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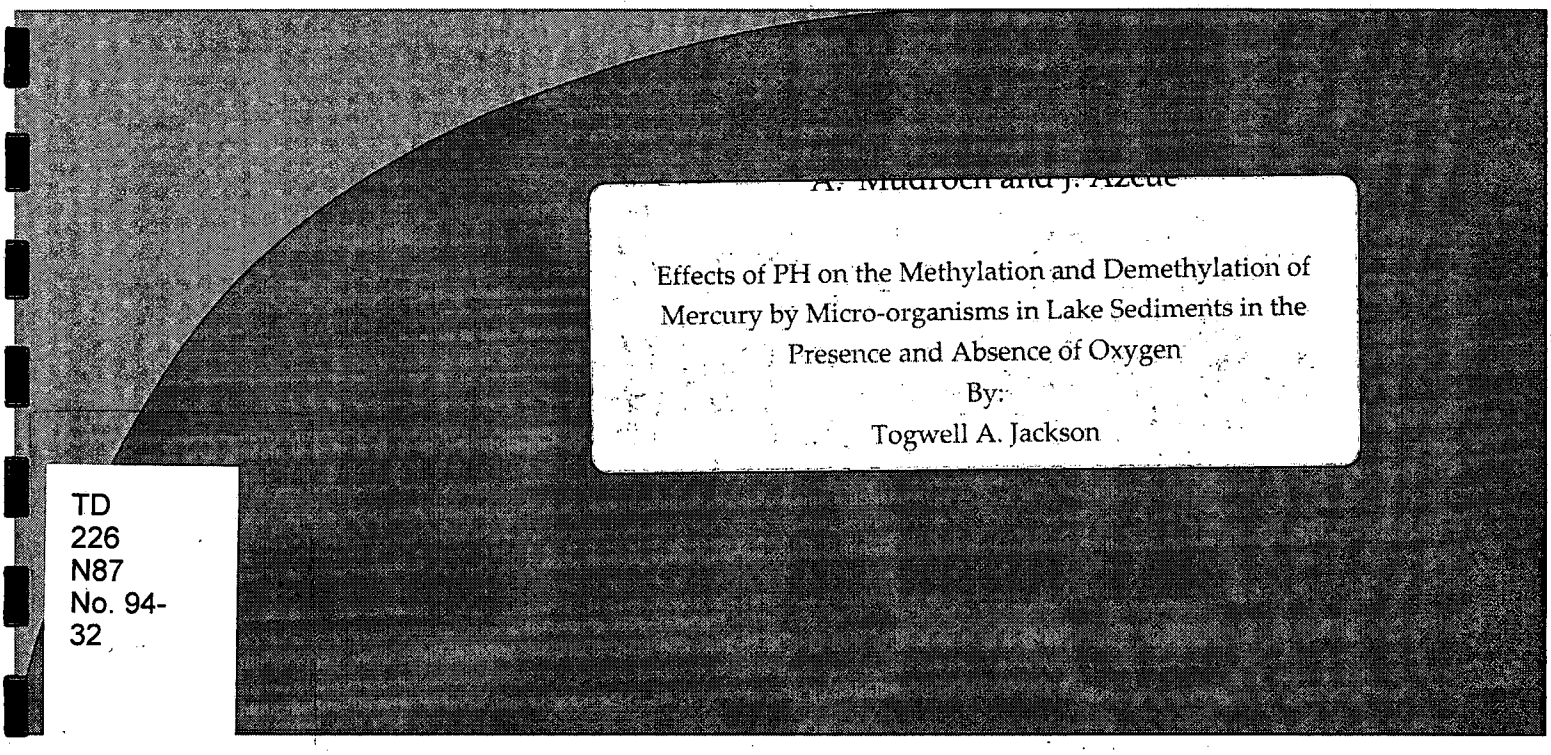

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Effects of PH on the Methylation and Demethylation of Mercury by Micro-organisms in Lake Sediments in the Presence and Absence of Oxygen

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MANAGEMENT PERSPECTIVE FOR "EFFECTS OF pH ON THE METHYLATION AND DEMETHYLATION OF MERCURY BY MICRO-ORGANISMS IN LAKE SEDIMENTS IN THE PRESENCE AND ABSENCE OF OXYGEN," BY T.A. JACKSON

25 August, 1994

An important, widespread environmental problem in Canada and other parts of the world is the general tendency of acid precipitation to cause an increase in the mercury content of fish in poorly buffered lakes. The result has been that fish in many remote lakes of the Canadian Shield have high mercury concentrations even though the lakes have not been affected by point-sources of mercury pollution. This problem has attracted considerable worldwide attention in recent years. Although hypotheses have been advanced to account for the problem, the cause of the phenomenon is still unknown. A possible explanation is that acidification somehow increases the rate at which micro-organisms in sediments and water produce methyl mercury (a highly toxic compound that is preferentially accumulated by fish tissues); but investigations of the phenomenon have yielded conflicting results, suggesting that the processes involved are complex and involve interactions of pH with a number of other factors.

This manuscript presents the results of a research project which may contribute to our understanding of the problem. The study was part of a larger Canada Water Act project undertaken by the author with the support of funds granted under the terms of the Canada-Manitoba Agreement on the investigation of mercury in recently formed hydroelectric reservoirs along the Churchill River diversion route in northern Manitoba. In a series of laboratory experiments employing the microfloras of lake sediments from northern Manitoba, effects of pH on the methylation and demethylation of mercury in the presence and absence of dissolved oxygen were examined. The methylation and demethylation rates were found to peak at pH values in the range ~6.0-7.5 (near neutrality) in both anoxic and oxygenated environments. At pH ~7 methylating activity was more intense under anoxic conditions than in the presence of oxygen, whereas demethylating activity was more intense in the presence of oxygen; for both of these reasons, the net rate of methyl mercury production at pH values near 7 was much greater in anoxic than in well oxygenated environments. However, under relatively acidic conditions (pH 4.5-6.0) the rates of both methylation and demethylation were the same in oxygenated environments as in anoxic environments. Thus, under sufficiently acidic conditions the rate of methyl mercury production was not affected by dissolved oxygen. These observations suggest that a *combined* effect of pH and dissolved oxygen (rather than an effect of pH alone) on the balance between the activities of methylating *and* demethylating micro-organisms may be responsible for an increase in the annual net rates of methyl mercury in poorly buffered lakes, resulting in the commonly observed rise in the mercury levels in fish following acidification. The results indicate avenues of future research which may lead to a fuller understanding of the effects of pH and other factors on methyl mercury production and may therefore provide a guide for management decisions which could ameliorate the mercury problem arising from acidification of lakes.

Abstract

Experiments were done to determine the effects of pH on the methylation and demethylation of mercury (Hg) by microbes in unpolluted Canadian Shield lake sediments in the presence and absence of O_2 (under air and N_2, respectively) over a sediment pH range of ~4.5-8.6. The methylation and demethylation rates peaked at pH values close to 7 (in the range ~6.0-7.5) under both air and N_2, decreasing progressively as conditions became increasingly acidic or alkaline. At pH ~7 methylating activity was more intense under N_2 than under air, but demethylating activity was more intense under air; thus, methyl Hg production was maximised in the absence of O_2. At pH values of ~4.5-6, however, the rates of both methylation and demethylation were the same under air as they were under N_2. These results suggest that the generally observed increase in the Hg content of fish in poorly buffered lakes following acidification may be caused not by low pH alone but by a combined effect of pH and dissolved O_2 which alters the balance between the activities of methylating and demethylating microbes, giving rise to an increase in the annual net rates of methyl Hg production in the lakes.

Introduction

Field studies involving many lakes in different geographical areas, together with controlled experiments, have established the important empirical generalisation that the mercury (Hg) content of fish in freshwater lakes usually increases as the pH, alkalinity, hardness, conductivity, and acid neutralising capacity of the water decrease (Jernelöv, 1972; Jernelöv *et al.*, 1975; Brouzes *et al.*, 1977; Scheider *et al.*, 1979; Wren and MacCrimmon, 1983; Håkanson *et al.*, 1988; Richman *et al.*, 1988; Lathrop *et al.*, 1989 and 1991; McMurty *et al.*, 1989; Cope *et al.*, 1990; Grieb *et al.*, 1990; Wiener *et al.*, 1990; Winfrey and Rudd, 1990; Ponce and Bloom, 1991; Wren *et al.*, 1991). Thus, acidification of poorly buffered, soft-water lakes is apt to cause a significant rise in the Hg concentrations of fish inhabiting these lakes, even if the lakes have no history of Hg pollution from point sources and therefore have only background traces of Hg in their sediments and water. Lakes of this nature are typical of regions throughout the world where the bedrock is composed mostly of silicate minerals, and they abound in the Canadian Shield. Acidification of such lakes has been occurring over wide areas because of acid rain (Schindler, 1988); a more localised process such as acid mine drainage could have a similar effect.

Although the inverse correlation between the pH of lake water and the Hg content of the fish living in the water is firmly founded on a large body of data, the underlying causes of this relationship are poorly understood. The observed effect may well be the net result of the complex interplay of a number of different physicochemical and biological phenomena (Wood, 1980; Richman *et al.*, 1988; Winfrey and Rudd, 1990; Ponce and Bloom, 1991). A direct effect of pH on the bio-accumulation of CH_3Hg^+ has been regarded as a possibility, but it has received only limited support from the available evidence and would seem to be, at most, a minor contributing factor (Bloom *et al.*, 1991; Ponce and Bloom, 1991). Probably a more compelling explanation is that pH variations affect the production of monomethyl mercury (CH_3Hg^+) by heterotrophic microbes in the surficial bottom sediments and water, but research in this area has yielded

seemingly contradictory results. Some publications claim that acidification increases the net rate of CH_3Hg^+ production (Jernelöv, 1972; Fagerström and Jernelöv, 1972; Beijer and Jernelöv, 1979; Jackson and Woychuk, 1980a, 1980b, 1981; Miskimmin *et al.*, 1992; Wood, 1980; Xun *et al.*, 1987; Winfrey and Rudd, 1990; Bloom *et al.*, 1991; Matilainen *et al.*, 1991; also see Jackson *et al.*, 1980), whereas other papers report evidence that acidification tends to inhibit CH_3Hg^+ production (Shin and Krenkel, 1976; Ramlal *et al.*, 1985; Jackson, 1987; Steffan *et al.*, 1988). This paradox probably indicates that the role of pH is complex and can be understood only by examining the combined effects of pH and other factors. Experimental data presented by Jackson (1987) suggest that investigation of the combined effects of pH and dissolved O_2 may help to solve the riddle, and observations reported by Jackson and Woychuk (1980a, 1980b, 1981) and by Matilainen *et al.* (1991), as well as literature surveyed by Winfrey and Rudd (1990), are consistent with this possibility. Furthermore, a distinction must be made between the rate of Hg methylation (a function of the activities of methylating microbes) and the net rate of CH_3Hg^+ production (the net result of the activities of methylating microbes and associated demethylating microbes).

The present paper reports the results of a series of experiments carried out to determine the effects of pH variations on the methylation and demethylation of Hg by microbes in sediments from two Canadian Shield lakes in the presence and absence of dissolved O_2. A brief account of this work was published elsewhere (Jackson, 1987) as part of a report on the biogeochemistry of mercury in recently formed hydroelectric reservoirs (also see Jackson, 1988a, 1988b, 1989, 1991a). Here the subject is discussed more fully.

Field Sites, Materials, and Methods

Description of the Field Area and Sampling Sites

The field area, which is situated in northern Manitoba, Canada, is characterised by low relief, moist sub-Arctic continental climate, podzolic soil,

and Boreal forest dominated by black spruce (*Picea mariana*). The bedrock in this region consists of Precambrian igneous and metamorphic rocks largely overlain by Pleistocene glacial deposits containing carbonate minerals (calcite and dolomite), which tend to keep the pH values of local surface waters close to neutrality (Jackson, 1988b; Jackson and Hecky, 1980). As the lakes are not acid-stressed in spite of their geographical location, their sediments provide suitable baseline environments for experimental study of the responses of Hg-transforming micro-organisms to acidification.

Sediment samples for analysis and experimental use were taken from East Mynarski Lake, a relatively productive pristine lake, and from a shallow bay (informally called Methyl Bay owing to the occurrence of intense Hg methylating activity there) within the near-shore zone of recently flooded land in Southern Indian Lake, a riverine lake which was artificially expanded to form a hydroelectric reservoir by impoundment and diversion of the Churchill River during the period 1974-1976. For a map of the field area showing the location of the sampling sites, see Figure 1 of Jackson (1988b).

