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RESEARCH ON THE INHIBITION OF BACTERIAL OXIDATION OF PYRITE AND THE CONCOMITANT ACID MINE DRAINAGE: PART 2. INVESTIGATIONS ON COAL WASTE DUMPS

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The report is presented in three parts:

Part 1. Investigations on gold mine sand dumps by MA Loos, JM Conradie, PA Whillier, J Maré and C Bosch (Department of Microbiology)

Part 2. Investigations on coal waste dumps by MA Loos, C Bosch and J Maré (Department of Microbiology)

Part 3. Development and testing of slow release systems by RD Sanderson and E Immelman (Institute of Polymer Science)

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RESEARCH ON THE INHIBITION OF BACTERIAL OXIDATION OF PYRITE AND CONCOMITANT ACID MINE DRAINAGE

PART 2. INVESTIGATIONS ON COAL WASTE DUMPS

EXECUTIVE SUMMARY

Little information is available on the extent and environmental impact of acid mine drainage from coal waste dumps in South Africa. The only two reported investigations, for river systems in Natal (Kemp, 1962; Rudd, 1973), show high concentrations of total dissolved solids, in particular sulphate, in rivers leaving coalfields, but limited declines in the pH of the initially mildly alkaline water and reductions in the salt concentrations downstream following dilution and self purification. However, with the large-scale development of open-pit mines, particularly on the Transvaal highveld for electricity generation and the mining of export coal, the acid drainage problem and its prevention must receive continuous attention. At all costs, the extensive acidification of rivers, as has occurred in the eastern U.S.A., must be avoided.

The following are the main findings and conclusions from our investigation of the possibility of inhibiting acid drainage-producing *Thiobacillus ferrooxidans* or other chemolithotrophic iron-oxidizing bacteria in coal waste dumps by treating the dumps with chemicals active against these bacteria. The initial basic laboratory studies (1 to 4 below) formed part of Phase I of the research, while the pilot scale dump and associated laboratory studies (5 to 8 below) formed Phase II. Phase II was conducted with the collaboration of the Chamber of Mines.

1. A limited study of coal waste dumps near Witbank showed the occurrence of acid drainage water in the lower parts of the dumps and in a pool alongside one of the dumps, associated with the presence of large populations of chemolithotrophic ferrous iron-oxidizing bacteria, presumed to be *T. ferrooxidans*. Thus a drainage water sample of pH 2,40 and three coal waste samples of pH 2,40 to 3,88 from the Douglas Colliery dump contained 4,64 x 10^3 to 2,83 x 10^6 iron-oxidizing bacteria/ml or g. These samples were from an acid drainage pool, a waterlogged bank near the drainage pool and from the bottom of a rubble slope. Four of seven samples from a dump on the Wolvekrans Section of the Douglas Colliery also contained iron-oxidizing bacterial populations (3,00 x 10^{-1} to 2,80 x 10^6/g). The four samples were coal waste of pH 2,85 to 7,54 from a flat drainage area at the toe of the dump, the bottom of the dump slope,

15 m (vertical height) up the slope and from ore with a high pyrite content on the top of the dump. No iron-oxidizing bacteria were detected in freshly deposited coal waste of pH 6,98 or drainage water of pH 7,99 from the Wolvekrans dump or in burned waste of pH 12,30 from the Douglas dump. If the iron-oxidizing bacteria and the associated oxidation of pyrite, which produces the acid mine drainage, are restricted mainly to the outer 25 to 30 cm of the dumps as is the situation elsewhere (Dugan, 1975; Good et al., 1970), the possibility exists for inhibiting acid mine drainage formation by the application of suitable antibacterial chemicals.

2. While developing a suitable experimental system for testing the inhibition of *T. ferrooxidans* cultures by chemicals in the presence of coal waste, we found that coal waste fractions of differing particle size up to 11,5 mm diameter, had a profound effect on the ferrous iron concentration and pH of the growth medium necessitating the inclusion in growth and inhibition experiments of uninoculated medium controls containing the coal fraction under study. The almost neutral (pH 6,34) coal waste fractions raised the pH of the medium and catalysed the disappearance of ferrous iron. Culture growth in the absence of inhibitors was adversely influenced by the pH increases caused by these fractions, but also by additional inhibitory effects of the coal waste.

3. In the presence of 500 g/l coal discard fractions of differing particle size (0,425 to 1,18, 2,00 to 2,80, 4,00 to 4,75 and 8,00 to 9,50 mm diameter), two *T. ferrooxidans* strains were inhibited by SLS at 70 to 90 mg/l, added at the beginning of culture incubation or during the exponential phase of culture growth. These concentrations were very much higher than the inhibitory concentrations of 2 to 4 mg/l SLS for cultures not containing coal waste. In the presence of the 2,00 to 2,80, 4,00 to 4,75, 8,00 to 9,50 mm coal waste fractions and a 10,0 to 11,5 mm fraction, sodium benzoate and sorbic acid were both inhibitory at 40 to 75 mg/l when added at the beginning or during the exponential phase of growth. In the absence of coal waste, the inhibitory concentrations of sodium benzoate were 15 to 30 mg/l and those of sorbic acid 15 to 20 mg/l. The various coal waste fractions had little or no differential effect on the inhibitory concentrations. These experiments showed that sodium benzoate and sorbic acid might be even better than SLS for the inhibition of *T. ferrooxidans* and the associated formation of acid in coal waste. Sodium benzoate has the additional advantage of being a much cheaper chemical than the

other two (about half the price of SLS and less than one third of the price of sorbic acid).

4. In inhibition experiments with no coal waste in the culture medium, fresh solutions of 1-bromo-3-dichloro-5,5-dimethylhydantoin (H900) inhibited *T. ferrooxidans* at 6 to 10 mg/l. Ageing had a detrimental effect on the inhibitory concentration, so that not even 14 mg/l of a 6-month-old solution was inhibitory. The instability of H900 over time makes it unsuitable for use in coal discard. Combinations of SLS with sodium benzoate, sorbic acid and H900 and of sodium benzoate with sorbic acid caused no substantial reductions in the inhibitory concentrations of any of the inhibitors tested.

5. The treatment of pilot-scale dumps containing 55 t of coal waste at the Wolvekrans mine by the application of 1 kg dissolved SLS and 0, 5, 10 or 15 kg natural or polyisoprene rubber pellets containing 35% SLS was inadequate to inhibit acidification, which occurred in all dumps within 3 months. Further treatment of the dumps after about 15 months with much higher doses of SLS and SLS-rubber pellets (5 kg dissolved SLS and 0, 10, 30 and 60 kg of the natural or polyisoprene rubber pellets with 35% SLS, to give total SLS treatments of 6,00, 11,25, 20,00 and 32,25 kg SLS/dump) failed to inhibit the acid, dissolved iron and sulphate production through the following 21 months. Very little of the applied SLS left the dumps and the concentrations in the effluents, which reached a maximum of only about 30 mg/l, were inadequate to prevent the survival or possibly development of high populations of chemolithotrophic iron-oxidizing bacteria in the effluent water.

6. Investigations of factors possibly responsible for the lack of success of the SLS treatments in controlling acidification of the pilot scale coal waste dumps, showed the presence in the dump effluents of populations of iron-oxidizing bacteria with resistance to 12 to 14 mg/l SLS, and the ability of cultures of *T. ferrooxidans* to greatly increase their resistance to SLS (from being inhibited by 2 or 4 mg/l to growing in medium with 8 or 18 mg/l, depending on the *T. ferrooxidans* strain). The acid effluents from the dumps also contained heterotrophic filamentous fungi and yeasts, among which SLS-degrading yeasts were detected. Slow or very slow release of SLS from the SLS-rubber pellets and a slow downward movement of the inhibitor in the dumps, presumably involving adsorption, resulted in only low concentrations of SLS in the coal waste (< 14 mg water-extractable SLS/kg) in the lower 0,7 to 1,5 m of dumps 2 and 3 with

natural rubber pellets and throughout the dumps with SLS-polyisoprene rubber pellets. These low SLS concentrations permitted the iron-oxidizing bacteria to establish populations of ca. 10^2 to 10^6/g in the coal waste. By contrast, in the upper 0,45 to 0,55 m of dumps 2 and 3, where the water-extractable SLS concentrations were > 40 mg/kg, no or very few iron-oxidizing bacteria were found. This result shows that application of SLS at a rate of 20 kg/dump or 0,6 kg/m^2 could inhibit iron-oxidizing bacteria in the coal waste to a depth of ca. 0,5 m, hence may be an adequate treatment for a dump with an oxygen barrier at ≤ 0,5 m depth. However, the cost of the SLS, excluding transport and application, for this treatment at the 1990 price would be R58 080/ha.

7. In a further pilot scale study, SLS and sodium benzoate were compared as inhibitors of acid drainage production by the 55-t coal waste dumps at the Wolvekrans mine. Treatments were the addition of 2 kg SLS or 0,2 or 2 kg sodium benzoate/dump every 2 weeks for ca. 10 months. From 188 days, the dumps receiving SLS and the high dose of sodium benzoate appeared to have their adsorption sites for the inhibitors saturated, as indicated by (with few exceptions) high levels of inhibitor in the effluents. Most of these effluents showed no iron-oxidizing bacteria, in contrast to high populations (mainly 10^2 to 10^6/ml) before 188 days and continuing high populations in the effluents from the dumps receiving the low benzoate dose or water only. The amounts of SLS and sodium benzoate giving the presumed saturation were, respectively, 600 and 500 mg/kg coal waste. If one third of these doses were applied to large dumps to saturate the outer 0,5 m of coal waste (to inhibit acidification outside the oxygen barrier) the application rates would be ca. 3 000 and 2 600 kg/ha and the cost of the chemicals (transport and application costs excluded) at 1990 prices R29 040 and R12 922/ha for SLS and sodium benzoate, respectively. However, none of the dumps showed clear evidence of reduced acidification resulting from the inhibitor treatments, although the dump receiving the high dose of benzoate yielded lower levels of dissolved iron and sulphate than the other dumps (unfortunately from the beginning of the experiment, with no change when the dump became saturated with benzoate).

8. Limited basic studies of the adsorption of SLS and sodium benzoate by coal waste showed higher levels of SLS adsorption than benzoate adsorption, except perhaps at SLS concentrations below 40 to 50 mg/100 g coal waste of diameter 2,0 to 10,0 mm in 100 ml water or HJJ medium. Under these conditions less SLS was adsorbed than remained in solution, but with higher SLS concentrations

supplied, the adsorption ranged from 72 to 86%. With a smaller coal fraction (1,18 to 2,00 mm), more than 68% of the SLS was adsorbed at all concentrations supplied. By contrast, less sodium benzoate was adsorbed by a 2,80 to 4,00 mm coal waste fraction than remained in solution with all sodium benzoate-coal waste combinations that we tested. Adsorption to coal waste can explain the effect of the waste on the minimum inhibitory concentrations of sodium benzoate for two *T. ferrooxidans* strains in the culture inhibition experiments, but not the much greater effect of the waste on the minimum inhibitory concentrations of SLS. Further comparative studies of the adsorption of SLS and benzoate to coal waste are required, as possible lower levels of adsorption of sodium benzoate may be another reason for choosing it as an inhibitor in preference to SLS.

9. The overall conclusion from these laboratory and pilot scale studies on coal waste is that sodium benzoate seems to have the best potential of the inhibitors studied to inhibit acidification of the pyrite. However, the 1990 price of sufficient chemical, without transport and application, would be about R12 922/ha and the effectiveness of the treatment remains to be proved.

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CONTENTS

16. GENERAL INTRODUCTION

Part 1 of this report (Loos et al., 1990) outlined in its General Introduction (section 1) the basic problem of acid drainage formation in mine waste dumps and specific acid drainage problems of the gold mining industry in South Africa. In part 2 attention is given to acid drainage from coal waste dumps in South Africa and our research on possible ways of controlling its formation by the application of antibacterial chemicals.

Limited information is available on the production of acid drainage as a result of coal mining in South Africa. Kemp (1962) and Rudd (1973) have reported on the pollution of Natal rivers in coal mining areas by high levels of total dissolved solids including high sulphate concentrations. Serious pollution is restricted to the smaller rivers in the coal fields themselves as the mild alkalinity of the Natal rivers and large volumes of water effectively neutralize and dilute polluted water draining into the larger rivers (Kemp, 1962). For example, the data of Rudd (1973) showed drops of the pH of the Buffalo and Mkuzi Rivers only to pH 6,5 and 5,0, respectively, and declines of the salt loads from their high levels near the pollution sources by dilution and self purification downstream. The acid coal mine waters studied by Thompson (1980) contained far less total dissolved solids (according to conductivity), including iron, than gold mine acid drainage water. The analyses suggest that acid drainage may be less of a problem in coal mining areas than on the gold mines, but it is a widespread, long-lasting problem (Henzen and Pieterse, 1978).

Quantitative estimates of the extent of acid drainage from above-ground coal wastes in South Africa do not seem to be available, but by analogy with the extent of the problem elsewhere, it must be regarded as a serious or potentially serious pollution problem if steps to control it are not taken. Together with outflows of acid water from abandoned mines, it constitutes a vast problem in the United States (Dugan, 1975; Good et al., 1970; Kleinmann et al., 1981; Kleinmann and Erickson, 1983; Walsh, 1978). Thus, in 1972 the United States Department of the Interior estimated that 10 000 miles (16 000 km) of streams and 29 000 surface acres (11 700 ha) of impoundments and reservoirs were seriously affected by surface mining operations and that deep mining added to these figures significantly (Lundgren et al., 1972). It was estimated that acid mine drainage from active and abandoned mines amounted to over 4×10^6 t (U.S.) ($3,6 \times 10^9$ kg) of acidity per year or 1×10^7 kg per day. Walsh (1978) noted that 90% of the pollutants in water discharged from mines, including 5×10^6 lbs ($2,3 \times 10^6$ kg) of acidity per day, were released from abandoned mines.

Different strategies for dealing with the problem of acid mine drainage have been developed, mainly in connection with acid drainage from coal mines and coal waste deposits. Attempts have been made to reduce the volume of acid drainage water by reducing the infiltration of water to mine workings (Kim et al., 1982; Walsh, 1978). Also, water is treated before discharge into rivers by liming (Bosman, 1983; Kim et al., 1982; Thompson, 1980; Van Staden, 1979) and can possibly be treated by passage through wetlands coupled with limestone treatment (Kleinmann, 1985), or by the addition of waste organic matter, such as sawdust, to the water in dams to promote the development of heterotrophic anaerobic sulphate-reducing bacteria (Tuttle et al., 1969). These bacteria produce hydrogen sulphide which reduces the ferric iron and precipitates it as ferrous sulphide (FeS). Alternative strategies involve suppression of ferrous iron oxidation by *T. ferrooxidans* at the sites in mines or dumps where it is producing the acid water. Abandoned sub-surface mines can be sealed to restrict the availability of oxygen for pyrite oxidation (Kim et al., 1982); however, Walsh (1978) has discussed serious problems with this approach. With strip mines the acid mine drainage can be effectively controlled by spreading topsoil over the mine dumps and establishing a vegetation cover (Atlas and Bartha, 1981). The activity of *T. ferrooxidans* can also be suppressed by various organic compounds (Erickson et al., 1985; Kleinmann, 1980; Kleinmann et al., 1981; Kleinmann and Erickson, 1983; Onysko et al., 1984a,b; Torma and Itzkovitch, 1976; Torma et al., 1976; Tuovinen, 1978; Tuttle and Dugan, 1976) and Kleinmann and co-workers (Erickson et al., 1985; Kleinmann, 1980; Kleinmann et al., 1981; Kleinmann and Erickson, 1982, 1983) have had considerable success with the inhibition of *T. ferrooxidans* in coal discard dumps under field conditions by the application of solutions of sodium lauryl sulphate (SLS) or SLS incorporated in rubber.

Our studies of acid drainage formation and the possibility of its inhibition in coal wastes by the application of antibacterial chemicals developed from similar investigations involving gold mine sand dumps and described in part I of this report. We first showed in a limited study that iron-oxidizing acid-generating bacteria had developed to large populations in and around two coal waste dumps on the Transvaal highveld near Witbank (section 17). As a prelude to studies on the inhibition of *T. ferrooxidans* in cultures containing inhibitory chemicals and coal waste, we investigated the effects of the coal waste itself on the pH and ferrous iron of the growth medium (section 18) and on the growth of *T. ferrooxidans* in the medium without inhibitors (section 19). The inhibitors studied in cultures containing coal waste were sodium lauryl sulphate (SLS), sodium benzoate and sorbic acid (section 20). Their inhibitory effects in combinations (pairs of inhibitors) with each other and of 1-bromo-3-chloro-5,5-dimethylhydantoin (H900) on its own and with SLS against *T. ferrooxidans* in cultures not containing coal waste were

also investigated (section 21). The studies of sections 17 to 21 were all part of Phase I (laboratory investigations) of the research.

Phase II, conducted with the collaboration of the Chamber of Mines, comprised a pilot scale investigation of SLS as a potential inhibitor of acidification of 55-t coal waste dumps at the Wolvekrans Section of the Douglas Colliery near Witbank. The SLS was applied in solution and as SLS-rubber pellets, providing for slow release of the SLS as described in detail in part 3 of this report (Sanderson and Immelman, 1990). Acidification of the dumps was followed by analysis of their drainage water (effluents) for pH, populations of iron-oxidizing bacteria, SLS, iron and sulphate concentrations (section 22). To explain the observed lack of inhibition of acidification, even after a second increased dosing of the dumps with SLS, supplementary investigations were conducted into the resistance to SLS developed by *T. ferrooxidans* and iron-oxidizing bacteria in the effluents (section 23), the degradation of SLS by heterotrophic microorganisms present in the effluents (section 24) and the adsorption of SLS to the coal waste in the dumps (section 25). Finally, the pilot scale study was extended to compare sodium benzoate and SLS, applied in solution at fortnightly intervals, as potential inhibitors of acid generation in the dumps (section 26), supplemented by basic laboratory studies of their adsorption to coal waste (section 27).

Part 2 of the report is presented as a direct continuation of part 1, with continuity of numbering of the pages, sections, tables, figures, appendices and appendix tables. The repetition of information in the two parts of the report is minimized as far as possible by referring in this part of the report to appropriate sections, tables, figures and appendix figures in part 1.

17. DISTRIBUTION OF IRON-OXIDIZING BACTERIA IN AND AROUND COAL WASTE DUMPS

17.1. Introduction

17.1.1. Iron-oxidizing bacteria of coal waste dumps

Iron- and sulphur-oxidizing chemolithotrophic bacteria of the genus *Thiobacillus* appear to be the most important factor in the oxidation of pyrite in coal waste dumps (see this report, part 1, section 1). The acidophilic iron- and sulphur-oxidizer, *T. ferrooxidans*, has been isolated regularly from coal mine runoff and seems to have the most important role (Belly and Brock, 1974; Colmer and Hinkle, 1947; Harrison, 1978; Lundgren et al., 1972; Olson et al., 1981; Tuttle et al., 1968; Walsh, 1978). Walsh (1978) and Walsh and Mitchell (1972) postulated that the less acidophilic iron-oxidizing bacterial genus, *Metallogenium*, might play a crucial role in the creation of the acidic conditions (pH < 3.5) necessary for significant activity of *T. ferrooxidans*, but the studies of Harrison (1978) and Kleinmann and Crerar (1979) do not confirm an essential role of *Metallogenium* in the pyrite oxidation-acidification process. *Leptospirillum ferrooxidans* (Balashova et al. 1974) could also play a role in the pyrite oxidation and acidification of coal waste resulting in the problem of acid drainage.

17.1.2. Environmental factors affecting distribution of iron-oxidizing bacteria in coal waste dumps

The distribution and activity of acid-generating iron-oxidizing bacteria in coal waste dumps is determined by environmental factors such as the amounts and availability of pyrite and other growth substrates, including oxygen, the moisture content and pH of the waste and the range of temperatures in the pile (Dugan, 1975; Maré, 1989; Torma, 1977; Whillier, 1987). These factors have been considered in general in part 1 of this report (section 2). Information specific to the distribution of the bacteria in coal waste dumps is considered briefly in the following sub-sections (17.1.2.1 to 17.1.2.6).

17.1.2.1. Pyrite substrate

Iron pyrite (FeS_2) is the main source of ferrous iron for oxidation by *T. ferrooxidans*. Pyritic minerals and shale are often found in intimate contact with coal. Several kinds of pyritic minerals are found but iron pyrite is the one most commonly encountered in association with coal (Dugan, 1975). The pyrite concentration in coal and associated

waste varies considerably. Thus, coal waste from Kentucky was divided by Kleinmann and Crerar (1979) into pyritic shale and sandstone overburden containing 0,6 and 1,6% sulphur, respectively. Two major coal seams at Colstrip, Montana, averaged only 1,07% and 1,87% sulphur (Olson et al., 1981), whereas the mean sulphur content of the dry coal studied by Harrison (1978), was 5,36% (3,56% pyrite sulphur, 1,34% organic sulphur and 0,46% inorganic sulphate sulphur). Dugan and Apel (1983) found Ohio coal refuse sulphur to range from 6 to 8%. Coal samples obtained by Tuovinen et al. (1980) from the Sun Spot Mine, Vermont, contained 3,09% iron, predominantly as iron pyrite.

Acid generation in most piles is directly related to the amount of pyritic material oxidized. Records for a coal pile in Ohio show an average acid production rate, where acidity was measured by its reaction with calcium carbonate, of 200 kg acidity/ha/day (Good et al., 1970). A coal pile in Pennsylvania, that was older than 80 years, required treatment at $7 500 per month for the control of acid drainage, according to Kleinmann and Erickson (1983). The age of the pile indicates that oxidation was slow, that pH can remain low for many years and that treatment of polluted water involves long-term expense.

Some forms of pyrite may not be susceptible to microbiological attack, even under favourable environmental conditions (Olson et al., 1981). Caruccio (1970) observed that differences in pyrite reactivity correlated with different pyrite morphologies. Framboidal pyrite was the most reactive pyrite form and the acid-producing potential of selected samples was related to the percentage of framboidal pyrite. Caruccio (1970) also showed that small particles of pyrite (2 to 15 μm) decomposed rapidly when exposed to the atmosphere while coarse pyrite particles (> 50 μm) remained stable.

17.1.2.2. Oxygen

The growth of *T. ferrooxidans* on ferrous iron has an absolute requirement for oxygen (Dugan and Lundgren, 1964). The prime source of oxygen is diffusion of the gas into the dump from the atmosphere. The rate of diffusion depends largely on the pore size between dump particles. It might be expected that coal waste, being porous (Tuttle et al., 1968) and loose (Harrison, 1978), is easily permeated by air and rain water which make it suitable for growth of iron- and sulphur-oxidizing bacteria. However, oxygen ordinarily does not penetrate into the pile beyond a depth of 20 to 30 cm, where its diffusion is limited by a zone designated as the oxygen barrier which results from the compaction of fine sediments (Dugan, 1975). Good et al. (1970) recognised three zones in an Ohio coal refuse pile. The first was the outer mantle, 2,5 cm thick, where most of

the oxidation of pyrite occurred. The second zone, which extended to a depth of 10 to 25 cm, had a low permeability but had discontinuities allowing water entry to the main body of the pile. The third zone was the main body of the pile with very little evidence of pyrite weathering. Good et al. (1970) reported that surface erosion continually uncovered active pyrite material. Kleinmann and Crerar (1979) concluded that *T. ferrooxidans* catalyzed pyrite oxidation in the zone of aeration of coal refuse piles, from the well oxygenated outer layer to the intermediate belt between this layer and the water table, for approximately 3 to 4 days after rainfall infiltration.

An examination of the gas mixtures in the pore spaces of a coal dump by Goodman et al. (1983) revealed that below 0,5 m the oxygen concentrations were extremely low and the carbon dioxide concentrations high. Samples from the surface of a coal refuse pile studied by Belly and Brock (1974), demonstrated maximal carbon dioxide uptake, apparently by chemolithotrophic bacteria, while only slight activity was demonstrated at depths below 8 to 10 cm.

17.1.2.3. Moisture

Belly and Brock (1974) recorded optimal oxidation rates of the pyrite in a Wisconsin coal pile at a moisture content of 23 to 35%. Olson et al. (1981) reported that pyrite in dry exposed spoil banks supported very little bacterial oxidaiton of pyrite. Moisture in waste dumps is dependent on the rainfall as coal overburden seldom becomes waterlogged and moisture evaporates quickly in dry weather. The decrease in pyrite oxidation during the dry months in Montana was believed by Olson et al. (1981) to be related to water stress. A water potential of -15 bars is the lower limit at which many soil bacteria can develop. *Thiobacillus ferrooxidans* was found to be completely inhibited at -23 bars.

Dugan (1975) used controlled sprinkling on a coal pile in Ohio to obtain a picture of the hydrology of the dump. He concluded that moisture content depended on the composition of the pile and showed 25% retention and 75% drainage of water following controlled sprinkling of a pile. This confirmed earlier estimations of 25% infiltration of precipitation into coal mine piles in the same area (Good et al., 1970). The latter study also detected the presence of a ground-water pool in the body of a large coal pile. Simulation experiments in ecological flasks showed that *T. ferrooxidans* would not be active in saturated ground-water environments (Kleinmann and Crerar, 1979), but with minimal water flow, following rainfall infiltration, the bacteria would be active for a limited time. The simulation experiments also suggested that *T. ferrooxidans* could survive extended drought periods.

17.1.2.4. pH

The pH of coal slag appears to vary considerably. Slag from strip mines in Montana is largely alkaline, in contrast to the more acidic waste in the eastern United States; however, the alkaline slag contains microzones of acidity (Olson et al., 1981). Wide pH variation over small areas, for example, from pH 2,0 to pH 5,5, was also recorded in an artificial coal pile in Missouri (Harrison, 1978) where the distribution of microbial species paralleled pH changes. Generally, at neutral to acid pH, the iron oxidisers are ecologically more important than the sulphur oxidisers, because the iron oxidation rate is ten times faster than sulphur oxidation. However, where alkaline mineral oxidation occurs, the sulphur-oxidising microorganisms may be more important than the iron oxidisers (Walsh, 1978).

Although there have been suggestions of pH-dependant bacterial successions that lower the pH of the environment to suit *Thiobacillus* in waste dumps, for example, the succession involving *Metallogenium*, (Walsh, 1978), there is evidence that *T. ferrooxidans* can colonise and acidify almost neutral environments (pH 6,9) without assistance (Kleinmann and Crerar, 1979). This work confirmed that *T. ferrooxidans* can survive above pH 6 in nature.

17.1.2.5. Temperature

In studies on Wisconsin coal piles, the optimal autotrophic carbon dioxide uptake occurred at ore sites with temperatures between 20 and 30°C (Belly and Brock, 1974).

Coal waste dumps may undergo spontaneous combustion. Bituminous coal wastes contain inflammable substances which cause waste heaps to flame when they burn (Colmer and Hinkle, 1947). The wastes may attain high temeperatures which could significantly affect the iron-oxidizing bacteria and the rate of acid production. In addition, the high temperatures combust sulphur-containing compounds with the production of sulphur dioxide, thereby polluting the air.

17.1.2.6. Dump age

A study on coal piles in Wisconsin indicated that chemo-autotrophic bacterial activity, measured by carbon dioxide uptake, was affected by the age of the dump (Belly and Brock, 1974). Activity was greatest in piles that were 2 to 3 years old, but was minimal in fresh piles and a pile that was 40 years old.

17.1.3. Acid drainage from coal waste in South Africa

Although acid drainage from coal mining areas has been demonstrated by the Natal river studies of Kemp (1962) and Rudd (1973) and the analyses of acid coal mine waters by Thompson (1980), no published information on acid production in South African coal waste dumps is available. The problem is known from the drainage from certain dumps of water of low pH in which precipitates of 'yellow boy' (products of ferrous to ferric iron oxidation) develop. As an initial step in our study of the possibility of controlling acid drainage from coal waste dumps by the application of antibacterial chemicals, we undertook a limited survey of the distribution of chemolithotrophic iron-oxidizing bacteria in and around two coal waste dumps near Witbank, Transvaal.

17.2. Materials and Methods

Samples were taken on 18 March 1985 from the northern side of the large coal waste dump at the Douglas Colliery near Witbank, not far from where the dump was smoking as a result of spontaneous combustion. Sample DC1 was water from an acid drainage pool on the northern side of the dump, while samples DC2 to 4 were coal waste samples from the base of the dump to about 15 m (vertical height) up the sloping side of the dump (full details are given under Results in section 17.3). The material at sampling point DC4 was warm from spontaneous combustion in the dump.

The dump on the Wolvekrans Section of the Douglas Colliery was also sampled on 18 March 1985. Sample WC5 was water from the pool at the southeastern toe of the dump. Coal waste samples were WC6 and 7 from the base of the dump, WC8 from about 15 m (vertical height) up the sloping side of the dump (this sample was from a warm area below a smoking zone of spontaneous combustion in the dump) and WC9 to 11 from the top of the dump, as detailed in full under Results (section 17.3).

The water samples were collected in sterile wide-mouthed, screw-capped bottles. The coal samples were removed by auger from depths shallower than 0,5 m and placed in sterile wide-mouthed plastic containers with screw-caps. All samples were stored overnight at 4°C.

Populations of iron-oxidizing bacteria in the samples were determined by the most probable number (MPN) method described in part I of this report (section 9.2.2), using three 50-ml wide-necked conical flasks per dilution, with 15 ml medium per flask. The MPN values were obtained from the table of de Man (1983) with correction for the moisture in the samples. The moisture and the pH of the samples were determined as for the soil samples from the gold mine sand dump seepage (this report, part I, section 3.2.1).

17.3. Results

Table 11 records the moisture, pH and populations of iron-oxidizing bacteria by MPN count in water and coal waste samples collected at the dumps of the Douglas Colliery and the Wolvekrans Section of the Douglas Colliery. The coal waste moisture contents were low (≤7,5%), except in the samples taken near the edge of the acid drainage pools (DC2, WC6). Among the samples with low moisture contents, the highest moisture content of sample DC3 was probably related to its location at the bottom of a rubble slope and the next highest of sample WC9 was because this sample was taken on the flattish top of the dump in a rain-collecting hollow which often contained a pool. A wide range of pH values (pH 2,40 to 12,30) was measured among the samples from the two dumps.

Significant populations of iron-oxidizing bacteria were found in the sample from the Douglas Colliery acid drainage pool (sample DC1), on the edges of the Douglas Colliery and Wolvekrans pools (samples DC2 and WC6) and on the lower slopes of the dumps above the drainage pools (samples DC3, WC7 and 8), even approaching a warm smoking surface in the case of sample WC8. The highest population was in sample WC7 at pH 5,31 and moisture 3,65%. No iron-oxidizing bacteria were found in the newly deposited coal waste (sample WC10, pH 6,98) close to the conveyor belt on the Wolvekrans dump nor in samples DC4 (coal waste) and WC5 (water) with a pH of about 8,0 or higher. Sample WC9, with pH 2,53 and moisture 7,10%, was well within the range of moisture and pH levels associated with high iron-oxidizing bacterial populations in other samples, but contained no detectable population of these organisms. Sample WC11, with a high visible pyrite content and high pH, showed iron-oxidizing bacteria at a very low level (MPN = 0,3/g).

TABLE 11. Moisture, pH and MPN counts of autotrophic acidophilic iron-oxidizing bacteria of water and coal waste samples collected at the dumps of the Douglas Colliery and the Wolvekrans section of Douglas Colliery near Witbank, Transvaal, on 18 March 1988

Coal waste dump and sample number	Habitat description	Percentage moisture[a]	pH[b]	Iron-oxidizing bacteria/ml or/g by MPN[c]
Douglas Colliery				
DC1	Water; acid drainage pool on northern side of dump		2,40	$3,88 \times 10^{4}$
DC2	Waterlogged bank, 1,5 m from pool	15,45	3,88	$2,83 \times 10^{6}$
DC3	Bottom of rubble slope 4 m from acid pool	7,50	3,65	$4,64 \times 10^{3}$
DC4	15 m (vertical height) up slope above sampling site DC2	6,42	(2,30	0
Wolvekrans Section				
WC5	Water; pool at south-eastern toe of dump		7,99	0
WC6	Coal waste in flat drainage area, 2 m from WC5	14,91	3,98	$2,80 \times 10^{6}$
WC7	Bottom of dump slope, 15-20 m from WC5	3,65	5,31	$9,65 \times 10^{7}$
WC8	15 m (vertical height) up slope above WC7	2,75	2,85	$2,46 \times 10^{3}$
WC9	Top of dump, rain-collecting hollow	7,10	2,53	0
WC10	Top of dump near conveyor belt discharge	2,41	6,98	0
WC11	Ore with high content of pyrite; top of dump	2,19	7,54	$3,00 \times 10^{1}$

a Moisture as percentage of wet coal waste mass; mean of duplicate determinations.

b Mean of duplicate determinations; coal waste pH was the pH of a 1:2,5 coal waste:water suspension.

c MPN/ml water or/g dry coal waste, determined from MPN tables of de Man (1983); 0 = no iron-oxidizing bacteria detected in flasks containing up to 10 g wet coal. See Maré (1989) for statistical categorization of results and theoretical 95% confidence limits.

17.4 Discussion

At the Douglas Colliery, the orange-brown colour of the drainage pool from which sample DC1 was taken, indicated the presence of oxidized iron in the water which was also very acid (pH 2,40). The high count of iron-oxidizing bacteria in sample DC1 was expected in the light of the high counts in similar water samples seeping from gold mine sand dumps (this report part 1, sections 3 and 9) and coal waste elsewhere in the world (Colmer and Hinkle, 1947; Dugan et al., 1970; Manning 1975; Millar, 1973; Tuovinen and Kelly, 1973; Tuttle et al., 1968, 1969). Coal waste samples DC2 and DC3 were taken not far from the drainage pool and, with a low pH as well as a relatively high moisture content, were habitats of large populations of iron-oxidizing bacteria. Sample DC4 was taken about 15 m (vertical height) up the slope of the dump above sampling site DC2. The coal waste here was warmer than 37°C and an area close by was smoking, indicating spontaneous combustion. The sampled dump material had the appearance of grey-white ash, suggesting that the coal waste had burned. The pH was very high (pH 12,30), which also indicated a changed chemical composition of the coal waste due to burning. No iron-oxidizing bacteria were found in this material, which was highly unfavourable for the usual iron-oxidizing bacteria species, *T. ferrooxidans*, in respect of the pH (Buchanan and Gibbons, 1974).

At the Wolvekrans Section of the Douglas Colliery, the pool at the south-eastern toe of the dump showed little evidence of oxidized iron in the water. The clear appearance of this water was more typical of a rain-collecting pool than an acid drainage pool. The water had a slightly alkaline pH (pH 7,99). Not surprisingly, sample WC5 taken from this pool showed no evidence of iron-oxidizing bacteria. Coal waste samples WC6 and WC7 were more acid (pH 3,98 and 5,31 respectively). They were taken from the vicinity of the pool and showed high MPN counts of iron-oxidizing bacteria. They consisted of fairly fine material dumped about one year previously and washed down the slope subsequently. Sample WC8 was taken from the side of the dump in a warm area downslope from a smoking area. The pH was 2,85. The iron-oxidizing bacterial population in this sample was relatively high. Sample WC9 was taken from the top of the dump in a rain-collecting hollow with a black muddy appearance and pH 2,53. Unexpectedly, no iron-oxidizing bacteria were found in this moist hollow. Material freshly dumped from the conveyor belt (sample WC10) had a neutral pH (pH 6,98) and a low moisture content. No iron-oxidizing bacteria were found at this sampling site. The chunk of pyritic material (sample WC11) from the top of the dump had a relatively low moisture content and a pH just above neutral; it contained a very low level of iron-oxidizing bacteria.

The foregoing results indicate that the most likely sites of bacterial oxidation of pyrite were in the coal waste in the lower parts of the dump where the pH of the waste had been lowered by the onset of acidification. The acidification and high populations of iron-oxidizing bacteria were possibly limited mainly to the outer mantle of the dump, to 10 to 25 cm depth, where Good et al. (1970) recognized the development of an impermeable oxygen barrier in an Ohio coal refuse pile. The iron-oxidizing bacteria in the acid drainage water from the Douglas Colliery dump were probably oxidizing dissolved ferrous iron that had been released from the pyrite in the dump (Kleinmann et al., 1981).

18. EFFECTS OF COAL WASTE FRACTIONS ON UNINOCULATED *T. FERROOXIDANS* GROWTH MEDIUM (HJJ MEDIUM)

18.1. Introduction

Initial attempted investigations of the inhibitory effects of SLS on *T. ferrooxidans* cultures containing coal waste suggested that the waste profoundly affected the experimental system. As a first step towards establishing the suspected effects of the coal waste, it was incubated (shaken) in uninoculated HJJ medium under the standardized conditions used for cultures. To define the experimental conditions even further and explore the effects of coal waste of differing particle size (and total surface area) on the medium, the coal waste was crushed and sieved into fractions of defined particle size. The changes monitored in the medium were pH and the disappearance of ferrous iron.

18.2. Materials and Methods

18.2.1. Coal waste fractions

Coal waste was obtained from the dump at the Wolvekrans Section of the Douglas Colliery. The moisture, pH and population of iron-oxidizing bacteria by MPN count were determined as described in section 17.2. The coal waste was crushed and sieved manually into 0,425 to 1,18 mm, 2,00 to 2,80 mm, 4,00 to 4,75 mm, 8,00 to 9,50 and 10,0 to 11,5 mm size fractions.

18.2.2. Effect on ferrous iron

Two coal waste fractions (unsterilized) with particle diameter 0,425 to 1,18 mm and 2,00 to 2,80 mm were added in separate experiments in duplicate to 250-ml Erlenmeyer flasks containing 100 ml HJJ (Harrison, Jarvis and Johnson) medium (this report, part 1, section 2) to give 10, 20, 30, 40 and 50 g coal waste per flask. In a subsequent experiment, the four smallest coal waste fractions were added to HJJ medium at 50 g/100 ml medium. Control flasks contained HJJ medium without coal waste. The flasks were incubated at 26°C with rotary shaking at 120 r.p.m. Samples of medium were removed periodically for determination of the disappearance of ferrous iron by the dichromate titration described in part 1 of this report (section 2.2.4). Ferrous iron disappearance from the flasks containing coal waste was corrected by subtraction of the mean ferrous iron disappearance from the duplicate control flasks without coal waste.

18.2.3. Effect on pH

In two separate experiments, all fractions and the four smallest fractions of the coal waste (unsterilized) were added in duplicate to HJJ medium at 50 g/100 ml medium in 250-ml Erlenmeyer flasks. The experiment with the four fractions was that of section 18.2.2 for determination of the effect of coal waste on the ferrous iron in the medium. Control flasks containing only HJJ medium were included. The flasks were incubated at 26°C with rotary shaking at 120 r.p.m. The medium was sampled periodically and the pH measured with a Beckman 7500 pH meter (Beckman Instruments, Inc., Berkeley, California) with a combination electrode (Series no. 39831).

18.3. Results

18.3.1. Coal waste

The coal waste used in these studies before being crushed had a pH of 6,34, moisture content of 1,60 g/100 g wet coal waste and no detectable population of iron-oxidizing bacterin in the MPN count.

18.3.2. Effect on ferrous iron

Figures 29 and 30 show that the unsterilized coal waste fractions with particle diameter 0,425 to 1,18 mm and 2,00 to 2,80 mm had a stimulatory effect on the disappearance of ferrous iron from HJJ medium. The first 25 h showed a rapid rate of ferrous iron disappearance from the flasks with 200 to 500 g/l coal; thereafter the rate of disappearance decreased. The rate of ferrous iron disappearance increased as the concentration of coal waste in the medium increased. The increases were greater with the smaller (0,425 to 1,18 mm) than with the larger (2,00 to 2,80 mm) coal waste particles. Only in the presence of 500 g/l 0,425 to 1,18 mm coal waste (Fig. 29) did the ferrous iron totally disappear (within 300 h).

The experiment with the four coal waste fractions (Fig. 31) showed that abiotic ferrous iron oxidation was the most stimulated in the presence of the smallest coal discard fraction (0.425 to 1.18mm), but was also considerably stimulated, to a similar extent, by the larger size fractions. The stimulation was particularly marked from 44 to 91 hours. However, after 404 hours the ferrous iron was not completely oxidized in any of the flasks.

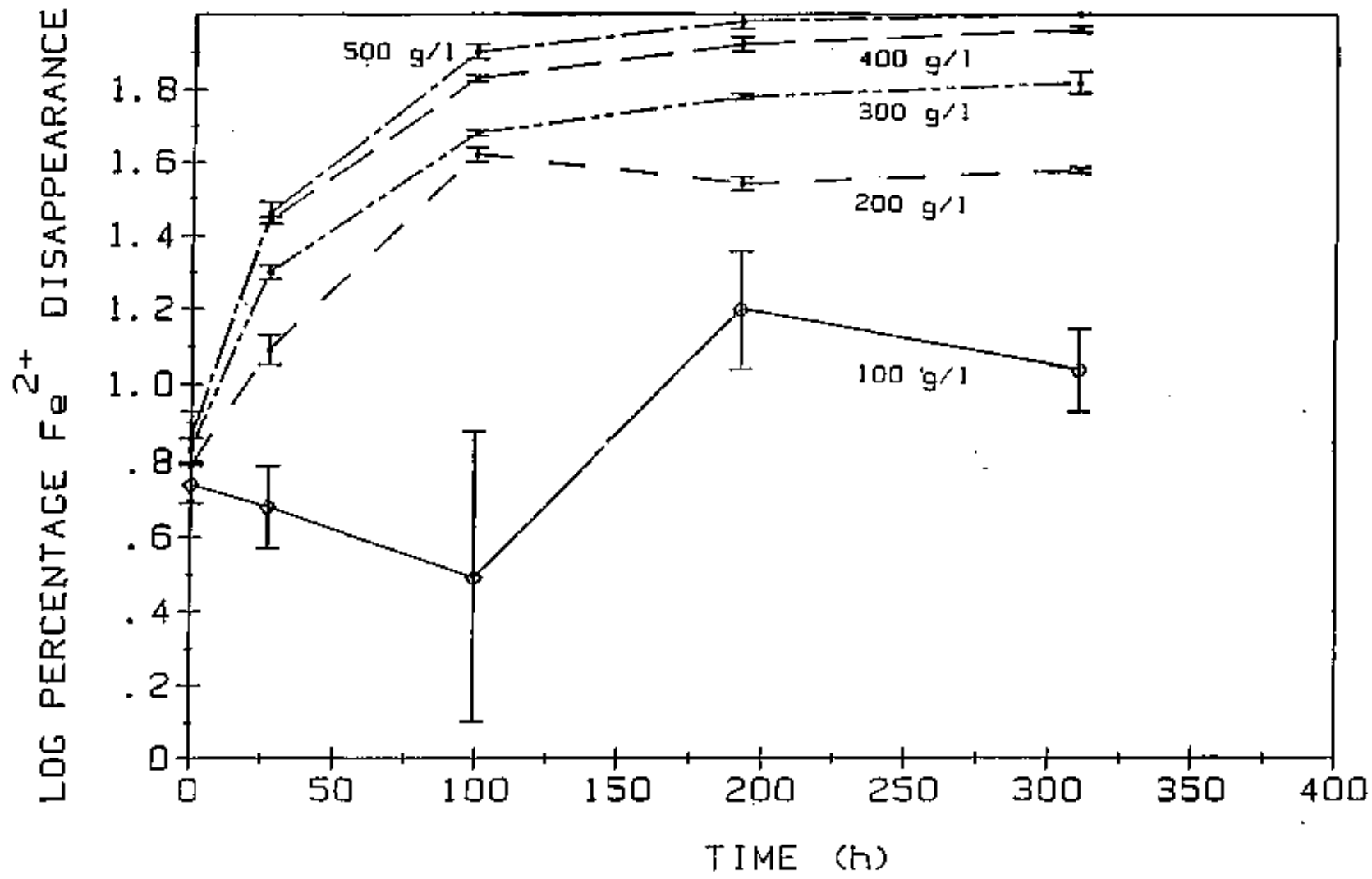


FIG. 29. Disappearance of ferrous iron from sterilized HJJ medium containing unsterilized coal waste (0,425 to 1,18 mm fraction). Figures alongside the curves are the coal waste concentrations. Plotted lines connect means of duplicate values shown by vertical bars.

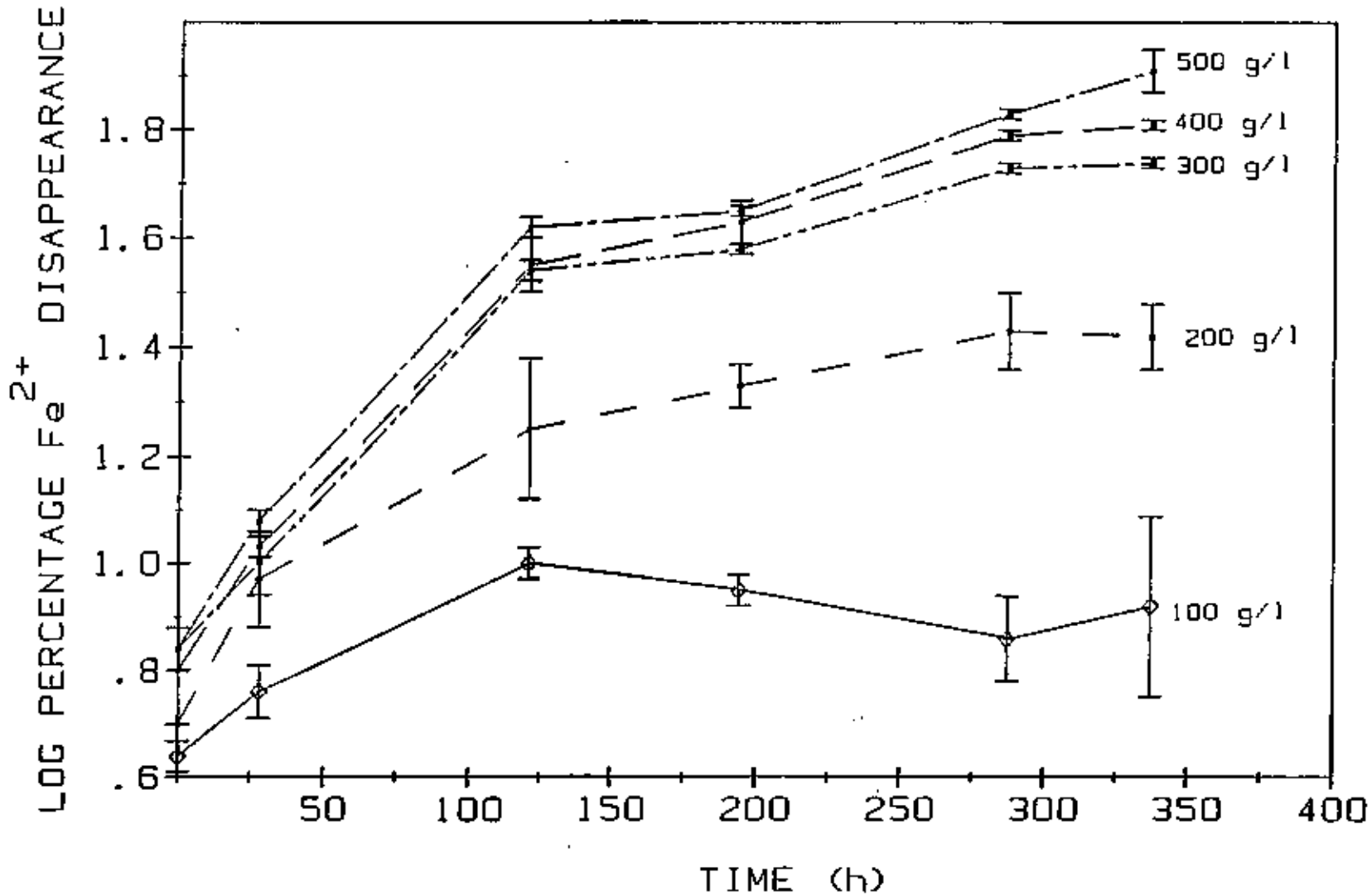


FIG. 30. Disappearance of ferrous iron from sterilized HJJ medium containing unsterilized coal waste (2,00 to 2,80 mm fraction). Figures alongside the curves are the coal waste concentrations.

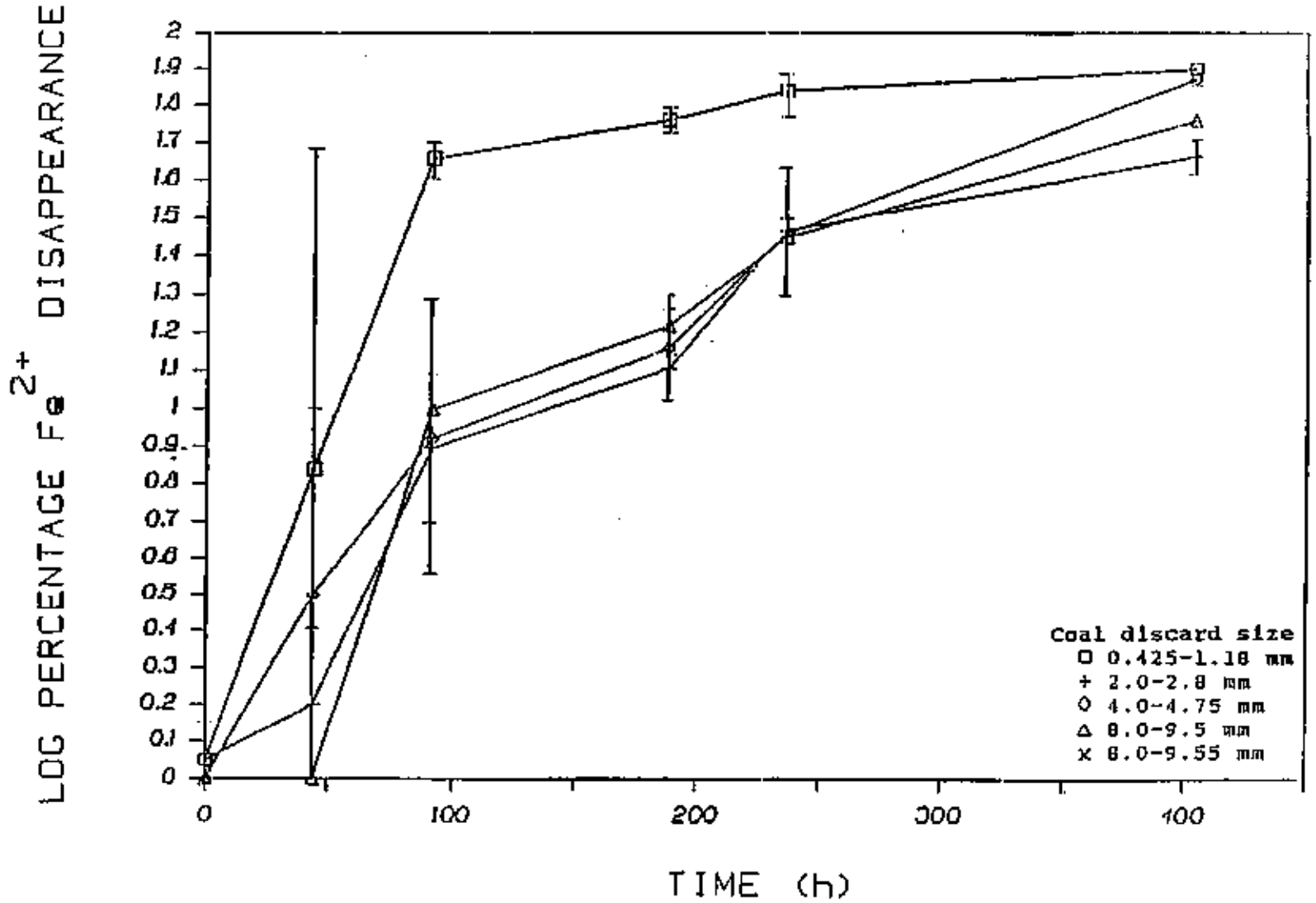


FIG. 31. Disappearance of ferrous iron from sterilized HJJ medium containing 500 g/l unsterilized coal discard of various size fractions.

18.3.3. Effect on pH

Figure 32 shows that 500 g/l crushed and sieved coal waste of diameter 10,0 to 11,5 mm had little effect on the pH of the HJJ medium medium within 100 h of incubation. However, fractions with smaller particles caused considerable increases in the medium pH to between ca. pH 3,0 and 5,5 (Fig. 32 and 33). The smaller the particle size the greater was the increase of pH. The pH increased in almost all the flasks to a maximum within the first 24 h. Thereafter it stabilized or declined, dropping to below pH 3,0 with all fractions during the long (400 h) incubation of the second experiment (Fig. 33).

18.4. Discussion

18.4.1. Effect on ferrous iron

The results of this investigation (Fig. 29, 30, 31) probably reflect the abiotic removal or transformation of ferrous iron as the coal waste contained no detectable iron-oxidizing bacteria. The mechanism of the ferrous iron removal or transformation is unknown, but it may be related to the surface area of the coal waste, as the smallest fraction caused more rapid removal than the larger fractions. It may involve adsorption and/or reactions, e.g. oxidation, catalyzed at the coal waste surface by compounds present in the coal waste. Growth experiments with *T. ferrooxidans* in HJJ medium containing coal waste, especially a fine fraction, should include an uninoculated control with the same coal fraction to obtain values for the spontaneous (abiotic) disappearance of ferrous iron under the specific experimental conditions for subtraction from the ferrous iron disappearance values obtained for cultures.

18.4.2. Effect on pH

The presence in the HJJ medium of 500 g/l of the different coal discard fractions (except the 10,0 to 11,5 mm fraction) caused a rapid increase in the medium pH within the first 30 hours; this rise was particularly high with the smaller fractions, reaching pH 5,2 (Fig. 32) or pH 4,1 (Fig. 33) with the 0,425 to 1,18 mm fraction. The pH increase may be ascribed to the release of alkaline compounds from the coal, which had a pH of 6,34, with more being released as the particle size decreased and the total surface area increased. The pH increase can partly explain the observed stimulation of ferrous iron disappearance caused by the coal waste fractions in HJJ medium (Fig. 29, 30, 31), as abiotic ferrous oxidation would be stimulated (Kleinmann et al., 1981). The subsequent

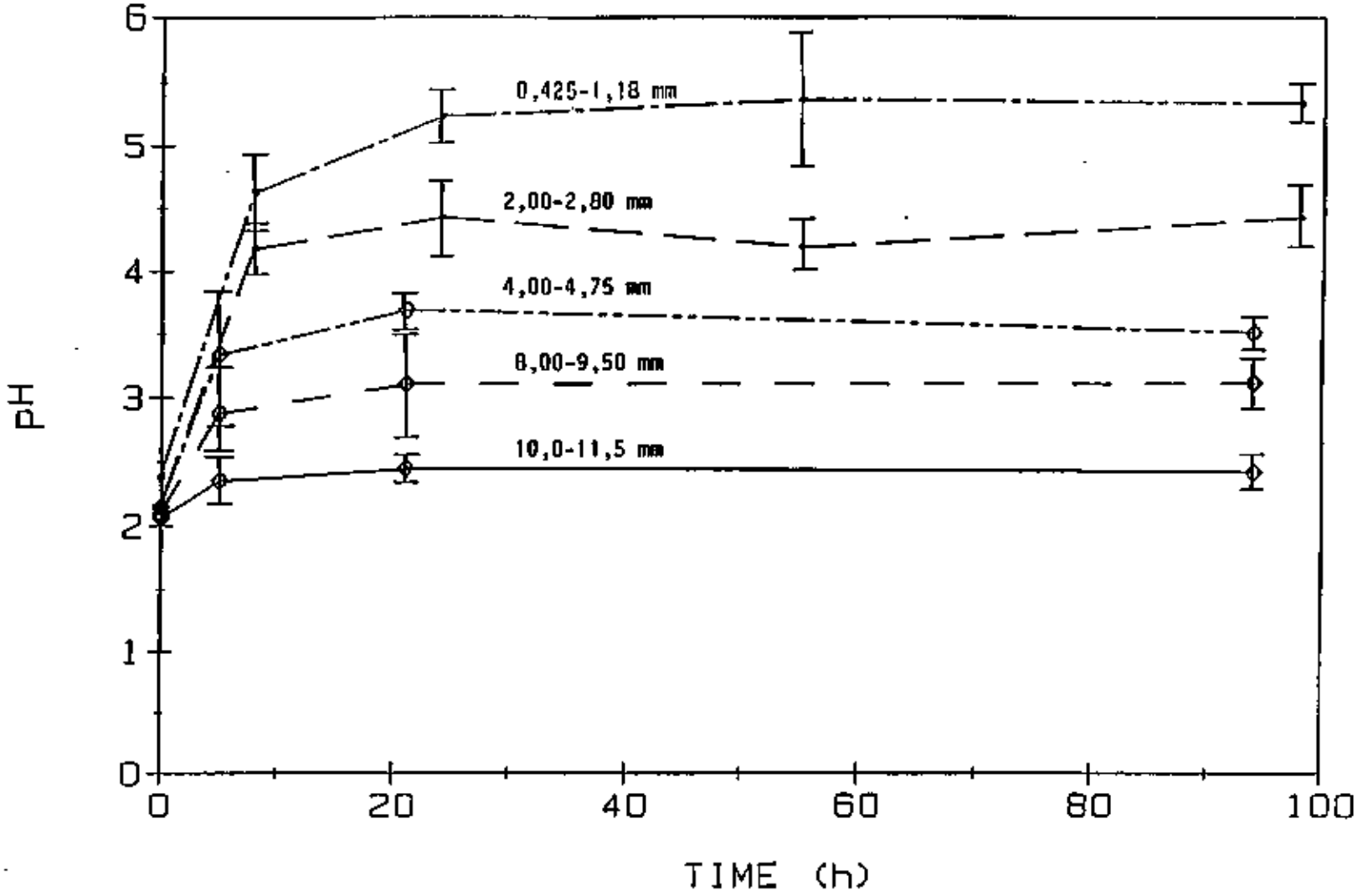


FIG. 32. Effects of 500 g/l unsterilized coal discard (various size fractions, indicated by values alongside curves) on the pH of sterilized HJJ medium.

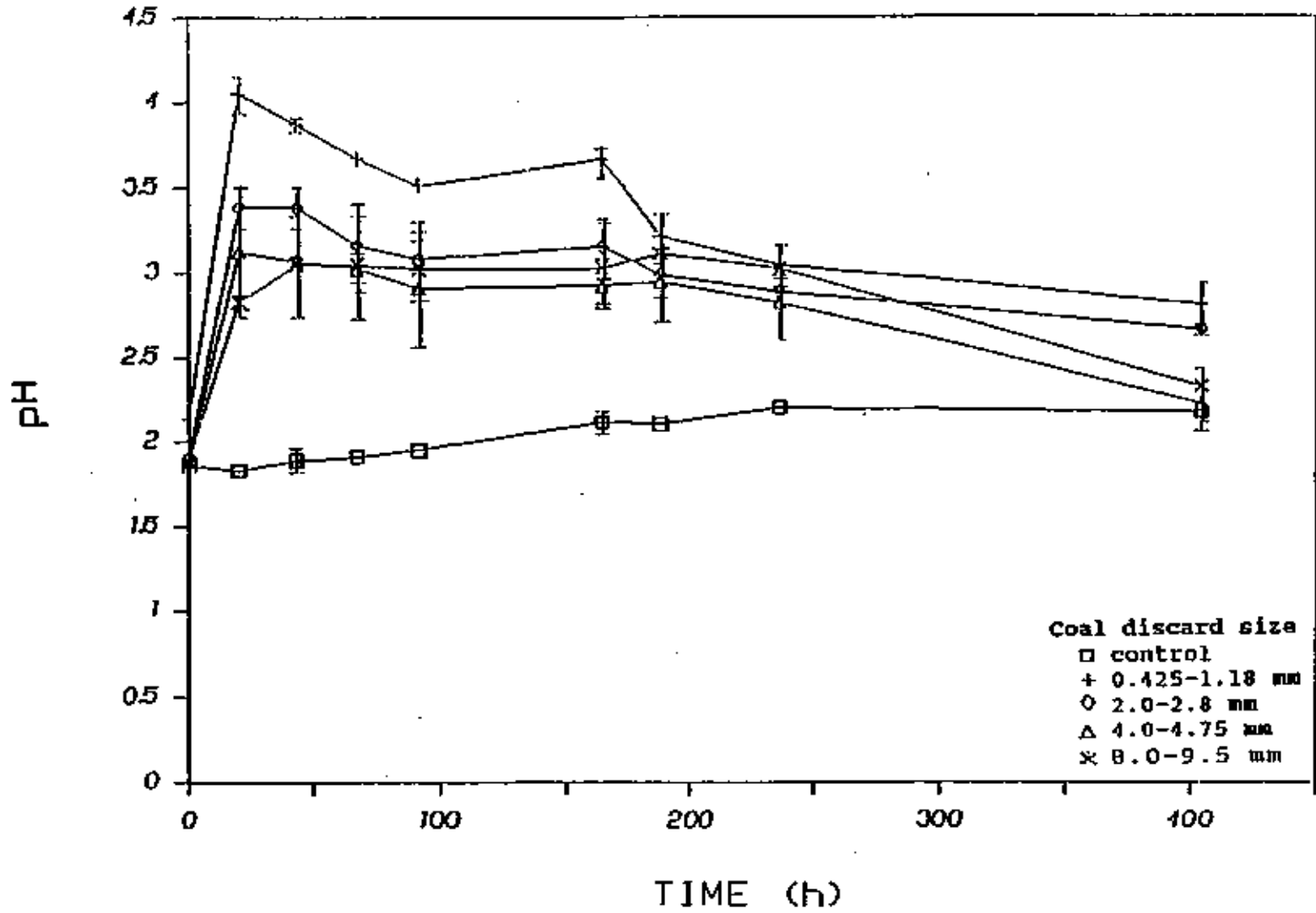


FIG. 33. Effect of 500 g/l unsterilized coal discard (various size fractions) on the pH of sterilized HJJ medium.

decrease in pH (Fig. 33) could be due to the oxidation of pyrite in the coal by ferric iron (Kleinmann et al., 1981), produced by this ferrous iron oxidation. The initial increase in the pH of HJJ medium containing coal wastes, particularly the smaller particle fractions, could have a retarding effect on the growth of *T. ferrooxidans*, as the optimum pH for *T. ferrooxidans* ATCC 19859 and WLR in HJJ medium is about pH 2,5 (this report, part 1, section 2).

19. EFFECTS OF COAL WASTE FRACTIONS ON CULTURES OF T. FERROOXIDANS IN HJJ MEDIUM

19.1. Introduction

Having established that coal waste fractions of defined particle size markedly affected the pH and ferrous iron content of HJJ medium (section 18), necessitating the inclusion of controls containing the appropriate coal waste fraction in all growth and inhibition studies with *T. ferrooxidans* cultures containing coal waste, we investigated the effects of two of the coal waste fractions on the growth of *T. ferrooxidans* in HJJ medium. The study was analogous to our previous investigation (this report, part 1, section 4) of the effect of sand from gold mine sand dumps on *T. ferrooxidans*, in which a marked retarding effect of the sand on culture growth had been shown. We conducted the experiments in a similar manner, investigating effects of increasing amounts of each coal waste fraction on the metabolism of ferrous iron by the bacteria and the pH of the cultures. As the effect of the coal waste on the pH of the medium (section 18.3.3) also manifested itself in the cultures, an experiment in which culture pH was kept approximately constant by daily adjustment to pH 2,0, was also conducted.

19.2. Materials and Methods

The effects of two coal waste fractions with particle diameters 0,425 to 1,18 mm and 2,00 to 2,80 mm (section 18) on *T. ferrooxidans* ATCC 19859 in HJJ medium were studied. Crushed, sieved coal waste in 0, 10, 20, 30, 40, and 50 g quantities was added in duplicate to 250-ml flasks containing 100 ml HJJ medium. Each flask was then inoculated (10% v/v) with a 2- to 3-day-old shaken (120 r.p.m., 26°C) culture of *T. ferrooxidans* ATCC 19859 in HJJ medium and incubated at 26°C with rotary shaking (120 r.p.m.). Duplicate uninoculated HJJ medium controls containing the appropriate coal waste fraction were included in the experiments. In the first experiment, the oxidation of ferrous iron was monitored using the potassium dichromate titration (this report, part 1, section 2.2.4). All results were corrected for abiotic ferrous iron oxidation by subtraction of the appropriate mean control value. In a repeated experiment, the pH of the medium was monitored regularly (section 18.2.3.) In a further experiment, the pH was measured every 24 h and where necessary adjusted to pH 2,0 by the addition of a 15% (v/v) sulphuric acid-15% (v/v) phosphoric acid solution. Ferrous iron oxidation was monitored in this experiment to show development of the bacteria in the presence of the coal waste under conditions of approximately constant pH.

19.3. Results

Figures 34 and 35 show an overall retarding effect of both the 0,425 to 1,18 mm and 2,00 to 2,80 mm fractions of crushed, sieved coal waste on the oxidation of ferrous iron by *T. ferrooxidans* ATCC 19859. The smaller particle fraction had a greater retarding effect than the larger particle fraction. However, during the first 25 h, the flasks containing the smaller coal particles showed a very rapid rate of ferrous iron oxidation, in fact, a stimulated oxidation when compared with that of cultures without coal waste. By contrast, in the flasks containing the larger particles of coal, the rate of ferrous iron oxidation during the first 25 h was retarded by comparison with the rate in the cultures without coal waste; however, the rate increased thereafter until a second phase of retardation began after 3 days.

When the experiments was repeated with pH instead of ferrous iron oxidation being monitored, the pH trends were as shown in Fig. 36 and 37. Marked increases in pH were observed with both coal waste fractions during the first 4 to 6 h, the increases depending on the coal waste concentration (the greater the concentration, the greater the pH increase) and particle size (the smaller coal particles caused greater pH increases than the larger particles at equivalent concentrations). By 10 h the pH values in the flasks with the smaller particle fraction were almost at their maximum values (the pH of the flasks with the larger particle fraction was not measured at this time) and after 20 or 23 h, the pH declined slowly in all flasks.

Ferrous iron disappearance in cultures of *T. ferrooxidans* ATCC 19859, containing the same two coal fractions but with the pH adjusted daily to pH 2,0, is shown in Fig. 38 and 39. With the small particle fraction (Fig. 38) the results were rather similar to those in the experiment where the pH was not adjusted (Fig. 34), except that the time for complete oxidation of the ferrous iron was shortened to about 230 h from almost 300 h or longer. With the larger particle fraction (Fig. 39) there was no evident change in the rate of ferrous iron disappearance from flasks with 100 and 200 g/l coal waste, but at the higher coal waste concentrations the initial phase of retarded ferrous iron disappearance was lengthened and the second retardation phase (after the phase of rapid ferrous disappearance) reduced or eliminated by adjustment of the pH.

