0126	73.5	149.7	223.2	32.9	125.5
DILUTION = 1/10	1.600	.0252	15.9	269.9	285.8	5.6	196.0
INCUBATION = 1 H	3.200	.0505	166.4	465.6	632.0	26.3	177.0
	8.000	.1262	52.2	1004.9	1057.1	4.9	265.0

continued . . .

Table 16. Continued

Site	A (µg/l)	U (MICROCURIE)	MEAN DISINTEGRATIONS/MIN RESPIRED	ASSIMILATED	(CORRECTED) TOTAL UPTAKE	PERCENT RESPIRED	Cut/c (HOURS)
7 (OCT.)	.800	.0126	71.6	318.8	390.4	18.3	71.7
DILUTION = 1/10	1.600	.0252	231.2	378.3	609.5	37.9	91.9
INCUBATION = 1 H	4.800	.0757	696.3	596.3	1292.6	53.9	130.0
	8.000	.1262	293.1	505.6	798.6	36.7	350.7
10 (OCT.)	.800	.0126	36.5	59.3	95.8	38.1	292.3
DILUTION = 1/10	1.600	.0252	95.2	114.1	209.2	45.5	267.7
INCUBATION = 1 H	3.200	.0505	156.5	169.3	325.7	48.0	344.0
	4.800	.0757	418.3	199.5	617.8	67.7	272.0
	8.000	.1262	52.6	254.8	307.4	17.1	911.2
	16.000	.2523	122.3	371.5	493.8	24.8	1134.6
A (OCT.)	.800	.0126	1040.1	3820.0	4860.1	21.4	5.8
DILUTION = 1/10	1.600	.0252	1200.4	4578.9	5779.3	20.8	9.7
INCUBATION = 1 H	3.200	.0505	1387.8	6147.7	7535.4	18.4	14.9
	4.800	.0757	1495.6	7384.5	8880.0	16.8	18.9
	8.000	.1262	1694.1	9314.4	11008.5	15.4	25.4
	16.000	.2523	2512.0	9646.8	12158.8	20.7	46.1
B (OCT.)	.800	.0128	146.0	170.7	318.7	46.1	88.4
DILUTION = 1/10	1.600	.0252	124.7	224.7	349.4	35.7	160.3
INCUBATION = 1 H	3.200	.0505	469.8	253.4	712.7	64.4	157.2
	4.800	.0757	169.4	279.8	449.1	37.7	374.2
	8.000	.1262	593.1	428.5	1021.6	58.1	274.2

continued . . .

Table 16. Continued

Site	A (µg/l)	U (MICROCURIE)	MEAN DISINTEGRATIONS/MIN RESPIRED	ASSIMILATED	(CORRECTED) TOTAL UPTAKE	PERCENT RESPIRED	Cut/c (HOURS)
4 (JAN.)	1.450	.0251	808.4	46.1	854.5	94.6	65.3
DILUTION 1/10	2.900	.0503	162.5	86.6	249.1	65.2	448.0
INCUBATION = 1 H	2.350	.0754	249.7	172.2	421.9	59.2	396.8
	7.250	.1257	453.2	178.3	631.5	71.8	441.8
	14.500	.2514	897.1	244.6	1141.7	78.6	488.8
	29.000	.5027	1280.5	801.2	2081.7	61.5	536.1
5A (JAN.)	2.900	.0503	145.6	224.7	370.3	39.3	301.4
DILUTION 1/2	4.350	.0754	219.4	447.0	666.4	32.9	251.2
INCUBATION = 1 H	7.250	.1257	330.7	507.6	838.4	39.4	332.8
	14.500	.2514	418.9	783.2	1202.1	34.8	464.2
	29.000	.5027	962.4	999.3	1961.7	49.1	568.9
7 (JAN.)	1.450	.0251	481.6	1748.9	2230.4	21.6	25.0
DILUTION = 1/2	2.900	.0503	318.2	2191.8	2510.0	12.7	44.5
INCUBATION = 1 H	4.350	.0754	325.5	3880.1	4205.6	7.7	39.8
	7.250	.1257	1219.9	5663.5	6883.4	17.7	40.5
	14.500	.2314	1739.4	7731.6	9471.0	18.4	58.9
	29.000	.5027	4740.6	5792.8	10533.4	45.0	106.0

continued . . .

Table 16. Concluded

Site	A (µg/l)	U (MICROCURIE)	MEAN DISINTEGRATIONS/MIN RESPIRED	ASSIMILATED	(CORRECTED) TOTAL UPTAKE	PERCENT RESPIRED	Cut/c (HOURS)
A (JAN.)	1.450	.0251	443.9	4665.2	5109.1	8.7	10.9
DILUTION = 1/5	2.900	.0503	1733.9	8352.1	10086.0	17.2	11.1
INCUBATION = 1 H	4.350	.0754	0.0	10030.5	10030.5	8.0	16.7
	7.250	.1257	1897.4	14153.7	16051.1	11.8	17.4
	14.500	.2514	1401.3	18832.5	20233.8	6.9	27.6
	19.030	.3299	3327.6	22145.6	25473.2	13.1	28.7
B (JAN.)	1.450	.0251	105.5	0.0	105.5	100.0	528.8
DILUTION = 1/10	2.900	.0503	9.2	29.0	38.2	24.2	2920.8
INCUBATION = 1 H	4.350	.0754	3.9	96.3	100.1	3.9	1671.7
	7.250	.1257	0.0	46.4	46.4	0.0	6017.2
	14.500	.2514	0.0	33.4	33.4	0.0	16704.6
	29.000	.5027	242.2	59.0	301.2	80.4	3705.6

[a] V_{max} and t_n were calculated by computer plot of Cut/c versus. A where $C = 2.22 \times 10^6$ disintegrations/min (DPM).

7.4 GC TRACES OF NEUTRAL COMPOUNDS FROM RIVER SEDIMENTS.

Traces of neutral compounds in sediments, of reference solutions, and of authentic compounds are summarized in Figures 8 to 16.

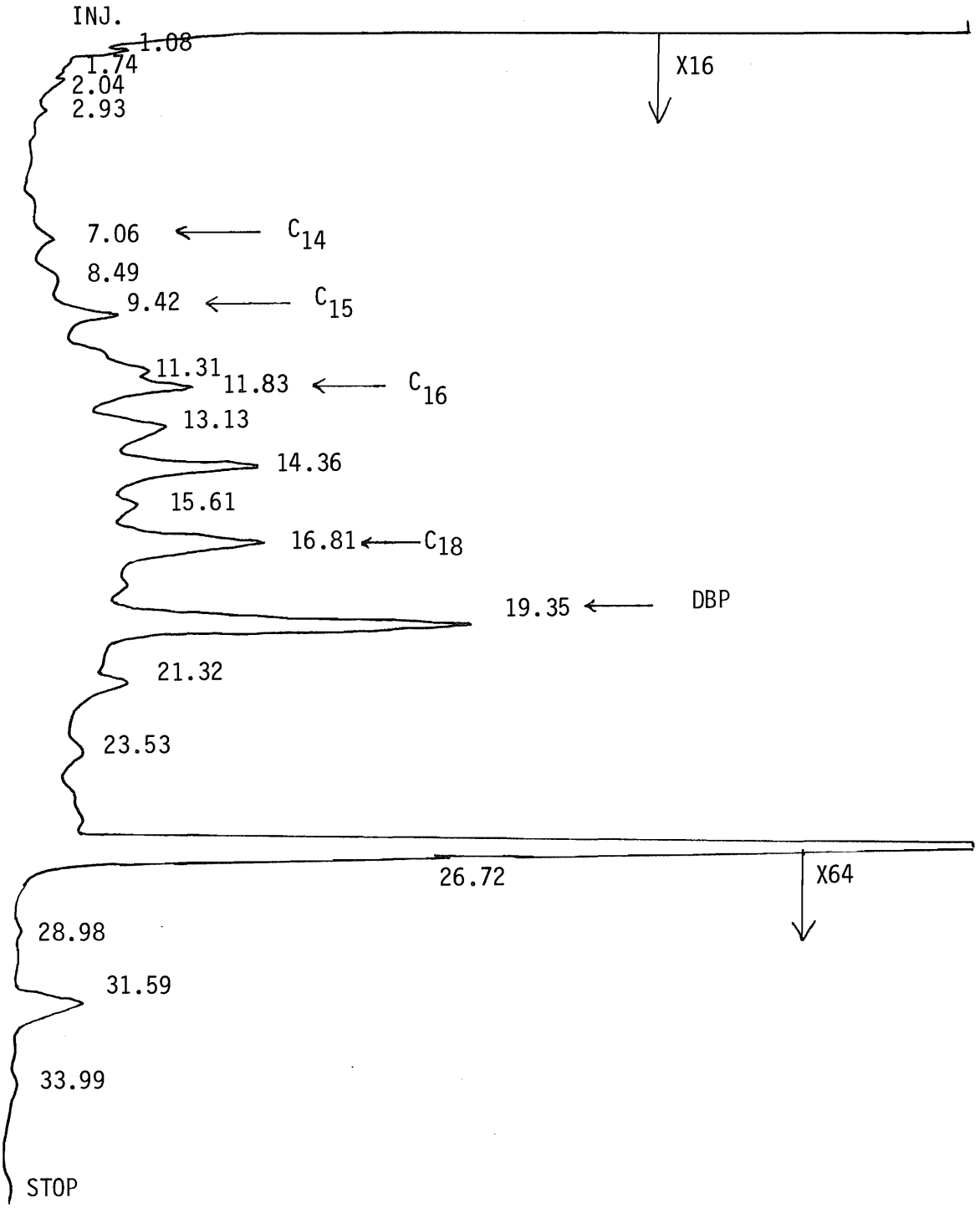


Figure 8. GC trace of neutral compounds in sediments 1A.

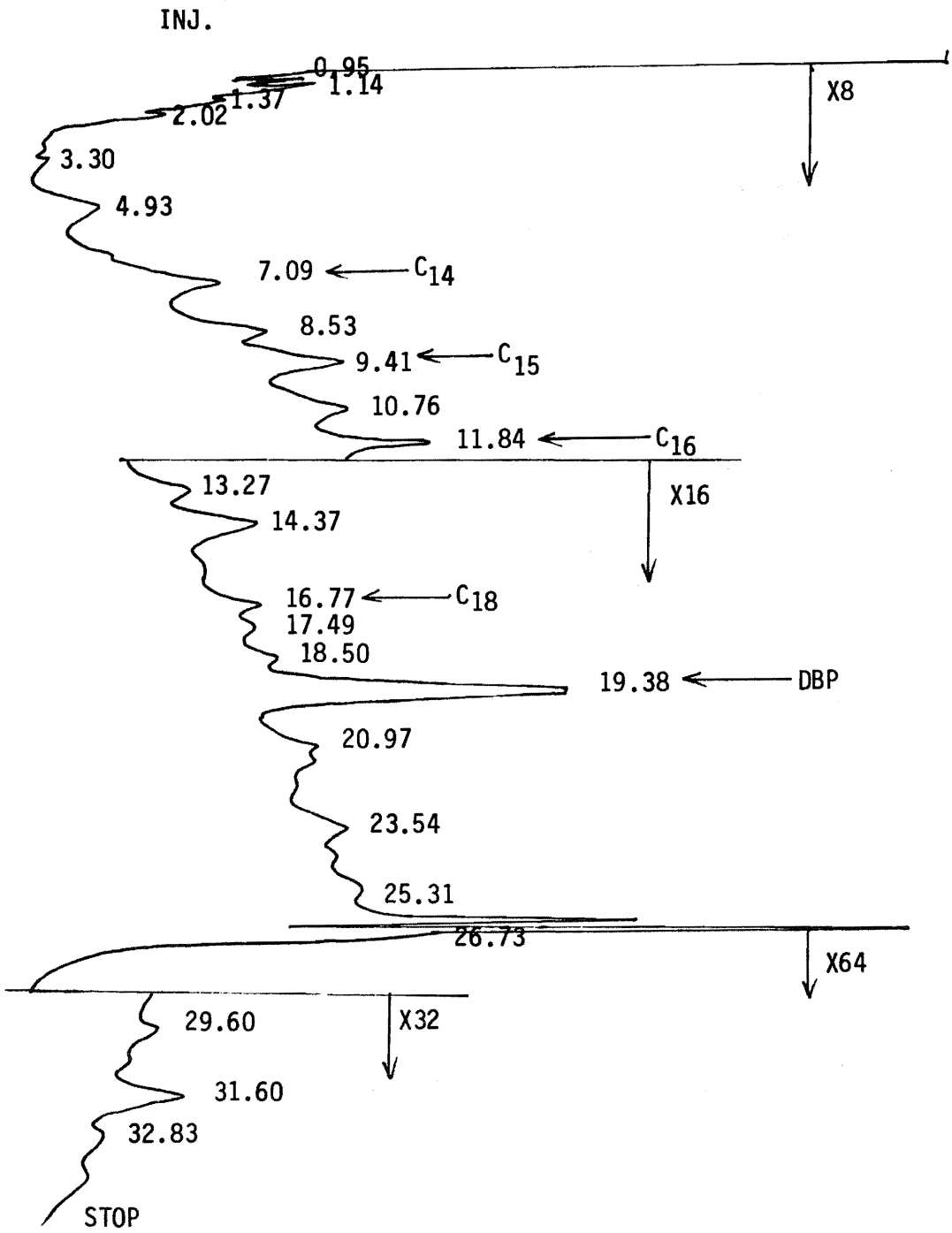


Figure 9. GC trace of neutral compounds in sediment 2.

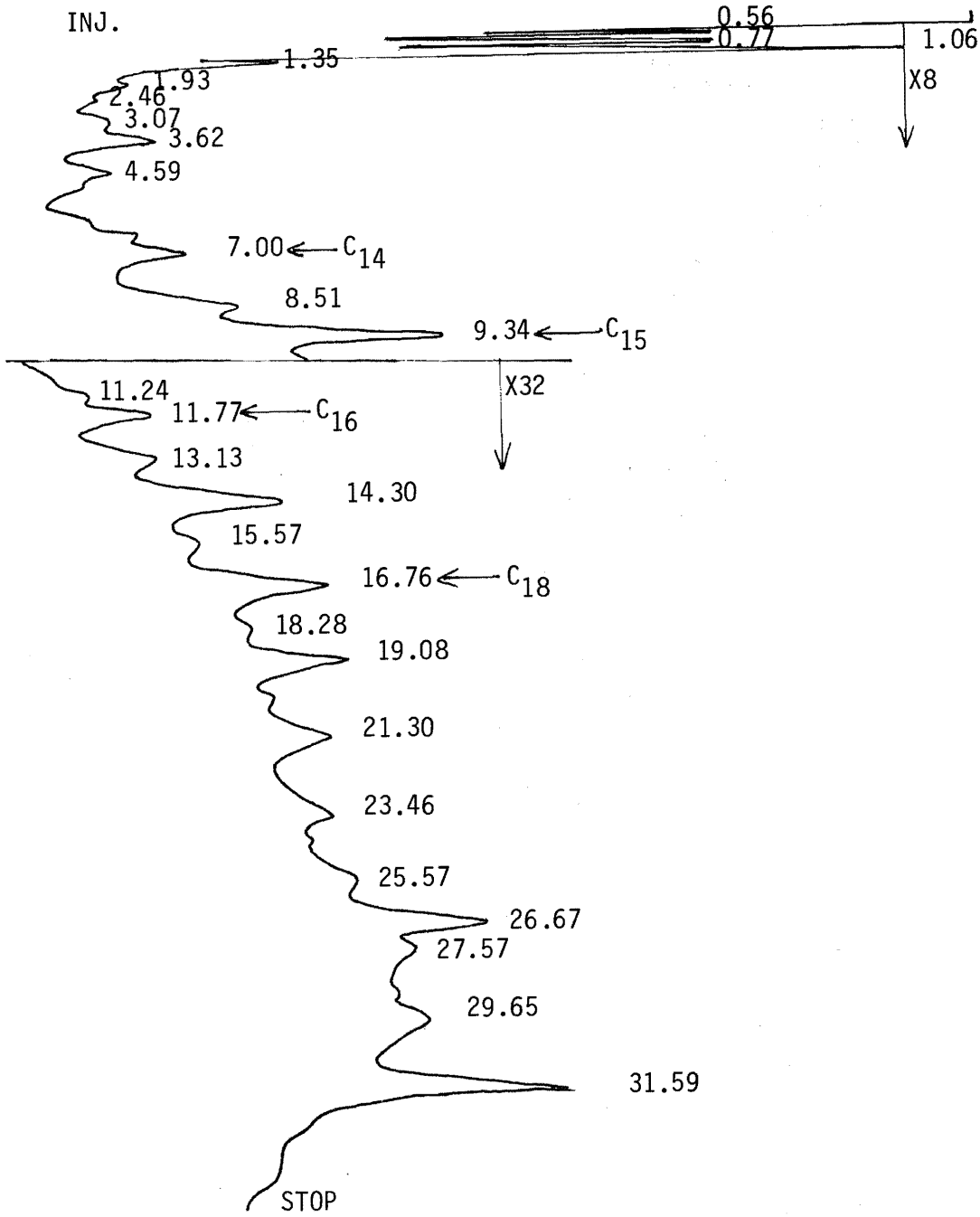


Figure 10. GC trace of netural compounds in sediment 4.