Field work and laboratory analyses

Grab samples of fine-grained offshore bottom sediments (approximately the top 10-15 cm), along with water samples and field data, were collected in August, 1983 and June, 1984. The East Mynarski Lake sediment consisted of grey mud with black mottling and had a faint sulfide odour; the sediment from Methyl Bay was made up of grey mud mixed with abundant plant detritus from the submerged forest, and it, too, had a slight sulfide odour. The samples were kept temporarily in a cool, dark storage space at a field camp and transferred a few days later to the laboratory, where they were stored in a 4°C cold room or in a freezer until required for analysis or experimentation. The sediments and water were analysed in detail (Jackson, 1988b), and sediment samples were subjected to laboratory experiments involving measurement of the production and decomposition of methyl mercury (CH_3Hg^+) and other indices of microbial activity, such as CO_2 and CH_4

production, under different conditions (Jackson, 1987, 1988b, 1989, 1991a, 1991b). The sediments reserved for experimental use were stored in the dark at 4°C in plastic bags from which air had been excluded. Compared with sediments from other lakes in the vicinity and from other sampling sites within Southern Indian Lake, the sediments from East Mynarski Lake and Methyl Bay were rich in labile organic matter. Consequently, they supported relatively intense heterotrophic microbial activity, resulting in copious production of both CH_3Hg^+ and CO_2 (and CH_4 in the case of Methyl Bay sediment) during incubation in the presence of bio-available inorganic Hg(II) under an inert atmosphere (N_2) (Jackson, 1987, 1988b).

Methylation and Demethylation Experiments

The methylation and demethylation experiments were performed in batches of 125 mL Pyrex Erlenmeyer flasks, each of which was fitted with a silicone stopper pierced by a tube connected to a three-way stopcock for sampling headspace gas. Replicate 10 g portions of homogenised sediment were weighed into the flasks, and other ingredients, such as buffers for maintaining desired pH values (see below), were added as needed. In several experiments 0.1000 g of air-dried sphagnum moss pulverised with a Cyclone sample mill (UD Corp.) was added to each flask to serve as a nutrient substrate for the microbes (sphagnum being a widespread, abundant component of the terrestrial vegetation in northern Manitoba, and therefore an important source of nutrients for Hg-methylating bacteria and other heterotrophic microbes in land areas submerged because of reservoir creation (Hecky *et al.*, 1987; Jackson, 1987, 1988b)). In methylation experiments the sediment in each reaction vessel was mixed with 20 mL of 10 μM $HgCl_2$ solution, but in demethylation experiments 20 mL aliquots of 100 nM CH_3Hg^+ acetate solution were used instead. If anoxic conditions were required, the slurries were purged with commercial grade (99.9% pure) N_2, and the headspace air in each flask was replaced with N_2; to create an oxygenated environment, ordinary air was used as the headspace atmosphere. The flasks, stoppers, and all solid experimental

materials except the sediment, which served as the source of live microorganisms, were autoclaved beforehand, and all solutions were sterilised by filtration through autoclaved 0.45 μm membrane filters. "Experimental" slurries containing buffers or other test substances affecting the pH were accompanied by "control" slurries lacking these additives but otherwise identical to the experimental systems. The experimental and control systems were incubated in the dark at room temperature (25 $\pm$ 1°C), with occasional swirling, either for 7 days or for varying lengths of time up to 14 days, after which the contents of the flasks were analysed. Starting conditions were ascertained by analysis of replicate experimental and control systems at the outset of the cycle of incubation.

For the investigation of the effects of ambient pH on the rates of microbial methylation and demethylation of Hg in the presence and absence of O_2, solutions of various buffers were used as the aqueous phases of the experimental slurries to fix the pH of each slurry at a desired value and keep it constant throughout incubation. The buffers employed were potassium hydrogen phthalate, potassium dihydrogen phosphate (KH_2PO_4), tris(hydroxymethyl)aminomethane, and borax ($Na_2B_4O_7{\cdot}10H_2O$). Stock solutions of these compounds were prepared beforehand and adjusted to pH values ranging from 4.00 to 10.00 by the addition of HCl or NaOH solution. Separate replicate sets of buffered and unbuffered slurries were incubated under air and N_2 for 7 days. Other experiments, in which the incubation time was variable, tested the reaction of anaerobic methylating microbes in sediments to the buffering action of reagent-grade $CaCO_3$ (1 g per flask), which maintained slurry pH values close to 7. Another experiment was performed to compare the effects of $CaCO_3$ and solutions of KH_2PO_4 and tris(hydroxymethyl)aminomethane adjusted to pH 7 over an incubation period of 7 days. In a related group of experiments involving incubation for varying lengths of time, slurries were mixed with calcareous silty clay from a varved Pleistocene glacial lake deposit on the shore of South Bay in Southern Indian Lake to determine the effect of the clay on methylating activity. Erosion of this material into the waters of the bay has had a significant impact on the local

aquatic environment, and there are grounds for thinking that it tends to inhibit CH_3Hg^+ production and the bio-accumulation of Hg (Jackson, 1987, 1988a, 1988b, 1991a). The mineral composition of the clay has been published elsewhere (Jackson, 1988b), and a small selection of total element concentrations has been reported as well (Hecky *et al.*, 1987); grain-size analysis showed that the clay is finer than 4 phi (in the silt-clay range) (Jackson, 1987). Before being employed in experiments, the clay was either passed through a 150 μm (100 mesh) screen or dispersed in deionised water, ultrasonified for 15 minutes, left standing for 1 hour to allow the coarser particles to settle out, and dialysed against deionised water to remove exchangeable salts and other adsorbed substances. One set of experiments involved clay which had been pickled in 1 M HCl (pH 1) for 16 hours and then dialysed, the purpose of the treatment being to remove carbonates and adsorbed substances. In the experiments involving clay, replicate slurries were mixed with varying amounts of clay (0-5 g) and incubated for 7 days or were mixed with a fixed quantity of clay (5 g per flask) and incubated for different lengths of time. The time-lapse experiment was repeated using slurries amended with reagent-grade $CaCO_3$ so that effects of noncarbonate components of the clay could be differentiated from effects due to buffering by the carbonate minerals in the clay. A more detailed and comprehensive treatment of the effects of clay minerals and other natural colloids on methylation and demethylation has been published elsewhere (Jackson, 1989).

After incubation, samples of the headspace gas were withdrawn with a syringe and analysed for CO_2 and CH_4 by gas chromatography (GC) using a Basic model 8000 GC unit (CA Instruments). The pH and Eh values of the slurries were then measured with a Corning Model 12 pH meter, employing a calomel reference electrode with Ag/AgCl and platinum electrodes for the pH and Eh readings, respectively. Finally, the CH_3Hg^+ content of each slurry was determined by (1) extraction of a weighed portion of homogenised slurry with $CuSO_4$ and $NaBr/H_2SO_4$ solutions and toluene, in which the CH_3Hg^+ dissolved as CH_3HgBr; (2) extraction of the CH_3Hg^+ into an aqueous, ethanolic $Na_2S_2O_3$ solution, in which it dissolved as a hydrophilic thiosulfate complex; (3) purification of the solution by

successive rinsings with benzene; (4) treatment of the purified solution with KI and benzene, converting the CH_3Hg^+ to CH_3HgI, which then dissolved in the benzene; and (5) analysis of the benzene solution with a Tracor Micro-Tek (MT) 220 GC unit employing a column of 10% Sp-1000 on 80/100 "Supelcoport" (Supelco Co.) with ultra high-purity N_2 as the carrier gas, and a Varian electron capture detector employing tritium (Uthe *et al.*, 1972). The validity of the CH_3Hg^+ data was checked by a confirmation test involving removal of all CH_3Hg^+ from the benzene solution by treatment with aqueous Ag_2SO_4 to convert it to a water-soluble sulfate, which was extracted into the aqueous phase (Jensen, 1969), followed by repetition of the GC analysis of the benzene solution. The detection limit for CH_3Hg^+ was 0.1-0.25 ng/g (wet weight), and the abundance of CH_3Hg^+ was expressed as total nanograms per flask.

Finally, it should be noted that experiments comparing sterilised and unsterilised samples of East Mynarski Lake sediment have demonstrated that all of the methylation and most of the demethylation occurring in the sediment are attributable to the activities of micro-organisms (Jackson, 1987, 1988b, 1989). In the presence of a viable sedimentary microflora, abiotic formation and decomposition of CH_3Hg^+ can be regarded as nonexistent or negligible.

Results and Discussion

Variation of anaerobic methylating activity with pH: preliminary experiments

Experiments employing various buffers

The results of an experiment to determine the effects of pH and various pH buffers on Hg methylation in a series of duplicate nutrient-enriched specimens of East Mynarski Lake sediment incubated under N_2 for 7 days are displayed in Figure 1. The figure, a plot of the total quantity of CH_3Hg^+ produced by day 7 against the pH of the slurry on day 7, shows that the CH_3Hg^+ level increased progressively and rather steeply with increasing pH over the entire range of pH values, which varied from 5.78 to 7.10; thus, the amount of CH_3Hg^+ generated at

pH 7.10 was 2.5 times the amount produced at pH 5.78. Evidently a neutral or weakly alkaline pH has a much more favourable effect than a mildly acidic pH on the production of CH_3Hg^+ by anaerobic microbes in the bottom sediments of the lake. Regression analysis affirmed the significance of the correlation between CH_3Hg^+ production and pH (Fig. 1); in addition, it revealed that the relationship is probably logarithmic, as regression of the logarithms of the CH_3Hg^+ data on the pH values yielded the highest correlation coefficient (r-value). Accordingly, the logarithms of the CH_3Hg^+ data were used to compute the regression line (Fig. 1). The inference that a logarithmic function most precisely defines the relationship implies that even a small change in ambient pH may cause a pronounced change in the rate of CH_3Hg^+ production; and it suggests a direct effect of pH on the growth rates of methylating microbes. Also noteworthy is the fact that there was remarkably little deviation of the points in the scatter diagram from the regression line (Fig. 1) despite the marked differences in the chemical composition of the slurries owing to the use of different buffers. Regression analysis showed that ~97% of the variation in the quantity of CH_3Hg^+ produced can be explained by variation in the ambient pH (r^2 = 0.966). Thus, CH_3Hg^+ production was virtually independent of any biological or physicochemical effects of the buffers other than the effects due to the variation in ambient pH. Moreover, there was excellent agreement between data from duplicate slurries (Fig. 1).

However, the pH values of slurries containing different buffers differed from one another more widely than expected, and in unforeseen ways, although this did not diminish the usefulness of the results. As the experiment was designed primarily to compare the effects of different buffers at the same pH (~7), the buffers selected for testing were $CaCO_3$ powder and solutions of KH_2PO_4 and tris(hydroxymethyl)aminomethane which had been adjusted to pH 7 before being mixed with sediment; but immediately after the two buffer solutions came into contact with the sediment, the pH values of the resulting slurries were found to lie in the range 6.05-6.81, whereas the slurries containing $CaCO_3$ had an initial pH of 7.16. During incubation the pH values of the $CaCO_3$-buffered and control

slurries showed hardly any change (the pH of $CaCO_3$-buffered slurries dropping from 7.16 to the range 7.08-7.10, and the pH of control slurries rising from 5.70 to 5.78); but the pH of the tris(hydroxymethyl)aminomethane-buffered slurry decreased from 6.81 to the range 6.09-6.16, whilst the pH of the phosphate-buffered slurry increased from 6.05 to the range 6.82-6.90. The relatively large changes in the pH values of the slurries containing buffer solutions presumably resulted from interactions of the buffers with the sediments (for instance, adsorption by clay or utilisation by microbes). The CH_3Hg^+ levels at day 7 correlated much more significantly with the final (day-7) pH than with the initial pH, and so the final pH was assumed to be a more meaningful parameter than the initial pH for the purpose of determining effects of pH on CH_3Hg^+ production in this experiment.

Experiments employing calcium carbonate or calcareous silty clay, or both

In a separate set of experiments, raising the pH values of East Mynarski Lake and Methyl Bay sediments from the range 5.81-6.75 to the range 6.87-7.23 by adding $CaCO_3$ boosted the production of CH_3Hg^+ in slurries from which O_2 had been excluded by as much as an order of magnitude (Fig. 2, A-C). The disparity between the CH_3Hg^+ yields in unbuffered and $CaCO_3$-buffered specimens of East Mynarski Lake sediment was much more extreme without the addition of an organic nutrient supplement (pulverised moss) (Fig. 2B) than with it (Fig. 2A), even though the difference between the pH values was no greater (and, in fact, was slightly less) without nutrient enrichment (Fig. 2A,B); evidently nutrient enrichment went far toward compensating for the negative effect of the somewhat acidic environment of the unbuffered sediment. In the absence of the nutrient substrate, the amount of CH_3Hg^+ produced was slightly but consistently greater in the unbuffered slurries for the first 3 days of incubation and then abruptly became more than tenfold higher in the buffered slurries (Fig. 2B), suggesting that a species of methylating microbe which had been quiescent or in the lag phase of its growth suddenly became an active, if not dominant, member of the microflora. A less dramatic example of the same phenomenon is discernible in the

Methyl Bay data (Fig. 2C).