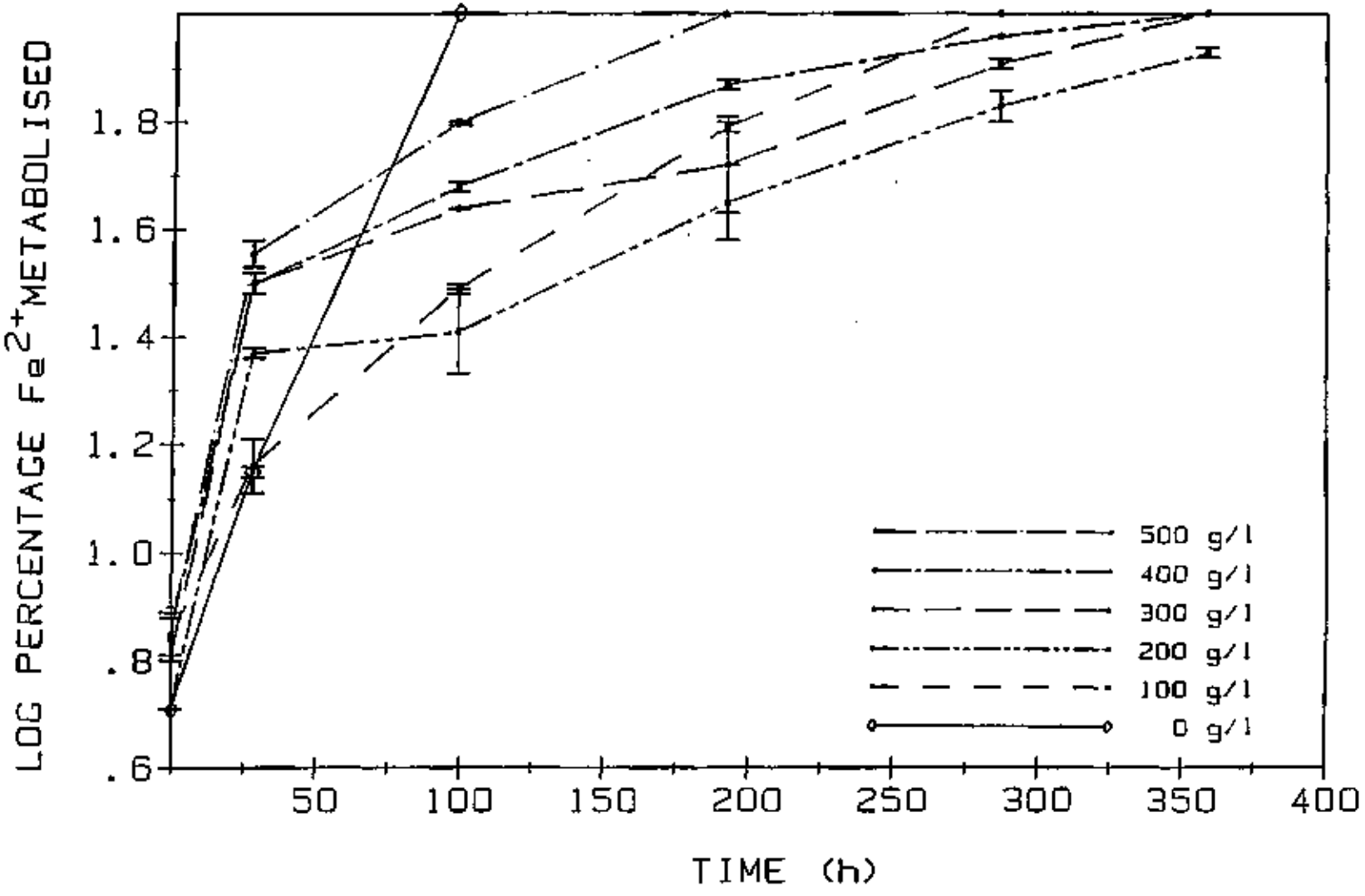


FIG. 34. Effect of coal waste (0,425 to 1,18 mm fraction) on ferrous iron oxidation by *T. ferrooxidans* ATCC 19859 in HJJ medium. Figures alongside symbols are g coal waste/l HJJ medium.

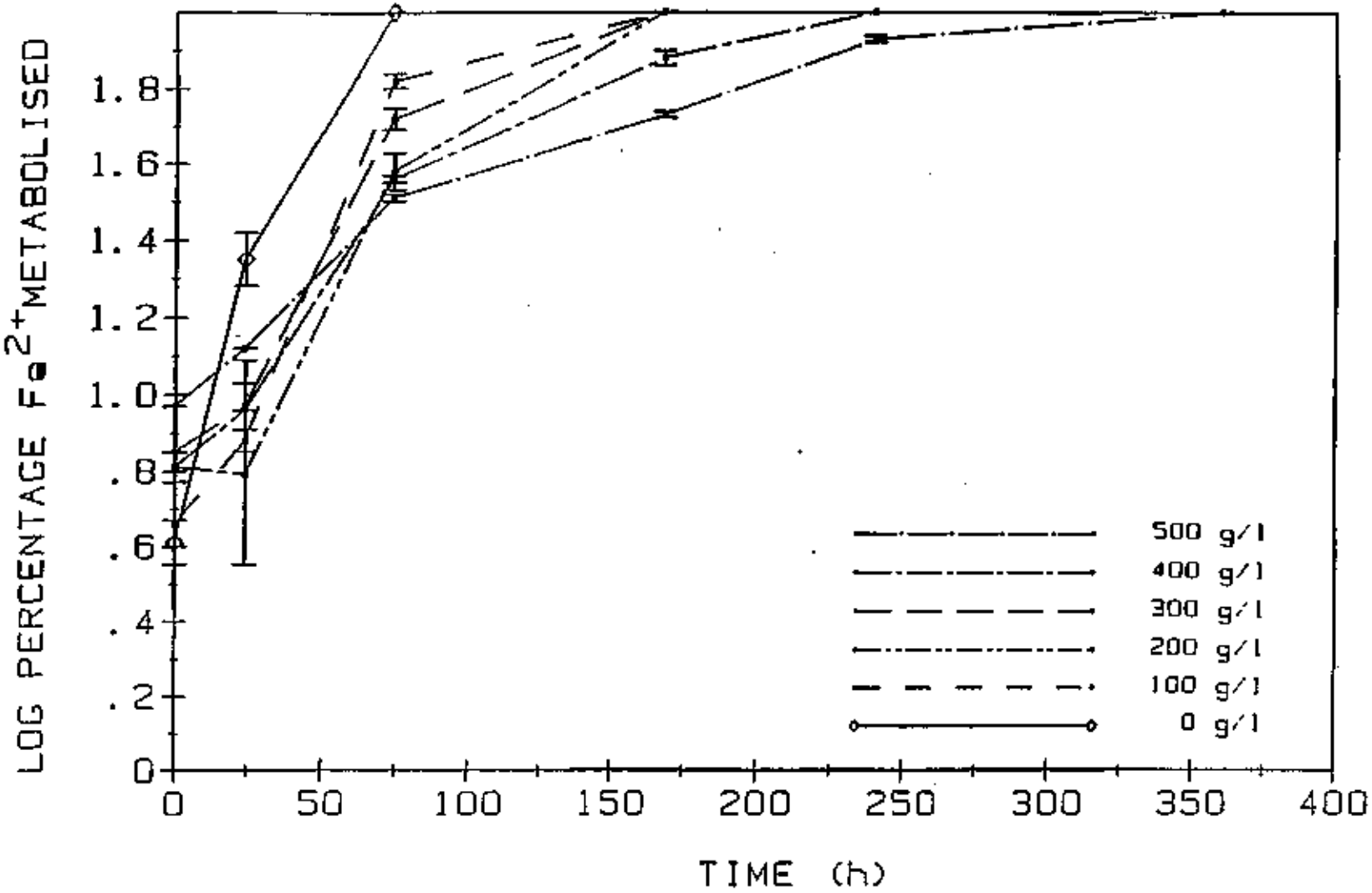


FIG. 35. Effect of coal waste (2,00 to 2,80 mm fraction) on ferrous iron oxidation by *T. ferrooxidans* ATCC 19859 in HJJ medium. Figures alongside symbols are g cola waste/l HJJ medium.

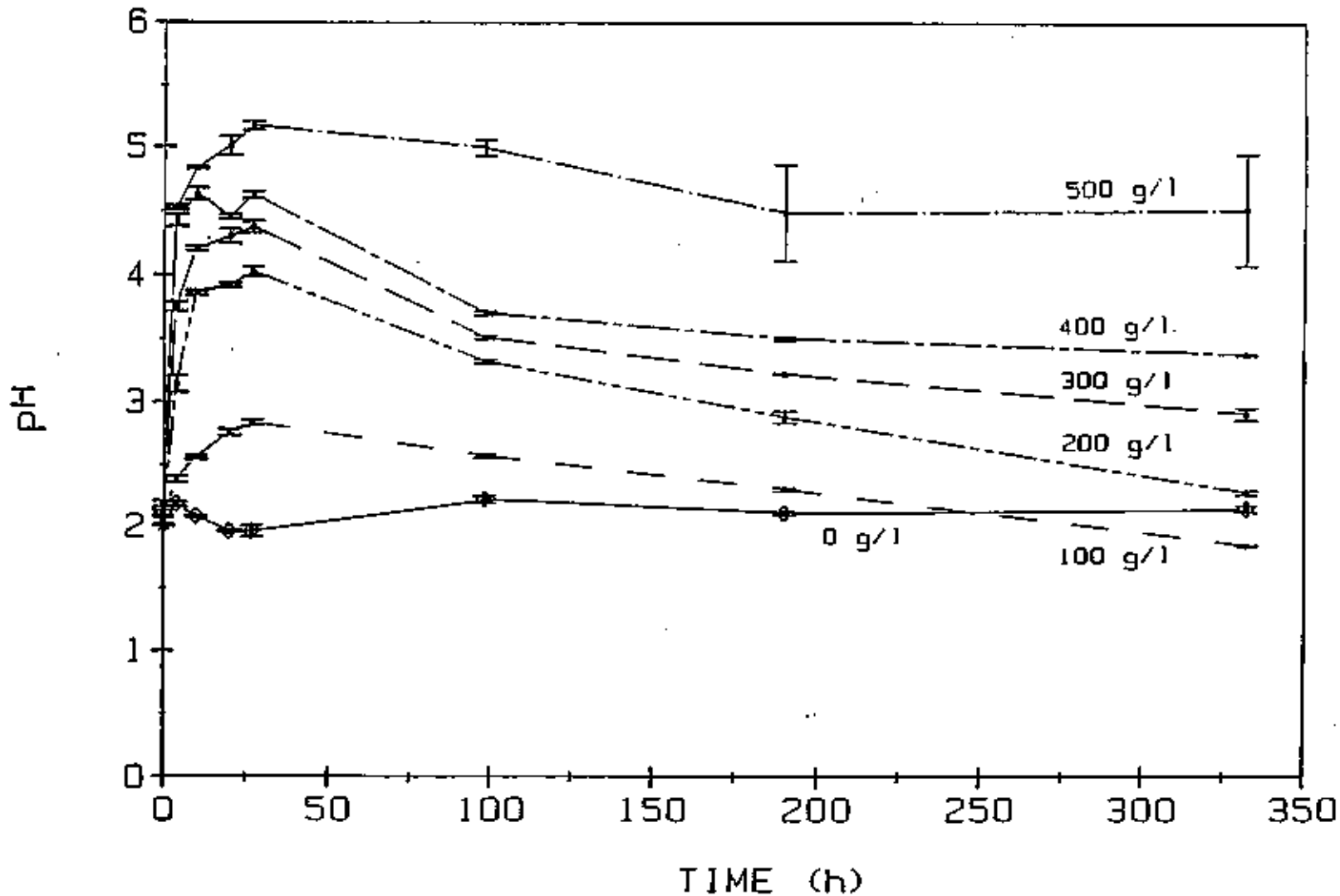


FIG. 36. Effect of coal waste (0,425 to 1,18 mm fraction) on the pH during ferrous iron oxidation by *T. ferrooxidans* ATCC 19859 in HJJ medium. Figures alongside curves are g coal waste/l HJJ medium.

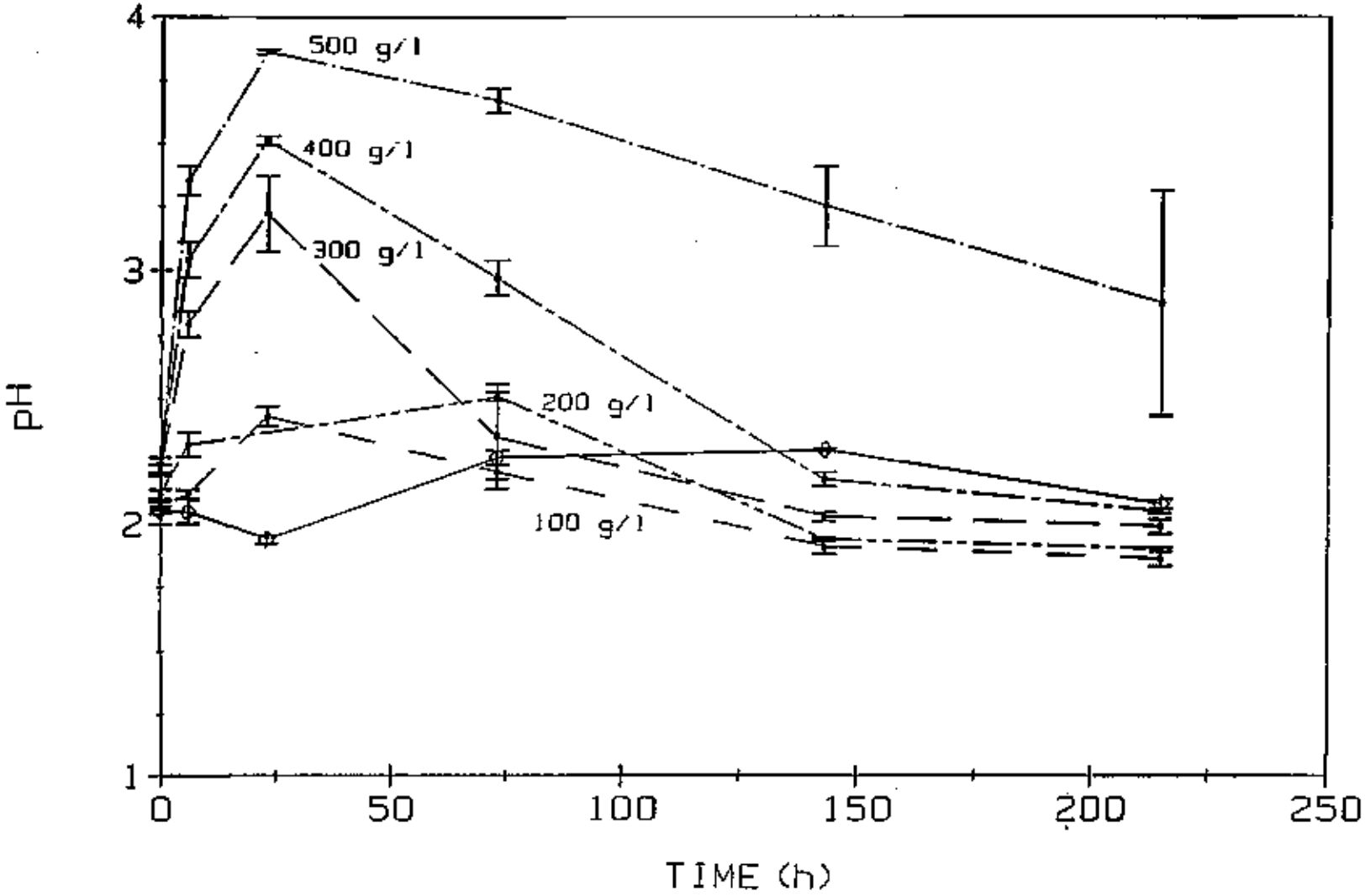


FIG. 37. Effect of coal waste (2,00 to 2,80 mm fraction) on the pH during ferrous iron oxidation by *T. ferrooxidans* ATCC 19859 in HJJ medium. Figures alongside curves are g coal waste/l HJJ medium.

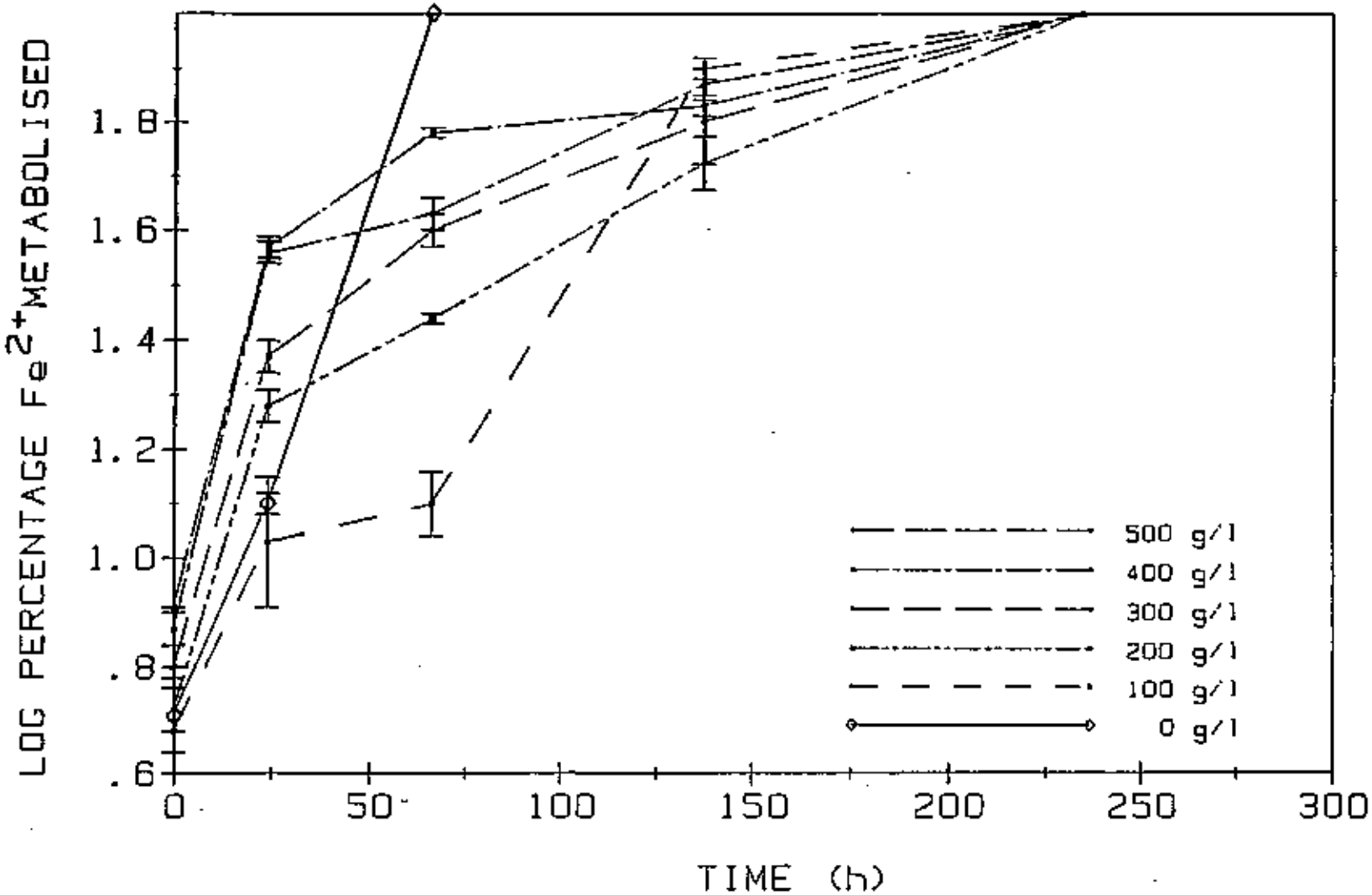


FIG. 38. Effect of coal waste (0,425 to 1,18 mm fraction) on ferrous iron oxidation by *T. ferrooxidans* ATCC 19859 in HJJ medium with daily pH adjustment to pH 2,0. Figures alongside symbols are g coal waste/l HJJ medium.

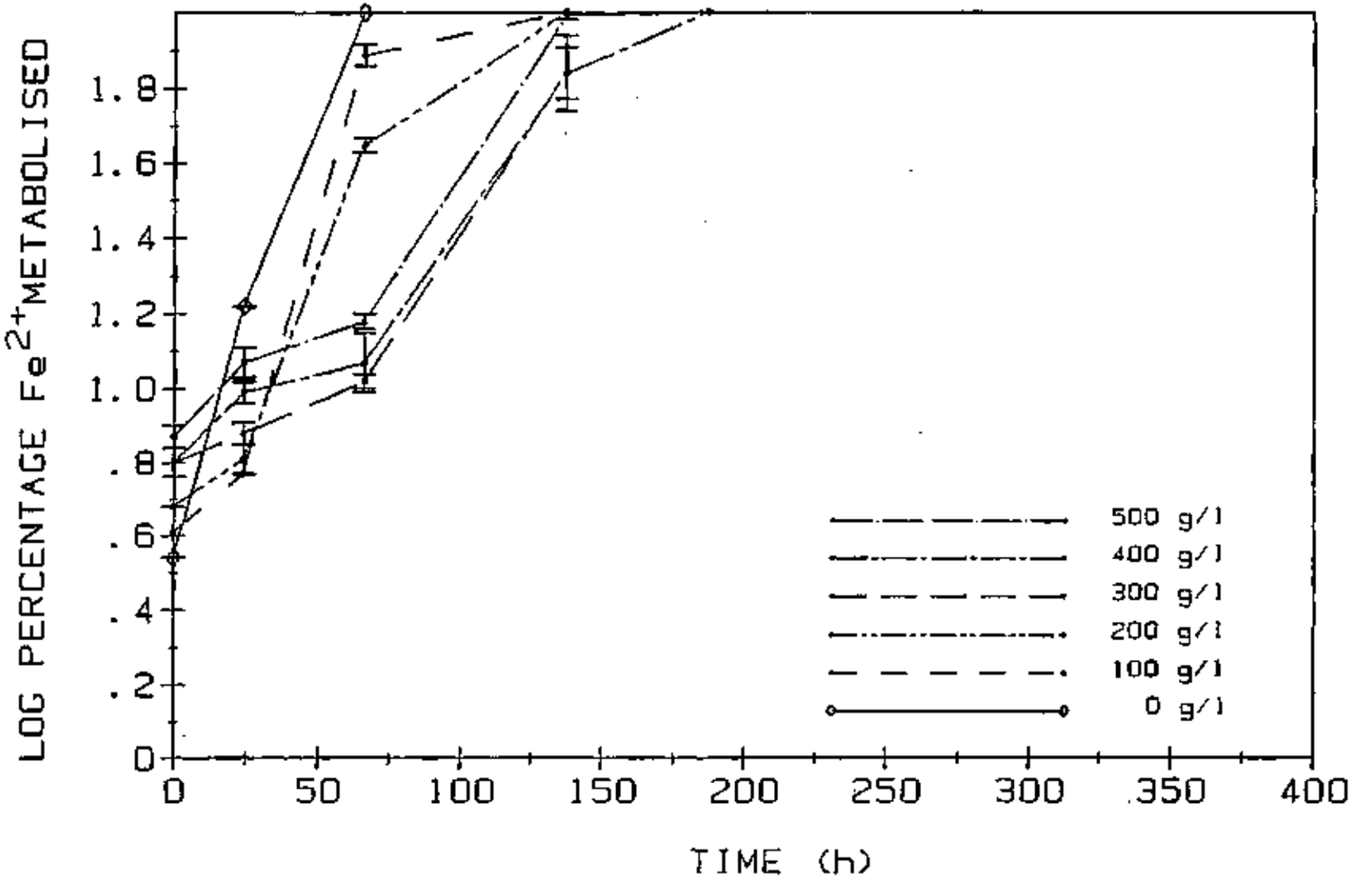


FIG. 39. Effect of coal waste (2,00 to 2,80 mm fraction) on ferrous iron oxidation by *T. ferrooxidans* ATCC 19859 in HJJ medium with daily pH adjustment to pH 2,0. Figures alongside symbols are g coal waste/l HJJ medium.

19.4. Discussion

The overall retarding effects of the 0.425 to to 1,80 mm and 2,00 to 2,80 mm coal waste fractions on ferrous iron disappearance from *T. ferrooxidans* ATCC 19859 cultures (Fig. 34 and 35) can be explained in part by the pH changes in these cultures as shown in Fig. 36 and 37. The pH rose rapidly in the cultures containing coal waste, according to the concentration of coal waste present, by less than a pH unit in the case of 100 g/l of coal waste (both fractions) to 3 pH units with 500 g/l of the 0,425 to 1,80 mm fraction and 2 pH units with 500 g/l of the 2,00 to 2,80 mm fraction. The pH values of 5,2 and 3,9, respectively, with these last two fractions after approximately 25 h were well above pH 1,75 to 2,50, which is the optimum pH range for growth of *T. ferrooxidans* ATCC 19859 (this report, part 1, section 2.3.3). In most of the flasks with the 2,00 to 2,80 mm coal waste fraction, it took about 8 days for the pH to decline again to about the optimum pH of 2,5, but with the 200 to 500 g/l concentrations of the 0.425 to 1,18 mm fraction the pH was still above this optimum pH after 14 days. The cultures with 500 g/l of the 0,425 to 1,18 mm fraction showed little decline in the pH after reaching the maximum (to only about pH 4,5). Unfavourable pH values, particularly in the flasks with the smaller particle fraction of the coal waste, can therefore explain the overall retardation of ferrous disappearance, to a greater extent with the smaller than with the larger particle fraction.

The repetition of the experiment, but with the pH adjusted daily to pH 2,0 to avoid retardation of the growth of the bacteria by an unfavourable pH, showed effects of the coal waste other than pH effects on the ferrous iron disappearance. The cultures with 200 to 500 g/l of the 0,425 to 1,18 mm coal waste fraction initially disposed of the ferrous iron at a faster rate than the culture with no coal waste, the rate increasing as the coal waste concentration increased (Fig. 38). This result was similar to that when the pH was not adjusted (Fig. 34); it can possibly be interpreted, with reference to Fig. 29 (section 18.3.2), as disappearance of the ferrous iron from the cultures by an abiotic mechanism, although abiotic removal of ferrous iron was compensated for, as far as possible, by subtraction of values for iron disappearance in uninoculated control flasks containing the appropriate coal waste fraction. After this initial rapid ferrous iron disapparance, the cultures showed inhibition of ferrous iron removal, to an increasing extent as the coal waste concentration increased. In the cultures with 100 g/l coal waste, the inhibition was overcome after 66 h and a rapid disappearance of ferrous iron was evident, suggesting that an active bacterial oxidation of ferrous iron took over at that stage. With the 2,00 to 2,80 mm fraction of the coal waste at the 300 to 500 g/l concentrations, there seemed to be a similar but slower initial abiotic removal of ferrous

iron, followed by a virtual cessation of this reaction when the reactive capacity of the coal waste was saturated or exhausted, then a renewed rapid disappearance of ferrous iron after 66 h (probably biotic). With this larger particle coal waste fraction at 100 and 200 g/l, the final rapid disappearance of the ferrous iron appeared to start earlier. These growth experiments with *T. ferrooxidans* ATCC 19859 confirmed the conclusion noted previously (section 18.4.1), that growth experiments with a coal waste fraction in HJJ medium should have as the uninoculated control, HJJ medium containing the same amount of coal waste of the same particle size fraction. This compensates for the abiotic effects of the coal waste on the ferrous iron in that only the ferrous oxidation caused by *T. ferrooxidans* is recorded following subtraction of the control value indicating total abiotic ferrous disappearance. Such a control was included in all the experiments investigating the inhibitory effects of SLS, sodium benzoate and sorbic acid on *T. ferrooxidans* ATCC 19859 and WLR in the presence of defined coal waste fractions, but the pH was not adjusted periodically in these experiments.

20. EFFECTS OF CHEMICAL INHIBITORS ON *T. FERROOXIDANS* IN HJJ MEDIUM CONTAINING COAL WASTE FRACTIONS

20.1. Introduction

The research described in this section involved the chemical inhibitors, sodium lauryl sulphate (SLS), sodium benzoate and sorbic acid. Their structures, mode of action as antibacterial agents and activity against *T. ferrooxidans* in the investigations of others as well as in our own research on the possibilities of inhibiting iron-oxidizing bacteria in gold mine sand dumps, have been described and discussed in part I of this report (SLS in section 5; sodium benzoate and sorbic acid in section 7).

The most effective inhibitor of *T. ferrooxidans* in HJJ medium in the absence or presence of gold mine dump sand was SLS. However, the sand had a detrimental effect on its inhibitory activity, increasing the minimum inhibitory concentration for *T. ferrooxidans* ATCC 19859 and WLR to 4 to 8 mg/l from 2 to 4 mg/l in the absence of sand. It was anticipated from the results of Kleinmann and Erickson (1983) that coal waste would raise the minimum inhibitory concentration considerably higher; solutions containing at least 25 mg/l SLS were required to kill *T. ferrooxidans* in coal refuse, i.e. about 10 times the concentration required to kill the bacteria in 9K medium. Kleinmann and Erickson (1983) and Onysko et al. (1984a) reported adsorption of, respectively, 45 and 200 mg SLS/kg coal waste, which would reduce the concentration available in solution for inhibition of the bacteria.

Sodium benzoate (or benzoic acid) and sorbic acid completely inhibited *T. ferrooxidans* in liquid culture at 15 to 30 mg/l in our experiments (in the absence of gold mine dump sand) and at 10 mg/l in the study of Onysko et al. (1984b). Although these concentrations are at least five times higher than the equivalent inhibitory SLS concentrations, benzoic acid delayed the acidification of coal waste for as long as the same concentrations of SLS and sorbic acid for longer (Erickson et al., 1985; Onysko et al., 1984a). The inhibitors were applied to the coal waste (200 kg) as single treatments of 60 and 600 mg/kg, by the addition of 500 and 5000 mg/l solutions. In flask experiments of Dugan (1987a), sodium benzoate at 75 and 100 mg/l inhibited pyrite oxidation in a 30% coal slurry for 22 and 18 days, respectively.

On the basis of such results, the inhibition of *T. ferrooxidans* ATCC 19859 and WLR by SLS, sodium benzoate and sorbic acid in HJJ medium containing 500 g/l coal waste (fractions of defined particle size) was investigated. The objective was a comparative

study of the effectiveness of the three inhibitors in the presence of defined coal waste fractions under standardized experimental conditions, in which the effect of coal waste of differing particle size on the activity of the inhibitors would also be evident.

20.2 Materials and Methods

20.2.1. General culture procedures

Growth and inhibition of two *T. ferrooxidans* strains, ATCC 19859 and WLR, were investigated in shaken cultures in HJJ medium containing, in separate experiments, a range of different unsterilized coal waste fractions (section 18.2.1) and a range of concentrations of SLS, sodium benzoate or sorbic acid as chemical inhibitor. In all experiments the bacteria were grown in 100 ml liquid HJJ medium in 250 ml flasks, with incubation on a rotary shaker (120 r.p.m.) at 26°C. Coal waste of the fraction used in the test was added at 50 g/flask. The inoculum was a culture of *T. ferrooxidans* ATCC 19859 or WLR grown for 2 to 3 days in HJJ medium on a rotary shaker (120 r.p.m.) a 26°C and supplied at a dose of 10% (v/v). Inhibitors were added from unsterilized stock solutions immediately after inoculation or during the logrithmic phase of culture growth. The oxidation of ferrous iron was monitored as an indicator of bacterial growth, using the potassium dichromate titration (this report, part 1, section 2.2.4). The abiotic disappearance of ferrous iron from uninoculated controls containing the same coal waste fraction was taken into consideration (subtracted) in all calculations of the bacterial oxidation of ferrous iron.

20.2.2. SLS

Different concentrations of SLS (supplied by Dr. Theodor Schuchardt, Munchen, West Germany) were tested in duplicate flasks against *T. ferrooxidans* ATCC 19859 and WLR. The SLS concentrations tested were 70,0, 80,0 and 90,0 or 60,0, 70,0 and 80,0 mg/l with strain ATCC 19859 and 60,0, 70,0 and 80,0 or 50,0, 60,0 and 70,0 mg/l with strain WLR. Separate experiments were conducted with coal discard of particle size 0,425 to 1,18 mm, 2,00 to 2,80 mm, 4,00 to 4,75 mm and 8,00 to 9,50 mm. Cultures of the *T. ferrooxidans* strains with the coal discard fractions but no SLS were also included in the experiments.

20.2.3. Sodium benzoate

Sodium benzoate (99% pure, supplied by Saarchem, Muldersdrift, Transvaal) was added in duplicate to cultures of *T. ferrooxidans* ATCC 19859 and WLR in HJJ medium containing 500 g/l unsterilized coal waste at the beginning of the experiment at concentrations of 0, 10,0, 20,0, 30,0 40,0, 50,0 and 60,0 mg/l or during the exponential phase of culture growth at concentrations of 0, 15,0, 30,0, 45,0, 60,0, 75,0 and 90,0 mg/l. Separate experiments were conducted with coal waste of particle diameter 2,00 to 2,80 mm, 4,00 to 4,75 mm, 8,00 to 9,50 mm and 10,0 to 11,5 mm in the cultures.

20.2.4. Sorbic acid

Sorbic acid (Analytical Reagent grade, supplied by E. Merck, Darmstadt, West Germany) was tested as an inhibitor of *T. ferrooxidans* ATCC 19859 and WLR in the same way as sodium benzoate, except for minor differences in the concentrations tested.

20.3. Results

20.3.1. SLS

The results of the experiments with the different *T. ferrooxidans* strains, coal waste fractions and times of SLS addition are shown in Appendix Fig. 59 to 66 and summarized in Table 12. Table 12 also summarizes results for SLS added to cultures without coal waste, presented in full in part 1 of this report (section 5.3.1; Appendix Fig. 1 to 4).

Appendix Fig. 59A, 60A and 61A show inhibition of *T. ferrooxidans* ATCC 19859 by 80 mg/l SLS, added at the beginning of incubation, in the presence of the 0,425 to 1,18 mm, 2,00 to 2,80 mm and 4,00 to 4,75 mm coal waste fractions. *Thiobacillus ferrooxidans* ATCC 19859 was inhibited by 70 mg/l SLS in the cultures with the 8,00 to 9,50 mm fraction (Appendix Fig. 62A). The same concentrations were inhibitory when added during the exponential phase of culture growth, except for the cultures with the smallest coal waste fraction where 90 mg/l SLS was inhibitory (Fig. 59B to 62B). Appendix Fig. 63 to 66 show that *T. ferrooxidans* WLR was inhibited by 70 mg/l SLS in the presence of the three larger coal discard fractions (2,00 to 2,80, 4,00 to 4,75 and 8,00 to 9,50 mm) and 80 mg/l in the presence of the smallest coal discard fraction (0,425 to 1,18 mm). The same concentrations were inhibitory when the SLS was added at the

TABLE 12. Effects of SLS on ferrous iron oxidation by cultures of *T. ferrooxidans* in HJJ medium in the absence and presence of coal waste fractions when the inhibitor was added at the beginning of the experiment or during the exponential phase of culture growth

Coal waste fraction (mm)	*T. ferrooxidans* ATCC 19859 with addition of inhibitor at				Appendix figure(s)	*T. ferrooxidans* WLR with addition of inhibitor at				Appendix figure(s)
	0 h		90-92 h			0 h		90-92 h		
	G[a] mg/l	I[b] mg/l	G mg/l	I mg/l		G mg/l	I mg/l	G mg/l	I mg/l	
0	2	4	1	2	1,2[c]	1	2	1	2	3,4[c]
0,425-1,18	70	80	80	90	59	70	80	70	80	63
2,00-2,80	70	80	70	80	60	60	70	60	70	64
4,00-4,75	70	80	70	80	61	60	70	60	70	65
8,00-9,50	60	70	60	70	62	60	70	60	70	66

[a]G = Highest concentration of SLS permitting oxidation of ferrous iron.

[b]I = Lowest concentration of SLS causing complete inhibition of ferrous iron oxidation.

[c]Appendix figures in part 1 of this report.

start of the incubations or during the exponential phase of growth of *T. ferrooxidans* WLR.

20.3.2. Sodium benzoate

The results of the sodium benzoate inhibition experiments with the various coal waste fractions in the HJJ medium are shown in Appendix Fig. 67 to 74. Those of corresponding experiments without coal waste in the medium are presented in part I of this report (section 7.3.1; Appendix Fig. 34 to 37). Both sets of results are summarized in Table 13, which indicates that:

(i) The concentrations of sodium benzoate needed to inhibit *T. ferrooxidans* ATCC 19859 and WLR in HJJ medium in the absence of coal waste were 15 and 30 mg/l for inhibitor added at the beginning of the experiment and during the exponential phase of culture growth, respectively.

(ii) In the presence of coal waste, the minimum inhibitory concentrations of sodium benzoate were higher, but the size of the coal waste particles seemed to have no differential effect on these concentrations. When the sodium benzoate was added at the beginning of the experiments, the inhibitory concentrations were 40 to 50 (with one exceptional result >60) and 40 to 60 mg/l for the ATCC 19859 and WLR strains, respectively; when the inhibitor was added during the exponential phase of culture growth, the corresponding inhibitory concentrations were 60 and 60 to 75 mg/l. The WLR strain thus seemed to be slightly more resistant to sodium benzoate than the ATCC 19859 strain.

20.3.3. Sorbic acid

The results of the sorbic acid inhibition experiments with the various coal waste fractions in the HJJ medium are shown in Appendix Fig. 75 to 82 and those of corresponding experiments without coal waste in the medium in part I of this report (section 7.3.2; Appendix Fig. 42 to 45). The summary of both sets of results in Table 14 indicates that:

(i) The concentrations of sorbic acid needed to inhibit *T. ferrooxidans* ATCC 19859 and WLR in HJJ medium in the absence of coal waste were 20 and 15 mg/l, respectively, when the inhibitor was added at the beginning of the experiment,

TABLE 13. Effects of sodium benzoate on ferrous iron oxidation by cultures of *Thiobacillus ferrooxidans* in HJJ medium in the absence and presence of coal waste fractions when the inhibitor was added at the beginning of the experiment or during the exponential phase of culture growth

Coal waste fraction (mm)	*T. ferrooxidans* ATCC 19859 with addition of inhibitor at				Appendix figure(s)	*T. ferrooxidans* WLR with addition of inhibitor at				Appendix figure(s)
	0 h		21-25 h			0 h		21-25 h		
	G[a] mg/l	I[b] mg/l	G mg/l	I mg/l		G mg/l	I mg/l	G mg/l	I mg/l	
0	10	15	20	30	36,37[c]	10	15	20	30	34,35[c]
2,00-2,80	30	40	45	60	67	30	40	45	60	71
4,00-4,75	30	40	45	60	68	40	50	60	75	72
8,00-9,50	60	>60	45	60	69	40	50	60	75	73
10,0-11,5	40	50	45	60	70	50	60	45	60	74

[a]G = Highest concentration of SLS permitting oxidation of ferrous iron.

[b]I = Lowest concentration of sodium benzoate causing complete inhibition of ferrous iron oxidation.

[c]Appendix figures in part I of this report.

TABLE 14. Effects of sorbic acid on ferrous iron oxidation by cultures of *Thiobacillus ferrooxidans* in HJJ medium in the absence and presence of coal waste fractions when the inhibitor was added at the beginning of the experiment or during the exponential phase of culture growth

Coal waste fraction (mm)	*T. ferrooxidans* ATCC 19859 with addition of inhibitor at				Appendix figure(s)	*T. ferrooxidans* WLR with addition of inhibitor at				Appendix figure(s)
	0 h		21-25 h			0 h		21-25 h		
	G[a] mg/l	I[b] mg/l	G mg/l	I mg/l		G mg/l	I mg/l	G mg/l	I mg/l	
0	15	20	15	20	44,45[c]	10	15	10	20	42,43[c]
2,00-2,80	40	50	45	60	75	30	40	30	45	79
4,00-4,75	40	50	30	45	76	40	50	60	75	80
8,00-9,50	50	60	45	60	77	40	50	60	75	81
10,0-11,5	60	>60	60	75	78	40	50	60	75	82

[a]G = Highest concentration of SLS permitting oxidation of ferrous iron.

[b]I = Lowest concentration of sorbic acid causing complete inhibition of ferrous iron oxidation.

[c]Appendix figures in part I of this report.

and 20 mg/l for both strains when it was added during the exponential phase of growth.

(ii) In the presence of coal waste, the minimum inhibitory concentrations of sorbic acid were higher, but the size of the coal waste particles had little or no differential effect on these concentrations. When the sorbic acid was added at the beginning of the experiments, the inhibitory concentrations were 50 to 60 and 40 to 50 mg/l for the ATCC 19859 and WLR strains, respectively; when the inhibitor was added during the exponential phase of culture growth, the inhibitory concentrations ranged from 45 to 75 mg/l for both strains.

20.4. Discussion

20.4.1. SLS

In the experiments without coal waste in the cultures of *T. ferrooxidans* ATCC 19859 and WLR (this report, part 1, section 5), SLS added at the beginning of incubation was completely inhibitory at 4 and 2 mg/l, respectively, and added during the exponential phase of culture growth, completely inhibited both strains at 2 mg/l. In the experiments with 500 g/l coal waste in the medium, the ATCC 19859 strain again showed greater resistance to SLS than the WLR strain, but the minimum inhibitory concentrations of SLS were greatly increased by the presence of the coal waste fractions tested (70 to 90 mg/l SLS were required to inhibit *T. ferrooxidans* ATCC 19859 and 70 to 80 mg/l to inhibit the WLR strain). The inhibition of ATCC 19859 by 70 mg/l SLS in the presence of the largest coal discard fraction and by 80 or 90 mg/l with the smaller size fractions may be related to the decrease in surface area and less adsorption of SLS as the particle size became larger, but this effect was not evident with the three largest coal discard fractions in the experiments with strain WLR. In cultures with the coal discard, the actively growing cells of both strains were no more sensitive to SLS than cells exposed to SLS from the start of culture incubation, in contrast to the cells of *T. ferrooxidans* ATCC 19859 in cultures without coal discard (this report, part 1, section 5). The slow utilization of ferrous iron by WLR in these inhibition experiments, even without SLS in the medium, may have resulted in part from the increase in pH caused by the coal waste (sections 18 and 19); however, the ATCC 19859 cultures were less influenced by any such effect of the coal waste, particularly in the presence of the larger particle fractions. The high concentrations of SLS required to inhibit the two test strains of *T. ferrooxidans* in the presence of coal waste suggest that the use of SLS to inhibit acidification of coal dumps is unlikely to be very economical.

20.4.2. Sodium benzoate

The antimicrobial activity of benzoic acid and its salts is related to pH, with the greatest activity at low pH (Jay, 1978). Inhibitor studies in the U.S.A. with cultures of *T. ferrooxidans* supplemented with benzoic acid indicated that 10 mg/l benzoic acid effectively inhibited bacterial ferrous iron oxidation (Onysko et al., 1984b). The results of the present studies (this report, part 1, section 7) showed greater concentrations of sodium benzoate than 10 mg/l to be necessary for the complete inhibition of *T. ferrooxidans* ATCC 19859 or WLR in HJJ medium, namely 15 mg/l when the inhibitor was added at the beginning of the experiment and 30 mg/l when it was added during the exponential phase of growth (Appendix Fig. 34 to 37). The higher concentrations of sodium benzoate required for complete inhibition of *T. ferrooxidans* ATCC 19859 and WLR when the addition was made during the exponential phase of growth, may have been related to the formation of a sparingly soluble precipitate with ferric iron (Onysko et al., 1984b), which would have been formed by the *T. ferrooxidans* cultures in the exponential phase of growth. However, such a precipitate may serve as a slow release source of benzoic acid for the control of *T. ferrooxidans* in acid mine water (Onysko et al., 1984a,b).

Differences of experimental technique are possibly responsible for the differences in the results of Onysko et al. (1984b) and those of our experiments. The inhibitory 10 mg/l benzoic acid of Onysko et al. (1984b) equals 11,8 mg/l sodium benzoate, thus this was not the reason. Differences in the *T. ferrooxidans* inoculum are possibly the main reason for the inhibitory concentrations of benzoate, added at the beginning of the experiment, being higher in our experiments than in that of Onysko et al. (1984b). They used a different *T. ferrooxidans* strain at an inoculation rate of 0,1% (v/v) compared with our 10% (v/v). Furthermore, the initial pH of their medium was pH 1,16 compared with our pH 2,0, and the compositions of the media were not identical.

Previous experiments (section 19) showed that coal waste in the HJJ medium retarded the growth of *T. ferrooxidans* ATCC 19859 in the absence of chemical inhibitors. The addition of sodium benzoate at concentrations of at least 20 or 30 mg/l had further retarding effects on the oxidation of ferrous iron, with complete inhibition of the ATCC 19859 strain at 40 to >60 mg/l and strain WLR at 40 to 60 mg/l in the presence of 500 g/l coal waste, when the inhibitor was added at the beginning of the experiment. Addition of sodium benzoate during the exponential phase of growth required concentrations of 60 to 75 mg/l for the complete inhibition of ferrous iron oxidation by

cultures of *T. ferrooxidans* ATCC 19859 and WLR in the presence of coal waste. However, the coal waste particle size had no or hardly any effect on the inhibitory concentration of sodium benzoate, even though Fig. 32 and 33 of section 18 suggest that the high pH caused by the smaller fractions could have contributed to the inhibition of the bacterial ferrous iron oxidation through an unfavourable pH effect. On the other hand, the high pH would lower the activity of the sodium benzoate. Possibly the two effects neutralized each other.

Comparison of Tables 12 and 13 indicates that the concentrations of sodium benzoate which inhibited the *T. ferrooxidans* test strains in the presence of the coal waste fractions varied from considerably lower than to similar to the inhibitory concentrations of SLS. As sodium benzoate is about half the price of SLS, it has the potential for providing more economical dump treatment to combat acidification than SLS (Onysko et al., 1984a).

20.4.3. Sorbic acid

Sorbic acid is most effective as an antimicrobial agent below pH 3, when more than 80% of the compound is undissociated (Jay, 1978). Undissociated molecules are essential for antimicrobial activity, preventing growth by inhibiting cellular uptake of substrate molecules. Onysko et al. (1984b) reported that 10 mg/l sorbic acid effectively inhibited the oxidation of ferrous iron by *T. ferrooxidans*. In our studies (this report, part 1, section 7) with *T. ferrooxidans* ATCC 19859 and WLR, 20 and 15 mg/l, respectively, were necessary for complete inhibition when the sorbic acid was added at the beginning of the experiment. When the sorbic acid was added during the exponential phase of growth, 20 mg/l completely inhibited the ferrous iron oxidation by both the ATCC 19859 and the WLR strains.

When 500 g/l coal waste fractions of different particle size were included in the HJJ medium, the retarding effect of the coal waste itself on the ferrous iron oxidation by the *T. ferrooxidans* strains in the absence of sorbic acid was noticed clearly. The addition of at least 10 mg/l sorbic acid at the beginning of incubation had further retarding effects with 40 to 60 mg/l being inhibitory. The addition of sorbic acid during the exponential phase of grwoth completely inhibited the bacterial activity at concentrations of 45 to 75 mg/l. The wide range over which this inhibition occurred may be due to the smaller particle fractions of the coal waste having influenced the growth conditions of the bacteria as a result of increasing the pH by 2 to 3 units in the media containing these fractions.

Erickson et al. (1985) conducted inhibition experiments with sodium benzoate and potassium sorbate in which 60 and 600 mg of each inhibitor were added as single doses to 1 kg coal. These doses retarded the development of acidity, in spite of weekly leachings with tap water, by, respectively, about 2 and 10 weeks (sodium benzoate) or 5 and 10 weeks (sorbic acid) beyond the 7 weeks required for the development of acidity in the control. In their experiments, as in ours (Tables 12 and 14), sorbic acid appeared to be a more effective inhibitor of acid-producing iron-oxidizing bacteria associated with coal waste than SLS. However, it is not a cheaper chemical than SLS and in our experiments was no more effective as an inhibitor than sodium benzoate (Tables 13 and 14), although it showed greater effectiveness in the study of Erickson et al. (1985). On the grounds of effectiveness plus cost, sodium benzoate appears to be potentially better than sorbic acid (which costs at least three times as much) for the treatment of coal waste dumps.

21. EFFECTS OF 1-BROMO-3-CHLORO-5,5-DIMETHYLHYDANTOIN (H900) AND MIXTURES OF INHIBITORS (H900, SLS, SODIUM BENZOATE AND SORBIC ACID) ON *T. FERROOXIDANS* IN HJJ MEDIUM

21.1. Introduction

The research of this section follows mainly from that of section 20 demonstrating the effectiveness of the inhibitors SLS, sodium benzoate and sorbic acid, against acid-generating iron-oxidizing bacteria in coal wastes, at concentrations which could provide economical treatments against acid mine drainage. In particular, benzoic acid or benzoate salts have an economic advantage (Onysko et al., 1984a).

This section deals with preliminary evaluations of a mixed dihalogenated dimethyl hydantoin, not yet demonstrated as a potential inhibitor of acid drainage from mine dumps, on its own and mixed with SLS, and mixtures of SLS, sodium benzoate and sorbic acid (as pairs of inhibitors). The 1-bromo-3-chloro-5,5-dimethylhydantoin (H900) is a highly active antibacterial compound as a result of the synergism of the bromo- and chloro-substituents which are released as the antimicrobial hypobromous and hypochlorous acids (Trueman, 1971). It was evaluated in view of an interest by a local supplier in its possible use for inhibiting the development of acid water in a coal-washing plant. The mixtures of inhibitors were investigated for possible synergistic effects, which might make them more effective and more economical than the single inhibitors.

Mixtures of SLS with sodium benzoate and an alkylbenzenesulphonate with sodium benzoate have been studied by Dugan (1987a,b) and Dugan and Apel (1983) for their ability to inhibit the acidification of coal waste. The mixture with each ingredient supplied at 100 mg/l prevented acidification of 30% coal slurries in flask cultures for 26 days, whereas the inhibitors on their own at 100 mg/l delayed the acidification process for only 12 or 18 days (Dugan, 1987a). Under 'simulated field conditions', with 341 kg high sulphur (6 to 8%) coal waste in almost horizontal shallow bins, and a series of four applications of the inhibitors at 20 or 100 mg/l of each in 3,78 l of water, the development of iron- and sulphur-oxidizing bacteria as well as the production of soluble iron, sulphate and acidity, were temporarily inhibited (Dugan 1987b; Dugan and Apel, 1983). However, the data from these experiments did not indicate whether the inhibition achieved by the mixtures was synergistic or additive.

In our preliminary evaluations of H900 and the mixtures of inhibitors, we examined the inhibition of two *T. ferrooxidans* test strains only in HJJ medium not containing coal waste.

21.2 Materials and Methods

21.2.1. General culture procedures

The test bacteria were *T. ferrooxidans* ATCC 19859 and WLR. Culture procedures were as described in section 20.2.1, except that coal waste was not added to the cultures. The combinations and concentrations of inhibitors in duplicate test cultures were as specified in the following sections (21.2.2 to 21.2.6). All inhibitors were added in appropriate volumes from stock solutions (1,0 g/l, except SLS and H900 at 2,0 g/l in the combination) at the start of the culture incubations.

21.2.2. H900

The single inhibitor H900 (supplied by Floccotan Pty. Ltd., Randburg, Transvaal) was added in HJJ medium to ATCC 19859 and WLR cultures at 8,0 to 14,0 mg/l and 5,0 to 15,0 mg/l, respectively, from a freshly prepared 1,0 g/l solution of H900 pellets dissolved in distilled water. It was also added to ATCC 19859 cultures at 2,0 to 10,0 mg/l from a 2,0 g/l solution supplied by Floccotan. The effect of storage of H900 in solution on its inhibitory activity was investigated by adding it from a 2,0 g/l solution that had been stored for about 6 months to give concentrations of 2,0 to 14,0 mg/l in WLR cultures.

21.2.3. SLS and H900

ATCC 19859 : SLS at 2,0, 2,5, 3,0 and 3,5 mg/l with H900 at 4,0 and 5,0 mg/l.

21.2.4. SLS and sorbic acid

ATCC 19859 : SLS at 1,0, 2,0 and 3,0 mg/l with sorbic acid at 10,0, 15,0, 20,0 and 25,0 mg/l; sorbic acid at 10,0 and 15,0 mg/l with SLS at 1,0, 2,0, 3,0 and 4,0 mg/l.

WLR : SLS at 1,0 mg/l with sorbic acid at 5,0, 10,0 and 15,0 mg/l; sorbic acid at 10,0 mg/l with SLS at 0,5, 1,0, 1,5 and 2,0 mg/l.

21.2.5. SLS and sodium benzoate

ATCC 19859 : SLS at 2,0 mg/l with sodium benzoate at 10,0, 12,0, 14,0 and 16,0 mg/l; sodium benzoate at 14,0 mg/l with SLS at 1,0, 2,0, 3,0 and 4,0 mg/l.

WLR : SLS at 1,0 mg/l with sodium benzoate at 5,0, 10,0, 15,0 and 20,0 mg/l; sodium benzoate at 10,0 mg/l with SLS at 0,5, 1,0, 1,5 and 2,0 mg/l.

21.2.6. Sorbic acid and sodium benzoate

ATCC 19859 : Sorbic acid at 20,0 mg/l with sodium benzoate at 4,0, 8,0, 12,0 and 16,0 mg/l; sodium benzoate at 10,0 mg/l with sorbic acid at 5,0, 10,0 and 15,0 mg/l; sodium benzoate at 14,0 mg/l with sorbic acid at 10,0, 15,0, 20,0 and 25,0 mg/l.

WLR : Sorbic acid at 10,0 mg/l with sodium benzoate at 5,0, 10,0, 15,0 and 20,0 mg/l; sodium benzoate at 14,0 mg/l with sorbic acid at 5,0, 10,0 and 15,0 mg/l; sodium benzoate at 5,0 mg/l with 5,0, 10,0 and 15,0 mg/l sorbic acid.

21.3. Results

21.3.1. General

Results establishing inhibitory concentrations of H900 and inhibitors in mixtures are shown in Appendix Fig. 83 to 104. The main results are indicated in the following sections (21.3.2. to 21.3.6).

21.3.2. H900

When added from a freshly prepared 1,0 g/l stock solution,H900 completely inhibited *T. ferrooxidans* ATCC 19859 and WLR at 8,0 and 10,0 mg/l respectively (Appendix Fig. 83). Appendix Fig. 84 shows that H900 (from a non-aged 2,0 g/l solution) at 6,0 mg/l completely inhibited *T. ferrooxidans* ATCC 19859 for at least 22 days and at concentrations of 2,0 to 4,0 mg/l retarded growth of the bacteria. The H900 (2,0 g/l solution) that had been stored for about 6 months was not inhibitory for WLR at 14,0 mg/l (Appendix Fig. 85).

21.3.3. SLS and H900

ATCC 19859: SLS at 2,0 to 3,5 mg/l did not inhibit ferrous iron oxidation with either 4,0 or 5,0 mg/l H900 (Appendix Fig. 86, 87), although the onset of oxidation was delayed completely for 266 hours by the H900 at 5,0 mg/l in combination with all the SLS treatments (Appendix Fig. 87).

21.3.4. SLS and sorbic acid

ATCC 19859: In two different experiments, SLS at 1,0 mg/l with 20,0 (Appendix Fig. 88) or 15,0 (Appendix Fig. 92) mg/l sorbic acid inhibited iron oxidation. Iron oxidation was inhibited by 2,0 and 3,0 mg/l SLS with sorbic acid at 15,0 to 20,0 mg/l (Appendix Fig. 89, 90) but not at 10,0 mg/l (Appendix Fig. 89 to 91).

WLR: SLS at 1,0 mg/l with sorbic acid at 15,0 mg/l inhibited iron oxidation (Appendix Fig. 93), but with sorbic acid at 10,0 mg/l, 1,5 and 2,0 mg/l SLS only retarded iron oxidation (Appendix Fig. 94).

21.3.5. SLS and sodium benzoate

ATCC 19859: SLS at 1,0 mg/l with 14,0 mg/l sodium benzoate (Appendix Fig. 96), or at 2,0 mg/l with 10,0 mg/l sodium benzoate (Appendix Fig. 95) inhibited iron oxidation completely.

WLR : SLS at 1,0 mg/l with sodium benzoate at 15,0 mg/l but not 10,0 mg/l belatedly inhibited iron oxidation (Appendix Fig. 97). Sodium benzoate at 10,0 mg/l with 2,0 mg/l SLS failed to inhibit iron oxidation completely (Appendix Fig. 98).

21.3.6. Sorbic acid and sodium benzoate

ATCC 19859 : Sorbic acid at 20,0 mg/l with sodium benzoate at 4,0 mg/l inhibited iron oxidation completely (Appendix Fig. 99). Sodium benzoate at 10,0 mg/l with sorbic acid also at 10,0 mg/l in a rather short (140-hour) experiment completely inhibited iron oxidation (Appendix Fig. 100), as did sodium benzoate at 14,0 mg/l with sorbic acid at 10,0 mg/l in a longer experiment (Appendix Fig. 101).

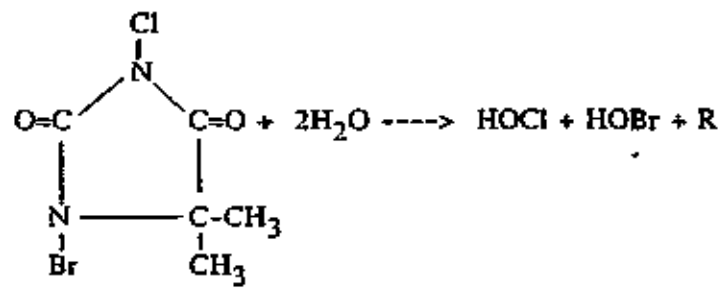
WLR : Sorbic acid at 10,0 mg/l with sodium benzoate at 10,0 mg/l inhibited iron oxidation (Appendix Fig. 102); iron oxidation was also inhibited by sodium benzoate at

14,0 mg/l with sorbic acid at 5,0 mg/l (Appendix Fig. 103) but not by sodium benzoate at 5,0 mg/l with sorbic acid at 15,0 mg/l (Appendix Fig. 104).

21.4. Discussion

21.4.1. H900

The inhibitory concentrations of non-aged H900 solutions (Appendix Fig. 83 to 85) for the two *T. ferrooxidans* strains (6,0 to 10,0 mg/l) were two to three times higher than the inhibitory SLS concentrations (2,0 and 4,0 mg/l) reported by Conradie (1984; also this report, part 1, section 5) and were considerably lower than the inhibitory concentrations of sodium benzoate and sorbic acid (15,0 and 20,0 mg/l) reported by Maré (1989; also this report, part 1, section 7). These concentrations refer to inhibitor addition at the beginning of culture incubations. However, aging of a solution of H900 for 6 months was clearly detrimental to its ability to inhibit *T. ferrooxidans*. This is not surprising as the antimicrobial action of H900 is related to the formation in water of highly reactive hypohalous acids (Trueman, 1971) which decompose spontaneously, particularly if exposed to light (Barnett and Wilson, 1957).



1-Bromo-3-chloro-5.5-dimethylhydantoin (H900)

$3HOCl \longrightarrow HClO_3 + 2HCl$

$2HOCl \longrightarrow 2HCl + O_2$

$HOCl + HCl \longrightarrow H_2O + Cl_2$

$5HOBr \longrightarrow HBrO_3 + 2Br_2 + 2H_2O$

$4HOBr \longrightarrow 2Br_2 + O_2 + H_2O$

They can also be expected to decompose by reaction with the ferrous iron in the culture medium or mine dumps, so are unlikely to remain effective inhibitors for long.

$HOCl \text{ (or } HOBr) + 2Fe^{2+} + 2H^{+} \longrightarrow 2Fe^{3+} + HCl \text{ (or } HBr) + H_2O$

These theoretical considerations together with the experimental results indicate that H900 would not be a satisfactory inhibitor for use against *T. ferrooxidans* in mine dumps over extended periods.

21.4.2. SLS and H900

The H900 added on its own was inhibitory at 6,0 mg/l but not at 4,0 mg/l. At all concentrations tested (Appendix Fig. 86, 87) the mixture of inhibitors was ineffective, as oxidation of the ferrous iron occurred in all duplicate flasks, albeit after a 266-hour delay when the H900 concentration was 5,0 mg/l. Some ferrous oxidation may have resulted from a chemical reaction between the ferrous iron and H900, which produces HOCl and HOBr; these might react with the ferrous iron oxidizing it to ferric. However, little of the ferrous iron in HJJ medium would be oxidized by 4,0 or 5,0 mg/l H900. More important is that the proposed reaction would remove the antibacterial HOCl and HOBr from the system. The inhibition of iron oxidation for 266 hours by the treatments with H900 at 5,0 mg/l suggests that such removal was not particularly rapid; however, there was no indication that the combination of H900 and SLS would be of any use for the treatment of mine dumps.

21.4.3. SLS and sorbic acid

ATCC 19859 : SLS added on its own at the beginning of culture incubation was completely inhibitory at 4,0 but not 2,0 mg/l (this report, part 1, section 5); sorbic acid on its own was inhibitory at 20,0 but not 15,0 mg/l (this report, part 1, section 7). The concentrations which were inhibitory in the mixtures, i.e. 1,0 mg/l SLS with 15,0 or 20,0 mg/l sorbic acid, or 2,0 and 3,0 mg/l SLS with 15,0 mg/l sorbic acid, thus indicate no substantial reduction of the minimum inhibitory concentration of the sorbic acid in the presence of three quarters to half the inhibitory concentrations of SLS (Appendix Fig. 88 to 92). It is not clear from the presented data how half the inhibitory sorbic acid concentration affects the minimum inhibitory SLS concentration. However, the use of such mixtures of inhibitors on mine dumps would depend on their remaining together to maintain a combination of inhibitory concentrations, without the loss of one by differential adsorption.

WLR : SLS on its own inhibited iron oxidation in HJJ medium at 2,0 but not 1,0 mg/l (this report, part 1, section 5) and sorbic acid at 15,0 but not 10,0 mg/l (this report, part 1, section 7). The inhibitory combination of 1,0 mg/l SLS and 15,0 mg/l sorbic acid (Appendix Fig. 93) thus represents no reduction in the inhibitory concentration of sorbic

acid in the presence of half the inhibitory concentration of SLS. However, the lack of total inhibition with 2,0 mg/l SLS and 10,0 mg/l sorbic acid (Appendix Fig. 94) is surprising. Possibly the WLR strain became more resistant to SLS with continued subculturing since it was tested by Conradie (1984; see also this report, part 1, section 5).

The sets of results with both strains give no indication that it would be beneficial to treat mine dumps with an SLS-sorbic acid mixture rather than with either inhibitor on its own.

21.4.4. SLS and sodium benzoate

ATCC 19859 : In the studies of Conradie (1984; this report, part 1, section 5) and Maré (1989; this report, part 1, section 7), SLS and sodium benzoate were inhibitory when added to HJJ medium (at the start of culture incubation) on their own at 4,0 and 15,0 mg/l, respectively. The inhibitory combination (Appendix Fig. 95) of 10,0 mg/l sodium benzoate with 2,0 mg/l SLS suggested that a reduction of the inhibitory concentrations of both chemicals by the use of the mixture might be achieved.

WLR : Inhibitory levels of SLS and sodium benzoate on their own, according to Conradie (1984; this report, part 1, section 5) and Maré (1989; this report, part 1, section 7), were 2,0 and 15,0 mg/l, respectively. With the SLS-sodium benzoate combinations, one of the inhibitors had to be at its inhibitory concentration for the combination to be inhibitory; thus, 1,0 mg/l SLS with 15,0 mg/l sodium benzoate (Appendix Fig. 97) or 2,0 mg/l SLS with 10,0 mg/l sodium benzoate (Appendix Fig. 98) were required for inhibition of *T. ferrooxidans* WLR. These results, in contrast to those with *T. ferrooxidans* ATCC 19859, give no indication of benefit from using an SLS-sodium benzoate combination rather than either inhibitor alone for the treatment of mine dumps.

Dugan (1987a) found the combination of SLS and sodium benzoate, both at 100 mg/l, to be effective for 26 days in inhibiting sulphate production from pyrite in coal in shaken flasks (125 ml of a 30 % slurry of coal). However the results of the present investigation suggest that both of the compounds were at or close to a concentration at which they would have been inhibitory on their own.

21.4.5. Sorbic acid and sodium benzoate

ATCC 19859 : Sorbic acid and sodium benzoate on their own were inhibitory at 20,0 and 15,0 mg/l, respectively (Maré, 1989; this report, part 1, section 7). Although the inhibitory combination of 10,0 mg/l of both inhibitors (Appendix Fig. 100) represents a substantial reduction over the inhibitory concentration of each acid on its own, the combination may not actually be more effective. If both inhibitors, as organic acids, have a similar mechanism of action, the inhibitory effect of the two together may be merely an additive organic acid effect. In this connection, the inhibitory concentrations of 20,0 mg/l sorbic acid or 15,0 mg/l sodium benzoate or 10,0 mg/l sorbic acid with 10,0 mg/l sodium benzoate represent 0,18, 0,11 and 0,16 mM monocarboxylic acid solutions, respectively, suggesting that when considered on a molar basis, sodium benzoate is a more effective inhibitor than sorbic acid at the pH of the HJJ medium.

WLR : Sorbic acid and sodium benzoate were both inhibitory at 15,0 mg/l when added on their own to WLR in HJJ medium (Maré, 1989; this report, section 1, part 7). In combination, 5,0 mg/l sorbic acid and 14,0 mg/l sodium benzoate (Appendix Fig. 103) were inhibitory, as were 10,0 mg/l sorbic acid with 10,0 mg/l sodium benzoate (Appendix Fig. 102); sodium benzoate at 5,0 mg/l with sorbic acid at 15,0 mg/l was not an inhibitory combination (Appendix Fig. 104). The additive millimolar concentrations of monocarboxylic acid in the three cases (0,14, 0,16 and 0,17 mM, respectively) do not correlate with this trend, suggesting, as do the results with the ATCC 19859 strain, that on a molar basis sodium benzoate may be a more effective inhibitor than sorbic acid.

These results with the sorbic acid-sodium benzoate combinations also suggest no potential benefit from the treatment of mine dumps with an inhibitory combination rather than with an inhibitory concentration of either inhibitor on its own.

22. PILOT SCALE STUDY OF SODIUM LAURYL SULPHATE (SLS) AS POTENTIAL INHIBITOR OF COAL WASTE ACIDIFICATION: ANALYSIS OF EFFLUENTS FROM SLS-TREATED AND UNTREATED DUMPS

22.1. Introduction

The crucial test of an apparently effective inhibitor of acid mine drainage formation, selected on this basis of laboratory studies, is whether it is effective in preventing acidification of mine wastes in the field under the prevailing conditions of dump construction, rainfall and temperature. Field trials of treatments can be conducted on existing waste dumps or on pilot scale dumps specially constructed for the trials. The latter approach was followed in Phase II of our research, to permit comparison of a range of different inhibitor treatments with controls and to make possible the collection of all effluent water from the coal waste for chemical and microbiological analyses.

The inhibitor investigated in the first pilot scale study was SLS. At the time when our pilot scale coal waste dumps were planned and constructed in 1985, SLS had emerged from research in the U.S.A., including tests on a pilot scale and by the large-scale treatment of existing coal waste dumps, as the leading inhibitor of acid mine drainage. This research was conducted mainly by Kleinmann and his group (Erickson et al., 1985; Kleinmann, 1979, 1980; Kleinmann et al., 1981; Kleinmann and Erickson, 1982, 1983; Onysko et al., 1984a) at the Research Center of the Bureau of Mines, Pittsburgh, Pennsylvania. Of particular significance was their development of SLS-rubber formulations in slab and pellet form for the long-term (up to 5 years) slow release of SLS into coal waste. Their research is reviewed briefly in part 1 of this report (section 5.1). The research proved so successful that Kleinmann and Erickson (1983) could report that 50 mining companies were using anionic surfactants on coal refuse, coal stockpile areas, unreclaimed mine spoil and waste sulphide rock, with varied success. The use of anionic surfactant-containing slow release pellets in combination with bactericide application in a liquid spray has since been commercialized by the BF Goodrich Co., Akron, Ohio, under the trade name ProMac System for combatting acid mine drainage from overburden, refuse piles, mine tailings and coal storage piles during active mining operations, as well as from old coal refuse during and following reclamation by grading, covering with soil and establishment of a vegetation cover. Examples of successful acid mine drainage reduction by this means, for a 4-year period following reclamation of coal refuse piles in Ohio and West Virginia, have been reported by Rastogi et al. (1989).

