Assessment of Water Pollution by a Rapid Microbiological Test

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To my parents,

to whom I belong.

Preface

This thesis is the original work of the author. He wishes to acknowledge that the financial support was provided by his parents for the past three years.

Taha A-G. Mulla-Ali

ADDITIONAL COURSES ATTENDED AS PART OF POSTGRADUATE TRAINING

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Quotation

For the next considerable effluent of the Clyde we have to pass the city of Glasgow. At Partick, just beyond the harbour, it is joined by the Kelvin, which brings to it the sewage of about one-eighth of the population of Glasgow, including the drainage of print works, paper works, and distilleries, together with much liquid refuse from chemical and print works on the Glazert, one of its tributaries above Kirkintilloch. Our samples taken on July 25th, 1870, and March 13th, 1872, show that the Kelvin contained in solution nearly five times as much organic impurity as was present in it above Kirkintilloch, and we have reason to believe that at the latter date the Kelvin, just above its junction with the Clyde, was less polluted than usual. In answer to our inquiries addressed to the authorities of Kirkintilloch, we are told: "The Kelvin was formerly quite pure, but for many years it has been little better than a noisome ditch, caused by pollution flowing in to the Glazert, and thence into the Kelvin. The Luggie is comparatively pure, but for about two weeks at the end of August in every year it is polluted by refuse from flax steeping. This renders the water unfit for use by man or beast, and kills all the fish." Lower down, as it passes through the outskirts of Glasgow, the Kelvin becomes very much more offensive; and when at length it joins the Clyde it is by far the most filthy of all the effluents which that river receives.

Sir Edward Frankland
Fourth Report of the River Pollution Commission
of 1868, pp.12-13 (England).

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SUMMARY

The ultimate aim of this project was to develop a rapid method for measuring organic pollution as an adjunct to water pollution control. Several parameters were examined in order to achieve this aim. First, the possibility of using the oxygen polarographic technique, for measuring the rate of degradation of organic compounds by microorganisms, was explored. Secondly, the preparation of a bacterial seed inoculum for the oxygen polarograph, which would be standardized, obtained in a large quantity and could be stored for a long period of time with the retention of maximum activity, and yielding reproducible results. Thirdly, study of any possible interfering factors on the O_2 uptake measurement in the polarograph. Fourthly, the application of the method for polluted waters was considered.

Extensive studies on the oxidation of several known organic substrate solutions were investigated with the use of (i) a variety of river-water microorganisms isolated from a river polluted with sewage effluents and (ii) Pseudomonas cepacia, both as pure and mixed cultures in the polarograph.

Escherichia

A river-water isolate of ↑ coli was found to give reproducible results. The uptake of oxygen was maximal with a concentration of E. coli cells at 3×10^{11} per ml. In each test glucose (0.3 ml of 1M) was used as a standard substrate with 0.1 ml of cell suspension and 2.7ml distilled water ↑. Subsequently, the organism was grown in a 15 litre batch-culture in a fermenter, standardized to the required concentration and stored at low temperatures of -25, -70 and -195.8°C or freeze-dried. Samples of E. coli suspension, sufficient to do 3,000 tests were obtained from one batch culture. The seed stored at -70°C or -195.8°C retained activity comparable to that of fresh seed even after 8½ months storage. Maximum activity in the test was obtained at a working temperature of 37°C and this temperature was used

* BOD_5	The amount of oxygen required, by microorganisms, for biochemical oxidation of organic material during 5 day incubation period at 20°C.
* QO_2	amount of oxygen consumed (µl) / mg dry weight of microorganism/hour

in all the tests. The test was not affected by pH over the range 6.5-8.5. The effect of 12 heavy metals and other toxic materials on the rate of O_2 uptake was studied. This effect could be reduced in the polarograph up to much higher concentrations than in the BOD_5^* test.

After standardization of test conditions river-water samples were examined over a period of two years and sewage samples from different stages of the treatment process over a period of six months. These were compared with the standard substrate solution and with the results to the corresponding BOD_5 determinations. Higher levels of O_2 uptake were found for higher BOD_5 values. In general with the very low BOD_5 values for river-water (below the government standard of 20 ppm) the QO_2^* values were clustered around 0-6. Increased levels of O_2 uptake were found with high BOD_5 sewage samples. It was possible to identify, by the rapid microbiological method, the crude sewage liquor, the effluent from the primary settlement tank, and the final effluents. The results paralleled those obtained by the BOD_5 test. A linear relationship was found between the results of QO_2 and BOD_5 for sewage samples and the standard substrate solution.

The statistical analysis results for QO_2 v BOD_5 results showed a significant relationship between the results of the rapid microbiological test and BOD_5 determinations for river-water samples, and a highly significant relationship for the results of the sewage samples and the standard substrate solution.

Subsequently, the rapid microbiological test proved to be a useful method for assessing organic pollution of samples within a very short time (3-5 min). The method is simple, economic, reproducible, has theoretical validity and its results are comparable to those of BOD_5 test. Therefore it could be used as a rapid, practical method in the field of pollution control.

OBJECT OF RESEARCH

The standard BOD_5 test has many shortcomings and disadvantages from a practical standpoint. Hence investigators have devoted much time and effort to the development of more appropriate means for measuring BOD. Although a number of workers have suggested different techniques, the BOD_5 has remained the technique most widely used. Therefore, there is still a need for a rapid, practical test for pollution monitoring.

The long period of 5 days required to obtain the result of the BOD_5 test has been a considerable problem for the river board scientists and sewage treatment plant operators, as any pollutional event is an indicator of a past event, and the river inspectors are forced to wait 5-7 days before they can decide about any waste-water.

The following were considered:-

1. To explore the possibility of using an oxygen polarographic electrode to measure within a very short time the rate of oxygen uptake for organic compounds in polluted water by a mass inoculum of bacteria.
2. The preparation of a bacterial seed inoculum to be used in the polarograph for oxygen uptake measurement. This inoculum could be standardized, give a rapid and reproducible result, could be obtained in a large quantity and stored for a long period of time with the retention of maximum activity.
3. Application of the method to measuring samples from a river-water polluted with sewage effluent, sewage itself at different stages of the treatment process and different known organic compounds.
4. The study of various factors which might affect the oxygen uptake measurement in the polarograph.

The main aim was to develop a rapid method for assessing organic pollution of fresh waters that would be simple, practical, reproducible, economic and have theoretical validity.

INTRODUCTION

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LIST OF ABBREVIATIONS

APHA	American Public Health Association
ATP	adenosine triphosphate
AWWA	American Water Works Association
BOD	biochemical oxygen demand
BOD_5	five days biochemical oxygen demand
COD	chemical oxygen demand
CRPB	Clyde River Purification Board
DO	dissolved oxygen
DW	distilled water
FPD	filter paper disc
FSIWA	Federation of Sewage & Industrial Wastes Association
HMSO	Her Majesty's Stationery Office
MF	membrane filter
mgd	million gallons per day
MLSS	mixed liquor suspended solid
NCTC	National Collection of Type Cultures
P	pressure
PV	permanganate value
QO_2	amount of oxygene consumed(µl)/dry weight of microorganism(mg)/hr.
R	constant
RW	river-water
SD	standard deviation
SS	suspended solid
SRC	Strathclyde Regional Council
STW	sewage treatment works
T	temperature
TbOD	total biological oxygen demand
TOC	total organic carbon
TOD	total oxygen demand
V	volume
WPCF	Water Pollution Control Federation
$\bar{x}$	arithmetic mean

INTRODUCTION

MICROFLORA OF NATURAL WATERS AND SEWAGE

The important microorganisms of fresh water are: bacteria, Fungi, algae, and protozoa. This review is primarily concerned with the bacteria, because they are mainly responsible for the biochemical oxidation of organic pollutants both in the rivers and in BOD tests.

A. The Bacterial Flora of Natural Waters

The bacterial flora of unpolluted water can be divided into two groups according to their origin (Topley and Wilson, 1975).

1 - Natural water bacteria

A - Bacilli:

1. Fluorescent

A - Gelatin liquefied (*Ps. fluorescens liquefaciens*)

B - Gelatin non-liquefied (*Ps. fluorescens non-liquefaciens*).

2. Chromogenic

A - Red pigment e.g. *Serratia indica*

B - Yellow pigment e.g. *Flavobacter ochraceum*

C - Orange pigment e.g. *Serratia aurescens* (pigmented variance of *Serr. marcescens*)

D - Violet pigment e.g. *Chromobacter violaceum*.

3. Non-Chromogenic

A - *Achromobacter* spp.

B - Gram (+) spore bearing bacilli

C - Members of the coli-typhoid group.

B - Cocci:

1. Chromogenic (generally yellow pigment)

2. Non-chromogenic (*Micrococcus candicans*).

C - Sarcinae:

e.g. *Sarcina lutea*.

2 - Soil bacteria

These organisms, though not normally inhabitants of water, are washed into water during heavy rain from the soil of the river bank. Most of them belong to the group of aerobic spore-bearing bacilli, such as Bacillus subtilis, B. megaterium and B. mycoides. Others are aerobic non-spore-bearing bacilli, which may be found on grain, plants and decaying vegetation, eg. Klebsiella aerogenes and Enterobacter cloacae, nitrifying bacteria such as Nitrobacter spp. and Nitrosomonas spp., and Streptomyces and Actinomyces.

B. The Microflora of Sewage

Many sewage organisms are normal inhabitants of the intestine of man and animals, others live chiefly on decomposing organic matter of either animal or vegetable origin, and some might also be pathogenic organisms.

The intestinal bacteria include Escherichia coli, Streptococcus faecalis and Clostridium perfringens. Pathogenic bacteria may also be found, eg. Salmonella typhi and Vibrio cholerae. Proteus vulgaris and anaerobic spore-bearing bacilli, eg Cl. sporogenes are also found in sewage.

The organisms of domestic sewage are mostly putrefying bacteria, such as Ps. fluorescens, Ps. aeruginosa, Proteus vulgaris, B. subtilis, B. cereus, Aerobacter cloacae and Zoogloea ramigera. In addition, there are varying numbers of many other physiological groups, particularly those which break down sugars, such as Clostridium butyricum, Staphylococcus aureus and Micrococcus roseus; starch, such as Bacillus subtilis, Actinomyces bovis and Act. viscosus; urea such as Yersinia

pseudotuberculosis, Y. enterocolitica, Proteus morganii, Klebsiella spp., Pasteurella ureae, Cl. sordelli, Cl. histolyticum, Mycobacterium tuberculosis and Micrococcus varians; and cellulose such as Cytophaga spp., Sporocytophaga spp., and Polyangium spp. The proportion of coliform bacteria is substantial, which is an important indicator of the pollution of the water by faecal material. The number of Escherichia coli in the sewage of the city of Kiel varied between several tens of thousands and several hundred thousands per ml (Hoppe, 1970). Frequently Aerobacter aerogenes was also found, which belongs like E. coli to the Enterobacteriaceae, along with Streptococcus faecalis, also an inhabitant of the human intestine.

In sewage rich in organic matter Chlamydobacteria are important, particularly Sphaerotilus natans, which is often referred to as sewage fungus. It is a typical sewage organism and often covers the bottom of strongly polluted waters as a thick lawn. Massive development of Sphaerotilus natans may occur between 5-20°C and a pH of 6-9, if the oxygen supply is limited as in stagnant water (Scheuring and Hohnl, 1956).

As nutrients mainly carbohydrates, certain organic acids and proteinaceous substances are utilized. Sphaerotilus natans also grows in effluent from cellulose industries and various food industries. Its vigorous growth is associated with considerable consumption of oxygen, hence, a critical situation often develops if great quantities settle in areas of stagnant water. This may very quickly result in complete disappearance of oxygen. The organisms eventually die and undergo putrefaction, and amongst other products H_2S is formed.

The formation of hydrogen sulphide is usually enhanced by bacterial sulphate reduction, as sewage often contains abundant desulphurizing bacteria such as Desulfovibrio desulfuricans.

Sewage also contains sulphur-oxidising bacteria, particularly Thiobacillus, Thiothrix and Beggiatoa species, which can multiply rapidly when H_2S is being produced. Denitrifiers, like Thiobacillus denitrificans, Micrococcus denitrificans are present in domestic and in some industrial sewage; various iron bacteria are also available in some industrial sewage, eg. Leptothrix ochracea and Thiobacillus ferrooxydans. In sewage waters containing oil, bacteria which break down hydrocarbons accumulate, these are mainly members of the Pseudomonas and Nocardia genera (Rheinheimer, 1974).

EFFECT OF ORGANIC DISCHARGES ON THE LEVEL OF OXYGEN IN A RIVER

Organic matter is one of the major constituents of industrial effluents, such as those from canneries, breweries, paper-mills, dairies and from the manufacture of synthetic organic compounds. Some organic substances of industrial origin are directly toxic to fish and other aquatic life, eg. phenol which is toxic for fish. Organic matter of vegetable or animal origin is usually non-toxic. Both types of material may cause pollution in a river and may interfere with the use of water for various purposes, for example, by causing difficulties at water-works and giving rise to unpalatable tastes in the treated water, by forming deposits of sludge on the river-bed and by stimulating the growth of sewage "fungus".

A. Oxygen Balance in a Stream

After discharge into a river, with few exceptions, organic substrates are oxidized by bacterial action, thus withdrawing oxygen from solution. However, when the level of dissolved oxygen falls

below the concentration that is in equilibrium with the atmosphere (about 10 ppm at 14°C), the water absorbs oxygen from the air. The extent to which the level of dissolved oxygen in the stream falls is determined by the relation between the rate of oxidation and the rate of re-aeration. The BOD test is a useful guide to the rate of oxidation of the constituents of an effluent, even though it was originally devised for assessing the quality of sewage effluents.

B. Factors which Influence the Dissolved Oxygen Level in a Polluted Stream

In order to assess what effect an effluent of a given BOD value will have on the distribution of oxygen in a stream several factors have to be taken into account, of which the following are usually the most important (Department of Scientific & Industrial Research, 1958).

1 - Degree of effluent dilution

The rate of oxygen removal will be related to the concentration of organic matter in the stream. The BOD is used as a measure of the concentration of organic matter, and the approximate BOD of the stream below the point of discharge of an effluent can be calculated arithmetically from the rates of flow of the two liquids, the flow rate of the effluent over the flow rate of the river. The higher the flow rate of the stream compared to the effluent the lower the drop in the DO content. In practice it is usually the lowest normal flow rate of the stream which is important since at this level the concentration of oxygen is likely to fall to its lowest level.

2 - Turbulence of stream

The greater the turbulence of a stream the greater is the

rate of oxygen solution from the air. The differences in this respect between the rates for fast streams and slow ones may be very great.

Work at the Water Pollution Research Laboratory, showed that in a small turbulent stream running down a hillside the rate of oxygen solution from the atmosphere was about 200 times greater than in stagnant water. This would mean that if both the turbulent stream and the stagnant water were of equal depth, approximately the same fall in oxygen content would be produced in nearly stagnant water if its BOD was increased by 1 ppm as in the turbulent stream if its BOD was increased by 200 ppm. Clearly, the turbulence of the stream can often be a major factor in determining the effect of a given effluent on the river (Department of Scientific & Industrial Research,1958).

3 - Reaeration inhibitors

Some substances, when present even in small concentrations in water, reduce the rate of absorption of oxygen from the air. Little is known about these substances, but some seem to occur in unpolluted waters. They are probably decomposition products of plants, and some occur in domestic sewage. In addition, sewage and sewage effluents may contain surface-active materials from synthetic detergents, which considerably reduce aeration. The effect of thin layers of oil, on the surface of the water, in reducing re-aeration appears to be much smaller than had been expected (Downing and Truesdale, 1955).

4 - Depth of river

The oxygen entering a river from the atmosphere passes from the surface to the depths of the river. Consequently, in the absence of other complicating factors, the rate at which the oxygen is renewed varies inversely with the depth. This is one reason why polluting

discharges often cause such a great depletion of oxygen in deep waters eg estuaries.

5 - River-water temperature

As the temperature increases there is a greater oxidation rate. A rise in temperature also increases the rate of oxygen solution from the air, but this is generally insufficient to compensate for the increased oxidation rate. Consequently, other conditions being constant, the oxygen level falls to a lower value in hot weather than found in cold weather.

6 - Suspended matter

Many organic effluents contain oxidizable matter in suspension and this may be deposited as silt below the sewage outfall. A large proportion of the total oxygen-absorbing capacity of the effluent will thus be concentrated in a short stretch of the river, and the level of dissolved oxygen in this region may fall to a very low value. The occurrence of anaerobic conditions in some stretches of a river may be due to the accumulation of sludge. The de-oxygenating effect of many effluents can be greatly reduced by removing the suspended matter, for example, on sand filters, the BOD of a sewage effluent can be reduced by 50-60 per cent (Evans and Roberts, 1952) in this way.

7 - Aquatic plants

River plants need, for their growth and development, both the gases oxygen and carbon dioxide, and dissolved mineral nutrients such as calcium, potassium, phosphate and nitrate. The dissolved oxygen and carbon dioxide are taken up from the water by submerged plants and algae. They use oxygen for respiration at night, while they produce oxygen during the day by photosynthesis. In some

circumstances, though, plants can actually decrease oxygenation, as when there is much decaying vegetative material (the microorganisms which use oxygen for the decomposition of the decaying material), or when much of a vegetation clump is too shaded by its upper shoots to allow photosynthesis (Haslam, 1978).

During the warm months of the year, when temperature and light are optimal, a phenomenon of blooming of algae may occur. This was proved fatal to other organisms in the river. It has a more complicated effect on the dissolved oxygen content of the river and on the other aquatic life, in that during the hours of sunlight the algae produce greater quantities of oxygen by photosynthesis than they consume, which may result in high pH values, lethal to fish, while in darkness their continued respiration may result in oxygen depletion and increased concentrations of carbon dioxide.

C. Importance of Dissolved Oxygen Level in a River

Apart from aesthetic objections that there may be to the presence of excessive quantities of organic substances in streams, the reduction in level of dissolved oxygen which they cause is very important. For example, fish cannot live in water when the oxygen concentration falls below a critical level eg. 4 ppm for trout. The concentration depends on the species of fish, being somewhat lower for coarse fish than for salmon and trout. The critical oxygen concentration level for fish is increased as the temperature rises.

Sub-normal concentrations of oxygen also increase the toxicity to fish of most poisonous substances. Another important consideration is that if the concentration of oxygen falls to zero a public nuisance

may be caused from the evolution of foul-smelling gases, particularly hydrogen sulphide (Department of Scientific & Industrial Research,1958).

SELF-PURIFICATION OF RIVERS

When polluting organic matter is discharged to a watercourse, a natural purifying action, involving biochemical oxidation, occurs. Biochemical oxidation is brought about by microorganisms which utilize the polluting substrates as a source of carbon, while consuming atmospheric oxygen, dissolved in the water, for respiration. This process removes pollutants from the water so that the river may become reasonably clean again some kilometres below the location of the sewage inlet.

Although physical and chemical processes like sedimentation and oxidation are involved in self-purification, the biological processes controlled by microorganisms are the most important. Numerous living creatures take part in the purification of river, from birds, and fish down to microorganisms. Various insect larvae, worms and protozoa take up smaller particles. The decisive role is played by bacteria and fungi for they can break down organic compounds, both suspended and in solution. They are thus able to effect complete mineralization of many organic pollutants. Protein, sugar and starch are broken down particularly quickly; fat, wax, cellulose and lignin more slowly and sometimes incompletely. With the progress of self-purification, the microbial population changes. Thus, where the pollution is due to domestic sewage, the proportion of proteolytic bacteria gradually decreases and that of organisms which decompose cellulose increases. With progressive self-purification of river, not only the concentration of pollutants but also the bacterial content diminishes, whilst

decreasing the oxygen consumption reduces the O_2 deficit. During the warm season of the year, the nitrite bacteria and cellulose-decomposing bacteria increase markedly. The final step of mineralization of organic nitrogen compounds, denitrification, reaches its peak only when the proteinaceous pollutants have been largely decomposed.

Waters differ a great deal in their power of self-purification. It is greatest where turbulence of the water causes a rapid distribution of the sewage and a brisk exchange of gases with the atmosphere. A really effective decomposition of pollutants is possible only in the presence of oxygen, which must be replenished continuously. These conditions are provided in most streams and rivers and in coastal waters with pronounced tidal movements or vigorous currents due to wind. In stagnant waters the sewage may precipitate and an insufficient oxygen supply leads to an early oxygen deficit and thereby to a collapse of self-purification.

The power of self-purification also varies with the time of year and is greater during the summer months than in winter, because bacterial activity is aided by the higher water temperature in summer, and in brighter light the phytoplankton supply more oxygen. The greater metabolic activity of most microorganisms during the warm season causes the nutrients, supplied with the sewage to be used up much more quickly.