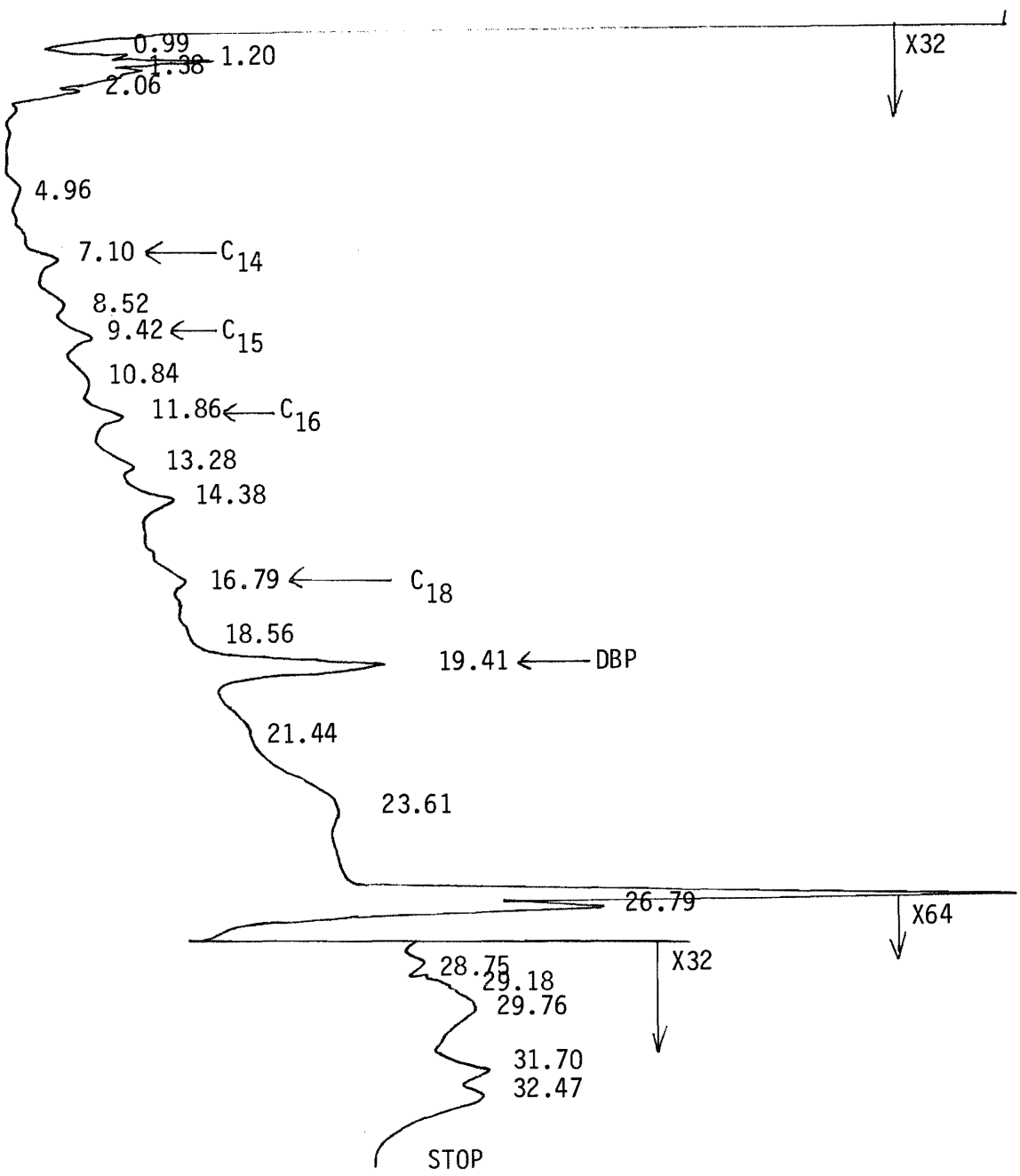


Figure 11. GC trace of neutral compounds in sediment 5A.

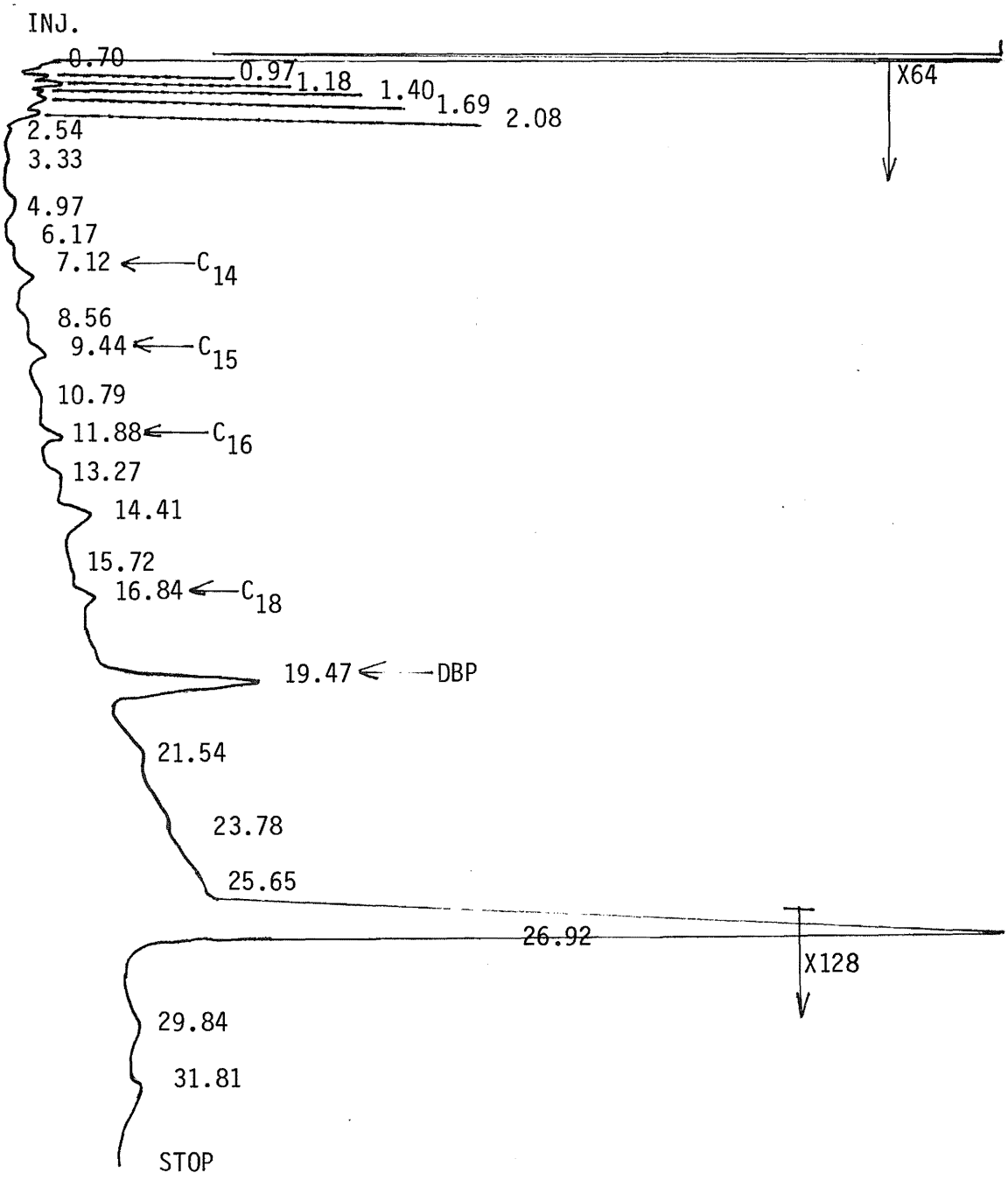


Figure 12. GC trace of neutral compounds in sediment 7.

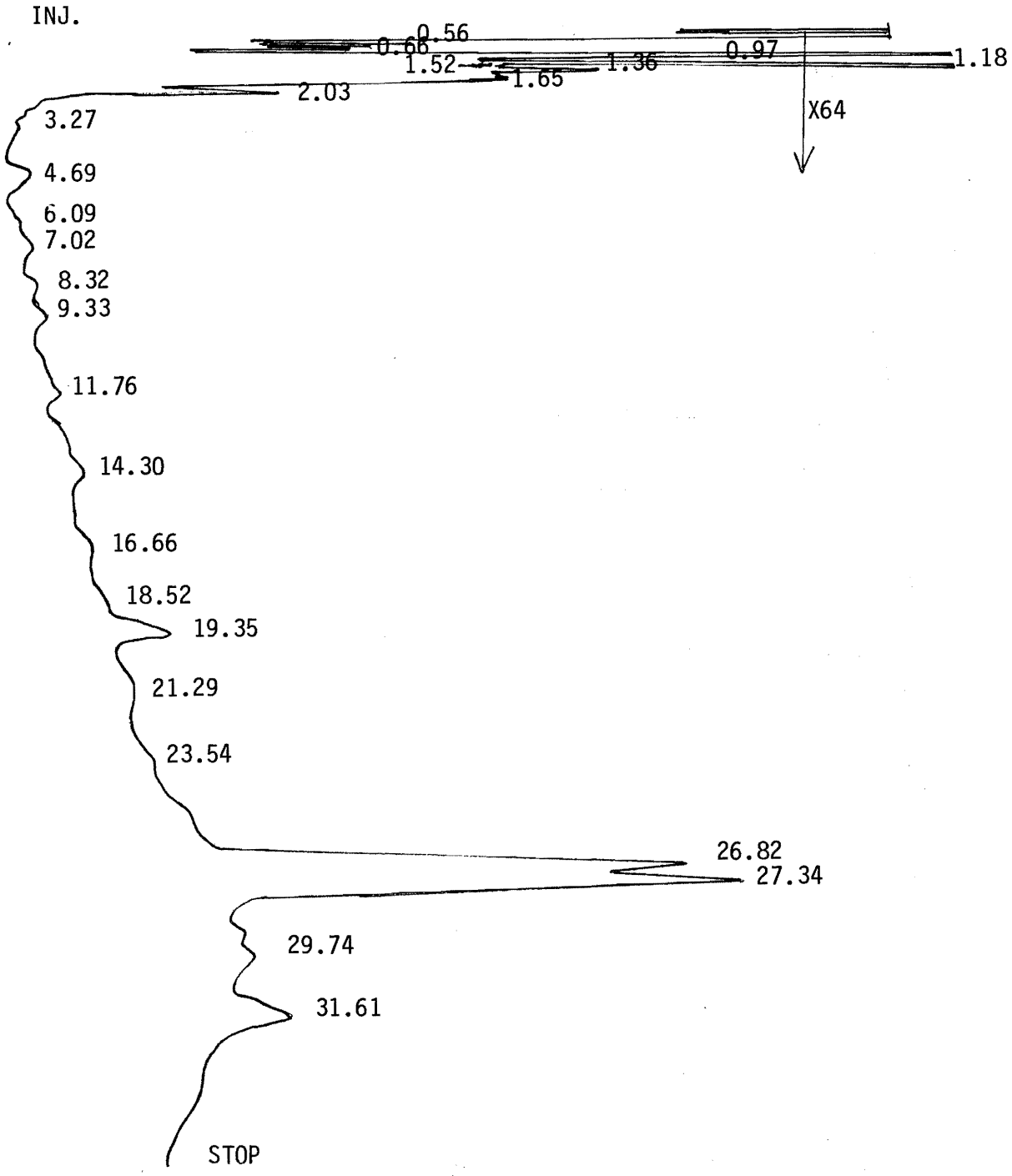


Figure 13. GC trace of neutral compounds in sediment 10.

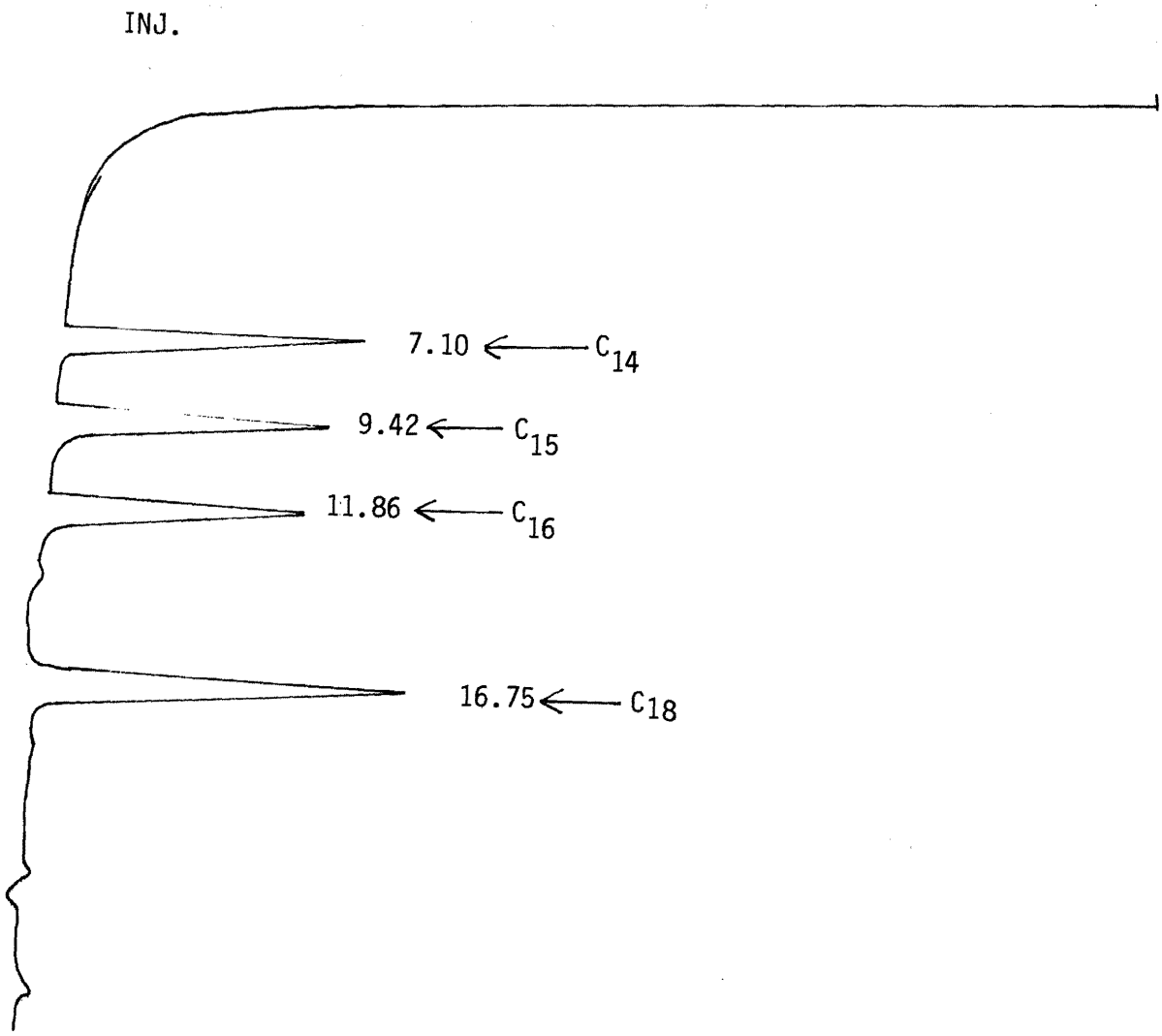


Figure 14. GC trace of reference solution (beginning of run).

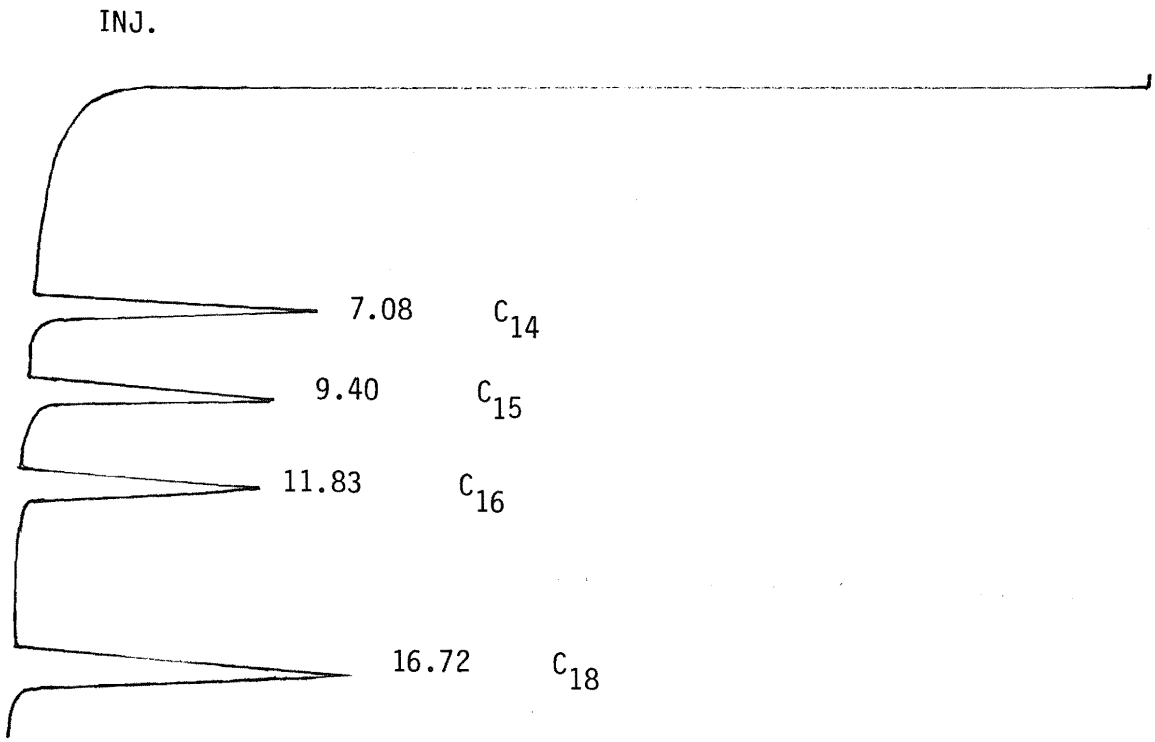


Figure 15. GC trace of reference solution (middle of run).