In our first pilot scale study, various concentrations of two SLS-rubber formulations in pellet form, in combination with a fixed dose of SLS in solution, were tested as potential inhibitors of acidification of 55-t coal waste dumps. The slow release pellets were formulations of SLS-natural rubber and SLS-polyisoprene rubber, which had been developed and studied in respect of their SLS release characteristics by Sanderson and Immelman of the Institute for Polymer Science, University of Stellenbosch. Their work was part of the present research and is described in full in part 3 of this report, adapted from the M.Sc. thesis of Immelman (1987). The pilot scale study lasted for 3 years, with re-dosing of the dumps during the second year when it became obvious that acidification was not being inhibited by the initial SLS applications. From that time, too, the supplementary studies described in the following three sections (sections 23 to 25) were undertaken as attempts to explain the apparent lack of success of the SLS treatments in preventing acid generation in the dumps.

22.2. Materials and Methods

22.2.1. Construction of pilot scale coal waste dumps

The study involved 10 dumps (test piles) constructed on the Wolvekrans Section of the Douglas Colliery near Witbank during 11 to 15 November 1985. The dumps were 7,5 m in length and 4,5 m wide, and each contained approximately 55 t of coal waste. The dumps were constructed in shallow basins which had a down slope of 1:40 and were lined with plastic sheeting to channel drainage water into a large (approximately 100 l) rubber trash bin at the lower end of the slope (Fig. 40). The coal waste was layered and levelled with a front-end-loader, then compacted with a 10-t mechanical compactor.

22.2.2. Dump treatments

Dumps 1 to 6 were treated at the time of their construction with 1 kg SLS in solution plus 18-mm-diameter rubber pellets containing 35% (m/m) SLS for slow release. The pellets were supplied by BTR SARMCOL, Induna Mills, Howick, Natal, and their SLS release properties were studied by Immelman (1987; this report, part 3). Two types of rubber pellet were compared, namely, natural rubber pellets on dumps 1 to 3 and synthetic polyisoprene pellets on dumps 4 to 6. Dumps 1 and 4 each received 5 kg pellets, dumps 2 and 5 each 10 kg and dumps 3 and 6 each 15 kg. The pellets were spread evenly over dumps which had been compacted to a height of 1,05 m and were then covered with a 0,5-m layer of coal waste. To each of the six dumps was then added 1 kg of SLS in 20 l water, spread evenly over the surface. Test pile 7 was treated

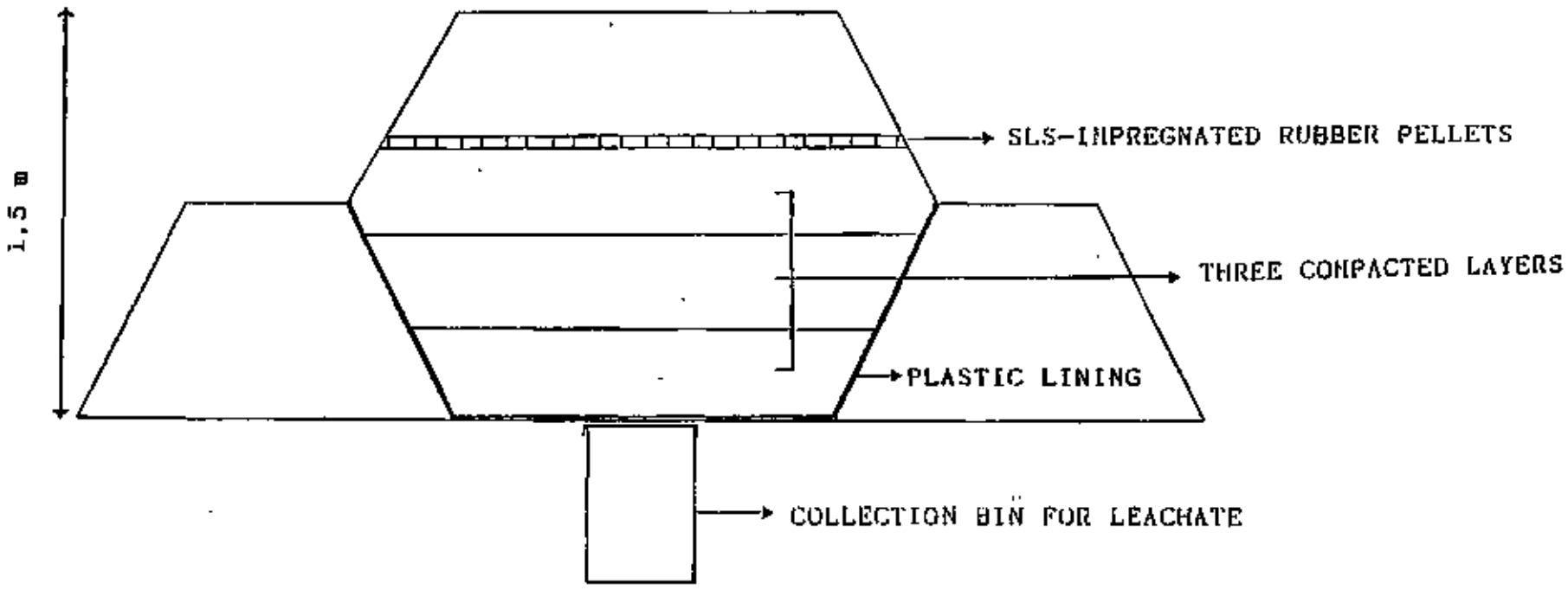


FIG. 40. Cross-sectional drawing of a test pile showing the compacted layers and the position of the rubber pellets containing 35% (m/m) SLS.

with only 1 kg SLS in 20 l water, while test pile 8 received no SLS treatment. Two further dumps (9 and 10) received no SLS treatment, but were inoculated with 200 l mine dump effluent as a source of *T. ferrooxidans*. All test piles were irrigated with 1200 to 1600 l water on 15 November 1985 and in addition, test pile 9 was irrigated fortnightly through rain-free periods with sufficient water to provide almost a collection bin full of drainage water.

When the results of a year of chemical and bacteriological monitoring of the effluent water (section 22.3) showed that the initial SLS treatments failed to prevent acidification of the test piles, the seven treated dumps were re-dosed with SLS on 3 February 1987 according to the schedule shown in Table 15. Dumps 1 to 3 again received natural rubber-SLS pellets and dumps 4 to 6 synthetic polyisoprene-SLS pellets, but at higher dose rates than initially. The pellets were applied just below the upper surfaces of the dumps. The additional 5 kg SLS in solution was applied to dumps 1 to 7 in the same way as the 1 kg applied initially.

22.2.3. Sampling and analyses

After irrigation of the dumps on 15 November 1985, the effluent from each dump was emptied from the rubber collection bin, measured and sampled in sterile, wide-mouthed, screw-capped bottles for MPN determinations of iron-oxidizing bacteria and chemical analyses. Subsequently, during the rainy season, the bins were emptied at approximately monthly intervals and the effluents measured and sampled. Midway between these monthly samplings, the irrigated test pile 9 was sampled further for chemical analyses and the collection bin emptied. The schedule of fortnightly sampling of this dump was continued through the dry seasons, when the dump was irrigated.

Rainfall was measured daily on the Wolvekrans mine about 2 km from the pilot scale dumps and the data supplied as totals for the approximately monthly periods during which effluent was collected in the rubber bins.

The water samples of 15 November 1985 were kept at the prevailing ambient temperature for 8 to 10 hours while being transported from Witbank to Stellenbosch by road and air. They were then stored for 2 days at 4°C before being used for MPN counts. Subsequent samples were transported from Witbank to Stellenbosch by air freight (South African Airways) and courier, exposed to the prevailing ambient temperature. The delay between sampling and the determinations of iron-oxidizing bacteria was 1 to 3 days. Longer delays were sometimes experienced with the chemical

TABLE 15. Dosages of SLS in solution and SLS-rubber pellets applied to test piles 1 to 10 in 1985 and 1987

Dump no.	SLS addition 1985		SLS addition 1987		Total SLS treatment 1985-87 (kg SLS/dump)
	Dissolved SLS (kg)	SLS-rubber pellets[a] (kg)	Dissolved SLS (kg)	SLS-rubber pellets[a] (kg)	
1	1	5	5	10	11,25
2	1	10	5	30	20,00
3	1	15	5	60	32,25
4	1	5	5	10	11,25
5	1	10	5	30	20,00
6	1	15	5	60	32,25
7	1	0	5	0	6,00
8	Treated only with mine dump effluent.				0,00
9	Treated only with mine dump effluent; irrigated fortnightly.				0,00
10	Completely untreated.				0,00

[a]SLS content 35 g/100 g pellets (test piles 1 to 3, natural rubber-SLS pellets; test piles 4 to 6, polyisoprene rubber-SLS pellets)

analyses, which were performed partly at Stellenbosch and partly at the Douglas Colliery near Witbank.

The pH of the effluents was measured as described previously in this report (part 1, section 3.2.1). Populations of iron-oxidizing bacteria were determined by the MPN procedure specified in section 17.2, using the appropriate MPN table of de Man (1983).

The SLS leached from the dumps in the effluents was determined by the colorimetric procedure described in part 1 of this report (section 12.2.2). For the determinations from 0 to 319 days, when the SLS concentrations tended to be low, 10-ml rather than 1-ml samples were analysed (maintaining the initial dilution to 100 ml).

Concentrations of total iron and sulphate in the effluent samples were determined in the Douglas Colliery Laboratory using atomic absorption spectroscopy.

22.3. Results

Rainfall recorded during the study period from the time of the construction of the dumps is shown in Fig. 41. The summer rains of late 1985 and early 1986 ceased by the beginning of May (193 days) and conditions were very dry until September (319 days). The period in the second rainy season from 25 November 1986 (375 days) to 1 April 1987 (502 days) was considerably wetter than the corresponding period during the first rainy season, and was succeeded by a third rainy season with heavy spring rains during September 1987 (661 to 717 days). However, the summer rainy season which started with these rains and continued until May, 1988 (902 days), was not as wet as the previous rainy season. The dry season of 1988 was shorter than any other dry season in the study period, consisting of only two dry months separated by a month with 29 mm of rain.

The pH measurements showed acidification in all dumps within 81 days (Fig.42). Test piles 1, 2 and 4 were acid from the beginning of the experiment. By 120 days, the pH of the efflent from all dumps was below pH 3,0. Thereafter it remained low (pH 1,7 to 3,2) with only slight fluctuations (Fig. 43, 44), even following retreatment of dumps 1 to 7 with high doses of SLS during February, 1987 (445 days), to the end of the study in November 1989 (1088 days).

Populations of iron-oxidizing bacteria in the effluents by MPN count increased during the first rainy season (Fig. 45) to ca. 10^7 to 10^9/ml (with the exception of test pile 4, where the iron-oxidizing bacteria decreased from ca. 10^8 initially to ca. 10^3). The

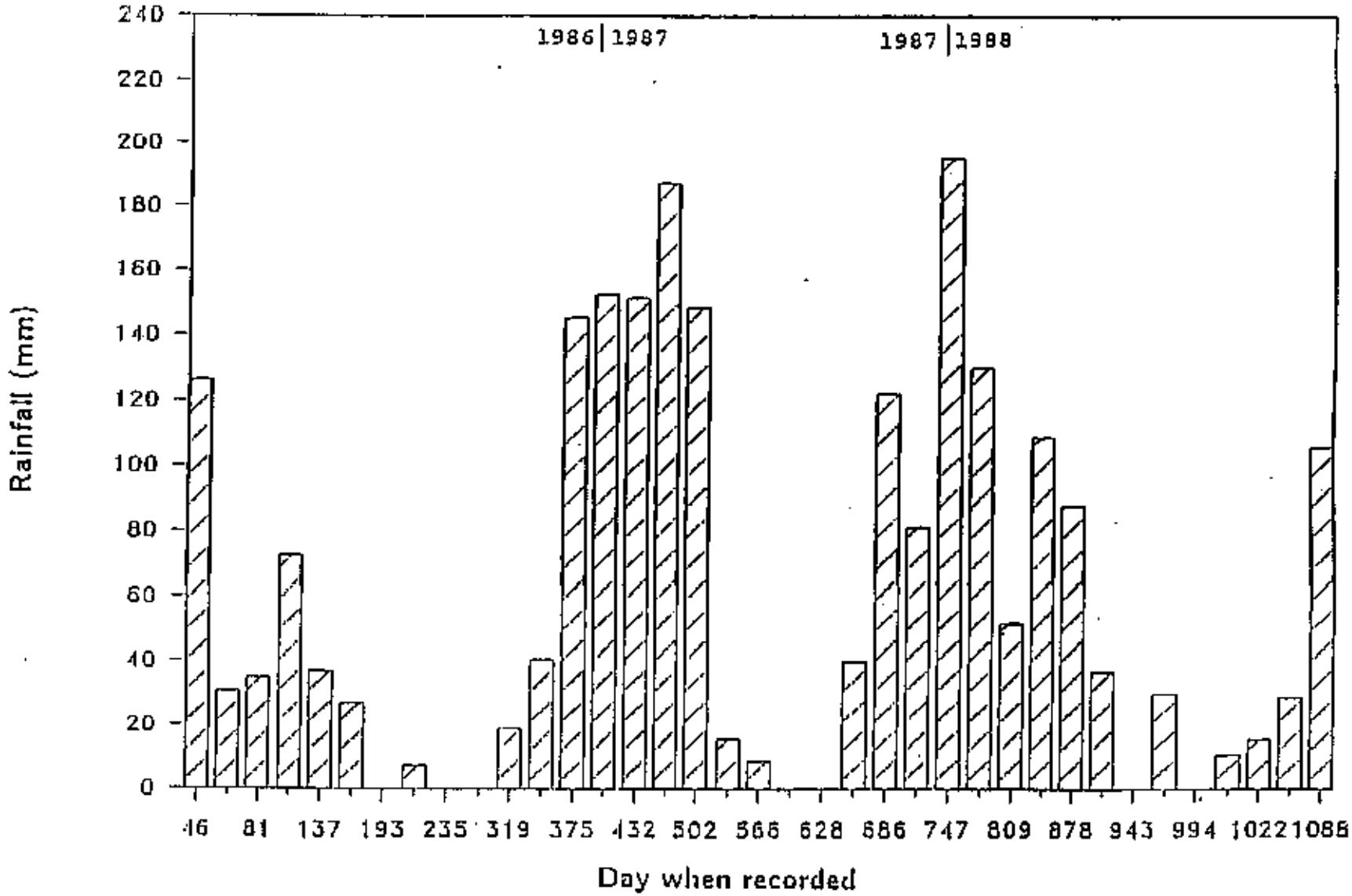


FIG. 41. Rainfall per month (approximately) at the Wolvekrans mine ca. 2 km from test piles from 15/11/85 (day 0) to 7/11/88 (day 1088).

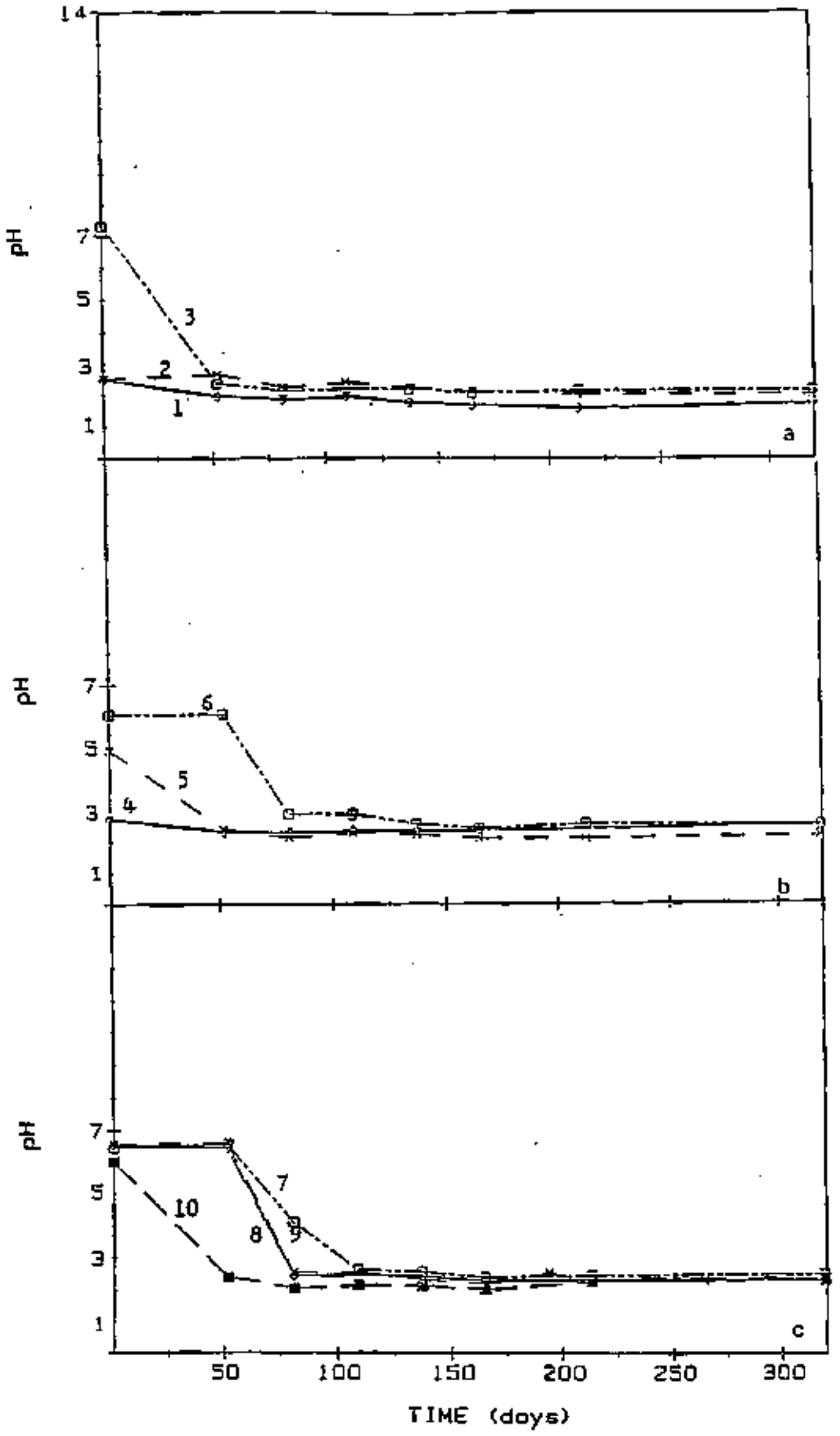


FIG. 42. Effluent pH of test piles (a) 1 to 3, (b) 4 to 6 and (c) 7 to 10 from 15/11/85 (day 0) to 30/9/86 (day 319). Numbers alongside plotted lines are dump numbers.

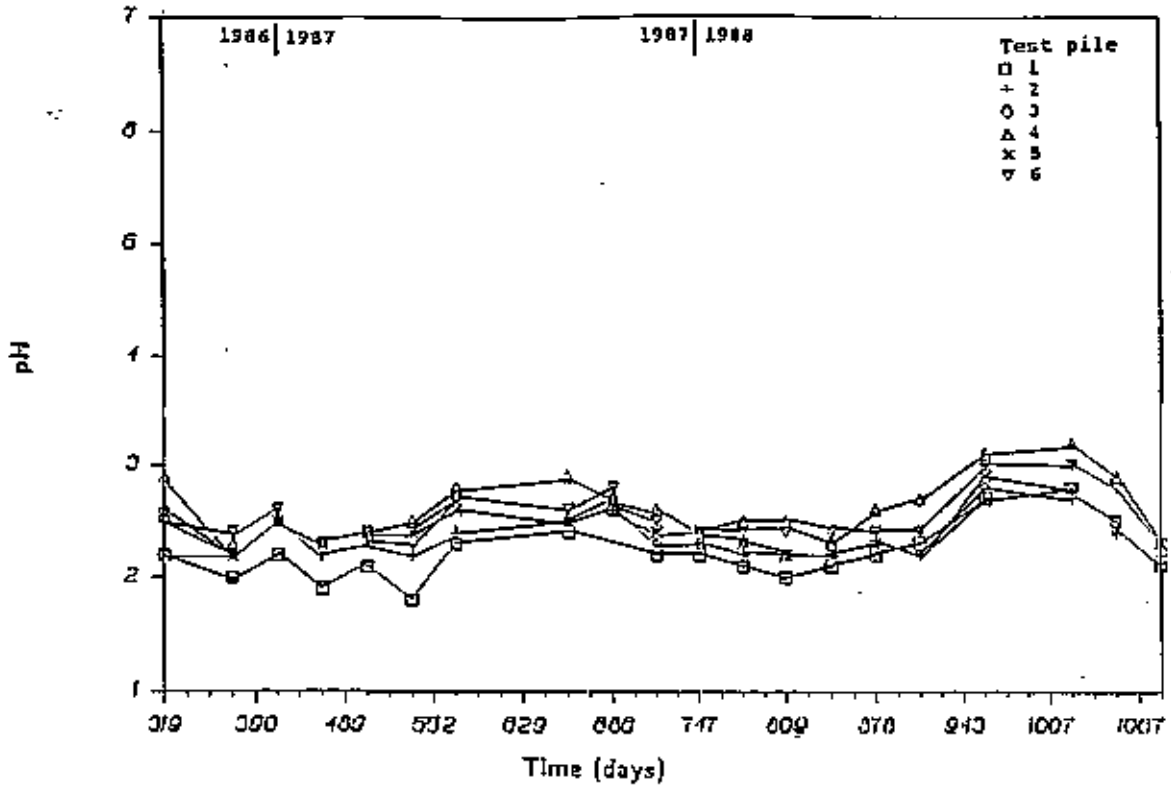


FIG. 43. Effluent pH of test piles 1 to 6 from 30/9/86 (day 319) to 7/11/88 (day 1088).

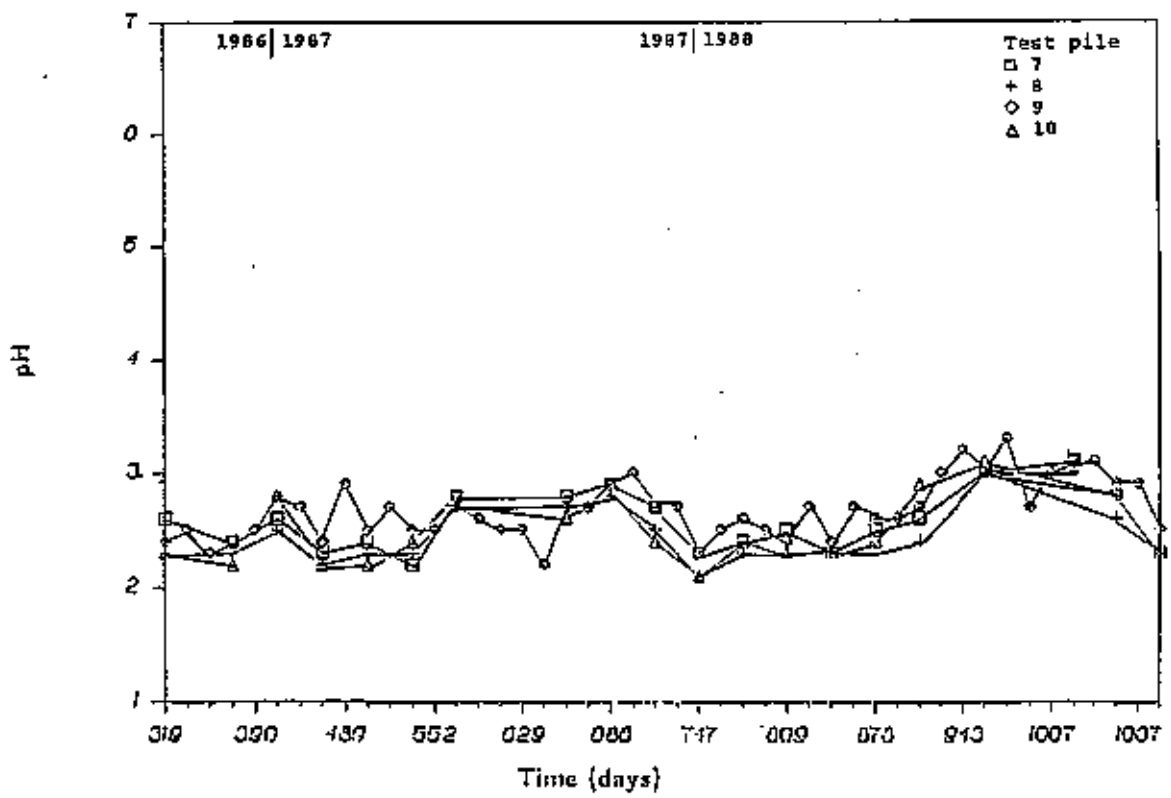


FIG. 44. Effluent pH of test piles 7 to 10 from 30/9/86 (day 319) to 7/11/88 (day 1088).

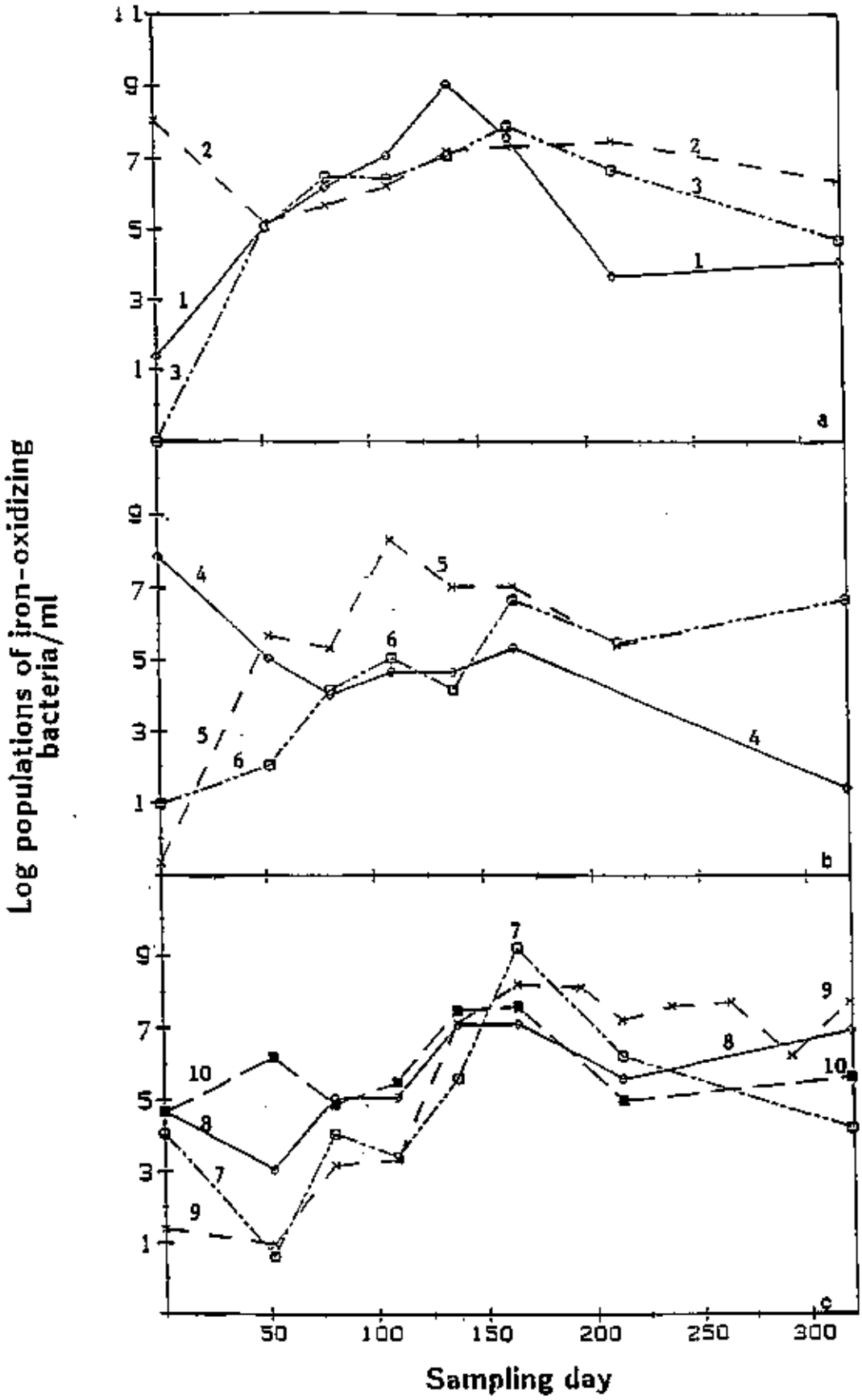


FIG. 45. Log populations (MPN) of iron-oxidizing bacteria in effluents from test piles (a) 1 to 3, (b) 4 to 6 and (c) 7 to 10 from 15/11/85 (day 0) to 30/9/86 (day 319). Numbers alongside plotted lines are dump numbers.

populations during the second and third rainy seasons fluctuated mainly in the range ca. 10^3 to 10^7/ml (Fig. 46, 47), to which they were declining by the end of the first rainy season. After the initial rains of the fourth rainy season (1007 day sample, August 1988) the populations were still high, in the range 10^4 to 10^7/ml. Three very pronounced but short-lived declines of the iron-oxidizing populations seemed randomly distributed among the test piles, and could not be related to prolonged dry conditions.

Sodium lauryl sulphate was leached from all treated dumps after the initial application (Fig. 48), but the concentrations of SLS in the effluents were low during the first rainy season, not exceeding 3,4 mg/l, from 0 to 213 days. In fact, all effluents showed a peak SLS concentration at 81 days of 2,5 to 3,4 mg/l for dumps 1 to 6 and 0,3 mg/l for dump 7. The other samples to 213 days contained less than 2,0 mg SLS/l. The very low concentrations of SLS leached from test pile 7 (0,0 to 0,3 mg/l), and the low cumulative amount leached by the end of the first rainy season (Fig. 48) indicate that 1 kg of SLS per dump was too low a dose to leave more than negligible amounts of SLS in solution following adsorption (see sections 25, 27) of most of the dose to the coal waste. The cumulative SLS leached from dumps 1 to 3 treated with the natural rubber pellets was higher than that from dumps 4 to 6 treated with corresponding concentrations of synthetic rubber pellets (Fig. 48).

The concentrations of SLS leached from the dumps from 319 days through the next two rainy seasons (Fig. 49), particularly after the additional dosing at 445 days, tended to be higher (mainly 5,0 to 30.0 mg/l), with fluctuations which may have been related to rainfall (Fig. 41). The high SLS concentrations were mainly associated with periods of low rainfall and the lower concentrations with periods of high rainfall. The effluents showed measurable SLS until 747 days, after which no SLS could be detected in any effluent. The cumulative SLS leached from the dumps by 747 days (Fig. 50) differed little among the various treatments, including dump 7 which received no pellets, indicating that the pellets possibly made little contribution to the SLS which leached from the dumps following their re-dosing.

The average daily iron release (calculated as the mean daily outflow since the previous sampling) from the test piles, even from the irrigated test pile 9, showed seasonal fluctuations, with the highest values during the warm, wet summer months (Fig. 51 to 54). Rates of iron release from most SLS-treated piles were higher during the third rainy season than during the first and second seasons, but test pile 7, which received the lowest SLS dose, and control piles 8 and 10 showed no obvious change in the release rates of iron from the second to the third season. More sulphate was released per day

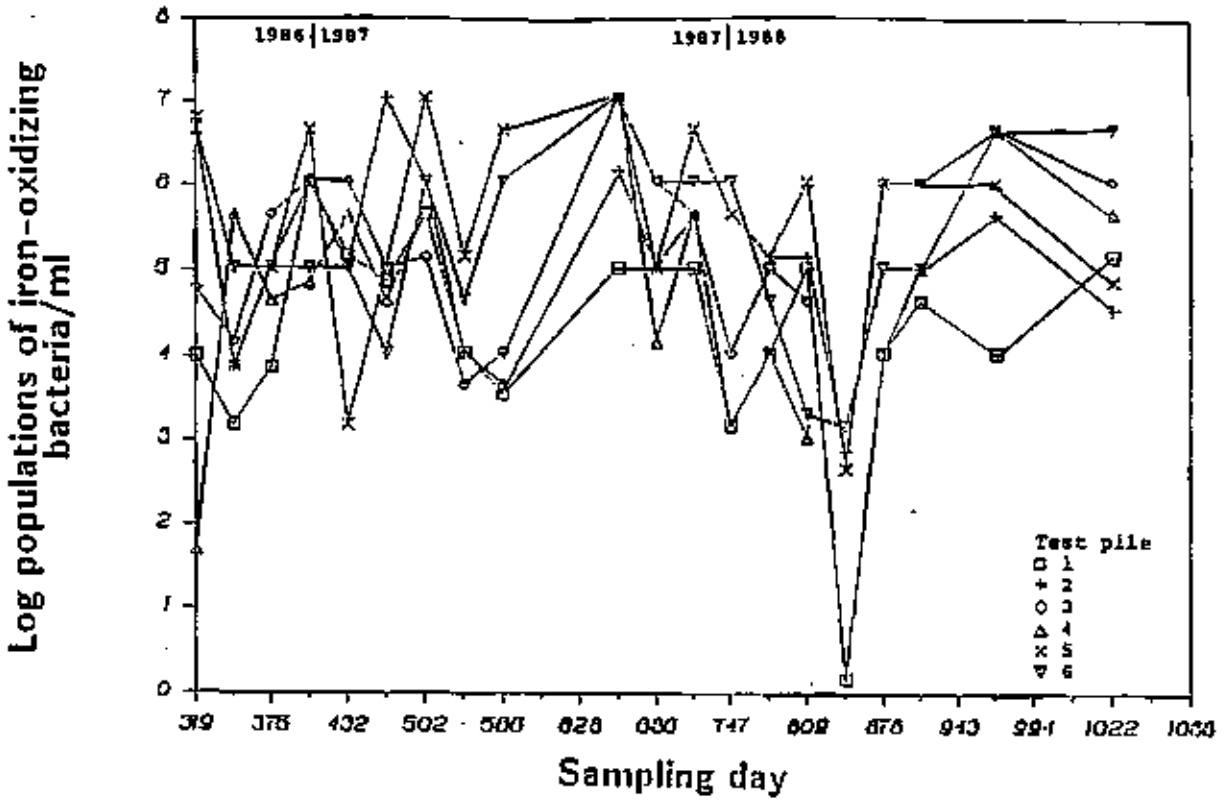


FIG. 46. Log populations (MPN) of iron-oxidizing bacteria in effluents from test piles 1 to 6 from 30/9/86 (day 319) to 7/11/88 (day 1088).

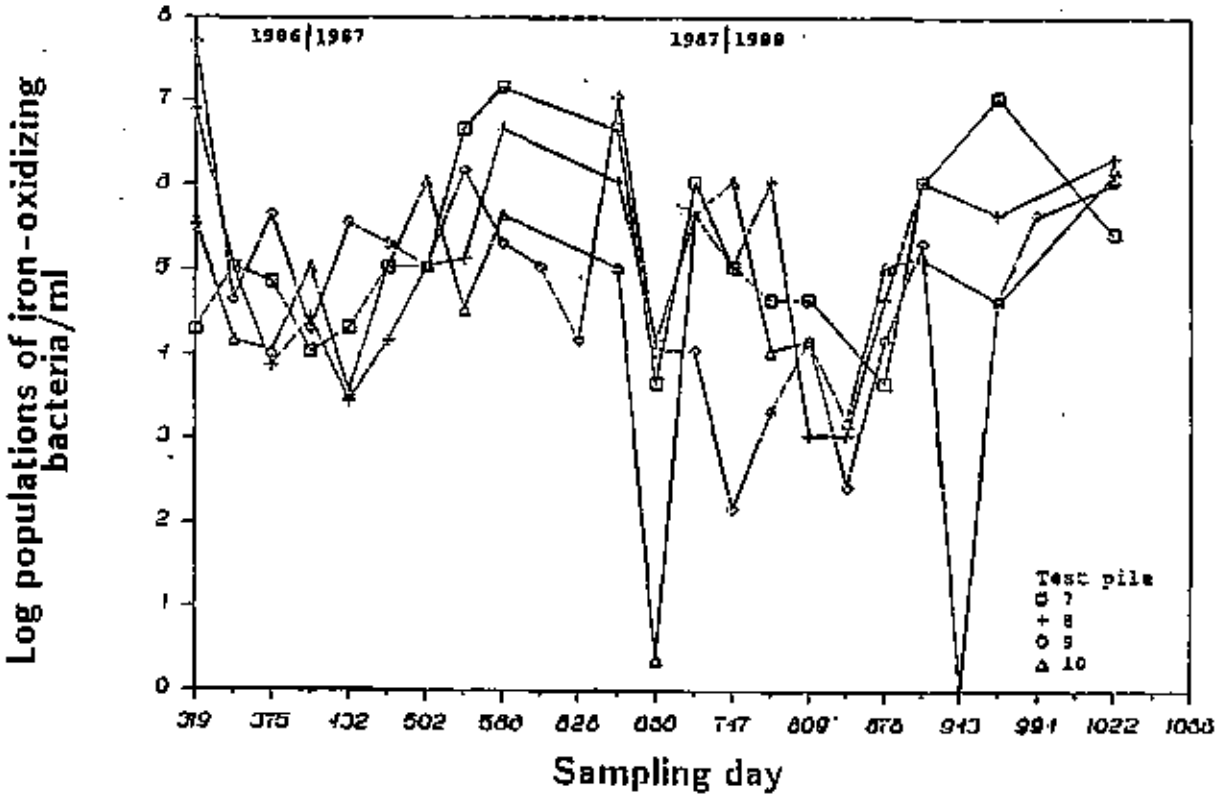


FIG. 47 Log populations (MPN) of iron-oxidizing bacteria in effluents from test piles 7 to 10 from 30/9/86 (day 319) to 7/11/88 (day 1088).

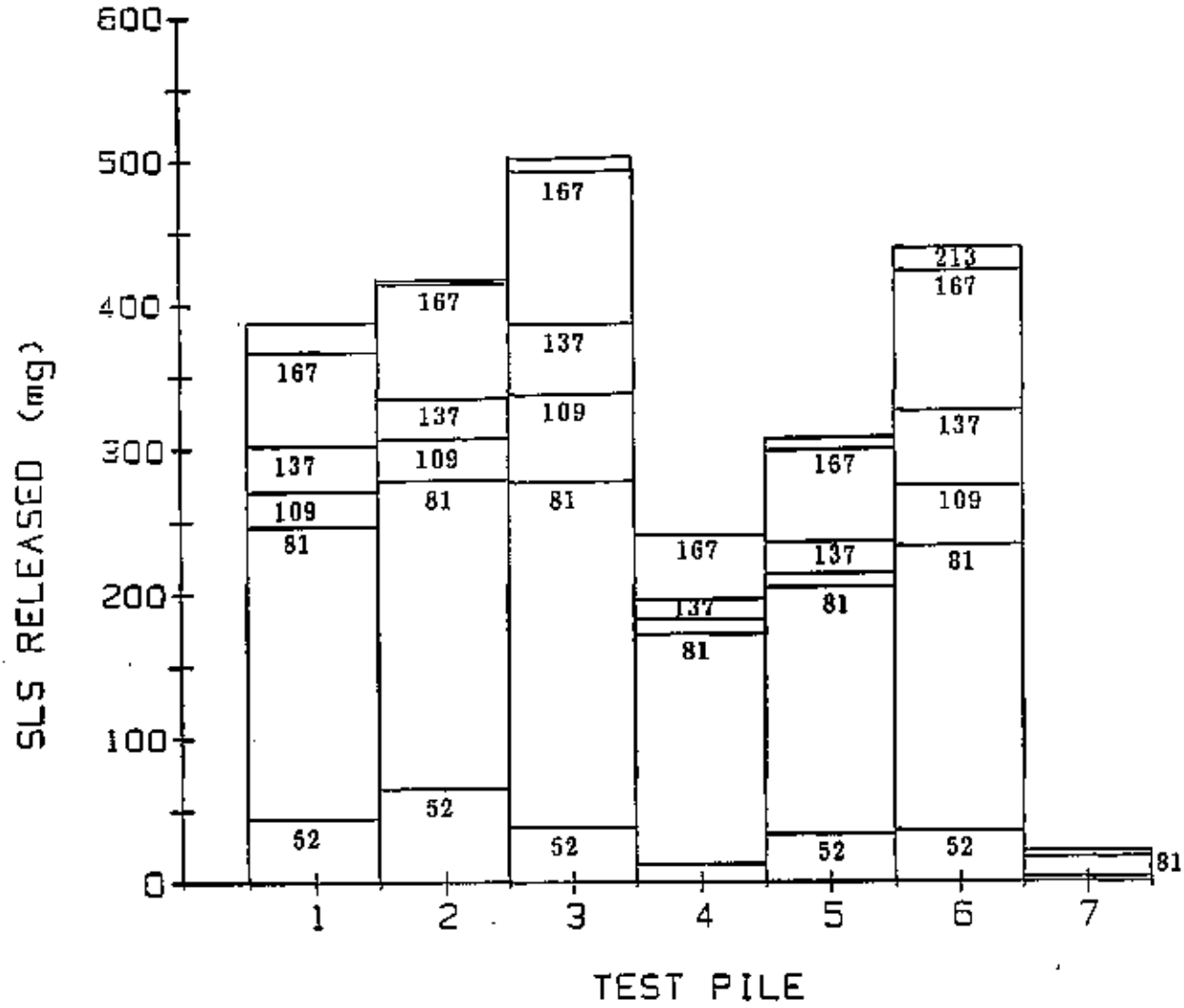


FIG. 48. Release of SLS at successive samplings (sections of columns, with sampling day indicated at top of each section) and in total from test piles 1 to 7 from 6/1/86 (day 52) to 16/6/86 (213 days).

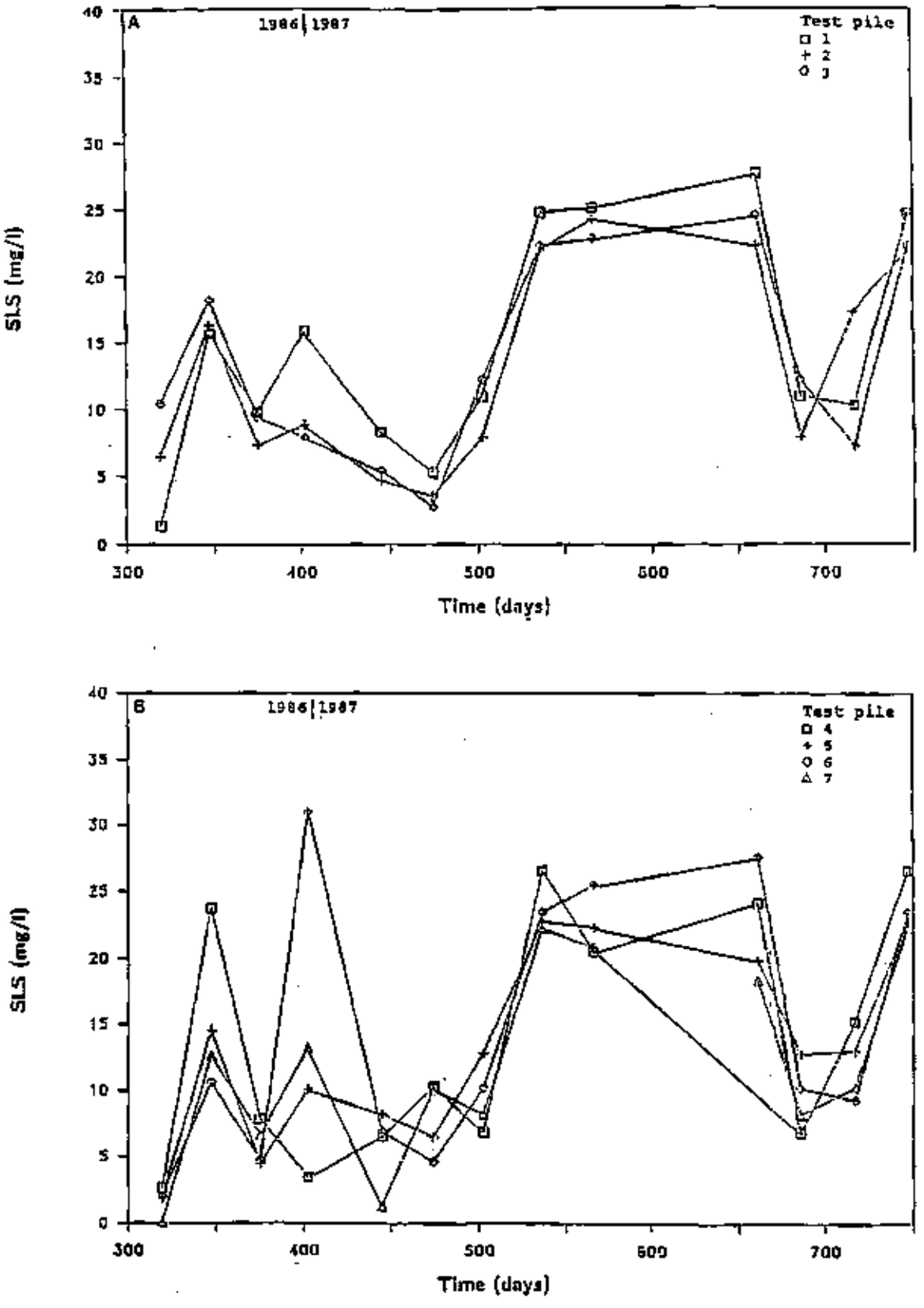


FIG. 49. Concentrations of SLS in effluents from test piles (A) 1 to 3 and (B) 4 to 7 from 30/9/86 (day 319) to 2/12/87 (747 days).

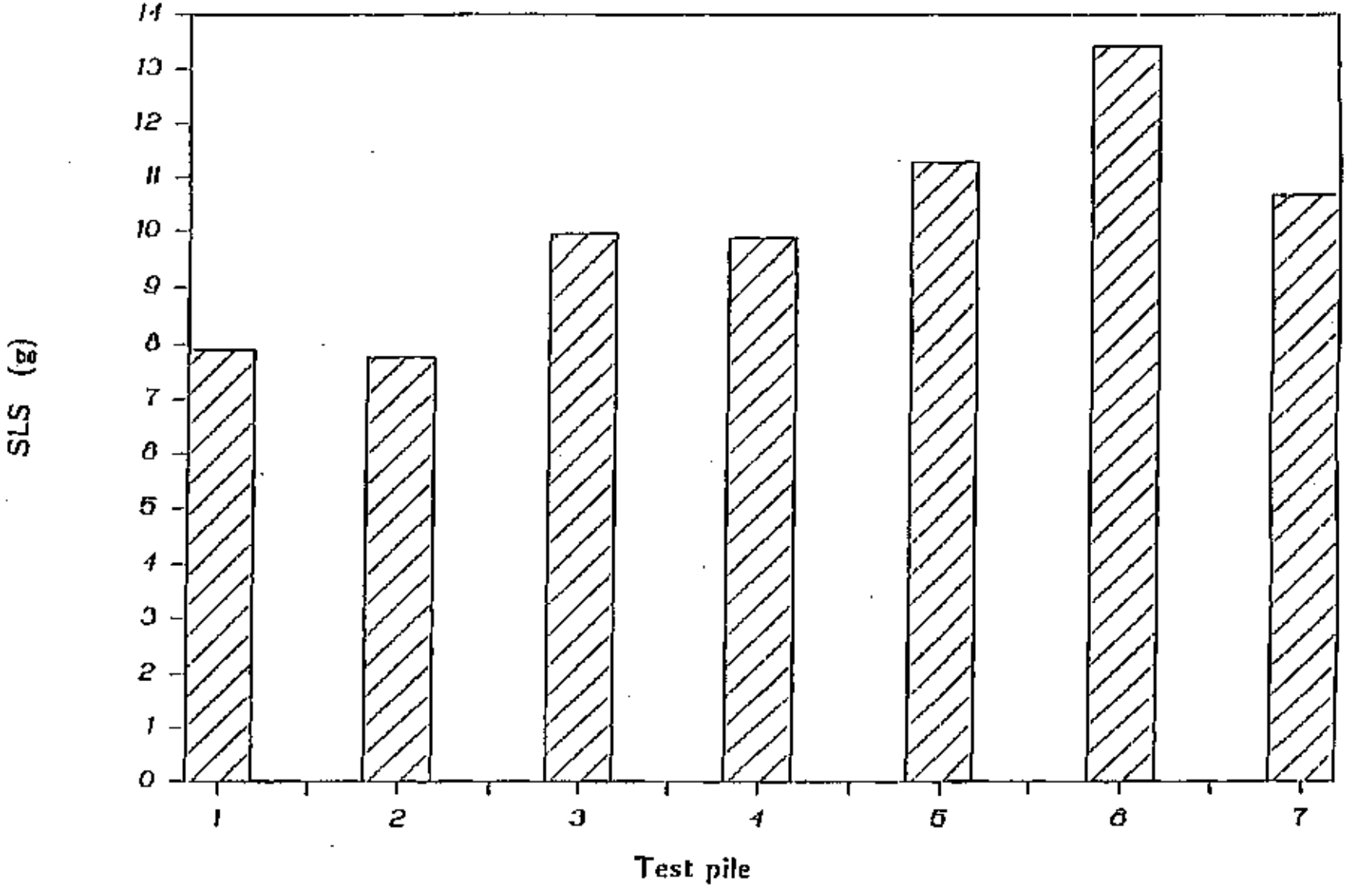


FIG. 50. Cumulative amounts of SLS leached from dumps 1 to 7 from 15/11/85 (day 0) to 2/12/87 (day 747).

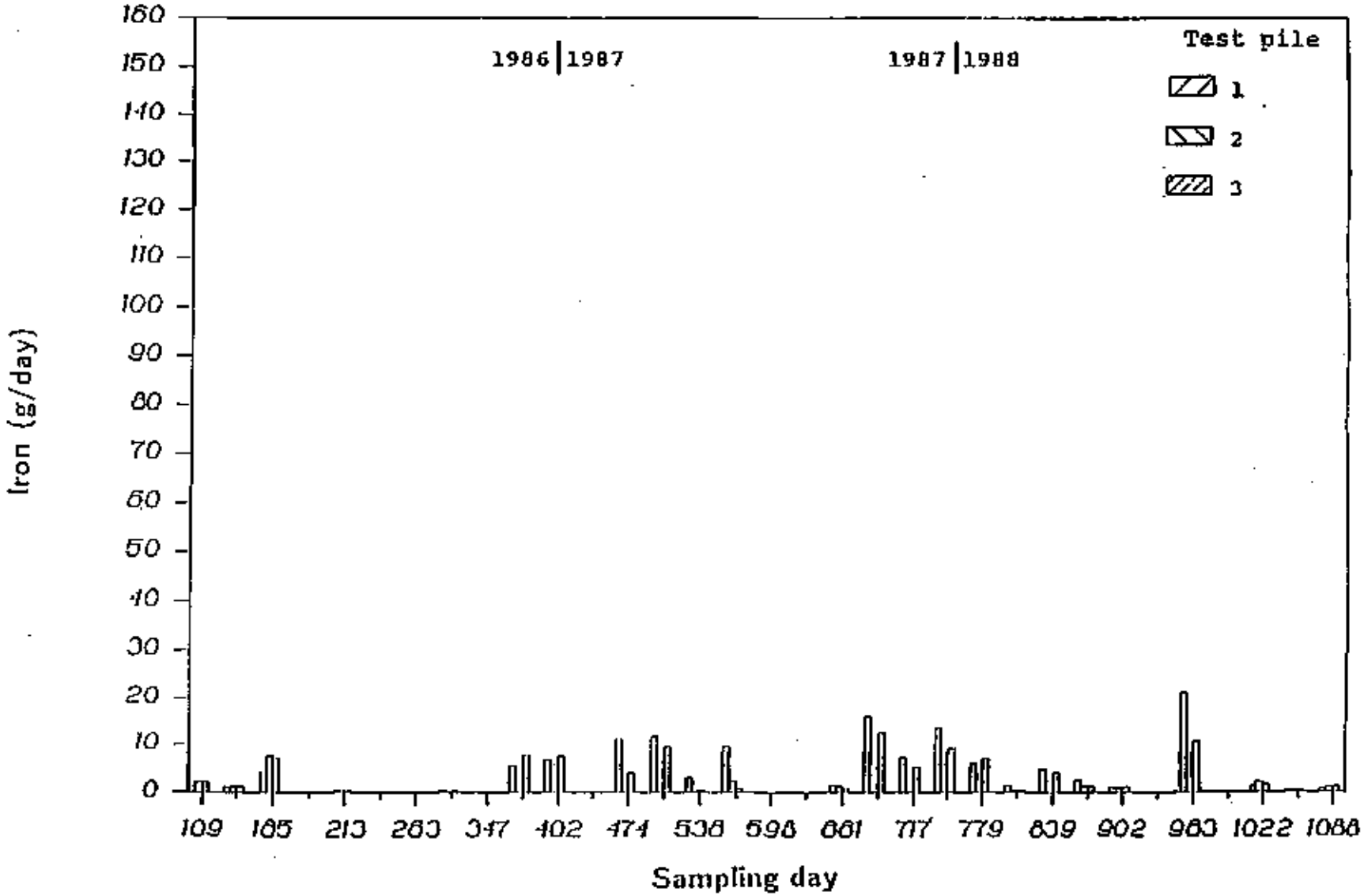


FIG. 51. Average daily iron outflow from test piles 1 to 3 during approximately monthly periods (preceding sampling days) from 15/11/85 (day 0) to 7/11/88 (day 1088).

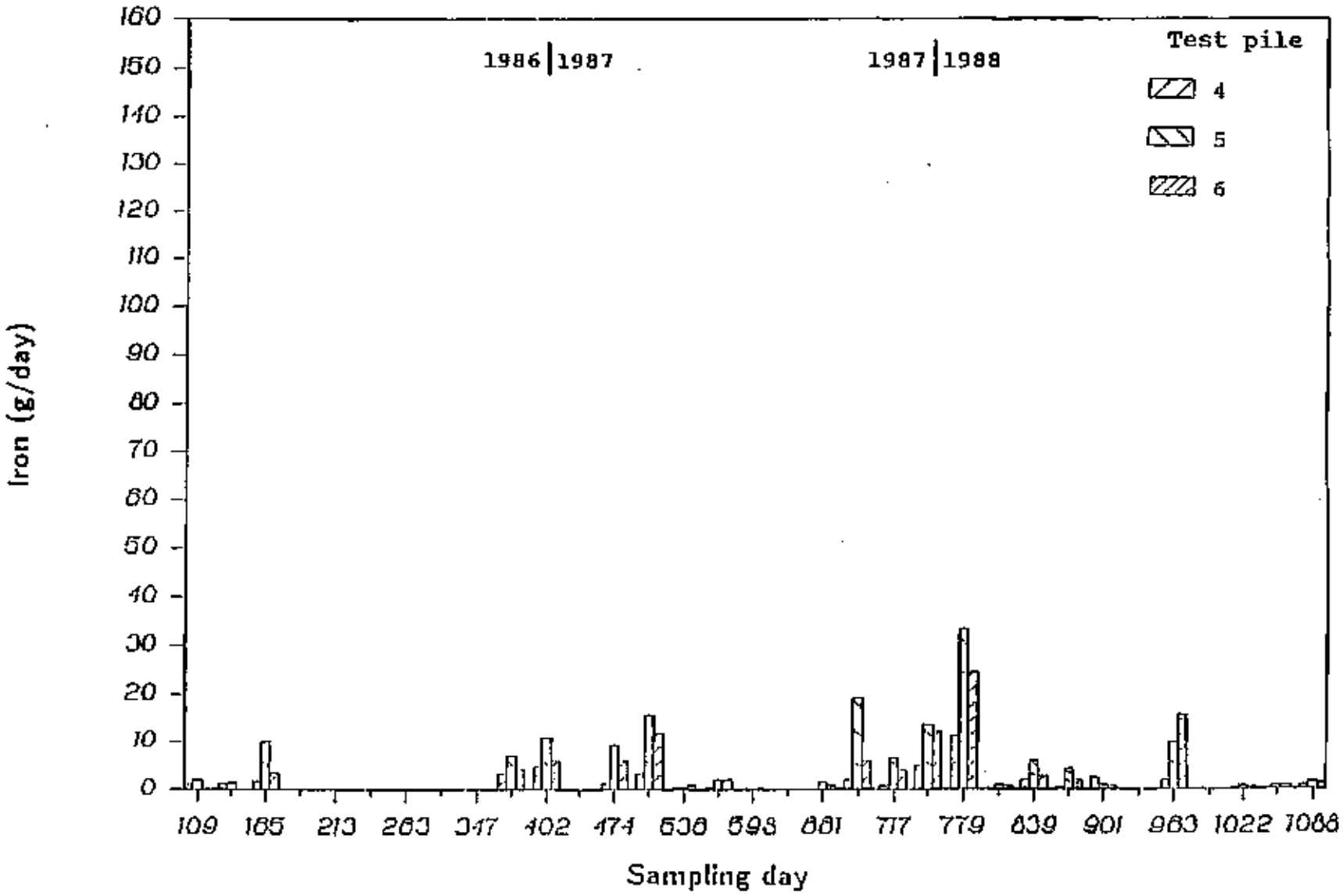


FIG. 52. Average daily iron outflow from test piles 4 to 6 during approximately monthly periods (preceding sampling days) from 15/11/85 (day 0) to 7/11/88 (day 1088).

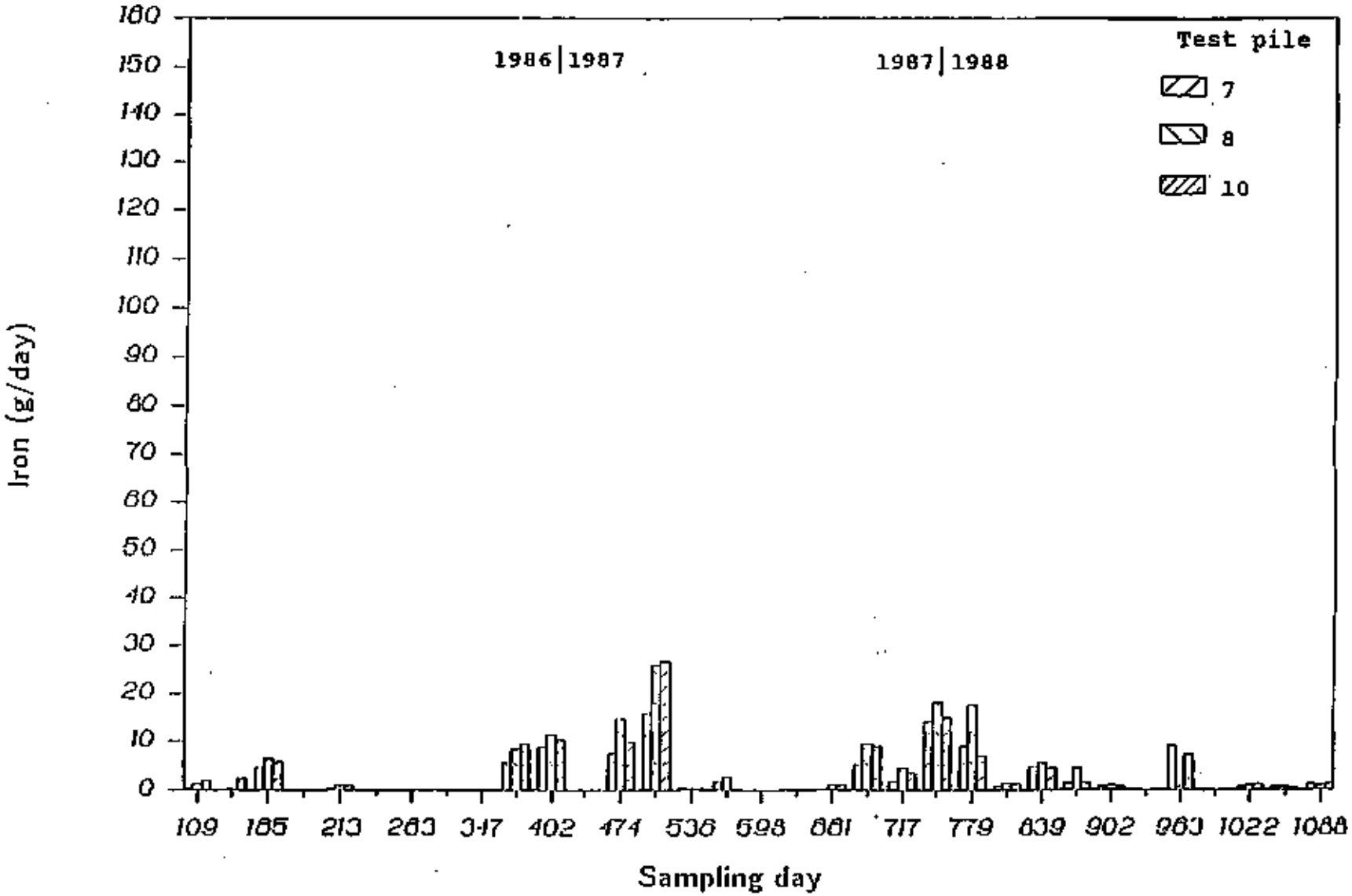


FIG. 53. Average daily iron outflow from test piles 7 to 10 during approximately monthly periods (preceding sampling days) from 15/11/85 (day 0) to 7/11/88 (day 1088).

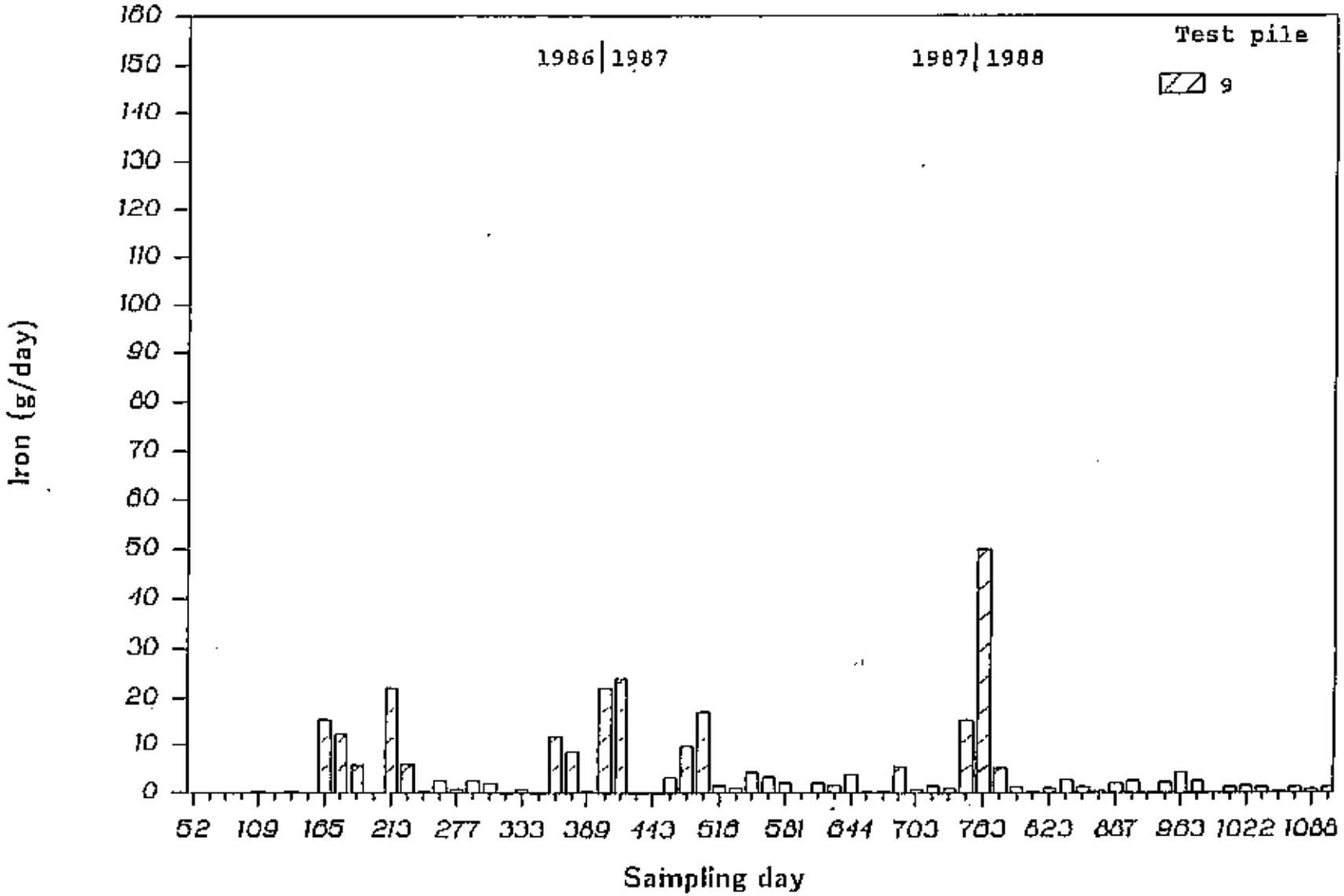


FIG. 54. Average daily iron outflow from test pile 9 during approximately fortnightly periods (preceding sampling days) from 15/11/85 (day 0) to 7/11/88 (day 1088).

than iron, but rates of sulphate release showed approximately the same patterns as the iron release rates, although with earlier peaks and declines (Fig. 55 to 58). The irrigated control test pile 9 also displayed seasonal fluctuations of sulphate release; however, the fluctuations were not as marked and well-defined as with the non-irrigated test piles.

Cumulative amounts of iron and sulphate leached from the test piles during the course of the experiment (to November 1988) are shown in Fig. 59. Control dump 9, which was irrigated regularly and thus not subjected to drying during the normal winter drought or drought periods in summer, released approximately the same amounts of iron and sulphate as the two other SLS-free control dumps (8 and 10), which were moistened only by the rainfall. These controls showed iron release within the range 3.8 to 4.2 kg and sulphate release within the range 19.0 kg to 25.0 kg per test pile. Among the SLS-treated piles, test piles 1 and 5 released about the same amounts of iron and sulphate as the controls, but the amounts of iron and sulphate released from the other SLS-treated piles were below those released from the controls. However, with the exception of test pile 4 the decreases were not marked. Test pile 4, in spite of the relatively low dose of SLS, consistently showed a much lower release of iron and sulphate than any of the other test piles. Even if this exception is ignored, there was no relationship between the amount of SLS added per pile and the amounts of iron and sulphate released. Any decrease in the production of soluble iron and sulphate in the dumps as a result of treatment with SLS was at best slight.

22.4. Discussion

Acidification seemed to be under way in the coal waste at the time of construction of the pilot scale dumps, as indicated by the acidity of the effluents of dumps 1, 2 and 4 (pH <3) at the 0-day sampling. In the other dumps, where the pH of the effluent water at the initial sampling was pH ≥6, acidification proceeded rapidly, so that the pH of all effluents had declined to pH <3 by 120 days. This pH monitoring provided the first indications that the initial SLS treatments were inadequate to prevent the bacterially catalysed oxidation of pyrite in the dumps. By the criterion of effluent pH, the subsequent SLS applications during February 1987 also seemed inadequate to prevent pyrite oxidation, as the pH of all effluents subsequently remained below pH ca. 3.