The seasonal variations in the numbers of saprophytes in sewage-loaded waters are not due solely to changes in the nutritional conditions. For example, the autolysis of bacteria and fungi is accelerated at higher temperatures, protozoa feeding on bacteria are more active, and in some places bright daylight may perhaps exercise a bactericidal effect. Correspondingly, the warm waters of the tropics show more rapid self-purification than the cold ones of the Arctic.

The breakdown of organic pollutants in the sea progresses more slowly than in comparable inland waters, even under favourable conditions of temperature and of oxygen supply. The self-purification of sea water polluted with domestic sewage takes twice the time of that of comparable fresh water (Mann, 1956). This is chiefly the result of the inhibitory action of sea water on many non-marine bacteria, and many marine bacteria having a lower metabolic activity than corresponding fresh water organisms.

The vigorous oxygen consumption often results in completely anaerobic zones where putrefying processes and bacterial sulphate reduction lead to the production of hydrogen sulphide. This in turn, causes the death not only of almost all higher organisms but also of many microorganisms, and a limited microbial population develops which can carry out only partial breakdown of the organic pollutants. The formation of iron sulphide leads to evil-smelling black sapropel which causes the death of the original fauna at the bottom. It is particularly dangerous if, in lakes and coastal waters, the H_2S-containing water from the deeper parts suddenly reaches the surface, for instance after strong storms; this may cause the death of fish on a large scale (Rheinheimer, 1974).

Disturbances in the self-purification process occur also through the direct introduction of poisonous substances, which may come with the sewage and refuse from industrial plants and cause the death of the organisms involved in the mineralization processes. This happens particularly with heavy metals, cyanide and organic poisons.

In the purification of oil-polluted waters, microorganisms play an important part. Hydrocarbon-decomposing bacteria and occasionally, fungi, accumulate in them. The oil components which are

soluble in water are broken down very quickly by microorganisms. Those bacteria are numerous in summer and very active at high water temperature. Their activity also depends on the concentration of inorganic nutrients, particularly phosphorus and nitrogen compounds (Gunkel, 1967). In oligotrophic waters, therefore, the microbial breakdown of oil may be helped by the supply of such compounds.

Organisms such as Nocardia rubra may play a role in the purification of sewage containing phenol. Phenol may come from the chemical industry and from hospitals.

BIOCHEMICAL OXYGEN DEMAND

The Biochemical Oxygen Demand (BOD) test measures the pollutional potential of a waste-water that contains an available organic carbon source for aerobic organotrophic microorganisms. The amount of oxygen required for the biochemical oxidation of biodegradable organic matter by microorganisms in a given time and at a constant temperature is determined. One reason for treating polluted water is to reduce this oxygen demand so that the residual organic matter will not seriously deplete the dissolved oxygen (DO) levels in the river. Dissolved oxygen is required to maintain a beneficial ecological balance in the water, as the reduction of dissolved oxygen below a certain level is deleterious to the aquatic life.

Therefore, the measurement of the pollutional potential of waste-water as an index of the depletion of the dissolved oxygen in a river is a reasonable and logical concept which provides a broad approach to the assessment of the strength of a waste-water.

Pollution control scientists and engineers find it advantageous

with organic wastes to use a colligative test, like the BOD, which directly assesses the likely impact on the environment. However, some other tests have been used to assess the pollutional strength of a waste water, such as Chemical Oxygen Demand (COD) and the direct measurement of organic carbon. Both of these are valuable methods used to measure the total amount of degradable and non-degradable organic material in a sample. It is better to determine the BOD of a waste since this directly measures the amount of oxygen used during microbial respiration, and during the continued metabolism by micro-organisms of the cellular components synthesized from the waste. Therefore, since the BOD test measures the amount of oxygen required for all the microbial metabolic reactions that result from the introduction of a particular amount of carbon source to the water, it cannot be compared directly with the other two analyses mentioned above.

Manometric procedures which permit the use of less dilute samples than required for the BOD test still measure biological oxygen demand. Such techniques can be used also to assess the oxygen requirement of microorganisms in the utilization of an organic substrate. None of these methods provides information about the behaviour of the pollutant in the river, or data which can be related to the kinetics of oxygen utilization in the river. On the other hand, the BOD test is supposed to depict kinetically the mode of deoxygenation in the river (Hoover, Jasewicz and Porges, 1953).

The BOD test has been used routinely by River Boards and by designers and operators of treatment plants, because the mechanism and kinetics of oxygen demand when considered with a reaeration coefficient in a differential equation can predict the course of depletion and recovery of the dissolved oxygen levels in the water.

The BOD test dates back to 1870 when Frankland measured the decrease in the dissolved oxygen content in bottles of polluted water, from the River Thames. He believed that oxidation of organic matter in streams was a purely chemical reaction (Frankland, 1870). From these studies he concluded there is no river in the United Kingdom long enough to effect the destruction of sewage by oxidation. Despite his lack of knowledge of the biochemical nature of the reactions, his data are comparable with present-day BOD results. It was merely the limited time taken from the source to the sea in the short streams of the United Kingdom that justified his conclusion (Phelps, 1944).

The modern concept of biochemical oxidation was founded in 1884, when Dupre showed that the decrease in the dissolved oxygen content of incubated samples was caused by the metabolic activity of the organisms present (Phelps, 1944). This concept resulted in many research studies during the last fifty years (Phelps and Winslow, 1907; Theriault, 1927; Ruchhoft et al, 1948; Ruchhoft, Placak and Etinger, 1948; Gotaas, 1948).

O'Brien and Clark (1962) credit Phelps with combining the early putrescibility test results, 'the putrescibility test was based on the process of degradation of organic materials', with the dissolved-oxygen depletion data. The latter has been the basic idea of the standard BOD_5 dilution test presented by the American Public Health Association (APHA, 1917) in their standard method (Bridie, 1969).

In earlier studies the BOD test was intended to assess the effect of waste-water discharges on the quality of receiving waters and for this particular purpose the test was useful. Today there is a need for a test with a much wider applicability; however, in this

respect the standard BOD_5 test shows several disadvantages. First, the conditions of the BOD_5 test may be quite different from those prevailing in the river, this is especially true when a) the concentration of pollutant in the samples requires more oxygen than is available from the dilution water, or b) the concentration of bacterial cells is appreciably higher. Secondly, the long period of incubation, 5 days, required for the test is impracticable for routine work in pollution control. The river is constantly receiving new water and/or pollutant, so the BOD will also change. Consequently, the BOD_5 value is an indication of a past pollutional event. Thirdly, no information is obtained until after five days. This makes it impossible to decide whether the same measurement might have been obtained after one day or whether the incubation period should have been continued for longer than five days. Moreover, this lack of information makes the test unsuitable for biological studies on the inhibition of oxidation by or toxicity of heavy metals and other toxic materials.

I. The Mechanism and Kinetics of Biochemical Oxygen Demand

A. Some effects of autotrophic metabolism

It is necessary to distinguish between O_2 uptake due to aerobic organotrophic metabolism of the carbon sources in the waste (Carbonaceous BOD) and that caused by the aerobic autotrophic organisms, for example, Nitrosomonas and Nitrobacter. These nitrifying bacteria, which may be present in certain well-aerated seed materials and in water treatment plant effluents, can cause an O_2 uptake which is sometimes termed the second-stage uptake or the Nitrogenous BOD.

In this case oxygen utilization is brought about by the oxidation of ammonia to nitrite by Nitrosomonas, and nitrite to nitrate

by Nitrobacter. Some of the chemical energy released during this progressive removal of electrons is trapped as biologically useable and highly transferable chemical energy, for example, as ATP. A reduced inorganic compound such as ammonia may also serve as an electron donor in the reductive fixation of inorganic carbon, eg carbon dioxide for which energy is supplied by ATP. The overall oxidation reaction can be written as the following equation

$$2\,NH_3^- + 4\,O_2 \_\_\_\_\_ 2\,NO^-_3 + 2H^+ + 2H_2O$$

The implication of this equation is that for each milligram/litre of NH_3, nearly 4 mg/l O_2 can be consumed. This can lead to a significant BOD in wastes containing large amounts of ammonia or nitrogenous organic matter.

Under the usual conditions of temperature, dissolved oxygen content, and the presence of sufficient number of heterotrophic bacteria, the BOD due to autotrophic metabolism is not important since it normally occurs after the 5 day incubation period. In general the number of nitrifying bacteria in the seed or sample is low and their growth is relatively slow, because it depends upon adequate supplies of both reduced inorganic nitrogen and inorganic carbon compounds. This type of O_2 uptake can occur in the receiving stream, but is unlikely to be a determinative factor in producing the minimal value of dissolved oxygen in a stream.

Studies of Streeter and Phelps (1925) led to the following conclusions concerning the course of biochemical oxidation of organic matter. The rate of biochemical oxidation of organic matter is proportional to the remaining concentration of unoxidized substance,

measured in terms of oxidizability. This law was expressed mathematically as follows

$$-\frac{dL}{dt} = K_1 L \qquad (1)$$

In integrated form

$$Lt = L_o e^{-Kl^t} \quad \text{or} \quad L_t = L_o\, 10^{-k_1 t} \qquad (2)$$

The symbol L_o represents the total oxidizability (BOD) of the organic matter initially present in the sample, that is, at zero time. This term has become known as the ultimate BOD. L_t is the amount of oxidizability (BOD) remaining to be expressed at the corresponding time, t. K_1 (natural logarithms) or k_1 (common logarithms) is the proportionality or velocity constant for the reaction.

Events which conform to this type of kinetic equation can be described by first-order kinetics. This formulation is also the kinetic mode of a monomolecular reaction. It states that the rate at which the amount of oxidizability or residual oxidizable material decreases becomes lower as the material is oxidized, eventually becoming asymptotic to some lower limit (Figure 1). The dotted line represents the same kinetic relationship in terms of oxidizability, or BOD; that is, $y = L_o - L_t$ at time t. Substituting for L_t in equation 2, the amount of BOD expressed, y, is given as

$$y_t = L_o\,(1-e^{-Kl^t}) \quad \text{or} \quad y_t = L_o\,(1-10^{-k_1 t}) \qquad (3)$$

Theriault (1927) in his report, observed that the course of the first stage, or carbonaceous "exertion" of BOD for Ohio river water conformed to the monomolecular law. Thus the dissolved oxygen "sag curve" equation of Streeter and Phelps (1925), which has become a widely used expression for predicting or estimating the assimilating capacity

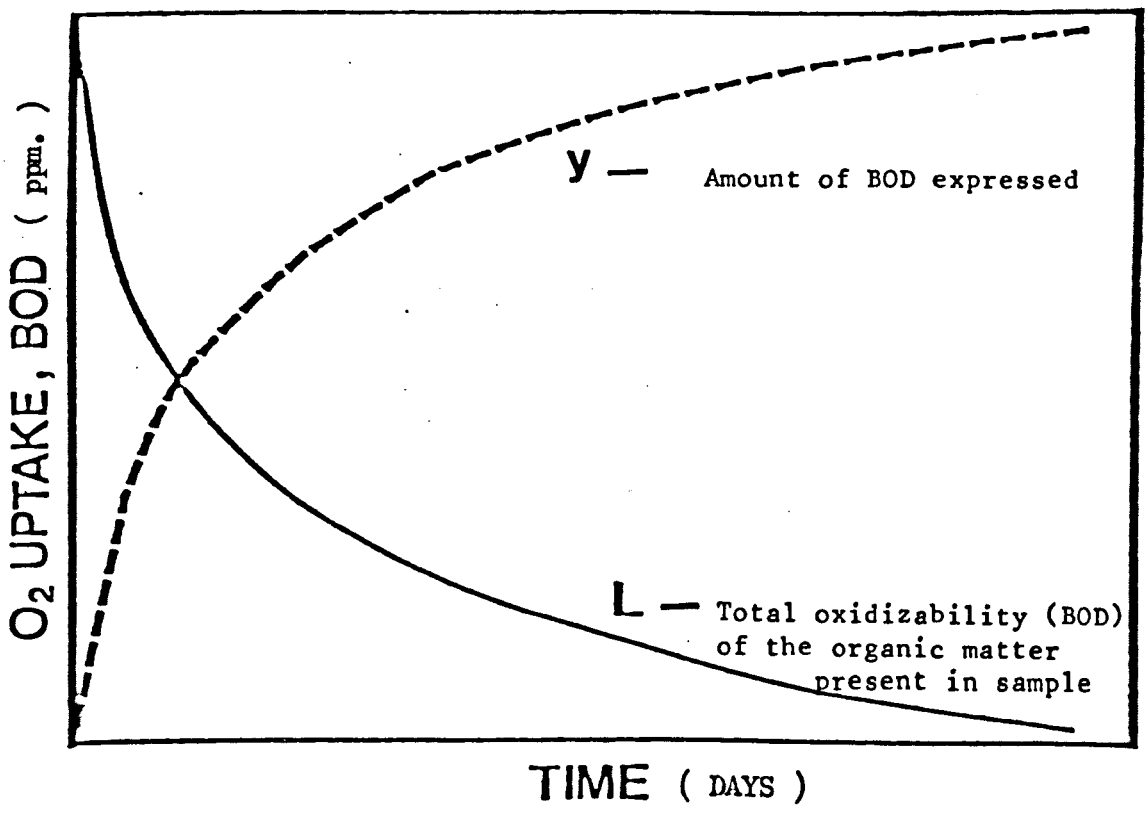


Figure 1. Exertion of biochemical oxygen demand in accordance with first-order decreasing rate kinetics (Monomolecular Law).

(from Gaudy, 1972)

of a receiving stream, is one in which two opposing first-order decreasing rate kinetic processes are combined. This equation is usually written as

$$\frac{dD}{dt} = K_1L - K_2D \qquad (4)$$

D represents the deficit of DO in the stream, that is, the difference between the DO saturation and the actual DO in the stream. K_2 represents the velocity or proportionality constant of the first-order decreasing rate expression depicting the course of physical reaeration of the stream. Integration of this equation with proportionality constants converted to base 10 leads to the sag equation:

$$Dt = \frac{k_1L_o}{k_2-k_1} (10^{-k_1t} - 10^{-k_2t}) + Do\ 10^{-k_2t} \qquad (5)$$

This equation has become one of the most widely quoted kinetic expressions in the field of pollution control.

One of the chief causes for concern with non-carbonaceous BOD arises from the estimation of the 'Ultimate BOD', that is, L_o. This term has been used synonymously with the 20-day BOD value, which survived from the early work on the BOD of municipal sewage where in the velocity constant, K_1 was approximately 0.1 day^{-1}. For this velocity constant, in accordance with monomolecular law, 99.0% of the carbonaceous BOD would be exerted by the twentieth day, hence the BOD_{20} sometimes equated with the ultimate BOD, L_o. Since O_2 uptake due to nitrification usually occurs during the second week of incubation, the nitrogenous exertion affects the values of L_o, but not the BOD_5.

A lack of agreement between actual measurement of BOD_{20} or L_o and calculation of L_o based on BOD_5 data may thus be observed. The

discrepancy is partially dependent upon the amount of non-carbonaceous BOD exerted. Therefore, L_o values may not represent the total carbonaceous BOD if they are based on BOD_{20} determinations.

Phelps (1944) expressed the view that, since the oxygen in NO^-_2 and NO^-_3 is available as a source of oxygen to some bacteria, the dissolved oxygen used in nitrification does not really represent a depletion of the total oxygen resource. It is important to realize that the bound oxygen in nitrite and nitrate is not available as an electron acceptor to most forms of life in the receiving stream, and that those microorganisms which can use this bound oxygen do not do so until the dissolved oxygen is low or extinct. Thus the bound O_2 would not be used until the stream was almost anaerobic.

If a healthy ecosystem is to be maintained in a stream dissolved free oxygen and not bound oxygen is required. Any agency which reduces free oxygen, should not be dismissed when assessing the effects of pollutants in the stream.

The nitrifying bacteria are not the only chemoautotrophic organisms which can increase BOD. The hydrogen bacteria (H_2 as electron donor), the colourless sulphur bacteria (H_2S, S, and $S_2O^{-2}_3$ as electron donors) and the iron bacteria (Fe^{+3} as electron donor) also possess this ability. However, these sources of energy are not usually present in sufficient quantities to cause serious oxygen depletion. One of the products of the oxidation of sulphur compounds is SO^{-2}_4 which, like NO^-_2 and NO^-_3 can be a source of bound oxygen for the strict anaerobe, Desulfovibrio. Therefore, the use of sulphate as an oxygen resourse is to be avoided, because it requires the presence of anaerobic conditions at the bottom of the receiving water course. It is emphasized, therefore, that aerobic autotrophic metabolism leading to

the production of bound oxygen (by nitrifiers and colourless sulphur bacteria) must ultimately cause depletion of the available oxygen resources in the receiving stream.

The photosynthetic production of oxygen, and oxygen utilization in the absence of light by algae is another factor, which can complicate observations during the course of exertion of carbonaceous BOD. Such complications are controlled in the laboratory with the use of dark bottles in the BOD test. Algae represent a deleterious factor in highly enriched streams, because they contribute to the organic loading (both suspended and those in the sediment). During the night the algal population may consume large amounts of oxygen. These factors contribute to the conclusion that any attempt by pollution control workers to consider photosynthetic oxygen production as an asset in calculating the dissolved oxygen resource in a stream is erroneous. In healthy streams, however, algae are present in relatively small numbers and are an asset, occupying a useful niche in the ecosystem (Gaudy, 1972)[11] cited in full from Gaudy (1972) because of the comprehensive explanation of the biochemical oxidation process of organic matter.

B. Carbonaceous biochemical oxygen demand

The biochemical and ecological reactions which occur during metabolism of organic carbon sources in the rather dilute aerobic environment in the stream and in the BOD bottle are different. In the BOD bottle and, to a greater extent in the stream, it seems essentially correct to consider the ecosystem as "closed". Consequently, the BOD bottle is similar in some respects to a microbial culture but with two important differences. First the concentration of nutrient supply is rather low in the BOD bottle because the aim of the technique is to

make the carbon source the limiting factor, and not the O_2 supply or any other nutrient source. Secondly, the microbial population is heterogeneous and "natural".

In a system (BOD bottle) inoculated with a relatively small number of cells in relation to the carbon supply, it was found that a typical microbial growth curve developed (Figure 2) (Gaudy, 1972). A curve of this shape is usually observed whether viable count, total count, biological solids concentration, or optical density is used to measure growth. The general shape of the O_2 uptake curve was similar, but it was not implied that growth and oxygen uptake curves for any system would be superimposable. The shape of both curves, however, would be those of an autocatalytic process; the inflection point would separate the first-order increasing rate portion (logarithmic growth) and the decreasing rate portion which sometimes may approximate first-order decreasing kinetics. The relative position of the inflection point which separates the two parts of the curve may vary, depending upon i) the procedure used to measure growth or oxygen uptake and ii) the microbial population whose growth is being assessed. The important point is that one would expect to observe both a logarithmic and a decline phase, regardless of whether growth or oxygen uptake is chosen as the parameter of measurement.

The initial lag period is not represented in Figure 2. If either a metabolic acclimation or an adaptation (population selection) is required before the log phase is entered, its extent (with regard to time) can be determined from a semilogarithmic plot of the data. At the end of the logarithmic phase, one would expect that the population had exhausted the available carbon source.

As our discussion progresses we will attempt to build upon

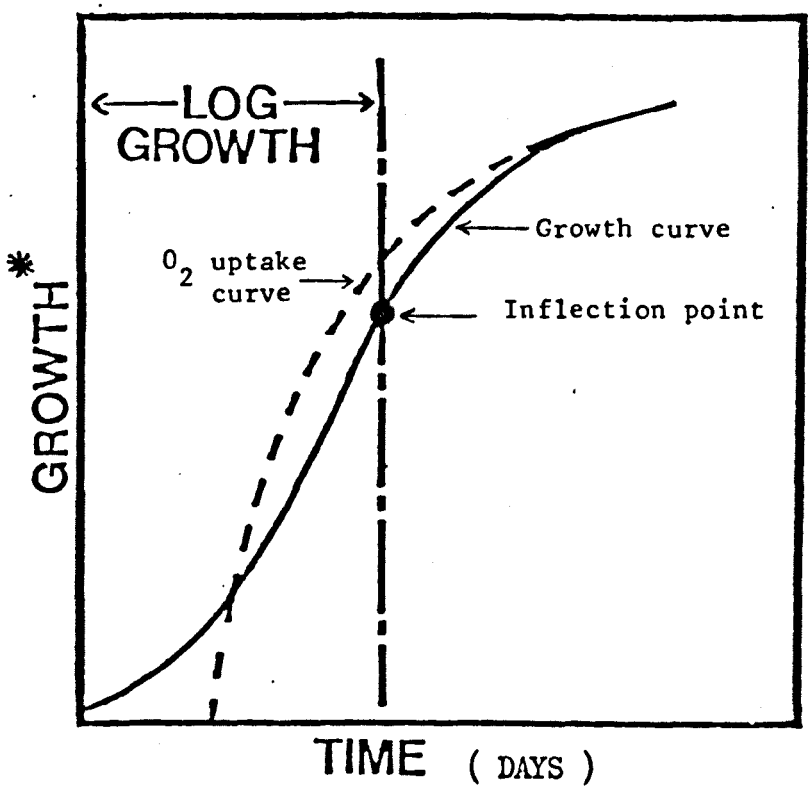


Figure 2. Typical autocatalytic growth curve (from Gaudy, 1972).