As with $CaCO_3$, the addition of small quantities of <150 μm calcareous silty clay (up to 0.1 g per flask) stimulated CH_3Hg^+ production by microbes in nutrient-enriched East Mynarski Lake sediment incubated for 7 days in the absence of O_2, apparently because the carbonate minerals in the clay raised the ambient pH appreciably (from ~6 in the control slurry to slightly more than 7 in the presence of 0.10 g of clay) (Fig. 3) (Jackson, 1987, 1989). (Both the initial pH (Fig. 3) and the final pH (not shown) showed virtually the same relationship with the amount of clay added.) With further increases in the abundance of clay up to 5 g, resulting in a further steady rise in the pH, the accompanying increase in the CH_3Hg^+ yield was sporadically interrupted by abrupt decreases suggesting upsurges in demethylating activity (Jackson, 1989); these anomalies can be attributed to changes in the species composition of the active microflora (ecological succession) in response to the progressive alteration of the physicochemical environment (Jackson, 1989). Such unpredictable effects are not surprising. The clay is highly heterogeneous, being a combination of carbonate and silicate minerals and other constituents, and its colloidal components have biologically important surface features such as ion exchange sites and iron oxyhydroxide (FeOOH) coatings on clay crystals. Such materials must have diverse and complex ecological effects, especially when acting upon entire microbial communities comprising many different species varying in their physicochemical requirements and limits of tolerance and interacting with each other as well as with environmental factors in various direct and indirect ways (Jackson, 1989).

In a pair of field experiments performed in limnocorrals installed in Southern Indian Lake, Hecky *et al.* (1987) independently examined effects of a few arbitrarily selected quantities of the same calcareous silty clay on CH_3Hg^+ production, which they measured indirectly by introducing $^{203}HgCl_2$ into the water and then monitoring the accumulation of ^{203}Hg by fish. Their results are in agreement with the observations reported here, as they demonstrate that the clay tended to promote CH_3Hg^+ production but was not consistent in its effects insofar as the relationship between the CH_3Hg^+ content of fish and the quantity of clay

added was concerned. Surprisingly, however, Hecky *et al.* (1987) made no attempt to interpret their results even though they had amassed enough information to develop a working hypothesis. Evidently it did not occur to them that pH buffering by the carbonate minerals in the clay might account for the stimulatory effect of the clay on methylation; yet their own raw data show that the clay is rich in carbonate and caused a rise in the pH and dissolved inorganic carbon content of the water paralleling the rise in the ^{203}Hg content of the fish (although the pH differential due to addition of clay was, for some reason, much greater in one of the experiments than in the other). Investigation of the phenomena responsible for their observations seems to have been beyond the scope of their regrettably superficial empirical study.

Figure 4A shows effects of dialysed clay and acid-treated clay (5 g of each per flask) on the production and subsequent decomposition of CH_3Hg^+ in nutrient-enriched East Mynarski Lake sediment incubated under N_2 for different lengths of time (also see Jackson, 1987). CH_3Hg^+ levels in sets of replicate experimental and control systems were plotted against incubation time, which varied from 0 to 14 days. In this experiment all slurries are designated as "unbuffered" because they contained no added buffer other than the calcite and dolomite naturally present in the clay (Jackson, 1988b). Another experiment of the same kind was performed, but this time all slurries were amended with $CaCO_3$ to eliminate the difference between the pH values of the experimental and control systems so that effects of noncarbonate constituents of the clay could be distinguished from the effects due to the buffering action of the carbonate minerals in the clay. The results are displayed in Figure 4B (also see Jackson, 1987). In both experiments the CH_3Hg^+ concentrations in the experimental and control systems rose progressively until day 7, whereupon methylation ceased and the CH_3Hg^+ content either decreased because of demethylation or levelled off because cessation of CH_3Hg^+ production was not followed by demethylation. In the unbuffered systems (Fig. 4A) the addition of clay to the sediment increased the net rate of CH_3Hg^+ production considerably, because the carbonates in the clay increased the pH values of the slurries from slightly acidic (5.81-6.16) to slightly alkaline

(7.15-7.43) (Fig. 4A). However, in the slurries amended with $CaCO_3$, which made the pH values of the controls (6.99-7.19) nearly the same as those of the systems containing added clay (7.10-7.35), the clay caused a small but consistent decrease in the net rate of CH_3Hg^+ production (Fig. 4B). Thus, the noncarbonate constituents of the clay tended to inhibit CH_3Hg^+ production slightly, but in the absence of $CaCO_3$ supplements, the beneficial effect of pH buffering by the carbonate minerals in the clay more than offset the negative effect of the noncarbonate minerals on the activities of methylating microbes. In both experiments the clay strongly promoted decomposition of the CH_3Hg^+ after day 7, whereas the acid-treated clay severely inhibited both methylation and subsequent demethylation; as these phenomena occurred regardless of whether the pH values of the slurries had been adjusted with supplementary $CaCO_3$, they can be attributed to noncarbonate constituents of the clay (Jackson, 1989). As with CH_3Hg^+, the CO_2 concentrations in the experimental and control systems increased with time for the first few days but declined steadily after day 7 in the presence of the clay (Jackson, 1987, 1989). This pattern of variation constitutes presumptive evidence for ecological succession in the microflora (replacement of the initially dominant Hg methylating, CO_2 producing microbes by demethylating, CO_2 consuming populations). Further experiments using citrate/dithionite-treated clay confirmed that removal of FeOOH coatings from the clay particles accounts for the strongly inhibitory effect of acid-treated clay (Jackson, 1989), although acidity may also have played a part in the unbuffered systems. In general, FeOOH tends to promote Hg methylation, and FeOOH coatings on clay crystals tend to protect both methylating and demethylating microbes from harmful effects of the clay, although the ecological functions of FeOOH coatings vary with other environmental factors such as nutrient levels (Jackson, 1989; also see Matilainen *et al.*, 1991). It should also be borne in mind that in a natural body of water the introduction of sufficiently large amounts of any kind of mineral detritus would probably tend to suppress the activities of methylating microbes by diluting and rapidly burying organic nutrient substrates, by interfering with exchanges of dissolved nutrients and metabolic waste products

between the microbes and the water, by inhibiting primary production of organic nutrient substrates, and perhaps by adsorbing dissolved nutrients (Jackson, 1993a). In any case, it is clear from the experimental results that the calcareous silty clay plays a complex role in the ecology of Hg methylating and demethylating microbes: It simultaneously promotes and inhibits microbial activities (*selectively* fostering certain activities whilst interfering with others), and its *net* effect is probably a function not only of the nature of the various constituents of the clay and their proportions in the clay but also the abundance of the clay, the particular combination of other physicochemical factors acting upon the microflora, and the characteristics and interrelations of the species that make up the microflora. In nature the net effect of the clay on the production and bio-accumulation of CH_3Hg^+ is probably a composite of many different effects, some negative and others positive, and a change in environmental conditions could shift the balance one way or the other.

Obviously any attempt to extrapolate experimental observations, such as those discussed above, to phenomena occurring in natural aquatic environments requires caution and must, above all, be backed by data from field studies. To determine the net effect of the calcareous silty clay in nature, a detailed comparative study of field samples of sediments and organisms from lake and reservoir environments of northern Manitoba was carried out (Jackson, 1987, 1988a, 1988b, 1991a). The samples were taken from localities where the aquatic environment has been affected to different degrees, or not at all, by clay eroded into the water from shoreline deposits. The results revealed that *in nature* the net effect of the clay is to depress the production of CH_3Hg^+ and the bio-accumulation of Hg (Jackson, 1987, 1988a, 1988b, 1991a). This may result both from inhibition of methylating activity and, perhaps especially, from strong stimulation of demethylating activity (Fig. 4B; Jackson, 1989). Investigation of a chain of Hg-polluted riverine lakes in Saskatchewan also led to the conclusion that clay and silt, which are transported into the lakes as fluvial detritus, inhibit CH_3Hg^+ production (Jackson, 1993); experiments performed on samples of lake sediment confirmed this inference (although in this case clay

inhibited methylation severely but did not foster subsequent demethylation) (Jackson, 1989). Similarly, field experiments performed by Rudd and Turner (1983) in Clay Lake, Ontario showed that silt and clay tended to suppress the accumulation of Hg by food chain organisms. Thus, there are grounds for believing that silt and clay generally tend to suppress the methylation and bio-accumulation of Hg. It is worth emphasising that the experimental results demonstrating positive effects of calcareous silty clay on CH_3Hg^+ production (Fig. 3, 4A) and bio-accumulation (Hecky *et al.*, 1987) have nothing to do with silt and clay as such and have everything to do with calcite and dolomite which happen to be present in this particular material. The apparent disagreement between the results of the field study in northern Manitoba and results of experiments demonstrating a net stimulatory effect due to the buffering action of the carbonate minerals could be explained by the fact that calcareous glacial deposits are so widespread in the field area that the waters of the region as a whole are well buffered and therefore have pH values ranging from nearly neutral to mildly alkaline regardless of whether major amounts of the clay are being washed into them from local shoreline deposits (Jackson, 1988b; Jackson and Hecky, 1980). In other words, the experiment involving slurries buffered with supplementary $CaCO_3$ (Fig. 4B) may be more relevant to the situation that exists in freshwater environments of northern Manitoba than the experiments performed on "unbuffered" slurries (Figs. 3, 4A; Hecky *et al.*, 1987). The quantity of clay introduced into the water at a particular site in a given period of time, the rate at which suspended clay settles out, and the effectiveness of fluvial currents in dispersing the clay may also be important factors. Be that as it may, the experiments of Hecky *et al.* (1987) were useless from the standpoint of elucidating the overall effect of eroded shoreline clay on CH_3Hg^+ production and bio-accumulation in the reservoirs of northern Manitoba; at the most, they provided limited information about certain natural processes that may occur in a particular set of circumstances such as the arbitrarily selected conditions of the limnocorral experiments. Accordingly, extrapolation of their conclusions from the limnocorral environments to the aquatic ecosystems of northern Manitoba

is unwarranted and could lead to eroneous conclusions. Unfortunately, Hecky *et al.* (1991) did, in fact, make this cardinal error, improperly using the results of their limnocorral experiments as a basis for speculation about the role of the clay under natural conditions. Consequently, their inferences about the effects of the clay on the biogeochemical pathways of Hg in hydroelectric reservoirs of northern Manitoba are unfounded. Hecky *et al.* (1991) compounded the error by overlooking or ignoring Jackson's previously published evidence demonstrating that eroded clay has a net inhibitory effect on Hg methylation and bioaccumulation in these reservoirs (Jackson, 1987, 1988a, 1988b). Clearly the simplistic speculations of Hecky and his coworkers are incorrect.

Experiments on the effects of pH on methylation and demethylation in the presence and absence of oxygen

The production and decomposition of CH_3Hg^+ by microbes in nutrient-enriched East Mynarski Lake sediment incubated for 7 days under atmospheres of N_2 and air varied in a remarkably regular manner as functions of pH over a wide range of pH values (Figs. 5 and 6). The patterns of variation were essentially the same regardless of whether the CH_3Hg^+ data were plotted against the initial or final (day 7) pH values, but the final pH values were judged to be more satisfactory because they yielded smoother curves and showed better agreement between the CH_3Hg^+ data for control slurries and experimental slurries of similar pH. The final pH values of the slurries ranged from 4.53 to 8.60. The pH values of the experimental (buffered) and control (unbuffered) slurries at the beginning and end of incubation, along with the pH values of the buffer solutions before coming into contact with the sediment, are listed in Table 1.

Under both N_2 and air the intensity of methylating activity increased steadily with rising pH, peaked at an optimum pH close to 7 (although the optimal pH value is impossible to determine precisely with the available information), and then declined steadily, forming simple bell-shaped curves (Fig. 5). Demethylating activity varied in a similar manner with respect to pH, and the

optimal pH, again, was close to 7 (Fig. 6). However, if we look beyond these superficial resemblances in terms of the shapes of the curves and the positions of the maxima, we are struck by the important fact that pH played a decisive part in determining what effect, if any, dissolved O_2 had on the formation and decomposition of CH_3Hg^+. At pH values close to 7 (in the range ~6-8) methylating activity was much more intense in the absence than in the presence of O_2 (Fig. 5). In contrast, demethylating activity was far more intense in the oxygenated environment than in the anoxic one (Fig. 6). For both of these reasons, the net rate of CH_3Hg^+ production at pH values near 7 was much higher in the absence of O_2 than in the presence of an ample supply of O_2. However, at pH $\leq$6 or $\geq$8 dissolved O_2 had no effect on the production and decomposition of CH_3Hg^+. For instance, at pH 5.4 the rate of demethylation was the same in an anoxic environment as in an O_2-rich environment (Fig. 6).