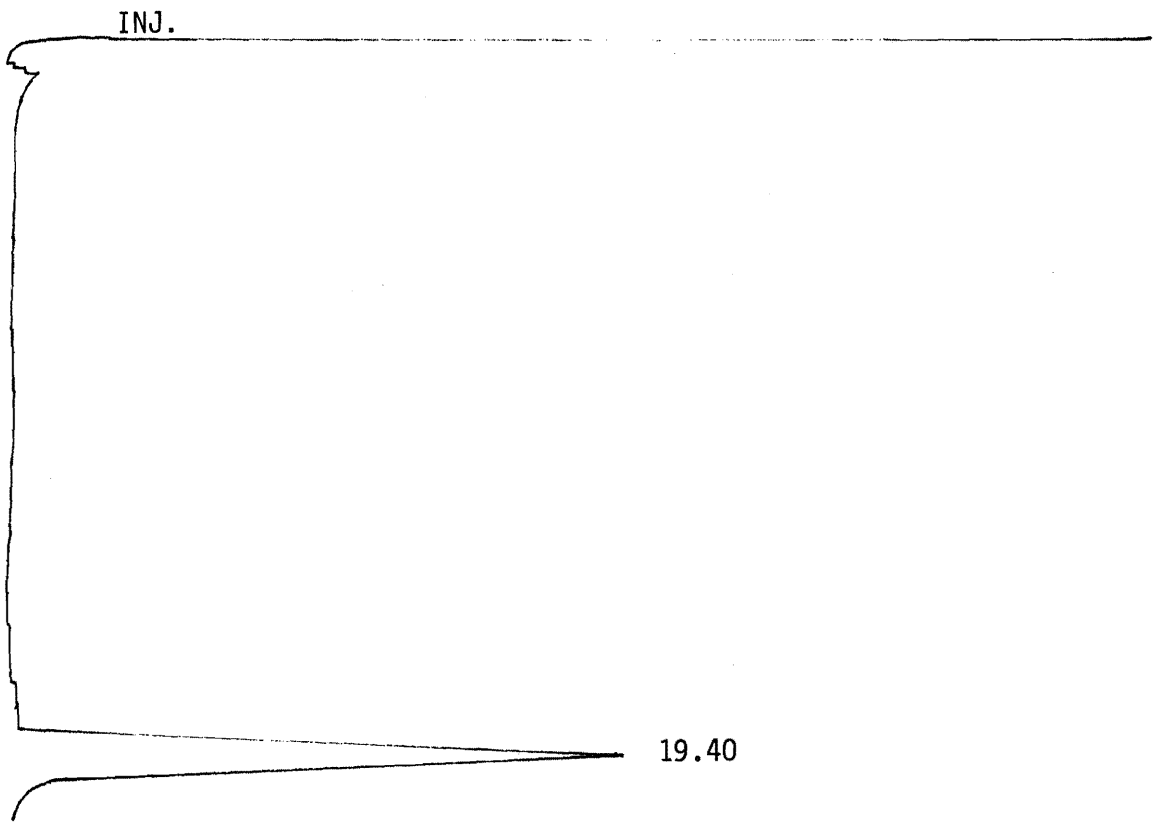


Figure 16. Authentic di-n-butyl phthalate.

7.5 GC TRACES OF ACIDIC COMPOUNDS FROM RIVER SEDIMENTS

Traces of organic acids as n-butyl esters in sediments are shown in Figure 17 to 22.

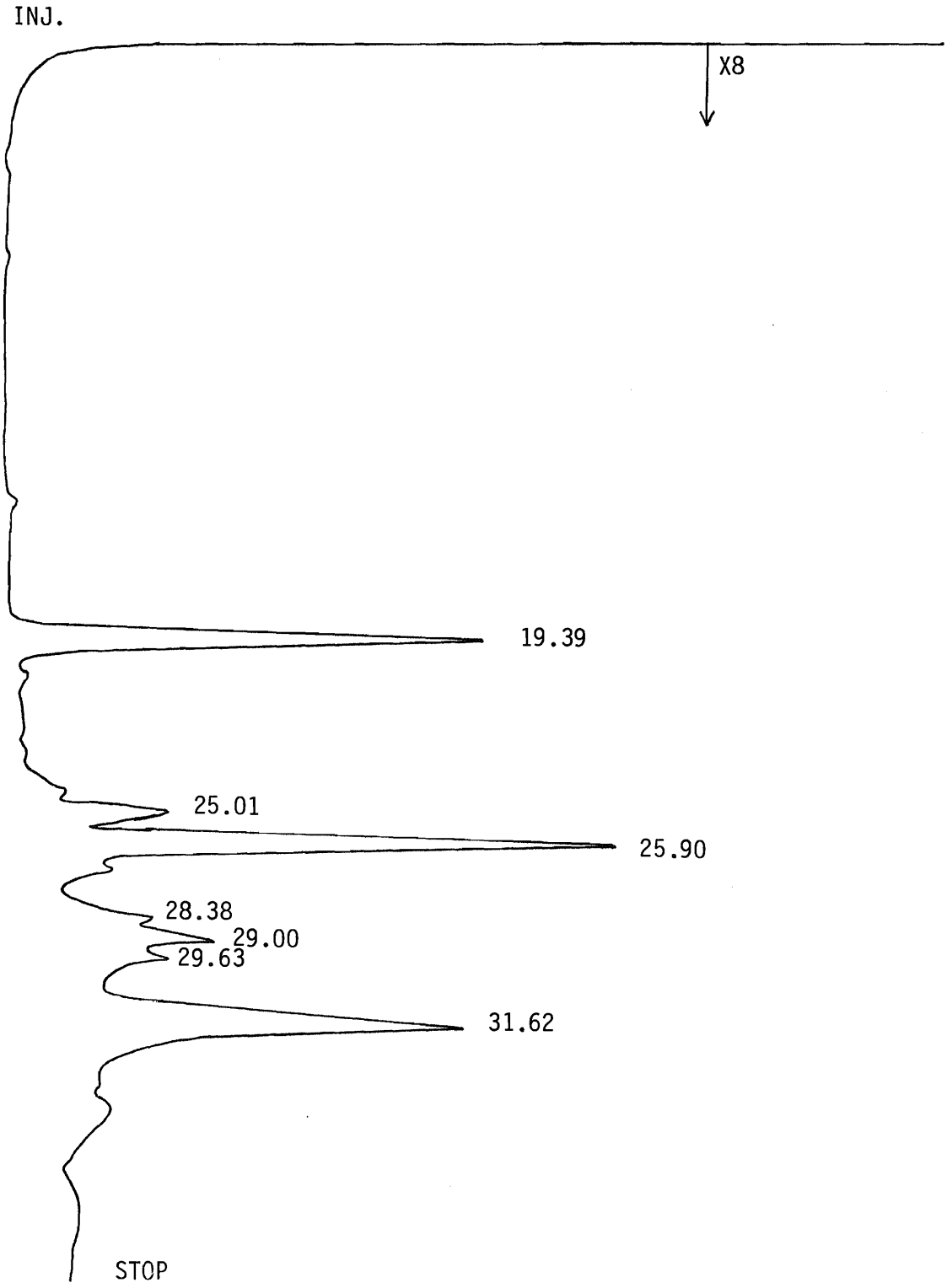


Figure 17. GC trace of organic acids (as n-butyl esters) in sediment 1A.

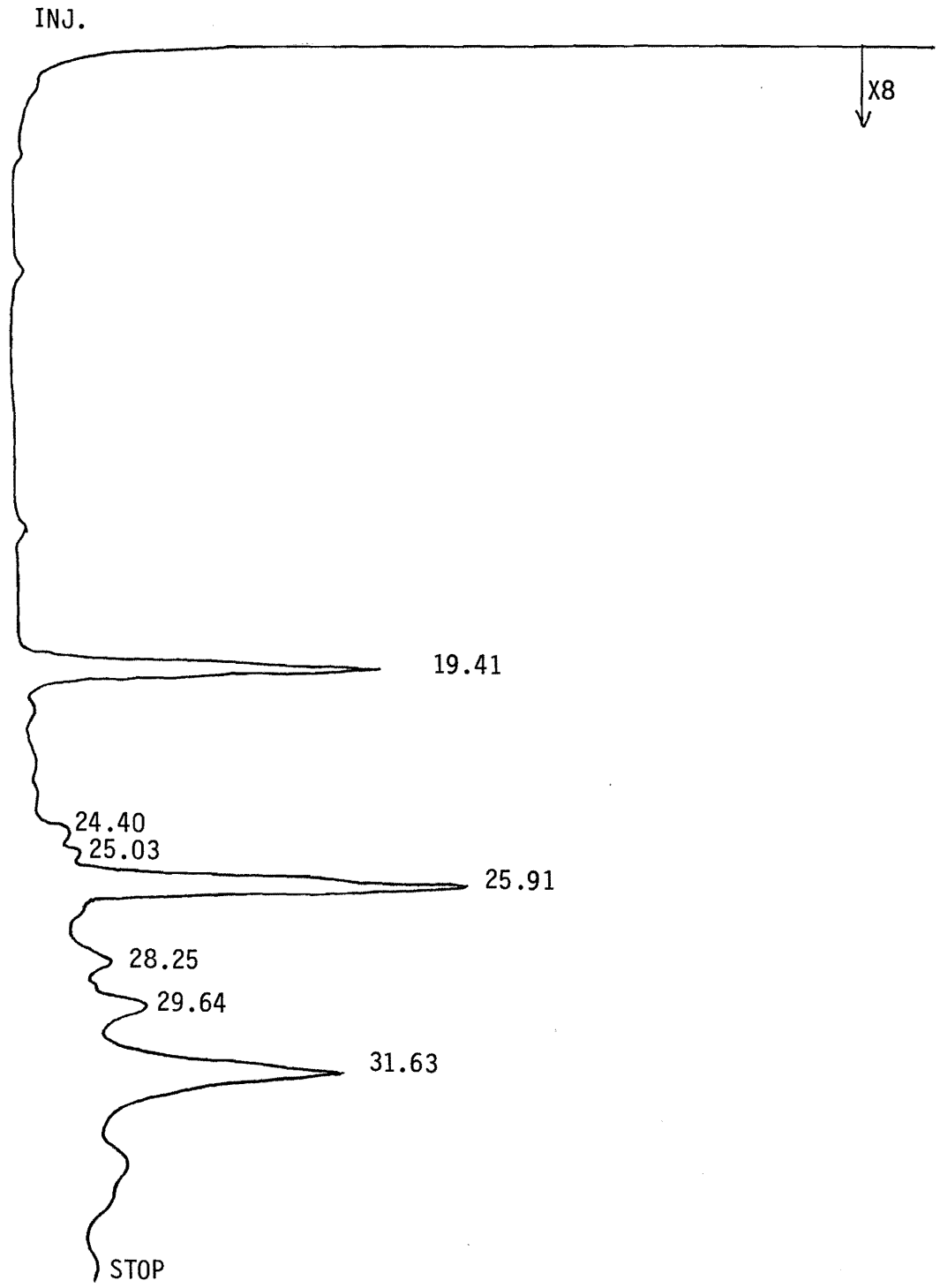


Figure 18. GC trace of organic acids (as n-butyl esters) in sediment 2.

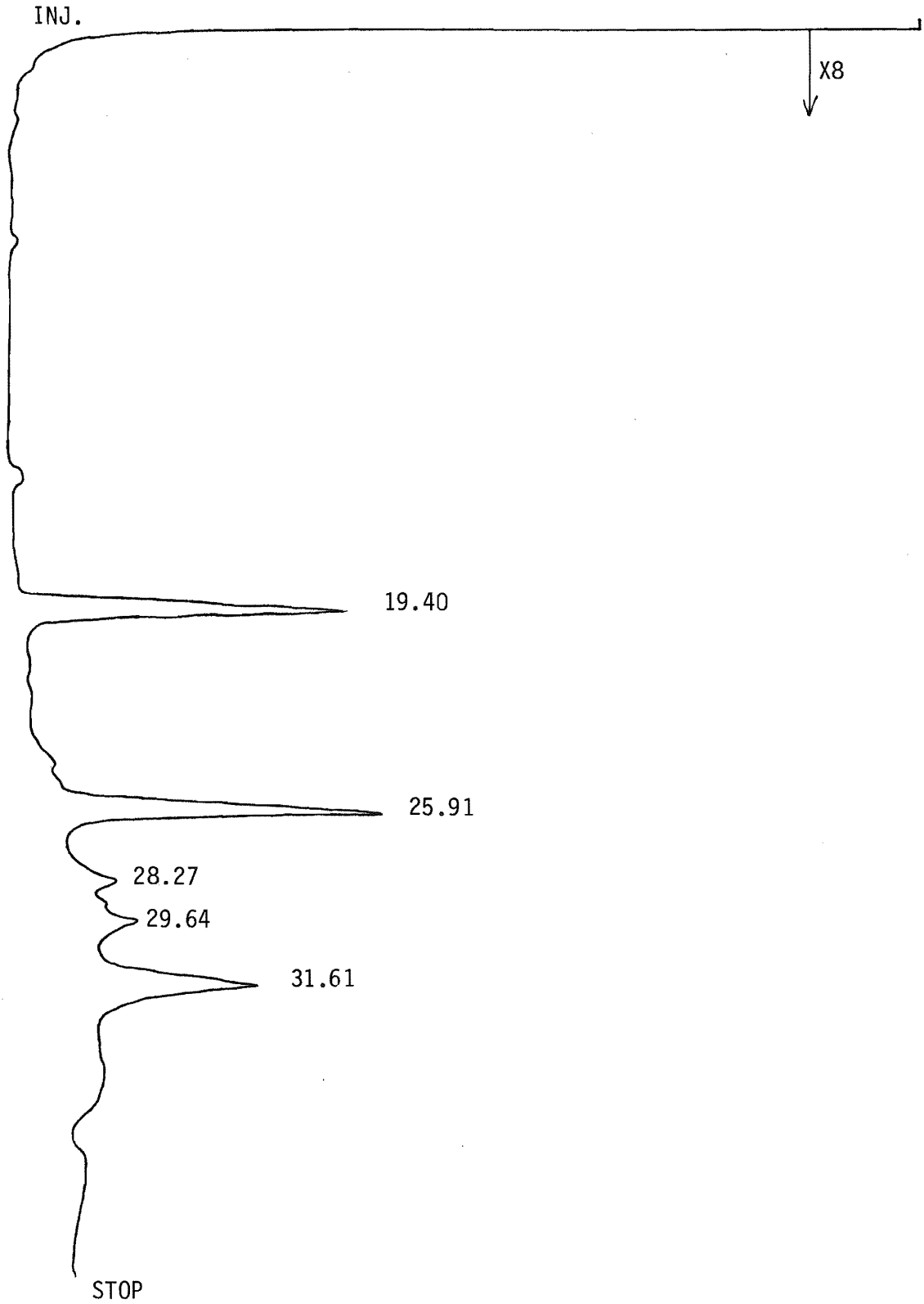


Figure 19. GC trace of organic acids (as n-butyl esters) in sediment 4.

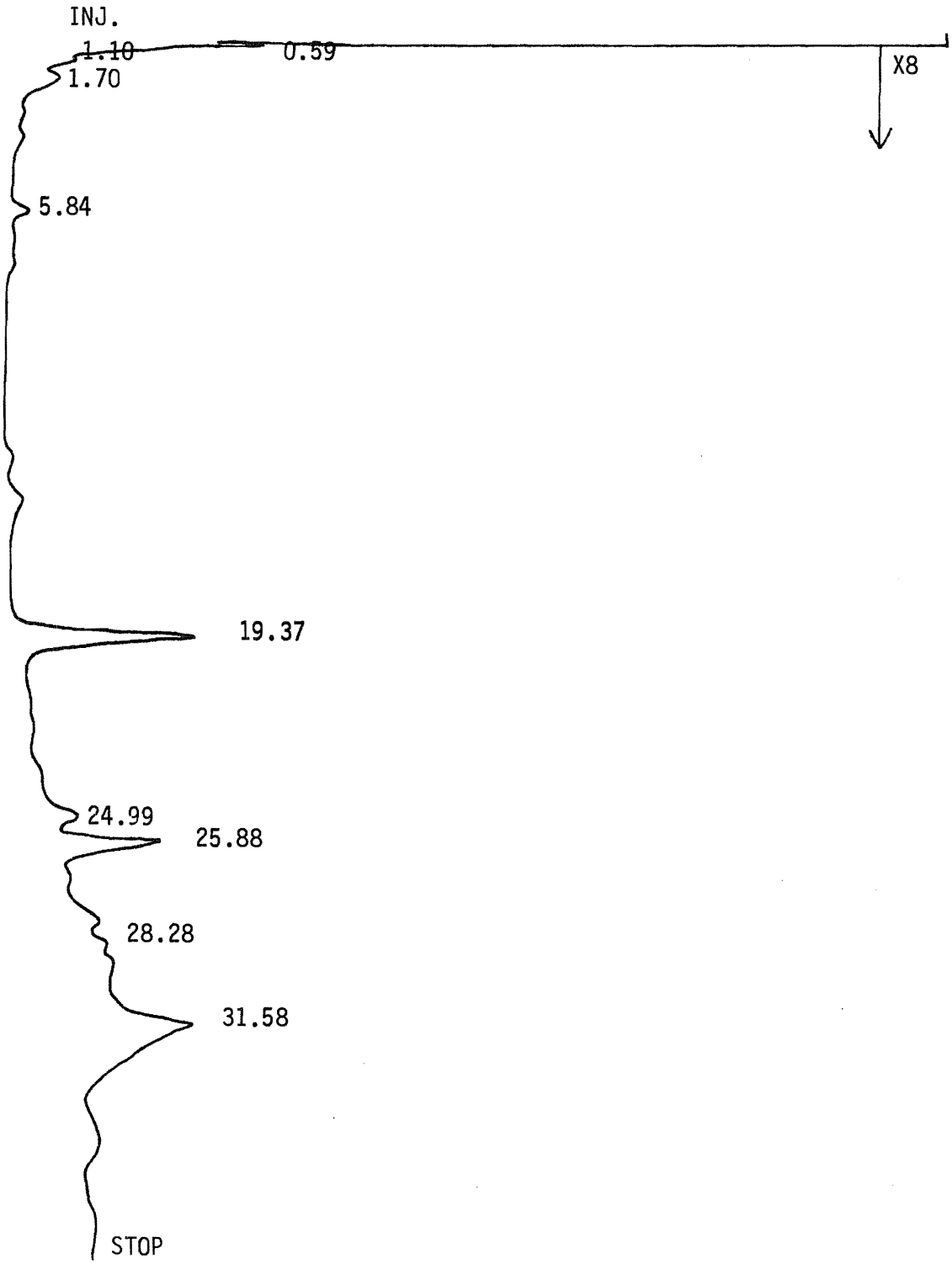


Figure 20. GC trace of organic acids (as n-butyl esters) in sediment 5A.