* Measurement of growth may be viable cell count, optical density, O_2 uptake, biological solids, total protein, or other parameters in specific cases.

this figure and fill in some of the other parameters of concern, such as the curve for removal of the carbon source which permitted the growth curve to be generated, events which occur after the removal of the carbon source, etc. But first, the curves of Figure 2 should be compared with the one produced by monomolecular law, which was shown in Figure 1. That curve disallowed a log phase of growth and O_2 uptake.

Gaudy (1972) stated that in attempts to "force" BOD curves into the monomolecular mould, the log phase is sometimes treated as a lag phase (dotted line, Figure 2). In employing this procedure one should be aware that the phase treated as a lag phase may actually be a log phase, and that there is a risk of discarding that portion of the data which are most critical from the standpoint of use in making a credible assessment of the critical dissolved oxygen in the receiving stream. On the other hand, it should be realized that the inflection point on the curve occurs early, much before the end of the standard 5-day incubation period. Therefore, for the curve shown, if the investigator is interested in keeping an historical record of the performance of a treatment plant or in assessing the BOD loading as a design criterion to size a trickling filter or activated sludge tank, it might make relatively little difference whether this early portion of the curve were treated as a lag or log phase.

Oxygen uptake can proceed under certain conditions without a log phase, for example, under severe nitrogen limitation, O_2 uptake may be initiated with either linear or decreasing rate kinetics, the latter may approximate to a first-order decreasing rate. Also endogenous respiration, that is, biological O_2 uptake due to oxidation of organic matter which has already been taken up by the organisms from

the external medium, may proceed with either linear or first-order decreasing kinetics. Often when one examines accumulated oxygen uptake curves under either of the conditions cited above, the early stages appear linear but, after tracing the O_2 utilization for a longer time, the decreasing rate of O_2 uptake becomes apparent.

It is likely that first-order decreasing rate kinetics was observed in the early studies on BOD because of a) nutrient deficiencies of b) the oxygen uptake being attributed to dense microbial populations utilizing organic components of the cells.

In the long term, O_2 uptake during the logarithmic phase of O_2 uptake may represent about 25% of the total amount of oxygen ultimately used by the ecosystem in the BOD bottle. Gaudy et al (1965) measured the O_2 uptake for different water samples daily, over a period of 20 days, and showed that the existence of a log phase of O_2 uptake might not be detected.

If a known substrate is used for measuring BOD exertion, it can be shown that only 35-45% of the theoretical oxygen demand has been exerted. The theoretical oxygen demand was defined as the amount of oxygen required to oxidize the substrate to CO_2 and water. For an actual waste sample the theoretical Chemical Oxygen Demand cannot be calculated, since the qualitative and quantitative compositions are unknown, but an approximation can be made by measurement of the COD. In neither case, known substrate or a waste sample, is theoretical oxygen demand equivalent to L_o, since equality would imply total biological oxidation of the waste. It is extremely unlikely that total oxidation in the BOD bottle would occur during any reasonable incubation time. An even greater discrepancy between theoretical chemical oxygen demand

and L_o, arises when a waste contains organic material which is relatively inert to biological oxidation.

When the organic substrates in a waste sample have been converted into cellular constituents, during the first stage of oxidation, the O_2 uptake curve roughly parallels the growth curve to the stationary phase (Figure 2). After this the viable count in the system usually decreases, and the continued rise in O_2 uptake cannot be attributed solely to endogenous metabolism (Gaudy et al, 1965; Bhatla and Gaudy, 1966).

The decrease in the number of viable bacteria may occur for various reasons, eg. death and autolysis, all of which lead to an increase in O_2 uptake.

After the external carbon source is exhausted in the ecosystem, the starvation conditions enhance competitive interactions, including predation. Thus, metabolism continues after the initial carbon source in the waste sample has been removed from the system, and the respiration associated with it is registered as carbonaceous BOD (quoted from Gaudy, 1972).

Butterfield, Purdy and Theriault (1931) studied the influence of the plankton on the biochemical oxidation of organic matters by pure and mixed cultures of bacteria in the presence and absence of plankton. They interpreted their experimental results (Figure 3; Table 1) in the form of a theory which ascribed to the protozoa a somewhat passive or indirect role in the exertion of BOD. According to these workers, the protozoa kept the bacterial population below a saturation point and thus provided suitable conditions for continuous bacterial multiplication, which in turn resulted in a more complete oxidation. This theory would be credible if, at the time the protozoa reduced the bacterial popu-

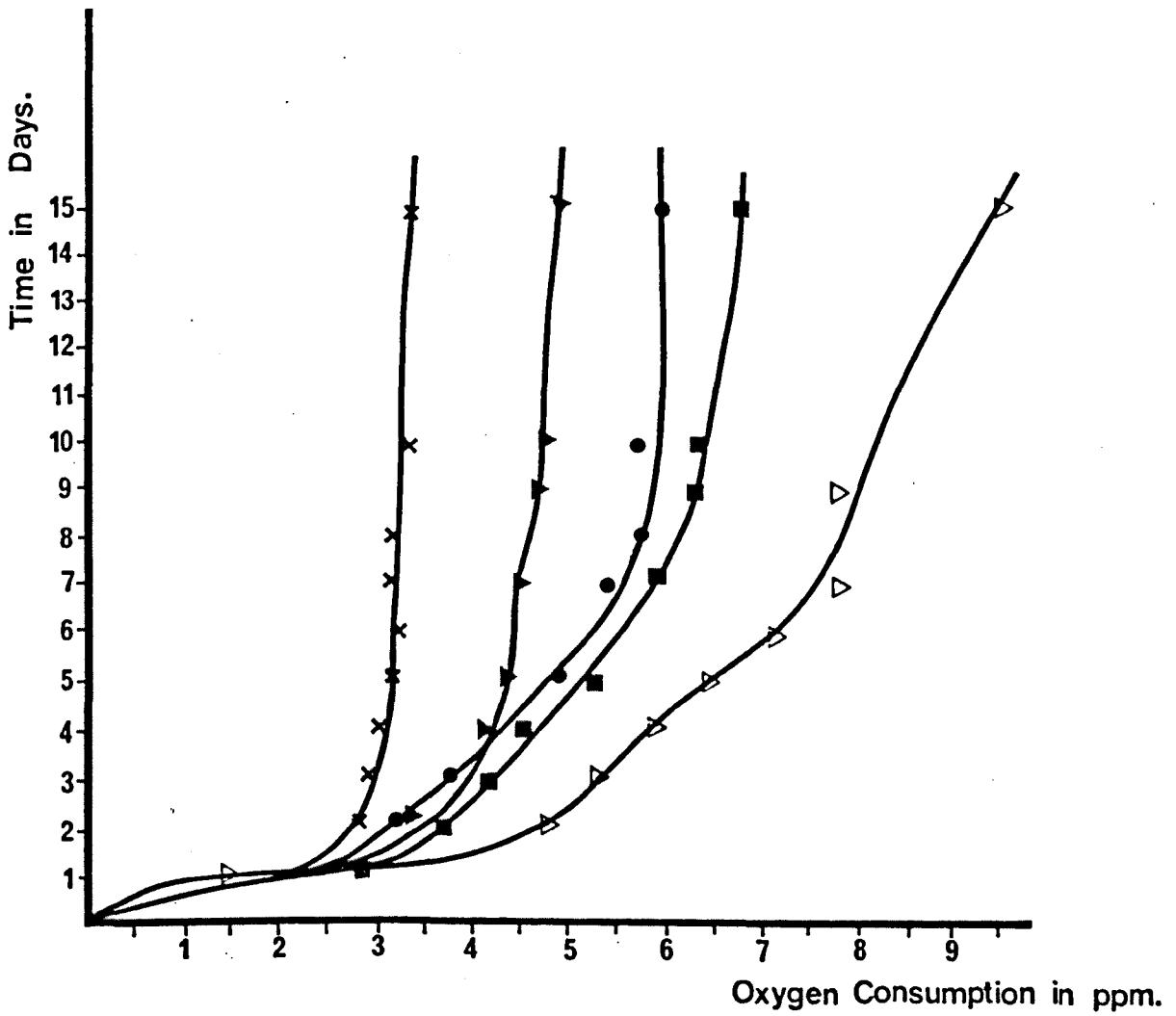


Figure 3. Oxygen depletions observed at 20°C when the dilute dextrose-peptone solution was seeded with inoculations which varied in their biological complexity

(Butterfield, Purdy and Theriault, 1931).

×—× Bact. aerogenes in pure culture.

▶—▶ mixed culture bacteria plankton free

●—● *Bact. aerogenes and Colpidium

■—■ mixed culture bacteria and Colpidium

▷—▷ all bacteria and plankton in river-water.

* Enterobacter aerogenes new name for Bacterium aerogenes

Time in days	Oxygen loss with varying biological inoculations, as follows:				
	Bact. aerogenes in pure culture	Mixed culture bacteria free from plankton	Bact. aerogenes plus Colpidium	Mixed culture bacteria plus Colpidium	All bacteria and plankton in raw river water
1	2.24	2.49	2.42	2.80	1.36
2	2.77	3.23	3.25	3.60	4.69
3	2.75	3.99	3.76	4.09	5.18
4	2.83	4.02	4.33	4.44	5.84
5	3.05	4.26	4.85	5.18	6.38
6	3.02	-	5.24	-	7.04
7	2.95	4.43	5.38	5.81	7.76
8	2.97	-	5.74	-	-
9	-	4.48	-	6.20	-
10	3.16	4.65	5.59	6.26	7.84
15	3.19	4.77	5.89	6.74	9.37
20	-	-	-	-	10.14
30	-	-	-	-	10.33

Table 1. Average oxygen depletions observed at 20°C when the dilute dextrose-peptone solution was acted upon by inoculations which varied in their biological complexity.

(Butterfield, Purdy and Theriatt, 1931)

lation, external carbon sources were still present in the medium so that bacteria could continue to grow and achieve a more complete oxidation of the waste. The theory of Butterfield, Purdy and Theriault (1931) was based largely on the surmise that some of the original carbon source, albeit a decreasing amount, was present in the medium throughout the incubation period. It has since been shown that the original external carbon source in the waste sample was depleted by the time the plateau is attained, before the protozoan population had increased to any great extent (McWhorter and Heukelekian, 1962; Grady and Busch, 1963; Bhatla and Gaudy, 1966). Consequently, there is insufficient external carbon source to sustain the multiplication of the reduced numbers of bacteria.

II. The Standard Dilution Method of the 5-Day Biochemical Oxygen Demand (BOD_5) Test

A. Definition and history

In 1898, a Royal Commission on sewage disposal was appointed to report on methods of waste treatment, the measurement of pollution, and to establish limits of pollution for rivers. The Commission showed a significant relationship between the DO content and the degree of pollution of river waters. In 1913, the Commission proposed in the eighth report that the amount of DO required for biochemical oxidation of organic material during the 5-day incubation period at 65°F (18.3°C) should be taken as the standard measurement of the quality of the liquid (McGowan, Frye and Kershaw, 1913). The temperature of 65°F was the maximum expected in British streams during the summer months. The

Commission included both long and short incubation periods but concluded that the 5-day test would allow a greater percentage of the waste to be oxidized and yet would not include the effect of nitrification. Also, five days was the maximum time required for any British river to flow from its source to the sea. Theriault (1927) standardized the current standard BOD_5 procedure, which was adopted in 1936 by the American Public Health Association (APHA) Standard Methods Committee. The principle of the BOD_5 method is the measurement of the dissolved oxygen content of the sample before and after incubation for 5 days at $20^{o}C$. The difference between the two values gives the BOD of the sample. Dissolved oxygen concentrations are measured by the Winkler Method (Winkler, 1888) or its modifications, such as the Rideal-Stewart (1901) and the Alkaline-Azide procedure (Institution of Water Engineers, 1960). The 5-day BOD test has remained as the standard method for monitoring pollution.

The BOD_5 test was first known as the Dissolved Oxygen Absorption Test or the Royal Commission Test; but biochemical oxygen demand superceded the earlier names. Oxidation is by no means complete in five days and for some purposes longer periods of incubation may be used. The number of days of incubation may be indicated by a suffix, eg BOD_5 or BOD_{20}. The result is expressed as milligrammes oxygen consumed per litre of sample.

The BOD_5 test requires the use of dark 250 ml bottles, to stop the interference of algal photosynthesis on the dissolved oxygen content of the sample. On the other hand, BOD determinations in dark bottles include the oxygen consumed by the respiration of algae. It is impossible to differentiate quantitatively the amount of oxygen consumed by the algae so the significance of the BOD of samples containing algae is dubious.

B. Development of the BOD_5 test

The early studies of the Royal Commission on sewage disposal made use of a 5-day incubation period. Some studies were made to determine whether this time (5 days) could be reduced, but it was generally concluded that the 5-day incubation period allowed time for the reaction to develop sufficiently (Gaudy, 1972).

Studies of the BOD technique (Sawyer and Bradney, 1946; Sawyer et al, 1950) led to the establishment of glucose and glutamic acid as primary standards of organic matter and the gradual realization of the dependence of the BOD_5 on the quantity and quality of the seed (Butterfield, Purdy and Theriault, 1931; Zehnpfennig and Nichols, 1953; Tidwell and Sorrels, 1955; Lee and Oswald, 1959b). Orford and Ingram (1953) showed the dependence of the k and L estimates on the time of observation or number of points used in calculating them and proposed a logarithmic equation

$$yt = S\ (0.85\ \text{Log}\ at + 0.41)$$

where

yt = BOD at any time t,

a = BOD rate constant,

t = time in days,

S = a constant = BOD at $\frac{5}{a}$ days

= 5 day BOD value which would have been obtained at standardized domestic sewage oxidation rates (a = 1.0), and 0.85 and 0.41 = constants.

These authors suggested that this equation had more biological significance and was better adapted to the interpretation of biological oxidation phenomena than the monomolecular reaction equation.

The calculation of BOD at any time agreed with the increase

in the standard BOD estimate with temperature increase, as the temperature variations were compensated, within the range 5-35°C (Gotaas, 1948).

Zehnpfennig and Nichols (1953) attempted to devise a BOD test which was not influenced by protozoa present in sewage and developed a BOD_2 test which gave reproducibility that was considerably better than that of the BOD_5 test.

The improved reproducibility of the BOD_2 test was confirmed by Tidwell and Sorrels (1955), but they did not agree that the bacterial inoculum, which was the supernatant of fresh settled sewage (1 ml/BOD_5 bottle), contained more bacteria capable of oxidizing sewage.

A series of analyses on raw sewage, using the BOD_2 and the BOD_5 tests were described by Tidwell and Sorrels (1956). They showed that the observed BOD values against BOD_2 values gave mean percentage errors of 5-10 per cent for BOD_2 tests and of 20-25 per cent for the BOD_5 tests. A series of raw sewage samples were also analysed for solids and the BOD_2 and BOD_5 were determined. Little or no correlation was shown between the solids determinations and either of the BOD readings. The BOD_2 and BOD_5 readings agreed reasonably well in 50% of the tests, while in the remainder the observed BOD_5 value was much lower than anticipated from the BOD_2 readings.

The effect of bacterial flora on deoxygenation was reviewed by Tidwell and Sorrels (1955). They compared the reproducibility of the special BOD_2 test of Zehnpfennig and Nichols (1953) with the standard BOD_2 and BOD_5 tests to see what changes took place in the microbial flora of the sewage as a result of filtration and incubation. They showed that in BOD incubation studies, with pure cultures and sterile synthetic sewage, some organisms (Aerobacter aerogenes,

Pseudomonas aeruginosa, Bacillus subtilis, Serratia marcescens and Proteus vulgaris, as well as a number of species isolated from sewage) could oxidize either carbohydrates or proteins but were unable to attack both; the inadequacies of particular species were not defined. While Tidwell and Sorrels (1955) stated that E. coli, Ps. aeruginosa, B. cereus, S. marcescens, P. vulgaris, Alcaligenes faecalis, Alc. metalcaligenes, Achromobacter liquefaciens, Achr. recti and Achr. bookeri were not satisfactory as inocula for the dilution test because even with those species capable of oxidizing the synthetic sewage used, the BOD obtained varied with the number of organisms in the inocula.

The special BOD_2 procedure showed a mean deviation of 2.2 per cent as compared to 4.3 and 5.7 per cent for the normal BOD_2 and BOD_5 test. The changes in the microbial flora occurring in the preparation of the inoculum for the special BOD_2 test were studied but no explicit explanation was given for the improved reproducibility of the special BOD_2 test. This study illustrated that the BOD of a given sewage varied with the amount of organic matter, and with the number of organisms capable of oxidizing the sewage, but not with the total number of organisms present.

A comparison was made by Orford and Matusky (1959) between the results of a short-term BOD test with those of the BOD_5 test. Tests were made for 1, 2, 3 and 5 days at 20°C and for 1 and 2 days at 37°C and it was found that the variation in the BOD results increased as the incubation was shortened and the temperature raised.

At 20°C the BOD_2 value was 25 per cent greater than the BOD_5 result, while for the BOD_1 test the variation was doubled. No improvement was obtained at 37°C because the variation at 1 and 2 days

was only slightly greater than the variation for the corresponding incubation periods at 20°C.

Orford and Matusky (1959) reviewed the short-term BOD test and concluded that for domestic raw and settled sewage, the BOD incubation period could be shortened to 2 days without seriously affecting the reproducibility of the results (Figure 4). The straight lines of best fit were determined by the method of least squares according to the equation

$$yt = m \log t + b \qquad (1)$$

For the BOD values at 20°C, the equation of the line is

$$yt = 75.04 \log t + 46.84 \qquad (2)$$

and for BOD observation at 37°C

$$yt = 75.04 \log t + 69.43 \qquad (3)$$

where

yt = ratio of BOD at time observed to BOD at 5 days 20°C, expressed as per cent, and

t = time of BOD observation, days.

Ratios computed from these equations should be reliable between 0.8 and 7 days at 20°C, and between 0.6 and 4 days at 37°C (Figure 4).

The relationship between the short-term and BOD_5 20°C values (Table 2) are given so that the 5 day 20°C BOD value can be estimated from a short-term test for raw and settled domestic sewage. It was found that the BOD_5 values estimated from BOD_1 results showed a three-fold variation.

Prokesova (1962) investigated the use of new apparatus to rotate the BOD bottles of known volume (1 litre) at different speeds, on a special holder, in a 20°C water bath in the dark. The bottles

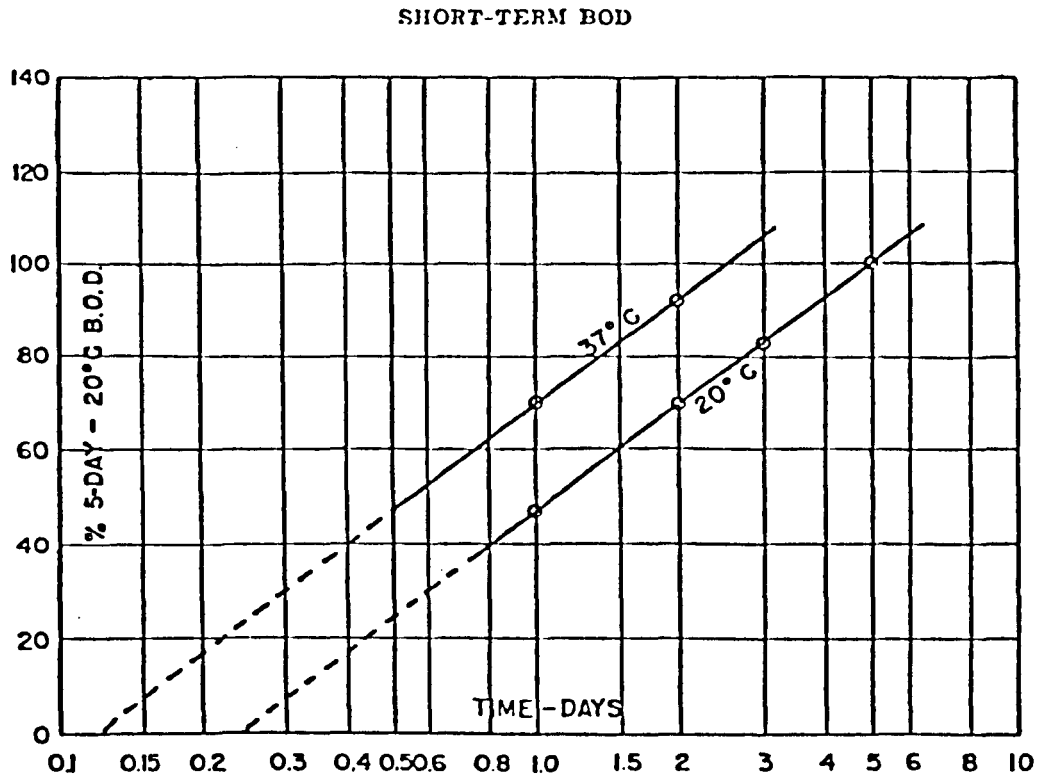


Figure 4 — Relationship between short-time BOD and standard 5-Day BOD of domestic sewage.