It was surprising how rapidly acidification occurred in dumps 3, 6, 7, 8, 9 and 10 in view of the high pH (pH ≥6) of the drainage water at the first one or two samplings. However, with the exception of test pile 3, all dumps showed detectable populations of iron-oxidizing bacteria in these effluents. *Thiobacillus ferrooxidans* has a maximum pH

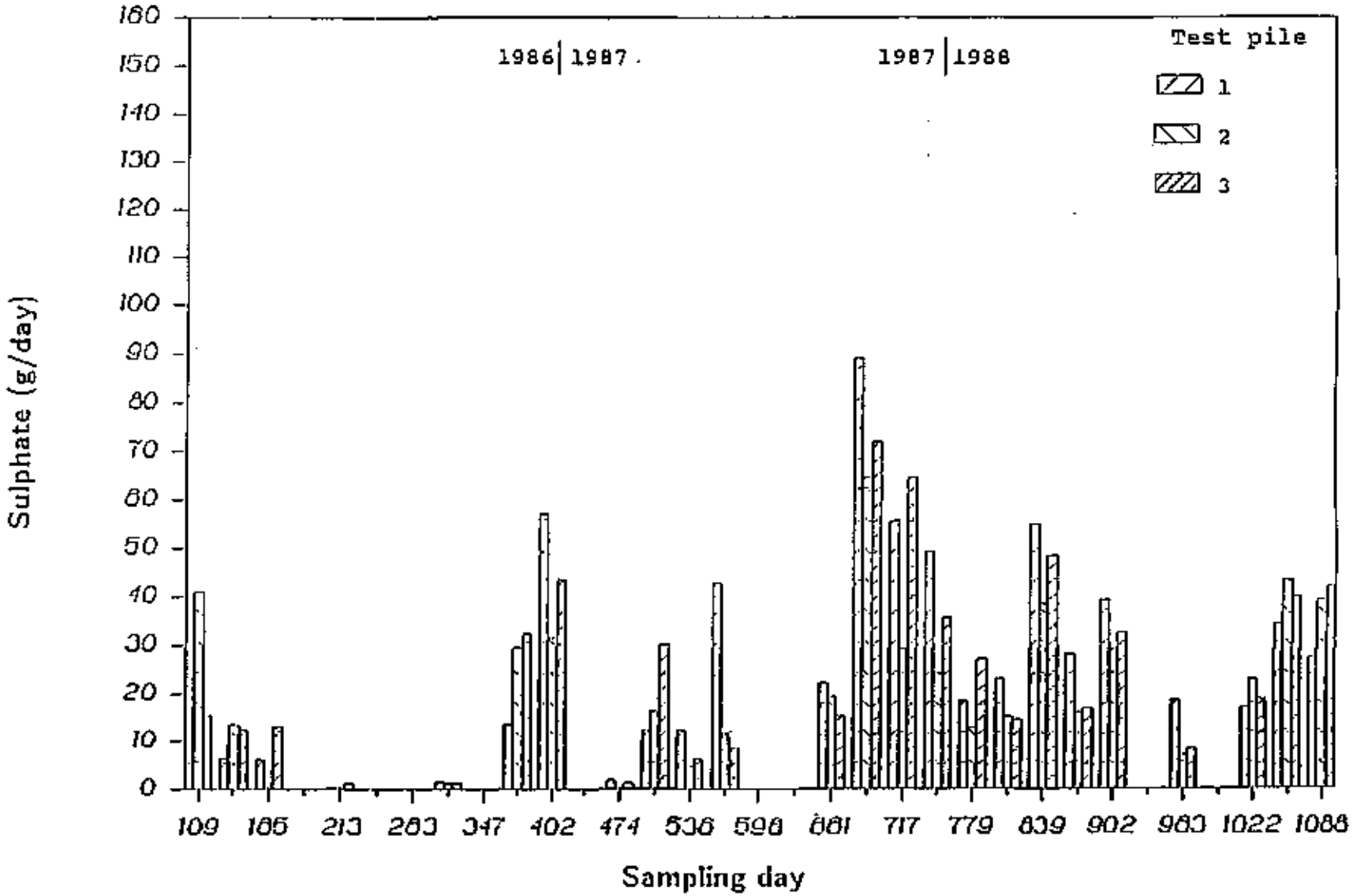


FIG. 55. Average daily sulphate outflow from test piles 1 to 3 during approximately monthly periods (preceding sampling days) from 15/11/85 (day 0) to 7/11/88 (day 1088).

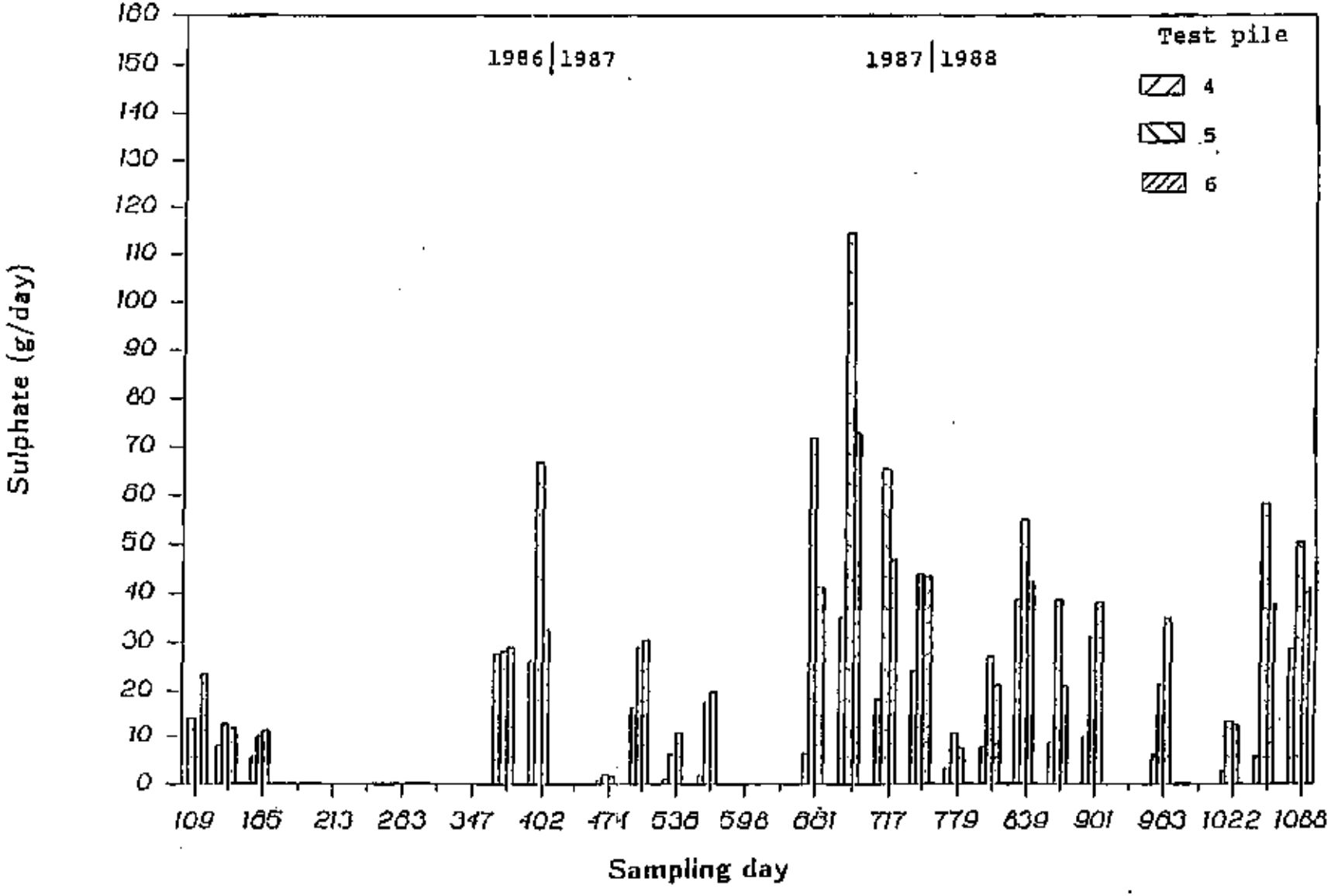


FIG. 56. Average daily sulphate outflow from test piles 4 to 6 during approximately monthly periods (preceding sampling days) from 15/11/85 (day 0) to 7/11/88 (day 1088).

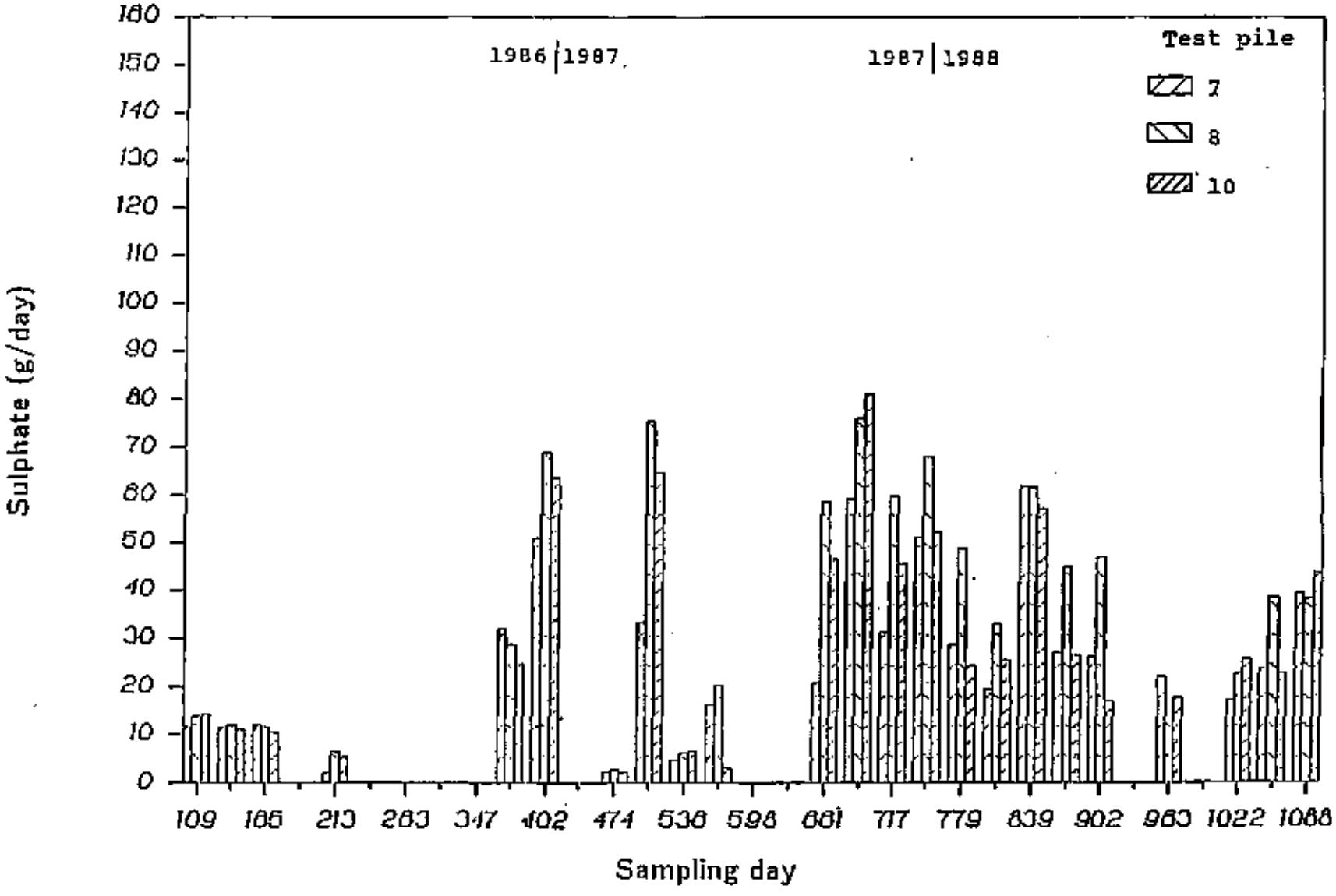


FIG. 57. Average daily sulphate outflow from test piles 7 to 10 during approximately monthly periods (preceding sampling days) from 15/11/85 (day 0) to 7/11/88 (day 1088).

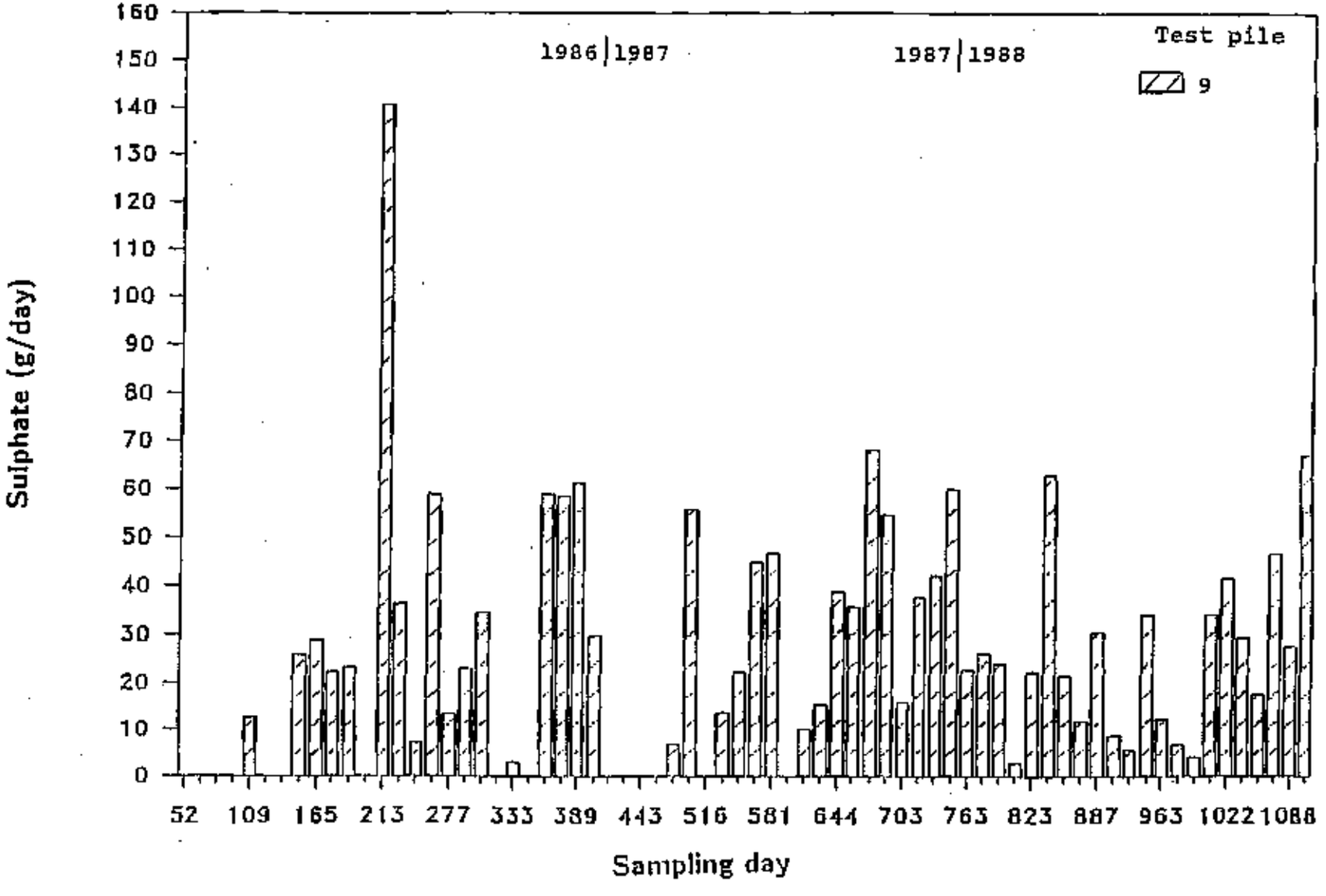


FIG. 58. Average daily sulphate outflow from test pile 9 during approximately fortnightly periods (preceding sampling days) from 15/11/85 (day 0) to 7/11/88 (day 1088).

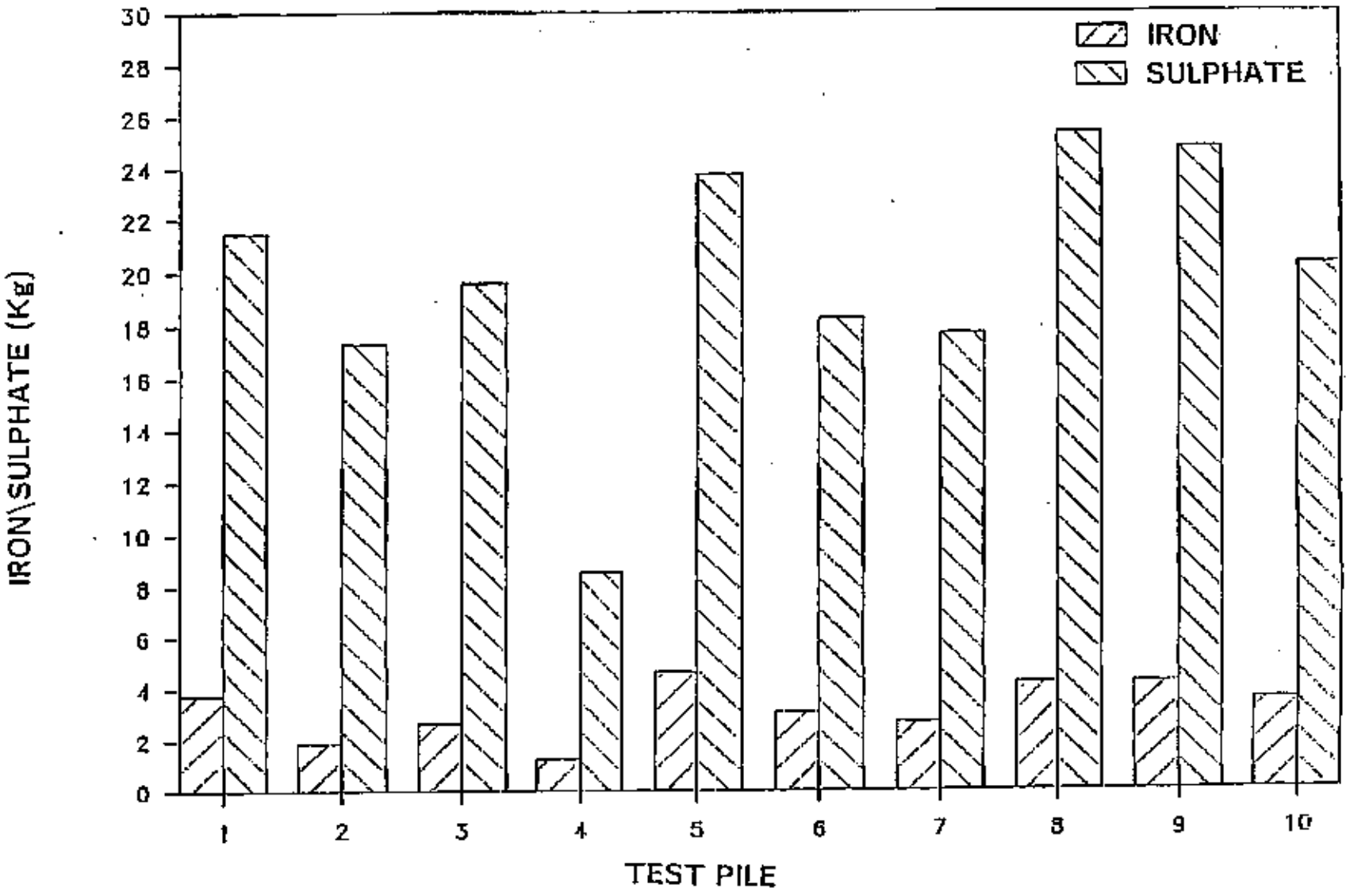


FIG. 59. Cumulative iron and sulphate leached from test piles 1 to 10 from 15/11/85 (day 0) to 7/11/88 (day 1088).

for growth of about pH 6 (Buchanan and Gibbons, 1974), but obviously in the complex environment of the higher pH dumps, iron-oxidizing bacteria were able to develop sufficiently rapidly to promote acidification, thus creating increasingly favourable conditions for their multiplication. They increased to very high populations of 10^7 to 10^9/ml effluent (except dump 4, where a decline was observed) during the warm, wet conditions of the first summer. Thereafter, following the 1986 winter, the populations were mainly in the range 10^3 to 10^7/ml, where they remained with few deviations until the end of the experiment. Probably stabilization of conditions in the dumps rather than the prolonged dry 1986 winter (Fig. 41) caused these trends, as the very high populations of the first summer were not found again in effluents during subsequent summers nor were population declines evident after subsequent winters.

It is not known whether the high MPN counts of iron-oxidizing bacteria in the effluent water from the test piles reflect the bacterial concentrations in the heaps or whether they resulted from growth of the bacteria on ferrous iron leached into the collection drums, which were emptied only once a month (except the drum of irrigated test pile 9 which was emptied fortnightly). However, whether the bacteria were growing in the heaps or drums, the SLS in the water draining from the test piles was insufficient to inhibit them.

All the test piles treated with SLS showed only low concentrations of leached SLS (< 3,4 mg/l) in the effluents during the first rainy season and mainly <30 mg/l thereafter. Kleinmann and Erickson (1983) determined the adsorptive capacity of coal for SLS to be about 45 mg SLS/kg coal. If SLS were adsorbed at this rate by the coal waste in the test heaps, a dosage of 2,47 kg SLS per heap would have just saturated the waste. The dose of SLS applied initially in solution (1 kg per heap) provided about 18 mg SLS/kg coal waste, thus could saturate an estimated 40% of the adsorptive capacity of the 55 t of coal waste. A portion of the SLS leached from the pellets applied initially to test heaps 1 to 6 would therefore also have been adsorbed by the coal; the calculated adsorbed amount of 1,47 kg per heap represents 84, 42 and 28% of the total SLS supplied in 5, 10 and 15 kg of pellets, respectively. However, if the adsorption rate were higher, for example, nearer the 200 mg/l reported by Onysko et al. (1984a), most of the SLS supplied in the pellets could be adsorbed. Also, the SLS from the pellets would have become available relatively slowly (Immelman, 1987; this report, part 3), and it is clear from the rapidity of acidification in the heaps, coupled with increases of iron-oxidizing bacterial populations in the effluents, that too little of the pellet SLS came into solution in any of the heaps, or it came into solution too slowly, for effective inhibition of the bacteria catalysing pyrite oxidation. The concentrations of < 3,4 mg SLS/l (usually less than 1 mg/l) in the effluents during the first rainy season after the initial SLS

applications probably reflect the concentrations of SLS in solution in the dumps and were obviously inadequate to inhibit the growth of the iron-oxidizing bacteria. A further possible problem was that with the construction of the dumps, the SLS-containing pellets were covered by coal waste to a depth of 0,5 m, which would have been treated only with the SLS in solution and not subsequently with SLS leaching from the pellets. Acidification could perhaps have occurred above the pellets even if it were blocked below that level by the concentrations of SLS supplied by the pellets.

During the second and third rainy seasons, particularly after the second applications of SLS, the SLS-treated test piles showed mainly higher but varying concentrations of SLS in the effluents until December 1987, after which no SLS could be detected in the samples (Fig. 49). The effluent concentrations of greater than 20 mg/l SLS far exceeded the 2,0 to 4,0 mg/l in solution which inhibited the development of pure cultures of *T. ferrooxidans* ATCC 19859 and WLR (this report, part 1, section 5). However, no inhibition of iron-oxidizing bacteria occurred. The reason could be a greater inherent and/or acquired resistance of the mixed populations in the effluent water towards SLS. A high level of resistance to SLS was displayed by enrichment cultures from the effluents (section 23), especially those from dumps treated with SLS, as well as *T. ferrooxidans* ATCC 19859 which was adapted to grow in the presence of 18,0 mg/l SLS. If *T. ferrooxidans* ATCC 19859 could grow at 18,0 mg/l after starting at 4,0 mg/l, the mixed cultures, which could grow at 14,0 mg/l SLS without training for resistance in the laboratory, could possibly be capable of growth at considerably higher concentrations. The resistance of the enrichment cultures showed trends coupled to the dosage applied. The highest minimum inhibitory concentration of SLS for the cultures was 14,0 mg/l, which is lower than many of the concentrations recorded in the effluents. Therefore, other as yet unknown factors in the effluents contributed to the resistance of the bacteria to the SLS.

The cumulative amounts of SLS leached from the test piles during the first wet season to 213 days (Fig. 48) show release of the highest quantities of SLS from the dumps treated with 15 kg pellets, followed by progressively lower amounts from the heaps treated with 10 and 5 kg of the corresponding pellets, then a much lower amount from test pile 7 which was treated only with the low dose of 1 kg SLS in solution. The SLS release rate from the natural rubber pellets was slightly higher than that from the polyisoprene rubber pellets, as could be expected from the work of Immelman (1987; this report, part 3). However, over the entire experiment, the cumulative amounts of SLS in the effluent water (Fig. 50) showed virtually no differences in the amounts from the different

treatments. Thus, the natural rubber-SLS pellets and polyisoprene-SLS pellets, with the possible exception of test pile 6, released no more SLS into their effluents than test pile 7, which received only the basal dose of SLS in solution. The SLS in the pellets thus remained therein or was adsorbed to the coal waste or was degraded. The last two mechanisms are suggested by the very low cumulative amounts (<14 g) of SLS which leached out of the heaps.

Although the populations of iron-oxidizing bacteria increased rapidly in most dumps with concomitant lowering of the pH during the first 120 days of the experiment, there was until about 150 days little release of iron to the effluents (Maré, 1989). Possibly there was considerable adsorbtion of iron on the coal waste or iron precipitation. Sulphate appeared earlier, in the first samples (109 days) in which it was analysed. Thereafter iron and sulphate were found regularly throughout the experiment but with their concentrations apparently varying with season. The seasonal variations can be related in the first instance (except in the case of the irrigated pile 9) to the wet summers and dry winters. The summer rains would have resulted in adequate moisture in the dumps for iron-oxidizing bacterial activity and chemical pyrite-oxidizing activity during this season (Fig. 51 to 58). Peaks of iron and sulphate release coincided with periods of high rainfall. The exceptional dip in iron and sulphate release shown by the 476-day samples, which accumulated in the dumps during a period of very high rainfall (188 mm), probably resulted from pronounced dilution of these materials and loss of effluent from the system, for example, by the collection drums overflowing or not collecting all effluent. Temperature also seemed to be an important ecological factor, as the release of iron and to a lesser extent sulphate from the irrigated pile 9 showed evidence of seasonality, with highest release rates during the summers. The rates of iron and sulphate release were higher in the SLS-treated piles during the third rainy season (1987-88) than earlier. This seems to indicate that a build-up of iron-oxidizing bacterial activity in the piles was taking place, coinciding with decreases of SLS in the effluent water to below detectable levels. Also the regular rains lasted longer than during previous wet seasons, allowing for more pyrite oxidation.

Besides being determined by climatic factors, the rates of production of acidity, soluble iron and sulphate may be related to permeability of the pyrite-containing material and the particle size of the pyrite. It was shown by Caruccio (1970) that small particles of pyrite (diameter 2 to 15 μm) rapidly decomposed when they were exposed to air and moisture and that coarse pyrite (diameter >50 um) remained stable. Such factors could have been important determinants of the rate of iron release from the coal waste test piles.

The cumulative iron and sulphate values of Fig. 59 show that the amounts released from the treated piles, with the exception of test pile 4, were similar to or slightly lower than the amounts released from the untreated piles. Differences in the cumulative amounts of iron and sulphate produced during the same sampling periods are approximately what is expected from the overall pyrite oxidation reaction, namely, 1 mol FeS_2 will yield 1 mol Fe^{2+}/Fe^{3+} and 2 mol SO_4^{2-}, i.e. iron and sulphate in a mass ratio of 56:192 = 1:3,4. No correlation with SLS treatment can be seen in the released amounts. The pattern of the slight decreases of iron and sulphate in the effluents was at such variance with the decreases expected for the different levels of treatment that it was not possible to ascribe the observed decreases to the SLS treatments. Rather these results appear to indicate that SLS did not inhibit acid formation in the test piles. The very low iron and sulphate values from test pile 4, with a low SLS treatment, indicate that non-homogeneity of the test piles was an important factor which could even have been responsible for all the observed differences in iron and sulphate production among the piles. Heterogeneity of the coal waste from one dump to another and within dumps is clearly shown by the analytical results in Appendix 4, but these data suggest no reason why the iron and sulphate release from test pile 4 should have been so much slower than the release from the other piles.

The pyrite sulphur content of the Wolvekrans coal waste was probably about 3% (J.D. Wells, Rand Mines (Mining and Services) Ltd., Johannesburg, personal communication), if not higher (Appendix 4). This pyrite would have provided each dump with approximately 165 kg of iron. No more than 5 kg iron was lost from any dump during the 3 years of the experiment. At this rate, only about 0,1% of the pyrite iron was being lost from the coal waste per year and the production of acid drainage water could possibly continue for 1000 years. This is a frightening prospect, emphasizing the necessity of finding effective means for the long-term inhibition of pyrite oxidation in waste deposits from coal mines.

23. PILOT SCALE STUDY OF SLS AS POTENTIAL INHIBITOR OF COAL WASTE ACIDIFICATION: RESISTANCE OF IRON-OXIDIZING BACTERIA IN TEST PILE EFFLUENTS AND INCREASE OF RESISTANCE OF *T. FERROOXIDANS* TO SLS

23.1. Introduction

Analysis of the effluents from test piles 1 to 7 in the pilot scale trial of SLS as a potential inhibitor of acidification of coal waste dumps (section 22), showed concentrations of SLS from 20 to 30 mg/l in many of the effluents (Fig. 49) after the re-dosing of the dumps with increased amounts of the SLS. In spite of these concentrations being about 10 times higher than the minimum inhibitory concentrations of SLS (2 to 4 mg/l) for *T. ferrooxidans* ATCC 19859 and WLR in HJJ medium (this report, part 1, section 5), high concentrations of chemolithotrophic iron-oxidizing bacteria were counted in the effluents (Fig. 45 to 47). One possible explanation could be that naturally occurring iron-oxidizing bacteria in the pilot scale dumps were considerably more resistant to SLS than the *T. ferrooxidans* ATCC 19859 and WLR strains. This possibility was suggested by the minimum inhibitory SLS concentration of 6 to 8 mg/l for iron-oxidizing bacteria cultured from acid seepage from gold mine sand dumps (this report, part 1, section 5.3). Another possible reason might be that on continued exposure to non-lethal levels of SLS, populations of iron-oxidizing bacteria might develop increased resistance to the inhibitor by mutation and/or selection (Franklin and Snow, 1981). We investigated both of these possibilities by (i) producing mixed enrichment cultures of iron-oxidizing bacteria from the effluents of the test heaps and determining for each the minimum SLS concentration, and (ii) culturing *T. ferrooxidans* ATCC 19859 and WLR in HJJ medium in the presence of progressively increasing concentrations of SLS to determine the highest concentrations to which they could become resistant.

23.2. Materials and Methods

23.2.1. Resistance of iron-oxidizing cultures from test pile effluent water

Water (10 ml) from test pile effluents was inoculated into 100 ml HJJ medium in 250-ml Erlenmeyer flasks. The cultures were incubated on a rotary shaker (120 r.p.m.) at 26°C until growth occurred. Subcultures were inoculated and grown in a similar way for 72 hours for use as inoculum for resistance tests. A 10% (v/v) inoculum and SLS at 2,0 to 14,0 mg/l were added to 250-ml Erlenmeyer flasks containing 100 ml HJJ medium. These cultures were incubated on a rotary shaker (120 r.p.m.) at 26°C and growth

monitored as ferrous iron metabolism as described previously (this report, part 1, section 2). This procedure was repeated with successive effluent samples from individual test piles.

23.2.2. Resistance of T. ferrooxidans ATCC 19859 and WLR grown in the presence of SLS

Stationary 1-week-old cultures of *T. ferrooxidans* ATCC 19859 and WLR grown at 26°C in 100 ml HJJ medium in 250-ml Erlenmeyer flasks were subcultured (10% v/v inoculum) in the same way in HJJ medium supplemented with 1,0 mg/l SLS. The cultures were incubated at 26°C until growth occurred. Further successive subcultures were made, each time increasing the concentration of inhibitor by 0,5 mg/l. A second series of subcultures, prepared in the same way, was incubated at 26°C on a rotary shaker (120 r.p.m.) .

23.3. Results

23.3.1. Resistance of iron-oxidizing cultures from test pile effluent water

Cultures of iron-oxidizing bacteria from the first three samplings seemed to show a difference between the SLS concentrations needed to inhibit the cultures from treated and untreated dumps (Table 16). Almost all cultures obtained during those samplings from test piles 2, 3, 5 and 6, which received the highest doses of SLS, were not inhibited by 6.0 mg/l SLS, whereas most cultures from the SLS-free control pile effluents (test piles 8, 9 and 10) were inhibited by 2,0 or 4,0 mg/l SLS. The cultures from the effluents of test piles 1 and 4, which received low doses of SLS, were inhibited by 6,0 mg/l or higher concentrations of SLS. However, several cultures from later samples (11/4/88 to 7/7/88) from control piles 8 to 10 showed minimum inhibitory SLS concentrations of 8,0 to 10,0 mg/l, i.e. in the range required for the inhibition of cultures from effluent from SLS-treated piles.

23.3.2. Resistance of *T. ferrooxidans* ATCC 19859 and WLR grown in the presence of SLS

By successive subculturing in HJJ medium containing increasing SLS concentrations (increments of 0,5 mg/l/subculture), *T. ferrooxidans* ATCC 19859 was adapted to grow in the presence of 18,0 (stationary cultures) and 18,5 (shaken cultures) mg/l SLS. The corresponding maximum SLS concentrations at which *T. ferrooxidans* WLR eventually

TABLE 16. Minimum inhibitory concentrations of SLS for iron-oxidizing bacteria in test pile effluents

Sampling date	Minimum inhibitory SLS concentration for effluent enrichment culture from test pile											
	1	2	3	4	5	6	7	8	9	10	Mean	Range
7/9/87	6	10	10	6	10	10	4	4	2	2	6,4	2-10
20/1/88	8	8	10	8	10	12	6	6	4	4	7,6	4-12
2/2/88	6	6	8	10	8	12	4	4	4	4	6,6	4-12
3/3/88	6	6	8	6	8	14	6	6	6	6	7,2	6-14
11/4/88	8	8	10	8	8	10	8	6	8	8	8,2	8-10
5/5/88	8	8	8	4	8	8	6	8	8	8	7,4	4-8
7/7/88	6	6	10	10	10	10	8	10	6	6	8,2	6-10
Mean	6,9	7,4	9,1	7,4	8,9	10,9	6,0	6,3	5,4	5,4		
Range	6-8	6-10	8-10	4-10	8-10	8-14	4-8	4-10	2-8	2-8		

grew, were 8,5 and 8,0 mg/l. Concentrations of SLS which inhibited these cultures initially (this report, part 1, section 5) were 4,0 (ATCC 19859, shaken) and 2,0 (WLR, shaken) mg/l.

23.4. Discussion

23.4.1. Resistance of iron-oxidizing cultures from test pile effluent water

The data in Table 16 show considerable resistance of iron-oxidizing bacteria in certain effluent samples from both treated and untreated test piles, to inhibition by SLS. High minimum inhibitory concentrations recorded for some cultures from treated piles may be the result of exposure to SLS in the test piles; however, there is no clear consistent pattern of greater SLS resistance among cultures from treated piles than among those from untreated piles. The highest SLS concentrations (12,0 to 14,0 mg/l) required to inhibit the mixed cultures of iron-oxidizing bacteria from the test piles were three to seven times as high as those required to inhibit pure cultures of *T. ferrooxidans* ATCC 19859 and WLR (this report, part 1, section 5), explaining resistance to and the possibility of growth in the presence of some of the SLS concentrations measured in the test pile effluents.

23.4.2. Resistance of *T. ferrooxidans* ATCC 19859 and WLR grown in the presence of SLS

Thiobacillus ferrooxidans ATCC 19859 developed the ability to grow in the presence of 18,5 mg SLS/l, which is 4,5 times higher than the initial minimum inhibitory SLS concentration (this report, part 1, section 5). The resistance of *T. ferrooxidans* WLR increased by a similar factor. Increases in the resistance of iron-oxidizing bacteria exposed to SLS in coal dumps can therefore be expected. Possibly, if they started with a requirement of 8 to 10 mg/l SLS for inhibition (section 23.3.1), these iron-oxidizing bacteria could develop resistance to the highest levels (ca. 25 to 30 mg/l) of SLS detected in the effluents. Even if they could not grow at these high concentrations, they might grow in the dumps in the presence of the protective coal waste (section 20) and be washed in high numbers into the effluent collection bins, remaining dormant, but alive, until counted.

24. PILOT SCALE STUDY OF SLS AS POTENTIAL INHIBITOR OF COAL WASTE ACIDIFICATION: DEGRADATION OF SLS BY HETEROTROPHIC MICROORGANISMS FROM TEST PILE EFFLUENT WATER

24.1. Introduction

Long-term control of acidification in coal waste dumps by treatment with SLS will depend not only on the dose applied to the dump and movement of the inhibitor through the coal waste to where the iron-oxidizing bacteria are catalyzing acid production, but also on the longevity of the SLS in the coal waste. As a widely used detergent, SLS is biodegradable, for example, by bacteria such as those studied by White et al. (1985) in an English river. Various heterotrophic microorganisms have been found, as apparent inhabitants, in acid water draining from coal waste dumps or in the wastes themselves; they include bacteria, yeasts and filamentous fungi (Belly and Brock, 1974; Colmer and Hinkle, 1947; Dugan, 1975; Dugan et al., 1970; Harrison, 1978; Lundgren et al., 1972; Millar, 1973; Tuttle et al., 1968, 1969; Wichlacz and Unz, 1981), some of which could possibly degrade SLS applied to the wastes. We investigated this possibility by isolating heterotrophic microorganisms (yeasts and filamentous fungi) from effluents from the test piles at the Wolvekrans mine and examining the abilities of the yeasts to grow in the presence of SLS and to use SLS as sole carbon source for growth. The degradation of SLS in liquid medium by six yeast isolates was determined quantitatively.

24.2. Materials and Methods

24.2.1. Isolation of acid-tolerant heterotrophs resistant to and able to utilize SLS

Inoculum (0,1 ml) from test pile effluent samples was spread on plates of medium M (White et al., 1985) of pH 4,5 to 5,0. The plates were incubated at 26°C until visible colonies (filamentous fungi and yeasts) appeared. The filamentous fungi were identified to form genus using the keys of von Arx (1974). The most abundant yeast colony type present on each plate was purified by picking single colonies from successive streak cultures on plates of medium M. Pure yeast cultures were streaked, using single colonies picked with a sterile loop, onto medium M plates containing 0,5 and 1,0 mM SLS. The plates were incubated at 26°C for 2 weeks and scored for the appearance of visible colonies. The ability of the yeast isolates to utilize SLS as sole carbon source was assessed using the carbon source assimilation technique of van der Walt and Yarrow (1984) with 0,5 mM SLS in the test medium. Physiological characteristics, namely, utilization of carbon and nitrogen sources for growth, growth at 25, 32 and 37°C and

formation of starch-like extracellular polysacchrides were assessed according to techniques described by van der Walt and Yarrow (1984). The yeasts were identified tentatively from the genus descriptions in Kreger-van Rij (1984).

24.2.2. Degradation of SLS by acid-tolerant yeast isolates

Selected yeast cultures able to grow well on medium M containing 0,5 or 1,0 mM SLS were inoculated, using a 10% inoculum from a 24-hour shaken culture, into 250-ml Erlenmeyer flasks containing 100 ml yeast extract-malt extract (YM) medium (van der Walt and Yarrow, 1984) supplemented with 0,5 mM SLS. The cultures were incubated at 120 r.p.m. on a rotary shaker at 26°C. Disappearance of SLS was determined by periodically analysing samples from the flasks for residual SLS by the method described in part 1 of this report (section 12.2.2).

24.3. Results

24.3.1. Isolation of acid-tolerant heterotrophs resistant to and able to utilize SLS

Many filamentous fungi and yeasts were isolated from test pile effluents. The most abundant filamentous fungi among the isolates were strains of *Penicillium*, *Aspergillus* and *Oidiodendron* or a similar genus. However, yeasts were the most abundant organisms on the medium M plates, with orange-, red- and yellow-coloured species more abundant than white species (Table 17). Physiological identification tests (Table 18) indicated that the coloured yeasts were strains of *Rhodosporidium*, *Cryptococcus*, *Sporidiobolus* and/or *Candida*. The abilities of the yeast isolates to grow on medium M plates in the presence of 0,5 mM and 1,0 mM SLS and to utilize SLS as sole carbon source are shown in Table 17. All of the isolates could grow in the presence of 0,5 mM SLS, but not all could grow in the presence of 1,0 mM SLS. Most isolates could utilize SLS as sole carbon source.

24.3.2. Degradation of SLS by acid-tolerant yeast isolates

Of six yeast isolates tested for SLS-degrading ability, four (isolates 9, 11, 12 and 33) were able to degrade SLS in YM medium (Fig. 60) and two (isolates 6 and 13) were not. The percentage of SLS degraded by the isolates varied from 20 to 34%. These limited degradation test results agree with the results of the study of utilization of SLS as sole C source by the six isolates (Table 17).

TABLE 17. Characteristics of acid-tolerant yeasts isolated from effluents from SLS-treated and control pilot scale coal waste dumps

Isolate no.	Test pile and sample (month/year)	Colour	Growth in presence of SLS		Growth on SLS as sole C source
			0.5 mM	1mM	
1	3(1/88)	orange	+	-	+
2	10(1/88)	white	+	-	+
3	4(1/88)	orange	+	-	-
4	4(1/88)	white	+	-	+
5	7(1/88)	orange	+	-	+
6	3(1/88)	red	+	-	-
7	9(1/88)	orange	+	+	+
8	10(1/88)	orange	+	+	+
9	8(3/88)	orange	+	+	+
10	9(4/88)	yellow	+	-	+
11	6(4/88)	orange	+	+	+
12	9(4/88)	orange	+	-	+
13	10(4/88)	orange	+	-	-
14	2(5/88)	white	+	-	-
15	3(5/88)	white	+	+	+
16	9(5/88)	white	+	-	+
17	7(5/88)	orange	+	-	+
18	10(5/88)	orange	+	-	+
19	10(5/88)	white	+	-	+
20	6(5/88)	white	+	+	-
21	5(5/88)	white	+	+	+
22	9(5/88)	yellow	+	-	+
23	9(5/88)	orange	+	-	+
24	4(5/88)	white	+	-	+
25	4(5/88)	white	+	-	-
26	5(6/88)	orange	+	+	+
27	6(6/88)	white	+	-	+
28	8(6/88)	orange	+	-	+
29	8(6/88)	yellow	+	-	+
30	3(6/88)	white	+	+	+
31	7(6/88)	orange	+	-	-
32	4(6/88)	orange	+	-	+
33	10(6/88)	orange	+	-	+

TABLE 18. Physiological characteristics of acid-tolerant yeasts isolated from effluents from BLS-treated and control pilot scale coal waste dumps

Physiological test	Isolate number																															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
Assimilation of																																	
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	-	-	-	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-
Melizitose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Mannitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Inositol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fermentation of																																	
Glucose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Nitrate as N source	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth at 25°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
30°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
37°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Starch formation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

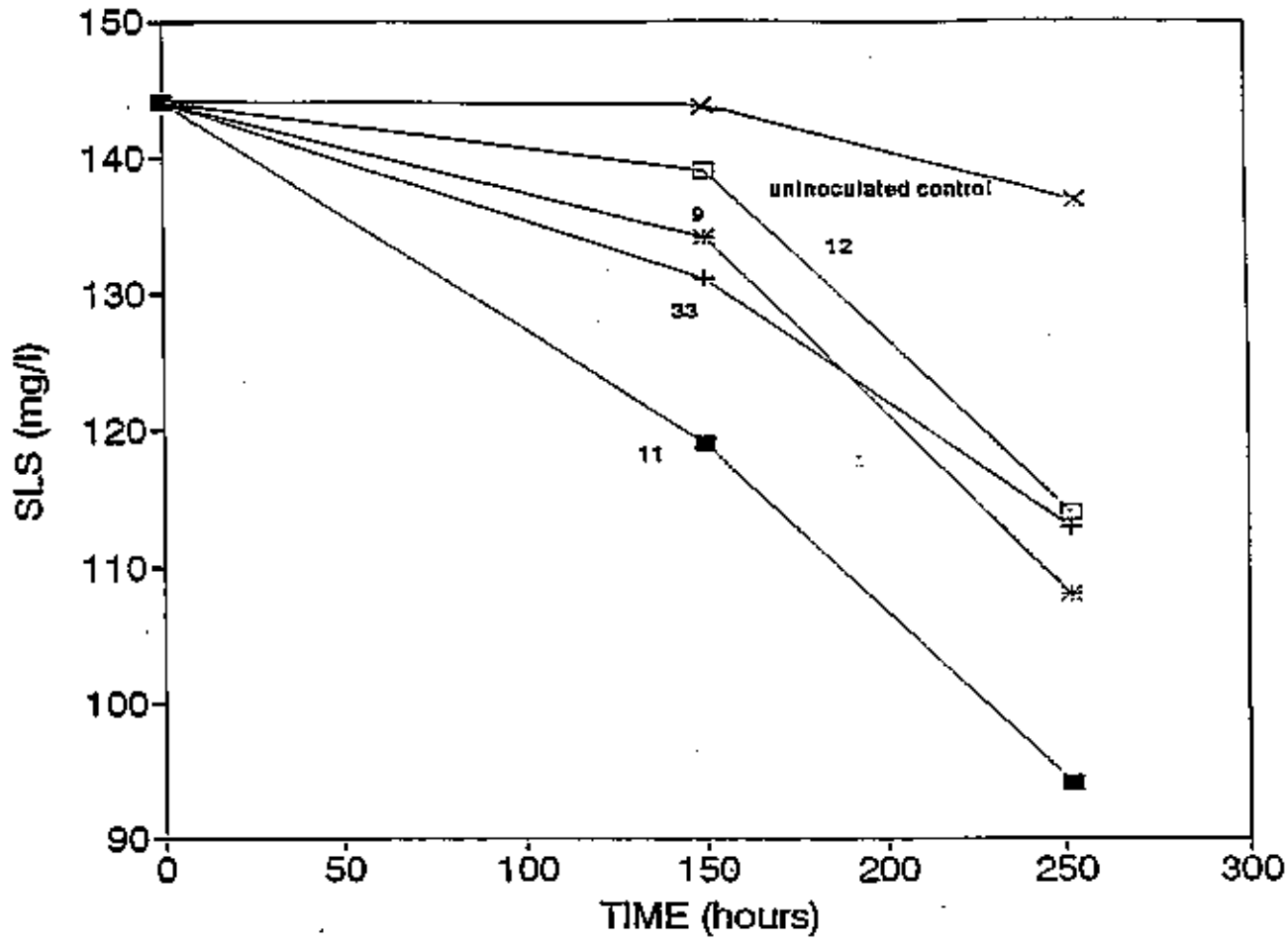


FIG. 60. Disappearance of SLS from cultures of four yeasts (coal waste effluent isolates 9, 11, 12 and 33) in yeast extract–malt extract medium supplied with 0,5 mM SLS.

24.4. Discussion

24.4.1. Isolation of acid-tolerant heterotrophs resistant to and able to degrade SLS

The abundance of filamentous fungi and yeasts on the isolation plates inoculated with effluents from the test piles, together with the absence of bacteria, indicates that fungi were the predominant hetrotrophs in the effluents and possibly also in the piles. This can be explained by the low pH of the test pile effluents, which are therefore unfavourable for most bacteria but not for fungi (Alexander, 1977). Of the yeasts isolated, those with orange, red or yellow pigment were the most abundant (Table 17). The physiological tests of Table 18 are inadequate for species and even genus identification, but assign the pigmented yeasts to the genera *Rhodosporidium*, *Cryptococcus*, *Sporidiobolus* and/or *Candida*.

White et al. (1985), in their survey of sections of a river in England, found SLS-resistant bacteria and bacteria containing alkylsulphatases in greater concentrations in SLS-polluted water than in non-polluted water. Although such results suggest that SLS in our coal waste piles could have favoured the development of SLS-resistant and SLS-assimilating microorganisms, both types of organism were common among the acid-tolerant yeasts isolated from untreated as well as treated piles (Table 17). Among the 18 isolates from SLS-treated piles, six (33%) were resistant to 1 mM SLS and 12 (67%) utilized SLS as sole carbon source; the corresponding percentages for the 15 isolates from the SLS-free control piles were 20% and 93%, respectively. These limited data suggest the possibility that SLS resistance among the heterotrophic yeasts associated with the SLS-treated dumps may have increased, but that the ability to utilize the SLS anion as a carbon source had not.

24.4.2. Degradation of SLS by acid-tolerant yeast isolates

Of the six isolates tested, four were able to degrade SLS; however, the SLS degradation was slow (Fig. 60). This could perhaps have resulted from the SLS degradation being tested in YM medium containing glucose as main carbon source. Thus, glucose would probably have been utilized first, then SLS. The degradation experiments showed that isolates from the test pile effluents were able to degrade SLS substantially and could thus possibly have reduced the concentration available in solution in the test piles for inhibition of the iron-oxidizing bacteria. However, whether such degradation of SLS actually occurred in the test piles, is unknown.

25. PILOT SCALE STUDY OF SLS AS POTENTIAL INHIBITOR OF COAL WASTE ACIDIFICATION: MOISTURE, pH, DISTRIBUTION OF SLS AND POPULATIONS OF IRON-OXIDIZING BACTERIA THROUGH PROFILES OF SLS-TREATED DUMPS

25.1. Introduction

Our analyses of the effluents of dumps 1 to 7 over the 3-year period of the pilot scale trial of SLS as a potential inhibitor of acidification of coal waste dumps (section 22), showed that little of the SLS applied in the treatments left the dumps (Fig. 50). Possible explanations include little leaching of SLS from the pellets (this would not apply to the 6 kg of SLS applied in solution to each dump), degradation of SLS by heterotrophic microorganisms in the coal waste (section 24) and adsorption. Adsorption was a likely contributing factor, as adsorption of 45 and 200 mg SLS/kg coal waste had been reported by Kleinmann and Erickson (1983) and Onysko et al. (1984a), respectively. After the analyses of the effluents of dumps 1 to 6 were stopped, we dug into the dumps to sample coal waste from different depths and analysed the samples for moisture, pH, SLS content and populations of iron-oxidizing bacteria. The aim of these analyses was to determine whether adsorption of SLS to the coal waste was retaining the SLS in the dumps and if so, whether the SLS was distributed unevenly through the dumps leaving zones (possibly the lower sections) with low SLS concentrations where iron-oxidizing bacteria could develop and acidification occur. Another aim was to determine to what depths in the compacted dumps conditions were favourable for the growth of iron-oxidizing bacteria.

25.2. Materials and Methods

Samples were taken on 17 January 1989 from the SLS-treated dumps 1 and 3, as well as on 20 March 1989 from dumps 2, 4, 5 and 6, at various depths to the bottom of the dumps, except dump 1 which was sampled only to 94 cm. Samples from dumps 1 and 3 were stored for 72 h and samples from dumps 2, 4, 5 and 6 for 24 h at 4°C until analysed. Moisture and pH of the coal waste samples were determined as outlined in section 17.2, but with a drying period of ca. 92 h for the moisture determinations. Water-extractable SLS in the samples was determined by shaking 100 g (wet mass) of the coal waste in 100 ml distilled water on a rotary shaker for 10 min at 120 r.p.m. The supernatant was removed and centrifuged at maximum speed in a Hettich bench top centrifuge for 10 min. A 10 ml sample was diluted with 90 ml distilled water in a separating funnel and analysed for SLS using the methylene

blue extraction procedure described in part 1 of this report (section 12.2.2). For MPN counts of iron-oxidizing bacteria, 500 g of the coal waste samples were added to 500 ml half-strength HJJ medium without ferrous sulphate. This 10^0 dilution was then diluted in the same medium to provide a tenfold dilution series to 10^{-8}. From each dilution, three 1-ml aliquots were pipetted into wide-mouth 50-ml Erlenmeyer flasks containing 20 ml HJJ medium and incubated at 26°C for ca. 30 days. The further procedure for the MPN determinations was as outlined in section 17.2. Where sufficient material was available, samples were also analysed at the Research Laboratories of the Anglo American Corporation of South Africa Ltd., Johannesburg, for carbon, sulphur and ash. Carbon and sulphur were analysed by the LECO procedure (LECO Corporation, St. Joseph, MI 49085, U.S.A.) involving conversion of the carbon and sulphur to carbon dioxide and sulphur dioxide, respectively, for infrared analysis, while ash was the residue following complete combustion of the sample.

25.3. Results

The moisture, pH, water-extractable SLS and log MPN count of iron-oxidizing bacteria recorded for the samples from different depths in test piles 1 to 6 are shown in Table 19 and the results of the carbon, sulphur and ash analyses in Appendix 4. The moisture in the dumps showed a tendency to increase with increasing depth. The pH of the coal waste samples varied from pH 1.70 to pH 4.17, with the highest pH values recorded for the uppermost three samples from dump 3. The SLS extracted in water from the coal discard samples varied from 0,56 to 46,52 mg/kg. In any dump, the highest concentrations of SLS were in the upper samples, with a sharp drop to the concentrations of the lower samples at a depth which in dumps 1 to 3 increased as the SLS dose applied to the dump increased. These dumps had been treated with SLS doses of 11.25, 20.00 and 32.25 kg/dump, respectively, with natural rubber as the carrier in the pellets. Corresponding samples from dumps 4 to 6, in which the SLS carrier in the pellets was polyisoprene rubber, yielded considerably lower concentrations of water-extractable SLS which did not show a correlation with dose. Iron-oxidizing bacteria were present in concentrations greater than 100/g in most of the dump samples, except in the top two or three samples from dumps 2 and 3, where none or few occurred. These were the samples to depths of 45 and 55 cm, respectively, with the highest concentrations of water-extractable SLS (> 40,00 mg/kg). The carbon, sulphur and ash analyses showed respective overall mean values of 28,60, 3,56 and 52,47 g/100 g coal waste but indicated clearly

TABLE 19. Percentage moisture, pH, water-extractable SLS and log iron-oxidizing bacterial count (MPN) of samples from different depths in pilot scale coal waste test piles 1 to 6

Test pile	Sample number	Sample depth (cm)	Percentage moisture[a]	pH in water[b]	SLS extracted from coal waste (mg/kg)	Log iron-oxidizing bacteria (MPN)/g[c]
1	1	10	7.30	1.83	17,50	2.20
	2	20	7.92	1.93	4,17	3.69
	3	30	8.69	1.74	2,11	4.08
	4	40	8.72	1.70	1,81	4.08
	5	50	8.53	1.76	1,61	4.36
	6	84	9.10	ND[d]	ND[d]	4.70
	7	94	8.36	1.83	2,69	4.07
2	1	5	5.72	2.62	43,10	0.00
	2	45	7.36	2.73	46,52	0.39
	3	70	9.96	2.46	1,28	2.08
	4	112	9.65	2.25	1,12	2.91
3	1	25	9.11	3.87	41,00	0.00
	2	40	8.69	4.17	46,21	0.00
	3	55	8.59	3.21	40,12	0.91
	4	75	8.38	2.42	10,70	2.08
	5	100	9.67	2.47	13,84	4.09
	6	112	10.64	2.31	3,65	3.71
4	1	10	7.30	2.41	1,39	2.07
	2	50	9.96	2.50	1,62	4.08
	3	85	7.22	2.50	1,13	5.07
	4	110	11.34	2.37	0,56	4.09
5	1	30	6.18	2.42	2,43	3.06
	2	67	9.50	2.51	0,89	5.08
	3	86	9.53	2.44	0,93	6.08
	4	112	8.34	2.34	1,56	6.08
6	1	8	6.47	2.30	1,19	3.49
	2	50	9.12	2.38	4,49	4.21
	3	80	9.94	2.18	0,65	5.22
	4	100	9.22	1.89	0,61	4.91

[a]Moisture as percentage of wet coal waste mass; mean of duplicate determinations.
[b]pH of a 1:2,5 coal waste:water suspension; mean of duplicate determinations.
[c]Log MPN/g dry coal waste, determined from MPN tables of de Man (1983); 0,00 = no iron-oxidizing bacteria detected in flasks containing organisms from up to 1 g wet coal waste.
[d]ND = not determined.

how variable was the composition of the coal waste from one test pile to another and within test piles.

25.4. Discussion

The relatively high moisture values recorded in the test piles indicate suitable moisture conditions throughout the dumps for the growth of iron-oxidizing bacteria should they not be inhibited by the chemical inhibitors. The pH levels would also have been favourable for *T. ferrooxidans* and acidification in all dumps, but also suggest some inhibition thereof in the upper 55 cm of test pile 3, dosed with 6,00 kg SLS in solution and 26,25 kg SLS in natural rubber pellets.

The SLS dosages applied to test piles 2 and 3 inhibited growth of iron-oxidizing bacteria to depths of 45 and 55 cm, respectively (slight growth occurred in the 45 and 55 cm samples from the respective test piles). This result suggests that SLS applied at a dose of 20 kg/test pile could have inhibited acidification of the coal discard had an oxygen barrier developed at ca. 30 cm depth as in large dumps elsewhere (Dugan, 1975). On an area basis, this dose rate is 20 kg/33,75 m^2 or 0,6 kg/m^2 or 6 000 kg/ha. The cost of the SLS at the 1990 price (transport and application excluded) would be R9,68 x 6 000 = R58 080/ha.

The levels of water-extractable SLS in the samples from the test piles explain entirely the pattern of inhibition of iron-oxidizing bacteria, the inhibition being associated with concentrations greater than ca. 40 mg SLS/kg coal discard. The effect of adsorption by the coal discard on the movement of SLS through the test piles can clearly be seen by the sharp decrease in SLS concentration below the upper sample (dumps 1, 4, 5 and 6) or the upper two (dump 2) or three (dump 3) samples. The natural rubber pellets released the SLS more effectively than the synthetic polyisoprene rubber pellets, in agreement with the results of Immelman (1987; this report, part 3), who showed the natural rubber pellets to have a faster SLS release rate than the polyisoprene rubber pellets. A relationship is also evident between the dose of SLS added to test piles 1 to 3 and the SLS recovered from the samples from the test piles. However, test piles 4 to 6 did not show this pattern, probably because of the much slower rates of SLS release from the polyisoprene pellets.

The distribution of SLS in the dumps raises the question of whether slow release rubber-SLS formulations are necessary at all. The apparent adsorption of high concentrations of SLS on the surface layers of the coal waste may itself function as a

slow release system, which could perhaps be formed merely by treating the coal waste with a high dose of SLS in solution.

The compaction of the dumps when they were constructed did not prevent growth of iron-oxidizing bacteria, which were present in large numbers in the basal layers of the coal waste. The compaction thus did not prevent the movement of adequate water and oxygen for growth of the bacteria into the lower regions of the dumps. Compaction tests on the dumps showed an increase in compaction from test pile 1 to 10, with none being compacted to accepted standards (Coetzee, 1986). There is no evidence that the increase in compaction from test pile 1 to 10 had any effect on the bacterial numbers. In the U.S.A., Erickson (1985) studied the movement of oxygen into coal discard at numerous sites on dumps and found oxygen penetration at concentrations > 2% to depths of 15 cm to > 10 m. Sites covered with a clay cap and vegetated showed seasonal fluctuations in oxygen content from ca. 0 to > 20% at a depth of 60 cm below the clay cap. This showed that vegetation or clay may not be highly effective in reducing oxygen diffusion into coal discard. A combination of inhibitors, compaction, clay cap and vegetation might control acidification. The inhibitors would be necessary as some authors (Brock and Gustafson, 1976; Goodman et al., 1983) have shown *T. ferrooxidans* to be capable of anaerobic growth. Further work will have to be done on the distribution and growth of iron-oxidizing bacteria in established mine dumps in South Africa to determine to what depth treatment with inhibitors is necessary.

The analyses of carbon, sulphur and ash showed the heterogeneity of the coal waste from dump to dump and within dumps, but show no relationship to the results in Table 19 nor to the patterns of iron and sulphate released from the dumps as reported in section 22.3.

26. PILOT SCALE COMPARATIVE STUDY OF SLS AND SODIUM BENZOATE AS POTENTIAL INHIBITORS OF COAL WASTE ACIDIFICATION

26.1. Introduction

In our study of the inhibition of two *T. ferrooxidans* test strains by SLS, sodium benzoate and sorbic acid in HJJ medium containing 500 g/l coal waste (section 20), sodium benzoate and sorbic acid showed lower minimum inhibitory concentrations (40 to 75 mg/l) than SLS (70 to 90 mg/l). Onysko et al. (1984a) found sodium benzoate comparable to SLS and potassium sorbate better in delaying the acidification of coal waste, while Dugan (1987a) found benzoic acid somewhat better than SLS in delaying the acidification of 30% coal waste slurries. Benzoic acid or benzoate salts are considerably cheaper than SLS, sorbic acid or sorbates (Erickson et al., 1985; Onysko et al., 1984a), being about half the price of SLS and less than a third of the price of sorbic acid. At the end of 1988, when it became evident that the SLS treatments were not preventing acid formation in our pilot scale coal waste dumps at the Wolvekrans mine, we decided to use dump 7, which had received only 6 kg of SLS in solution, and dumps 8 and 9, which had been controls, for a comparative study of SLS and sodium benzoate as inhibitors of acid generation. The application strategy was also changed to dosing dump 7 every 2 weeks with 2 kg SLS in solution and dumps 8 and 9 every 2 weeks with 0,2 and 2 kg, respectively, of sodium benzoate in solution. Dump 10 was kept as an untreated control. In this trial we aimed at saturating the adsorption sites of dump 7 with SLS and dump 9 with sodium benzoate to determine the amounts required for saturation and to study the inhibition of acidification when the coal waste was saturated with inhibitor.

26.2. Materials and Methods

Dumps 7, 8, 9 and 10 were treated on 16 January 1989 and fortnightly thereafter as follows: Dump 7 with 2 kg SLS (EMPICOL LZ with 89% SLS, Polycor Chemicals, Cape Town), dump 8 with 0,2 kg and dump 9 with 2 kg sodium benzoate (Heynes Mathew Ltd., Cape Town), while dump 10 was left untreated as a control. The inhibitor doses were dissolved in 100 l tap water and spread over the dump using 10-l buckets. During the rainy season, samples were collected from the rubber collection bins before each new addition of inhibitor solution. The water volume had to be increased from the beginning of the 1989 dry season, thus when no water was present in the collection bins on 13 March 1989 (day 56), 300 l water was added to each dump as 100 l water, 100 l water + inhibitor and 100 l water. However, on this

occasion, after addition of the water, 19,5 mm of rainfall was recorded near the test sites. The samples were taken the following day. As a similar watering procedure on 10 April 1989 did not produce drainage water in the bins for a sampling on the following day, a further 200 l of water were added to the dumps and samples taken on 12 April 1989 (86 days). In subsequent similar situations of no water in the collection bins, 500 l of water were added to each dump as 200 l water, 100 l water + inhibitor and a further 200 l water. Control dump 10 received only the appropriate volumes of water without any inhibitor.

All effluent samples from the collection bins were analysed for pH, iron-oxidizing bacterial numbers (MPN), total iron and sulphate as outlined in section 22.2.3. Effluents from dump 7 were also analysed for SLS as described in part 1 of this report (section 12.2.2) and effluents from dumps 8 and 9 for sodium benzoate by the Association of Official Analytical Chemists' (A.O.A.C.) procedure (Horwitz, 1980), adapted in the following way. A ca. 15-ml sample was centrifuged for 15 minutes at maximum speed in a Hettich bench top centrifuge. A 10-ml sample was taken from the centrifuged supernatant and diluted to 100 ml with saturated sodium chloride solution. The solution was made acid (pH <2) with hydrochloric acid. The acid solution was extracted successively with 35, 25, 20, 15 and 10 ml portions of ether, shaken well to ensure complete extraction. The combined ether extracts were washed with dilute (1:1000) hydrochloric acid and the aqueous washings discarded. The washed ether extract was diluted to 100 ml with ether and the absorbance of the extract determined, according to the A.O.A.C. method, on a Beckman DU8 spectrophotometer. Standards for determination of the sodium benzoate concentration were prepared as described in the A.O.A.C. method.

26.3. Results

Rainfall monitored during the study period is shown in Fig. 61. The first rainy season stopped in March 1989 (after 71 days). Thereafter a relatively long, mainly dry winter followed (to 276 days) before the start of the next summer rains.

The pH of the effluents remained mainly between pH 2,0 and 2,6, with temporary declines to below pH 2.0 at days 86 and 248 to 276 (Fig. 62). The pH of the effluents from the treated test piles 7 to 9 and the untreated control pile 10, showed no or only small differences, hence no clear inhibitory effect of any of the chemical treatments on acid generation in the dumps.

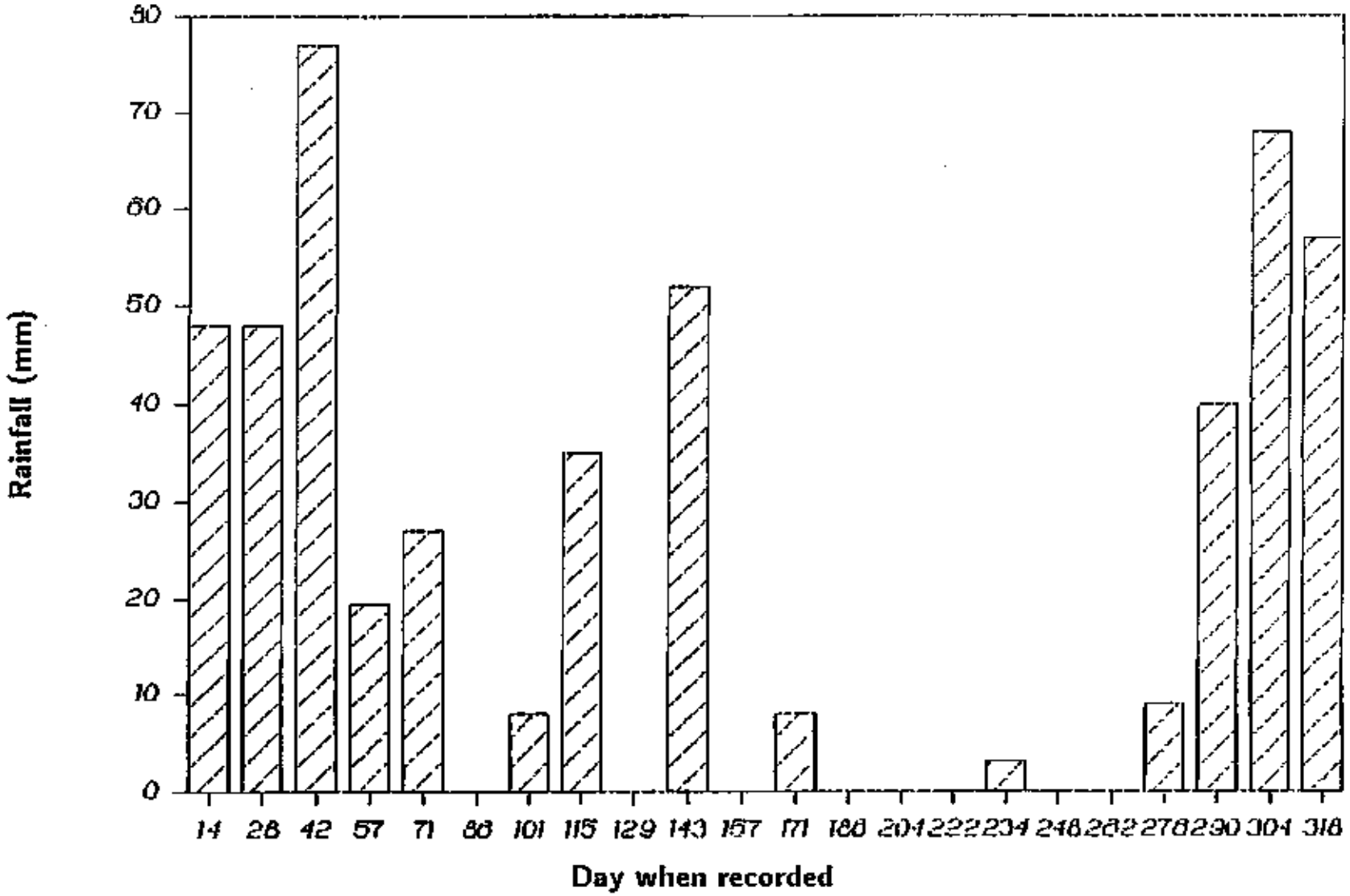


FIG. 61. Rainfall recorded at the Wolvekrans mine ca. 2 km from the test piles for approximately 14-day intervals from 1/16/89 (day 0) to 30/11/89 (day 318).