The observed variations in methylating and demethylating activity as functions of pH are generally consistent with the findings of several other workers (Shin and Krenkel, 1976; Ramlal *et al.*, 1985; Steffan *et al.*, 1988; Matilainen *et al.*, 1991). Moreover, the effects of dissolved O_2 (or the absence of it) at pH ~7 are in agreement with the results of other studies involving natural or experimental systems, in which the optimal conditions for CH_3Hg^+ production were shown to occur in anoxic environments or in the O_2-poor transition zone between anoxic and well oxygenated environments (Fagerström and Jernelöv, 1972; Jernelöv, 1972; Olson and Cooper, 1976; Compeau and Bartha, 1984; Callister and Winfrey, 1986; Jackson, 1987, 1988b, 1993a, 1993b; Jackson and Woychuk, 1980b; Matilainen *et al.*, 1991; Regnell and Tunlid, 1991; Mason *et al.*, 1993; Regnell, 1994; Watras *et al.*, 1994). Interestingly, Compeau and Bartha (1984) found that O_2 fosters demethylation at pH 6.8 (i.e. close to neutrality), whereas Matilainen *et al.* (1991) reported that O_2 appeared to have little effect on demethylation in a group of five lakes, four of which had acidic water (pH 4.7-6.4). These findings corroborate the experimental results reported here (Fig. 6). On the other hand, there is some literature which is ostensibly at variance with the experimental data. Thus, a number of workers have published

data suggesting that acidic conditions tend to promote CH_3Hg^+ production (Wood, 1980; Xun *et al.*, 1987; Bloom *et al.*, 1991; Miskimmin *et al.*, 1992), and Steffan *et al.* (1988) claimed that demethylating activity is independent of pH in the pH range 4.4-8.0, although it decreases sharply below pH 4.4. These apparent inconsistencies, however, do not necessarily cast doubt on the validity of any of the results that have been reported. A plausible explanation is that they reflect the complexity of the phenomena in question. Methylation and demethylation are mediated by many different kinds of coexisting micro-organisms and are affected in various ways by a multitude of environmental factors acting simultaneously; therefore, different sets of environmental conditions and different assemblages of microbial species could lead to altogether different conclusions.

Other kinds of microbial activity besides Hg methylation and demethylation, specifically, CO_2 production (a crude measure of total heterotrophic activity) and CH_4 production (a function of the activity of methanogenic bacteria), gave patterns of variation comparable to those obtained for the CH_3Hg^+ data except that the optimal pH values were not necessarily the same. CO_2 production peaked at mildly acidic pH values - close to pH 6 under anoxic conditions, but at a somewhat lower pH (~5.6) in the presence of O_2 (Fig. 7). Throughout the pH range of the experiment the CO_2 levels were consistently higher in the oxygenated systems than in anoxic ones of similar pH, but the disparity was small at pH >6, whereas it was very large at pH $\leq$6 (Fig. 7). As CH_3Hg^+ production has shown a strong positive correlation with CO_2 production in lakes and reservoirs of northern Manitoba, including East Mynarski Lake, reflecting the fact that labile organic matter stimulates the growth of heterotrophic methylating microbes (Jackson, 1987, 1988b), the displacement of the two maxima in Figure 7 may help to explain why the rates of aerobic and anaerobic CH_3Hg^+ production were the same at pH $\leq$6: Possibly the more intense heterotrophic microbial activity in the oxygenated systems raised the CH_3Hg^+ production rates to the same levels as the CH_3g^+ production rates in the corresponding anoxic systems, offsetting, to that degree, the unfavourable effect of the acidic conditions. However, this would

not account for the fact that the rates of aerobic and anaerobic CH_3Hg^+ production are virtually the same at pH $\geq$8 as well as pH $\leq$6.

CH_4 production, which, as might be expected, occurred only in the absence of O_2, was limited to a relatively narrow pH range (>6.1, <8.6) and peaked at a slightly alkaline pH (~7.5, or ~7.0-7.5 if estimated by extrapolating the flanks of the peak and assuming that the point where the two lines intersect marks the maximum) (Fig. 8). What relevance, if any, this has to the net production of CH_3Hg^+ is uncertain, as CH_4 synthesis may coincide with both methylating and demethylating activity (Wood *et al.*, 1968; Spangler *et al.*, 1973; Jackson, 1987, 1988b, 1989, 1991a, 1991b; Oremland *et al.*, 1991). Nevertheless, it is unlikely that methanogenic bacteria methylate Hg (McBride and Edwards, 1977; Compeau and Bartha, 1985), whereas a number of microbes are known to generate CH_4 as one of the end products of demethylation (Spangler *et al.*, 1973; Compeau and Bartha, 1985; Oremland *et al.*, 1991). Accordingly, CH_4 production in the anoxic slurries was probably linked directly to anaerobic demethylation.

In marked contrast to the microbial production of CH_3Hg^+, CO_2, and CH_4, the Eh of the slurry was inversely related to pH throughout the pH range of the experiments, decreasing from +300 mV at pH 4.5 to less than -200 mV at pH 8.1-8.6 (Fig. 9). This relationship can be attributed to biochemical oxidation-reduction reactions involving H^+ ions; reactions of this kind predominate in biological chemistry, and they lead to dependence of Eh on pH (Fruton and Simmonds, 1958). As would be expected, the Eh at a given pH was almost invariably lower in the anoxic slurry than in the oxygenated one (Fig. 9). The Eh data for these experiments plainly have no bearing on the problem of interpreting the effects of pH on the activities of methylating and demethylating microbes in the presence and absence of O_2. In experiments such as these, Eh can be used to estimate the overall intensity of microbial activity only if all experimental and control systems have the same pH.

Before going any further, let us digress briefly to consider an important question about the assumptions underlying the experiments represented by Figures 5-9: Considering that several different buffers were used to adjust and maintain

the various pH values of the slurries, can we be sure that no artifacts arose from biological or physicochemical effects of these substances other than the effects due to the variations in ambient pH? The following facts support the conclusion that the observed variations in the quantities of CH_3Hg^+ formed or decomposed in the presence of the buffers were due mainly to the variations in ambient pH and that any other effects of the buffers were of minor significance: (1) The curves showing variation of CH_3Hg^+, CO_2, CH_4, and Eh data with respect to pH are smooth and regular and show little scatter (Figs. 1, 5-9), whereas significant effects of the buffers other than their effects on the pH values would probably have resulted in considerable seemingly random variation; (2) as discussed above, a preliminary experiment involving slurries containing three altogether different buffers, including two of the four buffers used in the experiments represented by Figures 5-9, along with a control slurry, showed that ~97% of the variation in the CH_3Hg^+ data could be attributed to pH (Fig. 1); (3) the CH_3Hg^+, CO_2, CH_4, and Eh data for control slurries do not differ greatly from the corresponding data for buffered slurries of similar pH (Figs. 1, 5-9); (4) the bell-shaped curves representing variations in CH_3Hg^+, CO_2, and CH_4 production and CH_3Hg^+ decomposition as functions of pH (Figs. 5-8) are typical of curves showing effects of pH on microbial growth (Berkeley and Campbell, 1979) and enzyme activity (Fruton and Simmonds, 1958); and (5) the experimentally estimated pH optima (Figs. 5-8) fall in the typical range of pH optima for microbial growth (5-7.5) (Berkeley and Campbell, 1979).

More research is needed to determine the applicability of the relationships observed in the experimental systems (Figs. 5 and 6) to the biogeochemical cycling of Hg in natural aquatic environments. Nevertheless, these preliminary experimental results are highly suggestive, and they indicate avenues of future investigation which could lead to an explanation, or partial explanation, for the increase in the Hg content of fish with decreasing pH in poorly buffered lakes undergoing acidification. At first sight, the results of the experiments appear to be irrelevant to the problem of Hg bio-accumulation in acidic lakes, as they show that the optimal pH for Hg methylating activity is close to 7, declining

steadily with decreasing pH, in both anoxic and O_2-rich environments. However, if we consider the synergistic effects of pH and oxidation-reduction conditions, we come to the realisation that the results may, in fact, be highly relevant, for they suggest that the key to understanding the part played by pH in CH_3Hg^+ production and bio-accumulation in aquatic ecosystems is the combined effect of pH and dissolved O_2 (and other factors, perhaps) rather than pH alone. In the experimental model systems O_2 tended to suppress CH_3Hg^+ production at pH ~7 by inhibiting methylation and enhancing demethylation, but at pH <6 CH_3Hg^+ production was not affected by O_2 (Figs. 5 and 6). In an initially circumneutral lake whose bottom waters are well aerated for at least part of the year, acidification could, in theory, cause an increase in the net annual production of CH_3Hg^+ in the lake owing to a shift in the equilibrium between methylation and demethylation, resulting in higher overall rates of microbial CH_3Hg^+ production per year and therefore higher Hg levels in fish. For instance, exposure of the sediment-water interface to abundant dissolved O_2 might tend to suppress CH_3Hg^+ production at the interface if the water has a pH of 7 but not if the pH is 5 or 6. Assuming, moreover, that methanogenic bacteria contribute significantly to the demethylation of Hg in the absence of O_2, the strong preference of these bacteria for neutral to mildly alkaline pH values and the total suppression of methanogenic activity at pH $\leq$6.1 (Fig. 8) could tend to increase the net production of CH_3Hg^+ following acidification of an O_2-poor, nutrient-rich environment.

Investigation of the biogeochemistry of Hg in the Wabigoon River system of Northern Ontario yielded results that are consistent with the experimental observations and the author's interpretation of them (Jackson and Woychuk, 1980a, 1980b, 1981). The river system has been severely polluted with Hg and organic wastes from a chlor-alkali plant and associated pulp-and-paper mill in the town of Dryden. As expected, the total concentrations of Hg and organic matter (mainly decomposing wood chips) in surficial bottom sediments were found to decrease progressively with distance downstream from the source of pollution, but the abundance of CH_3Hg^+ varied independently of the total Hg content because

CH_3Hg^+ production was controlled by environmental factors affecting microbial activities and inorganic Hg availability, not by the total supply of inorganic Hg. Analysis of cores and grab samples of surficial organic bottom sediments (wood chips and slime) near the sources of pollution revealed the following information about the factors determining the vertical and horizonatal distribution of CH_3Hg^+: (1) CH_3Hg^+ concentrations tended to increase with decreasing pH within the observed pH range of 4.40-7.00 and also to increase with the total nitrogen concentration and the ratio of methionine to certain other amino acids in the sediment; and (2) the CH_3Hg^+ levels were generally highest, and the pH values lowest, at or near the sediment-water interface, whereas the total Hg concentrations (mainly representing inorganic Hg) were highest ~10-20 cm below the interface owing to burial of heavily Hg-contaminated sediment by younger sediment of lower Hg content (Jackson and Woychuk, 1980a, 1980b, 1981); in the top few cm of the sediment (the top 5-11 cm according to analysis of core slices) the mean pH was 5.70 and the range 4.40-6.50 (T.A. Jackson, unpublished data). The occurrence of maximum CH_3Hg^+ concentrations at or near the sediment-water interface can be explained by the existence there of optimal conditions for microbial growth in general (Kuznetsov, 1970) and the most favourable conditions for the growth of methylating microbes in particular owing to contact between O_2-rich river water and the anoxic sediment (Fagerström and Jernelöv, 1972; Mason *et al.*, 1993; Watras *et al.*, 1994), although reducing conditions prevailed even at or near the surface of the sediment (the range of Eh values being -340 to -70 mV (T.A. Jackson, unpublished data)). The pH minimum at the sediment-water interface may be ascribed to microbial oxidation of the wood fibres, resulting in the synthesis of organic acids as by-products. While insufficient to distinguish cause-and-effect relations from mere correlations, the data are consistent with the possibility that heterotrophic methylating microbes employing the methionine biosynthetic pathway for synthesis of CH_3Hg^+ (Landner, 1971), were dominant members of the microflora and that a combination of low pH and availability of O_2 at the sediment-water interface maximised the net rate of CH_3Hg^+ production. (Incidentally, it is also worth noting that the contrast

between the vertical distributions of CH_3Hg^+ and total Hg concentrations in the organic sediments provide a vivid illustration of the fact that CH_3Hg^+ production is generally controlled by environmental factors rather than total Hg abundance within a wide range of total Hg levels.)

Other workers, too, have suggested that the combined effect of pH and oxidation-reduction conditions may have important effects on rates of CH_3Hg^+ production in lakes. Thus, literature reviewed by Winfrey and Rudd (1990) purports to demonstrate that a drop in pH stimulates CH_3Hg^+ production in well aerated environments such as O_2-rich water and sediment-water interfaces exposed to oxygenated water but inhibits CH_3Hg^+ production in anoxic environments such as subsurface bottom sediments. Matilainen *et al.* (1991) emphasised that the combined effects of pH, oxidation-reduction conditions, and possibly other, related, environmental variables determine the net rate of CH_3Hg^+ production in sediments by controlling the state of balance between methylating and demethylating microbes.

In conclusion, the limited information available to date indicates that more research is needed to determine the combined effects of pH, dissolved O_2, and other factors on the methylating and demethylating activities of microbes and the net rates of microbial CH_3Hg^+ production and bio-accumulation in lakes. A particularly useful approach would be (1) a detailed, systematic comparison of many lakes representing a wide spectrum of environmental characteristics; (2) examination of temporal variations in the waters and sediments of individual lakes; and (3) controlled experiments designed to complement the field studies.

Acknowledgments

The field work was carried out by J. Tisdale with the assistance of D. Steinke, and E. DuVal. The experiments and associated analyses were done by A. Adams with technical guidance and assistance provided by senior technician K. Supeene. I also thank A. Furutani for permission to use his gas chromatograph for the gas analyses and for advice about experimental technique. The laboratory

work was carried out at the Freshwater Institute, Winnipeg, Manitoba. Financial support was provided by the governments of Canada and Manitoba through the agency of the Steering Committee for the Canada-Manitoba Agreement on the Study and Monitoring of Mercury in the Churchill River Diversion.