(Orford and Matusky, 1959).

Days	Temp (°C)	*V for BOD's Determined (per cent)	Ratio of Short-Term BOD to 5-Day BOD (per cent)	*V for Ratios of Short-Term BOD to 5-Day BOD (per cent)	*V for 5-Day 20°C BOD Computed from Short-Term BOD (per cent)
1	20	8.2	47.4	9.1	12.6
2	20	4.9	68.8	6.2	7.5
3	20	4.6	82.0	6.0	7.1
1	37	8.4	69.4	9.2	13.0
2	37	5.6	92.3	6.8	8.6
5	20	3.9	-	-	-

*Coefficient of variation

Table 2. Relationships Between Short-Time BOD and Standard 5-Day BOD of Domestic Sewage

(Orford and Matusky, 1959)

were filled, closed, then opened and 10 ml of water was withdrawn. They were then reclosed.

This method was used to determine the influence of aeration on BOD. The results showed that oxygen consumption in aerated bottles was lower than in those without aeration. The aeration seemed to lower the BOD of the first stage and support nitrification. With artificial sewage, aerated bottles showed lower BOD values than unaerated bottles.

A technique for studying the kinetics and mechanism of BOD exertion in dilute systems was suggested by Jennelle and Gaudy (1970). They compared the mechanisms and kinetic course of BOD exertion in both open and closed systems. The two open vessels were a simulated stream device and an open stirred vessel, and the closed systems consisted of standard BOD bottles and 2.4 litre vessels. In the closed systems, both static and shaking conditions of incubation were examined. The rate of oxygen uptake increased with the increasing concentration of carbon source. The relationship between specific O_2 uptake rate and substrate concentration approximated to a hyperbolic function similar to the Monod relationship for specific growth rate and substrate concentration.

C. Biochemical oxygen demand of organic compounds

Biochemical oxidation of organic compounds was investigated by several workers, e.g. Heukelekian and Rand, 1955; Busch, 1958; Busch and Myrick, 1961. An approach which differed from the arbitrary fixation of 1, 2, 3, 4 or 5 days incubation for BOD determination was sought by Busch (1958), who showed that when all the substrate was degraded oxygen consumption reached a highly reproducible maximum

plateau value. This value was about 41% of the theoretical value with glucose. When oxygen uptake was measured during the oxidation of glucose by microorganisms, it provided a measure of the carbon in glucose that was oxidized to form carbon dioxide. If all the carbon in glucose was oxidized to carbon dioxide, each milligram of glucose would consume 1.07 mg of oxygen, which is equivalent to the oxygen demand for glucose. When bacteria oxidized glucose oxygen consumption ceased after one hour and the plateau value was 0.38 mg of oxygen for 1.0 mg of glucose. If all the carbon was converted to carbon dioxide the plateau value would have been 1.07 mg of oxygen, therefore, more than half of the carbon was not oxidized to CO_2 but incorporated into cell components (Clifton, 1963a). The oxygen plateau value is only a part of the theoretical oxygen demand of the substrate. The oxygen demand of that portion of the chemical that has been assimilated into the cell is not measureable by this method or by the standard BOD method and remains an unknown factor (Busch, 1958; Busch and Myrick, 1961; Busch et al, 1962). Oxygen consumption in a BOD bottle, containing dilution water and bacterial seed, continued even after one and a half hours (Krishnamurty, 1964), due to bacterial endogenous respiration. When a massive inoculum of bacteria is introduced into a substrate the resulting oxygen consumption is due first, to endogenous respiration and secondly, to the breakdown of the substrate. The consumption of oxygen approaches zero when all the degradable substrate is broken down and converted to carbon dioxide or assimilated into cell components. When there is no further oxygen consumption due to substrate, the process reaches the plateau value.

Clifton (1963b) showed with E. coli and C^{14} labelled glucose (0.2 ml of 0.04M - 102.2 mμc) that in addition at the plateau value a

small part of the carbon, which was in the supernate, was gradually consumed with time. There is no way of determining the percentage of substrate assimilated or converted into carbon dioxide from the plateau values. Oxygen consumption at the plateau value is indicative of the expected oxygen demand when a part of the substrate is oxidized. Theoretically, if the assimilatory activity of bacteria is completely suppressed, all the carbon in glucose will be converted to carbon dioxide, and at the plateau the oxygen consumed would be 1.07 mg of oxygen per mg of glucose. The assimilatory activity could be eliminated if an enzyme system was used instead of living bacteria. If feasible the utilization of a complete enzyme system as the seed, instead of living bacteria, should provide higher BOD values. This procedure may be used to estimate the theoretical BOD value with a complex substrate of known composition, as it would give the oxygen utilized when there is no assimilatory activity by the cells. Later, Busch et al (1962) devised a short-term total oxygen demand test which measured the oxygen demand of the biomass produced as a result of cell synthesis during the oxidation of the substrates.

Clifton (1963b) reported that Escherichia coli cells grown on glucose agar assimilated about one-third of uniformly C^{14}-labelled glucose, a value less than the 50% reported by Clifton and Logan (1939) from manometric studies with nutrient agar grown cells. With nutrient agar grown E. coli cells Clifton (1963b) showed that the nature of the growth medium and cultural conditions influenced the subsequent metabolic activity of the cells. There were definite regulatory mechanisms within the cells which controlled the nature of their respiratory activities and the extent to which assimilation occurred.

Endogenous respiration appeared to be inhibited to a slight extent in the presence of an exogenous substrate. Later, Dietrich and Burris (1967) showed that endogenous respiration did not necessarily continue at an unchanged rate when a substrate was added to a seed, therefore, the usual practice of subtracting the oxygen consumption of the seed from that of the test suspension may not always be valid.

From the study of biochemical oxygen demand of organic chemicals by Oberton and Stack (1957), the interpretation of BOD results was limited by the environment created for the rapid growth of most aerobic microorganisms in the standard procedure. The pH, temperature, nutrients and trace elements are in the optimum range for rapid multiplication of microorganisms. Some of the organic material in water can not be utilized by microorganisms; the biochemical reactions that result in the oxidation of complex organic material are catalyzed by an enzyme system that is generally specific to a particular chemical structure. The microorganisms must possess a suitable enzyme system if they are to use a particular organic material. The organisms can modify their metabolic process in order to utilize most organic substrates, either by adaptation of the enzyme system to the new substrate or by a mutation. Hence, in rivers, lakes, trickling filters and activated sludge units, the microorganisms become acclimated to the existing food supply. Acclimation may proceed rapidly or may require a considerable period of time. In the standard BOD determination the general procedure is to prepare a dilution of the waste-water and seed the sample with domestic sewage. The organic material contained in the sample may be completely foreign to the microorganisms, yet the pattern of substrate decomposition may be different. With organic chemicals the results are difficult to

interpret. Oberton and Stack (1957) studied the biological oxidation of organic chemicals, and showed that an inflexible procedure such as BOD_5, may, for the reasons described, not express the true nature of biological oxidation of a given waste.

A true interpretation of BOD requires an understanding of toxicity, biochemical stability, the manner in which a BOD develops, and the influence that acclimation of microorganisms has on these factors.

In 1958, Busch presented a theory stating that the process of biological oxidation of soluble organic substrates exerted by a mixed culture of sewage organisms involves conversion of the material into cell components, and utilization of stored decomposition products and of cell substance. The latter phase involves both the oxidative bacteria and predators, eg. protozoa. The clear delineation of these two steps depends principally upon the microbial population present. A single strain of organisms or a predominance of substrate-consuming organisms over predator organisms yields the distinct two-stage curve of oxygen consumption, eg. see Figure 3. As the ratio of predators to substrate-consuming population increases, the separation of the two stages begins to blur.

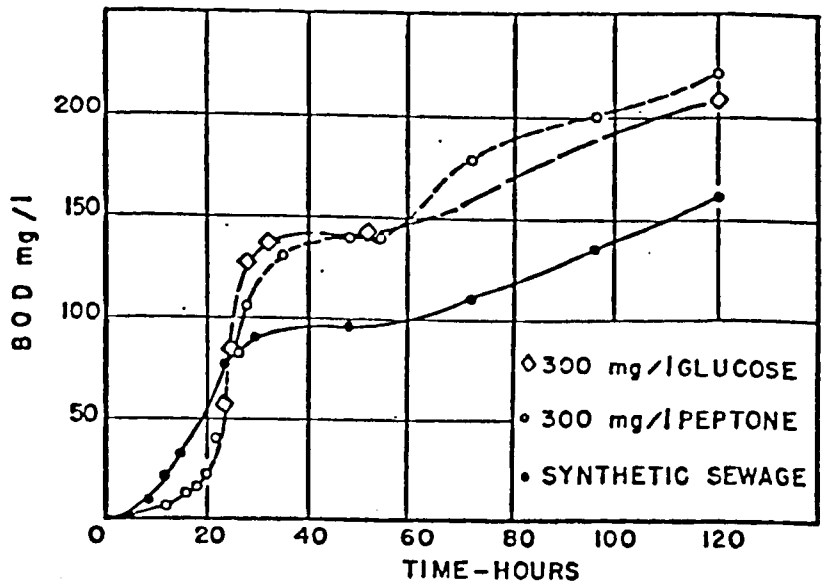
A plateau of oxygen utilization representing consumption of all substrate occurs generally in 12-48 hr depending on the lag and has a characteristic value for a given substrate. Theoretically, this plateau affords a much more valid point for determining the total load, for a waste-water on the receiving river, or ultimate oxygen demand than does the conventional 5-day incubation. In 1952 Busch and Sawyer noted that the 1-day BOD value was by far the most reliable result obtained in

tests with a soluble substrate of highly reproducible character, in this case a synthetic sewage. Independent observations by Garrett and Sawyer (1952) in studies on the activated sludge process supported these results. It was also noted that the progression of oxygen utilization did not adhere to the monomolecular reaction described previously (see Figure 1). These points were discussed by Busch and Sawyer (1952) and a tentative theory for the reaction was presented. It was shown that the reproducibility of the first day values was due to the nature of the substrate which was nearly all consumed after 24 hr incubation. It was further theorized that if practically all of the available substrate was converted into cell material after 24 hr, any further oxygen utilization was due to the activity of protozoa feeding on the bacteria.

Busch (1958), working with pure soluble organic substrates, was the first to emphasize the occurrence of a plateau between two distinct phases of oxygen uptake (Figure 5). He ascribed the first phase of oxygen uptake to assimilation of the soluble nutrients, and the second phase to the summation of endogenous respiration of the bacteria and the respiration of predators feeding on the bacteria. It was suggested that a higher initial number of protozoa in the seed would blur the separation of the two phases of oxygen uptake, and slightly smaller BOD values were obtained when the seed was treated to reduce the protozoan population. The major purpose of this work was the development of a short-term BOD test, and he proposed that the plateau value be used because he considered this to be more reproducible than longer-term oxygen utilization values.

With glucose as substrate McWhorter and Heukelekian (1962) observed the disappearance of the plateau at substrate concentrations

A. Typical BOD curves for glucose, peptone and synthetic sewage



B. Typical BOD curves for glucose and glutamic acid

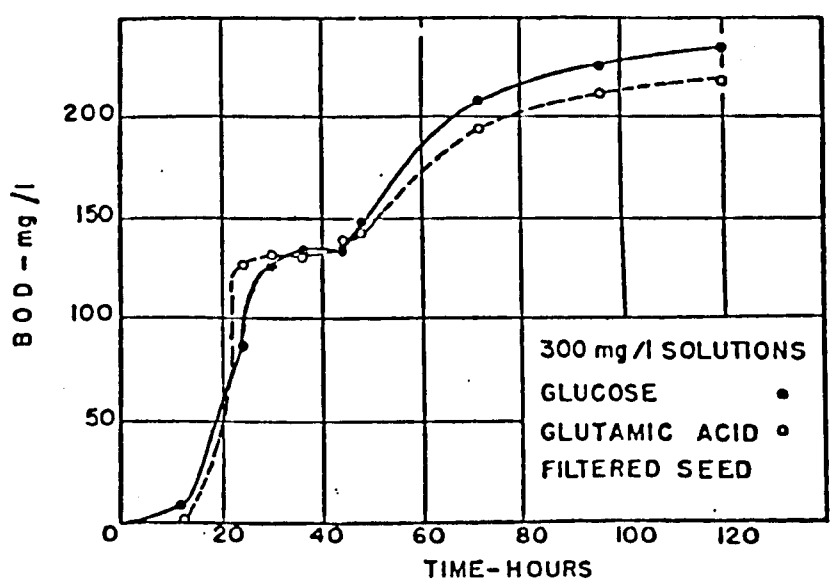


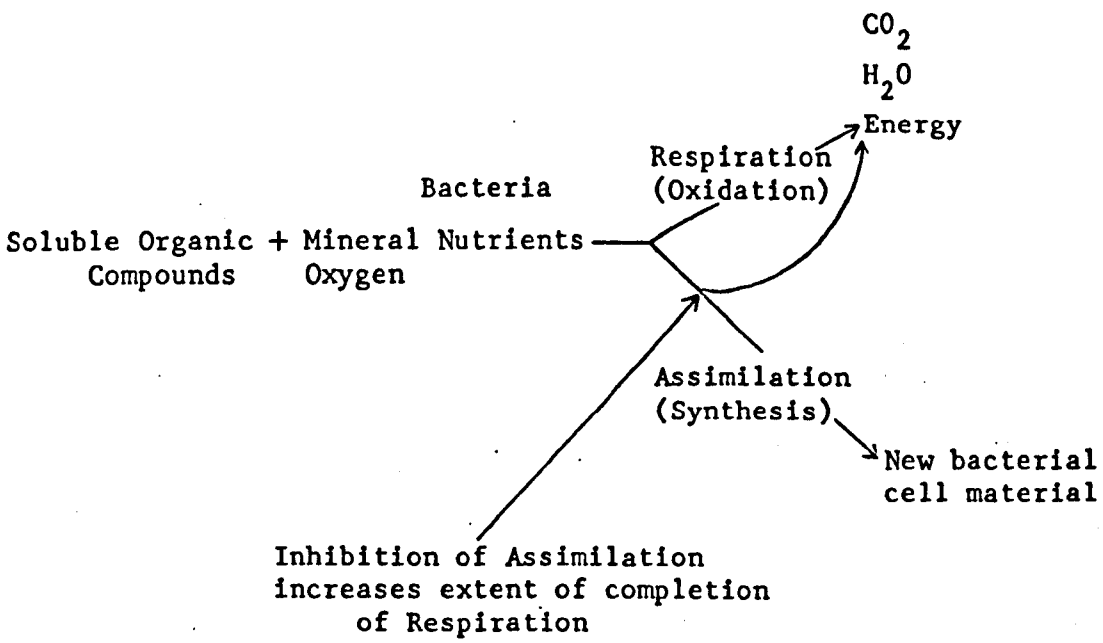
Figure 5. Typical BOD curves for different organic substrates and synthetic sewage

(from Busch, 1958).

of 1. 0 g/l and above. They used 0.5% settled sewage as the seed in both Warburg and dilution methods.

Zehnpfennig and Nichols (1953) observed a decrease of nearly 30% in the BOD_5 value when protozoa were removed from sewage seed by straining.

With the establishment of the validity of the first order stage plateau as a reproducible percentage of the theoretical BOD, a study was initiated to determine the possibility of increasing this percentage by selective poisoning of the bacterial assimilatory processes. Busch (1961) showed that blocking the assimilation of new bacterial cell material resulted in increased respiration and thus a higher ratio of plateau values to theoretical BOD. The relation between respiration and assimilation was presented diagrammatically as follows:



The relation between assimilation and respiration was studied by other workers (Clifton and Logan, 1939; Myrick and Busch, 1960; Clifton, 1963a,b)

Gaffney and Heukelekian (1961) studied the effect of substrate concentration on the rate of oxidation of acetate, propionate and butyrate. Acetate was oxidized less rapidly as the concentration increased, but the rate of oxidation of propionate and butyrate increased as the concentration increased. With all three, the lag period was greatest at higher concentrations. However, Busch (1958), using the dilution method and Bhatla and Gaudy (1966) using respirometers, found that the concentration of substrate (glucose or glutamic acid) had an effect on the rate and maximum levels of oxidation over wide ranges of concentration. The effect of substrate concentration on the respiration rate of activated sludge over short periods (10-30 min) was referred to by Lamb et al (1964). When milk was used as substrate, the respiration rate of the activated sludge increased rapidly at first with increasing concentration of milk, and then levelled off at a value which was presumably dependent on the concentration of sludge solids.

Bhatla and Gaudy (1965b) studied the evidence for the generation of the plateau level in oxygen uptake during exertion of carbonaceous biological oxygen demand by pure cultures. The Warburg method was used with glucose and phthalic acid as substrates. One organism, E. intermedia, exhibited a plateau, the formation of which depended upon the type of substrate and to some extent upon the initial cell concentration, but not upon substrate concentration (Gaudy et al, 1965).

The sequential metabolism of substrates by heterogeneous bacterial populations was reported by Bhatla and Gaudy (1965a). The phenomenon of oxygen uptake and substrate removal occurred at very dilute substrate concentrations, for example, 5 mg/l of glucose plus

5 mg/l of sorbitol, in the BOD bottles. Sequential metabolism of these substrates resulted in a diphasic curve of oxygen uptake and the two phases were separated by a discernible plateau (Figure 6). This study provided one possible explanation for the generation of discontinuity in the kinetic course of carbonaceous BOD exertion.

Bhatla and Gaudy (1966) indicated that the causation of diphasic oxygen uptake in high-energy systems was due to the release of cellular components during lysis of some bacterial cells. The need for an acclimatisation period before metabolism of these released components was not a general cause for the plateau. Also it appeared that the release of intermediate or end products during the metabolism of the original substrate was not a general cause for the plateau. These theories cannot be unequivocally eliminated for all systems and they do not provide areas for further investigation into general causation when heterogeneous bacterial populations are used in the oxidation of synthetic medium, containing 5 g/l of glucose.

From these studies it was noted that an increase in the initial substrate and in cell concentration produced a compressing effect on the plateau. This compression was observed with substrate concentrations varying from 100 to 1,000 mg/l and with weights of biological solids up to 500 mg/l. The concentration of neither substrate nor solids plays a decisive role in the occurrence of a plateau.