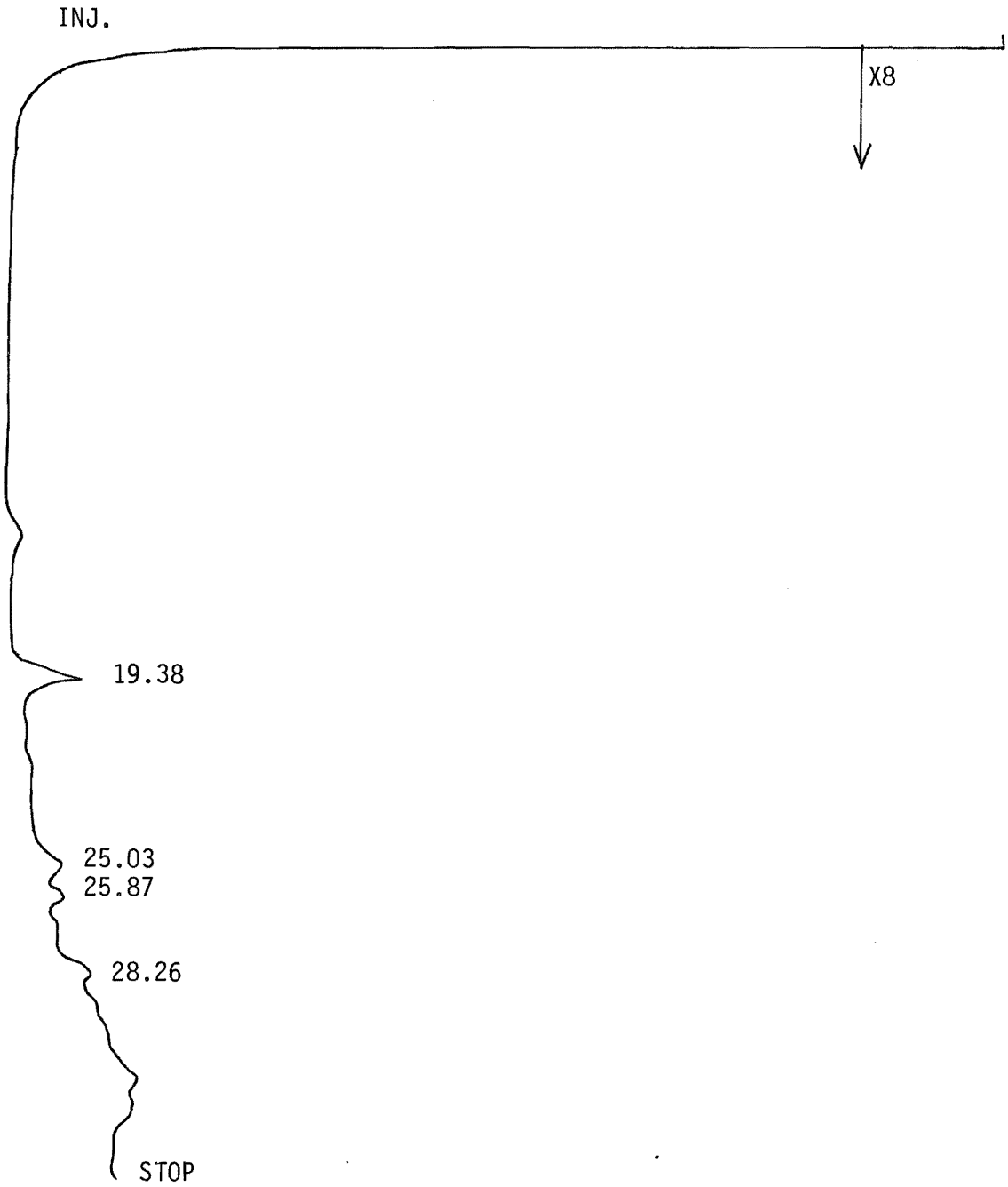


Figure 21. GC trace of organic acids (as n-butyl esters) in sediment 7.

Figure 22. GC trace of organic acids (as n-butyl esters) in sediment 10.

7.6 GC TRACES OF NEUTRAL COMPOUNDS FROM RIVER WATER.

Figures 23 to 27 illustrate traces of neutral compounds in acidified river water samples, reference solutions, and frozen river water samples.

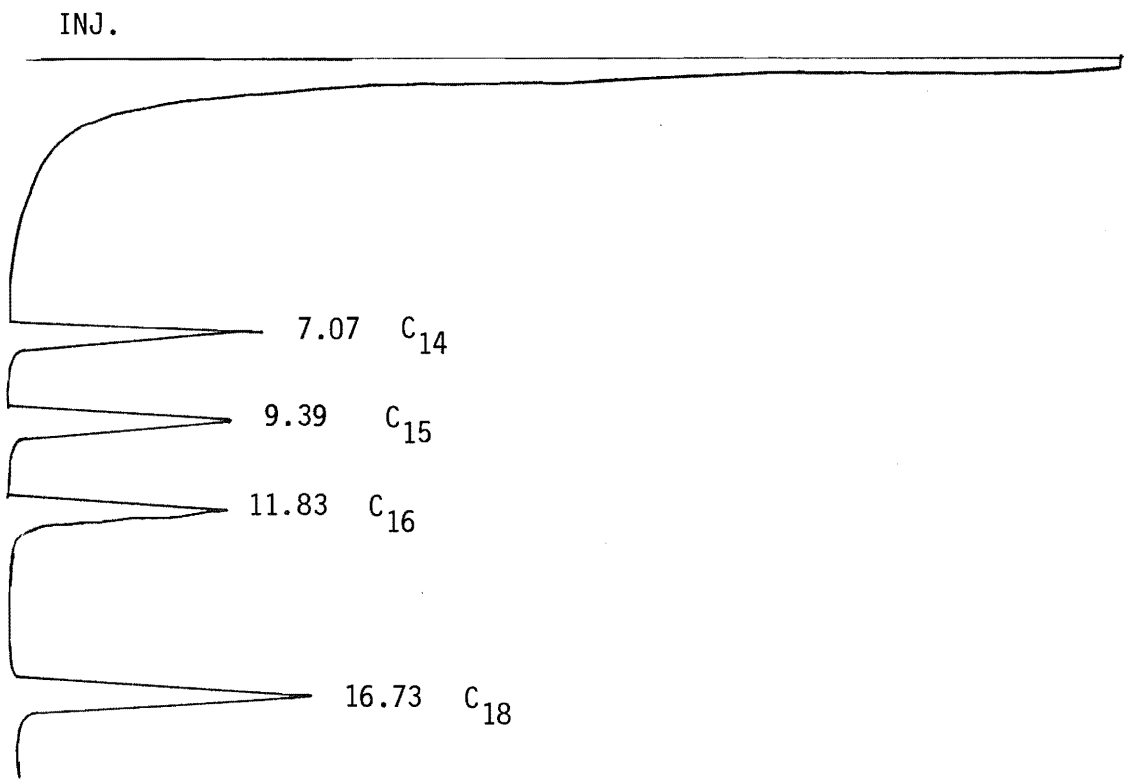


Figure 23. GC trace of reference solution.

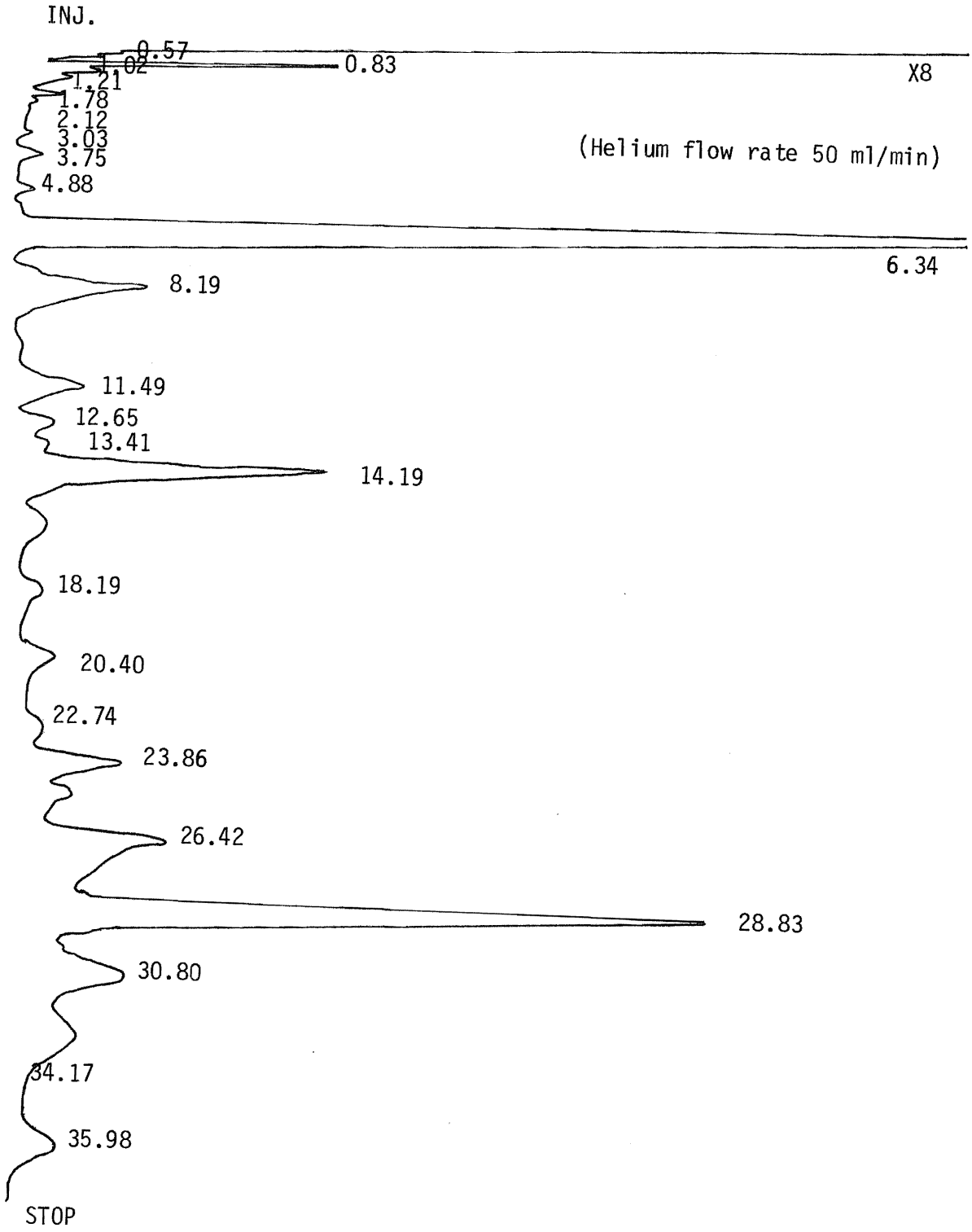


Figure 24. GC trace of neutral compounds in acidified water sample 1A.

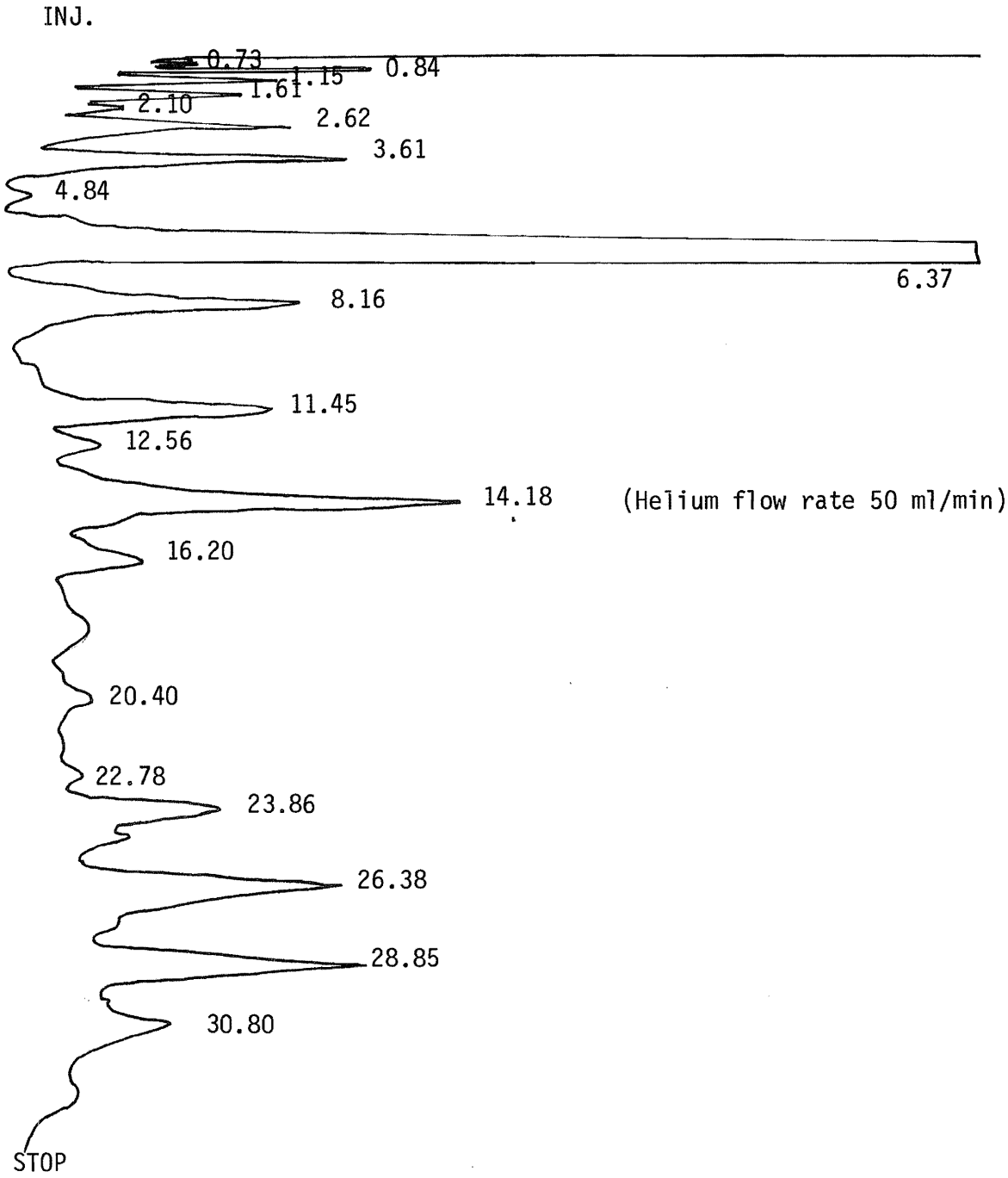


Figure 25. GC trace of neutral compounds in acidified water sample 5A.

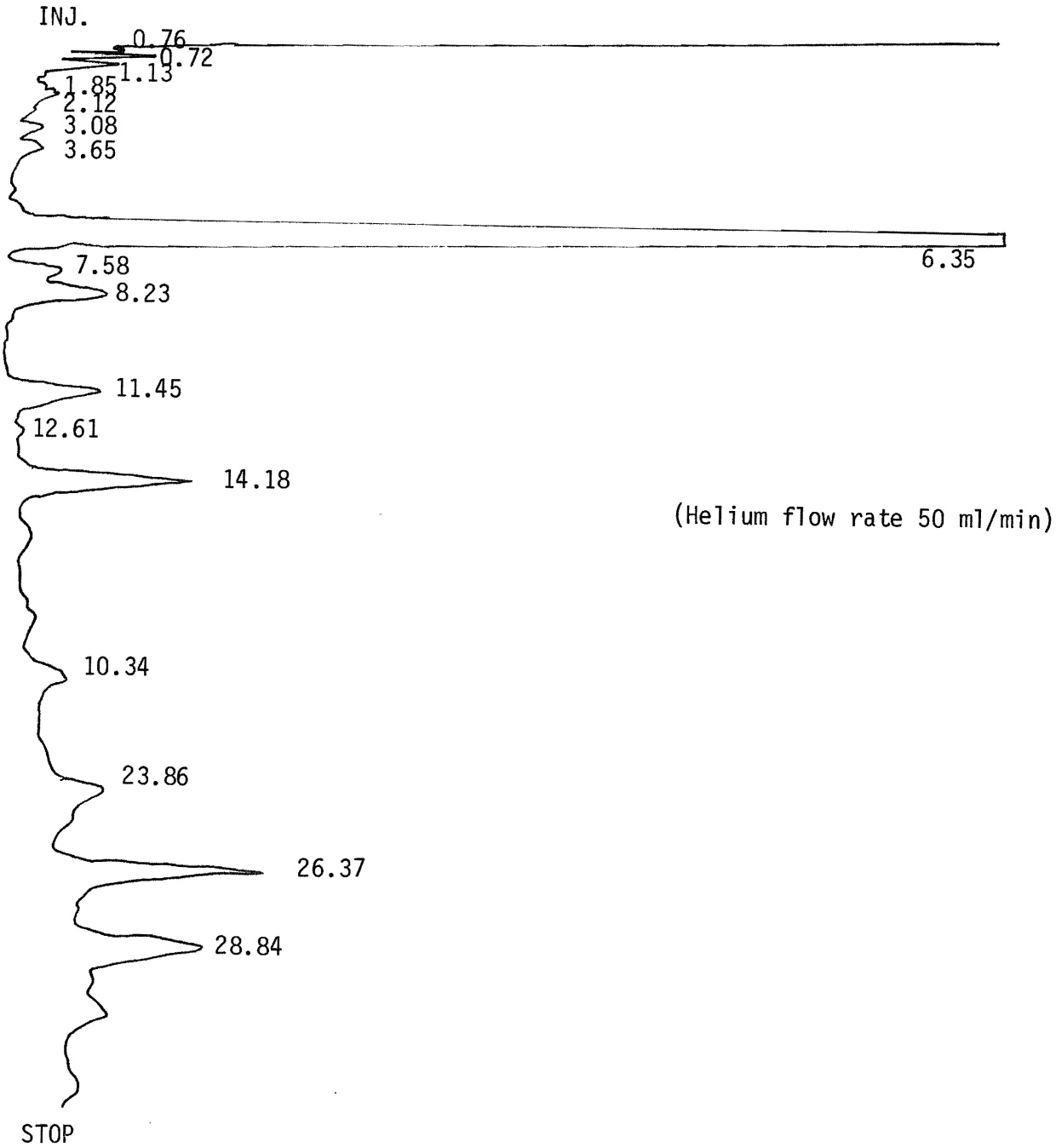


Figure 26. GC trace of neutral compounds in acidified water sample 10.