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TABLE 1. The pH values of the buffer solutions and the buffered and unbuffered slurries used in experiments on the methylation and demethylation of Hg by microorganisms in East Mynarski Lake sediment under atmospheres of N_2 and air. The pH values of the slurries were measured at the beginning and end of the 7-day incubation period.

Buffer	pH of buffer	pH of slurry			
		Under N_2		Under air	
		Initial	Final	Initial	Final
Potassium hydrogen phthalate + HCl	4.00	4.42-4.65	4.53-4.60	4.45-4.53	4.55-4.60
Potassium hydrogen phthalate + NaOH	5.01	5.06-5.20	5.33-5.43	5.10-5.19	5.33-5.50
Potassium dihydrogen phosphate + NaOH	5.94	5.69-5.86	6.10-6.21	5.72-5.82	5.85-5.86
Tris (hydroxy-methyl) aminomethane + HCl	6.88	6.13-6.17	6.58-6.68	6.15-6.25	6.00
Tris (hydroxy-methyl) aminomethane +HCl	7.85	7.45-7.56	7.35-7.48	7.65-7.79	7.09-7.15
Tris (hydroxy-methyl) aminomethane + HCl	8.93	8.23-8.25	8.11-8.14	8.98-8.99	7.93-7.97
Borax + NaOH	10.00	8.50-8.91	8.59-8.60	9.15-9.41	8.27-8.40
-----*	-----	5.65-5.70	6.50-6.54	6.27-6.28	5.51-5.80

*Control slurry. No buffer added.

FIGURE CAPTIONS

Fig. 1. Relationship between the total net quantity of CH_3Hg^+ produced and the ambient pH in nutrient-enriched East Mynarski Lake sediment slurries after incubation for 7 days under N_2 in the presence and absence of various buffers. Symbols identify buffer used or denote absence of buffer: KH_2PO_4, ◆; $CaCO_3$, ●; tris(hydroxymethyl)aminomethane, ■; unbuffered control, ▲.

Fig. 2. The quantities of CH_3Hg^+ produced by three different sets of sediment samples during incubation for different lengths of time under N_2 in the presence and absence of $CaCO_3$ (in "buffered" and "unbuffered" slurries, respectively), with ambient pH values noted in parentheses: A. East Mynarski Lake sediment collected in 1983 (nutrient-enriched); B. East Mynarski Lake sediment collected in 1984 (not nutrient-enriched); and C. Methyl Bay sediment collected from zone of flooded forest in 1984 (not nutrient-enriched).

Fig. 3. Effects of different quantities of calcareous silty clay on the ambient pH and production of CH_3Hg^+ in nutrient-enriched East Mynarski Lake sediment slurries incubated for 7 days under N_2.

Fig. 4. Effects of calcareous silty clay and acid-treated clay on CH_3Hg^+ production in (A) unbuffered and (B) $CaCO_3$-buffered nutrient-enriched East Mynarski Lake sediment slurries on incubation under N_2 for varying lengths of time, with ambient pH values noted in parentheses.

Fig. 5. Effects of pH on CH_3Hg^+ production in nutrient-enriched East Mynarski Lake sediment slurries incubated for 7 days under N_2 (black symbols, continuous line) and air (white symbols, dashed line). Buffered slurries: ●,○; unbuffered control slurries: ▲, △.

Fig. 6. Effects of pH on CH_3Hg^+ decomposition in nutrient-enriched East Mynarski Lake sediment slurries incubated for 7 days under N_2 (black symbols, continuous line) and air (white symbols, dashed line). Buffered slurries: ● ,○; unbuffered control slurries: ▲ , △ .

Fig. 7. Effects of pH on CO_2 production in nutrient-enriched East Mynarski Lake sediment slurries incubated for 7 days under N_2 (black symbols, continuous line) and air (white symbols, dashed line). Buffered slurries: ● , ○; unbuffered control slurries: ▲ , △ .

Fig. 8. Effect of pH on CH_4 production in nutrient-enriched East Mynarski Lake sediment slurries incubated for 7 days under N_2. Buffered slurries: ● ; unbuffered control slurry: ▲ .

Fig. 9. Variation of Eh with pH in nutrient-enriched East Mynarski Lake sediment slurries incubated for 7 days under N_2 (black symbols, continuous line) and air (white symbols, dashed line). Buffered slurries: ● , ○ ; unbuffered control slurries: ▲ ; △ .

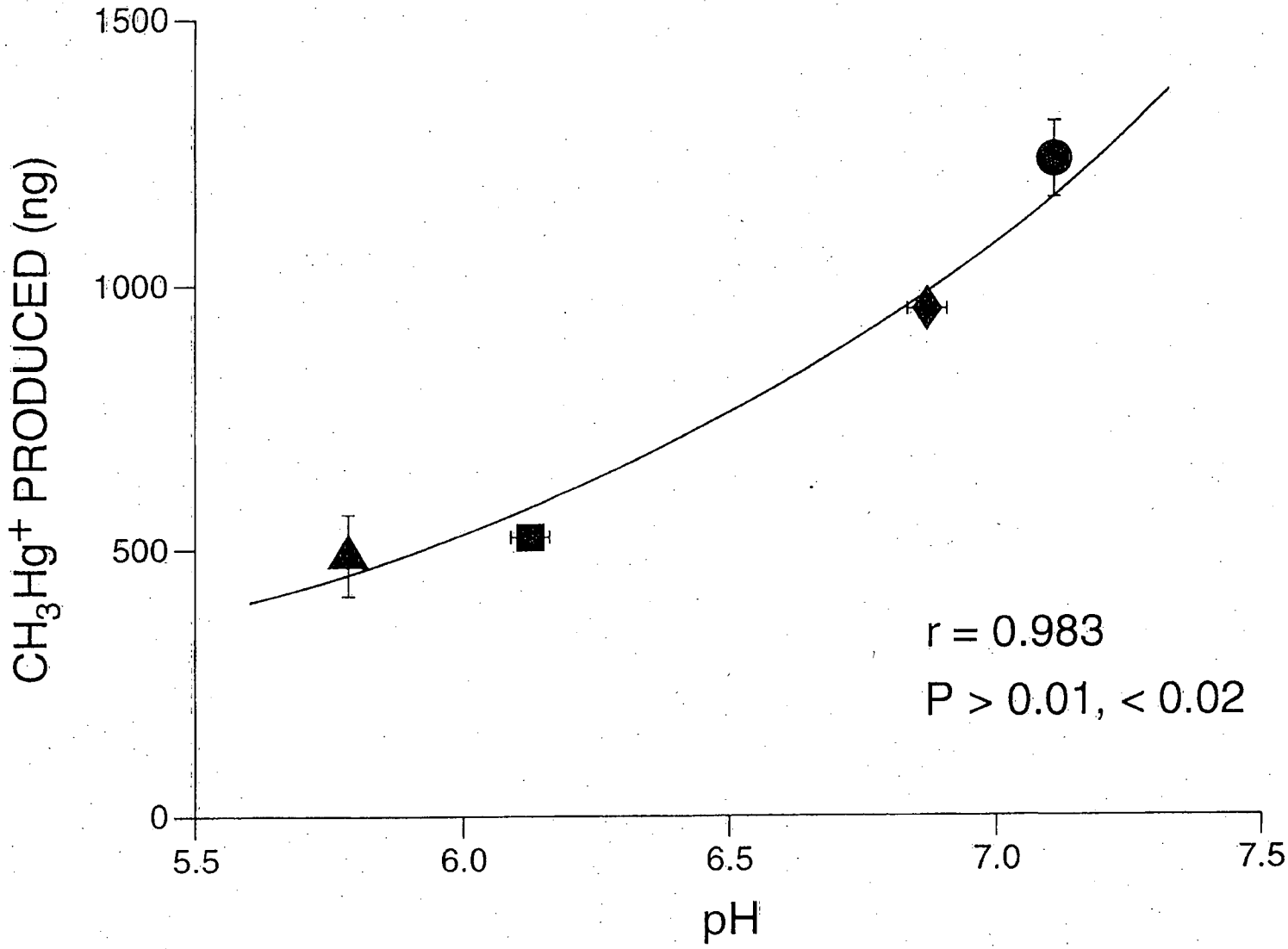
1500
1000
500
0
CH_3Hg^+ PRODUCED (ng)
5.5
6.0
6.5
7.0
7.5
pH
r = 0.983
P > 0.01, < 0.02

Fig. 1

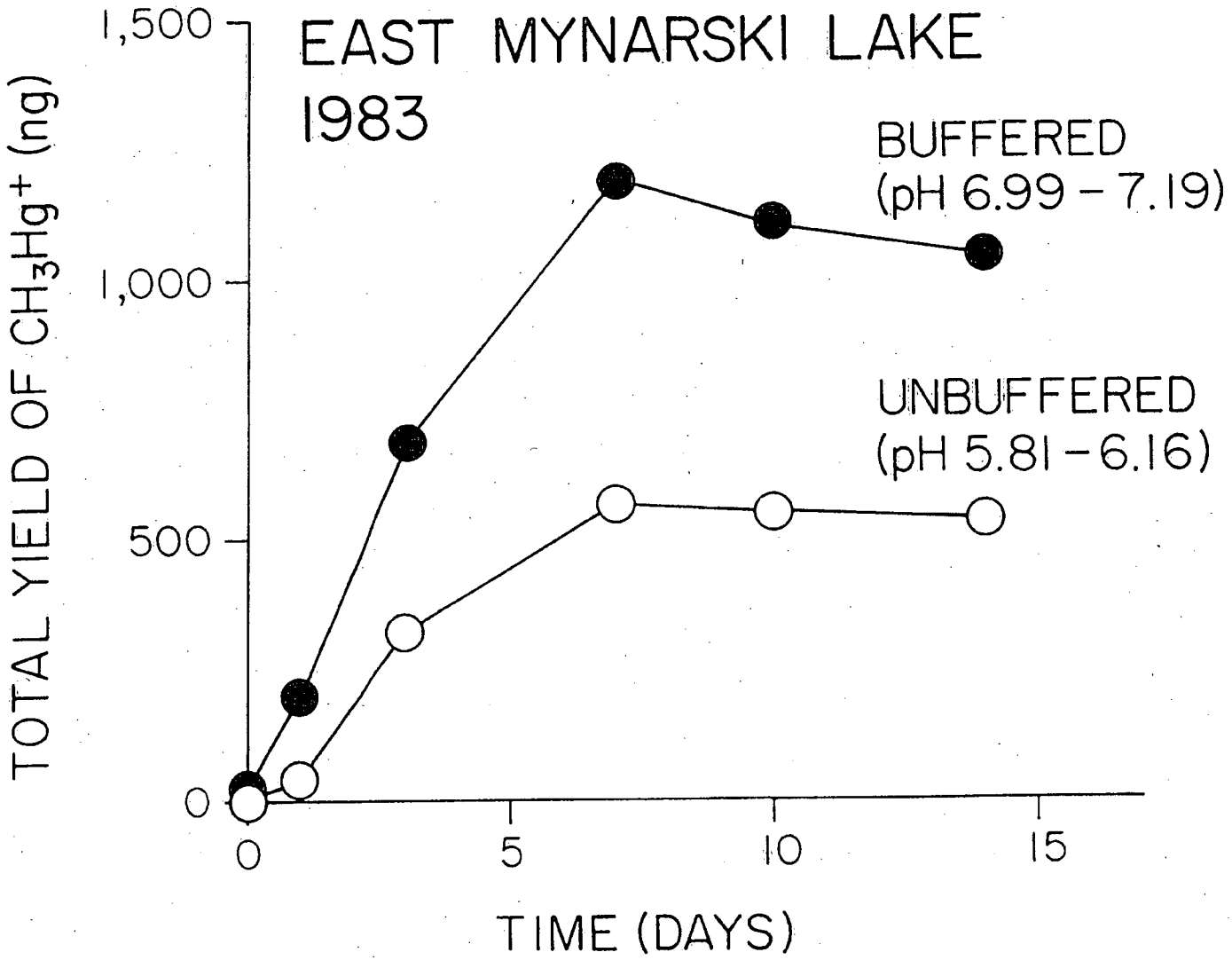
EAST MYNARSKI LAKE
1983
BUFFERED
(pH 6.99 – 7.19)
UNBUFFERED
(pH 5.81 – 6.16)
TOTAL YIELD OF CH_3Hg^+ (ng)
1,500
1,000
500
0
0
5
10
15
TIME (DAYS)