The biochemical oxidation of carbonaceous matter was studied by Stones (1970), with several amino acids at different concentrations and with an acclimatized seed prepared by inoculating the dilutions with settled domestic sewage. In every case it was found that the biochemical oxidation of amino acids over a period of twelve days occurred

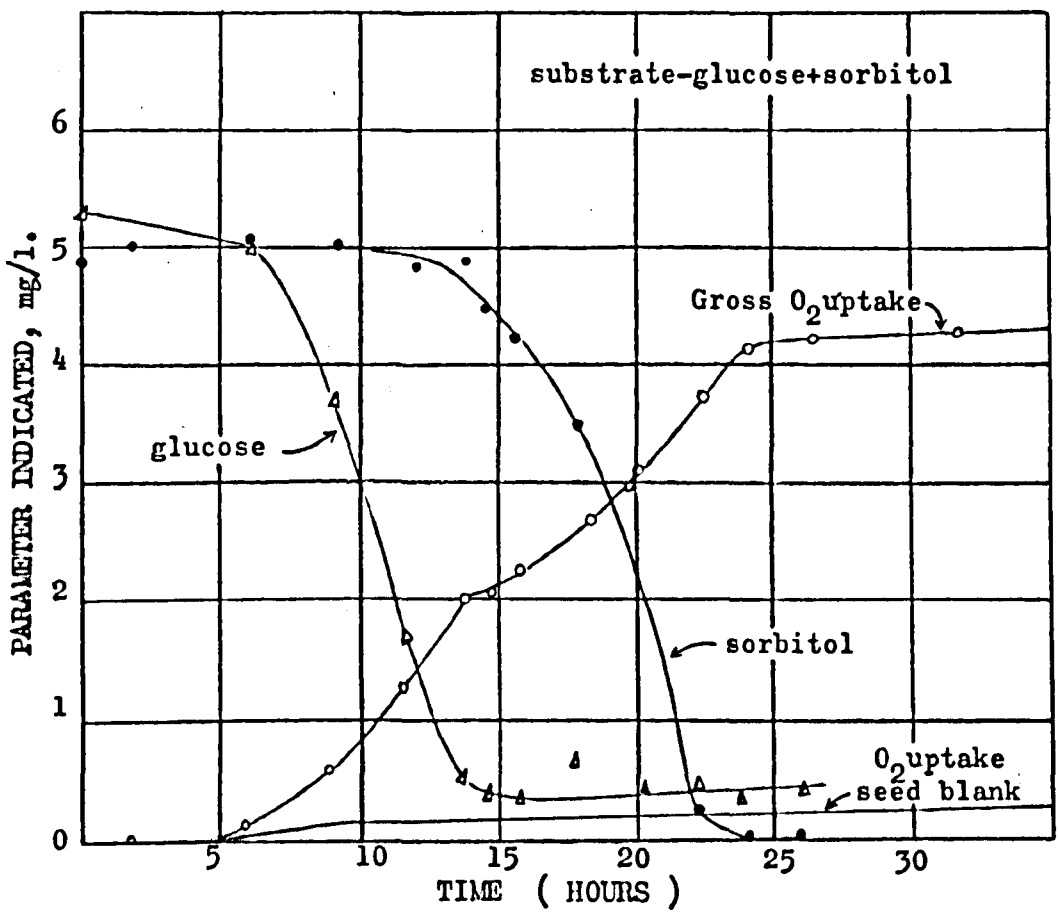


Figure 6 Oxygen uptake and substrate removal in the BOD bottle, with glucose-acclimated seed. (Bhatla and Gaudy, 1965a).

in two distinct stages. Complementary nitrogen determinations showed, there was no detectable nitrogenous oxidation, from which it followed that both stages of the biphasic curve were due to the oxidation of carbonaceous matter. Another series of experiments was done with solutions of various carboxylic acids and sugars. Ammoniacal nitrogen was added to provide for the metabolic requirement of the microorganisms and once again, complementary nitrogen determinations showed there was no detectable nitrogenous oxidation which only became evident after 15 days. The results were entirely due to the biochemical oxidation of carbonaceous matter and confirmed that this proceeded in two distinct stages which were independent of the molecular structure of the compound tested (Busch, 1958; Wilson and Harrison, 1960; Gaudy et al, 1965).

III. Alternative Methods for the BOD_5 Measurement

A. Manometric techniques of BOD measurement

Manometric methods were developed by Barcroft and Haldane (1902), Barcroft (1908) and Warburg (1923, 1926) in connection with investigations in microbiology and pathology.

They are based on the simple gas law

$$PV = RT$$

where

R = constant, P = pressure, V = volume and T = temperature.

If two of the three parameters are constant and the amount of gas in the system alters, the change in the variable parameter is a measure of the amount of gas consumed or released. The temperature (T) is most often the constant parameter, although it is possible to hold pressure (P) and volume (V) constant and measure changes in temperature, but the

procedure would be more complicated. There are three fundamental procedures; in each case the temperature is constant (Umbreit, Burries and Stauffer, 1972).

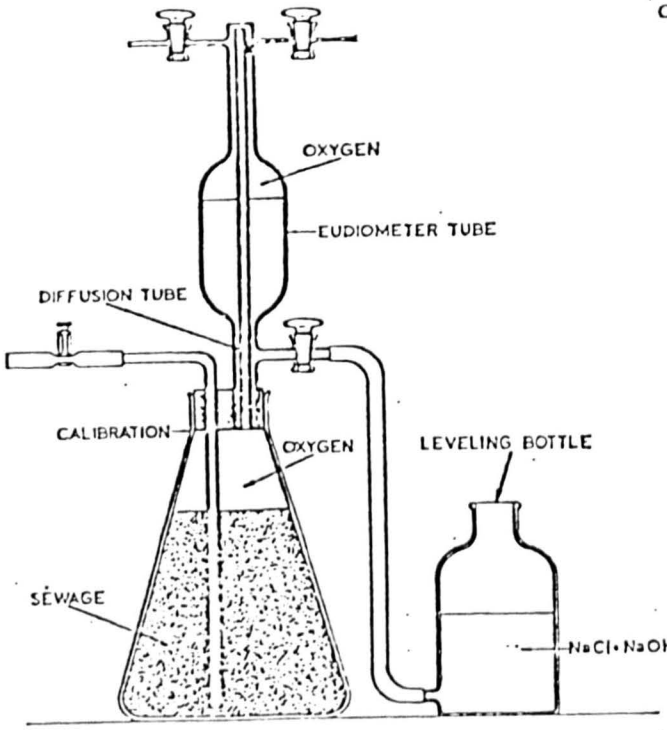
1 - The Warburg manometer

The volume is also constant and changes in the pressure are a measure of the amount of gas consumed or released. The Warburg respirometer (Warburg, 1926) was derived from the blood-gas manometer (Barcroft and Haldane, 1902; Brodie, 1910). The respirometer was based on the principle that at constant temperature and constant gas volume any alteration in the gas volume could be measured by changes in its pressure. This apparatus was commonly applied to measurement of oxygen uptake (Umbreit, Burries and Stauffer, 1972).

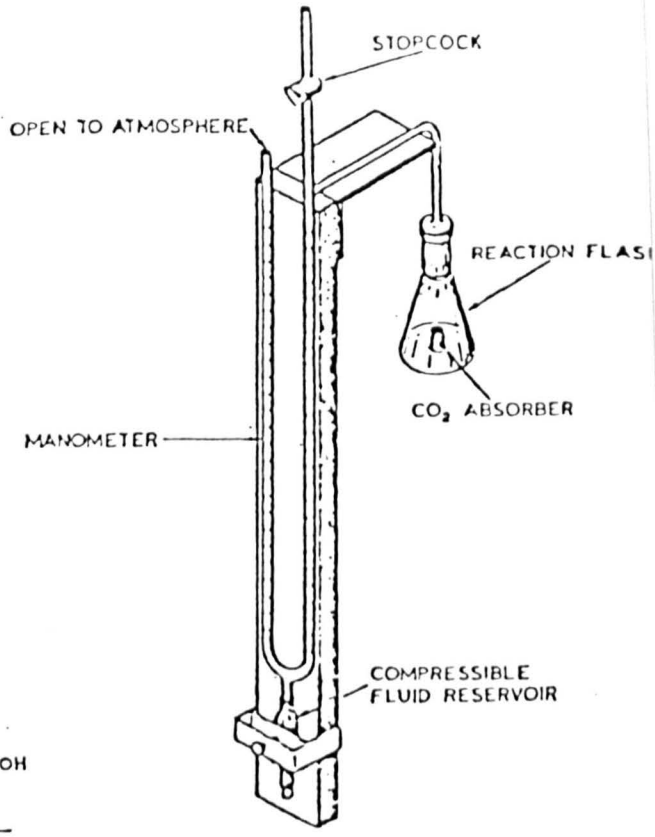
Warburg (Figure 7B) overcame the correction for CO_2 absorption by employing a cup of potassium hydroxide in the reaction flask. However, it used an open-ended manometer, so that the manometer readings had to be corrected for any change in barometric pressure, and where the temperature was not constant, for any changes in temperature (Umbreit, Burries and Stauffer, 1972).

2 - The constant pressure manometer

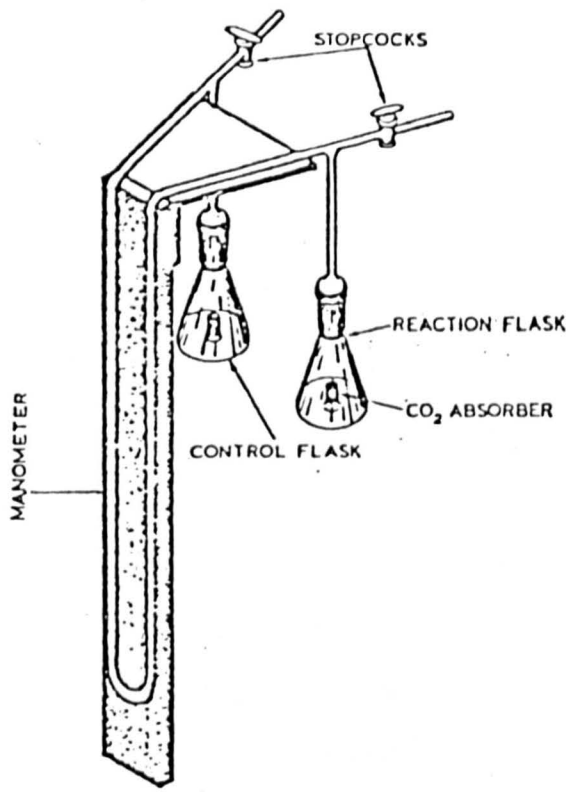
Under constant temperature and pressure changes in volume indicate the amount of gas consumed or released. Winterstein (1912, 1913) described the constant pressure respirometer. Various modifications and improvements were made on Winterstein's respirometer by Dixon (1943, 1951) and Scholander (1942a, 1942b, 1949). Despite the inherent advantages of the constant pressure apparatus, it did not challenge the popularity of Warburg and Barcroft respirometers for many



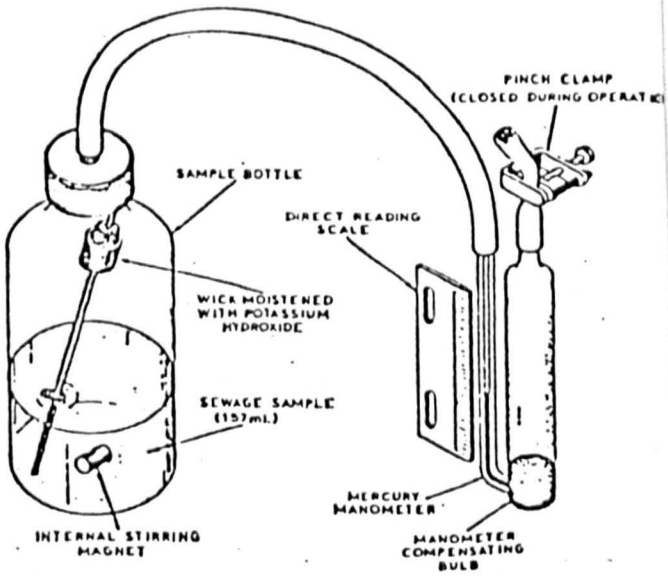
A – Diagram, Sierp Apparatus.



B – Diagram, Warburg Apparatus.



C – Diagram, Barcroft Apparatus.



D – Diagram, Hach Apparatus, One Cell.

Figure 7. Diagrams of four different manometric apparatus.

(from Tool, 1967)

years. At the time modifications made the constant pressure systems more popular (Scholander et al, 1952; Scholander and Iverson, 1958; Peterson, Freund and Gilmont, 1967; Gilmont and Finkle, 1969) (Umbreit, Burries and Stauffer, 1972).

3 - The Barcroft differential manometer

Both pressure and volume may be variables in the Barcroft manometer. Alterations in pressure and volume are measured, and from these measurements the amount of gas consumed or released is determined.

The differential respirometer or manometer was introduced by Barcroft (1908). The differential manometer was used in the same manner as the Warburg respirometer in determining oxygen uptake by the direct method. The Barcroft differential apparatus (Figure 7C) overcame one major flaw in the Warburg system. Although the manometer readings were no longer a function of barometer changes, the apparatus retained the other disadvantages of the Warburg. The Sierp apparatus (1928; Figure 7A) was dependent on the diffusion of carbon dioxide (CO_2) in to the eudiometer tube for its absorption is not effective, and corrections for unabsorbed CO_2 were applied by Falk and Rudolfs (1947) and Umbreit, Burries and Stauffer (1972).

Tool (1967) developed a manometric apparatus (Figure 7D) for measuring BOD. For most wastes the system was operated by taking one volume measurement and then direct reading of the manometer scale, which was calibrated as ppm. BOD. This apparatus is known as the Hach apparatus. A closed-end manometer was used so that the test was independent of any barometric or temperature change. The system was freed of CO_2 by a wick moistened with potassium hydroxide. The BOD of different sewage samples and a 50:50 (w/w) mixture of glucose and

glutamic acid were measured. The BOD values agreed within $\pm$ 5 per cent with the standard BOD method, and by increasing the incubation temperature to 35°C comparable results within $\pm$ 5 per cent, were obtained in 2.5 days for the BOD_5.

The work of Dixon (1943) stimulated the application of manometric methods to other fields. In 1936, the first application in the field of water pollution was made by Wooldridge and Standfast, and by Corbet and Wooldridge. In 1948, Caldwell and Langelier demonstrated the usefulness of manometric techniques, especially the Warburg method, for the study of sanitary engineering problems. The need to determine the entire oxygen consumption gave impetus to the adaptation of manometric techniques for BOD determinations by Ludwig, Oswald and Gotaas (1951). Lee and Oswald (1954) compared the Warburg method and the standard dilution method. They cited several objections to the Warburg apparatus - a, the initial cost of the apparatus and the running cost was about ten times more than that of the dilution method; b, both methods required a high degree of technical proficiency.

Lamb et al (1964) stated that the limited application of manometric techniques in the field of industrial waste and plant operation may be attributed to the amount of training and care required by staff in order to obtain meaningful results.

The frozen sewage seed used by Lee and Oswald (1954) for the manometric BOD determination was found to be unsatisfactory, because large numbers of bacteria were killed during freezing and storage.

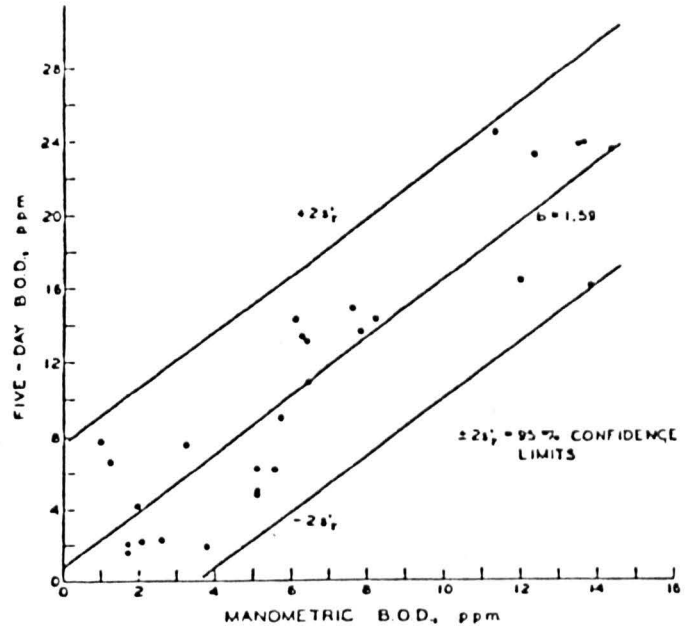
An increase in the density of seed was found by Dillingham, Knuth and Wessman (1958) to accelerate the stabilization of wastes. Their procedure was to use a massive pure culture inoculation in the evaluation of manometric techniques, as a rapid method for the

prediction of long-term dilution BOD values. To this end they proceeded to correlate short-term results with BOD_5 results for low value BOD waste-water. They concluded with spent sulphite liquors that 18 hours was the shortest practical reaction time for a manometric BOD test considering the precision of the technique, the slope of the regression and the length of the normal working day.

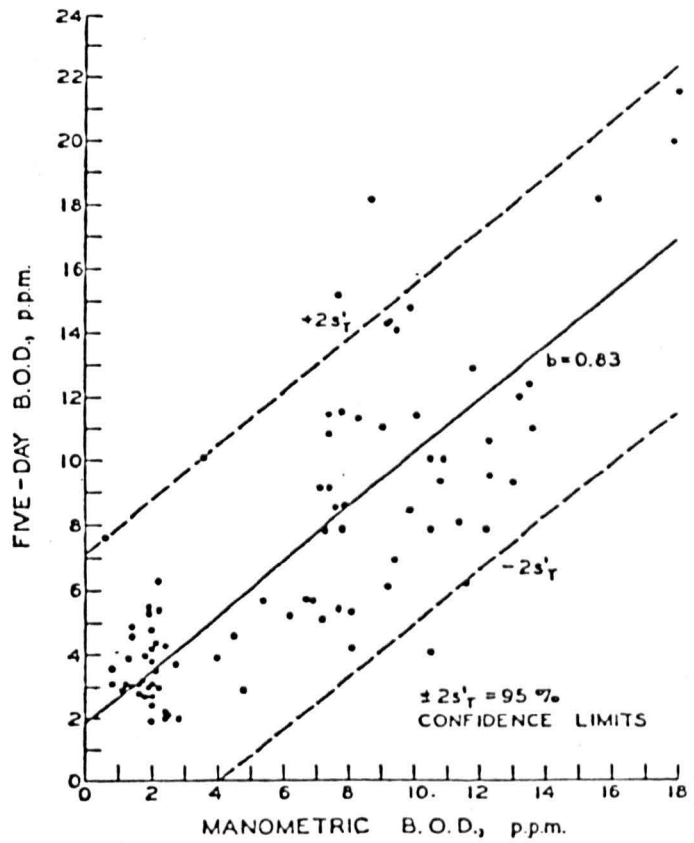
Dillingham and Jose (1960) measured the BOD of glucose, river-water and river-water plus glucose by Warburg manometry and the 5-day standard dilution method. All manometric BOD determinations were done at 37°C, with a standardized inoculum of Aerobacter aerogenes. The correlation curves for manometric and BOD_5 determinations of the glucose solutions are shown in Figure 8A and for both the river-water and river-water plus glucose samples are shown in Figure 8B. These authors concluded that the manometric technique provided a means of rapid BOD prediction in the range of 1 to 20 ppm.

A technique for the rapid determination of oxygen demand was described by Busch et al (1962). The procedure involved the measurement of BOD during the cell synthesis and calculation of the biomass produced during the oxidation of organic substrates (mg/l). The BOD plus the cells equivalent COD yield the total oxygen demand (TOD) value. The substrates used were glucose or glutamic acid, and a 1:1 mixture of these substrates in both Warburg and dilution tests. Warburg data showed a maximum TOD error of 4 per cent with substrate concentrations between 100-300 mg/l. The standard dilution method data showed a maximum error of 8 per cent with substrate concentrations between 12-16 mg/l.

The short-term BOD and TOD values represent the minimum and



A – The correlation of 5-day BOD and manometric BOD of glucose solutions.



B - The correlation of 5-day BOD and manometric BOD of river-water and river-water plus glucose

Figure 8. The correlation of BOD_5 and manometric BOD (Dillingham and Jose, 1960).

maximum oxygen requirements, respectively, for biological waste treatment and stream purification.

Busch (1961) proposed a test for total biological oxygen demand (TbOD) of a substrate by measuring the BOD plus COD to determine the TbOD. Three techniques for measuring the TbOD were presented by Grady and Busch (1963). An eight-hour BOD test using a heavy inoculum, aeration and COD was suggested by Hiser and Busch (1964). For the determination of 8-hour TbOD, raw or diluted waste was used as substrate for the aerobic microorganisms in a washed suspension. The COD for the culture and substrate were measured. Substrate was added to the aerating culture at zero time, allowed to mix for 1-3 minutes. Samples were withdrawn at intervals to follow the decrease in soluble COD with time; evaporation losses were adjusted with distilled water prior to sampling. COD's were set up on all samples, and the weight of cells in each sample was also determined to obtain the amount of Mixed Liquor Suspended Solids (MLSS). The TbOD calculated with this formula:

$$\text{TbOD} = \text{COD} \times \frac{a + b}{b}$$

a = initial culture volume (ml)

b = initial substrate volume (ml)

COD = calculated '0' mixed liquor COD - minimum mixed liquor COD (mg/l).

B. Studies of biochemical oxidation by direct methods

The term direct method is used to describe a method whereby the BOD is determined by placing the waste sample in an atmosphere of air or oxygen in a closed system under conditions of constant agitation and temperature. Oxygen utilization is measured at any given time interval by calculating the decrease in gas volume directly or from

changes in the pressure while maintaining a constant volume. These are the basic principles governing the use of the Sierp (1928) and Warburg (1926) direct method apparatus.

The direct method of oxygen utilization by microorganisms suggested by Sierp (1928), was improved and modified by other investigators (Buswell and Symons, 1929; Cromwell, 1930; Falk and Rudolfs, 1947; Heukelekian, 1947). Several authors developed specialized apparatus for studying oxygen utilization by sewage-activated sludge mixtures (Theriault and Butterfield, 1929; Grant, Hurwitz and Mohlman, 1930; Bloodgood, 1938; Sawyer and Nichols, 1939). Additional improvements were made by Gellman and Heukelekian (1950). They incorporated a small alkali vial to the Sierp apparatus to obtain complete CO_2 absorption (Gellman and Heukelekian, 1951).