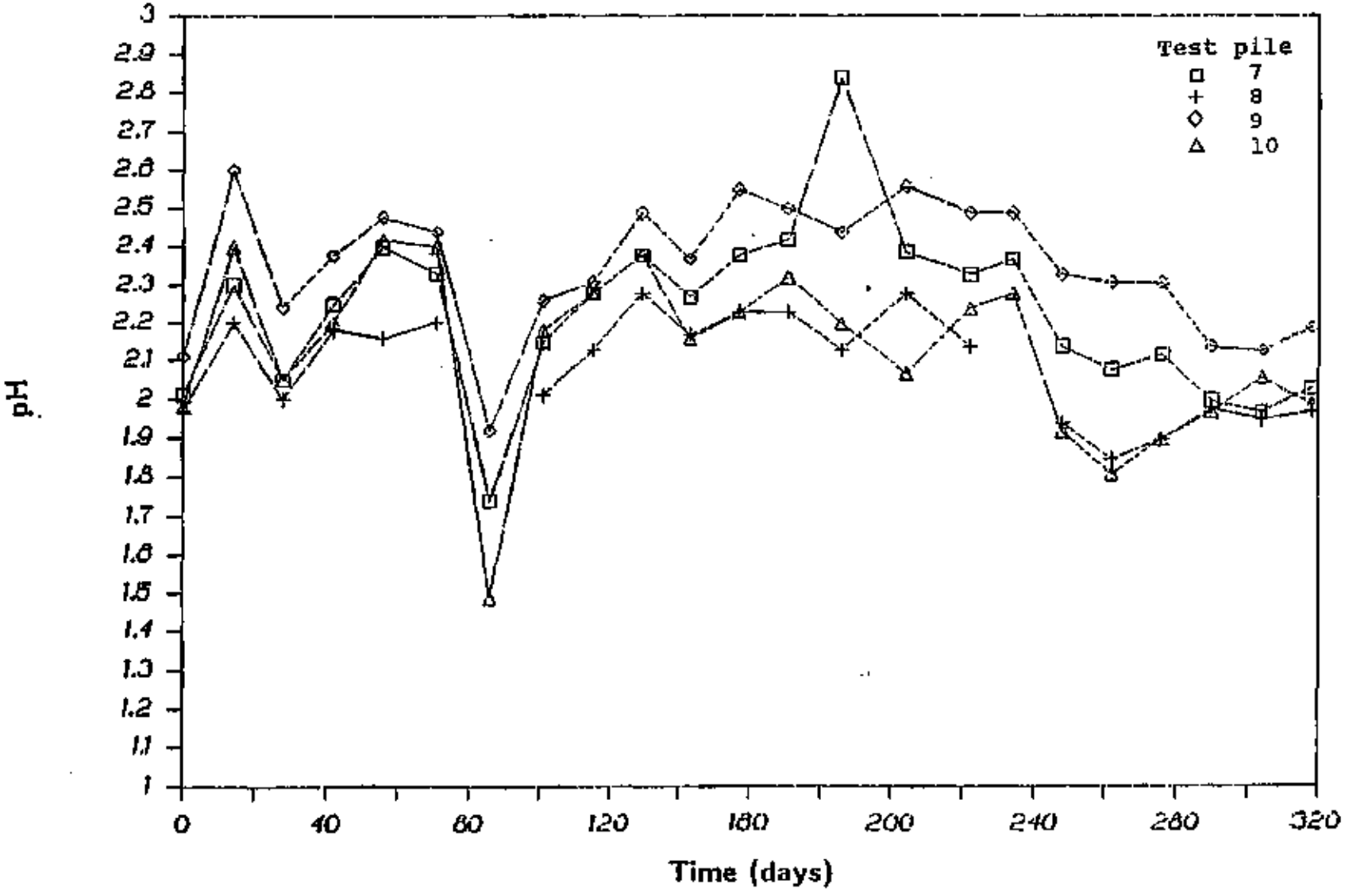


FIG. 62. pH of effluents from test piles 7 to 10 from 16/1/89 (day 0) to 30/11/89 (day 318).

Logarithms of the MPN counts of iron-oxidizing bacteria in the effluents from the test piles are shown in Fig. 63. Populations in the effluents from test piles 8 and 10 fluctuated but were always present, mainly between 10^3 and 10^6/ml. However, those in the effluents from test piles 7 and 9 decreased markedly after 157 days. No viable iron-oxidizing bacteria could be detected in the effluent from test pile 7 and most of the samples from test pile 9 from 188 days. The effluent samples from test pile 9 on day 234 and from day 290, showing ca. 10^2 to 10^3 iron-oxidizing bacteria/ml, were the exceptions.

Levels of SLS in the effluent water from dump 7 (Fig. 64) showed opposite trends to those of the iron-oxidizing bacterial populations. Only very low levels of SLS (mainly <0,5 mg/l) were detected at the samplings to day 172. From day 188 a large sustained flush of SLS from the dumps was evident, with concentrations of 18 to 498 mg/l measured in the effluents.

No or only traces of sodium benzoate were detected in the effluents from dumps 8 and 9 before 188 days (Fig. 65). A sustained flush of sodium benzoate from test pile 9, with concentrations mainly higher than ca. 100 mg/l, was evident from 188 days, with a maximum of 376 mg/l at 248 days. However, sodium benzoate was at low or trace levels in some of the effluent samples during that period, for example, on days 222 and 290 to 318. The concentrations of sodium benzoate in the effluent from test pile 8 remained low (< 30 mg/l) for the duration of the experiment.

The average daily outflows of iron and sulphate (Fig. 66, 67) from all the test piles fluctuated approximately in harmony, but overall gave no indication that the SLS added to dump 7 and the low dose of sodium bensoate added to dump 8 reduced soluble iron and sulphate production in the dumps. At most samplings, the average daily iron and sulphate outflows from dump 9 were considerably lower than those from the other dumps, but this was the situation from the first sampling at 14 days.

Cumulative iron and sulphate leached from the test piles are shown in Fig. 68. No significant reduction in iron and sulphate outflows from test piles 7 and 8, treated with SLS and the low dose of benzoate, respectively, is evident in comparison with the outflow from the control test pile 10, but the outflow from test pile 9, treated with the high dose of sodium benzoate, was less than half those from the other test piles.

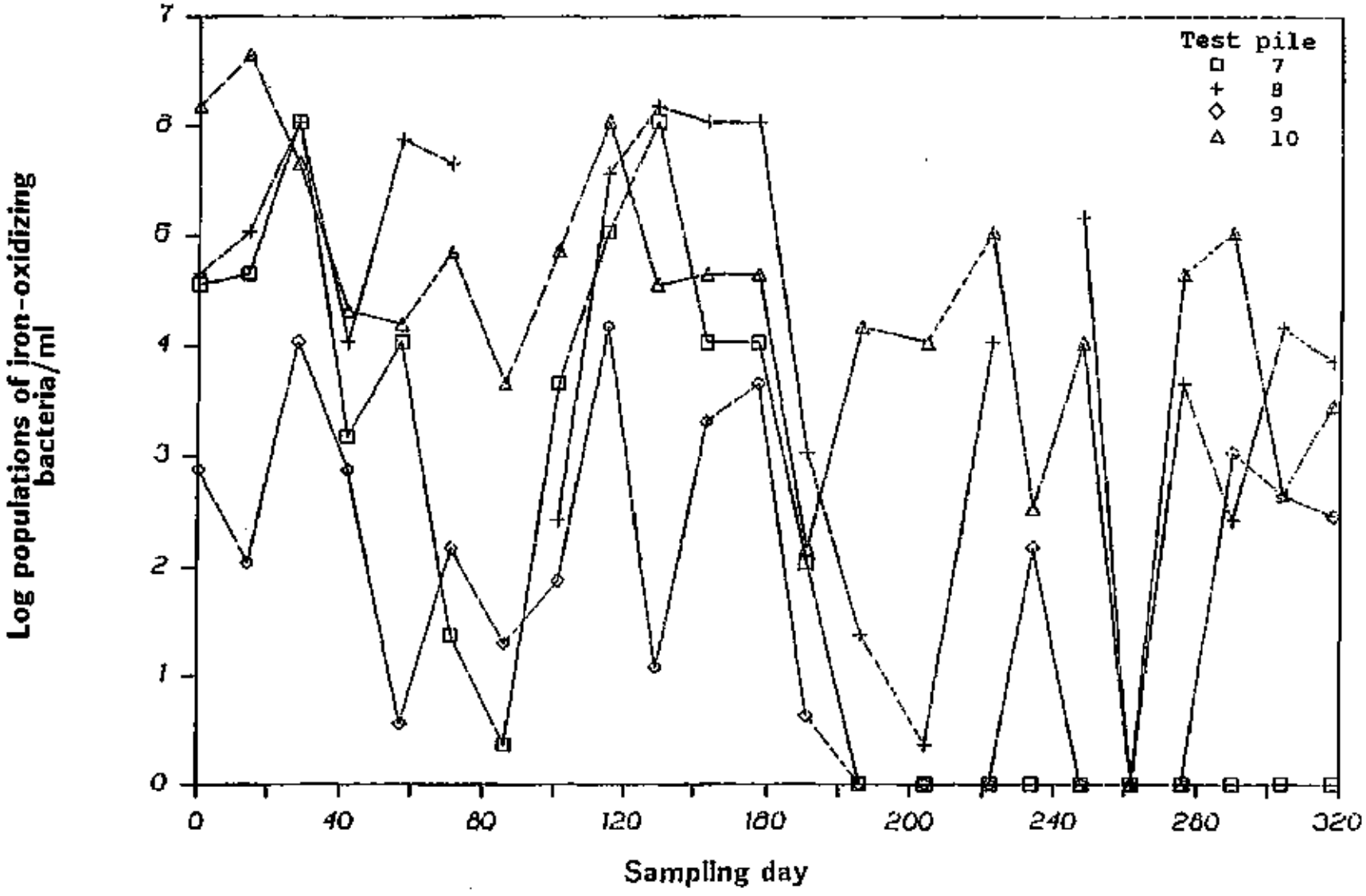


FIG. 63. Log populations (MPN) of iron-oxidizing bacteria in effluents of test piles 7 to 10 from 16/1/89 (day 0) to 30/11/89 (day 318).

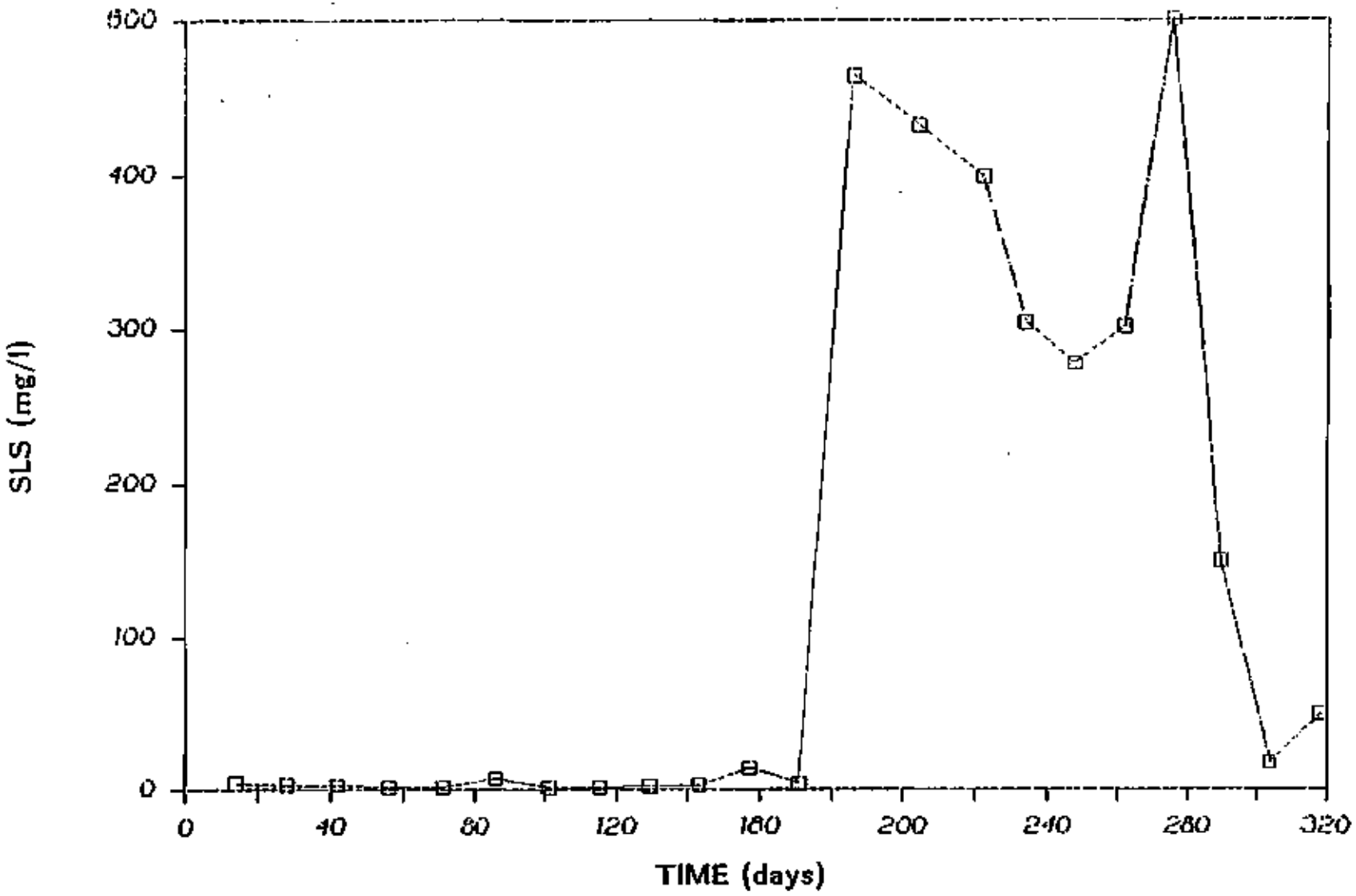


FIG. 64. Concentration of SLS in effluent from test pile 7 from 30/1/89 (day 14) to 30/11/89 (day 318).

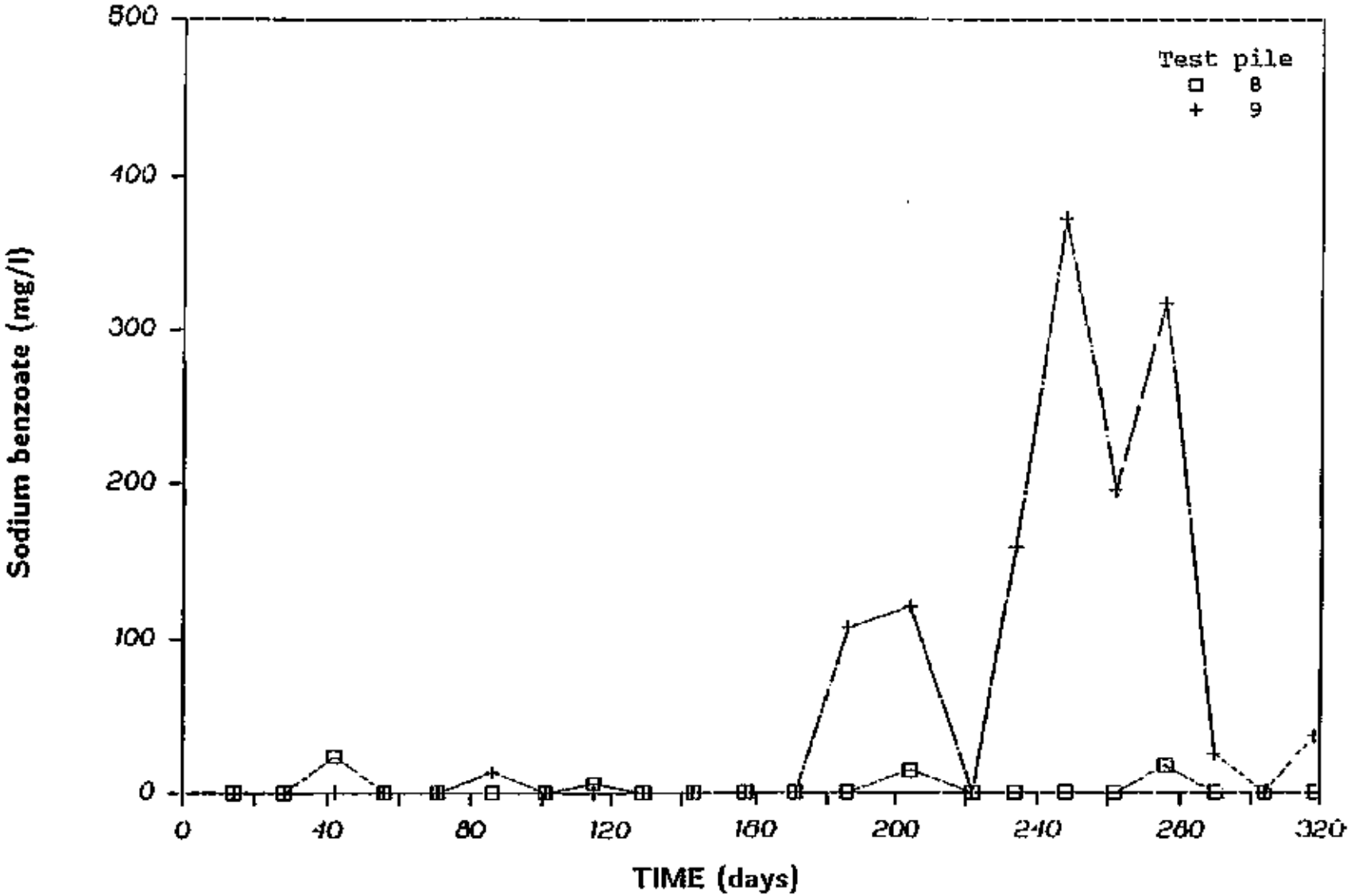


FIG. 65. Concentration of sodium benzoate in effluents from test piles 8 and 9 from 30/1/89 (day 14) to 30/11/89 (day 318).

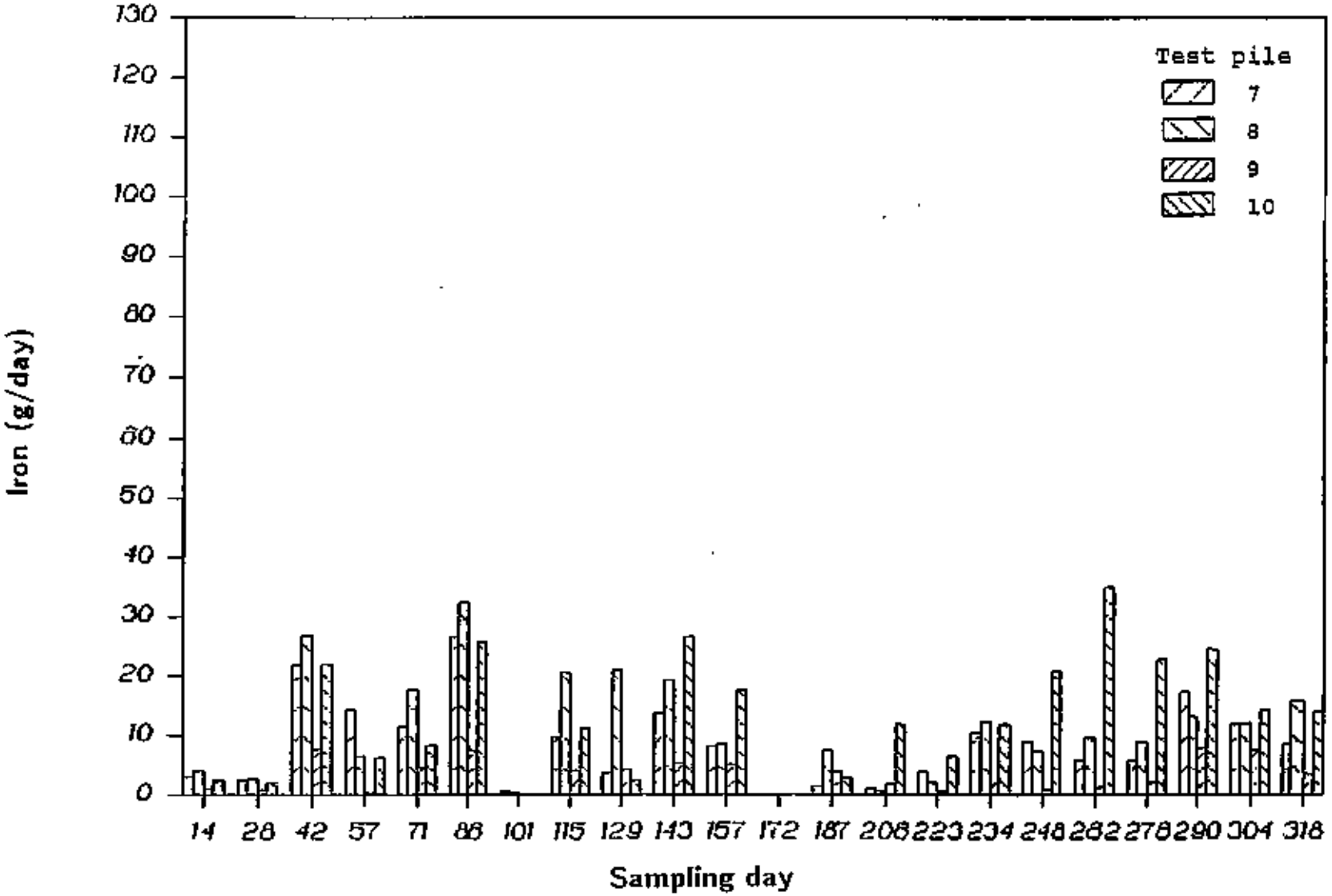


FIG. 66. Average daily iron outflow from test piles 7 to 10 during approximately 14-day periods (preceding sampling days) from 16/1/89 (day 0) to 30/11/89 (day 318).

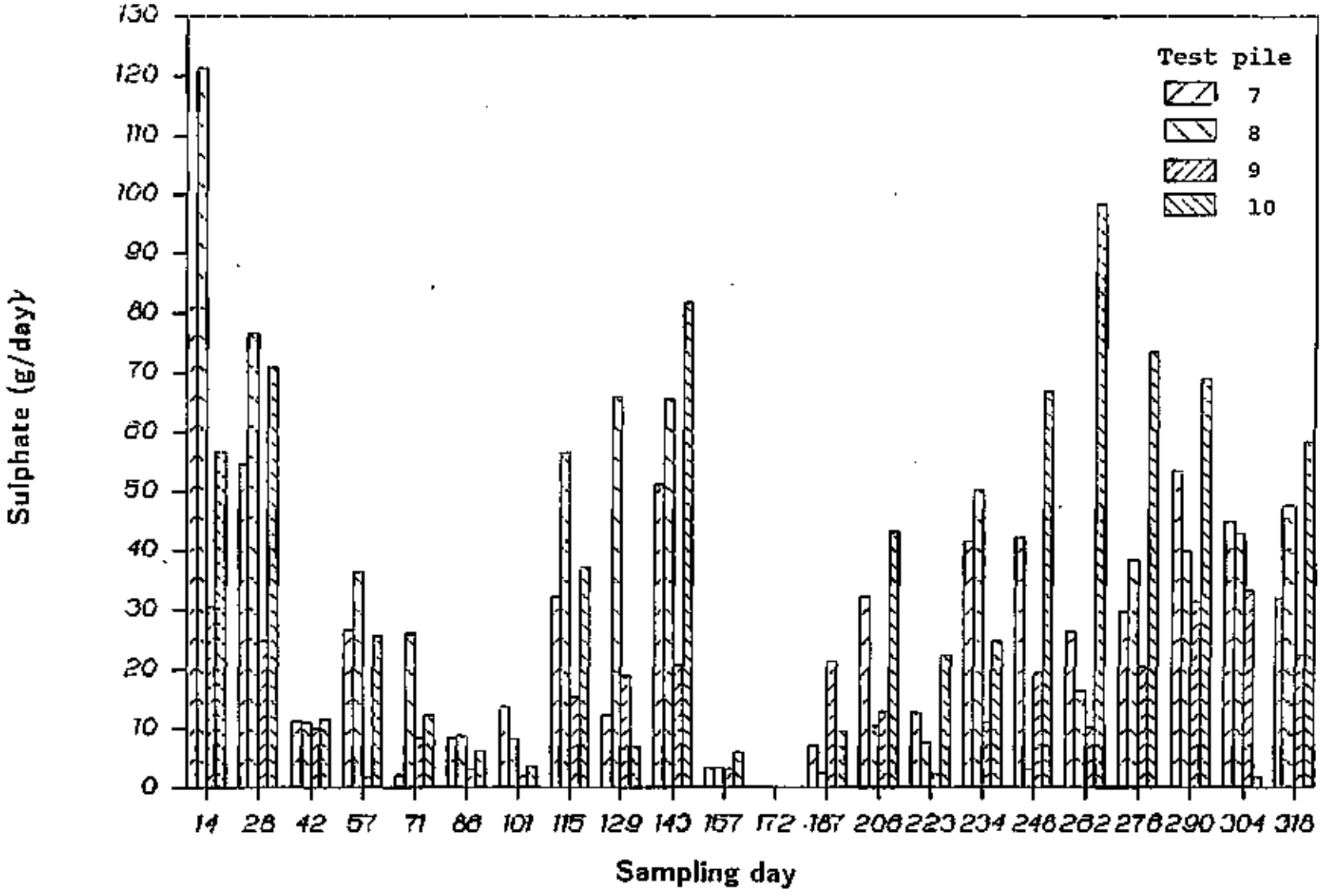


FIG. 67. Average daily sulphate outflow from test piles 7 to 10 during approximately 14 day periods (preceding sampling days) from 16/1/89 (day 0) to 30/11/89 (day 318).

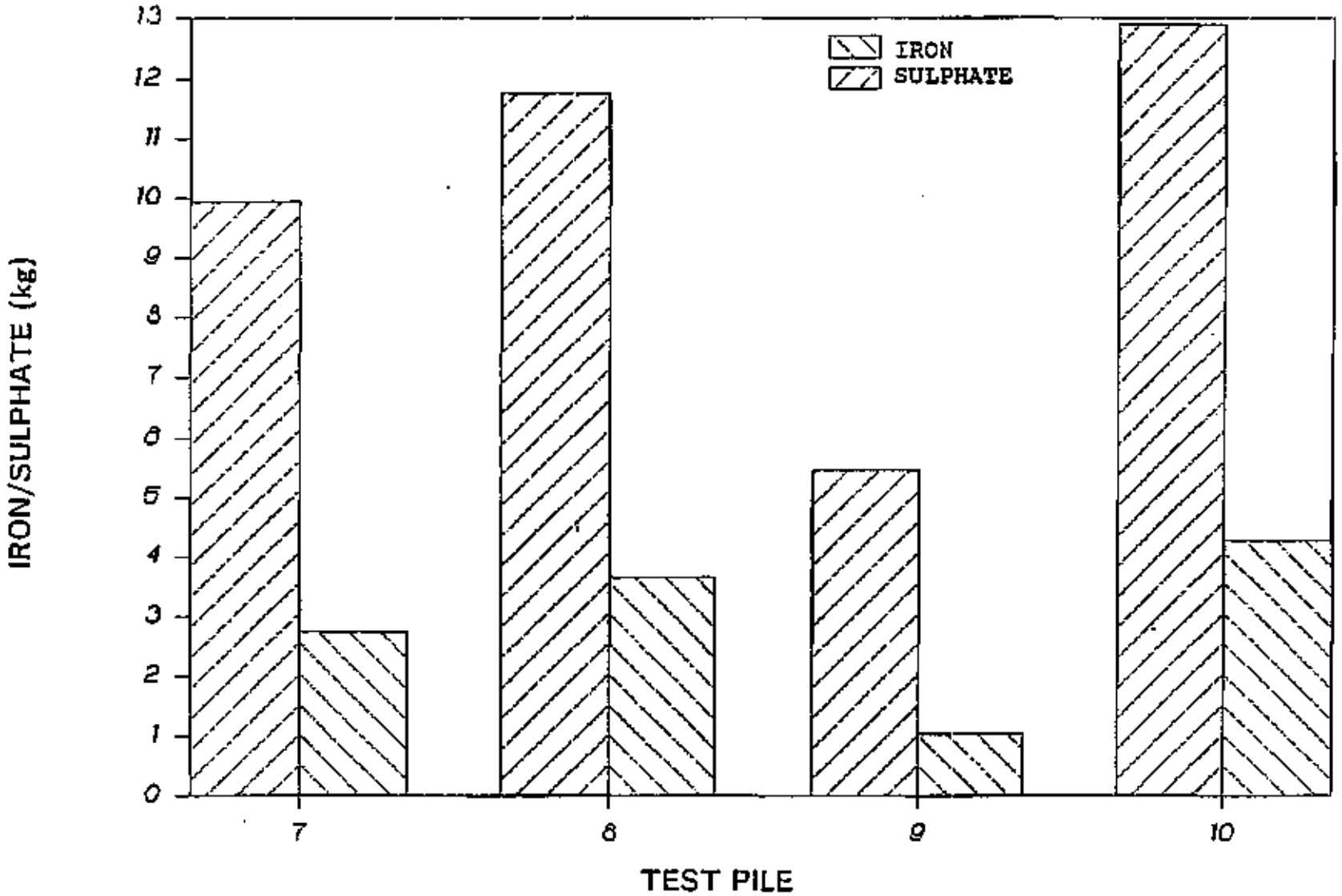


FIG. 68. Cumulative iron and sulphate leached from test piles 7 to 10 from 16/1/89 (day 0) to 30/11/89 (day 318).

26.4 Discussion

The rainfall in the test area during 1989 (Fig. 61) showed a long dry winter season from day 71 to 276, interrupted by sporadic rain giving substantial readings only at days 115 and 143. The long period of virtual drought necessitated the addition of 500 l of irrigation water to the test piles from day 86 until day 276, to obtain effluent in the collection bins.

The low pH (< pH 2,8) of the effluents from the test piles throughout the experimental period and the absence of any marked pH differences between the treated and untreated dumps (Fig. 62), give no indication of inhibition of acidification by any of the SLS or sodium benzoate treatments. However, it is possible that acidification could continue in the dumps abiotically (see this report, part 1, section 1, reaction 5) after inhibition of the activity of the iron-oxidizing bacteria.

Although the populations of iron-oxidizing bacteria in the effluents from the test piles (Fig. 63) showed considerable fluctuation, overall trends show marked population decreases in test piles 7 and 9 treated with SLS and the high dose of sodium benzoate, respectively, in contrast with little of no change in test pile 8 with the low dose of benzoate and the untreated control pile 10. The SLS treatment seems to have been the most effective, as no iron-oxidizing bacteria were detected in effluents from dump 7 after their initial complete disappearance at 188 days, whereas populations reappeared in some of the effluents from dump 9, particularly from 290 to 318 days, when sodium benzoate in the effluent was at low levels (Fig. 65). The reason(s) for the short-lived reappearance of iron-oxidizing bacteria in the effluent from dump 9 at 234 days, rather than at 223 days when the benzoate in the effluent was negligible, are unknown.

The low levels of SLS leached from test pile 7 until day 172 (Fig. 64) probably result from adsorption of most of the applied SLS to the coal waste (see section 25). At day 188, there was a marked increase in the amount of SLS in the leachate, probably due to the adsorption sites of the coal waste having become saturated with SLS as a result of the repeated addition of 2 kg SLS every 2 weeks. The decreases in the SLS concentration in the effluents after day 188 could possibly be due to other adsorption sites becoming available with the spread of the SLS through more regions of the dump. Rainfall (Fig. 61) also may have played a role in determining the low 290- to 318-day SLS concentrations in the effluents. Apart from direct leaching

effects, substantial rainfall could have influenced the SLS concentration by changing the sampling procedure (from the sampling of effluent that had collected during a 2-week period after the addition of inhibitor to the sampling of drainage water from irrigation, with the addition of inhibitor, the day before).

As 13 SLS applications to the dumps plus the 6 kg SLS applied previously (section 22.3.2) were necessary to achieve the postulated saturation of adsorption sites, the total SLS dose for saturation of 55 t coal waste appears to be 32 kg, or ca. 0,6 kg/ton coal waste. This is equivalent to 600 mg/kg coal waste, which is much higher than the 45 mg/kg determined by Kleinmann and Erickson (1983) or the 200 mg/kg determined by Onysko et al. (1984a) in small scale experiments. A saturation amount of 32 kg/dump also suggests that only about one third of the 32,5 kg SLS applied to dump 3 (6 kg SLS in solution and 26,6 kg in SLS-rubber pellets) moved into the rain water penetrating the dump, as most of the SLS was recovered from the coal waste in the upper one third of the dump (see section 25.2).

The absence of sodium benzoate, except in trace amounts, from the early samples of effluent from test piles 8 and 9 (Fig. 65), could possibly be due to both adsorption and the formation of a precipitate with iron as postulated by Onsyko et al. (1984a,b). The increase in the amount of sodium benzoate in the effluent of test pile 9 on day 188 seems to indicate that the adsorption/precipitation capacity of the dump in respect of benzoate was exceeded. Thereafter, sodium benzoate was detected at high levels in the effluent, except at day 223, until it declined sharply at day 290 after the start of the summer rains. The reasons for this decline may thus be related to rainfall and could possibly be the same as for the similar decline of effluent SLS. The reason for the absence of sodium benzoate from the effluent from test pile 9 at the 223-day sampling is unknown, but could possibly be related to where on the dump in relation to water channels the sodium benzoate was applied.

The postulated saturation of dump 9 with sodium benzoate was achieved after 13 doses of 2 kg inhibitor, thus 26 kg of sodium benzoate or ca. 0,5 kg/ton coal waste. This is a slightly lower total dose than the total dose of SLS needed to saturate a 55-t dump, and with the cheaper price of sodium benzoate, suggests that this compound should be the chemical of choice for combatting acidification of coal waste. However, the average daily iron and sulphate outflows from the test piles (Fig. 66, 67) show no clear evidence of inhibition of acidification in the treated dumps by either SLS or sodium benzoate. Test pile 9 regularly showed the lowest values, but these seemed to be merely a continuation of low initial values for the test pile. Test

pile 9, unlike dumps 7, 8 and 10, had been subjected to continuous fortnightly irrigation for the previous 3 years (section 22), which may have affected its performance in this study, although no more total iron and sulphate had been removed from dump 9 than from the others (Fig. 59). The low dose of sodium benzoate applied to test pile 8 and the SLS applied to test pile 7, seemed to have no inhibitory effect on the production of soluble iron and sulphate by the dumps. As in the case of acid production, iron and sulphate production from pyrite could possibly continue abiotically for an unknown period after inhibition of iron-oxidizing bacteria.

Compared with the cumulative iron and sulphate values for control pile 10, the cumulative iron and sulphate values for test pile 9 on their own suggest an inhibitory effect of the high dose of sodium benzoate (Fig. 68). However, the average daily iron and sulphate outflows discussed in the previous paragraph put this apparent inhibition in perspective and cast doubts on whether it was real inhibition. The treatments applied to test piles 7 and 8 did not seem to have had any inhibitory effect, according to Fig. 68.

Should saturation of coal waste dumps to a depth of about 0,5 m with SLS or sodium benzoate be effective in preventing acidification above an oxygen barrier, acidification control might be achieved with doses of ca. 10 kg SLS or 8,67 kg sodium benzoate/33,75 m^2, equivalent to ca. 3 000 kg SLS or 2 600 kg sodium benzoate/ha. At 1990 prices the cost of these materials (transport and application excluded) would be R9,68 x 3 000 = R29 040/ha for SLS and R4,97 x 2 600 = R12 922/ha for sodium benzoate treatment.

27. ADSORPTION OF SLS AND SODIUM BENZOATE BY COAL WASTE

27.1. Introduction

Several results from our research on the activity of inhibitors of iron-oxidizing bacteria in the presence of coal waste suggested that the adsorption of inhibitors by coal waste was an important factor. Thus, the activity of SLS was reduced 18- to 45-fold, and that of sodium benzoate and sorbic acid by two- to greater than fourfold (according to increases in the minimum inhibitory concentrations for two *T. ferrooxidans* strains) by the inclusion of 500 g/l coal waste in the HJJ culture medium (section 20). In the pilot scale field trial of SLS as a potential inhibitor of coal waste acidification, little of the applied SLS (6 kg/dump in solution and 0 to 26,5 kg in SLS-rubber pellets) had left the dumps in effluents by the end of the experiment (section 22). The distribution of SLS in the dumps at the end of the experiment (section 25) suggested adsorption by the upper layers of the coal waste of the SLS supplied in solution and in the SLS-natural rubber pellets of dumps 1 to 3. The amount of SLS retained in dump 7 when the saturation level seemed to have been reached, was 600 mg/kg, which is much higher than the 45 and 200 mg/kg adsorbed in the small scale laboratory studies of Kleinmann and Erickson (1983) and Onysko et al. (1984a), respectively. The corresponding value for sodium benzoate in dump 9 was 500 mg/kg. With such indications of the importance of adsorption in determining the activity of inhibitors in coal waste, but with little quantitative data on SLS adsorption and almost none on benzoate adsorption, we undertook limited basic laboratory investigations of SLS and sodium benzoate adsorption by defined fractions of the Wolvekrans coal waste used in the inhibitor studies of section 20.

27.2. Materials and Methods

27.2.1. SLS

Adsorption of SLS to 100 g of a 2,00 to 10,0 mm coal waste fraction was investigated by the solution deletion method of Parfitt and Rochester (1983). The SLS at concentrations of 100, 200, 500, 1000, 2000 and 3000 mg/l in 100 ml distilled water was shaken with the coal waste in 250-ml Erlenmeyer flasks on a Gerhardt RO 20 rotary shaker at 120 r.p.m. for 3 h. The aqueous phase was then clarified by filtration through Whatman no. 114 paper. The amount of SLS in the sample was determined by the colorimetric

measurement of methylene blue-SLS extracted into chloroform according to the modified Chamber of Mines procedure (this report, part 1, section 12.2.2). To investigate the effect of shaking time on the adsorption of SLS, a similar experiment was conducted with a shaking time of 24 h. Also the adsorption of SLS from 100 ml HJJ medium was investigated, using 100 g of the 2 to 10 mm coal fraction and 50 to 3000 mg/l SLS with shaking for 3 h.

Adsorption of SLS to 100 g of the 1,18 to 2,00 mm coal waste fraction was also investigated. The SLS at concentrations of 10, 20, 50, 100, 200, 300, 400, 500, 600, 700 and 800 mg/l in 100 ml distilled water was added to the coal waste and the suspension shaken at 120 r.p.m. for 3 h. After a further 10 to 15 minutes without shaking to permit the suspended coal to settle, clear supernatant was sampled and analysed for SLS.

27.2.2. Sodium benzoate

Adsorption of sodium benzoate by a 2,80 to 4,00 mm coal waste fraction was studied by the solution deletion procedure (Parfitt and Rochester, 1983). To 20 ml distilled water containing 5, 10 and 15 mg sodium benzoate in 50-ml Erlenmeyer flasks was added 1 g of coal waste. The mixtures were shaken for 1 h at 120 r.p.m. on a rotary shaker at 26°C. Supernatant (15 ml) was then removed and analysed for sodium benzoate as described in section 26.2. In a further experiment, the sodium benzoate was supplied at only 5 mg/20 ml and the coal waste concentration varied from 2 to 24 g/20 ml.

27.3. Results

27.3.1. SLS

To meet the needs of the present investigation, data were plotted as SLS adsorbed or SLS in solution against SLS supplied per 100 g coal waste in the 100 ml experimental system.

Figures 69 to 71 record SLS adsorption by the 2 to 10 mm coal fraction as determined using the solution deletion procedure. The shaking time (3 or 24 h) and adsorption of the SLS from HJJ medium rather than from water had little or no effect on SLS adsorption by the coal. With SLS supplied at 100 to 300 mg/100 ml, 72 to 86% of the SLS was adsorbed by the 100 g coal discard; with 5 to 50 mg/100 ml SLS supplied, the adsorption percentages were more variable, but $\leq$ 64% of the SLS was adsorbed. As the

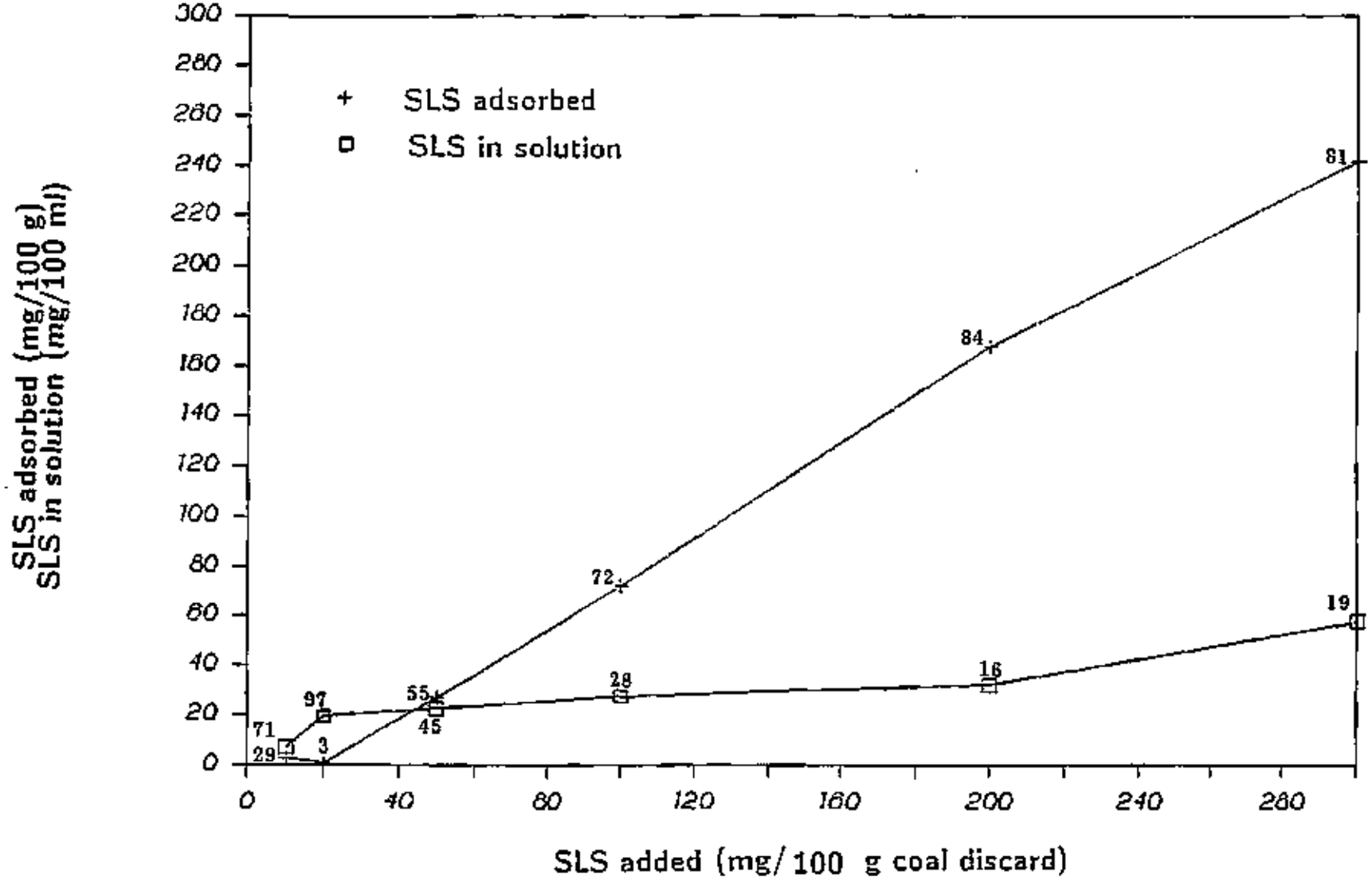


FIG. 69. Adsorption of SLS supplied at 10 to 300 mg/100 ml to 100 g coal waste (2,00 to 10,0 mm fraction) in 100 ml distilled water with shaking for 3 h. Figures alongside plotted points indicate percentage of supplied SLS that was adsorbed or remained in solution.

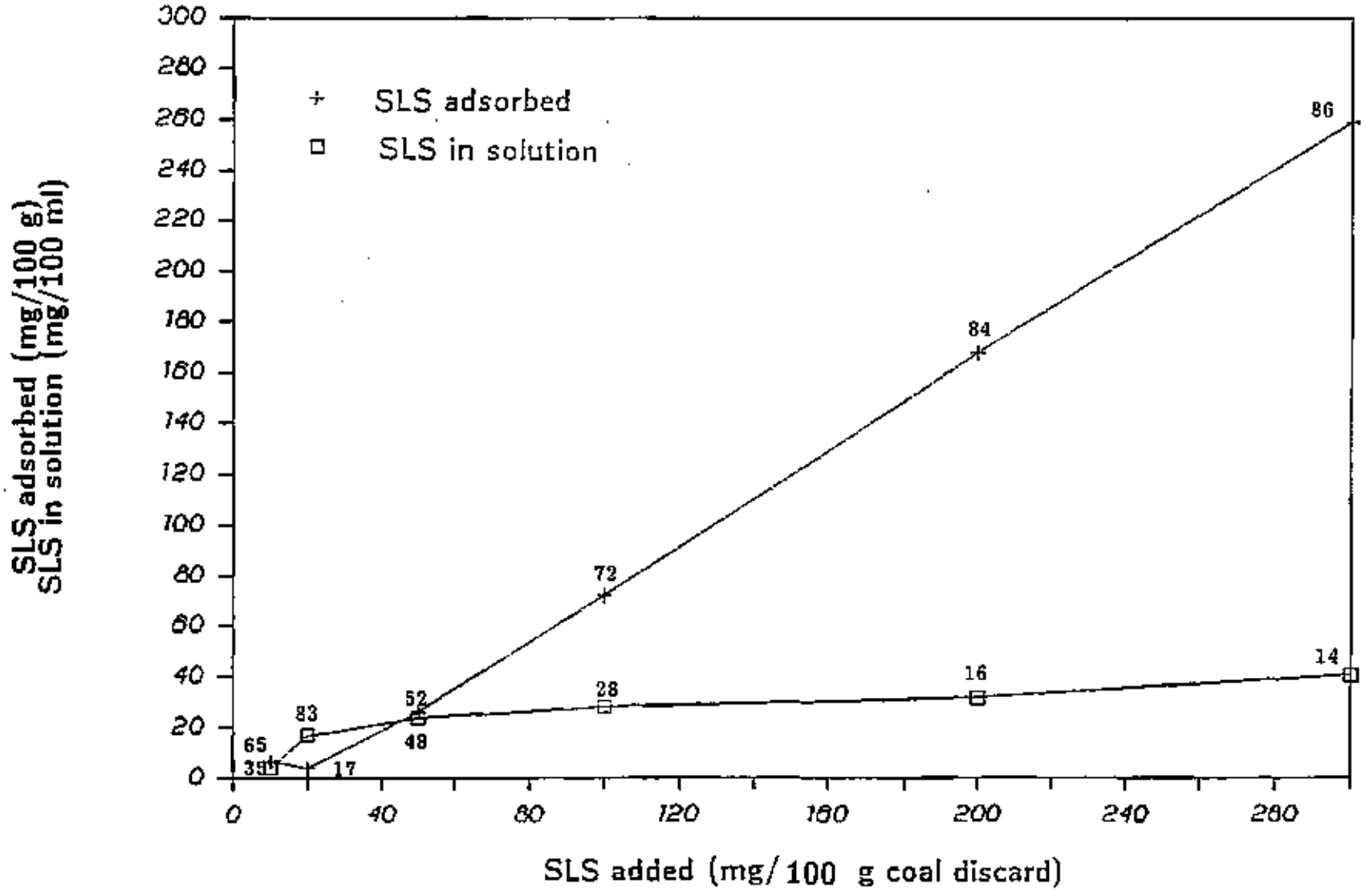


FIG. 70. Adsorption of SLS supplied at 10 to 300 mg/100 ml to 100 g coal waste (2,00 to 10,0 mm fraction) in 100 ml distilled water with shaking for 24 h. Figures alongside plotted points indicate percentage of supplied SLS that was adsorbed or remained in solution.

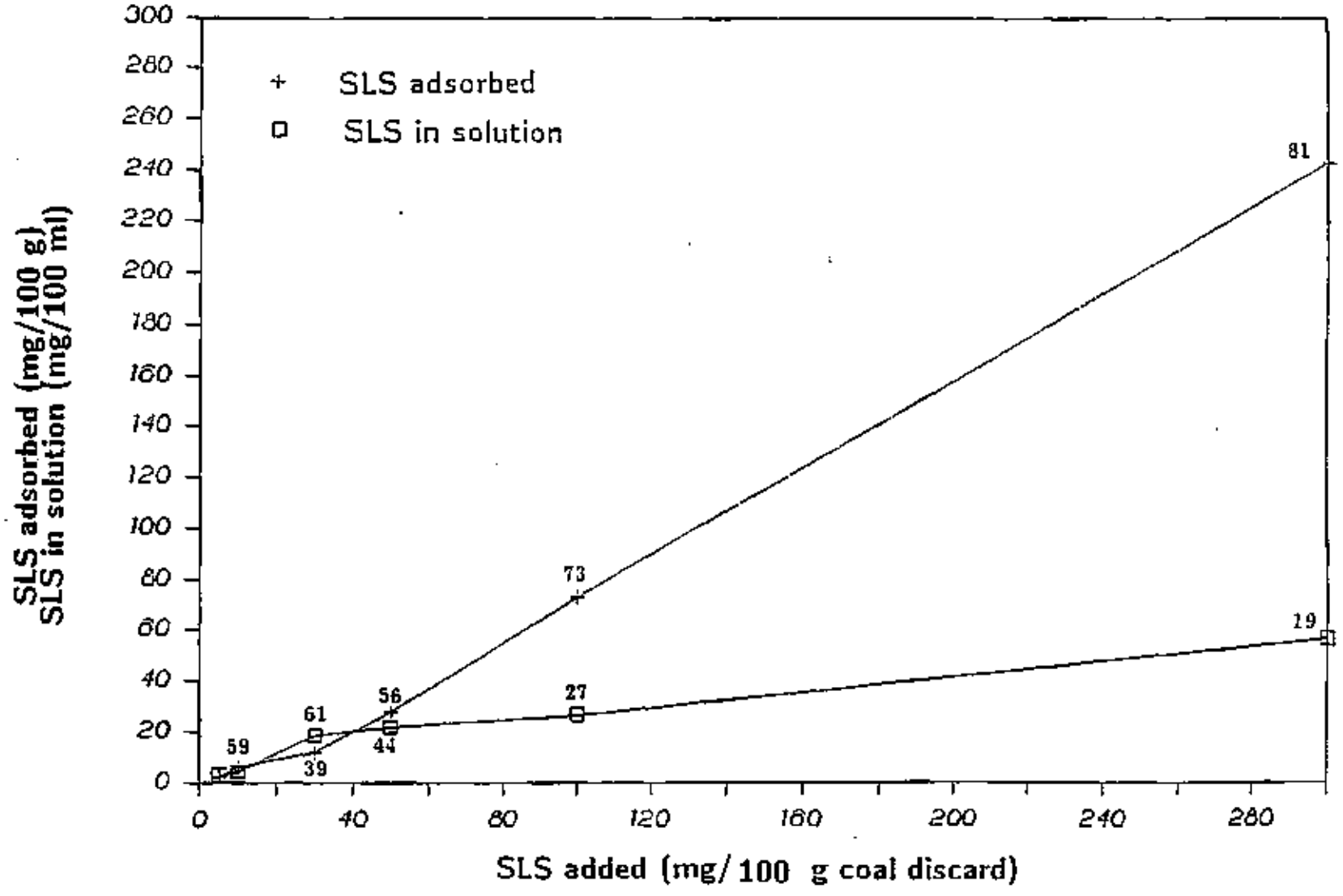


FIG. 71. Adsorption of SLS supplied at SLS 5 to 300 mg/100 ml to 100 g coal waste (2,00 to 10,0 mm fraction) in 100 ml HJJ medium. Figures alongside plotted points indicate percentage of supplied SLS that was adsorbed or remained in solution.

supplied SLS concentration decreased to below 40 to 50 mg/100 g coal waste, there was a 'cross-over' from most of the SLS being adsorbed to most remaining in solution.

With 100 g coal waste of particle size 1,18 to 2,00 mm and SLS supplied at 1 to 80 mg/100 ml, from 68 to 100% of the SLS was adsorbed (Fig. 72). This smaller particle fraction, unlike the 2 to 10 mm fraction, showd no 'cross-over' from most of the SLS being adsorbed to most remaining in solution as the SLS concentration decreased.

27.3.2. Sodium benzoate

In the adsorption study with 1 g coal waste (2,80 to 4,00 mm fraction) and 5 to 15 mg sodium benzoate in 20 ml distilled water, both the amount of sodium benzoate adsorbed and the amount in solution increased with increasing amounts of sodium benzoate supplied (Fig. 73). At all three sodium benzoate concentrations, more remained in solution (61 to 88%) than was adsorbed (12 to 39%); however, the percentage adsorbed increased and that in solution decreased as the sodium benzoate added to the system increased.

When the sodium benzoate in the system was kept constant at 5 mg/20 ml distilled water and the amount of coal waste varied (2 to 24 g/20 ml), the amount of sodium benzoate adsorbed increased as the amount of coal waste increased to 14 g/20 ml (Fig. 74). At coal waste concentrations of 14 to 18 g/20 ml, adsorbed sodium benzoate was at a maximum, then decreased when 20 to 24 g coal waste/20 ml solution was supplied. The amount of sodium benzoate in solution showed the opposite trends. However, at no coal waste concentration was more than half of the supplied sodium benzoate adsorbed. Plotting of the data as g sodium benzoate adsorbed/g coal waste versus g coal waste/20 ml solution yielded the curve shown in Fig. 75, i.e. as the coal waste concentration increased, the increments of sodium benzoate adsorbed/g coal waste became less and less.

27.4. Discussion

27.4.1. SLS

The data of Fig. 69 to 71 suggest a tendency for at least 20 mg SLS to remain in the 100 ml solution and most of the rest to be adsorbed to the coal waste (2,0 to 10,0 mm fraction) This was the situation whether the suspending medium was water, with the

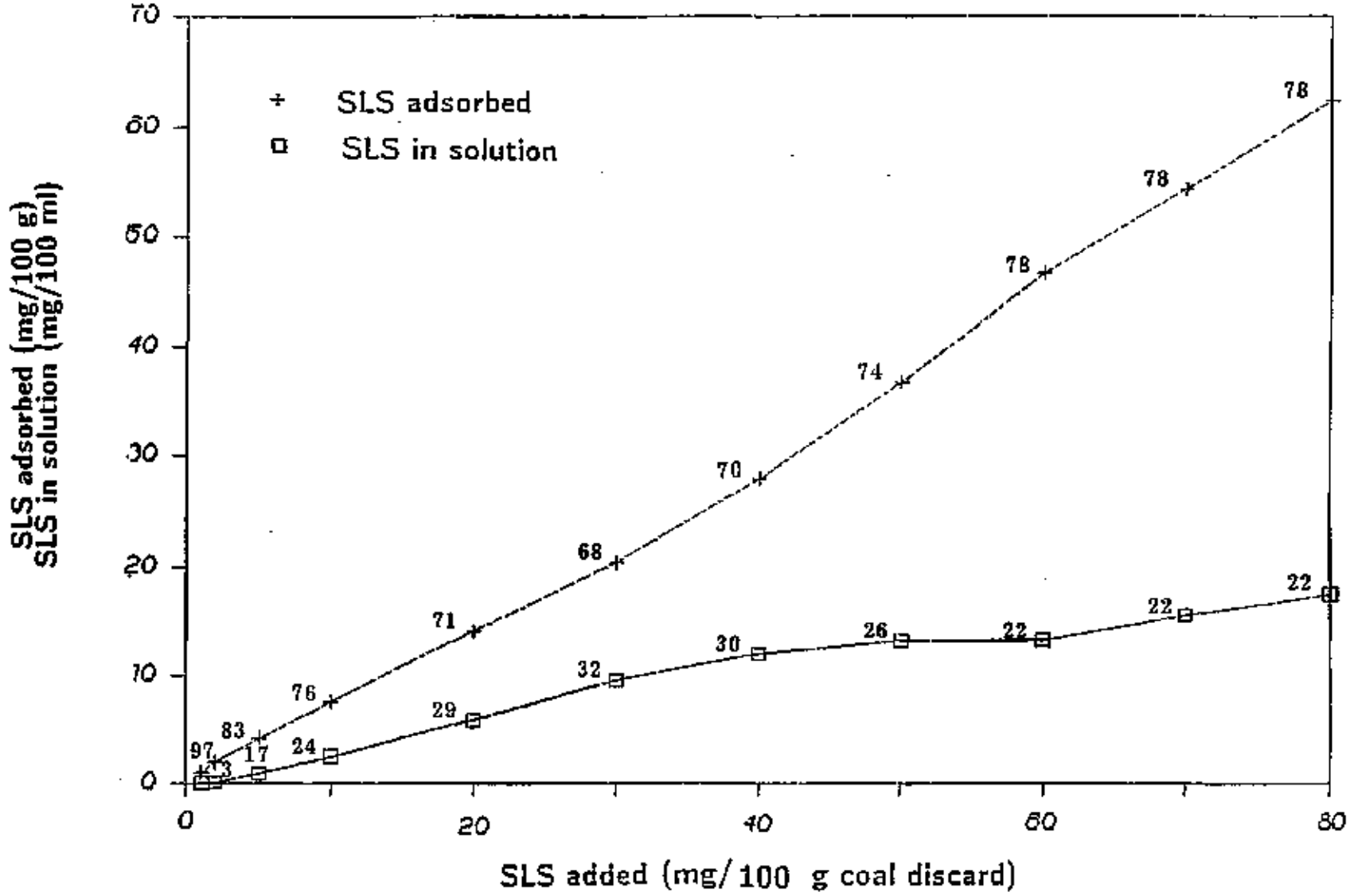


FIG. 72. Adsorption of SLS supplied at 1 to 80 mg/100 ml to 100 g coal waste (1,18 to 2,00 mm fraction) in 100 ml distilled water. Figures alongside plotted points indicate percentage of supplied SLS that was adsorbed or remained in solution.

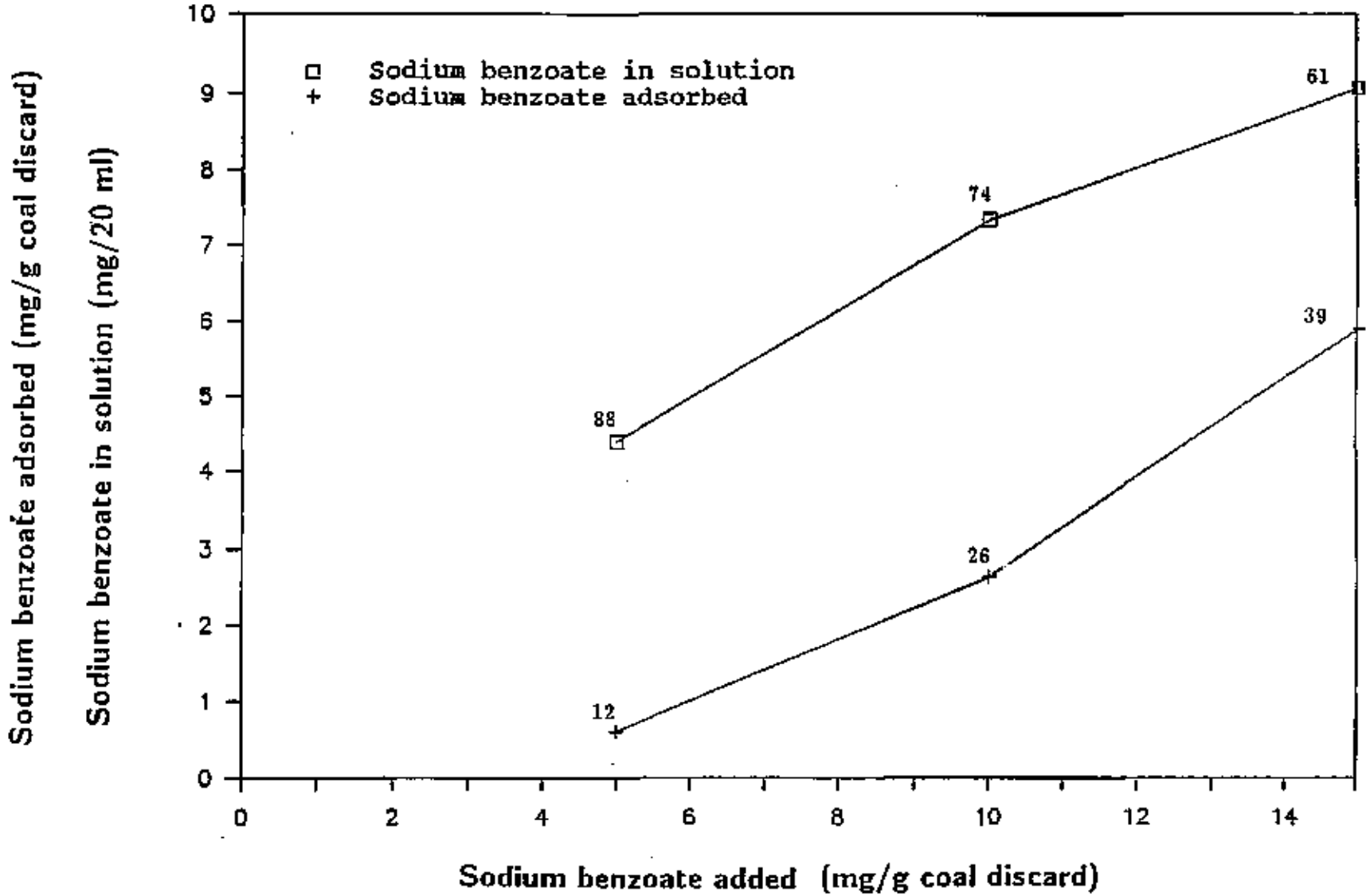


FIG. 73. Adsorption of sodium benzoate supplied at 5 to 15 mg/20 ml to 1 g coal waste (2,80 to 4,00 mm fraction) in 20 ml distilled water. Figures alongside plotted points indicate percentage of supplied sodium benzoate that was adsorbed or remained in solution.

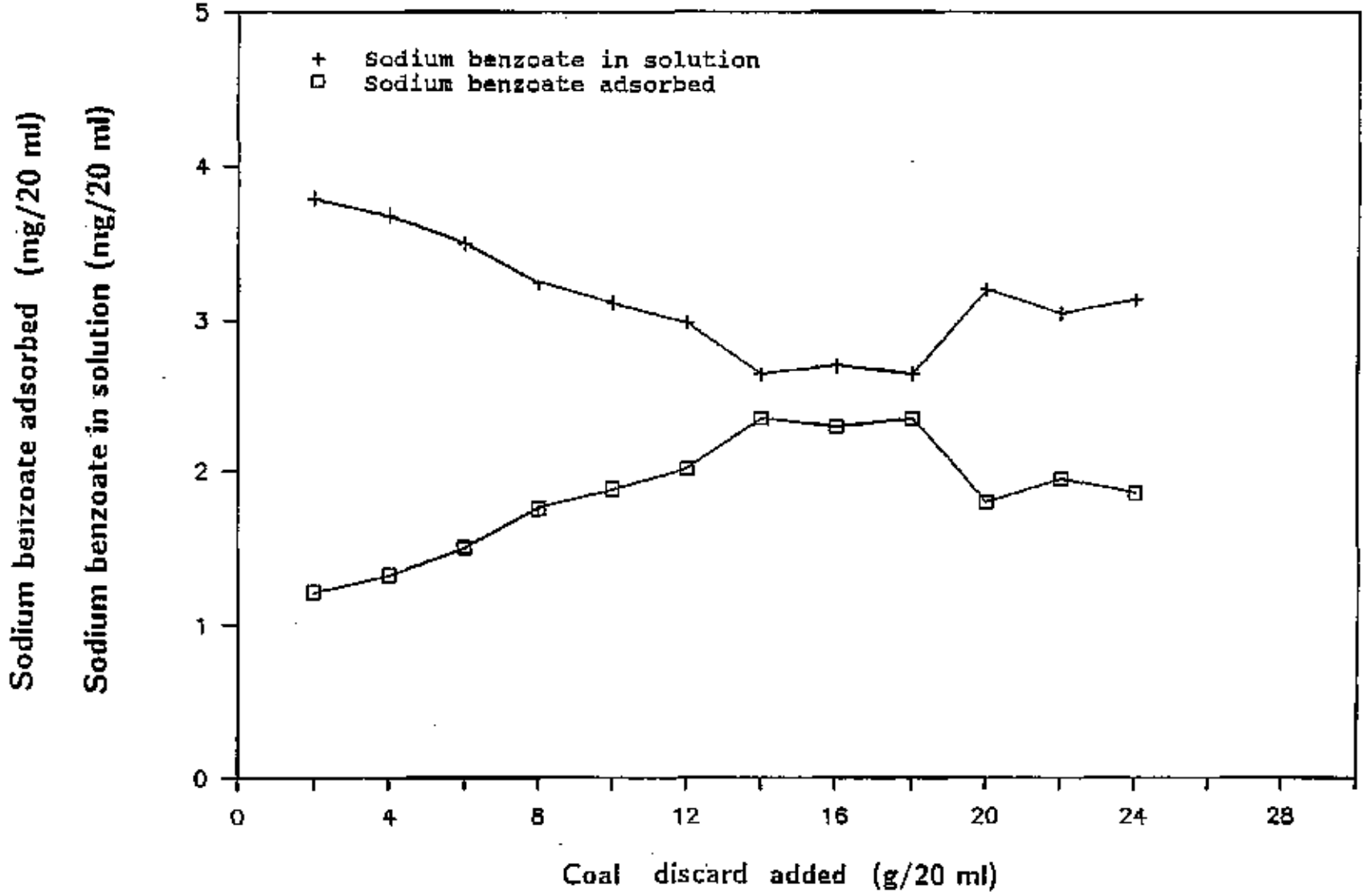


FIG. 74. Adsorption of sodium benzoate supplied at 5,00 mg/20 ml by 2 to 24 g coal waste (2,80 to 4,00 mm fraction) in 20 ml distilled water.