Fig. 2A

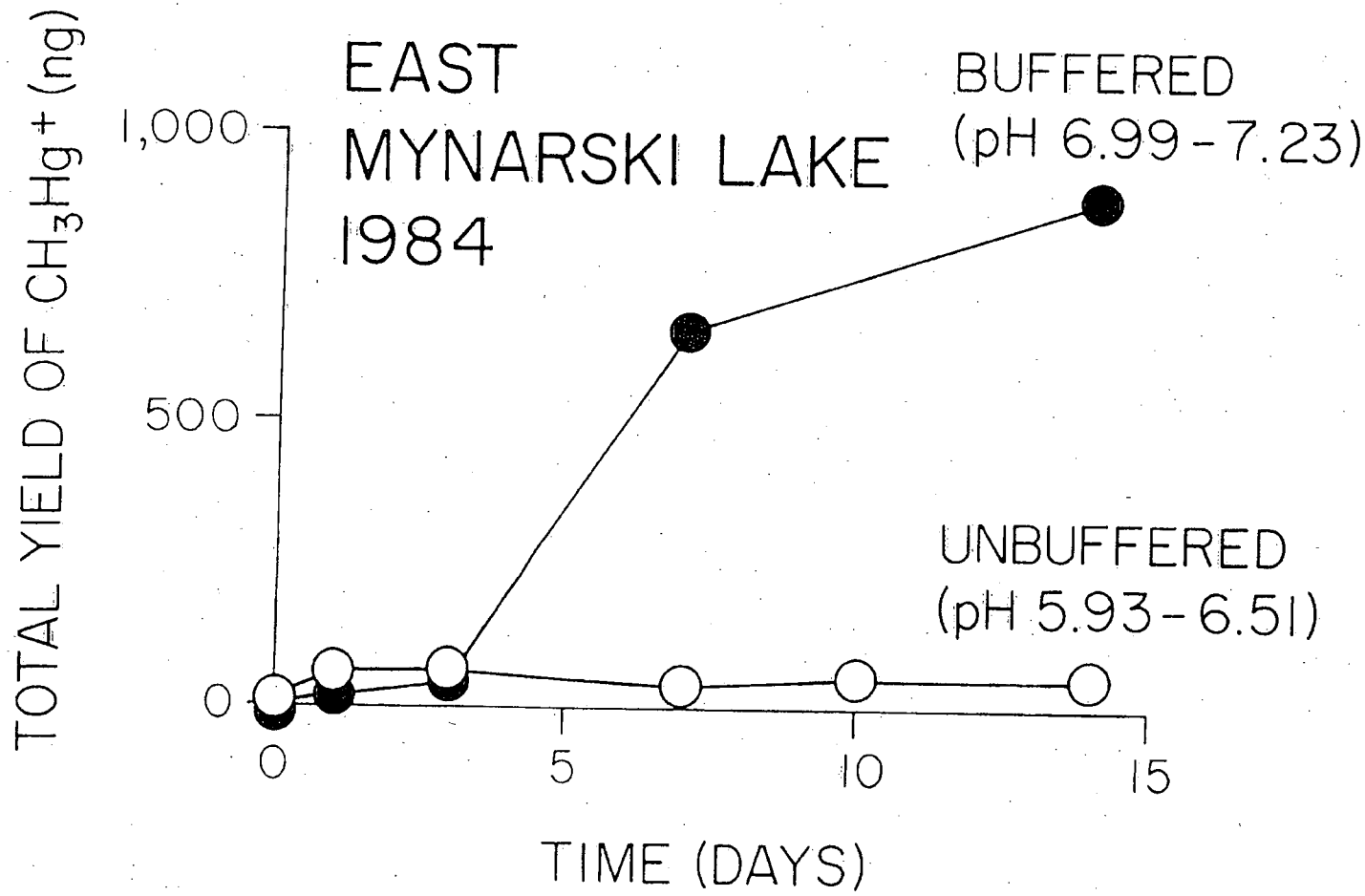
EAST
MYNARSKI LAKE
1984
BUFFERED
(pH 6.99 - 7.23)
UNBUFFERED
(pH 5.93 - 6.51)
TOTAL YIELD OF CH_3Hg^+ (ng)
1,000
500
0
0
5
10
15
TIME (DAYS)

Fig. 2B

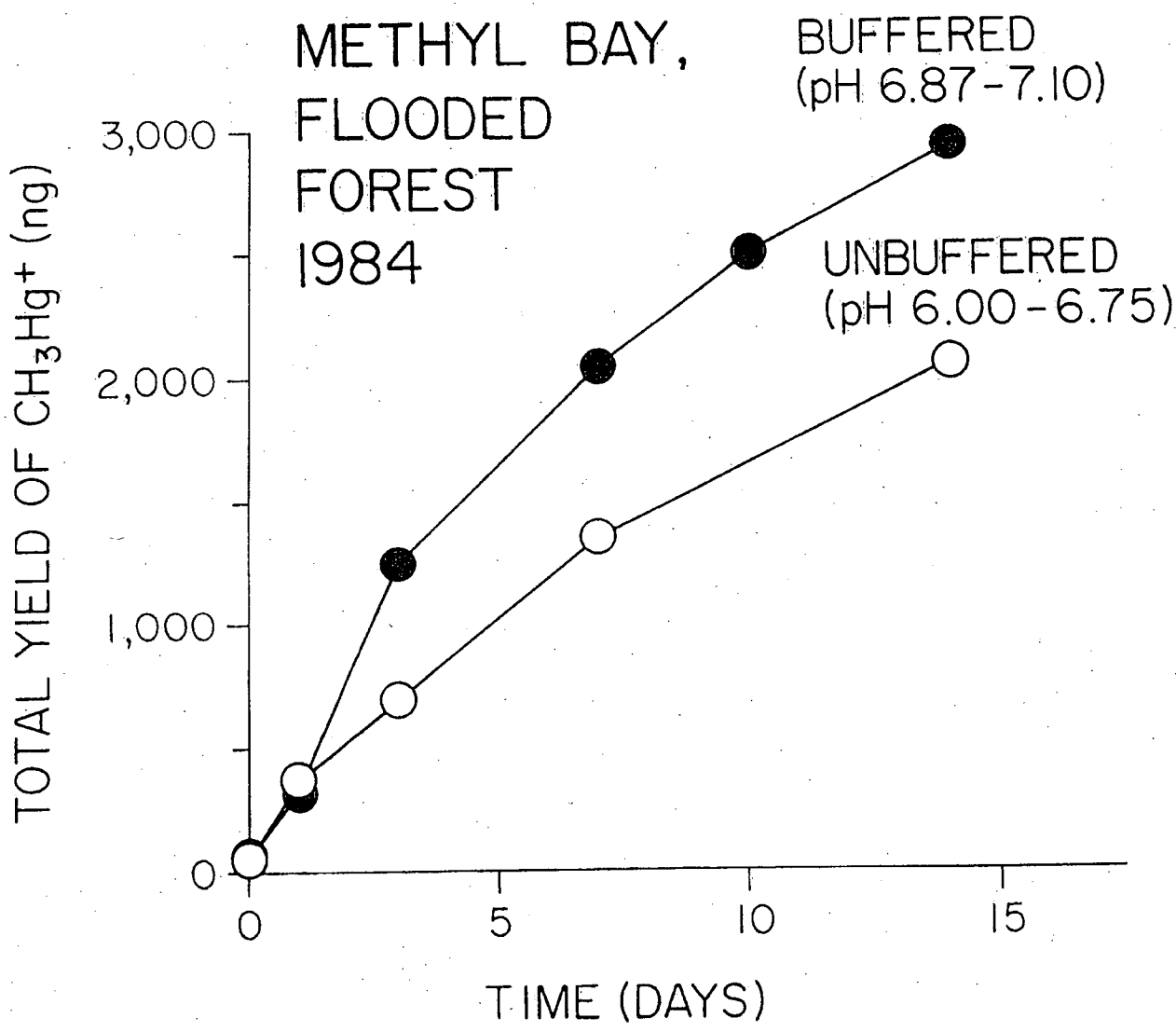
METHYL BAY,
FLOODED
FOREST
1984
BUFFERED
(pH 6.87-7.10)
UNBUFFERED
(pH 6.00-6.75)
TOTAL YIELD OF CH_3Hg^+ (ng)
3,000
2,000
1,000
0
0
5
10
15
TIME (DAYS)

Fig. 2C

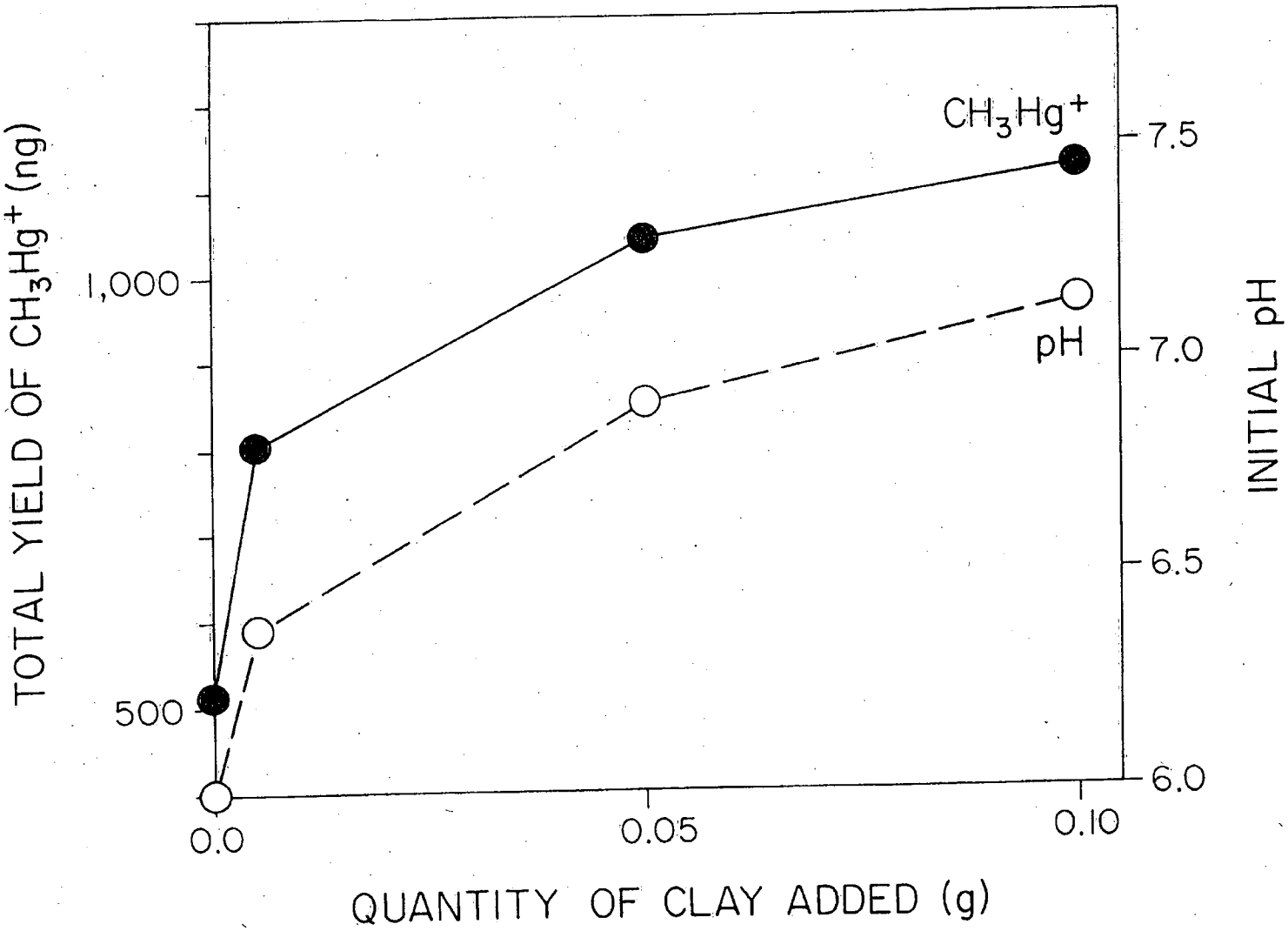
TOTAL YIELD OF CH_3Hg^+ (ng)
1,000
500
CH_3Hg^+
pH
7.5
7.0
6.5
6.0
INITIAL pH
0.0
0.05
0.10
QUANTITY OF CLAY ADDED (g)