Gellman and Heukelekian (1951) suggested a direct method for determining BOD of domestic sewage, industrial wastes and organic compounds with a modified Sierp apparatus incorporating small alkali vial to obtain complete CO_2 absorption. The results for wastes and organic compounds, measured with the direct and the standard dilution methods, are given in Table 3 a & b. Data for a group of organic compounds (Table 3b) shows that the Sierp and Warburg apparatus gave results consistently higher than the dilution method. In Figure 9, oxidation rates were plotted for two industrial wastes, candied fruit waste and rope cook liquor waste. The curves obtained by both methods for each waste were similar which indicated that there was little difference in the oxidation rate.

Heukelekian and Gellman (1951) studied the influence of various environmental factors on the biochemical oxidation of waste in

a.

Sample	Standard Dilution	Direct (Sierp)
Milk waste	1,090	1,100
Anti-biotics waste 1	1,450	1,095
Anti-biotics waste 2	840	750
White water 1	420	420
White water 2	580	585
White water 3	840	970
Yeast nutrients waste	720	940
Candied fruit waste	960	905
Rope cook waste	720	765
Waste sulfite liquor	1,155	1,215
Rice cooking waste	1,000	1,020

[1]Results expressed as p.p.m. oxygen demand

b.

Organic compound	Standard Dilution	Warburg	Sierp
Amyl alcohol	1,230	1,590	1,540
Sodium propionate	525	590	625
Sodium oleate	1,295	1,370	1,500
Starch	515	670	716
Dextrose	640	720	775
Casein	775	895	1,010

[1]Values based on 1,000 p.p.m. concentration

Table 3. Comparison of 5-Day Oxygen Demand Values[1] Obtained by Standard Dilution and Direct Oxygen Utilization Methods

(Gellman and Heukelekian, 1951)

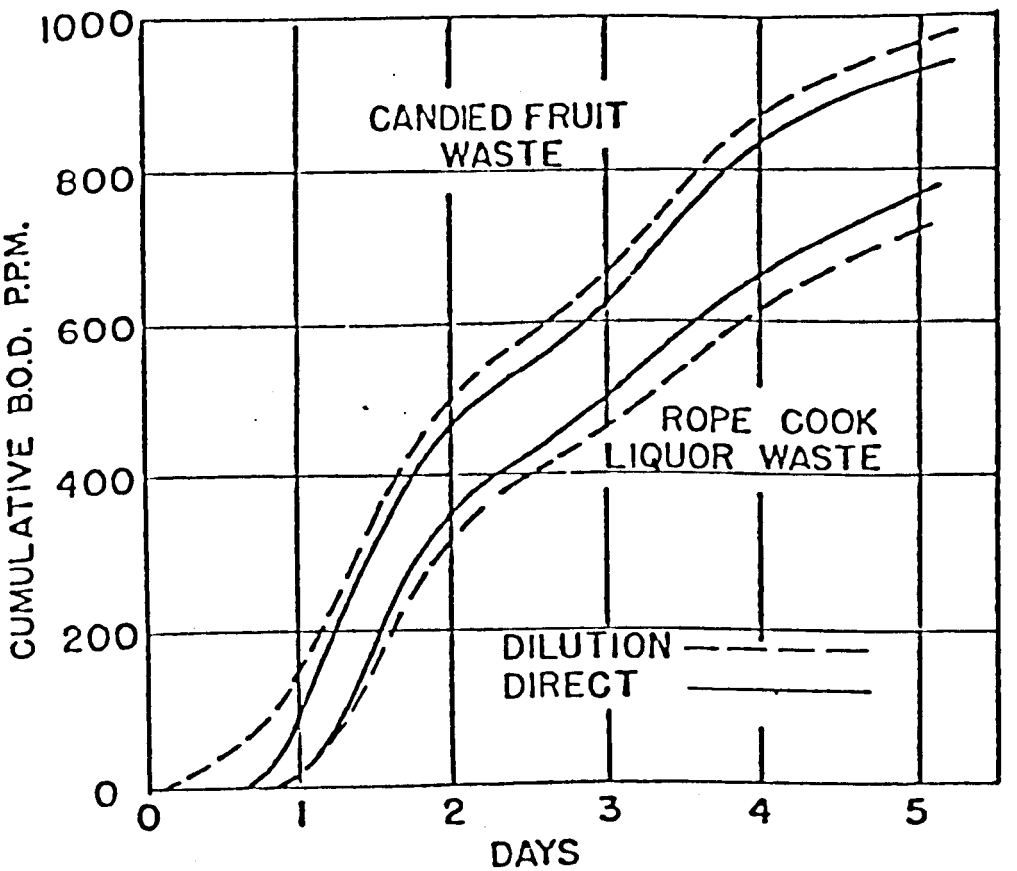


Figure 9 Comparison of oxidation rates of candied fruit waste and rope cook liquor waste measured by standard dilution and direct methods. (Gellman and Heukelekian, 1951).

the direct methods. The effect of pH, substrate concentration, the volume and source of sewage seed, and effect of seed adaptation on the oxidation of a number of industrial wastes was examined.

1. Adjustment of the initial pH to between 6.0-8.0 prevented interference with the oxidation. The effect of pH was more pronounced and the range was more restricted during shorter periods of incubation.

2. To prevent nitrogen deficiency from limiting the rate and extent of oxidation of wastes by microorganisms they concluded that a nitrogen source must be added to satisfy a BOD:N ratio between 17:1 and 22:1.

3. The reaction velocity constant 'K' for the oxidation of settled sewage was the same for the standard dilution and direct methods.

4. The oxygen demand values obtained by the direct method for sewage and industrial wastes were somewhat higher than those obtained by the standard dilution method. 5. The origin of the sewage used for seeding a dextrose peptone substrate had a minor influence on the rate of oxidation. The oxidation of wastes containing large numbers of organisms was not affected by variations in the volume or origin of the sewage seed. In the case of waste samples, which contained some antibiotics, little oxidation was obtained without seeding. The rate of oxidation increased proportionately with the volume of sewage seed.

6, By adaptation, a seed was produced that readily oxidized phenol concentrations as high as 2,000 ppm. With a non-adapted seed prolonged initial lag periods occurred which increased with the concentration of phenol.

Aeration is one factor that may effect the process of water purification in natural streams. The usual method of studying the rate and degree of water purification is to follow the course of oxygen consumption (BOD) of the examined water. In some manometric

methods, a constant volume of water is shaken in air (Caldwell and Langelier, 1948), and the amount of oxygen absorbed is measured volumetrically. This type of BOD determination can be applied only to grossly polluted water that has a rapid oxygen consumption.

The direct BOD determination without aeration can be used for water with a relatively low BOD (Prokesova, 1962).

C. Respirometric methods

A review of respirometric techniques showed that numerous techniques, based on the constant-pressure respirometer of Sierp (1928), with varying degrees of automation were published (Popel, Hunken and Steinecke, 1958; Snaddon and Harkness, 1959; Jenkins, 1960; Snaddon and Jenkins, 1964; Anderson, 1964; Arthur, 1965; Simpson, 1967; Wilson, 1967; Abson, Furness and Howe, 1967; Schulze and Hoogerhyde, 1967) (Montgomery, 1967).

An electrolytic respirometer was described by Bridie (1969) for routine BOD_5 determination and biodegradability studies over a wide range of substrate concentrations (15-220 mg/l). The electrolytic respirometer consisted of six measuring units, mounted in a thermostatic bath, which functions independently of changes in barometric pressure. Two determinations on compounds of known BOD_5 (glucose and glutamic acid, 50 mg/l) were done and the results were comparable to that of BOD_5 given in the standard methods. The shape of BOD curves, for the glucose and glutamic acid, obtained (Figure 10) were almost identical to that of Busch (1958) for glutamic acid and of Busch and Myrick (1961) for glucose. Continuous-flow respirometers were described by O'Brien and Clark (1967), Ribbons (1969), Thiele and Schmidt (1969) and Montgomery, Oaten and Gardiner (1971).

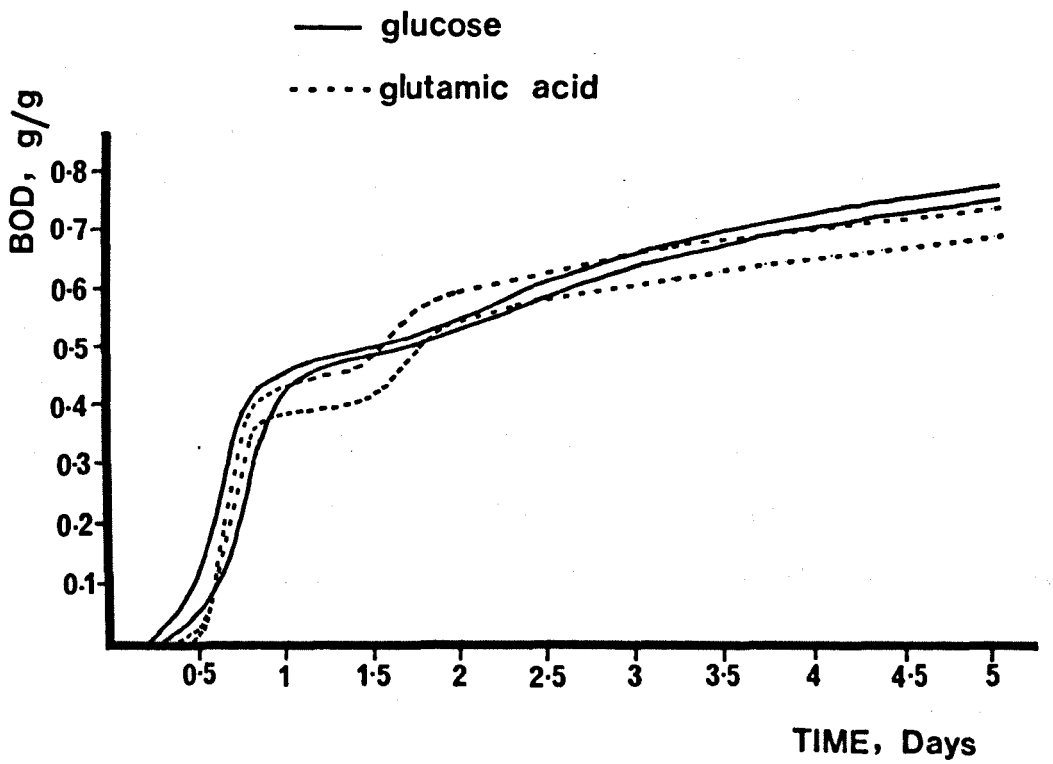


Figure 10. BOD of glucose and glutamic acid. 50 mg/l samples; respirometric technique (from Bridie, 1969).

Simpson and Nellist (1970) suggested the use of a large-volume automatic respirometer (1 litre flask) for the determination of BOD for pure organic substrates (glucose, sodium acetate, glycine and casein) over a period of 24 hr. Comparative results were obtained for 5-day BOD of the substrates (500-1000 mg/l) and their oxygen uptake as measured by the respirometric method.

The electrolytic respirometer was introduced by Clark (1960) and modified (Arthur, 1964, 1965; Young and Clark, 1965a; Young, Garner and Clark, 1965; Montgomery, 1967).

Montgomery, Oaten and Gardiner (1971) described an automatic electrolytic respirometer, based on that of Young, Garner and Clark (1965). This apparatus was designed for an investigation of seeding in a rapid oxygen demand test for sewage and some industrial effluents, and it was suitable for measuring oxygen consumption rates up to 200 mg/l/hr. Little application of this apparatus was shown for BOD measurements.

The automatic respirometer of Montgomery, Oaten and Gardiner (1971) was used by Montgomery and Gardiner (1971) in an attempt to develop a rapid test for waste waters, based on the oxygen uptake during several hours (6 hr) in the presence of a specially prepared inoculum. The inoculum was obtained by the aeration of settled sewage with organic additives. The concentration of inoculum applied was 100-400 mg/l. The plateau was reached in less than 11.5 hours with the pure organic compounds (sucrose, lactose, glucose, fructose, tyrosine, arginine, glutamic acid, hydroxyproline, acetic acid, succinic acid, citric acid and hippuric acid). More than 11.5 hours were needed with xylose, glycine, isoleucine, tryptophan, lysine and creatine.

No correlation was found between the results of respirometric oxygen uptake for sewage and the corresponding BOD_5 results.

A large-volume respirometer was suggested by Tebbutt and Berkun (1976), which utilized six Kilner preserving jars of 835 ml capacity fitted with screw caps as reaction vessels. The standard self-sealing caps were modified to provide two tapping points, one for connection to a mercury manometer and the other to provide for removal or addition of materials, for use in aeration of the flasks and during leak testing. Oxygen uptake for different sample volumes (100, 150, 200, 250 ml) was measured for crude domestic sewage daily, for a period of five days. Larger sample volumes produced higher oxygen uptake values. Harris (1954) indicated that the oxygen uptake of many bacteria was reduced in a carbon dioxide deficient atmosphere and this effect could be responsible for the sample volume dependence of the oxygen uptake results of the large-volume respirometer of Tebbutt and Berkun (1976). Five-day respirometric oxygen uptake data showed good correlation with conventional BOD_5 data but three-day data was insufficient to characterize the oxidation of a particular sample.

D. Polarographic measurement of dissolved oxygen and biochemical oxygen demand

(i) - Dissolved oxygen

Polarographic techniques have been used since 1922 as accepted methods for both cationic and anionic analyses as well as for the determination of dissolved oxygen in the field of water pollution. The technique was developed by Petering & Daniels (1938). Polarographic methods for the determination of dissolved oxygen in water, including wastes, were reviewed by Ballinger (1963).

Many investigators attempted to develop a polarographic

system for continuous dissolved oxygen measurements. Spoor (1948) developed a procedure using a dropping mercury electrode system for the continuous measurement of dissolved oxygen in flowing water. The water was maintained at a very low flow-rate to prevent interference with the mercury droplets.

Rotating and stationary solid-electrode systems were used for dissolved oxygen measurements and in many cases these were superior to the dropping mercury system. Kolthoff and Lingane (1941) showed that a rotating platinum microelectrode was preferable to a dropping mercury electrode for measurements of low oxygen concentrations because the diffusion current was greater with a rotating electrode.

Allen and Powell (1954) did not find a shift in potential for a rotating platinum electrode during reasonable lengths of operation. A linear relationship between the oxygen concentration and diffusion current existed; however, accurate temperature control was required.

Giguere and Lauzier (1945) investigated different types of electrode systems and found that the rotating and stationary types were extremely temperature dependent, as well as sensitive to the type of electrolyte used in the cell. Warshowsky and Schantz (1954) used a rotating platinum electrode to determine the DO of aerated and agitated culture media containing actively respiring bacteria. The DO values agreed with those determined by the Winkler procedure. The advocates of a rotating electrode cite its instantaneous adjustment to a steady state of current as one advantage. The spinning electrode, however, is difficult to maintain at a constant speed of rotation for the long periods required in BOD measurement (Eye, Reuter and Keshavan, 1961).

Clark et al (1953) used an oxygen permeable membrane cover on a stationary platinum electrode to measure oxygen tension in blood. The membrane, although permeable to oxygen, was much less permeable to other dissolved materials. The membrane was used to separate the measuring and reference electrodes, thereby causing high impedence characteristics for the system. Clark (1956) improved the system by combining the platinum-reference electrodes behind the membrane. Eye, Reuter and Keshavan (1961) devised a new platinum electrode system for measuring DO and BOD, but it lacked a thermocompensator for temperature correction.

Carritt and Kanwisher (1959) described an electrode with a thermocompensator which made rigid temperature control of the sample unnecessary. These improvements made polarography applicable to the routine measurement of dissolved oxygen.

(ii) - Polarographic determination of biochemical oxygen demand

A polarographic method for the measurement of dissolved oxygen in BOD determinations was shown by Busch and Sawyer (1952), to be applicable to sewage and industrial wastes. At an applied voltage of - 1.6 volts, the method was found to have an order of accuracy comparable to the standard Winkler technique. A considerable saving in time was obtained by the dropping mercury electrode method, as each DO determination required less than 1.5 minutes. This method had the disadvantage of the mercury interference with the BOD determination and no application was shown for the BOD determination.

Lemp (1960), suggested a polarographic instrument for measuring oxygen supply and demand during aerobic propagation of bacteria.

This method measured peak oxygen demand, critical oxygen concentration and oxygen supply during the actual mass propagation of an aerobic culture.

Morgan and Bewtra (1962) suggested a rapid polarographic method (Figure 11) for the determination of oxygen uptake for waste-water in an aeration tank. In this method the sample flowed continuously through the polarograph cell and the current, which corresponded to the DO of the sample, was recorded. The use of the Winkler chemical method was impossible in such a situation. This method was used to measure the oxygen uptake rate of a flowing waste-water, and not a measurement of the actual BOD.

Krishnamurty (1964) devised a rapid polarographic technique for BOD determination, in which the sample was diluted with aerated standard dilution water in a 300 ml bottle, and a large seed (100 mg wet weight/l) of enriched bacteria derived from sewage was added. The mixture was magnetically stirred throughout the experimental period of several hours, and the dissolved oxygen consumption was measured by means of a membrane-covered electrode, which was inserted into the 300 ml BOD bottles. The end-point of the test was reached when the rate of oxygen consumption by the diluted sample equalled that of the control bottle, which contained diluted seed material. The endogenous oxygen uptake for the seed was found from the control.

The author admitted that further work was required to control the endogenous respiration and variations in potency of the seed.

An improved apparatus for BOD determination was described by Young, Garner and Clark (1965). It consisted of a one litre, narrow-necked reagent bottle with a flat headed stopper, a CO_2 absorbent

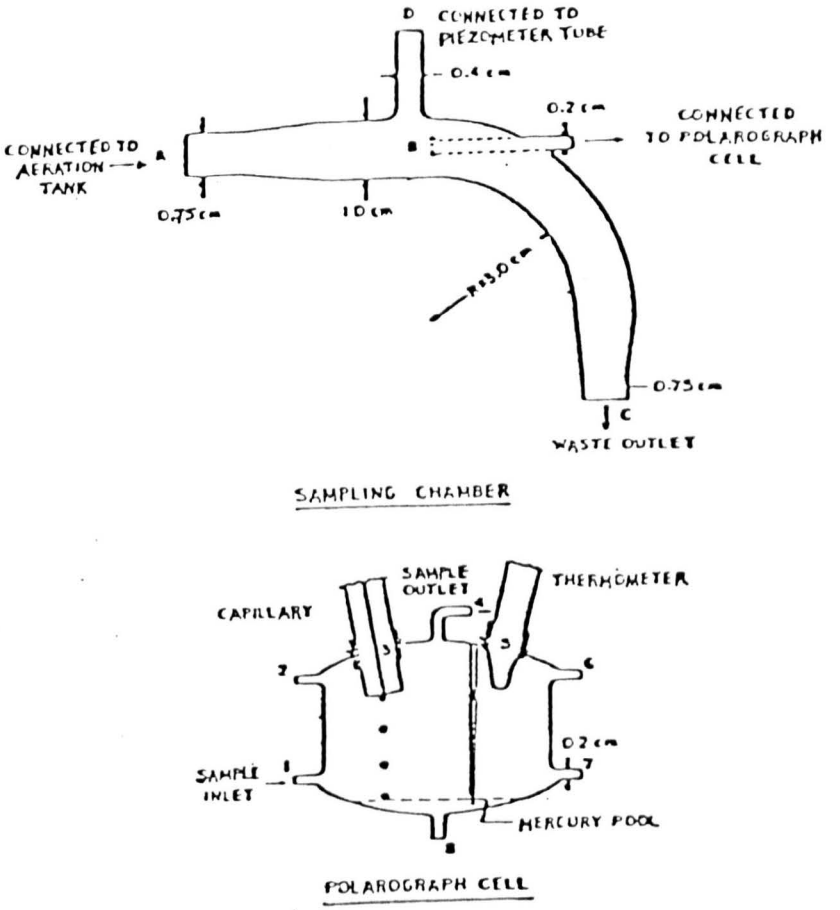


Figure 11 Details of sampling chamber and polarograph cell. (Morgan and Bewtra, 1962).

container and the electrolytic cell. This apparatus was an improvement on those described by Clark (1960, 1961). No application of the apparatus was shown for BOD measurement (Young, Garner and Clark, 1965). Eye and Ritchie (1966) suggested a membrane electrode system for measuring BOD. The system consisted of a membrane electrode mounted in a glass body which served as a seal for the custom-built 1500 ml BOD reaction flasks and a thermometer. The sample was magnetically stirred at a constant rate throughout the course of the 5-day BOD test. The samples were prepared in accordance with the standard methods (APHA, 1960; the dilution water was seeded with either settled domestic sewage or trickling filter effluents. The BOD for each sample was determined by both the membrane electrode procedure and the standard dilution technique daily. The values obtained were essentially the same. This method did not achieve any reduction in time for the BOD measurement.