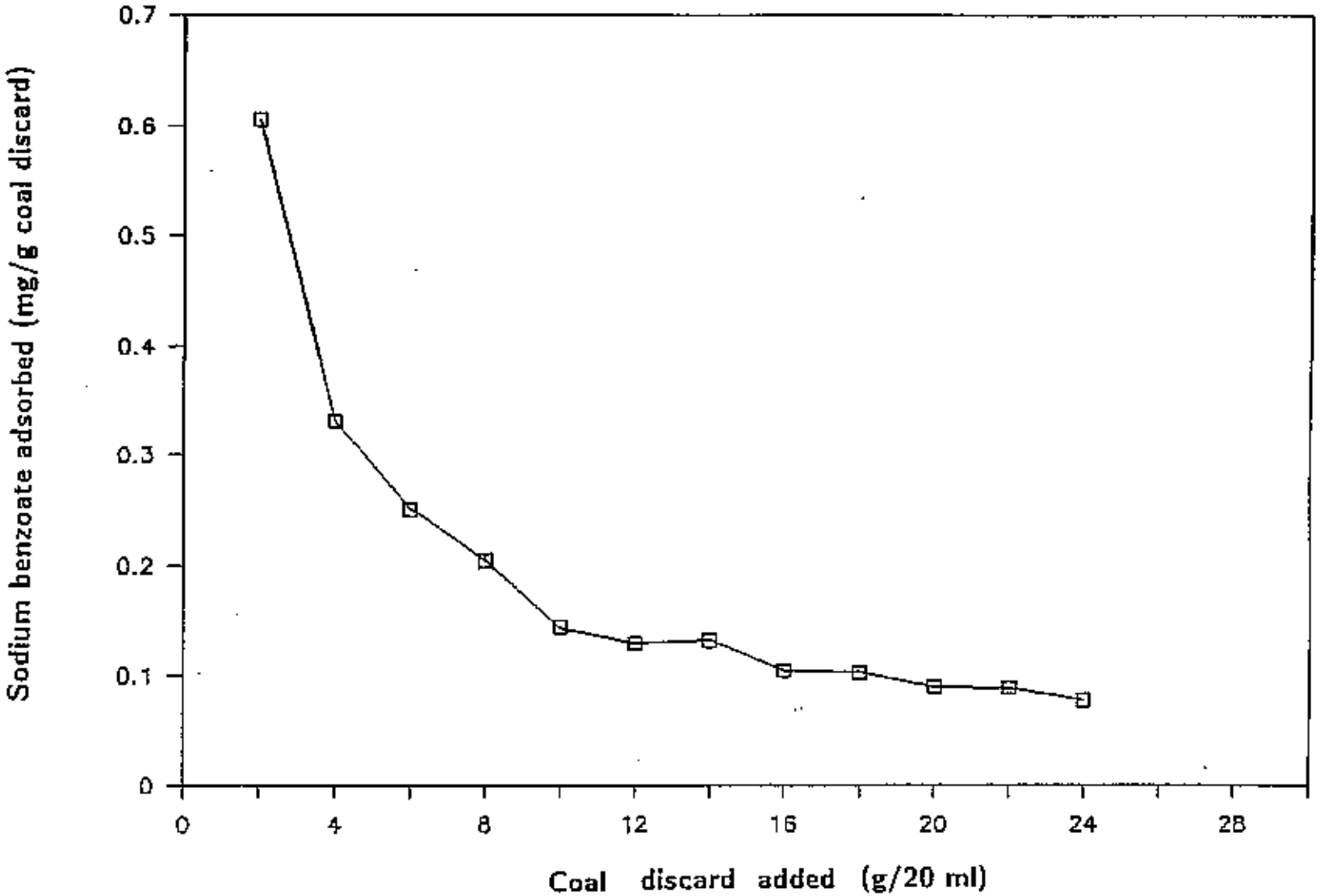


FIG. 75. Adsorption of sodium benzoate supplied at 5 mg/20 ml, by 2 to 24 g coal waste (2,80 to 4,00 mm fraction) in 20 ml distilled water, plotted as mg sodium benzoate adsorbed/g coal waste.

adsorption test mixtures shaken for 3 or 24 h, or whether it was HJJ medium. Thus, as the SLS in the system increased, the concentration in solution increased only slowly and the adsorbed SLS much more rapidly. This was reflected by increases in the percentage adsorbed and decreases in the percentage in solution as the concentration of SLS supplied increased. At the lowest concentrations of SLS supplied (50, 100 and 200 mg/l), the analytical data were rather variable, but the highest percentage of SLS adsorbed was 64% and adsorption of less than half the SLS was common among the samples. In the laboratory studies of SLS inhibition of *T. ferrooxidans* strains with coal waste fractions in the cultures (section 20), inhibitory SLS concentrations were between 50 and 100 mg/l (Table 12), i.e. in the concentration region where less than two thirds of the SLS should have been adsorbed by the larger fractions (> 2 mm). It is thus surprising that the minimum inhibitory concentrations of SLS were so high in the presence of these fractions, i.e. at least about 20 times as high as the minimum inhibitory concentrations in the absence of coal waste. This increase seems too high to be explained only by adsorption to the coal waste.

The results plotted in Fig. 72 show higher percentages of adsorption of SLS from water by the 1,18 to 2,0 mm coal fraction than by the 2,0 to 10,0 mm fraction at SLS concentrations of $\leq$ 80 mg/100 ml. At no SLS concentration was the amount adsorbed less than that in solution. Supplied SLS at 50 to 100 mg/l produced residual SLS in solution at concentrations of 9 to 24 mg/l. In the inhibition experiments of Conradie (1984; this report, part 1, section 5), 2 to 4 mg/l SLS in HJJ medium were adequate to inhibit the growth of *T. ferrooxidans* WLR and ATCC 19859. These concentrations should be achieved at much lower concentrations of SLS supplied than the 70 to 90 mg/l needed to inhibit the *T. ferrooxidans* strains in 100 ml HJJ medium containing 50 g coal waste (section 20, Table 12). This discrepancy confirms the conclusion reached in the previous paragraph, that other factors, besides adsorption to the coal waste, play a role in determining the marked decrease in effectiveness of SLS as an inhibitor of *T. ferrooxidans* in the presence of coal waste.

The observation from Fig. 69 to 71 that the adsorption mixtures seemed to have a requirement for ca. 200 mg/l SLS in solution before much adsorption took place and that thereafter the SLS in solution increased only slowly with increases in the SLS added to the system (to 409 to 580 mg/l for 3000 mg/l SLS supplied), may explain the levels of SLS in the effluent leaving test pile 7 when the inhibitor was flushing out (section 26, Fig. 64). From when the flush started at day 187, until day 276 before the onset of the

wet season and consequent more variable supply of water to the dumps, the SLS concentrations in the effluent samples were in the range 280 to 500 mg/l. Such concentrations, which are well above inhibitory levels, should be expected in the solution in SLS-saturated coal waste.

27.4.2. Sodium benzoate

Figure 73 shows that with a low concentration of coal waste in the adsorption mixture (5 g/100 ml) very little sodium benzoate (12%) was adsorbed from a solution containing 250 mg/l, rising to 39% as the sodium benzoate concentration increased to 750 mg/l. With higher concentrations of coal waste and with sodium benzoate supplied at 250 mg/l, the percentage adsorbed increased to a maximum of ca. 48% at coal waste concentrations of 70 to 90 g/100 ml (Fig. 74). At a coal waste concentration of 50 g/100 ml, as used in the inhibition studies in section 20, ca. 38% of the sodium benzoate was adsorbed. Although the sodium benzoate concentration was higher than the inhibitory concentrations of 40 to 75 mg/l for the two *T. ferrooxidans* test strains (Table 13), it seems likely that less than half of these concentrations would have been adsorbed on the coal waste. At least 20 to ca. 40 mg/l would thus have been available for inhibition of the bacteria, which agrees with the 20 to 30 mg/l shown to be necessary in experiments without coal waste in the cultures (this report, part 1, section 7).

The forms of the adsorption graphs in Fig. 74 and 75 indicate a decreasing benzoate adsorption/g coal waste as the coal waste concentration in the system increased (Fig. 75), to the extent that adsorption by the total amount of coal waste in the system passed through a maximum into negative adsorption (Fig. 74). This suggests that as the coal waste particles became tightly packed, they interacted to exclude the benzoate from adsorption. A possible model is that benzoate ions are weakly adsorbed through their aromatic ring to hydrocarbon moieties at the coal waste surfaces, in spite of repulsion of their carboxyl groups (if dissociated and negatively charged) by carboxyl groups on the coal surface. As the coal waste concentration increases, forcing the particles closer together, the intervening solution would be exposed to a greater concentration of coal waste carboxyl groups and an intensification of negative charge, which would eventually become adequate to exclude benzoate ions, increasing their concentration in the solution further removed from the coal. This behaviour of the benzoate is in marked contrast to that of SLS, which has a highly hydrophobic aliphatic hydrocarbon tail and is strongly adsorbed to the coal waste (section 27.3.1). The amount of sodium benzoate adsorbed

never exceeded 50% of that added (Fig. 74), whereas ca. 72% of the SLS added to suspensions of coal waste at 100 g/100 ml was adsorbed (section 27.3.1). The observation that at a similar coal waste concentration more than half of the added sodium benzoate remained in solution, makes sodium benzoate seem promising as an inhibitor for treatment of coal discard.

28. CONCLUSIONS

Little information is available on the extent and environmental impact of acid mine drainage from coal waste dumps in South Africa. The only two reported investigations, for river systems in Natal (Kemp, 1962; Rudd, 1973), show high concentrations of total dissolved solids, in particular sulphate, in rivers leaving coalfields, but limited declines in the pH of the initially mildly alkaline water and reductions in the salt concentrations downstream following dilution and self purification. However, with the large-scale development of open-pit mines, particularly on the Transvaal highveld for electricity generation and the mining of export coal, the acid drainage problem and its prevention must receive continuous attention. At all costs, the extensive acidification of rivers, as has occurred in the eastern U.S.A., must be avoided.

A limited study of coal waste dumps near Witbank showed the occurrence of acid drainage water in the lower parts of the dumps and in a pool alongside one of the dumps, associated with the presence of large populations of chemolithotrophic ferrous iron-oxidizing bacteria, presumed to be *T. ferrooxidans* (section 17). Thus a drainage water sample of pH 2,40 and three coal waste samples of pH 2,40 to 3,88 from the Douglas Colliery dump contained $4{,}64 \times 10^{3}$ to $2{,}83 \times 10^{6}$ iron-oxidizing bacteria/ml or g. These samples were from an acid drainage pool, a waterlogged bank near the drainage pool and from the bottom of a rubble slope. Four of seven samples from a dump on the Wolvekrans Section of the Douglas Colliery also contained iron-oxidizing bacterial populations ($3{,}00 \times 10^{-1}$ to $2{,}80 \times 10^{6}$/g). The four samples were coal waste of pH 2,85 to 7,54 from a flat drainage area at the toe of the dump, the bottom of the dump slope, 15 m (vertical height) up the slope and from ore with a high pyrite content on the top of the dump. No iron-oxidizing bacteria were detected in freshly deposited coal waste of pH 6,98 or drainage water of pH 7,99 from the Wolvekrans dump or in burned waste of pH 12,30 from the Douglas dump. If the iron-oxidizing bacteria and the associated oxidation of pyrite, which produces the acid mine drainage, are restricted mainly to the outer 25 to 30 cm of the dumps as is the situation elsewhere (Dugan, 1975; Good et al., 1970), the possibility exists for inhibiting acid mine drainage formation by the application of suitable antibacterial chemicals.

While developing a suitable experimental system for testing the inhibition of *T. ferrooxidans* cultures by chemicals in the presence of coal waste, we found that coal waste fractions of differing particle size up to 11,5 mm diameter, had a profound effect on the ferrous iron concentration and pH of the growth medium (section 18) necessitating the inclusion in growth and inhibition experiments of uninoculated medium

controls containing the coal fraction under study. The almost neutral (pH 6,34) coal waste fractions raised the pH of the medium and catalysed the disappearance of ferrous iron. Culture growth in the absence of inhibitors was adversely influenced by the pH increases caused by these fractions, but also by additional inhibitory effects of the coal waste (section 19).

In the presence of 500 g/l coal discard fractions of differing particle size (0,425 to 1,18, 2,00 to 2,80, 4,00 to 4,75 and 8,00 to 9,50 mm diameter), two *T. ferrooxidans* strains were inhibited by SLS at 70 to 90 mg/l, added at the beginning of culture incubation or during the exponential phase of culture growth (section 20). These concentrations were very much higher than the inhibitory concentrations of 2 to 4 mg/l SLS for cultures not containing coal waste. In the presence of the 2,00 to 2,80, 4,00 to 4,75, 8,00 to 9,50 mm coal waste fractions and a 10,0 to 11,5 mm fraction, sodium benzoate and sorbic acid were both inhibitory at 40 to 75 mg/l when added at the beginning or during the exponential phase of growth (section 20). In the absence of coal waste, the inhibitory concentrations of sodium benzoate were 15 to 30 mg/l and those of sorbic acid 15 to 20 mg/l. The various coal waste fractions had little or no differential effect on the inhibitory concentrations. These experiments showed that sodium benzoate and sorbic acid might be even better than SLS for the inhibition of *T. ferrooxidans* and the associated formation of acid in coal waste. Sodium benzoate has the additional advantage of being a much cheaper chemical than the other two.

In inhibition experiments with no coal waste in the culture medium, fresh solutions of 1-bromo-3-dichloro-5,5-dimethylhydantoin (H900) inhibited *T. ferrooxidans* at 6 to 10 mg/l (section 21). Ageing had a detrimental effect on the inhibitory concentration, so that not even 14 mg/l of a 6-month-old solution was inhibitory. The instability of H900 over time makes it unsuitable for use in coal discard. Combinations of SLS with sodium benzoate, sorbic acid and H900 and of sodium benzoate with sorbic acid caused no substantial reductions in the inhibitory concentrations of any of the inhibitors tested (section 21).

The treatment of pilot-scale dumps containing 55 t of coal waste at the Wolvekrans mine by the application of 1 kg dissolved SLS and 0, 5, 10 or 15 kg natural or polyisoprene rubber pellets containing 35% SLS was inadequate to inhibit acidification, which occurred in all dumps within 3 months (section 22). Further treatment of the dumps after about 15 months with much higher doses of SLS and SLS-rubber pellets (5 kg dissolved SLS and 0, 10, 30 and 60 kg of the natural or polyisoprene rubber pellets with 35% SLS, to give total SLS treatments of 6,00, 11,25, 20,00 and 32,25 kg SLS/dump)

failed to inhibit the acid, dissolved iron and sulphate production through the following 21 months. Very little of the applied SLS left the dumps and the concentrations in the effluents, which reached a maximum of only about 30 mg/l, were inadequate to prevent the survival or possibly development of high populations of chemolithotrophic iron-oxidizing bacteria in the effluent water.

Investigations of factors possibly responsible for the lack of success of the SLS treatments in controlling acidification of the pilot scale coal waste dumps, showed the presence in the dump effluents of populations of iron-oxidizing bacteria with resistance to 12 to 14 mg/l SLS (section 23), and the ability of cultures of *T. ferrooxidans* to greatly increase their resistance to SLS (from being inhibited by 2 or 4 mg/l to growing in medium with 8 or 18 mg/l, depending on the *T. ferrooxidans* strain). The acid effluents from the dumps also contained heterotrophic filamentous fungi and yeasts, among which SLS-degrading yeasts were detected (section 24). Slow or very slow release of SLS from the SLS-rubber pellets and a slow downward movement of the inhibitor in the dumps, presumably involving adsorption, resulted in only low concentrations of SLS in the coal waste (< 14 mg water-extractable SLS/kg) in the lower 0,7 to 1,5 m of dumps 2 and 3 with natural rubber pellets and throughout the dumps with SLS-polyisoprene rubber pellets (section 25). These low SLS concentrations permitted the iron-oxidizing bacteria to establish populations of ca. 10^2 to 10^6/g in the coal waste. By contrast, in the upper 0,45 to 0,55 m of dumps 2 and 3, where the water-extractable SLS concentrations were > 40 mg/kg, no or very few iron-oxidizing bacteria were found. This result shows that application of SLS at a rate of 20 kg/dump or 0,6 kg/m^2 could inhibit iron-oxidizing bacteria in the coal waste to a depth of ca. 0,5 m, hence may be an adequate treatment for a dump with an oxygen barrier at ≤ 0,5 m depth. However, the cost of the SLS, excluding transport and application, for this treatment at the 1990 price would be R58 080/ha.

In a further pilot scale study, SLS and sodium benzoate were compared as inhibitors of acid drainage production by the 55-ton coal waste dumps at the Wolvekrans mine (section 26). Treatments were the addition of 2 kg SLS or 0,2 or 2 kg sodium benzoate/dump every 2 weeks for ca. 10 months. From 188 days, the dumps receiving SLS and the high dose of sodium benzoate appeared to have their adsorption sites for the inhibitors saturated, as indicated by (with few exceptions) high levels of inhibitor in the effluents. Most of these effluents showed no iron-oxidizing bacteria, in contrast to high populations (mainly 10^2 to 10^6/ml) before 188 days and continuing high populations in the effluents from the dumps receiving the low benzoate dose or water only. The amounts of SLS and sodium benzoate giving the presumed saturation were, respectively,

600 and 500 mg/kg coal waste. If one third of these doses were applied to large dumps to saturate the outer 0,5 m of coal waste (to inhibit acidification outside the oxygen barrier) the application rates would be ca. 3 000 and 2 600 kg/ha and the cost of the chemicals (transport and application costs excleded) at 1990 prices R29 040 and R12 922/ha for SLS and sodium benzoate, respectively. However, none of the dumps showed clear evidence of reduced acidification resulting from the inhibitor treatments, although the dump receiving the high dose of benzoate yielded lower levels of dissolved iron and sulphate than the other dumps (unfortunately from the beginning of the experiment, with no change when the dump became saturated with benzoate).

Limited basic studies of the adsorption of SLS and sodium benzoate by coal waste (section 27) showed higher levels of SLS adsorption than benzoate adsorption, except perhaps at SLS concentrations below 40 to 50 mg/100 g coal waste of diameter 2,0 to 10,0 mm in 100 ml water or HJJ medium. Under these conditions less SLS was adsorbed than remained in solution, but with higher SLS concentrations supplied, the adsorption ranged from 72 to 86%. With a smaller coal fraction (1,18 to 2,00 mm), more than 68% of the SLS was adsorbed at all concentrations supplied. By contrast, less sodium benzoate was adsorbed by a 2,80 to 4,00 mm coal waste fraction than remained in solution with all sodium benzoate-coal waste combinations that we tested. Adsorption to coal waste can explain the effect of the waste on the minimum inhibitory concentrations of sodium benzoate for two *T. ferrooxidans* strains in the culture inhibition experiments of section 20, but not the much greater effect of the waste on the minimum inhibitory concentrations of SLS. Further comparative studies of the adsorption of SLS and benzoate to coal waste are required, as possible lower levels of adsorption of sodium benzoate may be another reason for choosing it as an inhibitor in preference to SLS.

The overall conclusion from these laboratory and pilot scale studies on coal waste is that sodium benzoate seems to have the best potential of the inhibitors studied to inhibit acidification of the pyrite. However, the 1990 price of sufficient chemical, without transport and application, would be about R12 922/ha and the effectiveness of the treatment remains to be proved.

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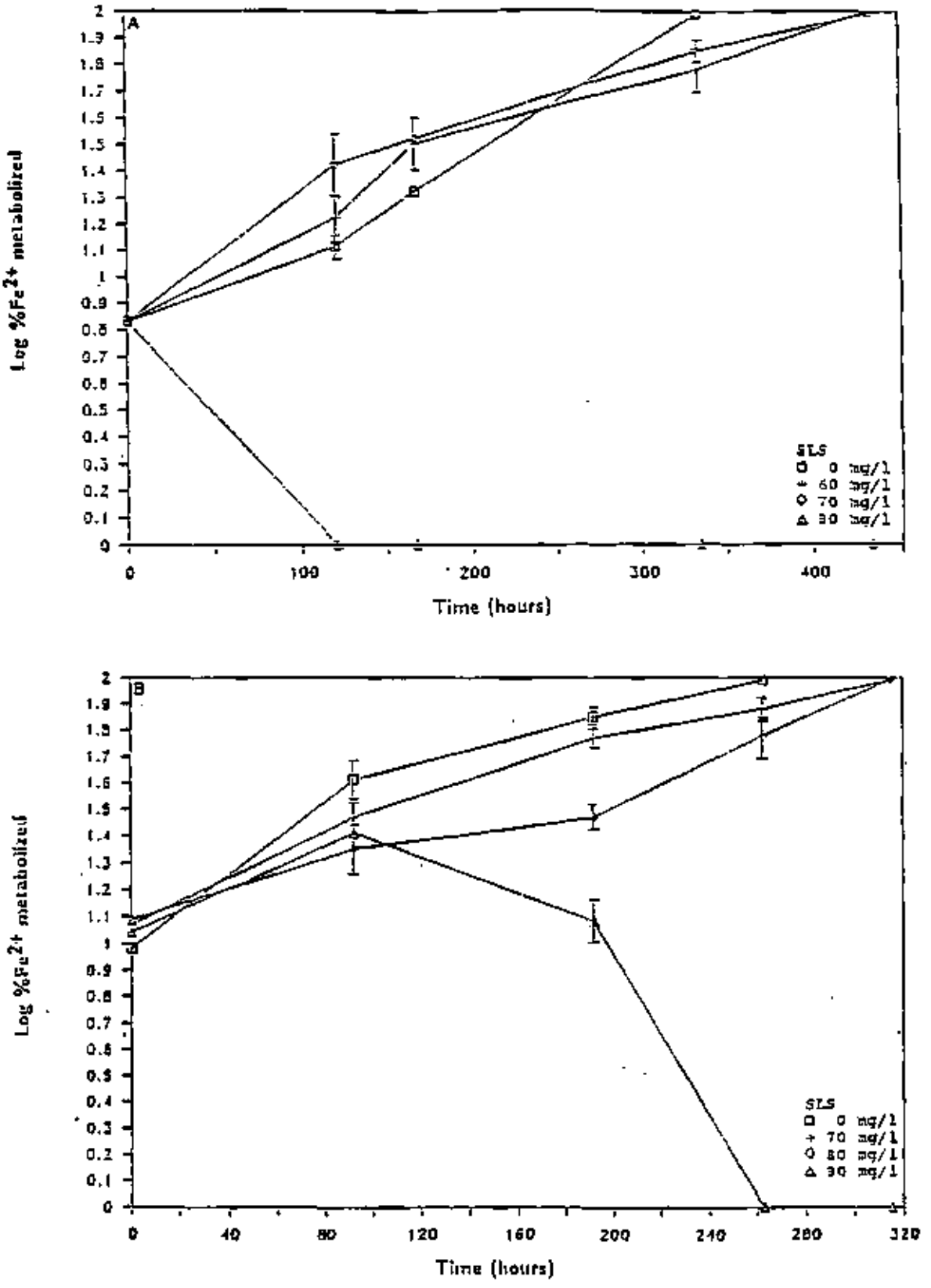
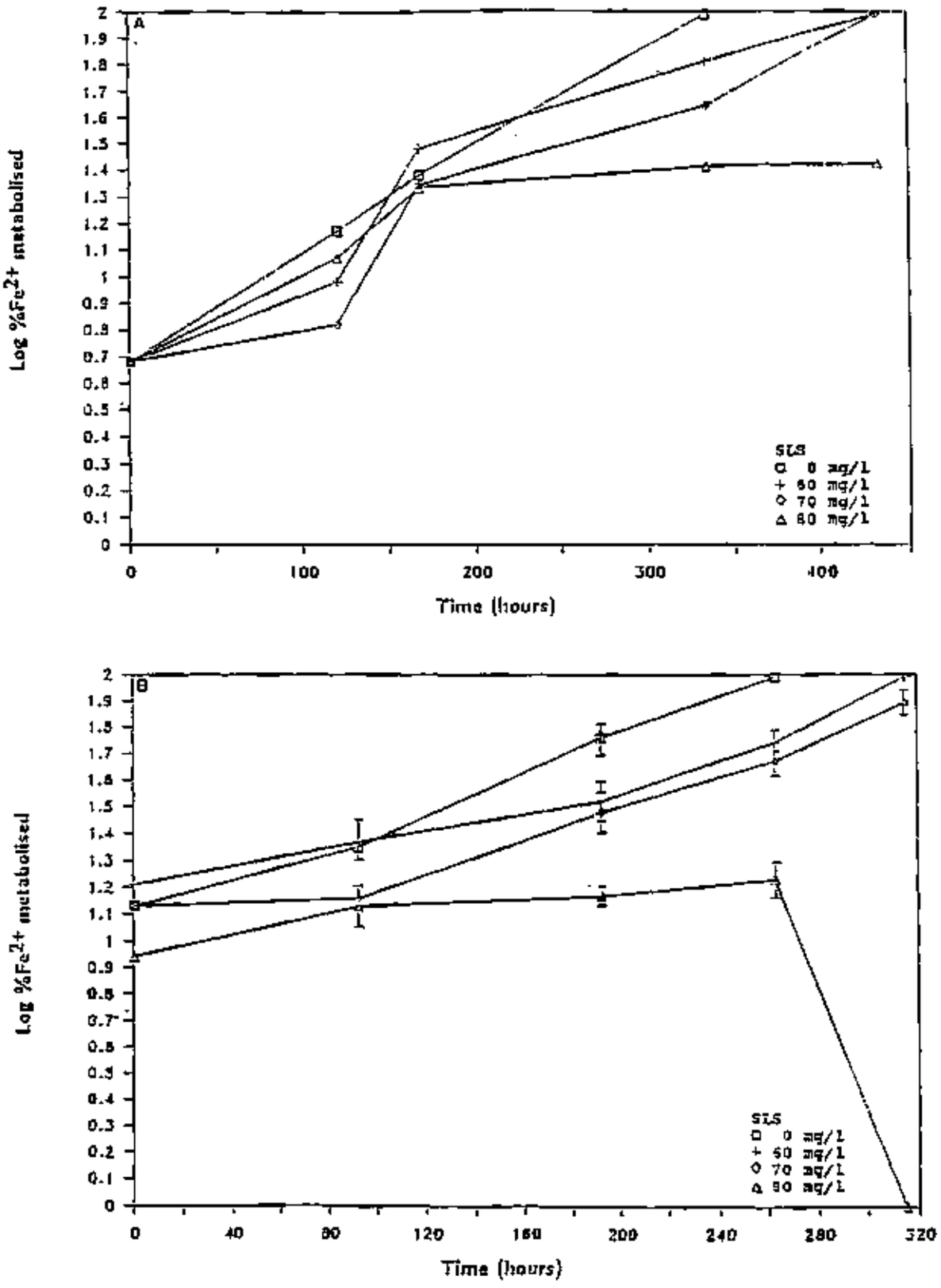
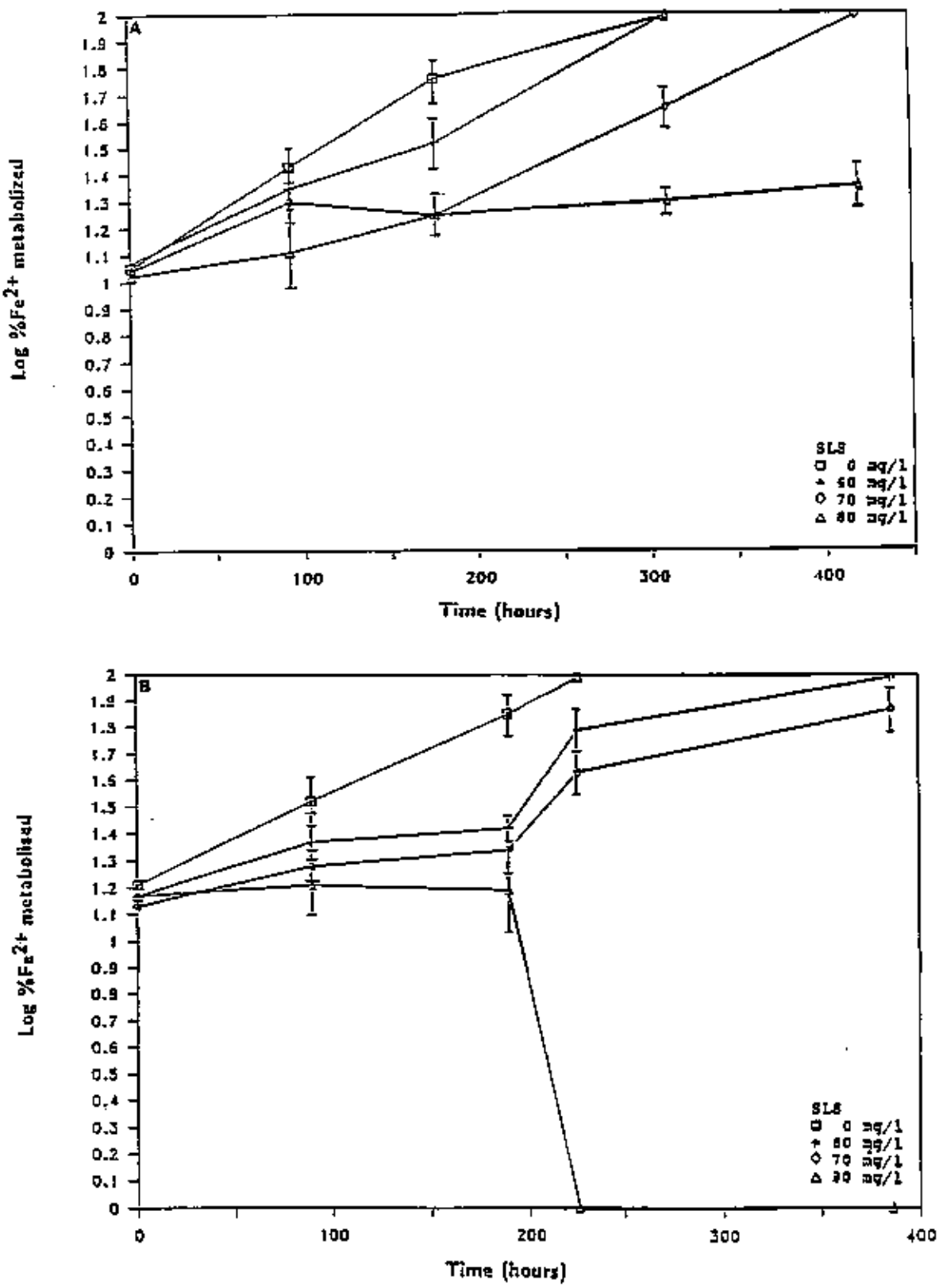
30. APPENDIX 2: COMPLETE RESULTS OF LABORATORY STUDIES OF CHEMICAL INHIBITION OF FERROUS IRON OXIDATION BY *T. FERROOXIDANS* IN HJJ MEDIUM CONTAINING COAL WASTE FRACTIONS



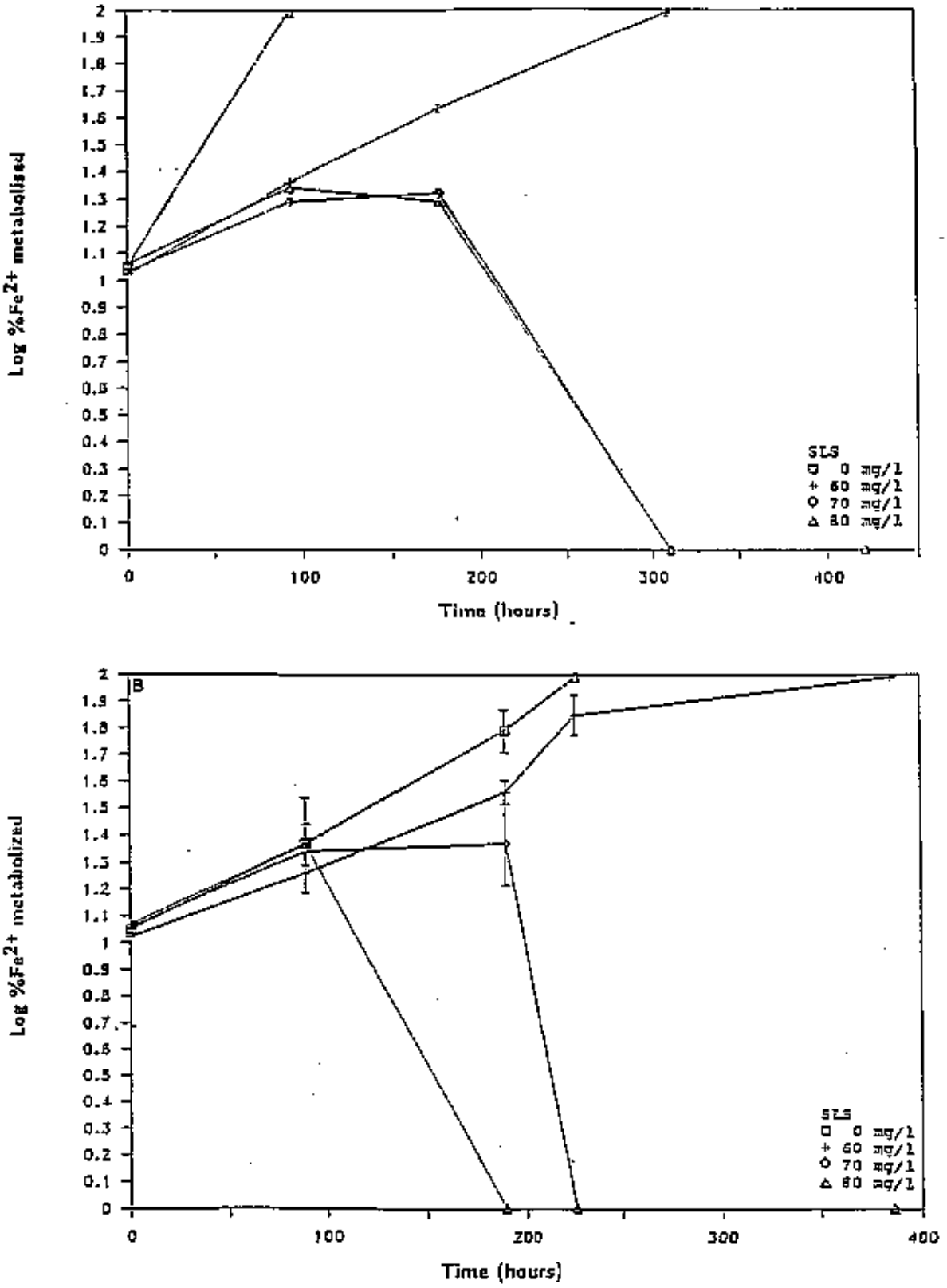
APPENDIX FIG. 59. Effect of SLS on ferrous iron oxidation by *T. ferrooxidans* ATCC 19859 in HJJ medium containing 500 g/l coal waste (0,425 to 1,18 mm fraction) when the inhibitor was added (A) at the beginning of incubation of the cultures or (B) during the exponential phase of culture growth (91 hours).



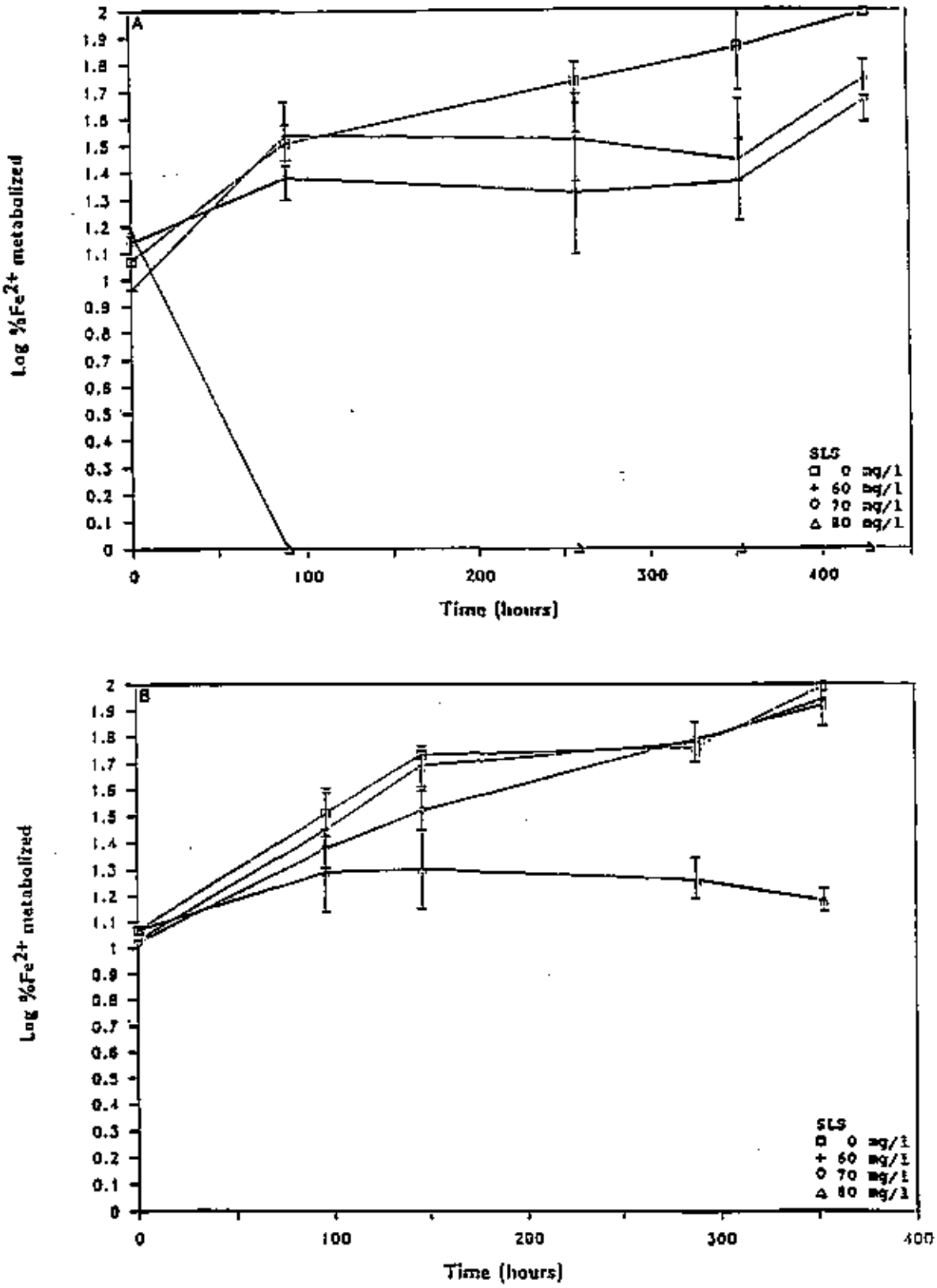
APPENDIX FIG. 60. Effect of SLS on ferrous iron oxidation by *T. ferrooxidans* ATCC 19859 in HJJ medium containing 500 g/l coal waste (2,00 to 2,80 mm fraction) when the inhibitor was added (A) at the beginning of incubation of the cultures or (B) during the exponential phase of culture growth (91 hours).



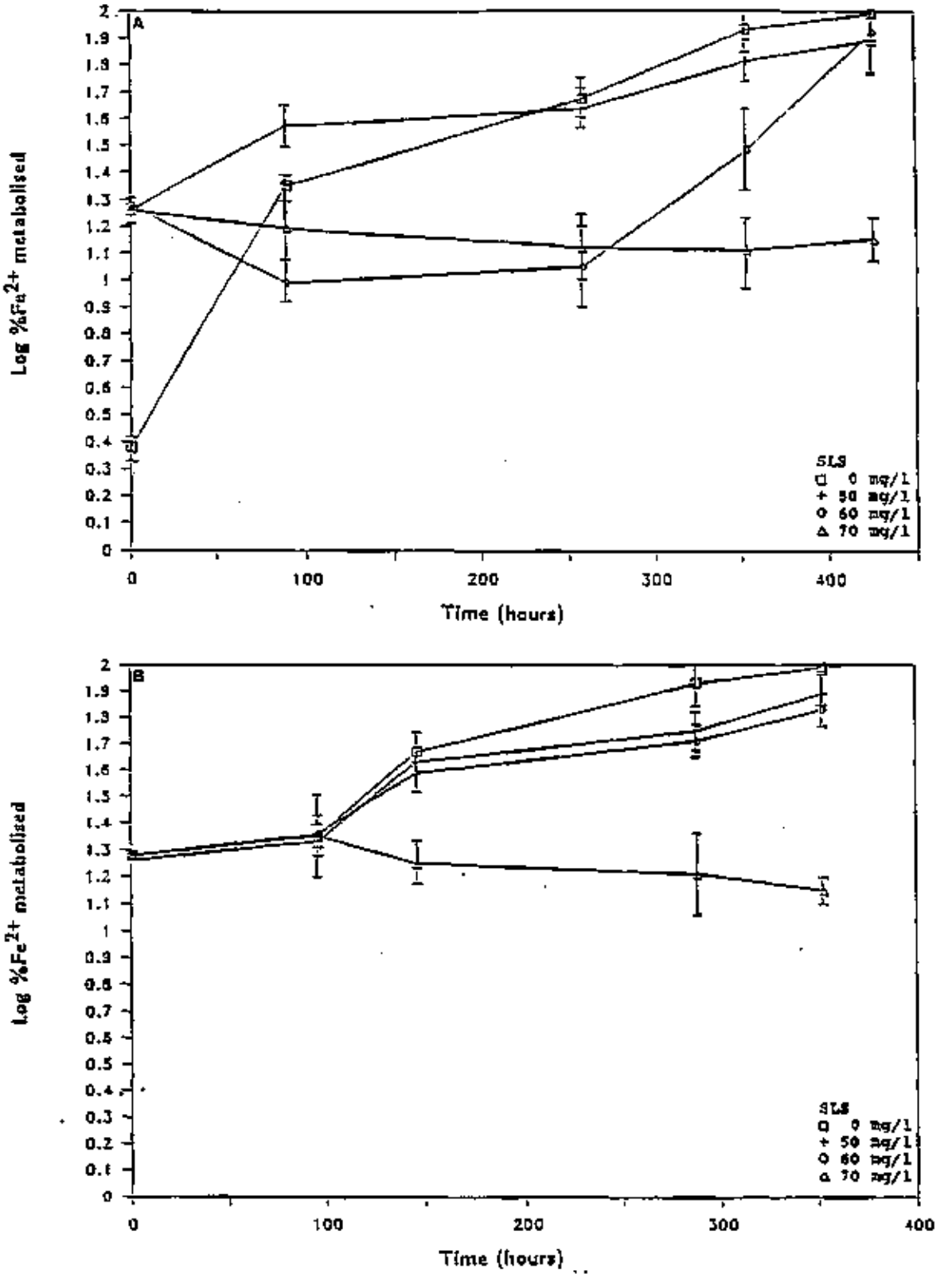
APPENDIX FIG. 61. Effect of SLS on ferrous iron oxidation by *T. ferrooxidans* ATCC 19859 in HJJ medium containing 500 g/l coal waste (4,00 to 4,75 mm fraction) when the inhibitor was added (A) at the beginning of incubation of the cultures or (B) during the exponential phase of culture growth (91 hours).



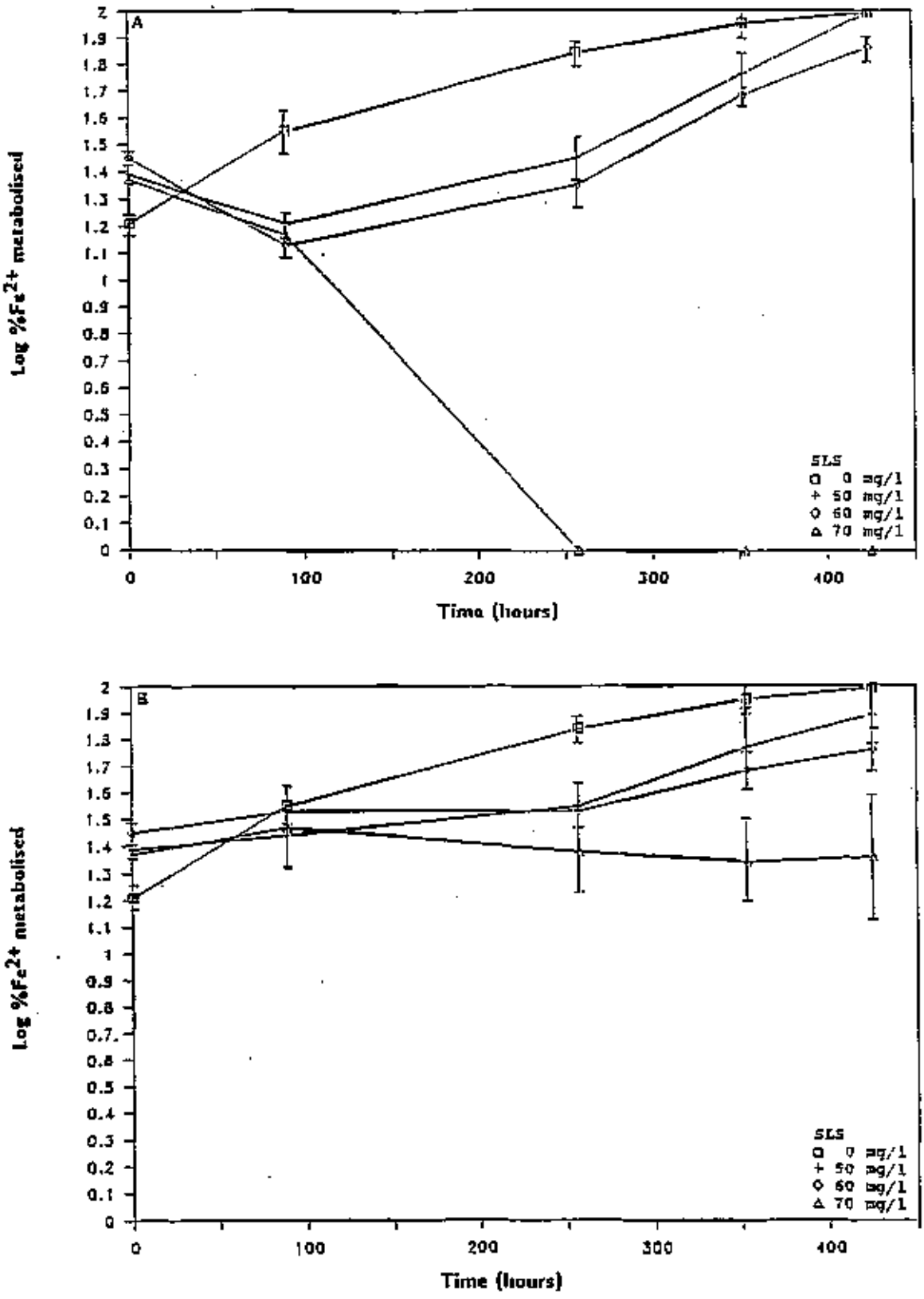
APPENDIX FIG. 62. Effect of SLS on ferrous iron oxidation by *T. ferrooxidans* ATCC 19859 in HJJ medium containing 500 g/l coal waste (8,00 to 9,50 mm fraction) when the inhibitor was added (A) at the beginning of incubation of the cultures or (B) during the exponential phase of culture growth (91 hours).



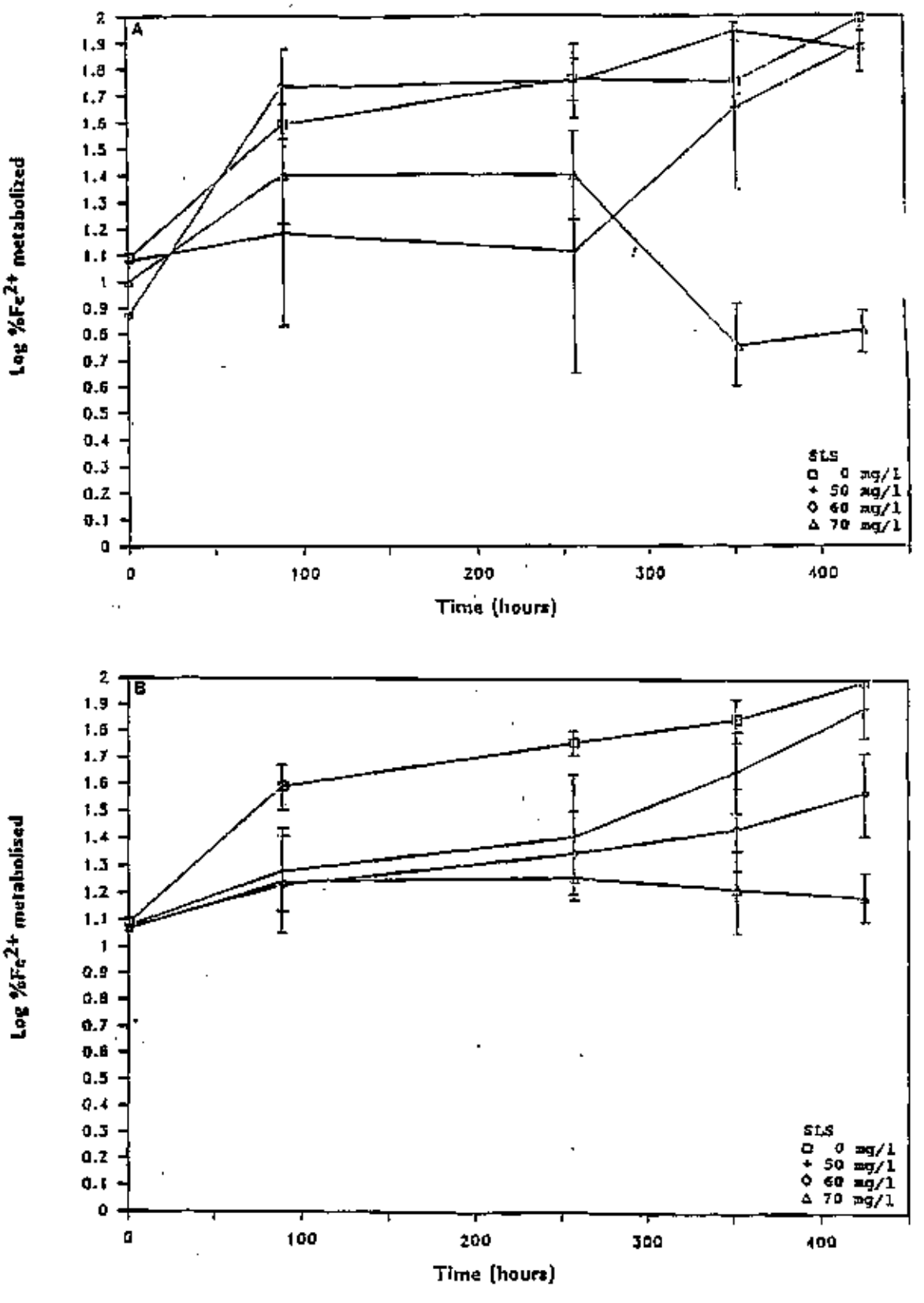
APPENDIX FIG. 63. Effect of SLS on ferrous iron oxidation by *T. ferrooxidans* WLR in HJJ medium containing 500 g/l coal waste (0,425 to 1,18 mm fraction) when the inhibitor was added (A) at the beginning of incubation of the cultures or (B) during the exponential phase of culture growth (91 hours).



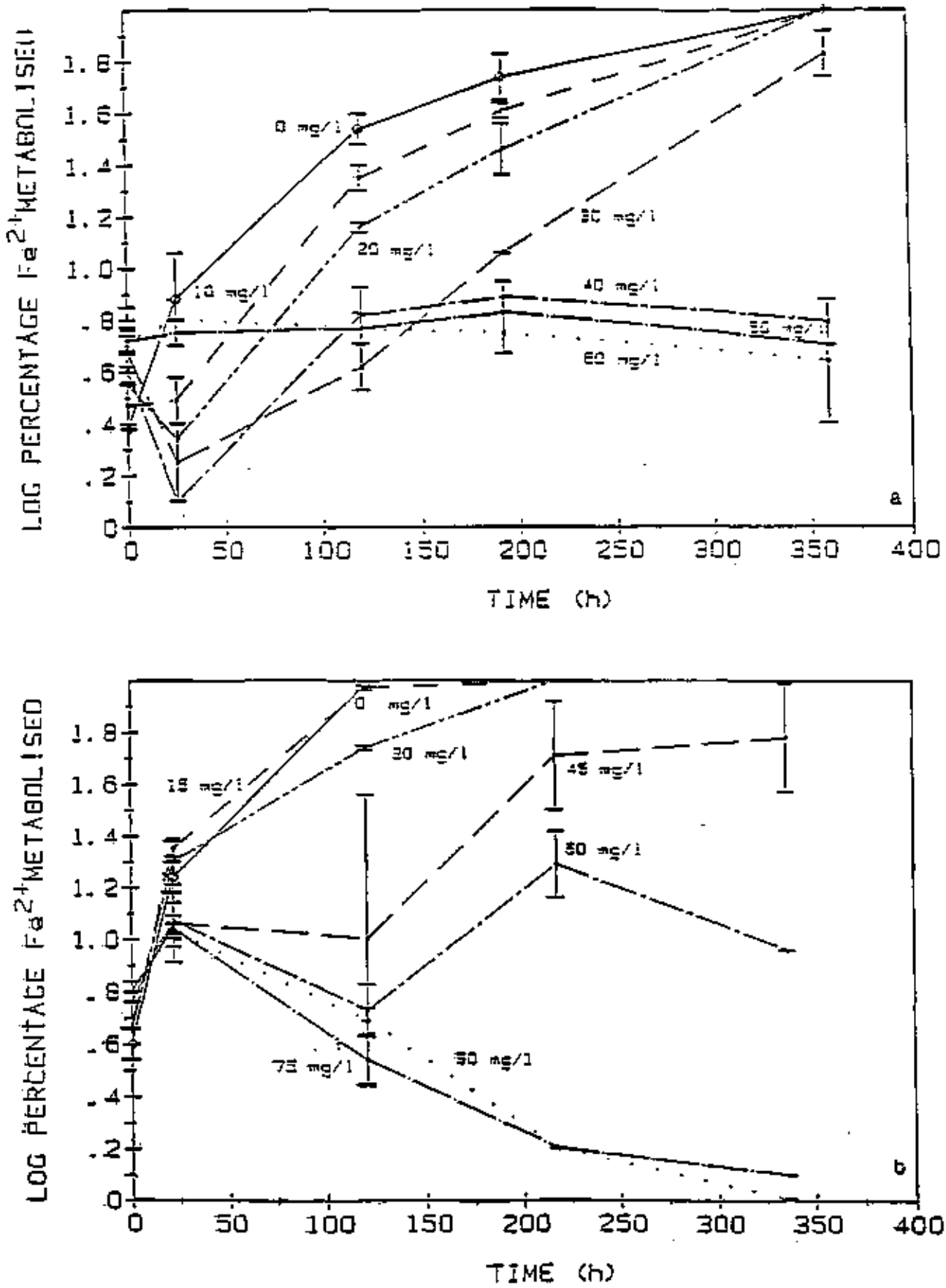
APPENDIX FIG. 64. Effect of SLS on ferrous iron oxidation by *T. ferrooxidans* WLR in HJJ medium containing 500 g/l coal waste (2,00 to 2,80 mm fraction) when the inhibitor was added (A) at the beginning of incubation of the cultures or (B) during the exponential phase of culture growth (91 hours).



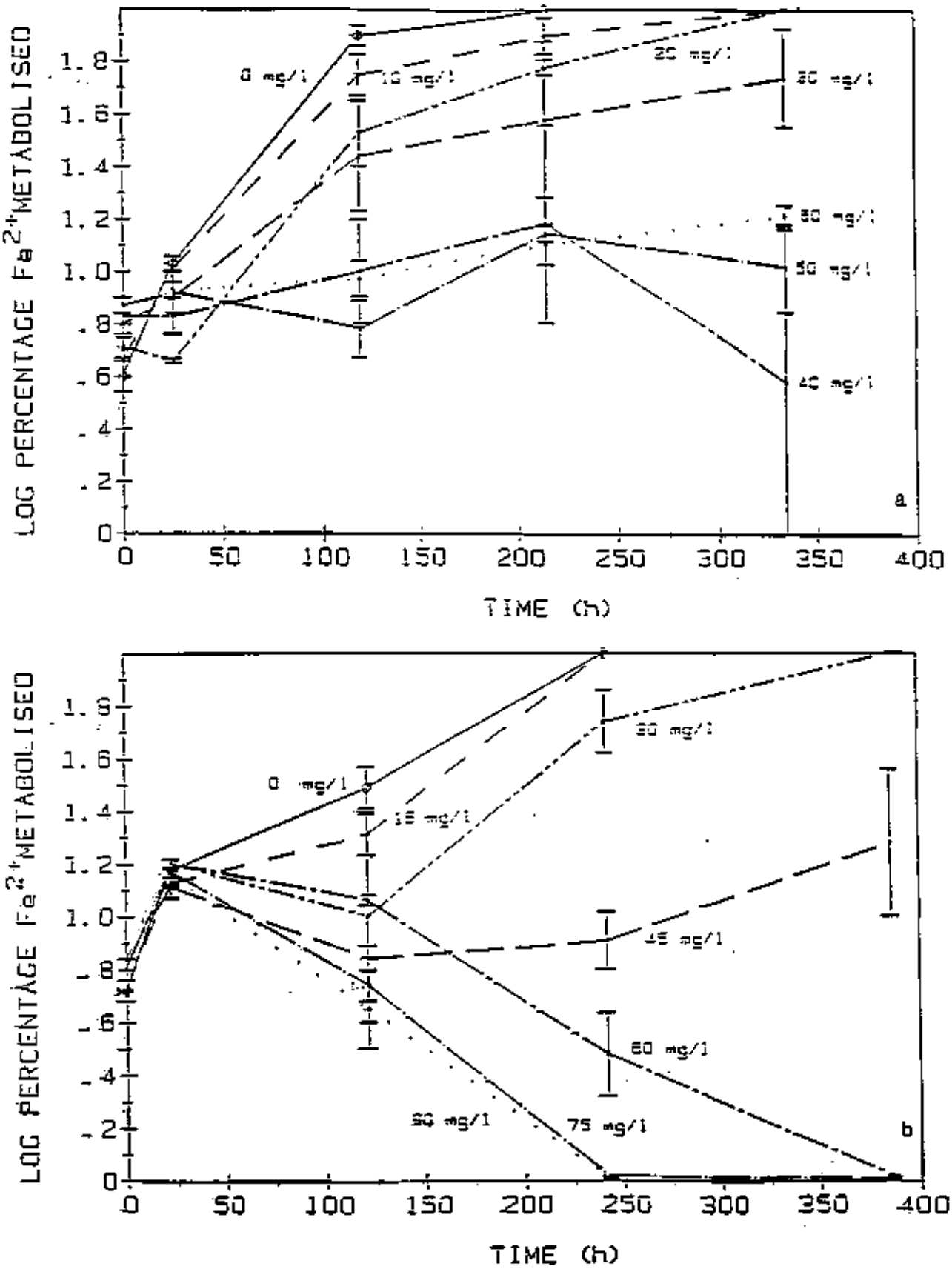
APPENDIX FIG. 65. Effect of SLS on ferrous iron oxidation by *T. ferrooxidans* WLR in HJJ medium containing 500 g/l coal waste (4,00 to 4,75 mm fraction) when the inhibitor was added (A) at the beginning of incubation of the cultures or (B) during the exponential phase of culture growth (91 hours).



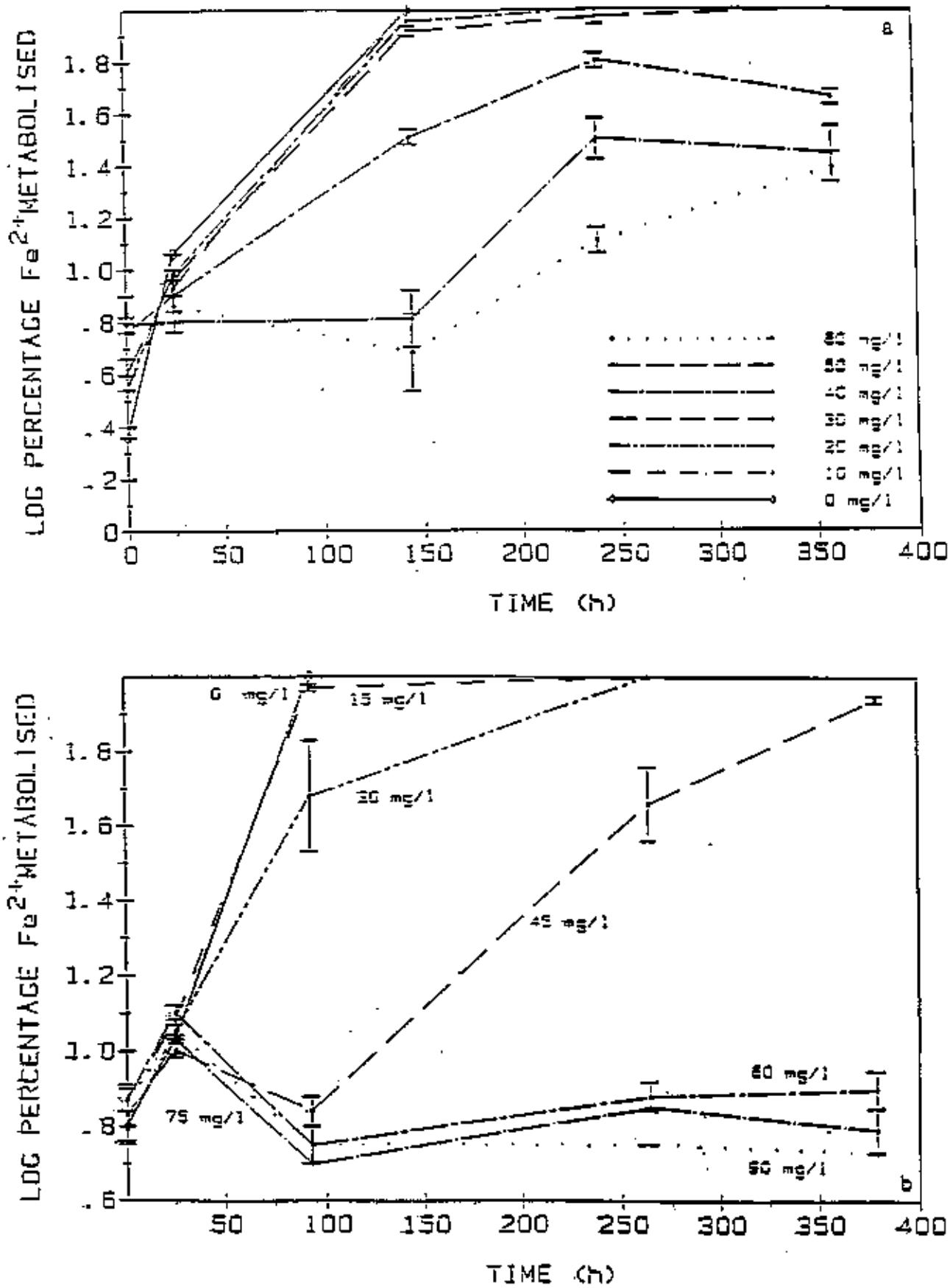
APPENDIX FIG. 66. Effect of SLS on ferrous iron oxidation by *T. ferrooxidans* WLR in HJJ medium containing 500 g/l coal waste (8,00 to 9,50 mm fraction) when the inhibitor was added (A) at the beginning of incubation of the cultures or (B) during the exponential phase of culture growth (91 hours).



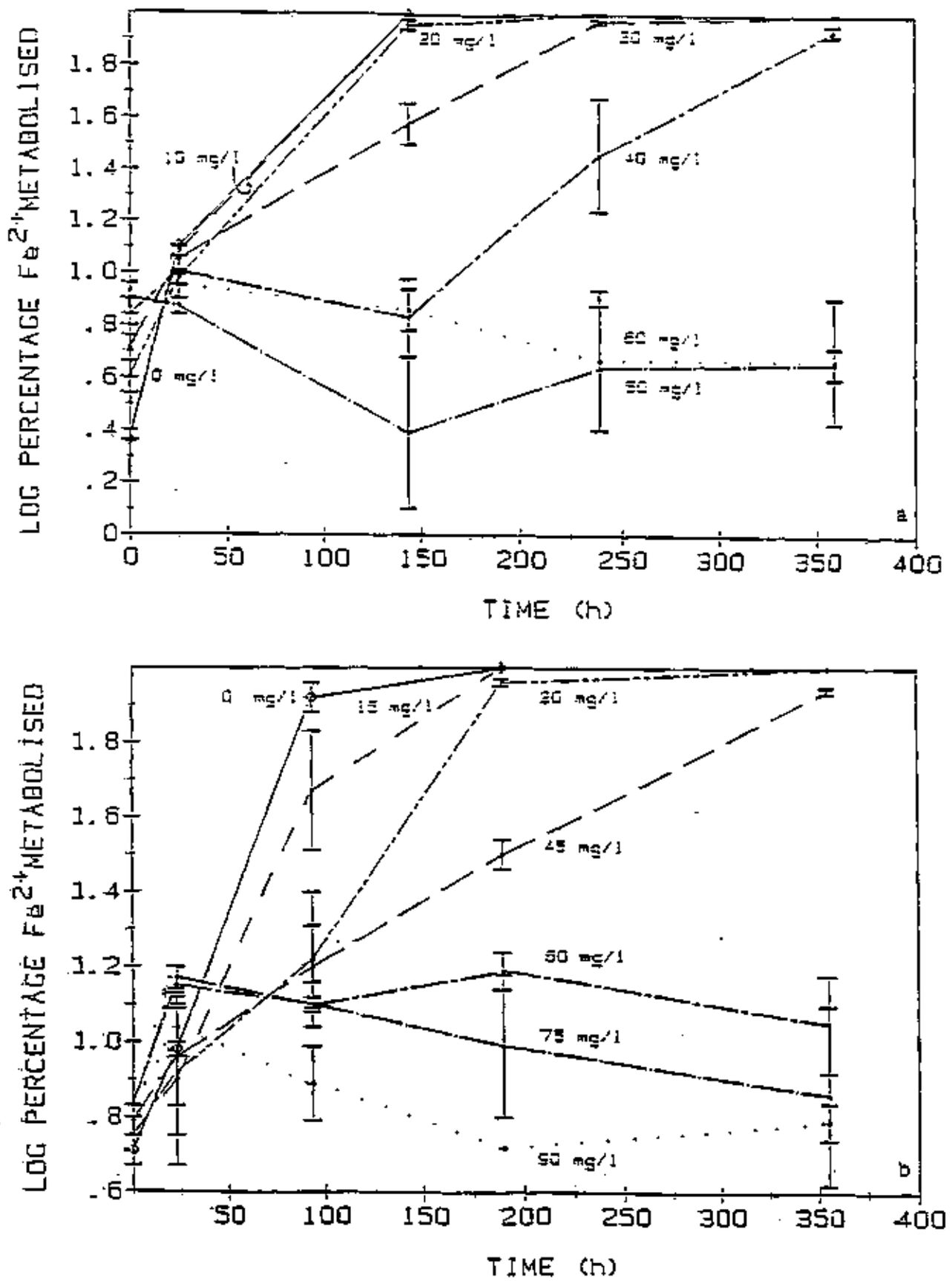
APPENDIX FIG. 67. Effect of sodium benzoate on ferrous iron oxidation by *T. ferrooxidans* ATCC 19859 in HJJ medium containing 500 g/l coal waste (2,00 to 2,80 mm fraction) when the inhibitor was added (a) at the beginning of incubation of the cultures or (b) during the exponential phase of culture growth (22h).