Fig. 3

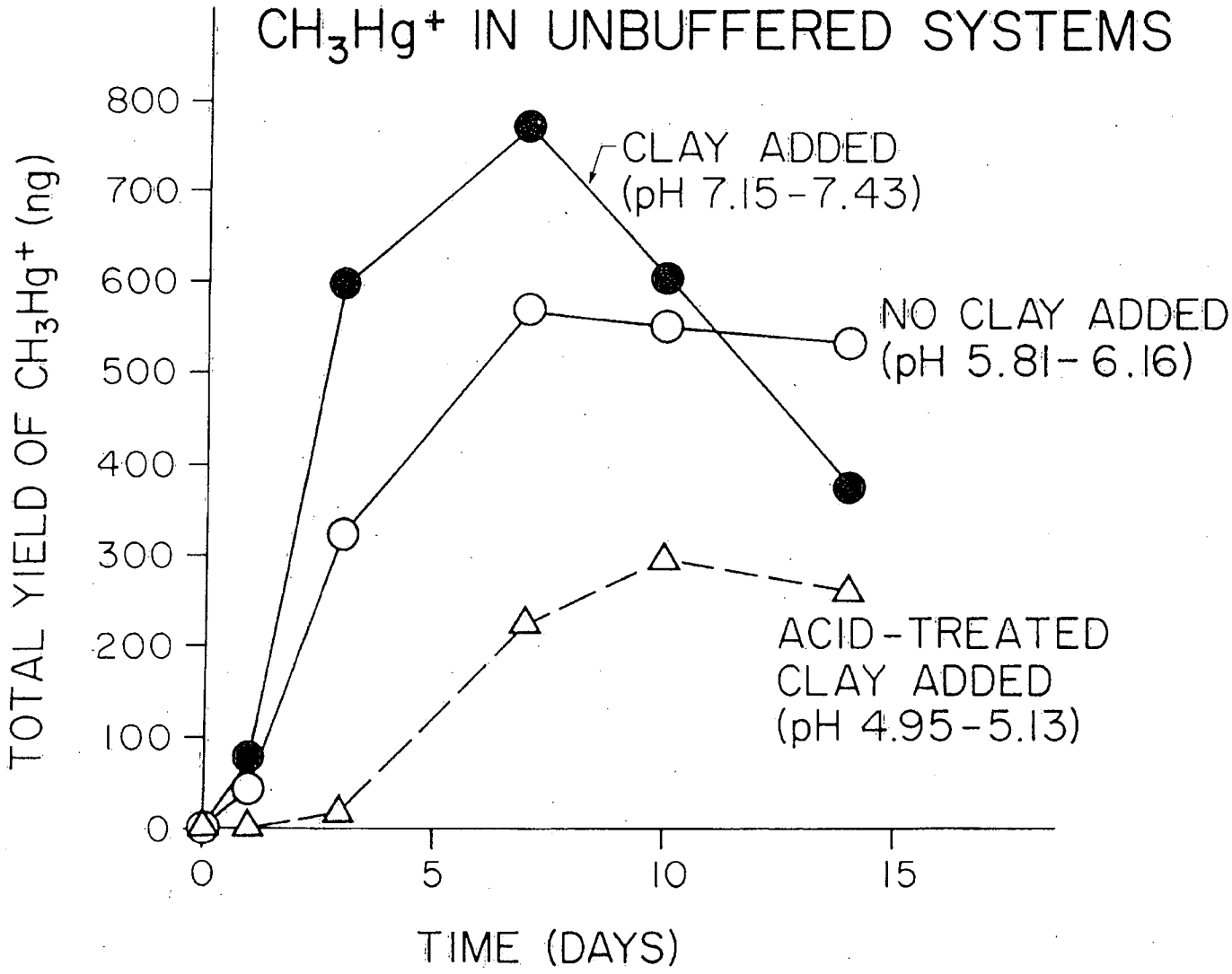
CH_3Hg^+ IN UNBUFFERED SYSTEMS
TOTAL YIELD OF CH_3Hg^+ (ng)
800
700
600
500
400
300
200
100
0
0
5
10
15
TIME (DAYS)
CLAY ADDED
(pH 7.15-7.43)
NO CLAY ADDED
(pH 5.81-6.16)
ACID-TREATED
CLAY ADDED
(pH 4.95-5.13)

Fig. 4A

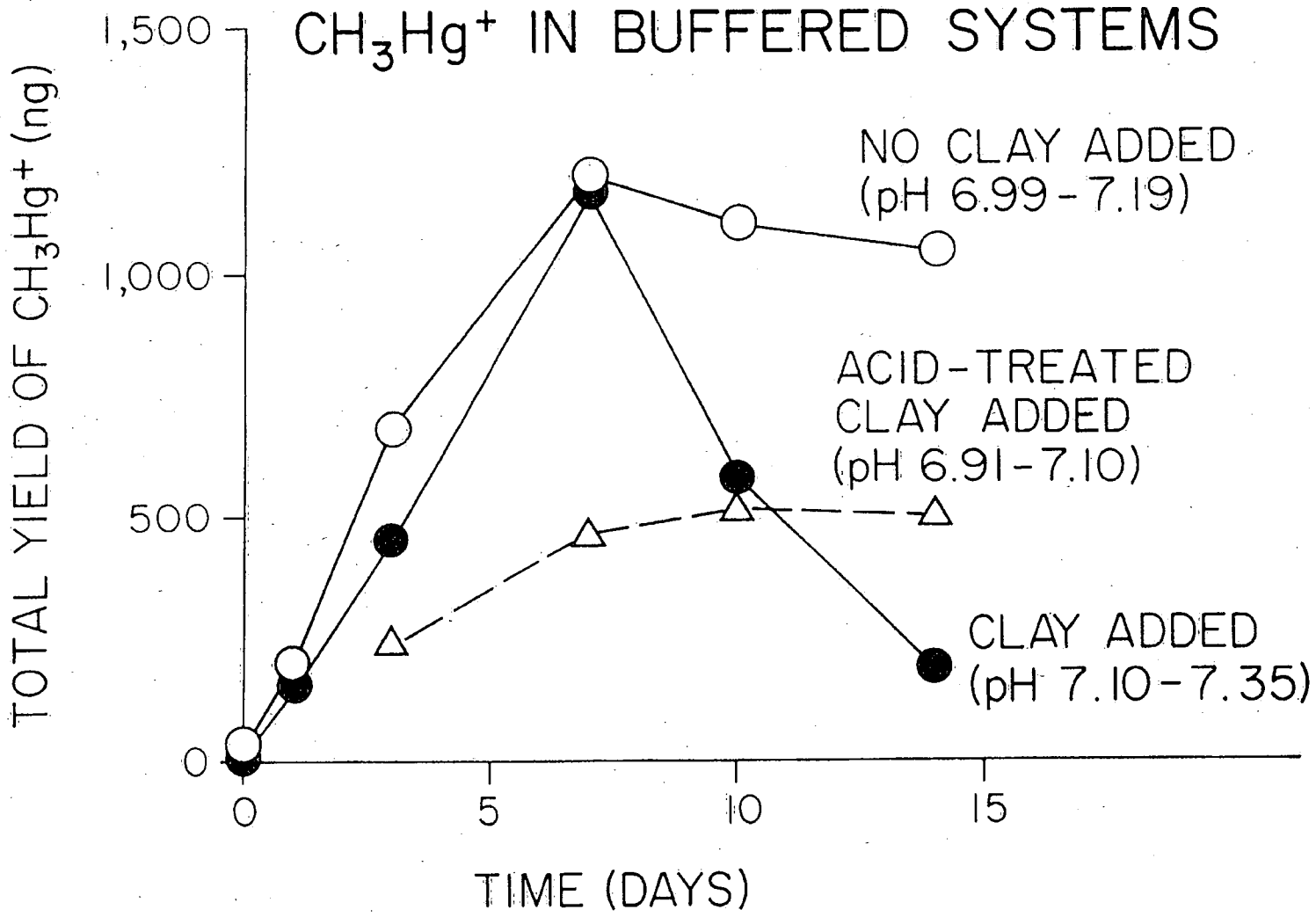
CH_3Hg^+ IN BUFFERED SYSTEMS
TOTAL YIELD OF CH_3Hg^+ (ng)
1,500
1,000
500
0
0
5
10
15
TIME (DAYS)
NO CLAY ADDED
(pH 6.99-7.19)
ACID-TREATED
CLAY ADDED
(pH 6.91-7.10)
CLAY ADDED
(pH 7.10-7.35)