Since the commencement of this study, a system for the rapid estimation of BOD_5 values was described by Karube et al (1977). Micro-organisms immobilized in polyacrylamide gel were used with an oxygen electrode for the determination of dissolved oxygen. The electrode current decreased linearly with time, when the electrode was inserted into a solution of organic substrate. The rate of the decrease was dependent upon the concentration of substrates and the current increased proportionately with the number of microorganisms in gel. The rate of decrease was not affected by pH in the range 6.6-7.4. As the temperature was raised there was an increase in the current, which varied linearly with the BOD of the standard solution. No decrease in the respiration of the immobilized microorganisms, isolated from soils and activated

sludges, was observed over a 10-day period.

The BOD of waste-water in an alcohol factory was estimated with this system. The reproducibility was found to be within 6% (relative standard deviation) for the standard solution. However, the authors concluded that it will be difficult to use this system to estimate the BOD of waste-waters which contain organic compounds which could not be degraded by the gel-enclosed microorganisms which had been isolated from soil.

A considerable amount of work, time and expensive substances were involved in this method, i.e. for the preparation of the immobilized microorganisms in polyacrylamide gel. This made the method impractical and uneconomic for pollution control, and insufficient application of the method was shown.

IV. Heavy Metals and Other Toxic Substances Affecting the BOD Test

All of the heavy metals possess some antimicrobial properties but most of them are only weakly bactericidal. The most active are the salts and organic complexes of mercury, tin and silver, and to a lesser extent, copper. Mercury, tin and silver compounds are both antibacterial and antifungal, but the copper compounds are employed mainly for their antifungal properties.

Increasing concentration of heavy metal ions with consequent inhibition of microbial oxidation.	Lowering of BOD value due to inhibition of microbial oxidation. This leads to erroneous BOD results for waste water.

The inhibitory mechanism appears to be similar for all metals, but the concentrations at which inhibition occurs vary considerably. It is a function of their ability to combine with the

proteins, especially enzymes, of the bacterial cell. The ability to form these complexes depends upon the presence of free metal-containing ions, but is independent of the molecular concentration of the salt. The concentrations at which the different metals exhibit antimicrobial activity varied from 1-3 ppm for mercury and silver to 10 ppm for aluminium and cobalt.

Some metals are essential for the growth and metabolism of microorganisms, mainly those of low atomic weight, for example sodium, potassium, calcium and magnesium. Metals with higher atomic weights, such as copper, cobalt, zinc, molybdenum and vanadium, are equally important for some bacteria at low concentrations. However, each metal has a limiting concentration of tolerance beyond which it becomes increasingly toxic, exhibiting first inhibitory and finally lethal properties. Thus many of the metals function initially as cell stimulants, and at higher concentrations they reverse this function and become growth antagonists and cell poisons (Sykes, 1965).

Natural waters contain trace metals in differenc chemical forms, such as copper, lead, cadmium and zinc. Florence (1977) showed that copper in rivers and reservoirs was associated mainly with organic matter, probably organic colloids. Lead was divided between stable inorganic and organic forms. Cadmium existed almost entirely in the labile ionic form. Zinc was found as the labile ionic species and the stable inorganic form, but very little zinc was associated with organic colloids. This suggested that the trace metals were not combined with inorganic colloids.

The toxicity of trace metals towards aquatic organisms in natural water depends on the chemical form of the metal. A water with

a high total metal concentration may in fact be less toxic than another water with a lower concentration (Allen, 1976). For example, many workers (Steeman and Wium-Andersen, 1970; Black, 1974; Pagenkopf, Russo and Thurston, 1974) concluded that ionic copper was far more toxic towards aquatic organisms than complexed copper; the more stable the copper complex the less toxic it was. Similar conclusions were reached for lead (Davies et al, 1976), and zinc (Pagenkopf, 1976).

Rudolfs et al (1950) reviewed the literature on the effect of toxic substances on sewage treatment processes, streams and BOD determinations. The inhibitory effect of toxic metals in industrial wastes on the biochemical oxidation during the treatment process and the BOD test was measured.

The effect of copper on the BOD of sewage determined by the dilution method, was noted by Scott (1930). Lower BOD values were obtained in the presence of copper at a concentration as low as 0.1 ppm.

Kalabina et al (1944) reported that 0.5 ppm of copper was toxic to all microorganisms and 0.1 ppm only to bacteria. Lead was toxic for microbial oxidation of organic matter at 0.1 ppm, for nitrifying bacteria at 0.5-1.0 ppm, and for other bacteria at 1.0 ppm.

Heukelekian (1947) determined the toxicity of mercuric bichloride, copper sulphate, cobalt chloride, potassium cyanide and sodium arsenate. Oxygen utilization decreased in sewage in the presence of various concentrations of these compounds (see Figure 12). Placak, Ruchhoft and Snapp (1947) reported that copper at a concentration 0.01 ppm prevented the determination of the true BOD. Chromates in amounts greater than 0.3 ppm caused depression of the true BOD values. Experiments done by Krieger and Moore (1949) indicated that both trivalent and hexavalent chromium were toxic. Although both

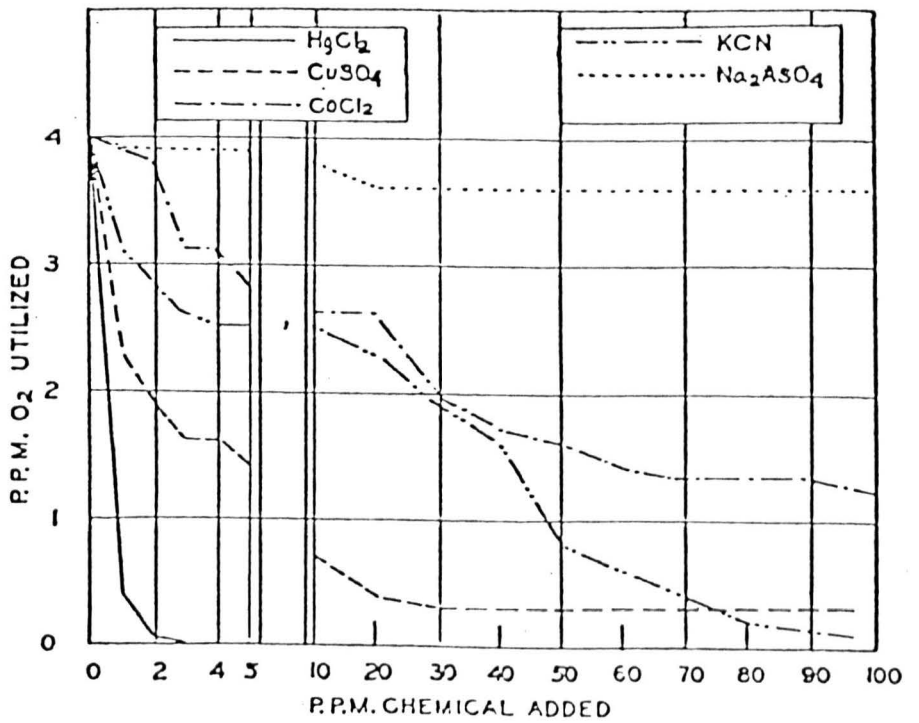


Figure 12. The effect of various concentrations of chemicals on the 5-day oxygen utilization of sewage as determined by the dilution method.

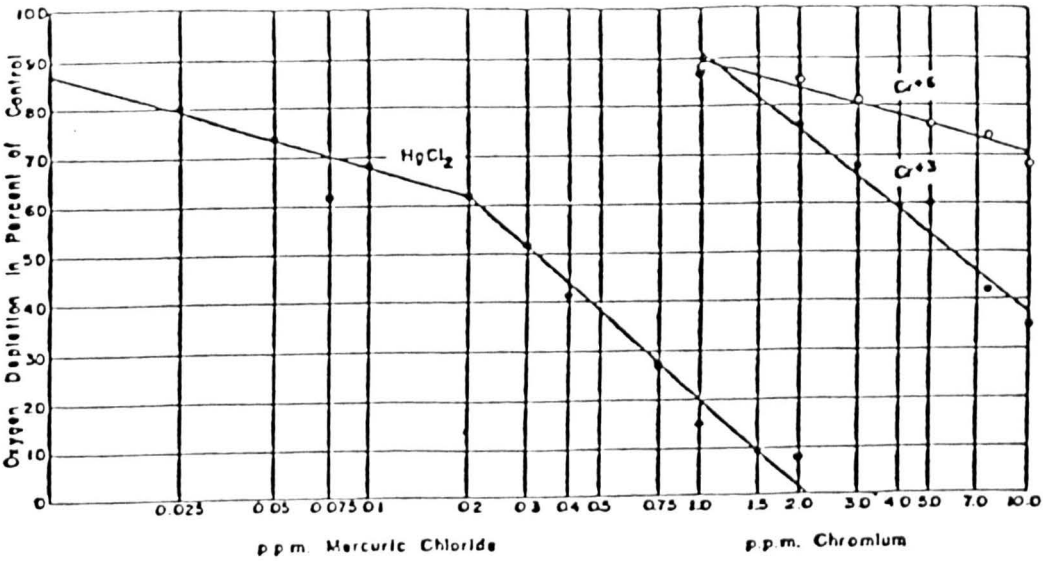
(Heukelekian, 1947).

forms of chromium induced a BOD lag with 1.0 ppm concentration, the trivalent form showed a more consistent inhibition as the chromium concentration increased. Hexavalent chromium reached a plateau in its effect on the BOD values of sewage, at a concentration of 3 ppm; further increases to 10 ppm had no significant effect.

It was reported by FSIWA the subcommittee on toxicity of industrial waste, section II (Ingols, 1954) that the toxicity of mercuric chloride increased slowly from 0.02 to 0.2 ppm, and beyond 0.2 ppm. there was a sharp rise in the toxicity until at approximately 2.0 ppm bacteriostasis occurred, or the absence of a BOD within the 5-day period. Chromium was much less toxic than mercury, with the chromic ion (Cr^{+3}) being rather more toxic than the chromate ion (Cr^{+6}) in the range 1.0 to 10.0 ppm in the standard dilution BOD test (Figure 13A).

The effect of copper, nickel, zinc, cadmium, trivalent chromium, hexavalent chromium and cobalt on the oxidation of sewage and activated sludge-sewage mixtures was studied using the Warburg respirometer by Heukelekian and Gellman (1955). The range of metal ion concentrations used was from 1.0 to 100 ppm. The order of decreasing metal toxicity from the BOD_5 values was nickel, copper, cobalt, cadmium, trivalent chromium, zinc and hexavalent chromium (Table 4). The concentration required to retard oxidation of the sewage substrate increased proportionately as the time interval (hr) increased.

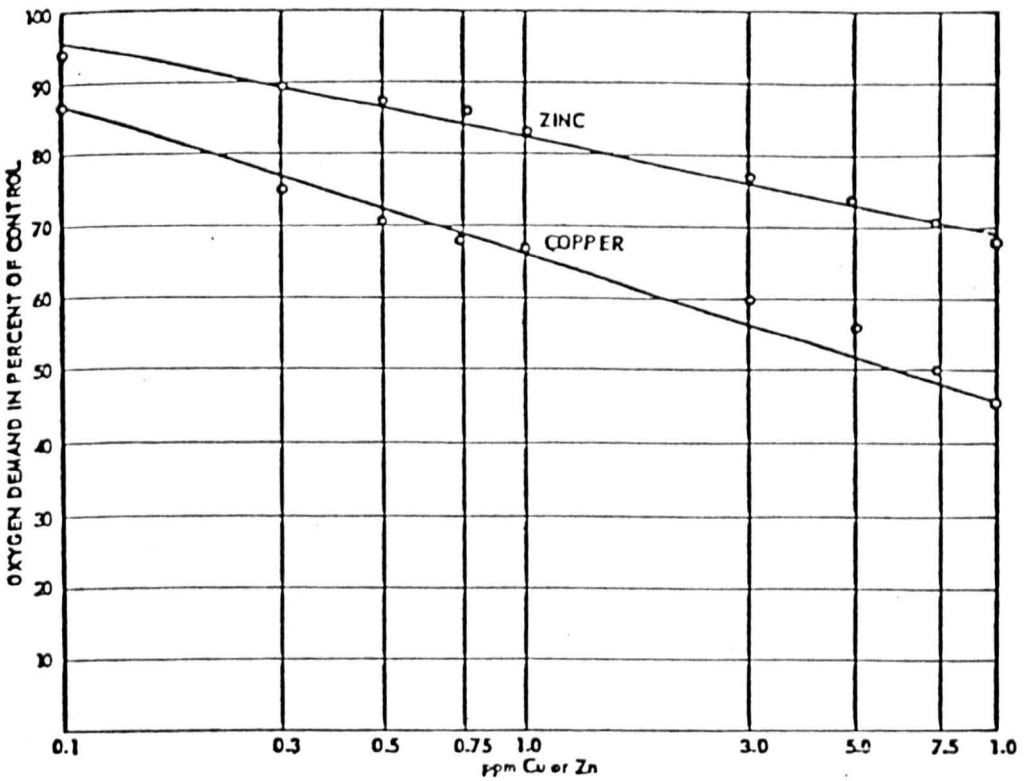
In the second report of the FSIWA subcommittee on toxicity of industrial wastes, section II (Ingols, 1956) the toxicity of copper and zinc ions in the dilution BOD test was reported. The results



A– Average values from 58 individual runs comparing the B.O.D. of sewage alone and sewage with various amounts of mercuric chloride, and of 20 runs comparing sewage alone and sewage with various amounts of chromium in the form of chromic sulfate and sodium chromate.

(from Ingols, 1954)

TOXICITY IN B.O.D. TEST



B– A comparison of the toxicity of copper and zinc in the dilution B.O.D. test (average of 25 tests).

(from Ingols, 1956)

Figure 13. Toxicity of mercuric chloride, chromic sulphate, sodium chromate, copper and zinc in the dilution BOD test. (Ingols, 1954 and 1956)

Time (days)	Copper Concentration (p.p.m.)						
	0	1	2.5	5	10	25	50
1	59	54	39	6	2	2	5
1.25	65	59	54	33	10	4	5
2	85	64	62	57	51	33	5
3	96	70	67	64	61	56	5
4	98	75	70	66	64	62	5
5	100	78	72	68	66	66	5

a —Effect of Copper Concentration on Biochemical Oxidation[1] of Domestic Sewage

Time (days)	Nickel Concentration (p.p.m.)				
	0	5	10	25	50
1	57	8	8	8	5
1.25	63	23	11	8	5
2	80	62	48	8	5
3	90	70	61	8	5
4	96	74	65	8	5
5	100	76	68	8	5

b —Effect of Nickel Concentration on Biochemical Oxidation[1] of Domestic Sewage

Time (days)	Zinc Concentration (p.p.m.)						
	0	5	10	25	50	75	100
1	59	50	45	33	8	5	5
1.25	65	58	58	51	35	12	6
2	85	66	67	60	53	46	29
3	96	77	77	67	61	57	49
4	98	85	85	73	68	63	56
5	100	88	88	76	72	65	60

c —Effect of Zinc Concentration on Biochemical Oxidation[1] of Domestic Sewage

Time (days)	Cadmium Concentration (p.p.m.)					
	0	5	10	25	50	75
1	57	46	32	8	4	4
1.25	63	56	51	31	4	4
2	80	63	61	58	4	4
3	90	70	67	68	22	4
4	96	77	73	66	48	6
5	100	84	78	67	61	8

d —Effect of Cadmium Concentration on Biochemical Oxidation[1] of Domestic Sewage

Time (days)	Trivalent Chromium Concentration (p.p.m.)				
	0	10	25	50	75
1	56	55	20	18	18
2	82	81	47	34	18
3	92	90	72	58	18
4	97	97	81	67	18
5	100	100	86	73	18

e —Effect of Trivalent Chromium Concentration on Biochemical Oxidation[1] of Domestic Sewage

Time (days)	Hexavalent Chromium Concentration (p.p.m.)					
	0	10	25	50	75	100
0.75	47	40	32	26	18	12
1	55	49	50	43	39	34
2	79	68	62	56	56	56
3	87	81	67	61	61	61
4	94	90	81	71	65	64
5	100	97	90	85	70	67

f —Effect of Hexavalent Chromium Concentration on Biochemical Oxidation[1] of Sewage

Time (days)	Cobalt Concentration (p.p.m.)				
	0	5	10	25	50
0.75	48	18	8	4	5
1	56	30	17	5	5
2	80	56	47	8	6
3	89	64	56	25	5
4	95	70	60	42	5
5	100	76	64	50	6

g —Effect of Cobalt Concentration on Biochemical Oxidation[1] of Sewage at Varying Time Intervals

Table 4. Effect of copper, nickel, zinc, cadmium, trivalent chromium, hexavalent chromium and cobalt concentrations on biochemical oxidation of sewage. (Heukelekian and Gellman, 1955).

showed that copper was more toxic than zinc in all concentrations from 0.1 to 10 ppm. The BOD_5 values were reduced at concentrations of 0.3 to 1.0 ppm of copper, less reduction was shown within the same concentration range for zinc (Figure 13B).

Mowat (1976) studied the toxicities of 12 common metals and cyanide in the standard BOD test for 5 per cent raw waste water. The concentration range was from 0.01 ppm to 20 ppm. Decreasing levels of BOD were found as the metal concentration increased. Mercury was found to be the most toxic metal (Figure 14A); complete toxicity occurred at 1.0 ppm in BOD_5 tests. Silver was the next most toxic metal, in 5 days there was a 30 per cent uptake of dissolved oxygen at the 1.0 ppm level (Figure 14B). The dissolved oxygen uptake in 5 days in the presence of 10 ppm cyanide decreased by 70 per cent, and at 2.5 ppm by 45 per cent (Figure 14C). The 5 day toxicities at 20 ppm were roughly in the following order:

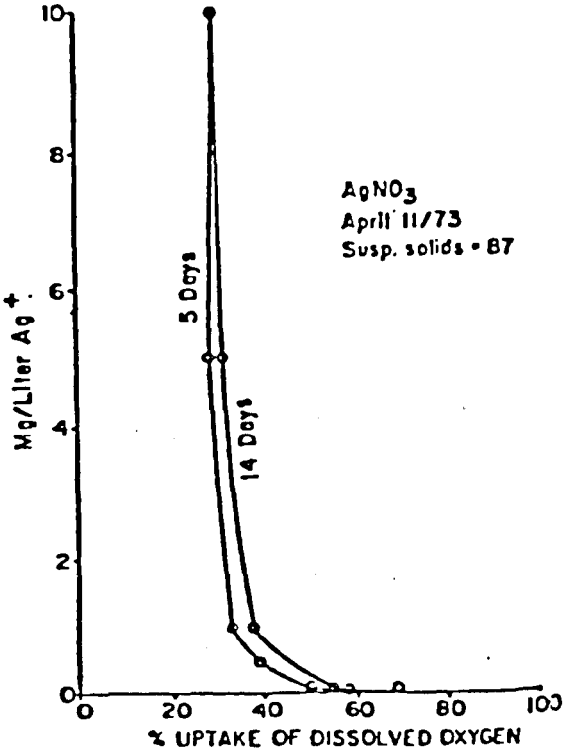
$$Hg^{+2} > Ag^{+1} > Cr^{+3} > Al^{+3} > Fe^{+3} > Cu^{+2} > CN^{-} > Ni^{+2} > Cd^{+2} > Co^{+2} > Cr^{+6} > Sn^{+2} > Zn^{+2}.$$

A series of 5 day BOD at low concentration, yielding toxicities roughly in the following order:

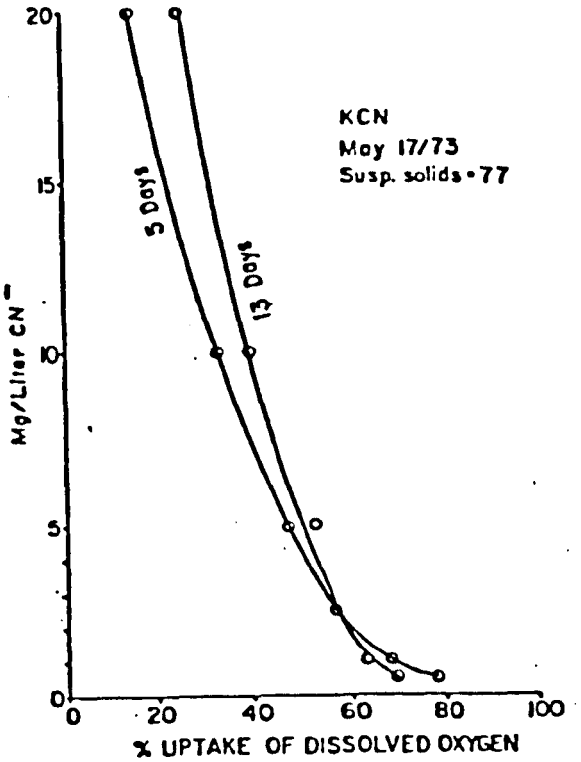
at 0.25 ppm $Ag^{+1} > Hg^{+2}$;

at 2.5 ppm $Cu^{+2} > CN^{-} > Cd^{+2} > Co^{+2} > Ni^{+2} > Cr^{+3} > Cr^{+6} > Al^{+3}$ (Table 5).