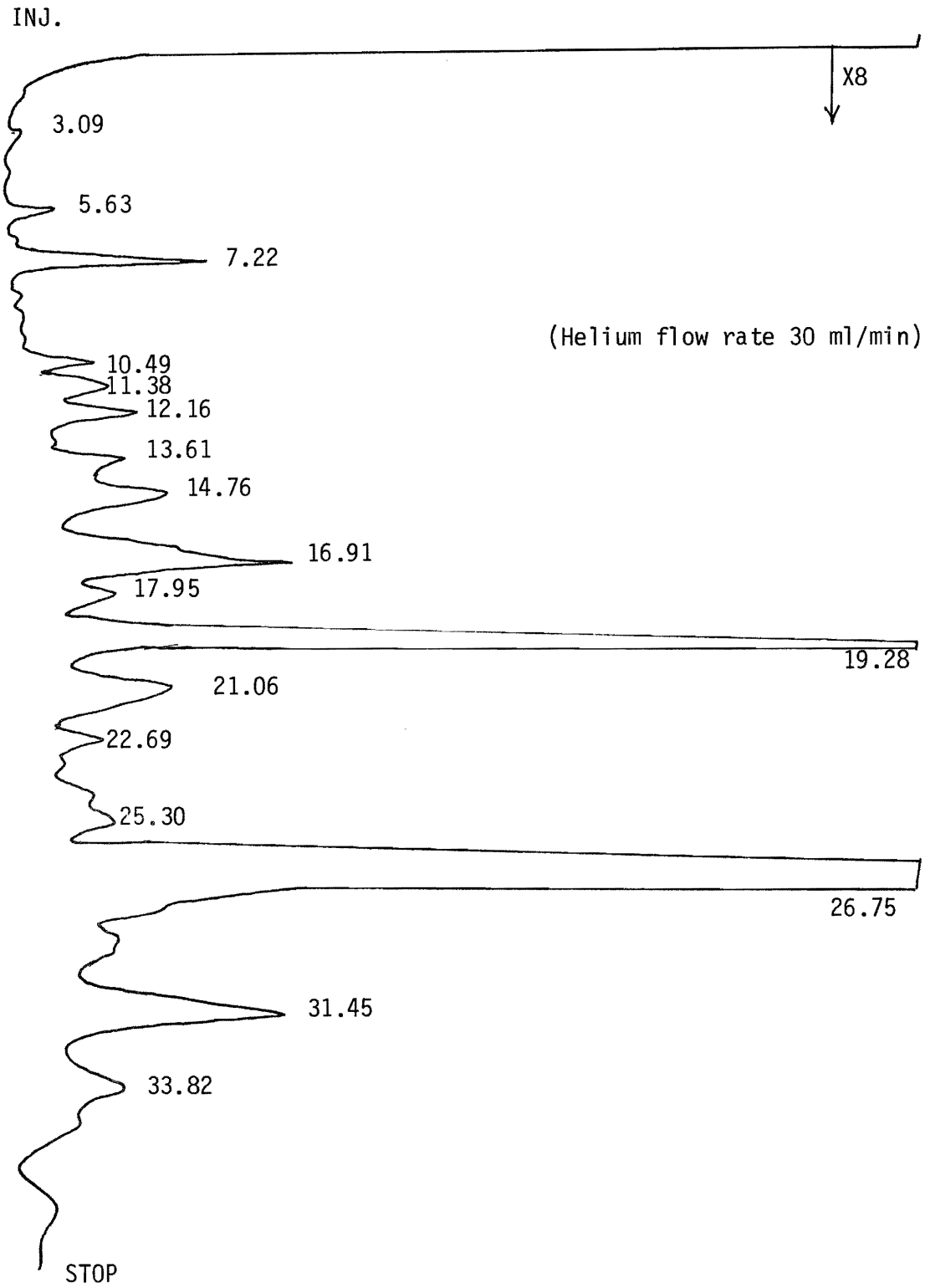


Figure 27. GC trace of neutral compounds in water sample 4.

7.7 GC TRACES OF ACIDIC COMPOUNDS FROM RIVER WATER.

Figures 28 to 30 illustrate traces of organic acids as n-butyl esters in acidified river water samples.

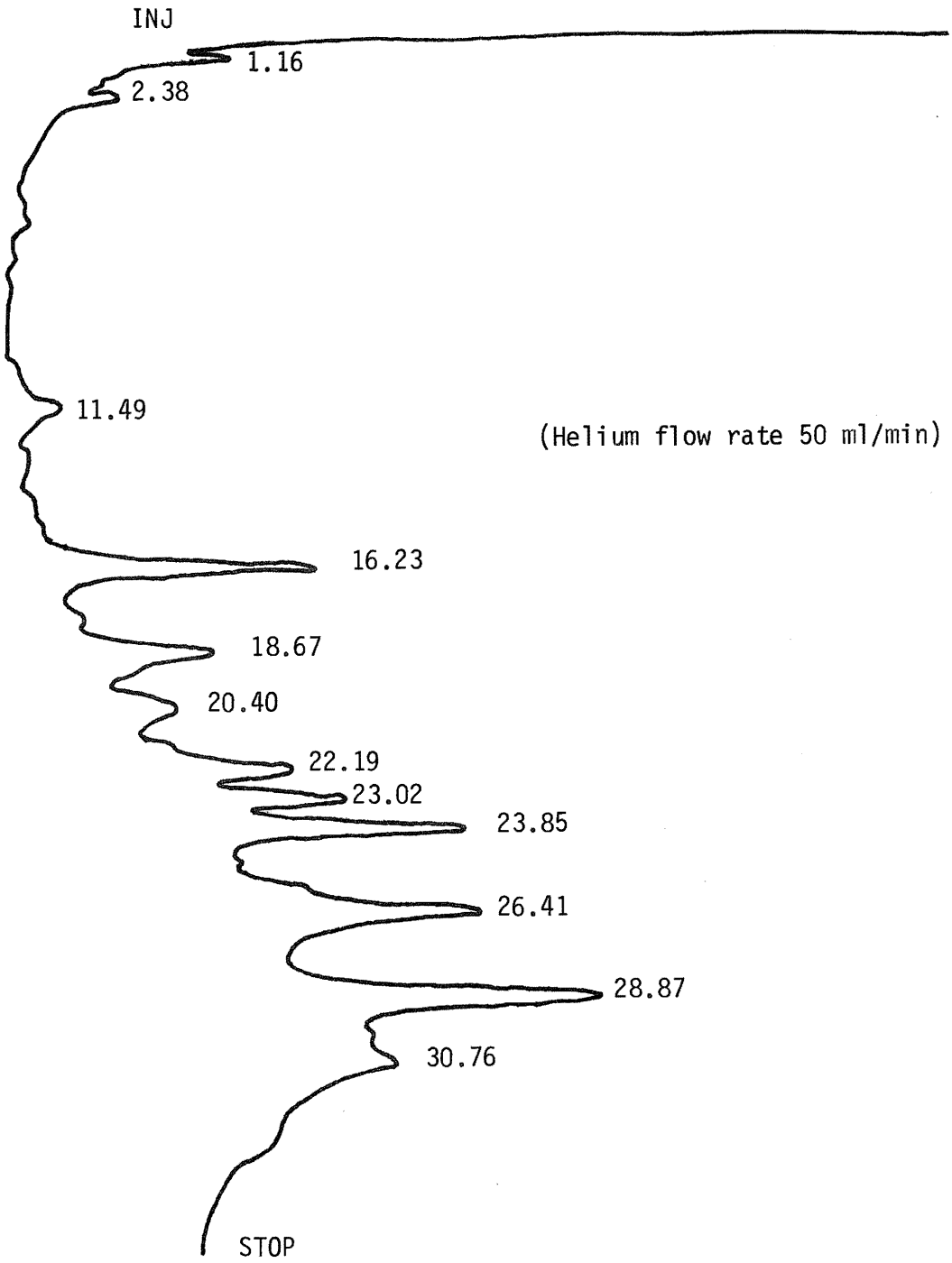


Figure 28. GC trace of organic acids (as n-butyl esters) in acidified water sample 1A.

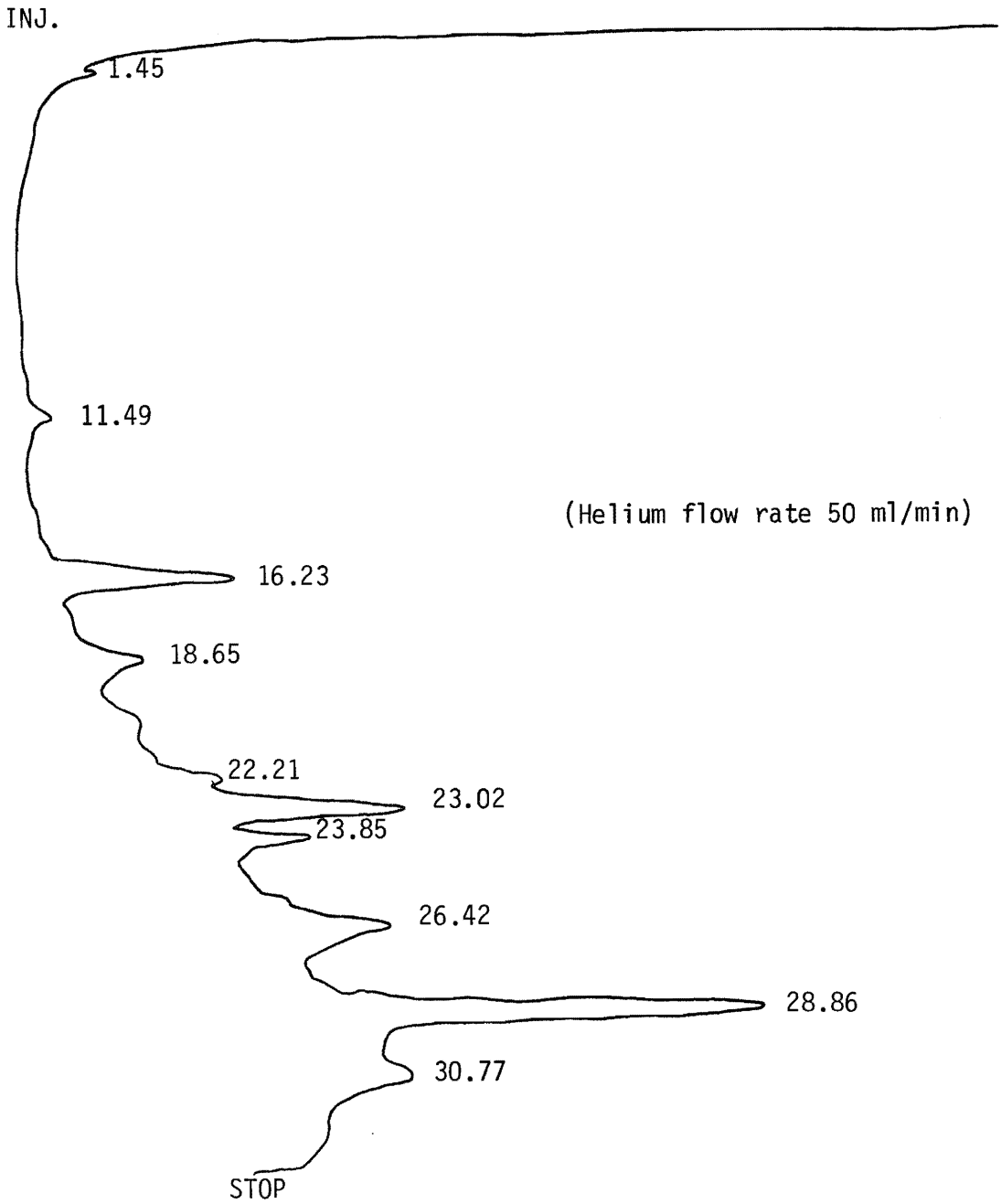


Figure 29. GC trace of organic acids (as n-butyl esters) in acidified water sample 5A.

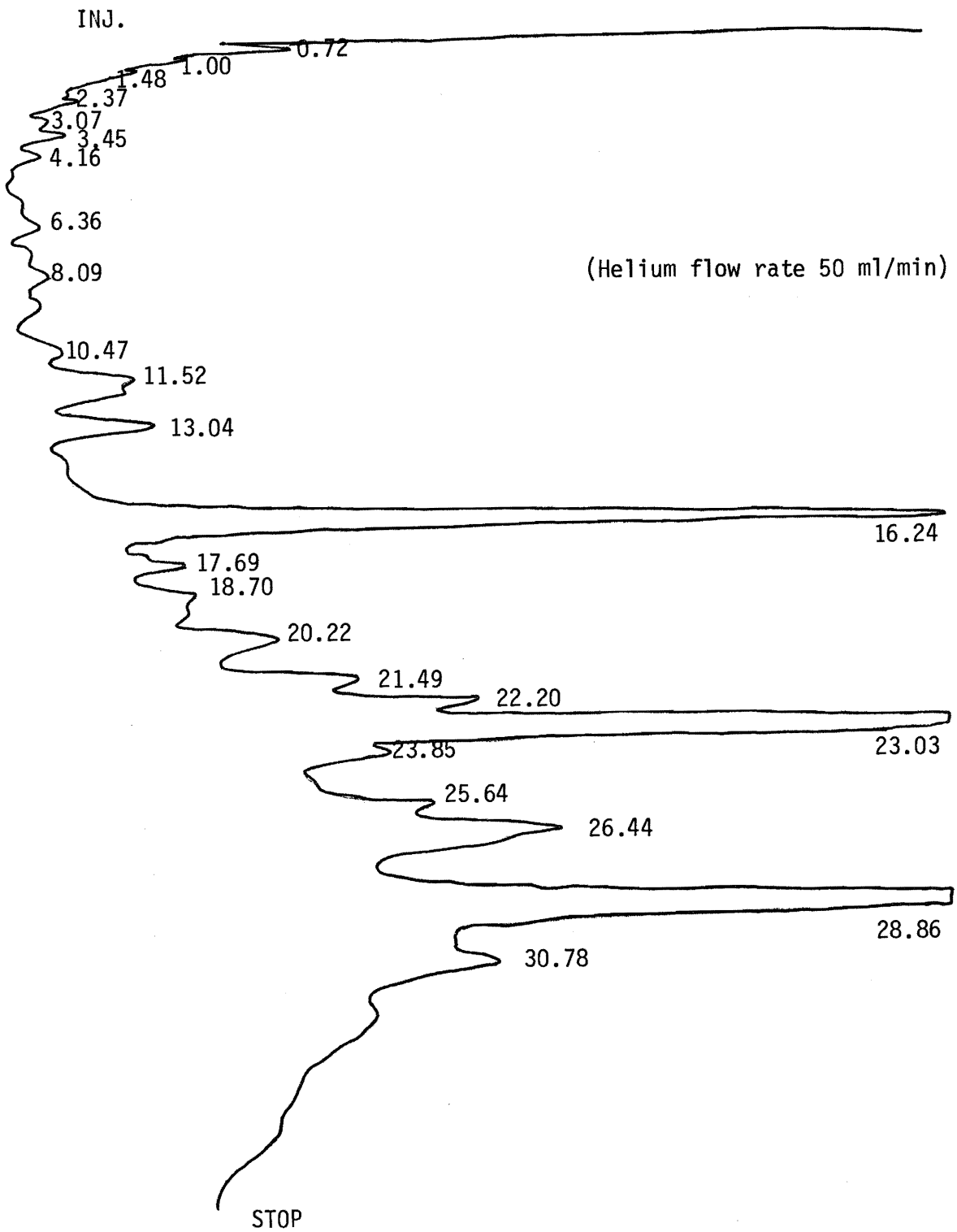


Figure 30. GC trace of organic acids (as n-butyl esters) in acidified water sample 10.

7.8 Annotated Bibliography

The following annotated bibliography is principally concerned with documenting the use of ^{14}C-carbon uptake studies in determining heterotrophic activity in fresh water systems. A brief section which describes other methodologies has been included although it cannot be considered a comprehensive review. In addition, a section has been added that deals mainly with the use of epifluorescence microscopy in counting cell numbers. Two relatively new assays for bacterial biomass, muramic acid and lipopolysaccharide, are also included in this section.