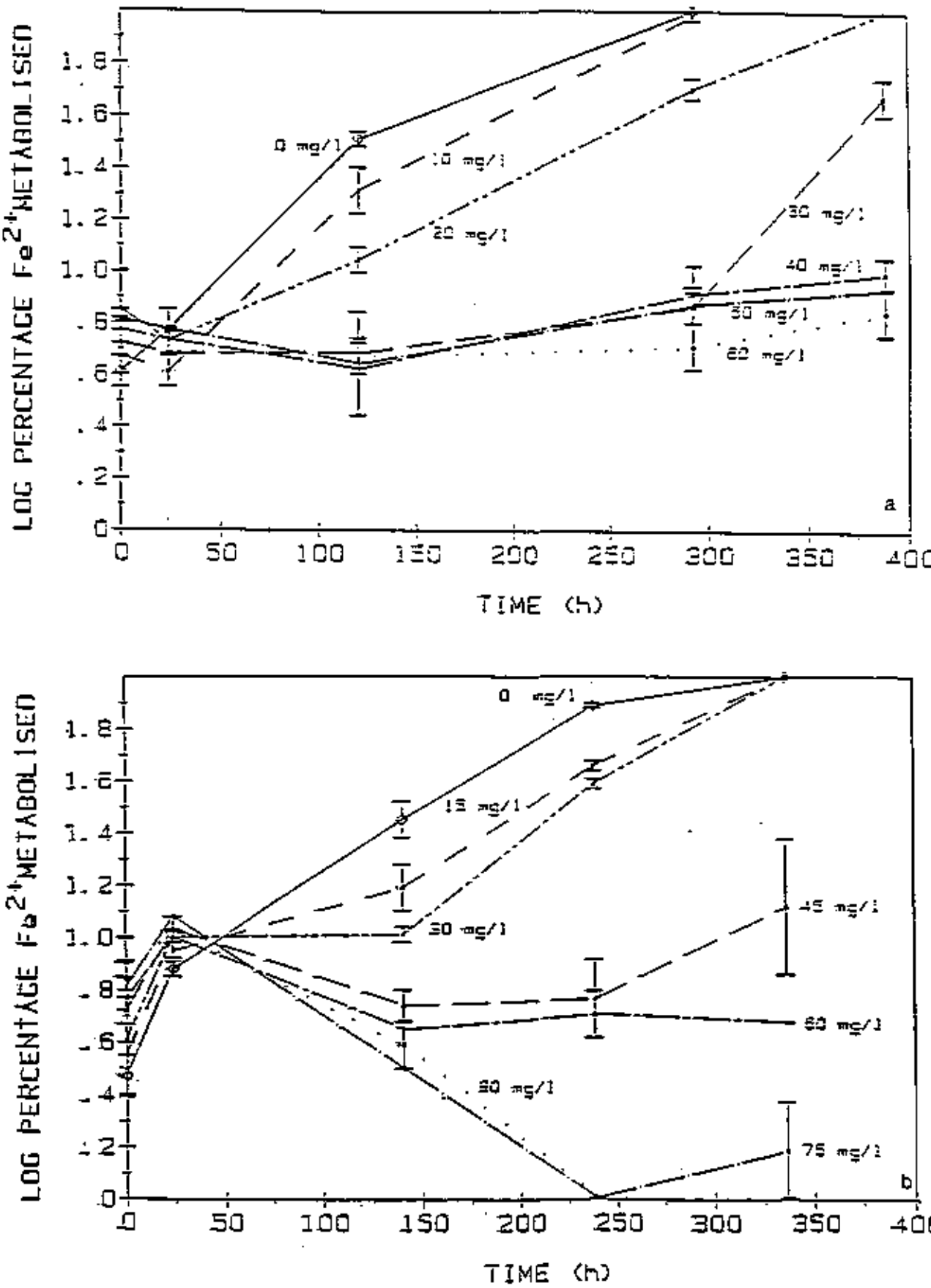
APPENDIX FIG. 68. Effect of sodium benzoate on ferrous iron oxidation by *T. ferrooxidans* ATCC 19859 in HJJ medium containing 500 g/l coal waste (4,00 to 4,75 mm fraction) when the inhibitor was added (a) at the beginning of incubation of the cultures or (b) during the exponential phase of culture growth (23h).



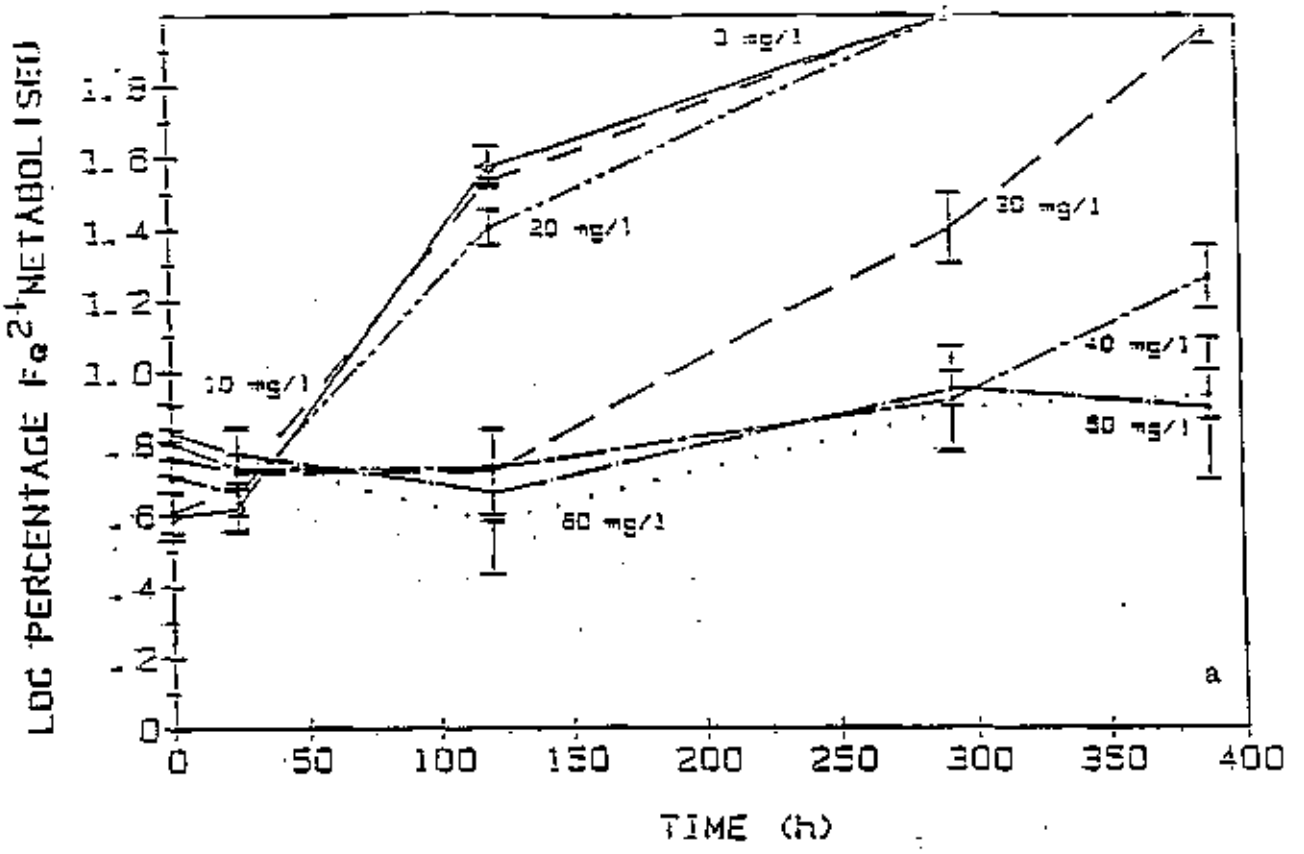
APPENDIX FIG. 69. Effect of sodium benzoate on ferrous iron oxidation by *T. ferrooxidans* ATCC 19859 in HJJ medium containing 500 g/l coal waste (8,00 to 9,50 mm fraction) when the inhibitor was added (a) at the beginning of incubation of the cultures or (b) during the exponential phase of culture growth (25h).

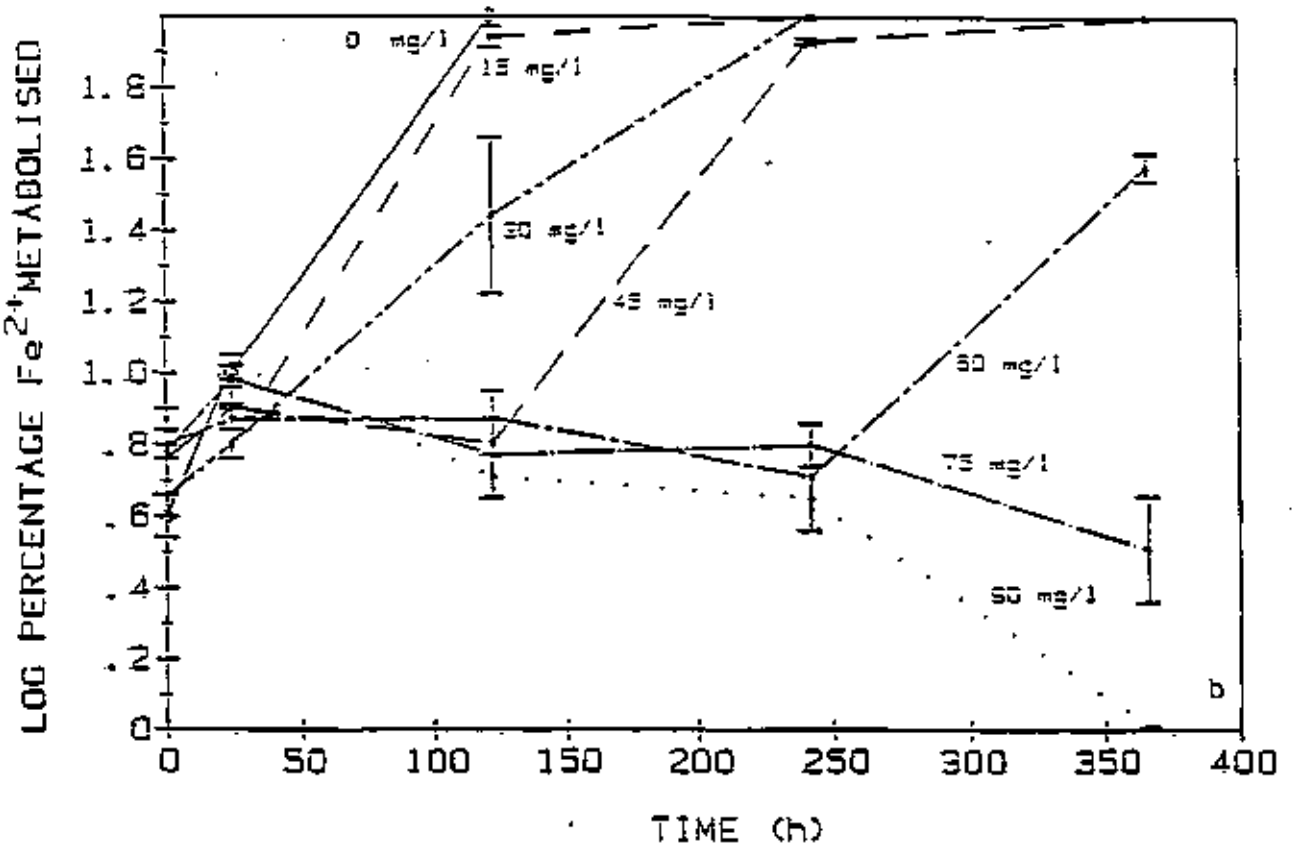


APPENDIX FIG. 70. Effect of sodium benzoate on ferrous iron oxidation by *T. ferrooxidans* ATCC 19859 in HJJ medium containing 500 g/l coal waste (10,0 to 11,5 mm fraction) when the inhibitor was added (a) at the beginning of incubation of the cultures or (b) during the exponential phase of culture growth (23h).

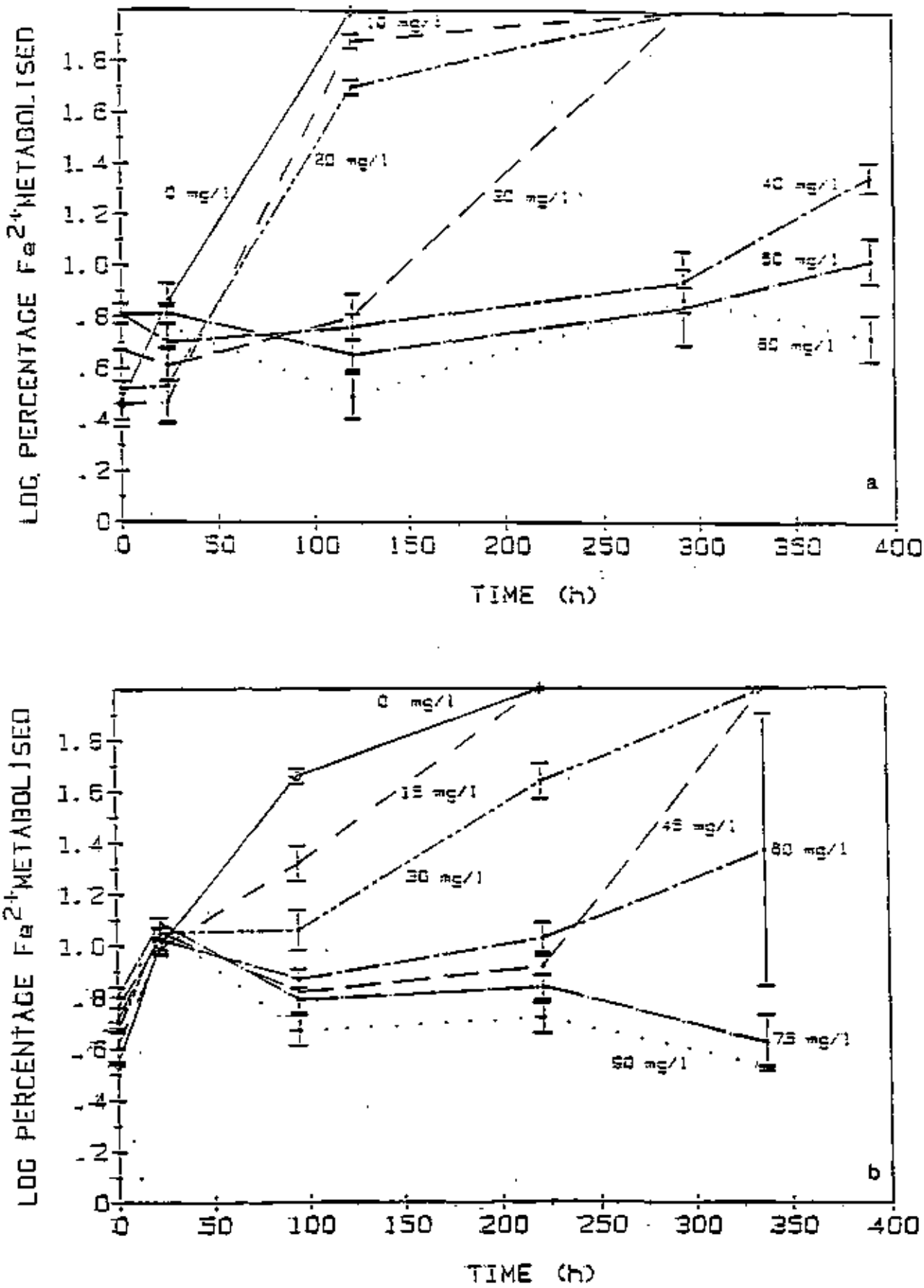


APPENDIX FIG. 71. Effect of sodium benzoate on ferrous iron oxidation by *T. ferrooxidans* WLR in HJJ medium containing 500 g/l coal waste (2,00 to 2,80 mm fraction) when the inhibitor was added (a) at the beginning of incubation of the cultures or (b) during the exponential phase of culture growth (24h).

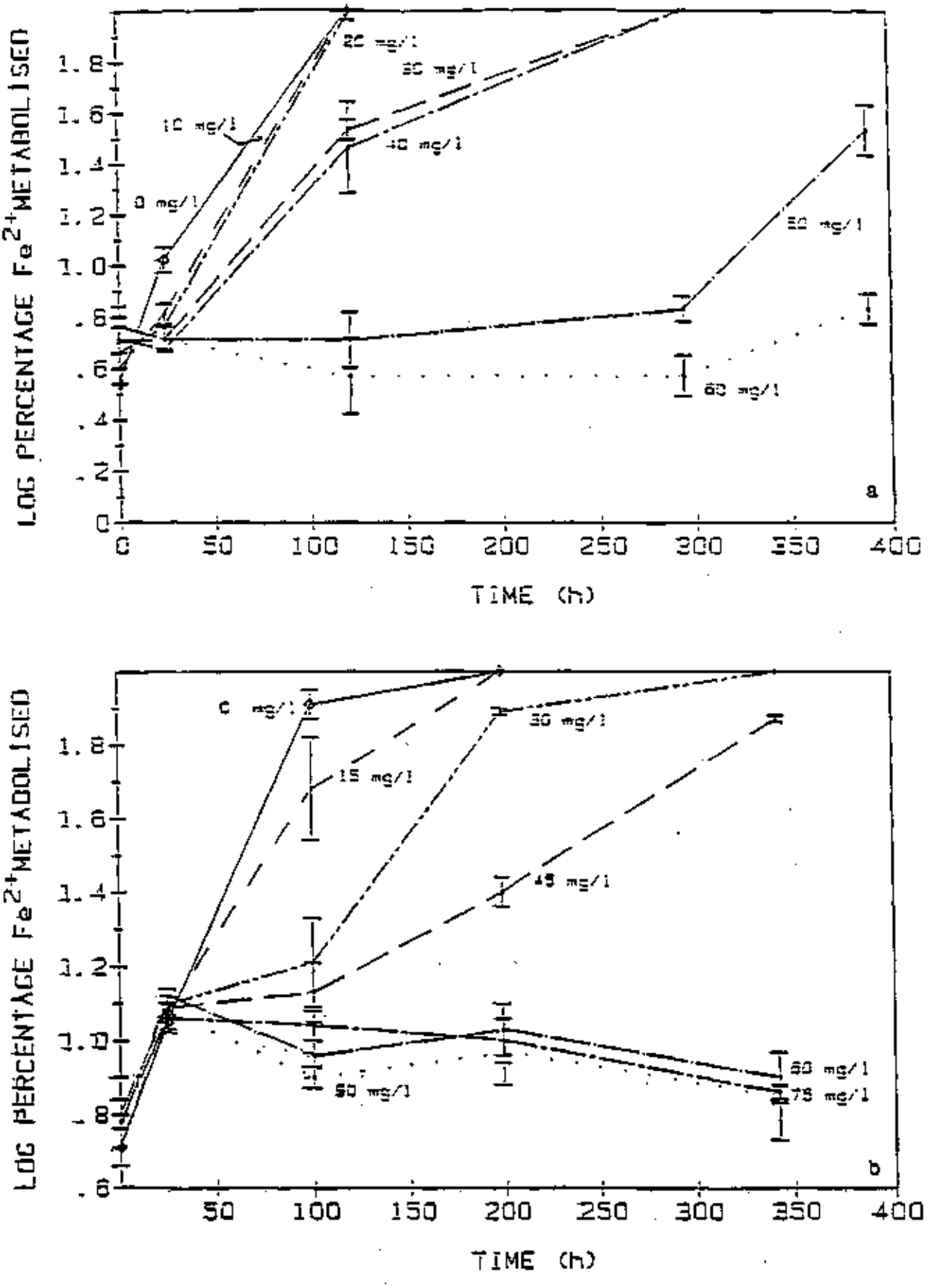




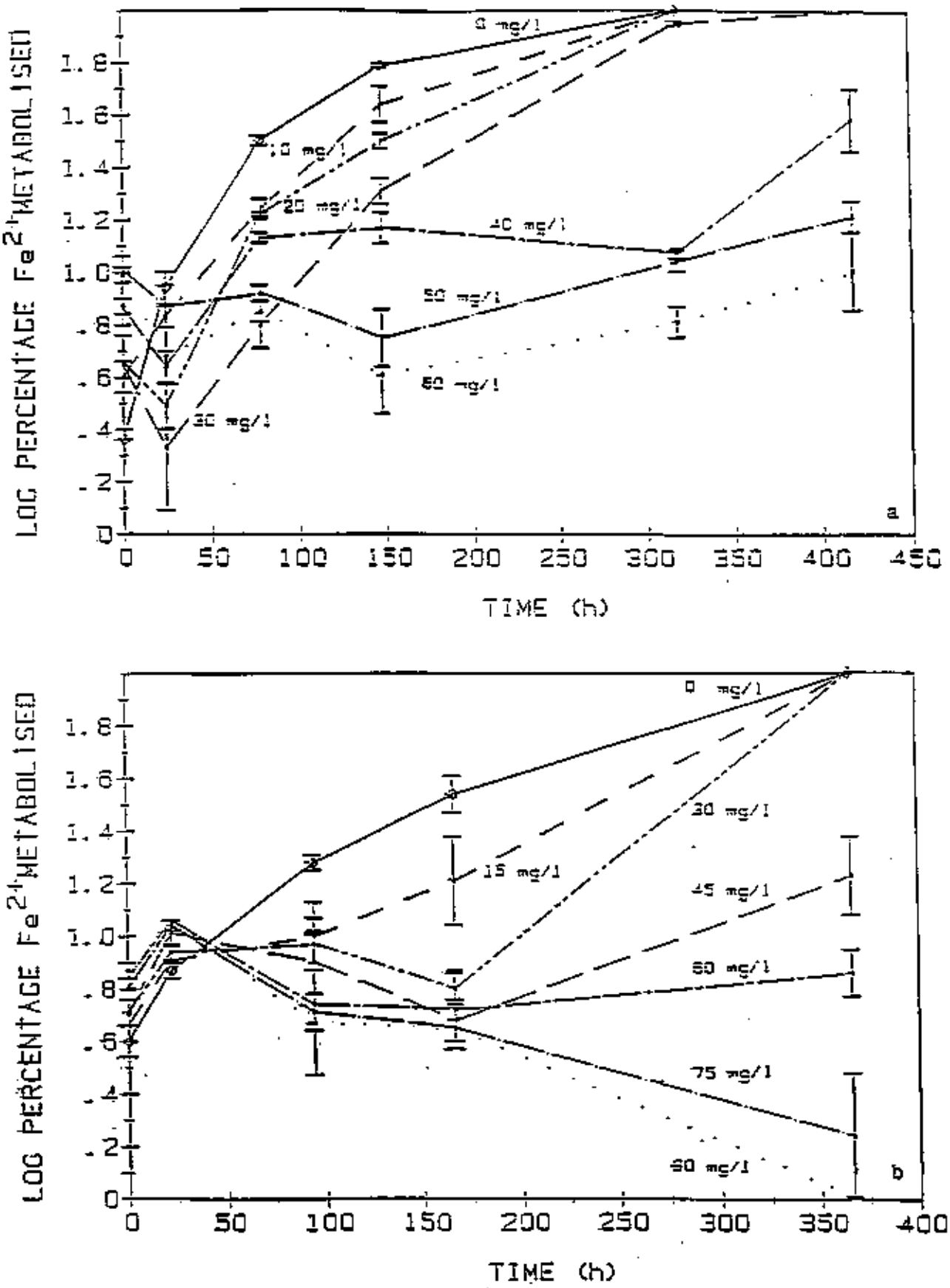
APPENDIX FIG. 72. Effect of sodium benzoate on ferrous iron oxidation by *T. ferrooxidans* WLR in HJJ medium containing 500 g/l coal waste (4,00 to 4,75 mm fraction) when the inhibitor was added (a) at the beginning of incubation of the cultures or (b) during the exponential phase of culture growth (24h).



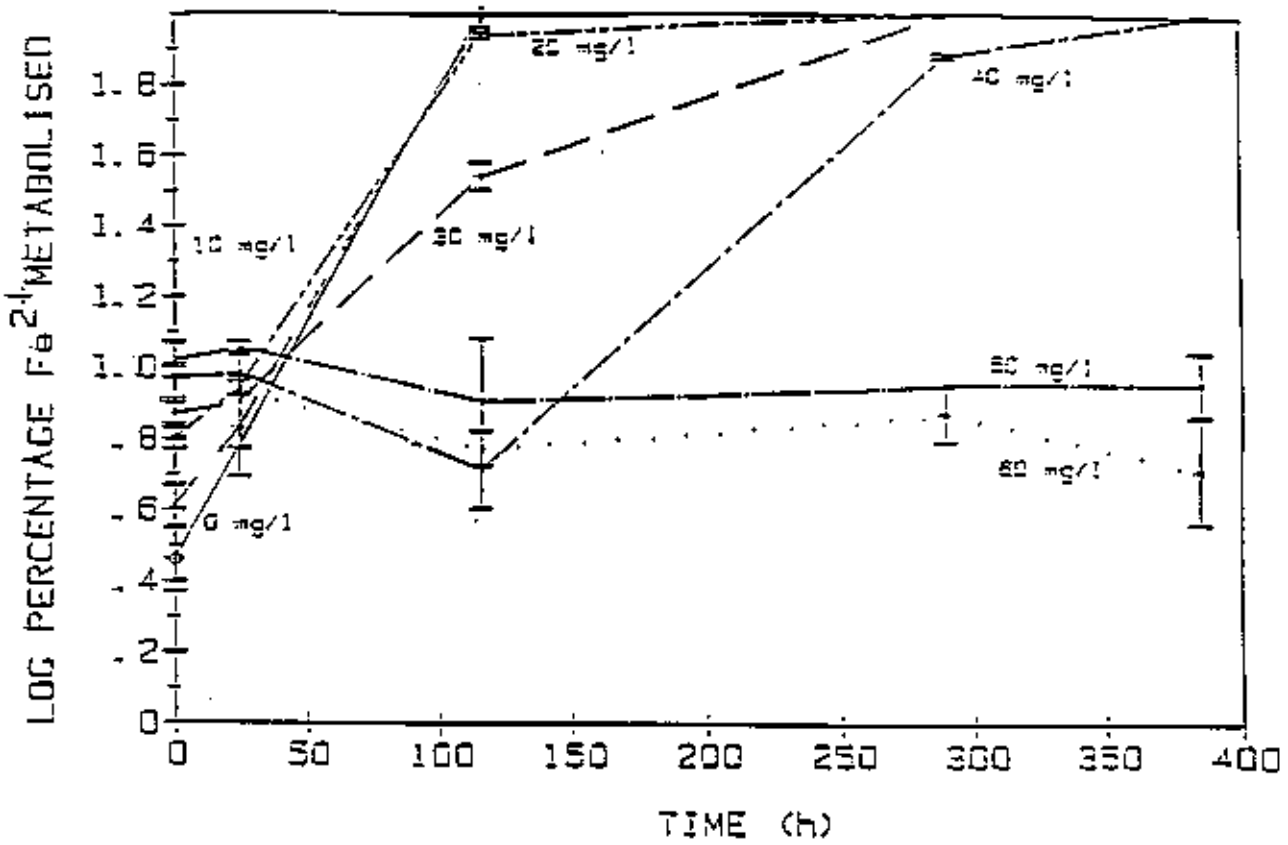
APPENDIX FIG. 73. Effect of sodium benzoate on ferrous iron oxidation by *T. ferrooxidans* WLR in HJJ medium containing 500 g/l coal waste (8,00 to 9,50 mm fraction) when the inhibitor was added (a) at the beginning of incubation of the cultures or (b) during the exponential phase of culture growth (23h).

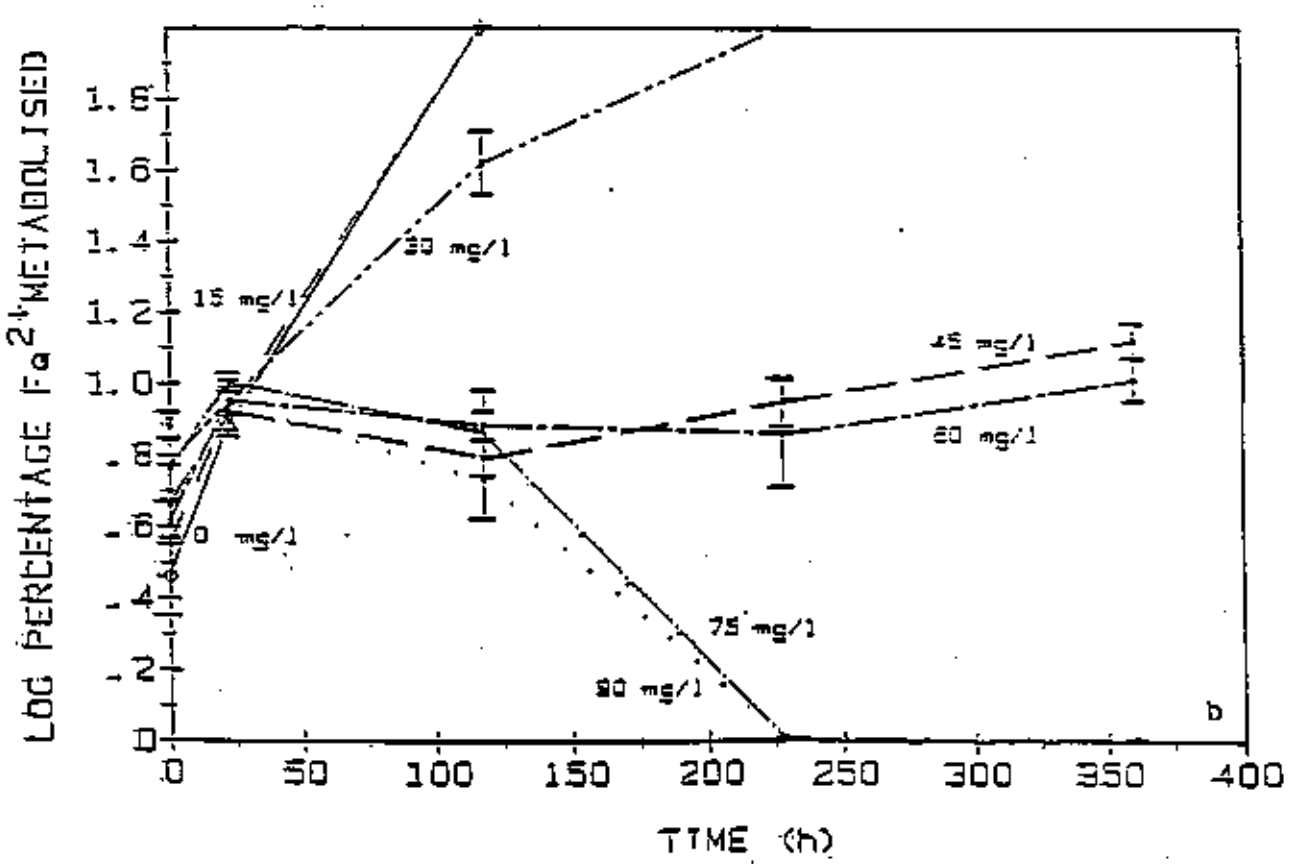


APPENDIX FIG. 74. Effect of sodium benzoate on ferrous iron oxidation by *T. ferrooxidans* WLR in HJJ medium containing 500 g/l coal waste (10,0 to 11,5 mm fraction) when the inhibitor was added (a) at the beginning of incubation of the cultures or (b) during the exponential phase of culture growth (25h).

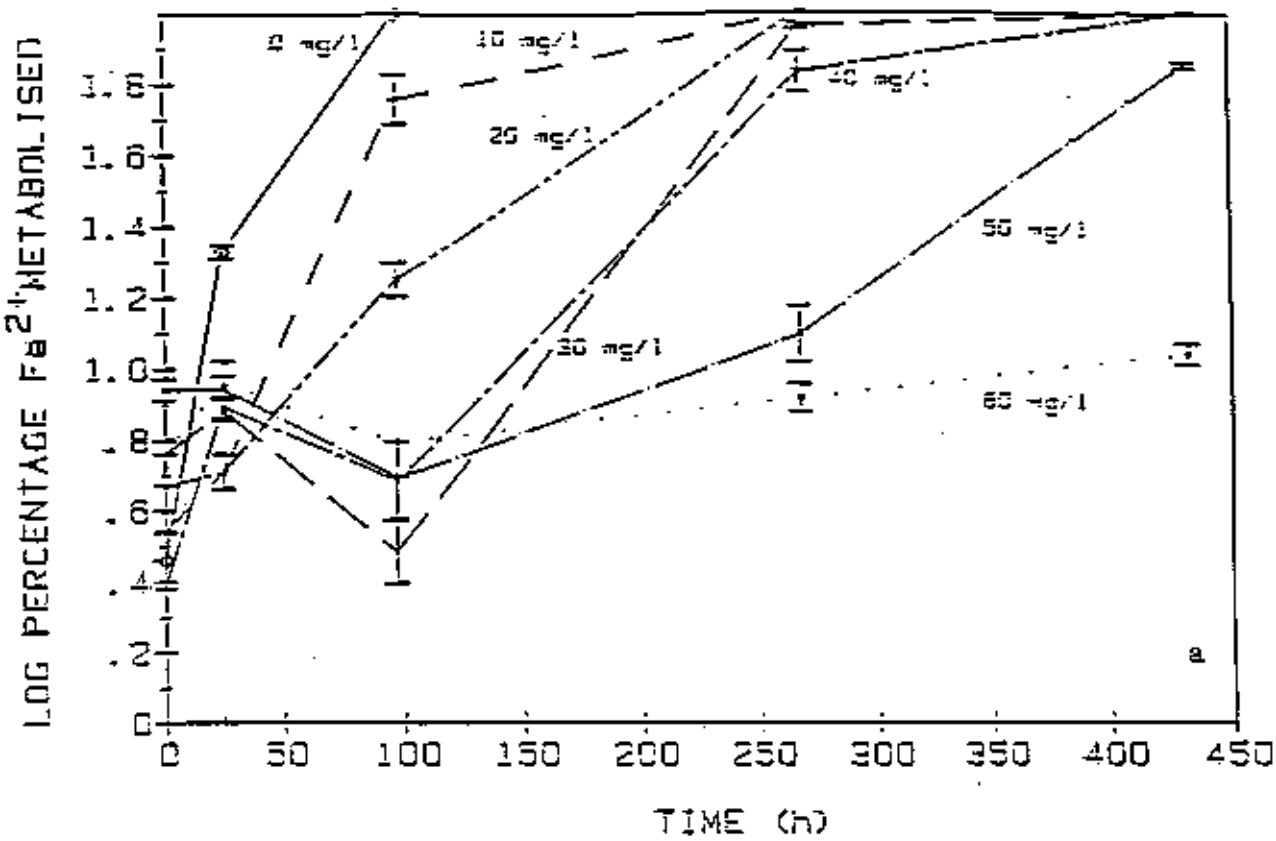


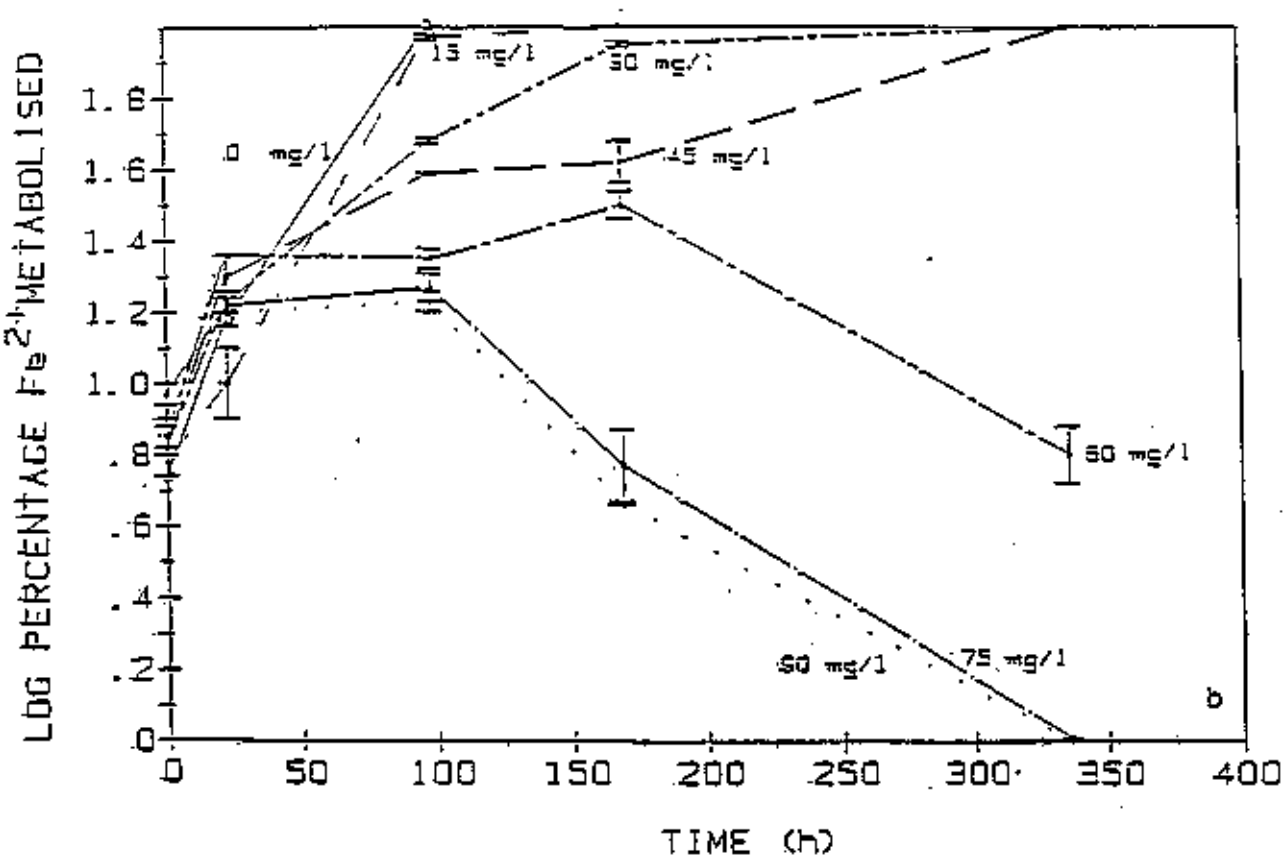
APPENDIX FIG. 75. Effect of sorbic acid on ferrous iron oxidation by *T. ferrooxidans* ATCC 19859 in HJJ medium containing 500 g/l coal waste (2,00 to 2,80 mm fraction) when the inhibitor was added (a) at the beginning of incubation of the cultures or (b) during the exponential phase of culture growth (22h).



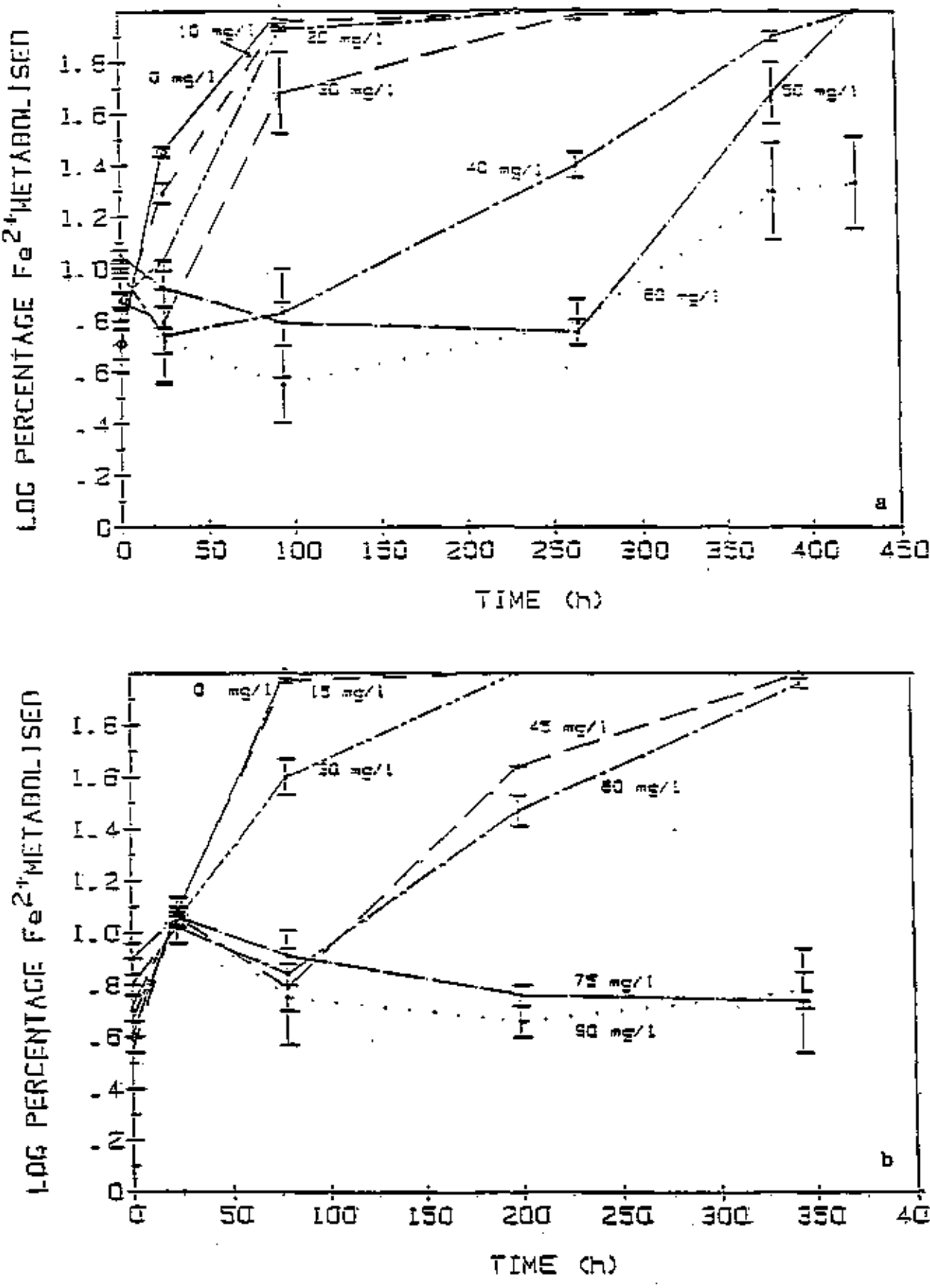


APPENDIX FIG. 76. Effect of sorbic acid on ferrous iron oxidation by *T. ferrooxidans* ATCC 19859 in HJJ medium containing 500 g/l coal waste (4,00 to 4,75 mm fraction) when the inhibitor was added (a) at the beginning of incubation of the cultures or (b) during the exponential phase of culture growth (22h).

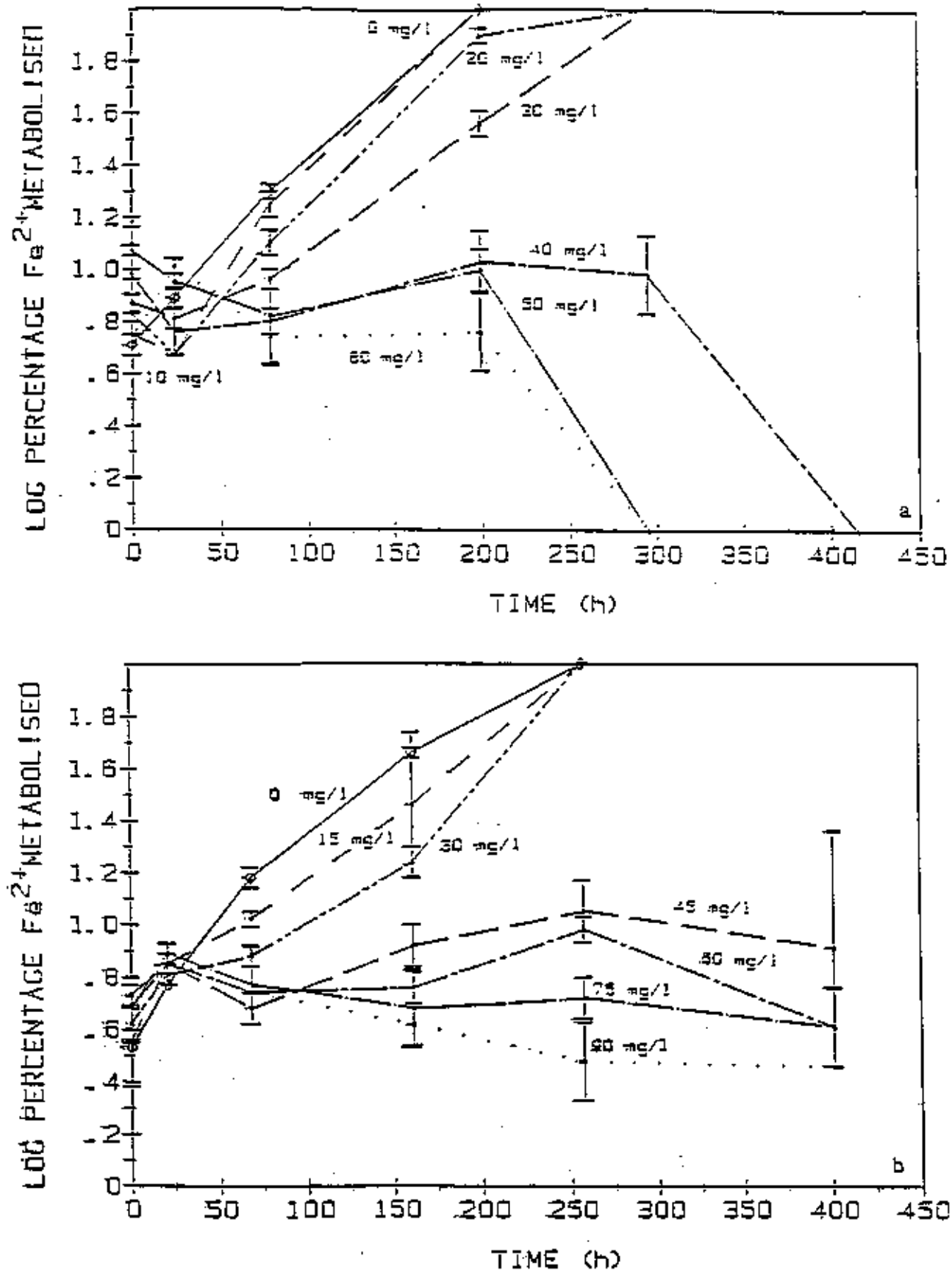




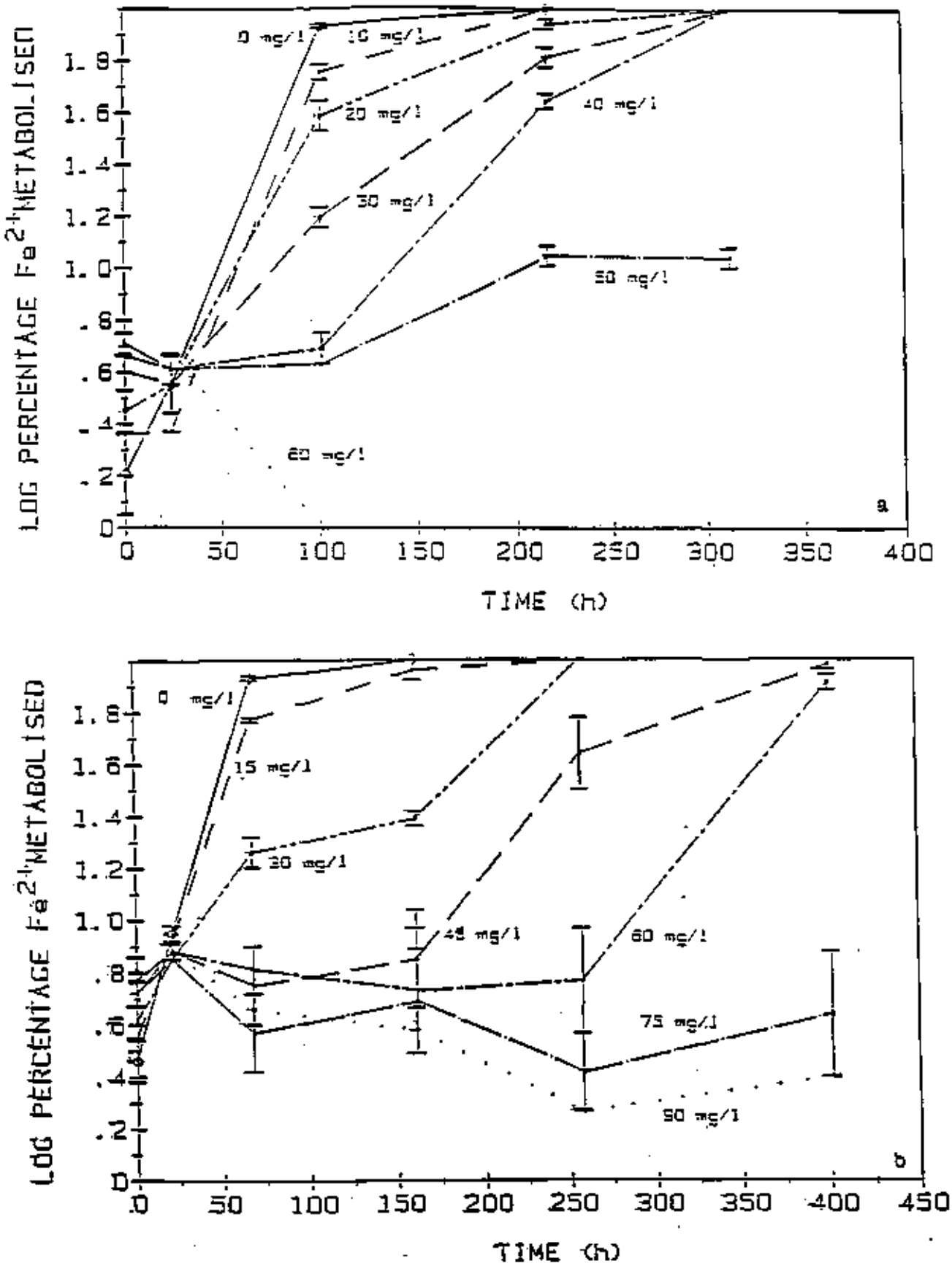
APPENDIX FIG. 77. Effect of sorbic acid on ferrous iron oxidation by *T. ferrooxidans* ATCC 19859 in HJJ medium containing 500 g/l coal waste (8,00 to 9,50 mm fraction) when the inhibitor was added (a) at the beginning of incubation of the cultures or (b) during the exponential phase of culture growth (23h).



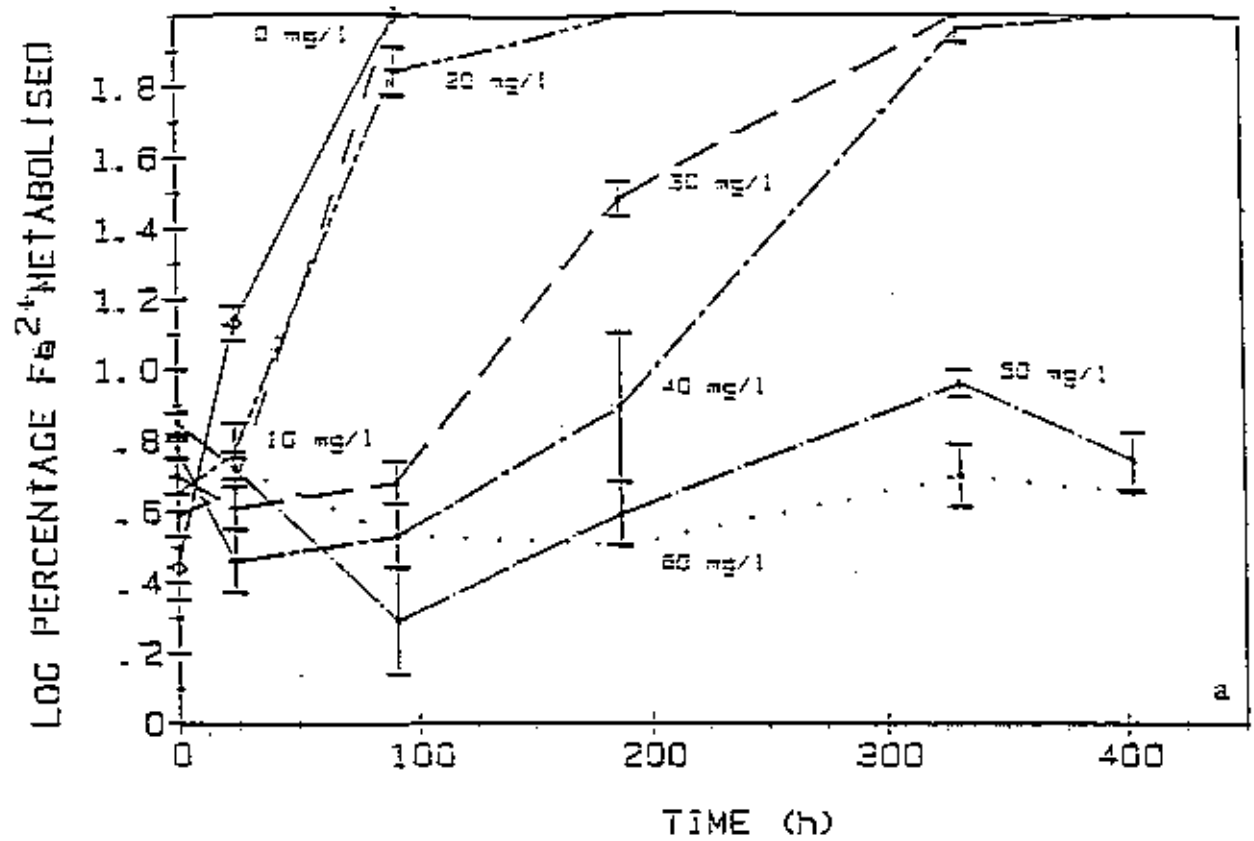
APPENDIX FIG. 78. Effect of sorbic acid on ferrous iron oxidation by *T. ferrooxidans* ATCC 19859 in HJJ medium containing 500 g/l coal waste (10,0 to 11,5 mm fraction) when the inhibitor was added (a) at the beginning of incubation of the cultures or (b) during the exponential phase of culture growth (23h).

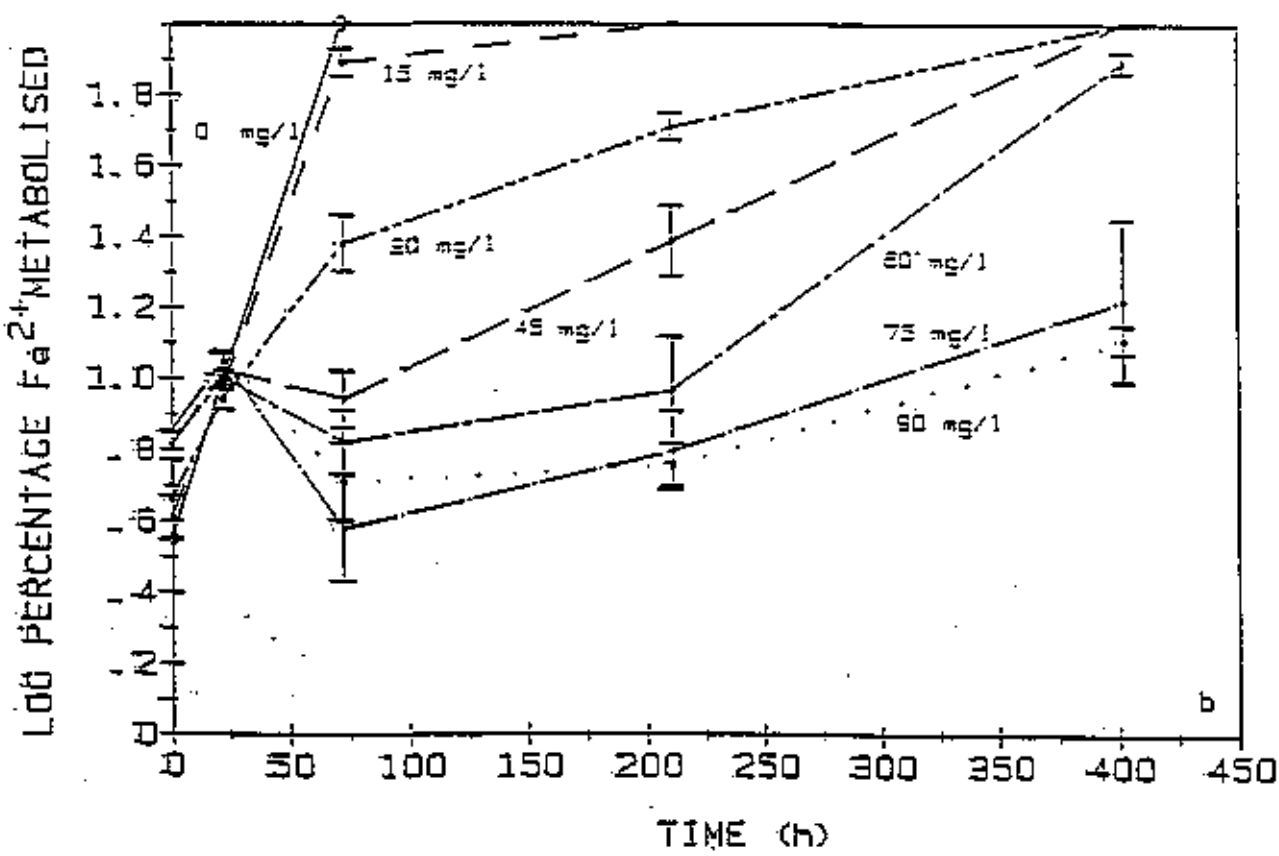


APPENDIX FIG. 79. Effect of sorbic acid on ferrous iron oxidation by *T. ferrooxidans* WLR in HJJ medium containing 500 g/l coal waste (2,00 to 2,80 mm fraction) when the inhibitor was added (a) at the beginning of incubation of the cultures or (b) during the exponential phase of culture growth (21h).

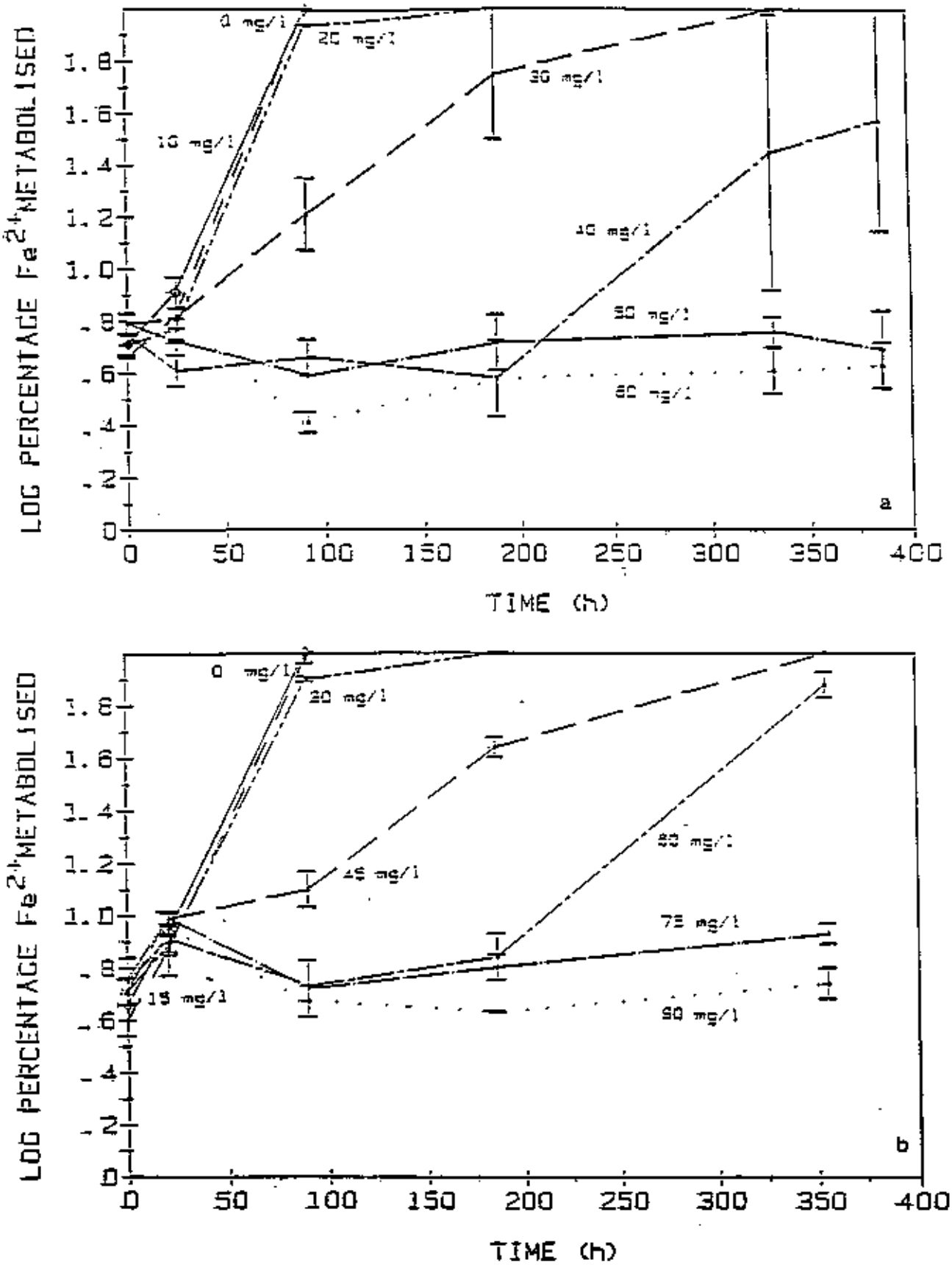


APPENDIX FIG. 80. Effect of sorbic acid on ferrous iron oxidation by *T. ferrooxidans* WLR in HJJ medium containing 500 g/l coal waste (4,00 to 4,75 mm fraction) when the inhibitor was added (a) at the beginning of incubation of the cultures or (b) during the exponential phase of culture growth (21h).



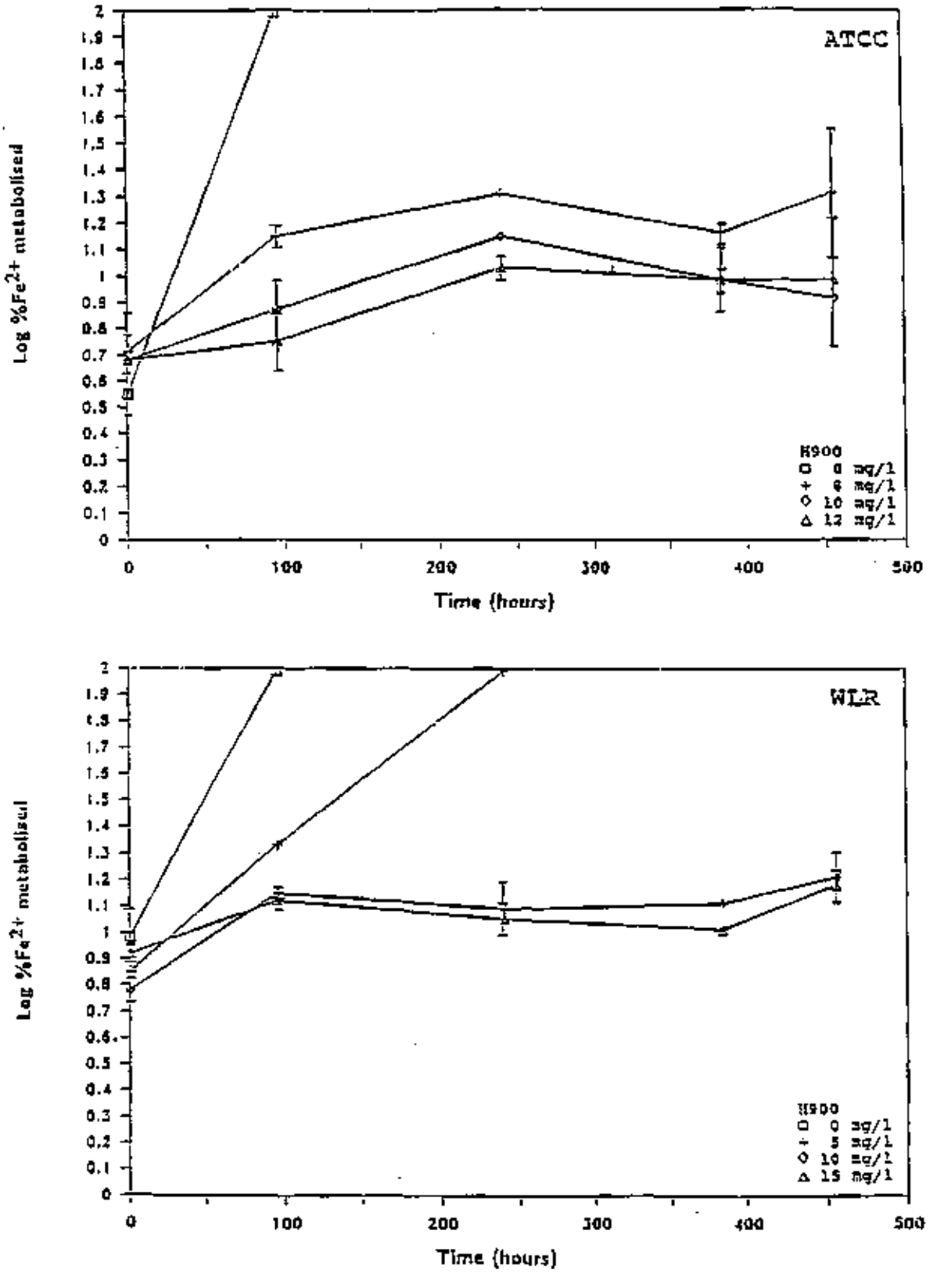


APPENDIX FIG. 81. Effect of sorbic acid on ferrous iron oxidation by *T. ferrooxidans* WLR in HJJ medium containing 500 g/l coal waste (8,00 to 9,50 mm fraction) when the inhibitor was added (a) at the beginning of incubation of the cultures or (b) during the exponential phase of culture growth (22h).