Fig. 4B

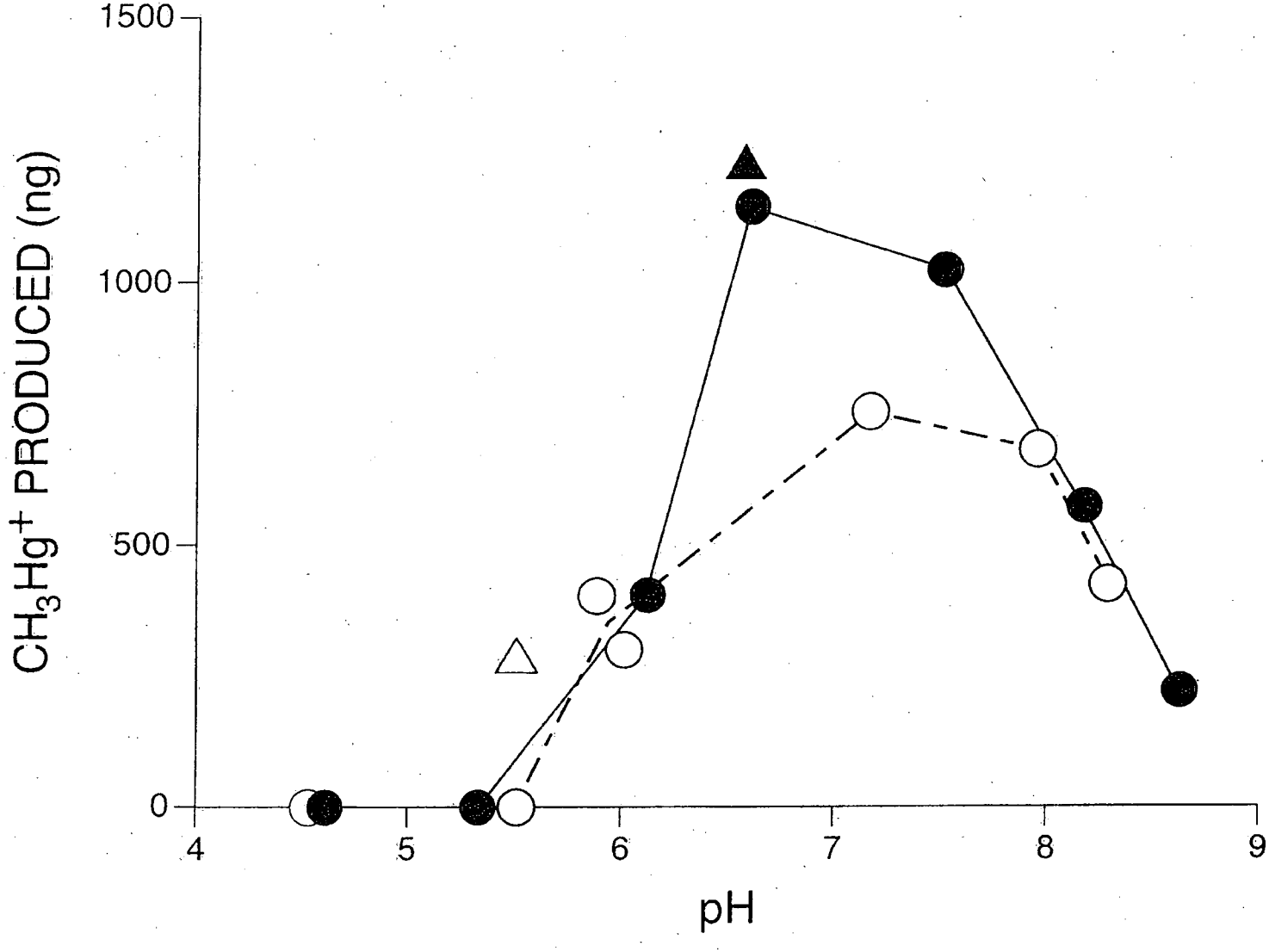

Fig. 5

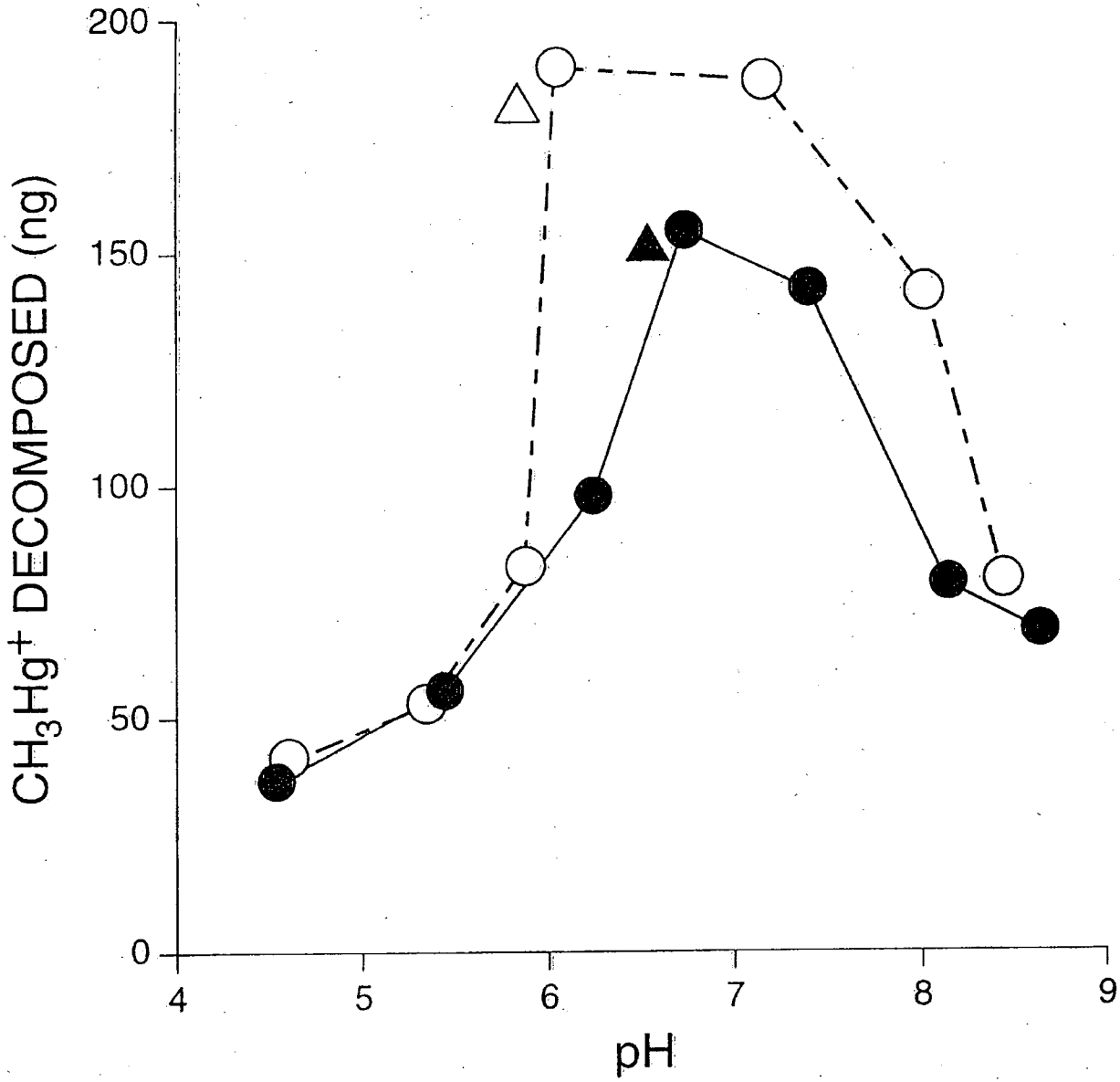

Fig. 6

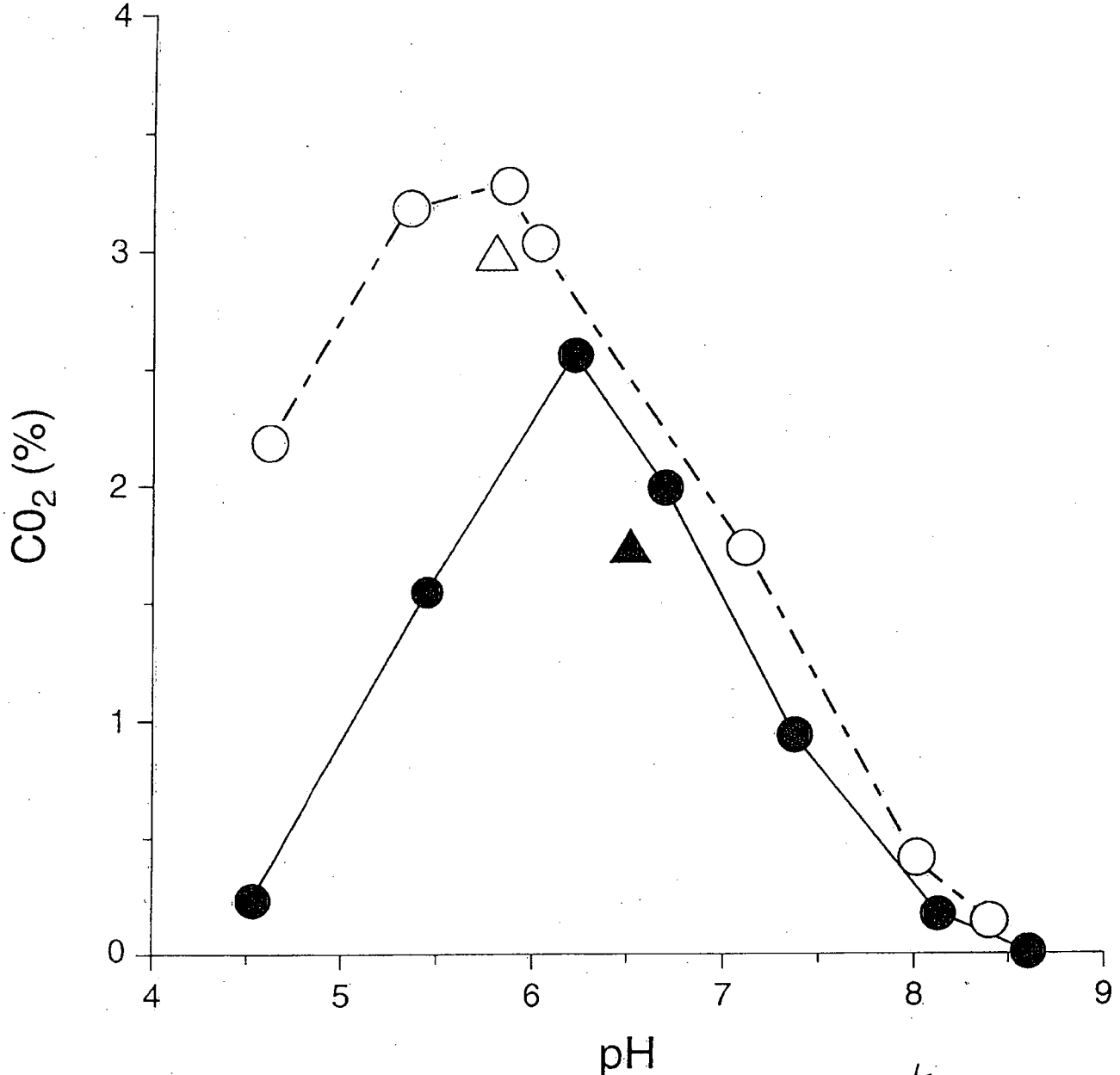

Fig. 4

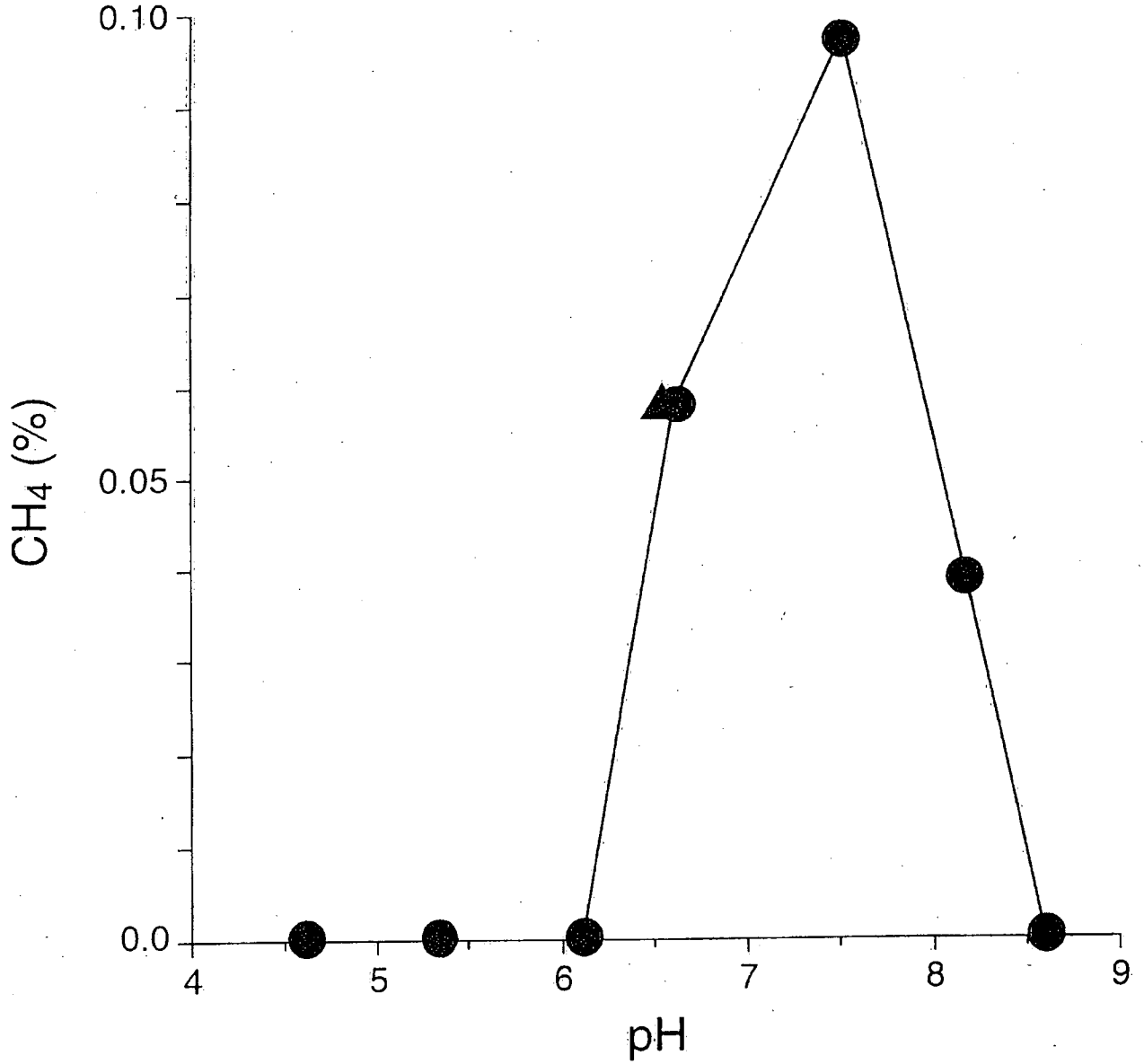

Fig. 8

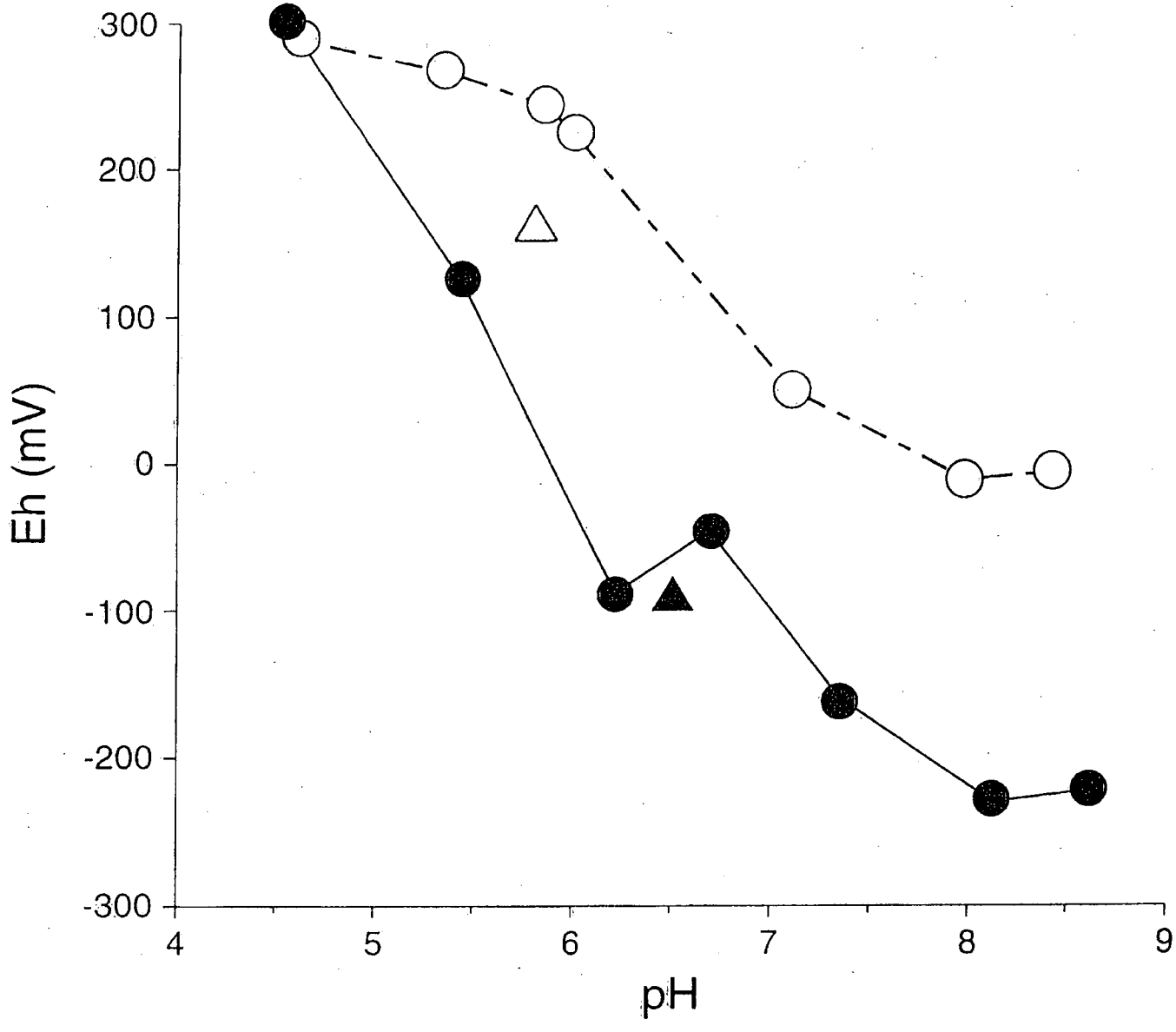

Fig. 9

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