7.8.1 Some Methods for the Study of Aquatic Bacteria

Atlas, R.M., and R. Bartha. 1972. Degradation and mineralization of petroleum in sea water: limitation by nitrogen and phosphorous. Biotechnology and Bioengineering, Vol. XIV, Pages 309-318.

"Biodegradation and mineralization of petroleum, added at 1% (v/v) to freshly collected sea water, were measured using gas-liquid chromatographic, residual weight, and carbon dioxide-evolution techniques. Only 3% of the added petroleum was biodegraded and 1% was mineralized in unamended sea water after 18 days of incubation. Added individually, nitrate ($10^{-2}M$) or phosphate ($3.5 \times 10^{-4}M$) supplements caused little improvement, but when added in combination, they increased petroleum biodegradation and mineralization to 70% and 42%, respectively. Attempts to clean up oil spills with the aid of microorganisms should take into consideration the nutritional deficiencies of sea water".

Azam, F., and O. Holm-Hansen. 1973. Use of tritiated substrates in the study of heterotrophy seawater. Marine Biology. 23:191-196.

"An improved method is described for the study of heterotrophic utilization of dissolved organic substrates by marine microorganisms. The method is based on the use of ^{3}H-labelled organic substrates of very high specific activity, rather than the conventionally used ^{14}C-labelled substrates. Direct measurement of the rate of tracer uptake at near ambient concentration can thus be made instead of extrapolation using the Michaelis-Menton equation.

The method also permits comparison between the rates of tracer uptake in sub-samples exposed to different physico-chemical conditions (temperature, light, pollutants, etc.) without the necessity of determining the ambient substrate concentration. The method was applied to the determination of D-glucose uptake by nearshore and pelagic natural microbial populations, and was found to be sensitive and convenient".

Baskett, R.C., and W.J. Lulves. 1974. A method of measuring bacterial growth in aquatic environments using dialysis culture. J. Fish Res. Board Can. Vol. 31. No. 3, 372-374.

"An inexpensive, easy-to-use method was developed for studying microbial growth and behaviour in situ. Dialysis tubing suspended from a styrofoam flotation ring was filled with sterile distilled *Brevibacterium* sp. The viable population within the tubing increased significantly within 96 h. indicating that nutrients in the pond water necessary for growth and multiplication of *Brevibacterium* sp. diffused through the dialysis membrane".

Boling, H.B., E.D. Goodman, and J.A. Van Sickle. 1975. Towards a model of detritus processing in a woodland stream. Ecology 56:141-151.

"A state-space model for the microbial and abiotic decomposition of detritus in a temperate zone woodland stream is developed. Detritus, defined as nonliving, particulate organic matter and its associated microbial populations, is classified according to particle size and extent of microbial colonization as reflected by community respiration. These categories form a matrix whose entries are detrital biomass; detritus processing in the stream is simulated using transition function to move biomass through the categories. The model can be coupled to population models of stream invertebrates via removal of detritus from the matrix for detritivore feeding. Simulation results are presented and compared with field data".

Hoppe, H.G. 1976. Determination and properties of actively metabolizing heterotrophic bacteria in the sea, investigated by means of micro-autoradiography. Marine Biology. 36:291-302.

"Substrate transformation and microbial biomass production in aquatic ecosystems depend mainly on the total number of actively metabolizing heterotrophic bacteria. The most common methods used concern the determination of either the colony-forming bacteria or the total number of bacteria including autotrophs and inactive organisms. A micro-autoradiographic method is presented which enables the substrate uptake of single bacteria by means of ^{3}H-amino-acid mixture and Nuclepore filters to be determined. The standardization procedure revealed the greatest success after 3 h incubation with 10 µCi/ml tritiated amino-acid mixture and an exposure of 14 days to the X-ray film. Preliminary experiments showed inactivation of an active fresh-water population from 100% to .6% within 3 h at 28%S. With increasing distance from the shore, the number of colony-forming units decreases from 6 to .01% of the total number of active heterotrophic bacteria. It is concluded from the results that the fraction of very small heterotrophic bacteria which cannot be cultured on nutrient media is responsible for the continuous breakdown of organic matter in off-shore regions of the sea".

Jannasch, W.H. 1969. Current concepts in microbiology. Verh. Internat. Verein. Limnol. 17:25-39.

This paper is taken from an address by Dr. Jannasch at the Edgardo Baldi Memorial lecture. In it he summarizes and comments on current ideas and techniques in aquatic microbial ecology. He questions the usefulness of methods such as plate counts and explains that the lack of quantitative information on various microbial transformations (e.g., assimilation of organics) is basically due to deficiencies in methodology. He emphasizes the need for studies that are carried out under conditions as close to in situ conditions as possible, especially with respect to substrate concentrations.

King, J.D., D.C. White, and C.W. Taylor. 1976. Use of lipid composition and metabolism to examine structure and activity of estuarine detrital microflora. Appl. and Environ. Micro. 33:1177-1183.

"Earlier studies have shown that the activity of the estuarine detrital microflora measured by various enzyme activities, muramic acid and adenosine 5'-triphosphate (ATP) content, heterotrophic potentials, and respiratory activities correlates with the incorporation of ^{14}C and ^{32}P into the microbial lipids. In this study, these lipids were reproducibly fractionated into neutral lipid, glycolipid, and phospholipid classes. Distinct differences between the active microflora of oak leaves, sweet gum leaves, and pine needles were evidenced both in the rate of lipid synthesis and in the proportions of neutral lipids, glycolipids, and phospholipids. Successional changes in the microflora of leaves incubated in the semitrophical estuary, previously suggested by ATP-to-muramic acid ratios and scanning electron micrography, were reflected in changes in the proportions of ^{14}C in major lipid classes when analyzed from the same type of detritus. Short incubation times with ^{14}C gave lipid compositions rich in phospholipids that are typical for the faster-growing bacterial populations; longer incubation with ^{14}C gave lipid compositions richer in neutral and glycolipids, more chacacteristic of slower-growing eukaryotes or morphologically more complex prokaryotes. The metabolism of the of the lipids of the estuarine detrital microflora was examined by a pulse-chase experiment with ^{14}C. Glycolipids lost ^{14}C at a rate equal to the loss of ^{14}C of the slow component of muramic acid. Individual phospholipids lost ^{14}C from their backbone glycerol esters at different rates".

Ramsay, A.J. 1973. The use of autoradiography to determine the proportion of bacteria metabolizing in an aquatic habitat. J. Gen. Micro. 80:363-373.

"Autoradiographic methods have been developed to determine the proportion of metabolizing bacteria on leaves of *Elodea canadensis* . Detection with a light microscope of tritium labelling of pure cultures of bacteria was optimal when samples were incubated with 20 μCi of (^{3}H) glucose or (^{3}H) thymidine/ml

for 1.75 to 2 h and the bacteria exposed to K^2 emulemulsion for 1^4 days. Two fluorescent pseudomonads did not form autoradiograms when labelled with ^3H thymidine. For autoradiograms of bacteria from *E. canadensis*, L^4 emulsion was more suitable than K^2 emulsion.

"High-resolution autoradiograms were prepared of a pure culture of a pseudomonad and of bacteria from moribund leaves of *Elodea canadensis*. Counts of labelled and unlabelled cells in light-microscopic and high-resolution autoradiograms suggested that valid counts of labelled bacteria could be obtained from light-microscopic autoradiograms. Direct counts of bacteria from *E. canadensis* leaves of different ages revealed greater increases as leaves matured and died than did indirect methods. Autoradiographic data from young leaves indicated that the percentage of bacteria labelled with (^3H)glucose that was similar to the percentage of the total population which was estimated by the plate method. On the mature and moribund leaves larger proportions of the populations were labelled with (^3H)glucose than were included in the plate counts".

Stanley, P.M., and J.T. Staley. 1977. Acetate uptake by aquatic bacterial communities measured by autoradiography and filterable radioactivity. Limnol. Oceanogr. 22:26-37.

"In a pulp mill waste aeration lagoon with a high organic content including measurable amounts of acetate, community uptake of acetate followed Michaelis-Menton kinetics at concentrations of added acetate up to 30 μg ml. The measured in situ substrate level, S_n, was 6% to 46% of the $K + S_n$ value calcuated from the Michaelis-Menton equation.

"Autoradiography was used to determine the uptake kinetics of individual bacterial cells. Two morphologically distinct bacterial types followed Michaelis-Menton kinetics, but one was at least four times more active in situ. Comparison of several different cell types indicated a minimum uptake activity range of 20-fold. A few cell types did not absorb acetate. Different cells of the same type showed a 10-fold range of activity, and the activity per cell did not follow a Poisson distribution".

Walker, J.D., and R.R. Colwell. 1975. Microbial petroleum degradation: application of

computerized mass spectrometry. Can. J. of Micro. 21:1760-1769.

"An analytical procedure is presented for obtaining detailed characterization of petroleum hydrocarbons which undergo microbial degradation. The procedure includes column chromatography separation and characterization of the resulting fractions by mass spectrometry and gas chromatography. The use of computerized low-resolution mass spectrometry is offered as a method for assessing microbial degradation of petroleum. This method provides information which cannot, at the present time, be obtained by other available analytical methods. Use of this method to evaluate degradation of South Louisiana crude oil by a mixed culture of estuarine bacteria revealed that asphaltenes and resins increased by 28% after degradation, while saturates and aromatics decreased by 83.4% and 70.5%, respectively. Most of the normal and branched-degradation was observed by gas-liquid chromatography. Susceptibility of cycloalkanes to degradation was less as the structure varied, i.e., 6-ring > 1-ring > 2-ring > 3-ring > 5-ring > 4-ring. Susceptibility of aromatic components to degradation decreased with increase in the number of rings, viz., monaromatics > diaromatics > triaromatics > tetraaromatics > pentaaromatics. Aromatic nuclei containing sulphur were twice as refractory as non-sulphur analogs".

Weiser, W., and M. Zech. 1976. Dehydrogenases as tools in the study of marine sediments. Marine Biology. 36:113-122.

"A method is outlined for the measurement of $NADH^2$-dependent dehydrogenase activity in whole samples as well as in fractions of carbonate sediments. The method is based on extraction of enzyme activity with phosphate buffer containing 0.12% Triton X-100, dialysis, and a photometric assay which permits initial velocity determinations of the enzyme reaction under controlled conditions of pH, temperature, and substrate concentration. The dehydrogenases extracted from carbonate sediment and subtropical beach at Bermuda are characterized by low Km values (0.03 to 0.7 mdM $NADH^2$/1), pH maximum around 8.5, and temperature maxima between 35^0 and 40^0C. Their vertical distribution in the sediment agrees with what is known about the distribution of biomass on beaches of this

type, maximum activity occurring in the top-most centrimetre. Approximately 80 to 90% of the total electron transport activity is contained in the grain fraction, the remainder in the interstitial fraction of a sediment sample. From this it can be inferred that the interstitial fauna contributes only little to the total energy budget of this type of sediment, which is dominated by the "Aufwuchs" on the sand grains. The electron transport activity (ETA) measured by means of the dehydrogenase assay is taken to represent the maximum transport capacity (ETC) of all the organisms living in the sand ecosystem. From published evidence it is inferred that oxygen consumption of sediment samples and of individual organisms measured in vivo usually accounts for 10 to 20% of the maximum electron transport capacity measured in vitro with saturating concentrations of substrates and with Triton X-100 as part of the enzyme assay. It is suggested further that the ratios of dehydrogenase activities in different fractions or zones of sediment, or of different organisms, may represent one of the best indicators, so far available, of energy relationships in marine sediments".

Westlake, D.W.S., A Jobson, R. Phillippe, and F.D. Cook. 1974. Biodegradability and crude oil composition. Can. J. of Micro. 20:915-928.

"Four crude oils (Prudhoe Bay, Norman Wells, Atkinson Point, and Lost Horse Hill) of different chemical composition were tested as to their biodegradability under mesophilic and psychrophilic conditions. Changes in bacterial numbers and chemical composition of the oils were monitored using a plate count and chromatographic techniques respectively. Populations induced under psychrophilic conditions readily metabolized similar quality oils under mesophilic conditions. Mesophilic populations, however, only showed a limited metabolic capability on similar quality oils under psychrophlic conditions. Gram-negative rods were predominant in all the populations obtained under these experimental conditions. The ability of the mixed populations to use crude oil as a sole carbon source was dependent not only on the composition and amount of the n-saturate fraction but also on that of the asphaltene and NSO (i.e.,

nitrogen-sulphur-, and oxygen-containing) fraction. Growth on an oil which lacked a normal n-alkane component indicated that the aromatic fraction of oil was capable of sustaining bacterial growth. Oil quality and temperature of incubation affected the generic composition of populations obtained which would use crude oil. The isoprenoids, phytane and pristane, while readily used under mesophilic conditions, were more resistant to bacterial metabolism under psychrophilic conditions".

Zeikus, J.G., and T.D. Brock. 1971. Effects of thermal additions from the Yellowstone Geyser Basins on the bacteriology of the Firehole River. Ecology 53(2): 283-290.

"A series of stations was established on the Firehole River where it flows through the main geyser basins of Yellowstone Park, and temperature, pH, alkalinity, conductivity, and phosphate were measured. The temperature of the water gradually increased as it flowed through the thermal area, at its warmest averaging about 16 ^{0}C higher than in the cool area (10 ^{0} to 26^{0}C). Alkalinity, conductivity, pH, and phosphate also increased markedly. The growth rates of periphytic bacteria measured in situ on glass slides were virtually identical at different stations despite the wide differences in temperature. The effect of temperature on the incorporation of ^{14}C-glucose by benthic bacterial populations at the different stations demonstrated that the temperature optimum for this process increased in parallel with the temperature of the habitat. It is thus concluded that the bacteria are near-optimally adapted to the temperature of their habitat and that despite wide differences in temperature of the rate of bacterial activity is the same.

"In another study, samples of water were placed on agar media and incubated at temperatures of 25^{0}, 55^{0}, and 70^{0}C to obtain estimates of the number of bacteria able to grow at these temperatures. The thermophilic organisms developing at 55^{0}C were primarily *Bacillus stearothermophilus* and those at 70^{0}C primarily *Thermus aquaticus* . Thermophiles were not present in water which had not received thermal additions, but both kinds of thermophiles were found in the warm water and numbers increased progressively as more thermal water entered. In the recovery one downstream from the thermally heated region, the

the numbers of thermophilic bacteria decreased markedly. The possible value of these thermophilic bacteria as indicators of thermal pollution is discussed. It is suggested that the Firehole River is an excellent model for long-term studies on the ecological consequences of thermal pollution".

7.8.2 Assimilation Studies Using ^{14}C-Uptake Methods

Anderson, R.S. and M. Dokulil. 1977. Assessments of primary and bacterial production in three large mountain lakes in Alberta, Western Canada. Int. Revue ges. Hydrobiol. 62(1):97-108.

"Preliminary studies on production by phytoplankton and bacteria in three large mountain lakes in Alberta, Canada (two in Waterton Lakes National Park and one in Jasper National Park) were concluded mainly through the use of the ^{14}C technique. The main experiments were conducted in August 1974, and some were repeated in August 1975. Net primary production rates varied little from 1974 to 1975, even though there was drastic changes in the phytoplankton composition. Production in the largest lake (max. depth 135m; mean phytoplankton production 206.5 $mgCm^{-2}d^{-1}$) was approximately twice that for each of the two smaller lakes (max. depth 19 and 27 m; average phytoplankton production 109 $mgCm^{-2}d^{-1}$). Bacterial production estimates averaged 3.8 times those for the phytoplankton production, after a proportionately large error in the dark-uptake technique was subtracted. High production rates in the largest lake are probably due to enrichment. Bacterial production rates are comparable to those in smaller oligotrophic lakes in Europe".

Baross, J.A., F.J. Hanus, R.P. Griffiths, and R.Y. Morita. 1975. Nature of incorporated ^{14}C-labelled material retained by sulphuric acid fixed bacteria in pure cultures and natural aquatic populations. J. Fish. Res. Board Can. Vol. 32(1):1876-1879.

"The levels of ^{14}C-substrates (glutamic acid, alanine, glucose, and uracil) retained by aquatic bacteria in pure cultures and natural marine samples alter fixation with 2 N sulphuric acid (sulphuric acid to reduce pH to 2) were found to be identical to the amount of ^{14}C-labelled material associated with cold 10% trichloroacetic acid (TCA) precipitates collected on filters. Both TCA and sulphuric acid were also equally effective in driving carbon dioxide from solution. In heterotrophic potential studies, therefore, the ^{14}C activity associated with natural bacterial populations collected on membrane filters after sulphuric acid fixation represents macromolecules only, and not free substrate pools or low molecular weight metabolites".

Boylen, C.W., and T.D. Brock. 1973. Bacterial decomposition processes in Lake Wingra sediments during winter. Limn. Oceanogr. 18:628-634.

"Throughout winter, viable counts of bacteria from sediments of Lake Wingra (Madison, Wisconsin) at several different incubation temperatures were always higher at 25°C than 4°C, although the temperature of the sediments remained below 4°C for over 3 months. All of the organisms isolated and initially cultivated at 4°C grew better at 25°C; no obligately psychrophilic bacteria were found. Isotope studies to measure the temperature optima of the resident bacterial flora showed that the optimum temperature for incorporation of ^{14}C-glucose into cell material and conversion of ^{14}C-glucose into ^{14}C-carbon dioxide remained at 25° or greater all winter. A true psychrophilic flora does not develop in these sediments in winter, and bacterial decomposition processes occur at a much slower rate in winter than in summer".

Burnison, B.K., and R.Y. Morita. 1973. Heterotrophic potential for amino acid uptake in a naturally eutrophic lake. Appl. Micro. 27: 488-495.