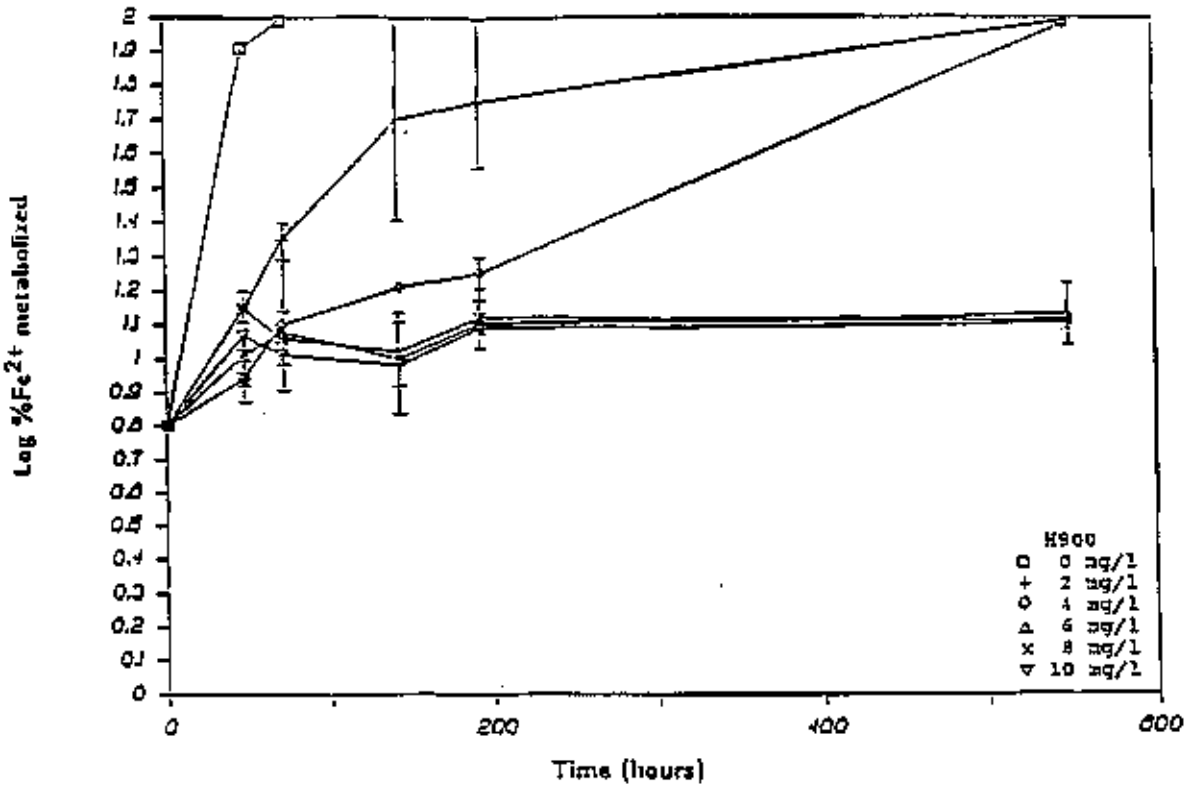


APPENDIX FIG. 82. Effect of sorbic acid on ferrous iron oxidation by *T. ferrooxidans* WLR in HJJ medium containing 500 g/l coal waste (10,0 to 11,5 mm fraction) when the inhibitor was added (a) at the beginning of incubation of the cultures or (b) during the exponential phase of culture growth (21h).

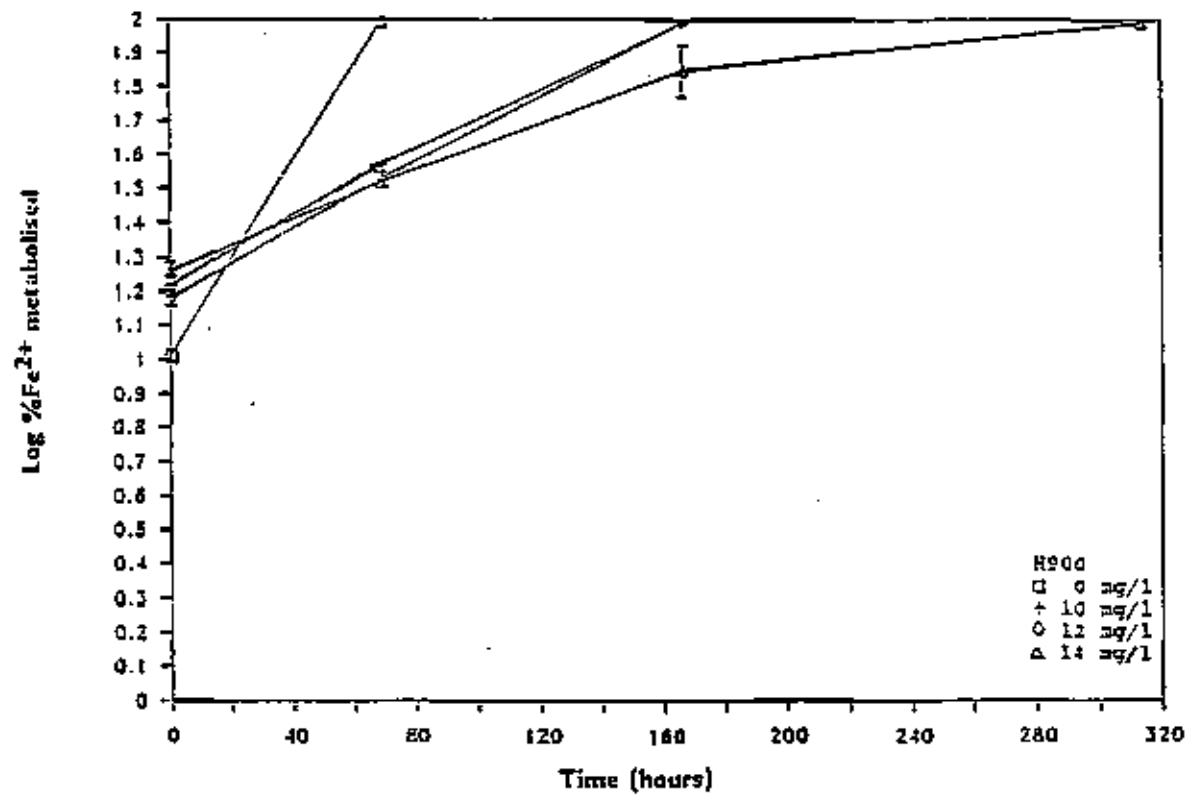
31. APPENDIX 3: COMPLETE RESULTS OF LABORATORY STUDIES OF CHEMICAL INHIBITION, ESPECIALLY BY MIXTURES OF INHIBITORS, OF FERROUS IRON OXIDATION BY *T. FERROOXIDANS* IN HJJ MEDIUM



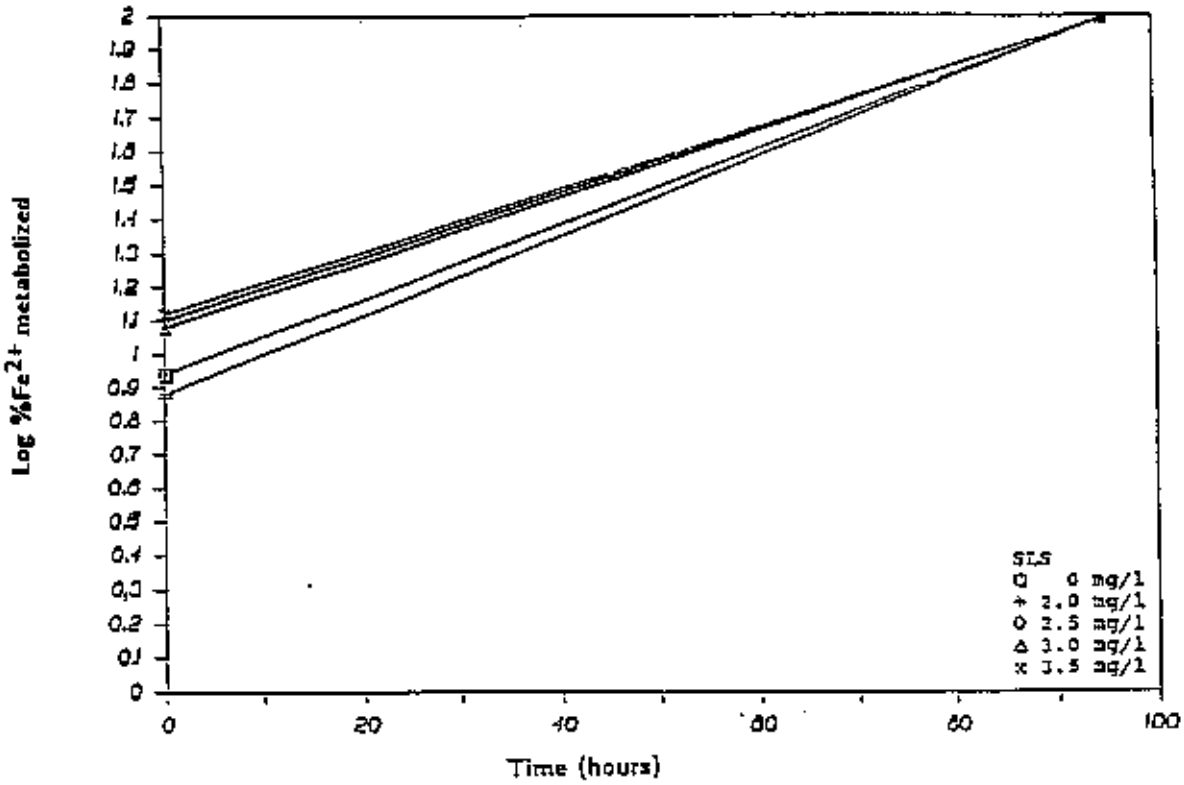
APPENDIX FIG. 83. Growth of *T. ferrooxidans* ATCC 19859 (above) and WLR (below) in the presence of H900 (from freshly prepared 1,0 g/l solution) added at the beginning of incubation.



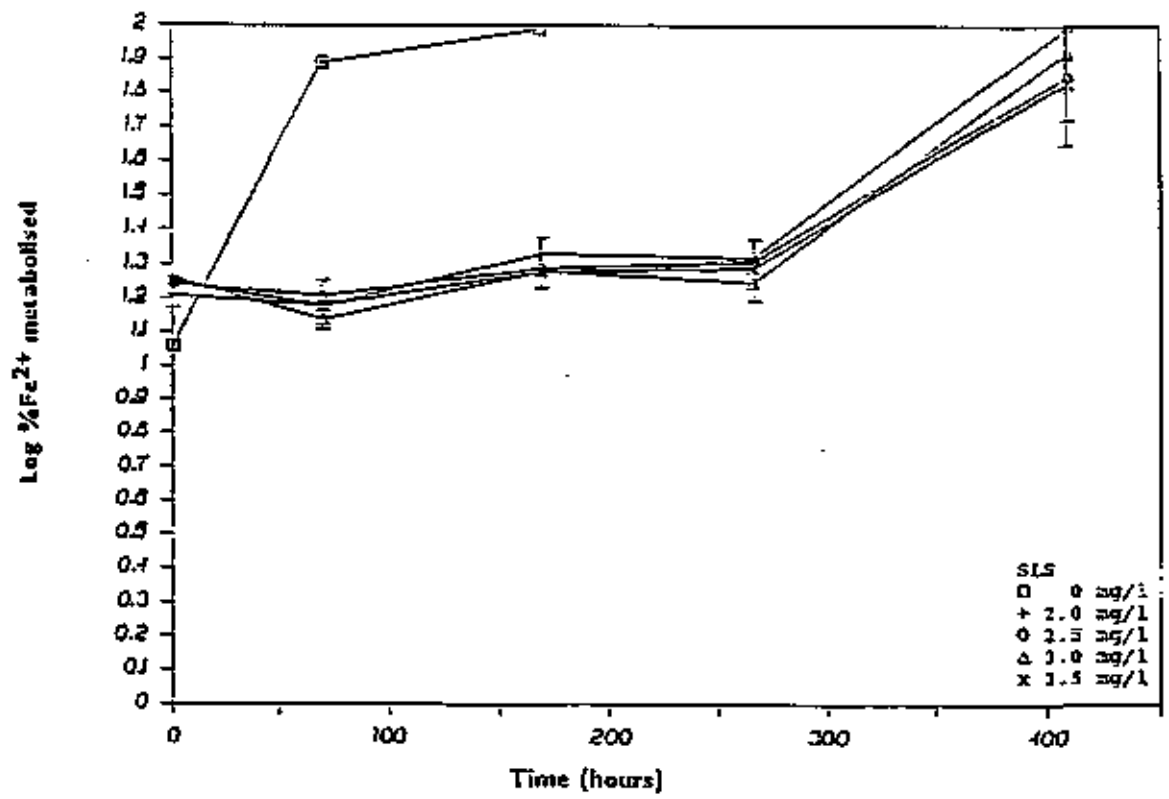
APPENDIX FIG. 84. Growth of *T. ferrooxidans* ATCC 19859 in the presence of H900 (from non-aged 2,0 g/l solution) added at the beginning of incubation.



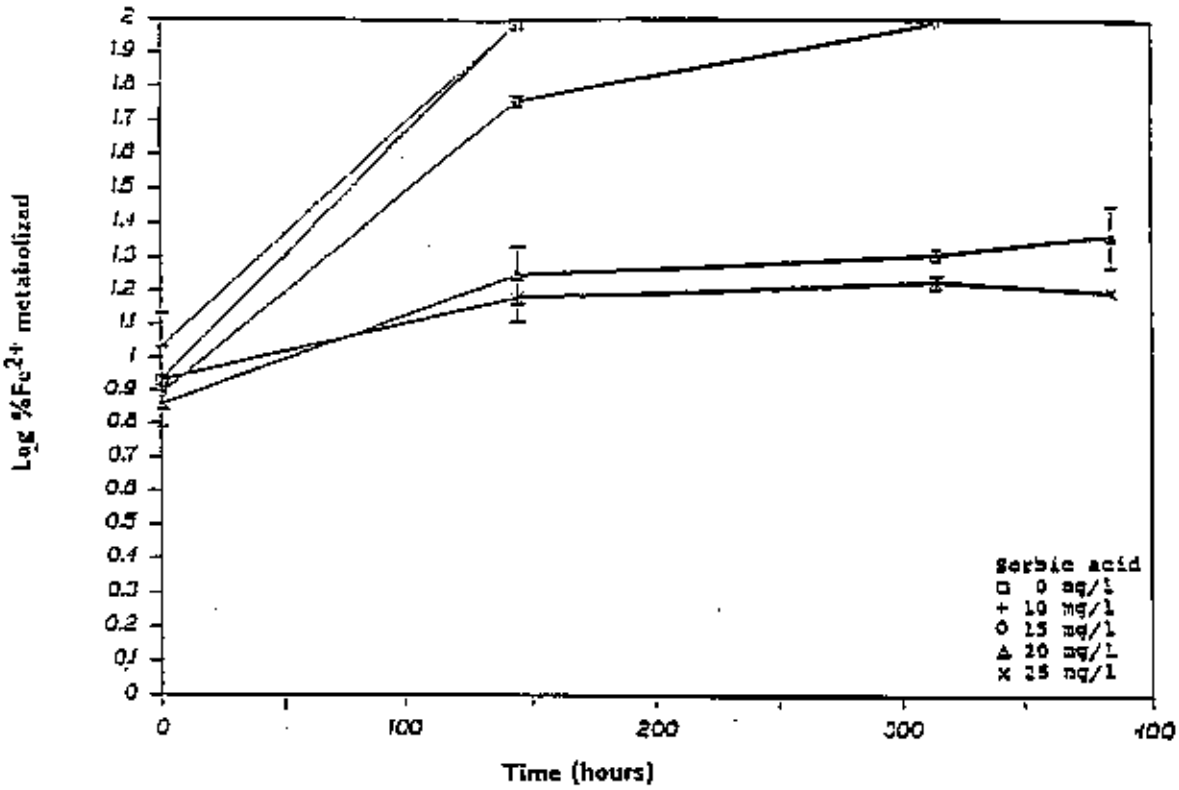
APPENDIX FIG. 85. Growth of *T. ferrooxidans* WLR.in the presence of H900 (from 6-month-old 2,0 g/l solution) added at the beginning of incubation.



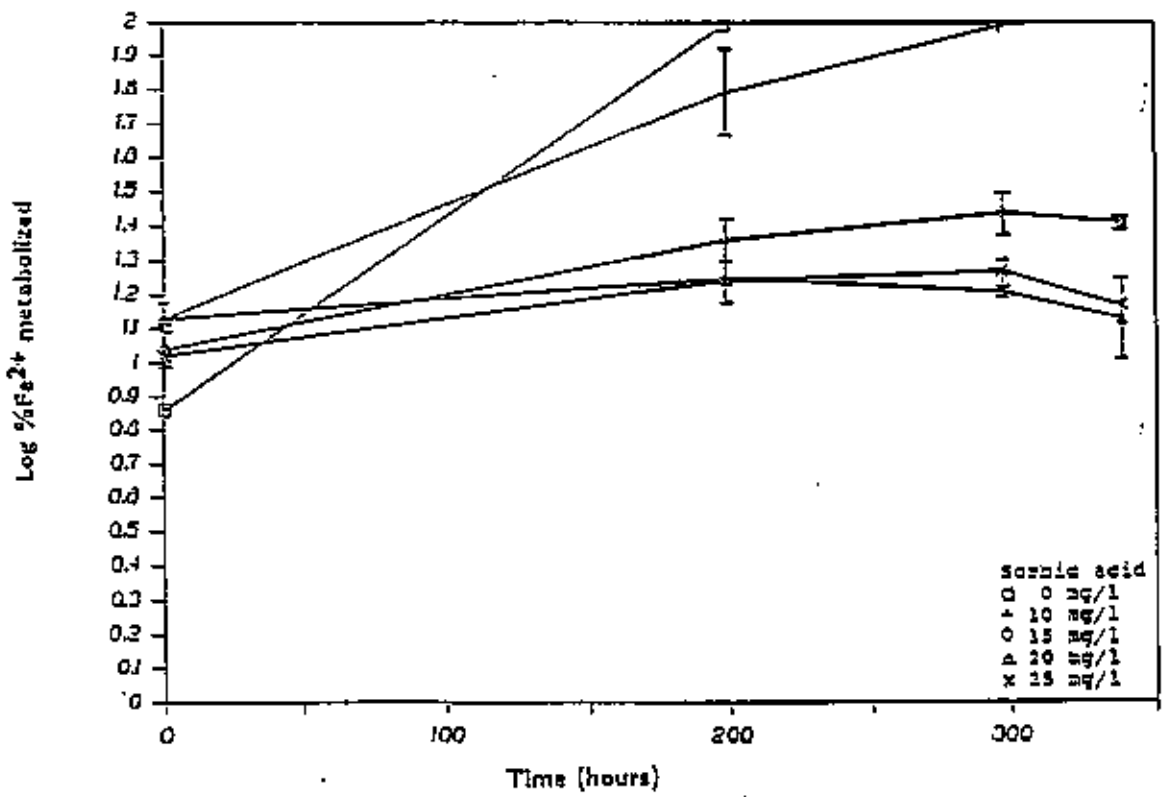
APPENDIX FIG. 86. Growth of *T. ferrooxidans* ATCC 19859 in the presence of SLS (2,0 to 3,5 mg/l) and H900 (4,0 mg/l) added at the beginning of incubation.



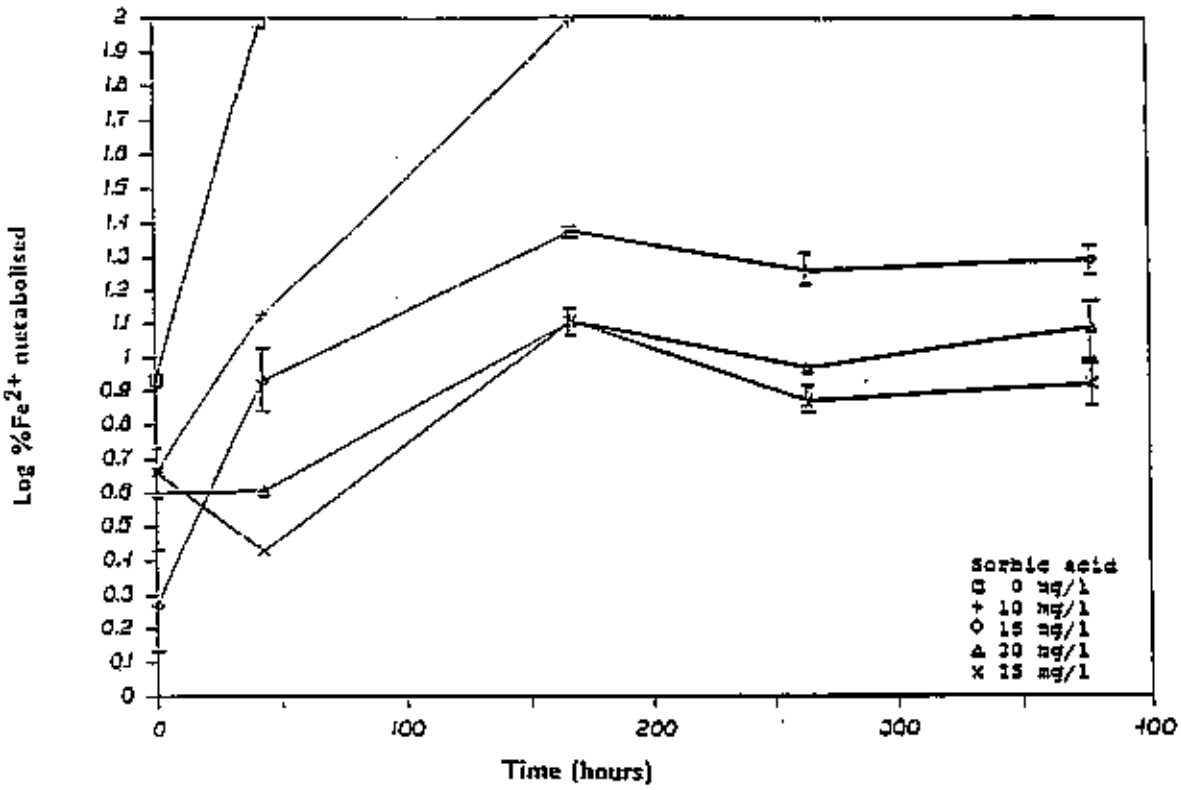
APPENDIX FIG. 87. Growth of *T. ferrooxidans* ATCC 19859 in the presence of SLS (2,0 to 3,5 mg/l) and H900 (5,0 mg/l) added at the beginning of incubation.



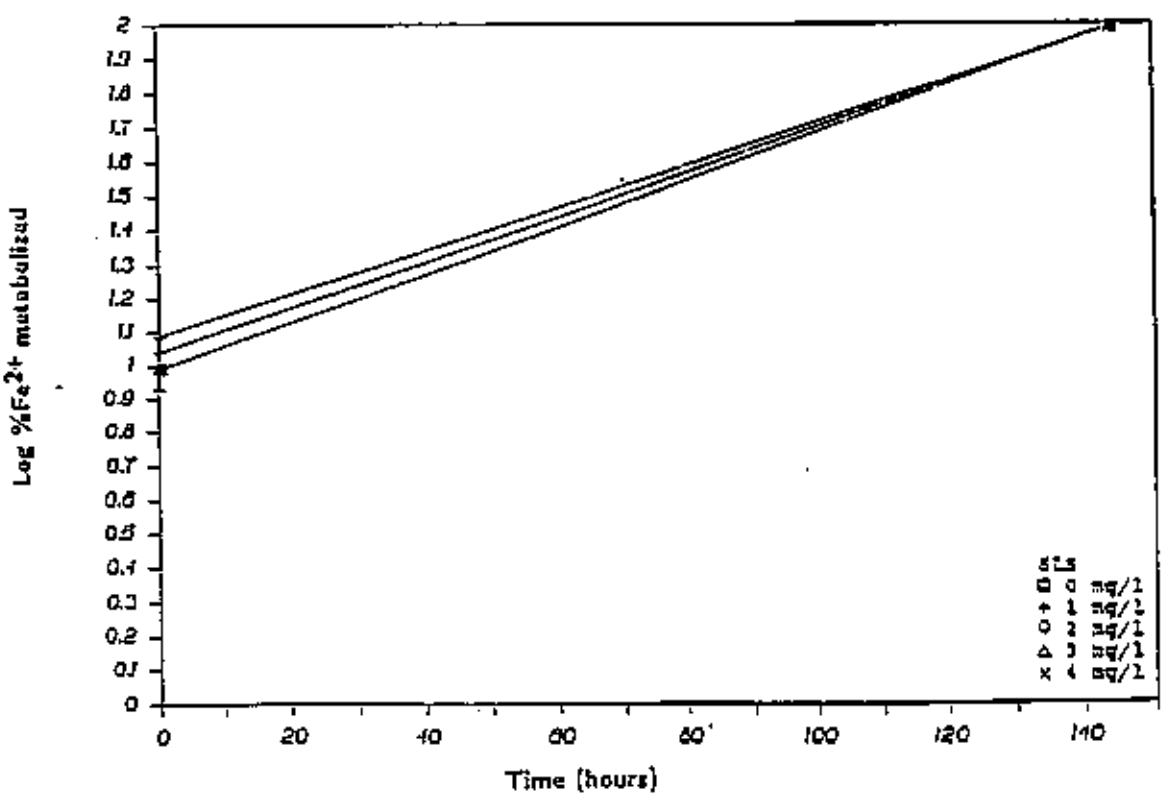
APPENDIX FIG. 88. Growth of *T. ferrooxidans* ATCC 19859 in the presence of SLS (1,0 mg/l) and sorbic acid (10,0 to 25,0 mg/l) added at the beginning of incubation.



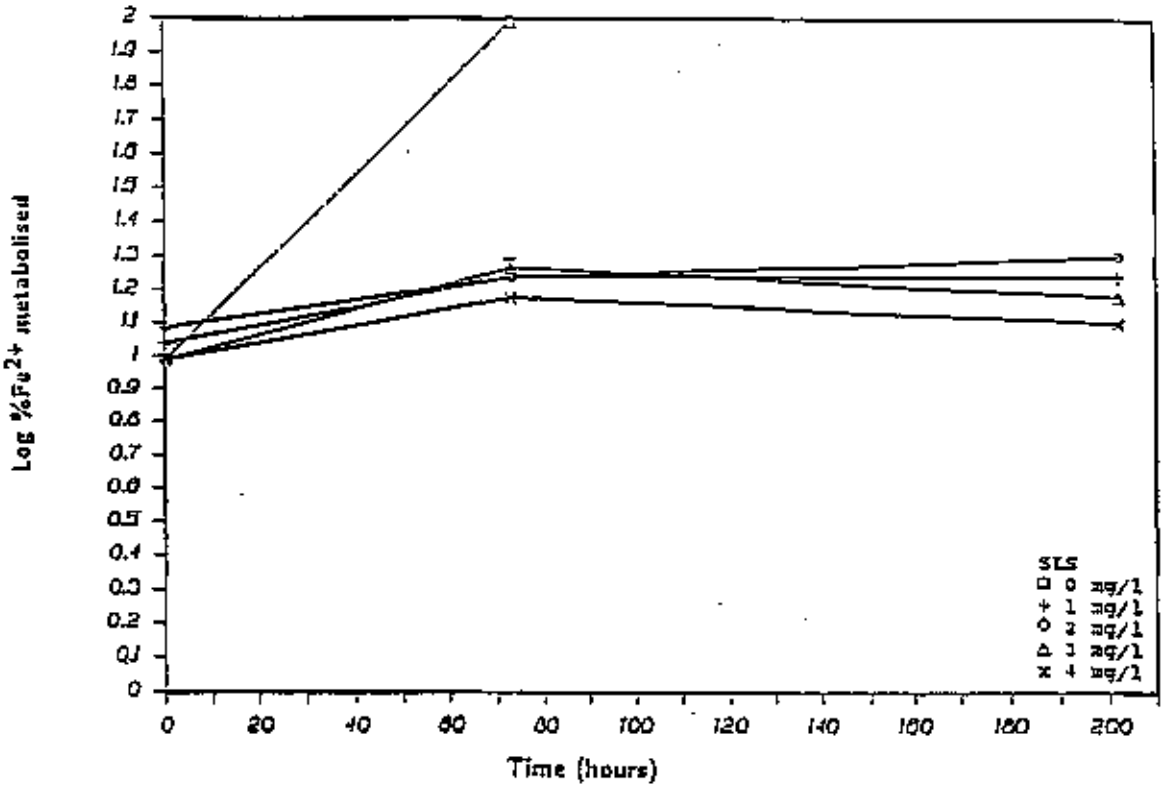
APPENDIX FIG. 89. Growth of *T. ferrooxidans* ATCC 19859 in the presence of SLS (2,0 mg/l) and sorbic acid (10,0 to 25,0 mg/l) added at the beginning of incubation.



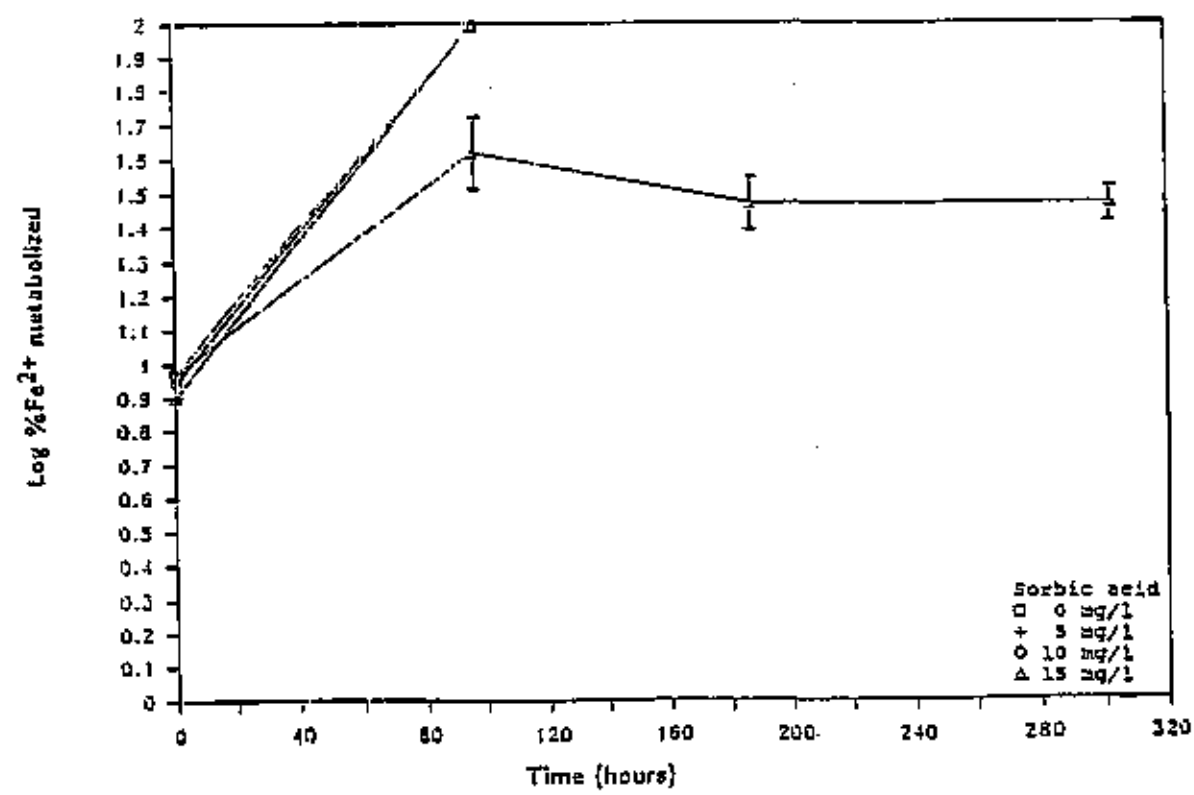
APPENDIX FIG. 90. Growth of *T. ferrooxidans* ATCC 19859 in the presence of SLS (3,0 mg/l) and sorbic acid (10,0 to 25,0 mg/l) added at the beginning of incubation.



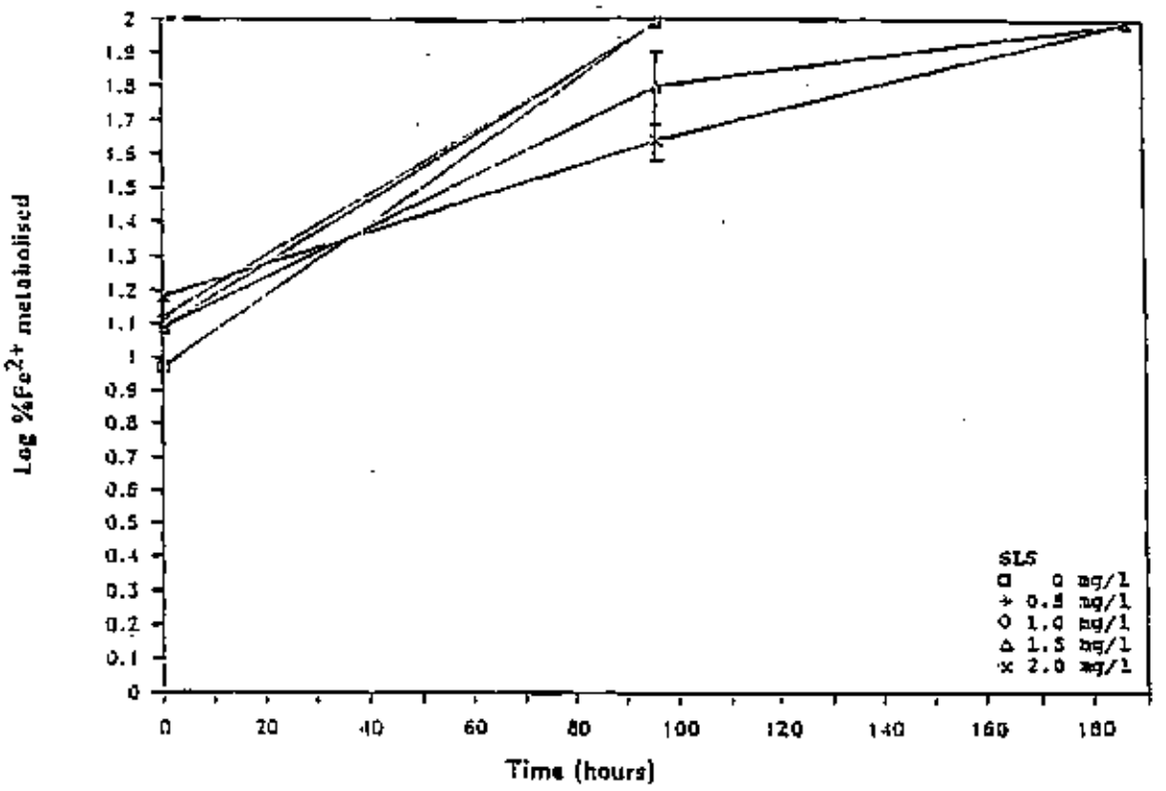
APPENDIX FIG. 91. Growth of *T. ferrooxidans* ATCC 19859 in the presence of SLS (1,0 to 4,0 mg/l) and sorbic acid (10,0 mg/l) added at the beginning of incubation.



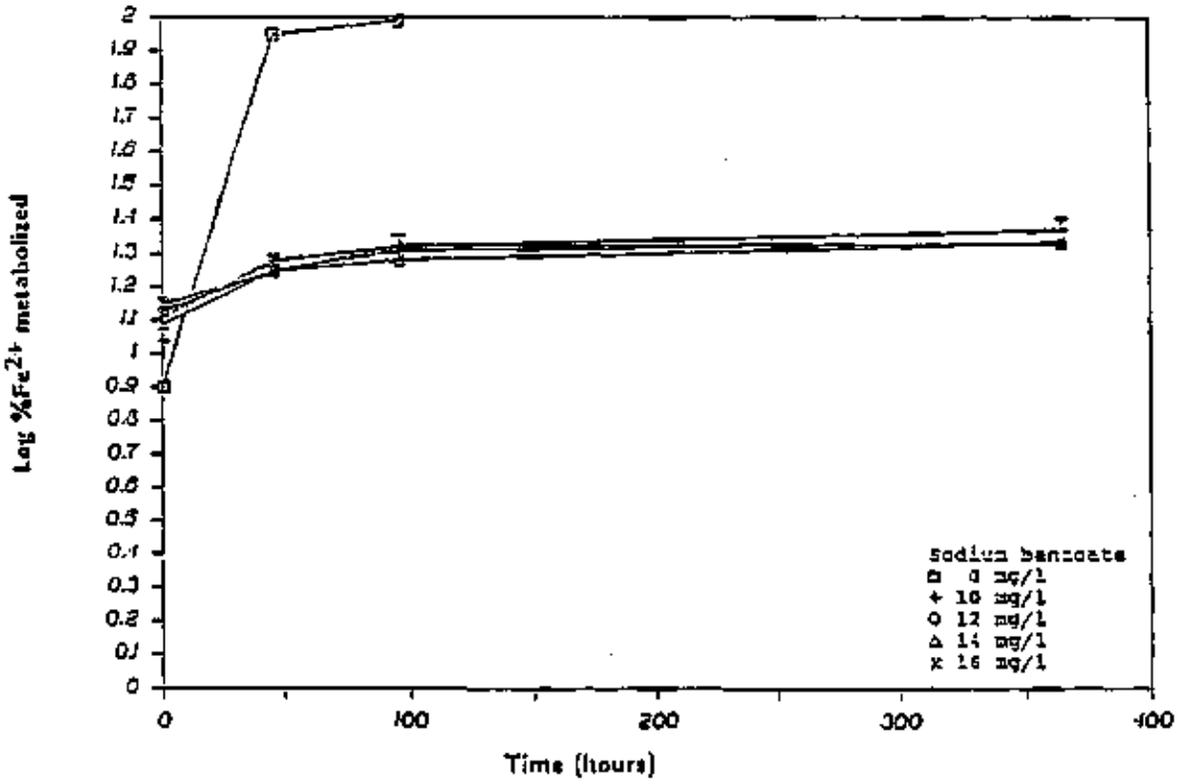
APPENDIX FIG. 92. Growth of *T. ferrooxidans* ATCC 19859 in the presence of SLS (1,0 to 4,0 mg/l) and sorbic acid (15,0 mg/l) added at the beginning of incubation.



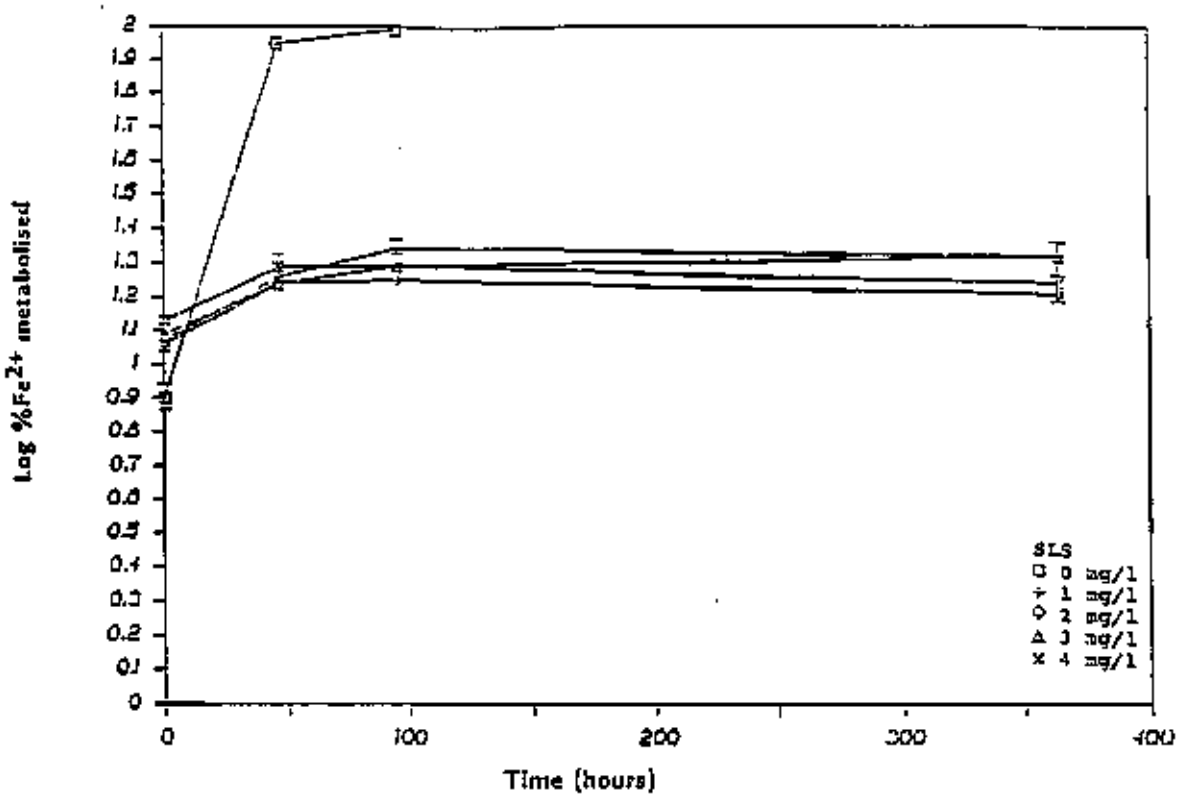
APPENDIX FIG. 93. Growth of *T. ferrooxidans* WLR in the presence of SLS (1,0 mg/l) and sorbic acid (5,0 to 15,0 mg/l) added at the beginning of incubation.



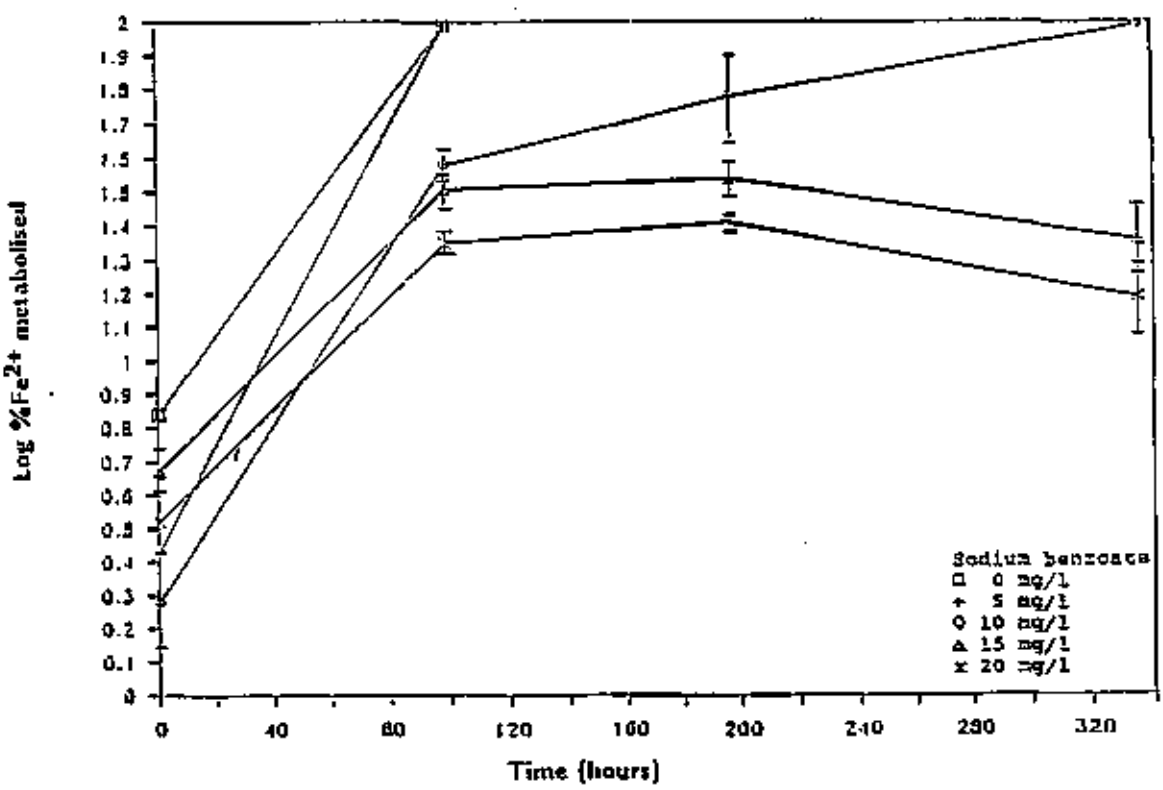
APPENDIX FIG. 94. Growth of *T. ferrooxidans* WLR in the presence of SLS (0,5 to 2,0 mg/l) and sorbic acid (10,0 mg/l) added at the beginning of incubation.



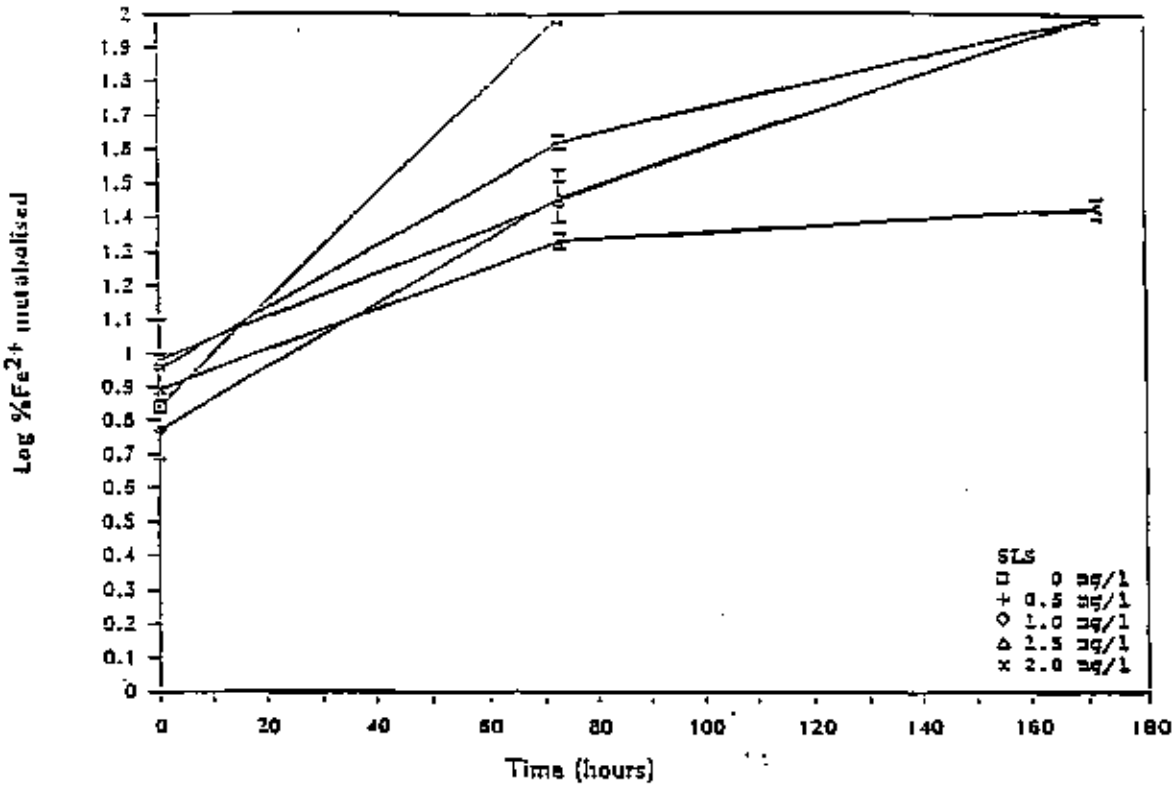
APPENDIX FIG. 95. Growth of *T. ferrooxidans* ATCC 19859 in the presence of SLS (2,0 mg/l) and sodium benzoate (10,0 to 16,0 mg/l) added at the beginning of incubation.



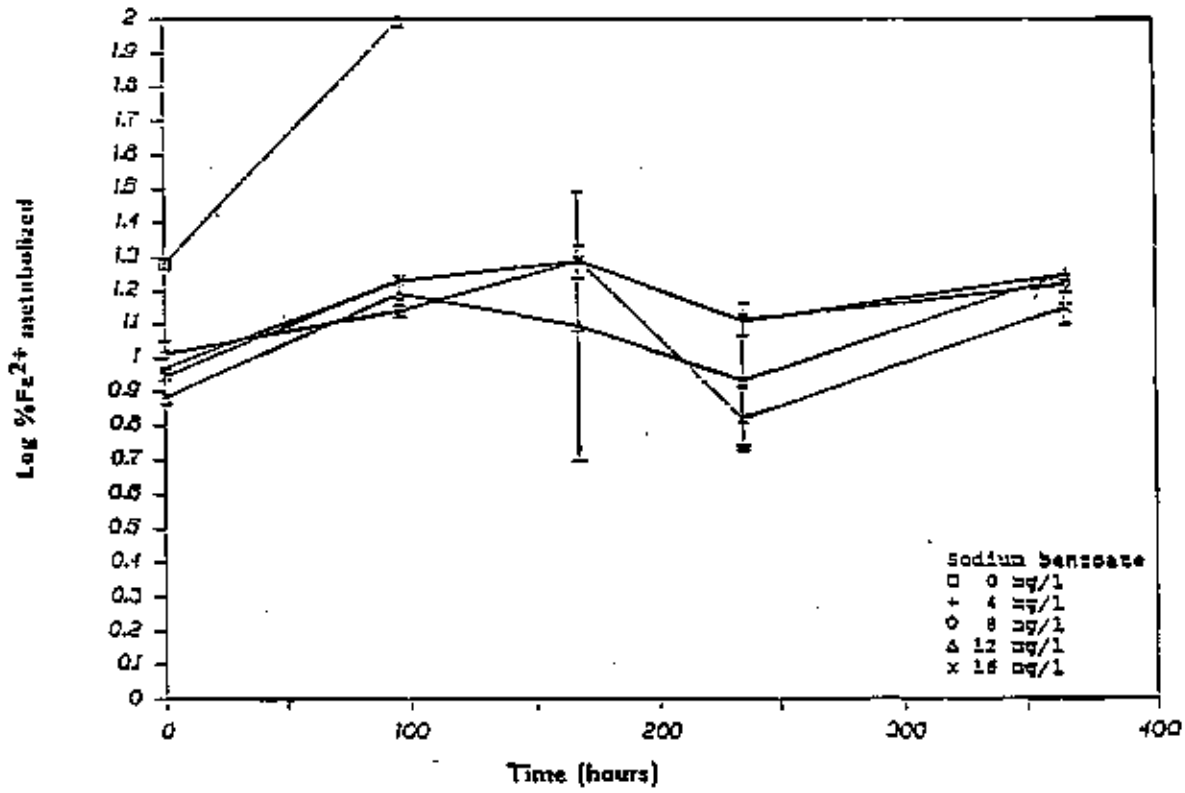
APPENDIX FIG. 96. Growth of *T. ferrooxidans* ATCC 19859 in the presence of SLS (1,0 to 4,0 mg/l) and sodium benzoate (14,0 mg/l) added at the beginning of incubation.



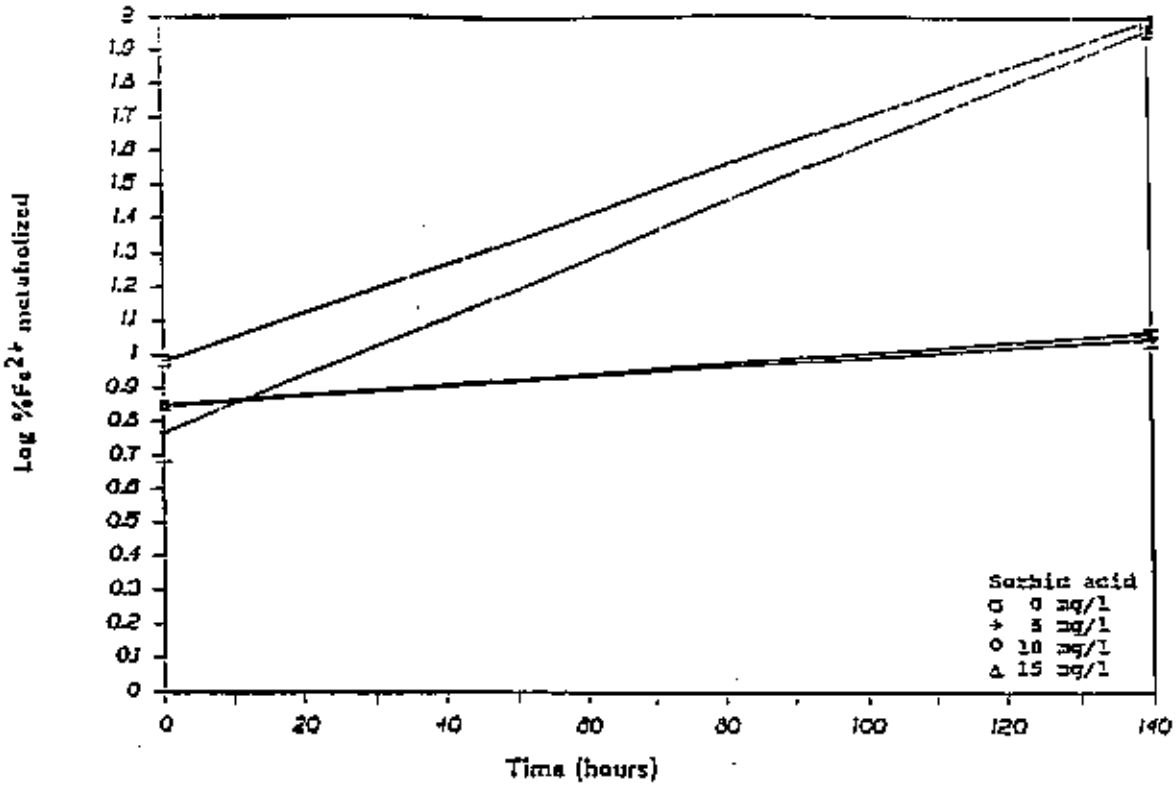
APPENDIX FIG. 97. Growth of *T. ferrooxidans* WLR in the presence of SLS (1,0 mg/l) and sodium benzoate (5,0 to 20,0 mg/l) added at the beginning of incubation.



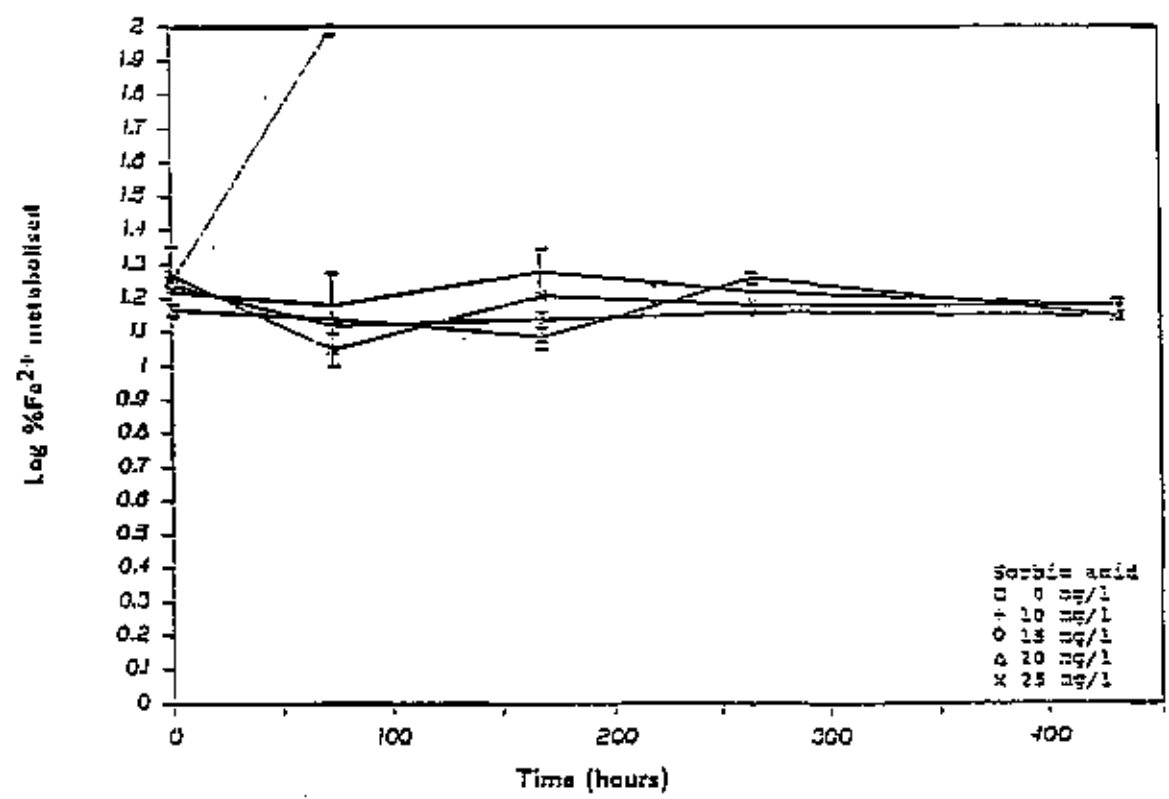
APPENDIX FIG. 98. Growth of *T. ferrooxidans* WLR in the presence of SLS (0,5 to 2,0 mg/l) and sodium benzoate (10,0 mg/l) added at the beginning of incubation.



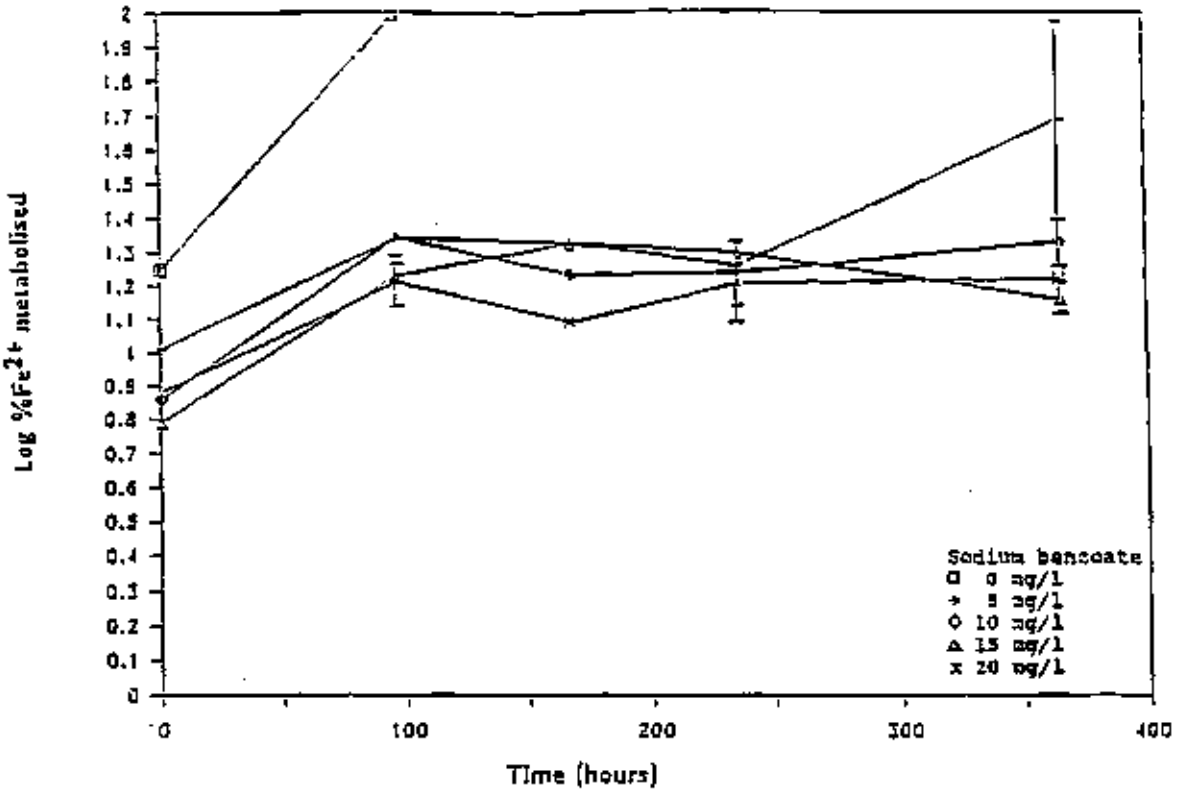
APPENDIX FIG. 99. Growth of *T. ferrooxidans* ATCC 19859 in the presence of sorbic acid (20,0 mg/l) and sodium benzoate (4,0 to 16,0 mg/l) added at the beginning of incubation.



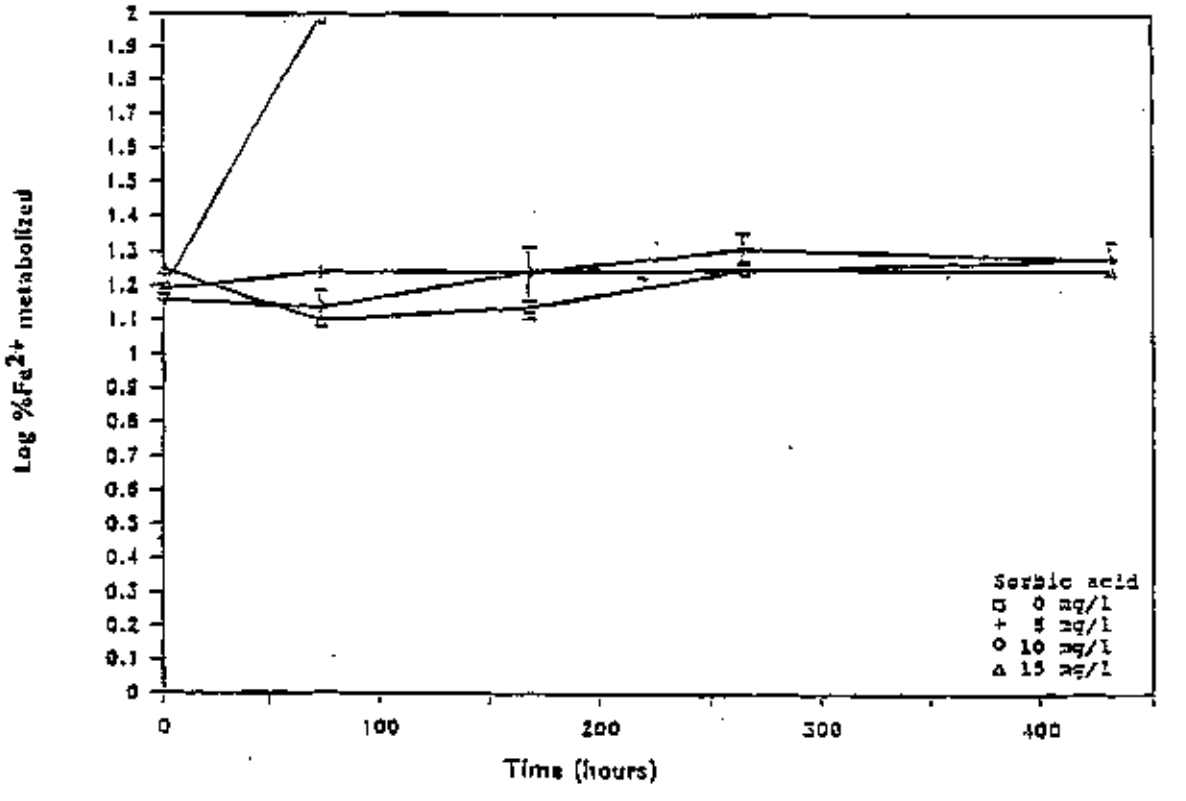
APPENDIX FIG. 100. Growth of *T. ferrooxidans* ATCC 19859 in the presence of sorbic acid (5,0 to 15,0 mg/l) and sodium benzoate (10,0 mg/l) added at the beginning of incubation.



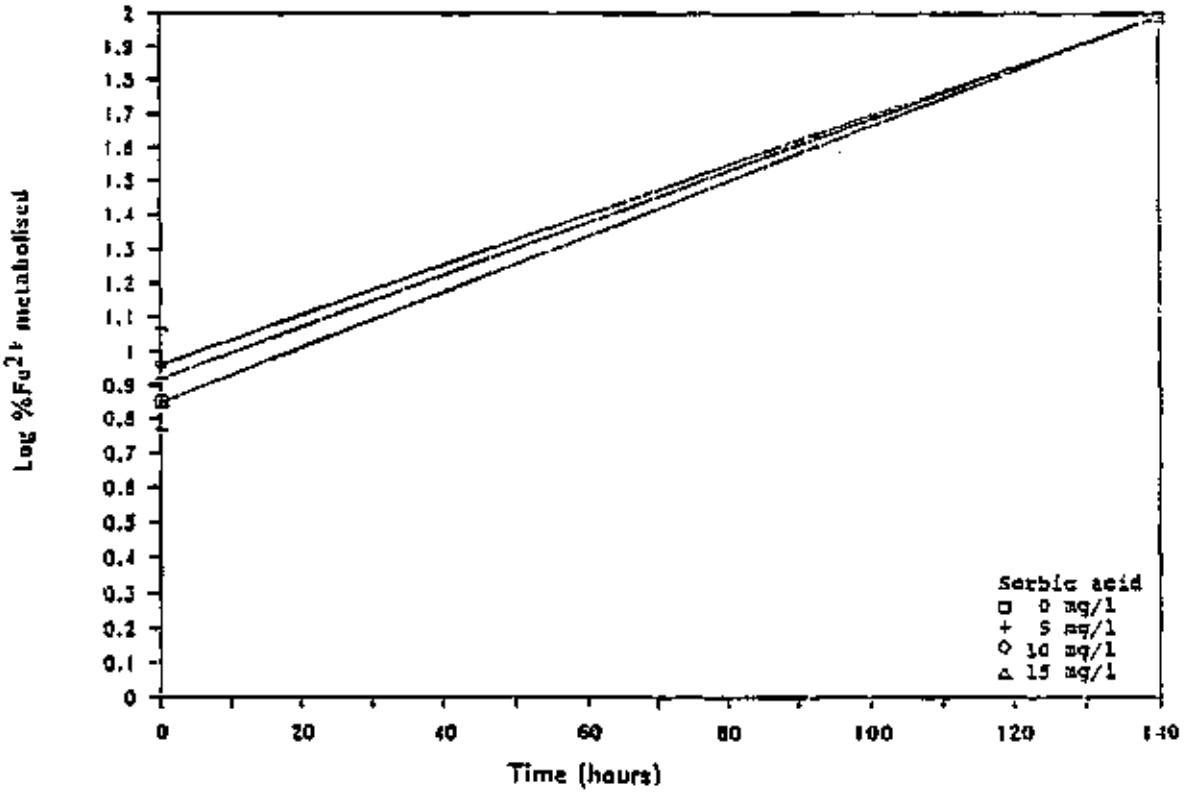
APPENDIX FIG. 101. Growth of *T. ferrooxidans* ATCC 19859 in the presence of sorbic acid (10,0 to 25,0 mg/l) and sodium benzoate (14,0 mg/l) added at the beginning of incubation.



APPENDIX FIG. 102. Growth of *T. ferrooxidans* WLR in the presence of sorbic acid (10,0 mg/l) and sodium benzoate (5,0 to 20,0 mg/l) added at the beginning of incubation.



APPENDIX FIG. 103. Growth of *T. ferrooxidans* WLR in the prsence of sorbic acid (5,0 to 15,0 mg/l) and sodium benzoate (14,0 mg/l) added at the beginning of incubation.



APPENDIX FIG. 104. Growth of *T. ferrooxidans* WLR in the presence of sorbic acid (5,0 to 15,0 mg/l) and sodium benzoate (5,0 mg/l) added at the beginning of incubation.

32. APPENDIX 4: TABLE OF CARBON, SULPHUR AND ASH ANALYSES OF SAMPLES FROM DIFFERENT DEPTHS IN PILOT SCALE COAL WASTE TEST PILES 1 TO 6[a]

Test pile	Sample number	Sample depth (cm)	Carbon (g/100g)	Sulphur (g/100g)	Ash (g/100g)
1	2	20	28,60	3,50	55,80
	3	30	21,80	2,62	55,60
	5	50	28,10	2,31	49,00
	7	94	35,70	3,26	52,10
	Mean		28,55	2,92	53,13
2	1	5	38,20	2,27	28,60
	2	45	43,30	2,87	37,00
	3	70	19,10	2,36	60,40
	Mean		33,53	2,50	42,00
3	1	25	30,10	5,60	53,00
	2	40	33,10	4,81	49,20
	3	55	15,40	1,96	69,30
	4	75	25,70	2,14	57,70
	Mean		26,08	3,63	57,30
4	1	10	22,30	1,47	78,30
	3	85	28,40	3,96	51,90
	Mean		25,35	2,72	65,10
5	2	67	22,00	11,50	47,80
	3	86	19,50	2,20	65,90
	Mean		20,75	6,85	56,85
6	1	8	46,30	4,20	27,90
	Mean		46,30	4,20	27,90
	Overall mean		28,60	3,56	52,47

[a]The analyses were conducted by the Research Laboratories of the Anglo American Corporation of South Africa Ltd., Johannesburg, as outlined in Section 25. Only coal waste material with a diameter less than approximately 10 mm was analysed; larger particles were removed from the samples by hand.