"The uptake of sixteen ^{14}C-labelled amino acids by the indigenous heterotrophic microflora of Upper Klamath Lake, Oregon, was measured using the kinetic approach. The uptake velocity, V_{max}, of all the amino acids was proportional to temperature. The maximum total flux of amino acids by the heterotrophic microflora ranged from 1.2 to 11.9 μmol of ^{14}C per litre per day (spring to summer). Glutamate, asparagine, aspartate, and serine had the highest V_{max} values and were respired to the greatest extent. The percentages of the gross (net + respired) uptake of the amino acids which were respired to carbon dioxide ranged from 2% for leucine to 63% for glutamate. Serine, lysine, and glycine were the most abundant amino acids found in Upper Klamath Lake surface water; at intermediate concentrations were alanine, aspartate, and threonine; and the remaining amino acids were always below 7.5 X 10^{-8}) M (10 μg/l). The amino acid concentrations and determined chemically appear to be the sum of free and adsorbed amino acids, since the values obtained were usually greater than the (K_t + S_n) values obtained by the heterotrophic uptake experiments".

Crawford, C.C., J.E. Hobbie, and K.L. Webb. 1974. The utilization of dissolved free amino acids by estuarine microorganisms. Ecology 55:551-563.

"The importance of bacteria in the cycling of carbon in the Pamlico River estuary was studied by measuring the rates of uptake of organic compounds. Our methods allowed analysis with the Michaelis-Menton kinetics equations, and both the rates of uptake of dissolved free amino acids (DFAA) and glucose as well as the percentage of carbon subsequently respired as carbon dioxide were determined. In addition, the concentrations of the amino acids in the water were determined using ion exchange chromatography. Other tests included measurements of primary productivity and of the effects of the other amino acids in the water upon the uptake of one amino acid.

"There was considerable variation in the heterotrophic activity over time and distance probably caused by patchiness in distribution of plankton and dissolved compounds in the water. Although there is some competition between amino acids being taken up, the effect upon kinetics measurements is probably negligible. Tests made every 3 h showed a coefficient of variability (CV) of the measured maximum velocity of uptake (V_{max}) of aspartic acid to be only 26%, and a similar CV was found for daily samples. In several instances the uptake of one amino acid was found to be competitively inhibited by the presence of another amino acid, but the concentrations necessary to inhibit were far above natural concentrations and such effects are probably unimportant in nature. Mutual inhibition was found between the similar amino acid pairs, glutamic acid and aspartic acid, threonine and serine, glycine and alanine, and leucine and alanine.

"Highest V_{max} values were found during the summer months and early fall and ranged from a high of 69.42 µg C/l-h for alanine in August to less than 0.20 µg C/l-h for most of the substrates tested in the colder months. The V_{max} values for glucose uptake (0.06 to 9.64 µg C/l-h) indicate that this estuarine system is one of the most microbially-active environments tested.

"The DFAA were present in the water at concentrations of from 10 to 30 µg C/l; over half of

this was ornithine, glycine, and serine. The DFAA were only about 0.2% of the total dissolved organic carbon in the water. Further, seasonal variations of DFAA concentrations, generally paralleling those of primary productivity, suggested that the amino acids originated from algal excretion and the decay of algal cells. The orders of abundance and concentrations of individual amino acids were similar to those reported for other bodies of water.

"When the natural concentration of a substrate is known the actual velocity of uptake (V_n) or flux for that substrate may be found. Flux rates were only 1%-10% of the V_{max} values in the coldest months; the highest values were found in the warmest months.

"At each experimental concentration of amino acid, a certain amount was taken up, and a percentage of this amount was oxidized to carbon dioxide. This percentage was constant for a particular amino acid in spite of varying experimental times, substrate concentrations, and temperatures. Leucine had the lowest percent respired (13%) while aspartic and glutamic acids had the highest (50%). Failure to correct uptake data for this respiratory loss introduces significant underestimation. The production of particulate material was calculated by correcting total uptake figures for each amino acid by its characteristic respiration percentage. Over 60% of the particulate production from amino acids was by uptake of alanine, leucine, valine, serine, glycine, aspartic acid, and glutamic acid. Such particulate production averaged 0.79 µg C/l-h for the year and ranged from 0.06 to 2.37 µg C/l-h; this is about 10% of the rate of production by algae during the summer months. This amount of particulate organic material is a significant contribution to this estuarine food chain".

Griffiths, R.P., J. Hanus, and R.Y. Morita. 1974. The effects of various water-sample treatments on the apparent uptake of glutamic acid by natural marine microbial populations. Can. J. Microbiol. 20:1261-1266.

"The apparent glutamate utilization by natural marine microbial populations was shown to be greatly influenced by the method used to terminate the uptake reaction. There was a significant difference in the amount of labeled material associated with the

microorganisms when treated with either acid or buffered formaldehyde before the sample was assayed. A comparison of the data collected from acidified and non-acidified water samples resulted in significant differences in the following calculated values: percentage respired, the maximum velocity of uptake (V_{max}), the turnover time (T_t), and the transport constant and natural substrate concentration ($K_t + S_n$). The rates of radioactivity incorporation into carbon dioxide and acidified and non-acidified cells were followed with time and were found to be non-linear after extended incubation.

"Data is presented which indicate that the labeled material that is released during acidification is that associated with the glutamate pool. The effects of these observations on the interpretation of data collected during heterotrophic potential studies are discussed".

Hall, J.H., P.M. Kleiber, and I. Yesaki. 1972. Heterotrophic uptake of organic solute by microorganisms in the sediment. Proceedings of the IBP-UNESCO Symposium on detritus and its role in aquatic ecosystems. Mem. Inst. Ital. Idrobiol., 29 Suppl.:441-471.

"The net uptake and mineralization of acetate, glucose and glycine by sediment microorganisms in Marion Lake, British Columbia were investigated. Temperature, light, particle size, fertilization and depth of sediment affect assimilation of the solutes by mixed sediment systems. The data were analyzed using modified Michaelis-Menton enzyme kinetics. Undisturbed sediment cores did not show saturation enzyme kinetics at low solute concentrations. Uptake was approximately an order of magnitude slower in the undisturbed system than in mixed sediment samples".

Hall, K.J., and K.D. Hyatt. 1974. Marion Lake (IBP) - from bacteria to fish. J. Fish. Res. Board Can. 31:893-911.

"The microbial-detritus food chain in the sediment of Marion Lake, British Columbia, is an important pathway for the cycling of organic matter derived from both allochthonous (leaf litter) and autochthonous (phytoplankton, aquatic macrophytes, and benthic algae) sources. The activity of the sediment

microbial community (approximately 10^9 - 10^{10} cells/g dry wt) as estimated by oxygen consumption and solute uptake is greatest during the summer months, when water temperatures are higher than 10^0C. Solute uptake is also influenced by sediment depth, nutrient enrichment, sediment particle size, and oxygen concentration. Organic matter from natural sources such as algal excretion, interstitial water, and leaf detritus is complex and is not assimilated as rapidly as the simpler organic compounds.

"Rainbow trout (*Salmo gairdneri*) and kokanee (*Oncorhynchus nerka*) in Marion Lake are characterized by small average size and slow groth rates. Fish production, although highly variable, is at the low end of the range compared to other salmonid producing systems. These characteristics are related to the inefficiency of trout and kokanee in cropping the benthic food supply. Experiments with individual predators and their prey have identified the nature of many of the interactions controlling the availability of benthic prey to the predators. Construction of simulation models shows a promise as a means to quantify the importance of such interactions".

The author points out some of the problems with many "conventional" techniques used to assess the rate of assimilation. Oxygen consumption rates, for example, cannot evaluate anaerobic processes and control tests are usually treated with antibiotics which do not completely eliminate bacterial activity. Many bacterial counting methods are also deficient; plate counts are obviously inadequate and even method such as ATP analysis and direct counts (nonfluorescent) and leave much to be desired.

Values for assimilation rates (v) in sediments using radioisotope labelled glycine, acetate, and glucose were less than 2, 1-20, and 1-20, µg/l/h respectively.

Harrison, M.J., R.T. Wright, and R.Y. Morita. 1971. Method for measuring mineralization in lake sediments. Appl. Micro. 21:698-702.

"A method is described for measuring the mineralization of an organic solute (^{14}C-glucose) by the heterotrophic indigenous bacteria in lake sediments. Since there is no suitable procedure for the determination of in situ microbial activities in

sediments, the procedure described is probably the best devised so far and may serve as a base for a more definitive procedure".

Hobbie, J.E., N.C. Raleigh, and R.T. Wright. 1968. A new method for the study of bacteria in lakes: description and results. Mitt. Internat. Verein. Limnol. 14:64-71.

"The study of the ecology of aquatic bacteria is a very difficult one indeed. The methods in general use today give a quantitative picture of the total numbers of bacteria (direct counts on membrane filters) and of the numbers of certain organisms that can be cultured on enriched media. Yet, little is known of the dynamics of these populations; turnover rates and generation times. Some idea of the importance of a dynamic approach comes from the work of Hayes & Phillips (1958) with radioactive phosphorous. These authors found that the bacteria were extremely important in the rapid turnover of nutrients and were able to give some quantitative data on turnover times in an ecosystem.

"As noted by Heukelekian & Dondero (1964, p. 446), an extension of isotope tracer techniques seems desirable as a supplement to other methods of studying aquatic bacteria. A number of workers have attempted to use radioactive organic compounds, such as glucose-C14, but the interpretation of the results appears to be very difficult. In this present paper, we will discuss some of the methods used by previous workers and describe a new method. This method gives information on the potential heterotrophic ability of the bacteria, measures the turnover rate for some organic compounds, indicates the population's size, and gives a value for the maximum concentration of the substrate".

Hobbie, J.E. and R.T. Wright. 1965. Competition between planktonic bacteria and algae for organic solutes. Pages 177-185 in C.R. Goldman, ed. Primary productivity in aquatic environments. Mem. Ist. Ital. Idorbiol., 18 Suppl., University of California Press, Berkeley. 278 pp.

"New methods are presented to measure the uptake of organic solutes by planktonic microorganisms. By measuring the velocity of uptake of radioisotopes over a range of substrate concentrations, two

mechanisms of uptake can be differentiated. One of these, attributable to the bacteria, appears to be a transport system that is very effective at substrate concentrations below 100 µg/l. The other, in the algae, follows the kinetics of simple diffusion and is effective only at higher substrate concentrations (above 500 µg/l). In natural waters the bacteria are so effective that they keep the substrate concentration below 20 µg/l and so drastically limit the heterotrophy of the algae".

Hobbie, J.E. 1971. Heterotrophic bacteria in aquatic ecosystems; some results of studies with organic radioisotopes. Pages 181-195 in J. Cairns Jr., ed. The structure and function of fresh-water microbial communities. The American Microscopical Society Symposium, Blacksburg, Virginia. 300 pp.

"The rates at which heterotrophic bacteria transform organic material are virtually unknown in aquatic ecosystems. One experimental approach is to add very small quantities of radioactive organic compounds and measure the uptake into the bacteria after short incubations. The kinetics of the uptake can be studied by adding different concentration of substrate and analyzing by the Michaelis-Menton formula. Maximum velocities of uptake are highest after spring and late summer algal blooms and may drop to a tenth of those values during the winter. The concentrations of the substrates, such as glucose, acetate, or amino acids, remain at low levels throughout the year as the rate of supply to the dissolved organic pool appears to be balanced by the rate of removal by the bacteria. In polluted systems, the bacteria may remove all of the substrate with 1/2 hour; in oligotrophic systems it may take over 1000 hours. When respiration is calculated and the actual concentration of substrate known, the flux of carbon through these bacteria can be estimated. In one estuarine system, the flux of amino acids alone accounted for close to 10% of the primary productivity. In autotrophic systems the bacteria can exist upon the dissolved organic carbon and are always actively taking up substrate. In oligotrophic systems, the transport systems have to be induced before uptake of the substrate occurs".

Hobbie, J.E. 1973. Using kinetic analyses of uptake of Carbon-14 to measure rates of movement of individual organic compounds into aquatic bacteria. Bull. Ecol. Res. Comm. (Stockholm) 17:207-214.

"The most difficult question of microbial ecology concerns the rate of microbial processes in the natural milieu. This is an extremely hard question to answer because every manipulation we make, every change we introduce in order to make this measurement of activity, changes the environment. Usually, the change is so great that the measurement is worthless as it can not be extrapolated back to the natural undisturbed situation. I suggest that an activity measurement must fulfill two conditions: 1) that it does not appreciably disturb the organisms' environment and 2) that it can be extrapolated back to the natural conditions. It follows, therefore, that a tremendous effort must go into studying the effects of the conditions of measurement on the organisms and on their environment.

"In terrestrial studies, so far only measurements of carbon dioxide evolution fulfill these criteria although perfusion studies are also acceptable under some conditions. Unfortunately, bacteria in aquatic systems are so dispersed that studies of gas evolution or uptake do not work except in rich sediment. The long-term incubations necessary to obtain measureable changes always completely alter the systems. Some information on activity of a part of the aquatic bacterial population can be obtained, however, through short-term incubations with various radio-isotopes. The techniques described here have been developed for studying the activity of heterotrophic aquatic bacteria. They could be used equally for terrestrial studies although here there are great problems of interpretation.

"The technique stems from the remarkable discovery of Parsons & Strickland (1962) that the uptake of organic compounds, such as glucose and acetate, could be described by the same equations that describe uptake by bacterial cultures. Evidently, many of the different types of bacteria possess similar transport systems (permeases) for organic compounds. These systems can be described by meaurring the uptake velocities at a number of different substrate concentrations. The Michaelis-Menton equation yields V_{max}, the half-saturation constant K, and the velocity of uptake at the natural substrate level (v).

"In the discussion that follows, it is important to remember that the natural levels of individual dissolved organic compounds are only a few micrograms per litre. For example, the total dissolved free amino acid pool in aquatic systems varies from 15 to 80 µg/l (Andrews & Williams, 1971) and glucose is always between 1 and 10 µg/l. Other simple organic compounds are likely in the same range".

Horowitz, A., and R.M. Atlas. 1977. Response of microorganisms to an accidental gasoline spillage in an arctic freshwater ecosystem. Appl. and Environ. Micro. 33:1252-1258.

"The response of microorganisms to an accidental spillage of 55,000 gallons of leaded gasoline into an arctic freshwater lake was studied. Shifts in microbial populations were detected after the spillage, reflecting the migration pattern of the gasoline, enrichment for hydrocarbon utilizers, and selection for leaded-gasoline-tolerant microorganisms. Ratios of gasoline-tolerant/utilizing heterotrophs to "total" heterotrophs were found to be a sensitive indicator of the degree of hydrocarbon contamination. Respiration rates were elevated in the highly contaminated area, but did not reflect differences between moderately and lightly contaminated areas. Hycrocarbon biodegradation potential experiments showed that indigenous microorganisms could extensively convert hydrocarbons to carbon dioxide. In situ measurement of gasoline degradation showed that, if untreated, sediment samples retained significant amounts of gasoline hydrocarbons including "volatile components" at the time the lake froze for the winter. Nutrient addition and bacterial inoculation resulted in enhanced biodegrative losses, significantly reducing the amount of residual hydrocarbons. Enhanced biodegradation, however, resulted in the appearance of compounds not detected in the gasoline. Since the contaminated lake serves as a drinking water supply, treatment to enhance microbial removal of much of the remaining gasoline still may be advisable".

Robinson, G.G.C., and L.L. Hendzel. 1973. A relationship between heterotrophic utilization of organic acids and bacterial populations in West Blue Lake, Manitoba. Limno. Oceanogr. 18:264-269.

"The relationship between the concentration of nine organic acid substrates (lactic, pyruvic, fumaric, malic, acetic, succinic, glycolic, citric, and formic) and their velocities of uptake has been investigated in West Blue Lake, Manitoba. Maximum uptake velocities were ranked and compared with bacterial plate counts on minimal media supplemented with single organic acids. For pyruvic, fumaric, malic, acetic, glycolic, and citric acids an excellent correlation ($r = 0.98$) existed between plate counts and acid uptake indicating that for these substrates maximum velocities of uptake are indeed indicative of actual bacterial populations".

Sorokin, J.I. 1965. On the trophic role of chemosynthesis and bacterial biosynthesis in water bodies. Pages 189-205 in C.R.Goldman, ed. Primary productivity in aquatic environment. Mem. Ist. Ital. Idrobiol., 18 Suppl., University of California Press, Berkeley. 278 pp.

"Dark bacterial biosynthesis is in principle secondary production because it derives its energy from organic matter built during photosynthesis. It is however, in some respects more nearly primary than secondary production because it proceeds mostly by the utilization of organic matter which would otherwise be practically lost for the direct feeding of animals. This is particularly true in the case of some inland waters and certain oligotrophic ocean waters where bacterial biosynthesis through the oxidation of allochthonous material is more important than photosynthesis. Experiments show that the trophic role of chemosynthesis is the utilization of energy bound in the end products of anaerobiosis, and that chemoautotrophes serve to connect the destructive processes in the sediments with biological productivity in water masses".

Sorokin, Y.I., and H. Kadota. 1972. Techniques for the assessment of microbial production and decomposition in fresh waters. I.B.P. Handbook No. 23. Blackwell Scientific Publications. 235 pp.

This text is a valuable summary of methods that have been used to assess the growth of bacterial cells in fresh water and the corresponding decomposition of organic molecules. This activity has a direct influence on the productivity of aquatic organisms at the various trophic levels.

Techniques such as oxygen uptake, radioisotopic uptake using organic as well as inorganic (carbon dioxide) molecules, dehydrogenase activity, gas evolution, and substrate utilization are fully discussed although a critical evaluation is not always provided. Bacterial counting techniques (plate counts, Most Probable Number, fluorescent counts, A.T.P., electron microscopic, etc.) are also explained in some detail.

Using many of these techniques, the authors describe various methods of assessing bacterial production. They remark that the utilization of allochthonous materials is essentially a type of primary production since it "increases the total amount of energy used by the aquatic community".

Walker, J.D., and R.R. Colwell. 1975b. Measuring the potential activity of hydrocarbon-degrading bacteria. Appl. and Environ. Micro. 31: 189-197.

"(^{14}C) hydrocarbons were utilized as a means of estimating the hydrocarbon-degrading potential of bacteria in estuarine and marine environments. Evaporation of the hydrocarbons must be considered in estimates of oxidation. Amount of mineralization of (^{14}C) hexadecane can be equated with the total number of petroleum-degrading bacteria and the percentage of the total heterotrophic population, which they represent. Mineralization activity was found to be related to the activity of the bacterial populations during in situ incubation. Rates of mineralization were observed, as follows, for (^{14}C)hexadecane > (^{14}C) naphthalene > (^{14}C)toluene > (^{14}C)cyclohexane. Increased rates of uptake and mineralization were observed for bacteria in samples collected from an oil-polluted harbor compared with samples from a relatively unpolluted, shell-fish-harvesting area, e.g., turnover times of 15 and 60 min for these areas, respectively, using (^{14}C)-hexadecane".

Ward, D.M., and T.D. Brock. 1975. Environmental factors influencing the rate of hydrocarbon oxidation in temperate lakes. Appl. and Environ. Micro. 31:764-772.

"Rates of hydrocarbon biodegradation were estimated by following oxygen uptake during mineral oil oxidation or oxidation of (1-^{14}C)hexadecane to

^{14}C carbon dioxide, when these substrates were added to natural water samples from Wisconsin lakes. A lag phase preceded hydrocarbon oxidation, the length of which depended on population density or on factors influencing growth rate and on the presence of non-hydrocarbon organic compounds. Hydrocarbon oxidation was coincident with growth and presumably represented the development of indigenous hydrocarbon-degrading microorganisms in response to hydrocarbon additions. In detailed studies in Lake Mendota, it was found that, despite the continued presence of hydrocarbon-degrading microorganisms in water samples, seasonal variations in the rates of mineral oil and hexadecane oxidation occurred which correlated with seasonal changes in temperature optimum for oil biodegradation remained at 20 ^{o}to 25^{o}C throughout the year, so that temperature was the main limiting factor during winter, spring, and fall. During summer, when temperatures were optimal, nutrient deficiencies limited oil biodegradation, and higher rates could be obtained by addition of nitrogen and phosphorus. The rates of hydrocarbon biodegradation were thus high only for about one month of the ice-free period, when temperature and nutrient supply were optimal. Nutrient limitation of oil biodegradation was also demonstrated in 25 nutrient-poor lakes of northern Wisconsin, although in almost every case oil-degrading bacteria were detected. Knowledge of temperature and nutrient limitations thus will help in predicting the fate of hydrocarbon pollutants in freshwater".

Wetzel, R.G. 1975. Limnology. W.B. Saunders Co., Toronto. 743 pp.

In this chapter, Dr. Wetzel points out that an analyses of utilization rates of organic substrates under in situ conditions with natural populations have been applied in only a few cases, and employing only a few simple substrates such as glucose, other sugars and isomers of glucose, acetate, glycolate, and amino acids. Maximum velocities of uptake are quite variable among lakes and substrates, but generally occur after spring and late summer algal maxima. Rates decrease by about an order of magnitude during winter in temperate lakes.

He also notes that in general the turnover time (t_{η}) for amino acids in lakes is less than 20 hours. At low substrate concentrations (S_{η}), algae always consume less than 10% of the total uptake of substrate.

Williams, P.J. LeB. 1973. The validity of the application of simple kinetic analysis to heterogeneous microbial populations. Limn. Oceanogr. 18:159-164.

"It is shown by use of a mathematical model that heterogeneous populations do not adhere to the Michaelis and Menton equation; at low substrate concentration, observed rates are higher than predicted ones. The discrepancy between observed and predicted values increases as the population becomes more diverse. If kinetic analysis based on the Michaelis and Menton equation is used to determine the rate of turnover of substrates in natural waters, the rate may be underestimated unless measurements are made at very low added substrate concentrations".

Wright, R.T., and J.E. Hobbie. 1965. The uptake of organic solutes in lake water. Limn. Oceanogr. 10:22-28.

"Basic changes are made in an existing method using radioactive organic compounds for measuring the uptake of organic solutes by planktonic microorganisms. The changes give greater experimental flexibility and enable the maximum uptake velocity, V, to be found. Measurements over a range of substrate concentrations appear to show uptake by at least two mechanisms, by a transport system that becomes saturated at very low substrate concentrations and by simple diffusion, which shows a fairly constant increase in velocity of uptake with increasing substrate concentration. Because of this, the technique of measuring uptake at a single substrate concentration to give "relative heterotrophic potential" is questioned. The best estimate of heterotrophic potential may be V, the maximum uptake velocity attainable by planktonic populations with a transport system functioning according to the Michaelis laws. It is suggested that bacterial populations are responsible for uptake by means of transport systems and that algal populations show uptake by diffusion".

Wright, R.T., and J.E. Hobbie. 1966. Use of glucose and acetate by bacteria and algae in aquatic ecosystems. Ecology 47:447-464.

"New methods employing C^{14}-labeled organic compounds measure the uptake of organic solutes by

planktonic microorganisms. By testing uptake over a wide range of substrate concentrations, two separate mechanisms can be differentiated and their kinetics measured. Using filtration and evidence from experiments with laboratory cultures of planktonic bacteria and algae, specific transport systems effective at very low substrate concentrations were traced to the bacteria, and a diffusion mechanism, effective only at higher substrate concentrations, to the algae.

"Studies have shown that V, the maximum velocity of uptake by bacterial transport systems, gives information about the size and function of the bacterial populations. A diffusion constant, Kd, gives information on the rate of uptake of solutes by the algal populations. Turnover times, derived from kinetic parameters, indicate that the algal uptake of glucose and acetate in Lake Erken, Sweden, is always less than 10% of the bacterial uptake, even though the algal biomass may be orders of magnitude greater than the bacteria.

"Two new types of bioassay employ the kinetics of bacterial uptake systems as the measuring reaction. Acetate and glucose were found in 1-10 μg/l concentrations in the several natural waters tested. At these very low concentrations, algal uptake of glucose and acetate is so low that effective heterotrophy is impossible. In contrast, the bacteria effectively remove substrate from solution at these low levels and probably keep the substrate at these low concentrations. By doing this, the bacteria may prevent heterotrophic growth of algae in nature".

7.8.3 Epifluorescence Microscopy and Other Methods to Determine Cell Numbers

Bowden, W.B. 1976. Comparison of two direct-count techniques for enumerating aquatic bacteria. Appl. and Environ. Micro. 33:1229-1232.

"Planktonic bacteria from an estuary were concentrated on membrane filters and counted with both a scanning electron microscope and an epi-illuminated fluorescent microscope. Counts on 0.2 μm Nucleopore filters (polycarbonate) were significantly higher ($P < 0.001$) than counts on 0.2 μm Sartorius filters (cellulose). In contrast, there was not a statistically significant difference between the two techniques when Nuclepore filters were used ($0.5 < P < 0.9$). The average cell volume from this study area was 0.047 μm^3. The estimated number of bacteria ranged from 10^6 to 10^7 bacteria per ml, representing from 4 to 40 mg of C per m^3".

Buckley, E.N., R.B. Jones, and F.K. Pfaender. 1976. Characterization of microbial isolates from an estuarine ecosystem: relationship of hydrocarbon utilization of ambient hydrocarbon concentrations. Appl. and Environ. Micro. 32:232-237.

"Water collected at 12 sites in the Neuse River estuary of North Carolina was analyzed for total counts on three isolation media (Trypticase soy agar (TSA), marine agar 2216, Sabouraud agar) and total hydrocarbons by fluorescence spectroscopy. Counts of 3.9×10^1 to 3.8×10^3 cells/ml were found for total heterotrophs, well within the range commonly reported for marine and estuarine waters. Generally, marine agar 2216 gave higher counts than TSA at stations with salinities greater than 6.0 mg/ml; TSA gave higher counts than marine agar 2216 at sites with salinities less than 4.0 mg/ml. The microbial species isolated on the three media agree well with those previously reported for estuarine microbial communities. Water analyses, using XAD-2 resin and fluorescence spectroscopy, revealed petroleum hydrocarbon concentrations in the range of 5 to 79 ng/ml. Representatives of the microbial species isolated from these communities were tested individually for their ability to grow using kerosene as a sole source of carbon and energy. At but two stations, the majority of the species isolated were able to grow on hydrocarbons, indicating that

hydrocarbons, indicating that this ability is widespread even in environments not subjected to high levels of hydrocarbon pollution".

Daley, R.J. and J.E. Hobbie. 1975. Direct counts of aquatic bacteria by a modified epifluorescence technique. Limn. Oceanogr. 20:875-882.

"Various excitation and emission filters, microscope lamps, membrane filters, and field storage techniques used for direct counting of bacteria by epifluorescence microscopy have been compared. A rapid, simple modification of the Acridine Orange procedure using a new light filter combination, Sartorius cellulose membrane filters, and distilled water rinse gave the brightest cell fluorescence, darkest background, least fading, and highest counts for both estuarine and freshwater samples. Field fixation with Formalin permits storage of bacterial samples for 1 to 2 weeks without changes in bacterial numbers".

Francisco, D.E., R.A. Mah, and A.C. Rabin. 1973. Acridine orange-epifluorescence technique for counting bacteria in natural waters. Trans. Amer. Micros. Soc. 92(3):416-421.

"Total direct counts of aquatic bacteria from natural samples were estimated by epifluorescence microscopy after acridine orange staining. Cells were concentrated by membrane filtration on black membrane filters and counted directly. Using this method, bacterial counts of pure filters corresponded to those obtained by the Petroff-Hauser and Coulter counter methods. Epifluorescence counting is the method of choice for ecological studies of the natural distribution of bacteria in aquatic environments since it permits ready discrimination of bacteria from detritus and does not rely on the adequacy of culture methods to elicit growth of all viable organisms. Total counts obtained in this way were related to glucose and acetate uptake by resident microbial populations of lake water sample. Viable counts of the same samples were not related".

Jones, J.G. 1974. Some observations on direct counts of freshwater bacteria obtained with a fluorescence microscope. Limn. Oceanogr. 19:540-543.

"The use of different fluorochromes for direct counts of bacteria in water was compared. The two dyes in most common use, acridine orange (AO) and fluorescein isothiocyanate (FITC) were compared with an acridine derivative, euchrysine 2GNX (E-2GNX). Minor changes in technique could produce significant differences in counts. The acridine based dyes were easier to apply than FITC, and of these, E-2GNX gave consistently higher counts, all at a final dye concentration 5 mg/l with a contact time of 3 min. Details of the methodology should be consulted since changes can produce opposite results".

Jones, J.G., and B.M. Simon. 1975. An investigation of errors in direct counts of aquatic bacteria by epifluorescence microscopy, with reference to a new method for dyeing membrane filters. J. Appl. Bact. 39:317-329.

"A number of methods for observing freshwater bacteria by epifluorescence (incident light fluorescence) microscopy are examined. The suitability of each method for quantitative studies using black membrane filters is assessed. In spite of inadequacies it was considered that the use of acridine-based fluorochromes provided the best available estimate of the bacterial population. The errors which may arise when these stains are used were examined and it was noted that small changes in methodology could cause significant differences in the resuls obtained. The largest errors were associated with changes in volume of sample filtered and the pore size of the membrane used. A procedure for sample treatment is suggested and a new method for dyeing membrane filters is given which allows the use of 0.22 µm pore size membranes of the cellulose ester and polycarbon type".

King, J.D., and D.C. White. 1976. Muramic acid as a measure of microbial biomass in estuarine and marine samples. Appl. and Environ. Micro. 33:777-783.

"Muramic acid, a component of the muramyl peptide found only in the cell walls of bacteria and blue-green algae, furnishes a measure of detrital or sedimentary procaryotic biomass. A reproducible assay involving acid hydrolysis, preparative thin-layer chromatography purification, and colorimetric analysis of lactate released from muramic acid by alkaline

hydrolysis is described. Comparison of semitropical estuarine detritus, estuarine muds, and sediments from anaerobic Black Sea cores showed muramic acid levels of 100 to 700 μg/g (dry weight), 34 μg/g, and 1.5 to 14.9 μg/g, respectively. Enzymatic assays of lactate from muramic acid gave results 10- to 20-fold higher. Radioactive pulse-labeling studies showed that (^{14}C)-acetate is rapidly incorporated into muramic acid by the detrital microflora. Subsequent loss of ^{14}C, accompanied by nearly constant levels of total muramic acid, indicated active metabolism in procaryotic cell walls".

Larsson, K., C. Weibull, and G. Cronberg. 1977. Comparison of light and electron microscopic determination of the number of bacteria and algae in lake water. Appl. and Environ. Micro. 35:397-404.

"Determinations of the number of microorganisms in lake water samples with the bright-field light microscope were performed using conventional counting chambers. Determinations with the fluorescence microscope were carried out after staining the organisms with acridine orange and filtering them onto Nucleopore filters. For transmission electron microscopy, a water sample was concentrated by centrifugation. The pellet was solidified in agar, fixed, dehydrated, embedded in Epon, and cut into thin sections. The number and area of organism profiles per unit area of of the sections were determined. The number of organisms per unit volume of the pellet was than calculated using stereological formulae. The corresponding number in the lake water was obtained from the ratio of volume of solidified pellet/volume of water sample. Control experiments with pure cultures of bacteria and algae showed good agreement between light and electron microscopic counts. This was also true for most lake water samples, but the electron microscopic preparations from some samples contained small vibrio-like bodies and ill-defined structures that made a precise comparison more difficult. Bacteria and small blue-green and green algae could not always be differentiated with the light microscope, but this was easily done by electron microscopy. Our results show that transmission electron microscopy can be used for checking light microscopic counts of microorganisms in lake water".

Watson, S.W., T.J. Novitsky, H.L. Quinby, and F.W. Valois. 1976. Determination of bacterial number and biomass in the marine environment. Appl. and Environ. Micro. 33:940-946.

"Three techniques for the measurement of bacterial numbers and biomass in the marine environment are described. Two are direct methods for counting bacteria. The first employs an epifluorescence microscope to view bacteria that have been concentrated on membrane filters and stained with acridine orange. The second uses a transmission electron microscope for observing replicas of bacteria that are concentrated on membrane filters. The other technique uses Limulus amebocyte lysate, an aqueous extract from the amebocytes of the horseshoe crab, *Limulus polphemus* , to quantitate lipopolysaccharide (LPS) in seawater samples. The biomass of gram-negative (LPS containing) bacteria was shown to be related to the LPS content of the samples. A factor of 6.35 was determined for converting LPS to bacterial carbon".

8. AOSERP RESEARCH REPORTS

1.		AOSERP First Annual Report, 1975
2.	AF 4.1.1	Walleye and Goldeye Fisheries Investigations in the Peace-Athabasca Delta--1975
3.	HE 1.1.1	Structure of a Traditional Baseline Data System
4.	VE 2.2	A Preliminary Vegetation Survey of the Alberta Oil Sands Environmental Research Program Study Area
5.	HY 3.1	The Evaluation of Wastewaters from an Oil Sand Extraction Plant
6.		Housing for the North--The Stackwall System
7.	AF 3.1.1	A Synopsis of the Physical and Biological Limnology and Fisheries Programs within the Alberta Oil Sands Area
8.	AF 1.2.1	The Impact of Saline Waters upon Freshwater Biota (A Literature Review and Bibliography)
9.	ME 3.3	Preliminary Investigations into the Magnitude of Fog Occurrence and Associated Problems in the Oil Sands Area
10.	HE 2.1	Development of a Research Design Related to Archaeological Studies in the Athabasca Oil Sands Area
11.	AF 2.2.1	Life Cycles of Some Common Aquatic Insects of the Athabasca River, Alberta
12.	ME 1.7	Very High Resolution Meteorological Satellite Study of Oil Sands Weather: "a Feasibility Study"
13.	ME 2.3.1	Plume Dispersion Measurements from an Oil Sands Extraction Plant, March 1976
15.	ME 3.4	A Climatology of Low Level Air Trajectories in the Alberta Oil Sands Area
16.	ME 1.6	The Feasibility of a Weather Radar near Fort McMurray, Alberta
17.	AF 2.1.1	A Survey of Baseline Levels of Contaminants in Aquatic Biota of the AOSERP Study Area
18.	HY 1.1	Interim Compilation of Stream Gauging Data to December 1976 for the Alberta Oil Sands Environmental Research Program
19.	ME 4.1	Calculations of Annual Averaged Sulphur Dioxide Concentrations at Ground Level in the AOSERP Study Area
20.	HY 3.1.1	Characterization of Organic Constituents in Waters and Wastewaters of the Athabasca Oil Sands Mining Area

21.		AOSERP Second Annual Report, 1976-77
22.	HE 2.3	Maximization of Technical Training and Involvement of Area Manpower
23.	AF 1.1.2	Acute Lethality of Mine Depressurization Water on Trout Perch and Rainbow Trout
24.	ME 4.2.1	Review of Dispersion Models and Possible Applications in the Alberta Oil Sands Area
25.	ME 3.5.1	Review of Pollutant Transformation Processes Relevant to the Alberta Oil Sands Area
26.	AF 4.5.1	Interim Report on an Intensive Study of the Fish Fauna of the Muskeg River Watershed of Northeastern Alberta
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45.	VE 3.3	Interim Report on Physiology and Mechanisms of Air-Borne Pollutant Injury to Vegetation, 1975 to 1978

46.	VE 3.4	Interim Report on Ecological Benchmarking and Biomonitoring for Detection of Air-Borne Pollutant
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53.	HY 3.1.2	Baseline States of Organic Constituents in the Athabasca River System Upstream of Fort McMurray
54.	WS 2.3	A Preliminary Study of Chemical and Microbial Characteristics of the Athabasca River in the Athabasca Oil Sands Area of Northeastern Alberta.
55.	HY 2.6	Microbial Populations in the Athabasca River

These reports are not available upon request. For further information about availability and location of depositories, please contact:

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