A —Typical results for mercury.



B —Typical results for silver



C —Typical results for cyanide.

Figure 14. Effect of heavy metal ions and cyanide on the O_2 uptake by microorganisms in the BOD_5 test. (Mowat, 1976).

Compound	Date	SS	Percent Oxygen Uptake for Given Concentration in mg/l							
			2.5	1	0.5	0.25	0.1	0.05	0.01	BOD
$AgNO_3$	4/4/74	110	—	—	—	51	69	80	93	125
	4/12/74	84	—	—	—	31	39	67	87	66
	4/17/74	78	—	—	—	47	53	62	100	67
$HgSO_4$	4/4/74	110	—	—	—	47	66	82	91	125
	4/12/74	84	—	—	—	51	62	74	92	66
	4/17/74	78	—	—	—	50	59	71	97	67
$CuSO_4 \cdot 5H_2O$	11/28/73	144	55	59	71	—	100	—	—	140
	3/21/74	121	62	67	80	—	89	—	—	110
	3/28/74	96	—	66	72	—	89	93	96	103
	5/9/74	107	51	59	66	—	91	89	93	106
	5/16/74	97	56	66	70	—	84	92	100	91
	5/23/74	131	49	65	74	—	90	94	95	120
KCN	5/9/74	107	59	72	80	—	95	96	100	106
	5/16/74	97	58	68	78	—	93	—	—	91
	5/23/74	131	68	74	81	—	100	—	—	120
$CdCl_2 \cdot 2\frac{1}{2}H_2O$	4/4/74	110	55	75	82	—	91	—	—	125
	4/12/74	84	72	82	82	—	93	—	—	66
	5/9/74	107	72	85	93	—	96	—	—	106
	5/30/74	118	58	81	85	—	100	—	—	105
$K_2Cr_2O_7$	11/14/73	139	84	86	95	—	—	—	—	146
	4/25/74	81	78	91	93	—	—	—	—	103
	5/2/74	117	86	84	86	—	97	—	—	116
$CrCl_3 \cdot 6H_2O$	11/14/73	139	62	84	90	—	—	—	—	146
	4/25/74	81	71	91	91	—	93	—	—	103
	5/2/74	117	74	92	92	—	93	—	—	116
$Al_2(SO_4)_3 \cdot 16H_2O$	11/14/73	139	70	92	98	—	—	—	—	146
	3/21/74	121	91	97	—	—	—	—	—	110
	3/28/74	96	80	95	—	—	—	—	—	103
$CoCl_2 \cdot 6H_2O$	11/28/73	144	76	73	95	—	98	—	—	140
	3/20/74	160	72	90	92	—	—	—	—	108
	3/28/74	96	72	89	99	—	—	—	—	103
$Ni(NO_3)_2 \cdot 6H_2O$	11/28/73	144	63	84	88	—	98	—	—	140
	3/20/74	160	75	86	91	—	—	—	—	108
	3/28/74	96	70	81	93	—	93	—	—	103

Table 5. The effect on O_2 uptake in the BOD_5 test of the toxicities for microorganisms of metals and cyanide at low concentration. (Mowat, 1976).

Note that in the presence of compounds toxic to microorganisms the BOD_5 values are much lower when compared to the control value obtained in a test without the toxic inhibitor.

MATERIALS AND METHODS

THE RIVER KELVIN

The River Kelvin (Figure 15) is about 22 miles in length and runs from its source in the Campsie Fells at Kelvinhead through farmland, small burghs and part of the City of Glasgow before it reaches the River Clyde.

For this study, 16 different sampling sites were chosen along the length of the Kelvin. These sites were chosen to provide water samples of variable quality and because they were used by the Clyde River Purification Board (CRPB) for their routine pollutional survey.

The sites included all the burns which run into the Kelvin, Luggie Water, Glazert Water, Allander Water, Dock Water, Board Burn and Bishopbriggs Burn. Some of these burns carry sewage effluents from the 15 sewage works that discharge their effluent into the river. These sewage works provide the major source of pollution for the river.

At its source (station 1; 757783; Plate 1a) the river is about 4-6 feet wide and is stagnant, with a mat of vegetation of the surface. It flows in a southwesterly direction through farmland and, before it reaches site 2 at Auchinstarry Bridge (719770; Plate 1b), it receives the sewage effluent from the town of Dullatur (746773); this effluent, which is fairly small (0.1 mgd) is satisfactory and causes little pollution. By Auchinstarry Bridge the river is shallow and fast flowing but at Queenzieburn Bridge (698763; Plate 3b) it is visibly polluted. This is due to the sewage effluent from the town of Kilsyth (720775) which flows in the Dock Water (701765; Plate 2b). The polluted Dock Water joins the main river at site 3; plate 2a. Station 3 was sited above and Site 4 below Dock Water (Plate 3a).

Just downstream of this point, two tributaries enter the river

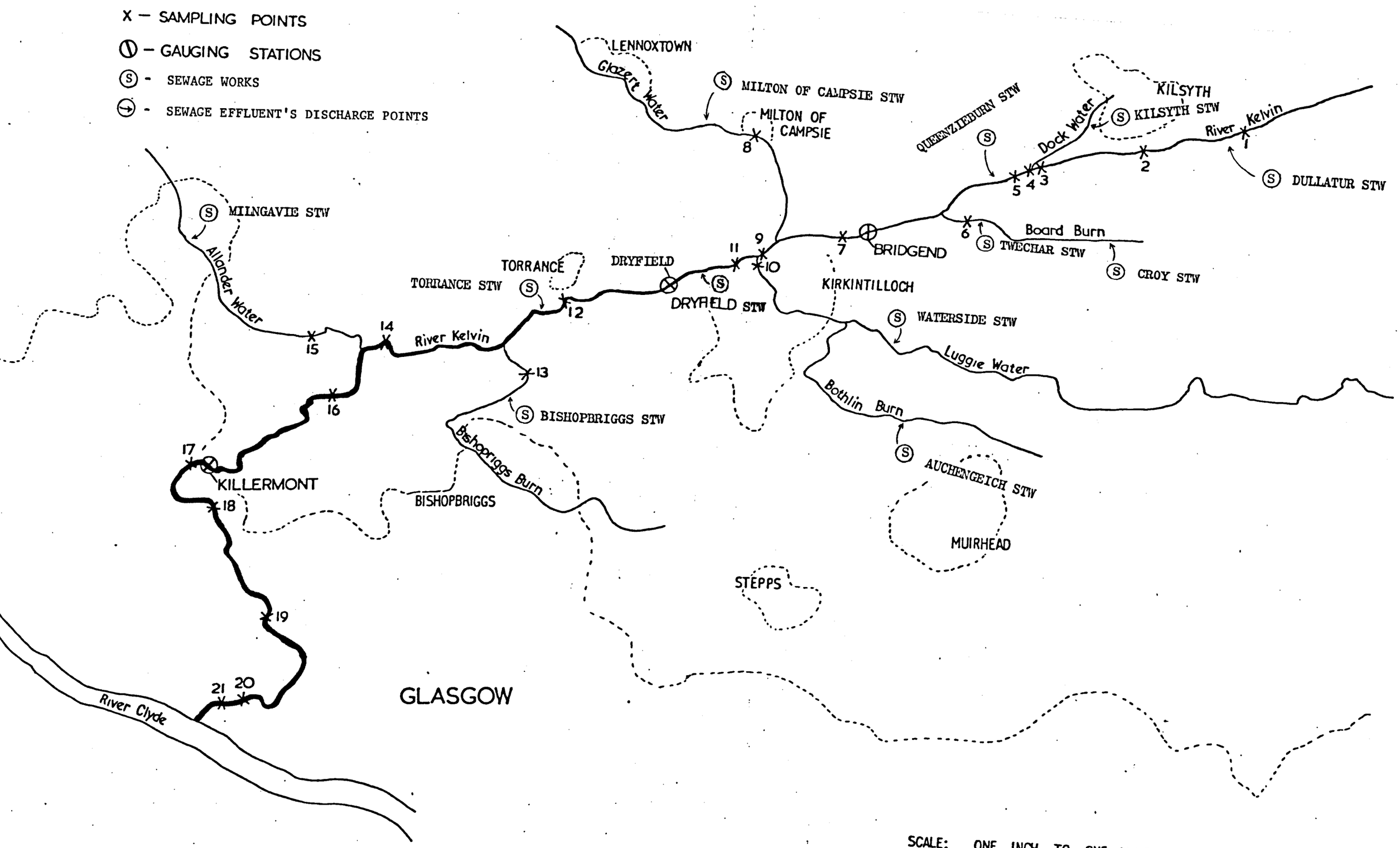


Figure 15 - The River Kelvin, showing sampling points, gauging stations, sewage works and the discharge points.

Plate 1a. Station 1 (757783) River Kelvin near the source

Plate 1b. Station 2 (719770) River Kelvin,

Auchinstarry Bridge.

Plate 2a. Station 3 (701765) River Kelvin (above Dock Water)

Plate 2b. Station 4 (701765) Dock Water

Plate 3a. Station 3 & 4 (701765) The junction of Dock Water with the River Kelvin.

Plate 3b. Station 5 (698763) River Kelvin, Queenzieburn Bridge.

within one mile of each other. The first is the Queenzie Burn (695762) which carries a satisfactory effluent from the town of Queenzieburn. The second is the Board Burn (685694; Plate 4a), a reasonably clean stream carrying a high quality effluent from Croy sewage works (Site 6). Soon after the confluence of the Board Burn with the Kelvin the effluent from Twechar sewage works enters the river; this has no apparent pollutional effect on the river. Beyond Inchbelly Bridge (Station 7, 668749; Plate 4b) the river becomes slower-moving and has a canal-like appearance for approximately three miles. In this stretch it is joined by the Glazert Water (Station 8, 656747; Plate 5a) a fairly clean river which carries sewage and industrial effluents (0.68 mgd). The industrial effluent comes from the Universal Pulp Containers works at Milton of Campsie; the sewage effluents originate from Lennoxtown, Lennox Castle Hospital and Milton of Campsie. This effluent has, in the past, been a major cause for concern, but the new comprehensive drainage system alleviated this problem.

At station 9 (654746; Plate 5b) which is about 100 yards from the junction of the Glazert Water with the Kelvin, zonation in the river is noticeable, where the turbid water of the Kelvin has not mixed with the clean Glazert water.

The Luggie Water (654774; Plate 6a) from the town of Cumbernauld joins the Kelvin at station 10. This tributary receives effluent of borderline quality from the sewage works at Condorrat and Waterside and it has three branches. The first of these, the Cameron Burn, carries unsatisfactory sewage effluent from the town of Greengairs (785705). The second, the Mollins Burn, carries ferruginous mine-water from the Bedlay Colliery (721706). At times of low rainfall more than half of the flow of the Luggie Water results from sewage effluent. The third tributary of the Luggie Water is the Bothlin Burn which carries sewage effluent from Auchengeich.

Plate 4a. Station 6 (685694) Board Burn

Plate 4b. Station 7 (668749) River Kelvin, Inchbelly Bridge.

Plate 5a. Station 8 (656747) Glazert Water

Plate 5b. Station 9 (654746) River Kelvin, B757 Road Bridge.

The river at station 11 (650743; Plate 6b) looks turbid and flows at a fairly normal speed in a widening river. Approximately 100 yards downstream of the CRPB's gauging station at Dryfield, the effluent from Kirkintilloch sewage works is discharged into the river. This sewage works produces a satisfactory effluent and there is no marked deterioration in river water quality. One and a half miles further downstream the sewage works at Torrance discharges its satisfactory effluent into the river. Station 12 is Torrance Bridge (619737; Plate 7a).

One mile from Torrance the Bishopbriggs Burn (609728; Plate 7b), which receives the effluent from Bishopbriggs sewage works, enters the Kelvin. This effluent is generally unsatisfactory and seriously pollutes the burn because of insufficient dilution. The river at Bardowie Bridge (station 14, 588729; Plate 8a) becomes slower-moving and runs through farmlands. Before it reaches Balmuildy Bridge (station 16, 579718) it receives effluent from the Allander Water (Station 15, 584725; Plate 8b). The Allander Water is a fairly clean stream which, on occasions, has been polluted by the effluent from Milngavie sewage works.

From Balmuildy Bridge the Kelvin flows sluggishly through the outskirts of Glasgow to Killermont Bridge (Station 17, 554706; Plate 9a) and the Garscube Estate. This estate owned by the University of Glasgow, which is used in part as an experimental farm. During the summer abundant growth of weed and long ropes of filamentous algae can be seen (Plate 9b & 10a). This growth is encouraged by the presence of nutrients, essential minerals, added by the sewage effluent, the sluggish flow of the river, the presence of higher concentration of light, which is required for photosynthesis, than some other parts of the river, and by the slightly higher temperature of the water in the city.

Once the Kelvin reaches Dawsholm Bridge (Station 18; 559697) it is faster-flowing and there are a number of short falls before it

Plate 6a. Station 10 (654774) The Luggie Water.

Plate 6b. Station 11 (650743) River Kelvin, Springfield Farm Bridge.

Plate 7a. Station 12 (619737) River Kelvin, Torrance Bridge.

Plate 7b. Station 13 (609728) Bishopbriggs Burn.

Plate 8a. Station 14 (588729) River Kelvin, Bardowie Bridge.

Plate 8b. Station 15 (584725) Allander Water.

Plate 9a. Station 17 (554706) River Kelvin, Killermont Bridge.

Plate 9b. River Kelvin, Lady Campbell Bridge (552704)
Garscube Estate.

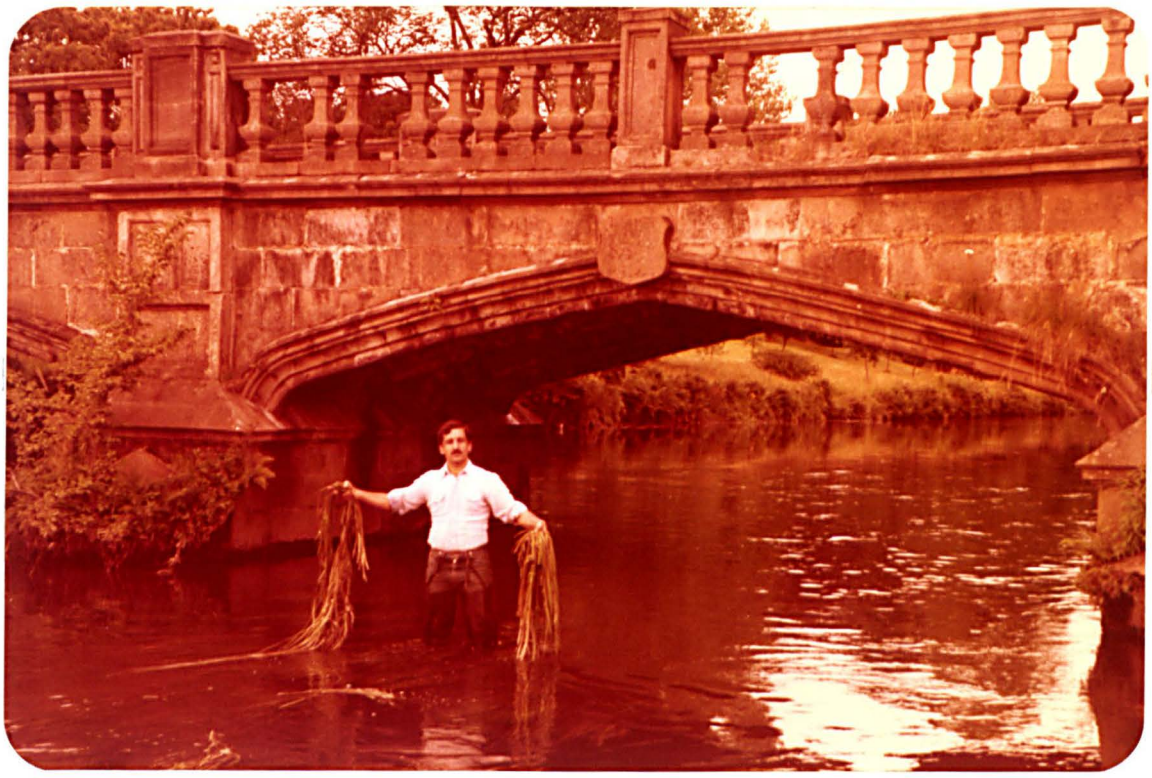


Plate 10a. River Kelvin (Garscube Estate) Weed growth and long ropes of filamentous algae.

Plate 10b. The River Kelvin and discharge of rubbish.

reaches the river Clyde at Partick. At the Botanic Gardens (Station 19; 569676), although the river is slow-flowing and muddy some vegetation can be seen on the river-bed. A feature of the river at this site and throughout the city is the prevalence of rubbish on the river-bed (Plate 10b) eg. prams, bicycles, wrecked cars and spades. At Partick Bridge (Station 20; 565664) the Kelvin is cloudy and polluted. This is a particular problem after heavy rainfall due to the discharges from the twenty-three storm-water outlets found between station 17 and station 21 (Kelvin/Clyde junction). Below Partick Bridge the River Kelvin widens considerably and joins the River Clyde. The quality of the water improves throughout the lower reaches of the river due in part to the aeration caused by the waterfalls which assist in the oxidation of the organic pollutants.

The river Kelvin with its average flow of 152 mgd carries a large amount of sewage effluent and a moderate amount of industrial effluent and these contribute largely to the unsightly condition of the river. The river also carries a large percentage of undissolved solids, possibly due in part to the boulder clay and calciferous sandstone deposits on the bed of the river.

During 1977 there was further general improvement in the biological condition of the Kelvin with the exception of the adverse effects of the polluted Dock Water and Bothlin Burns, and also the influence of the Bishopbriggs Burn resulting from the discharge of sewage during storms. The pattern of pollution in the Glazert Water remains the same, with the sewage effluent discharge from Lennox Castle Hospital the main source of pollution. Although the river remained in good condition, both biologically and chemically, for most of the year, a serious fish kill occurred in July 1977 when some 5,000 brown trout were killed (Plate 11) by a discharge of acid waste into the Glazert following a boiler-cleaning operation at Lennox Castle Hospital.

Plate 11. The discharge of acid wastes resulted in a major fish kill in the Glazert Water at Lennoxtown.

(from the CRPB's report, 1977)

The biological improvement in the Luggie Water showed a slight improvement in chemical quality, although the impact of the Bothlin Burn which receives the effluent from Auchengeich sewage treatment works is still significant. Two fish kills occurred during 1977. The first was in May; 50 trout were found dead due to an unknown cause. The second occurred in June when 100 trout were also killed due to an unknown cause (CRPB report 1977). Figure 16 shows the plotted curves of the summarized results of BOD_5, suspended solid, amonical nitrogen, and dissolved oxygen of the monthly sampling over the river Kelvin (CRPB, 1977).

During 1978, there was an overall improvement in the biological condition of the River Kelvin. The Dock Water, however, continued to exert a significant polluting load on the river as illustrated in Figure 17, the CRPB Annual report (1978). A serious pollution incident in the Dock Water during February was due to drainage from the former Dumbreck Coke Oven at Kilsyth. The impact on the river of the poor quality effluent from Kirkintilloch sewage-works is shown in Figure 17. The effect of storm discharges from Bishopbriggs sewage works on the Bishopbriggs Burn were minimised by constructional improvements to the storm overflow system at the works (CRPB, 1978).

The pattern of pollution in the Glazert Water remained unchanged, with the inferior quality effluent from the Lennox Castle Hospital sewage treatment works forming the major polluting load. A significant aesthetic improvement in the Glazert was noted further downstream resulting from the connection of trade effluent from Universal Pulp Containers to the public sewer. The Glazert's general condition remained satisfactory, both biologically and chemically (CRPB, 1978).

During 1979 the Kelvin continued to be polluted by the Dock Water and by the effluent from Kirkintilloch sewage works. It should be mentioned that the CRPB was consulted by the Strathclyde Regional Council

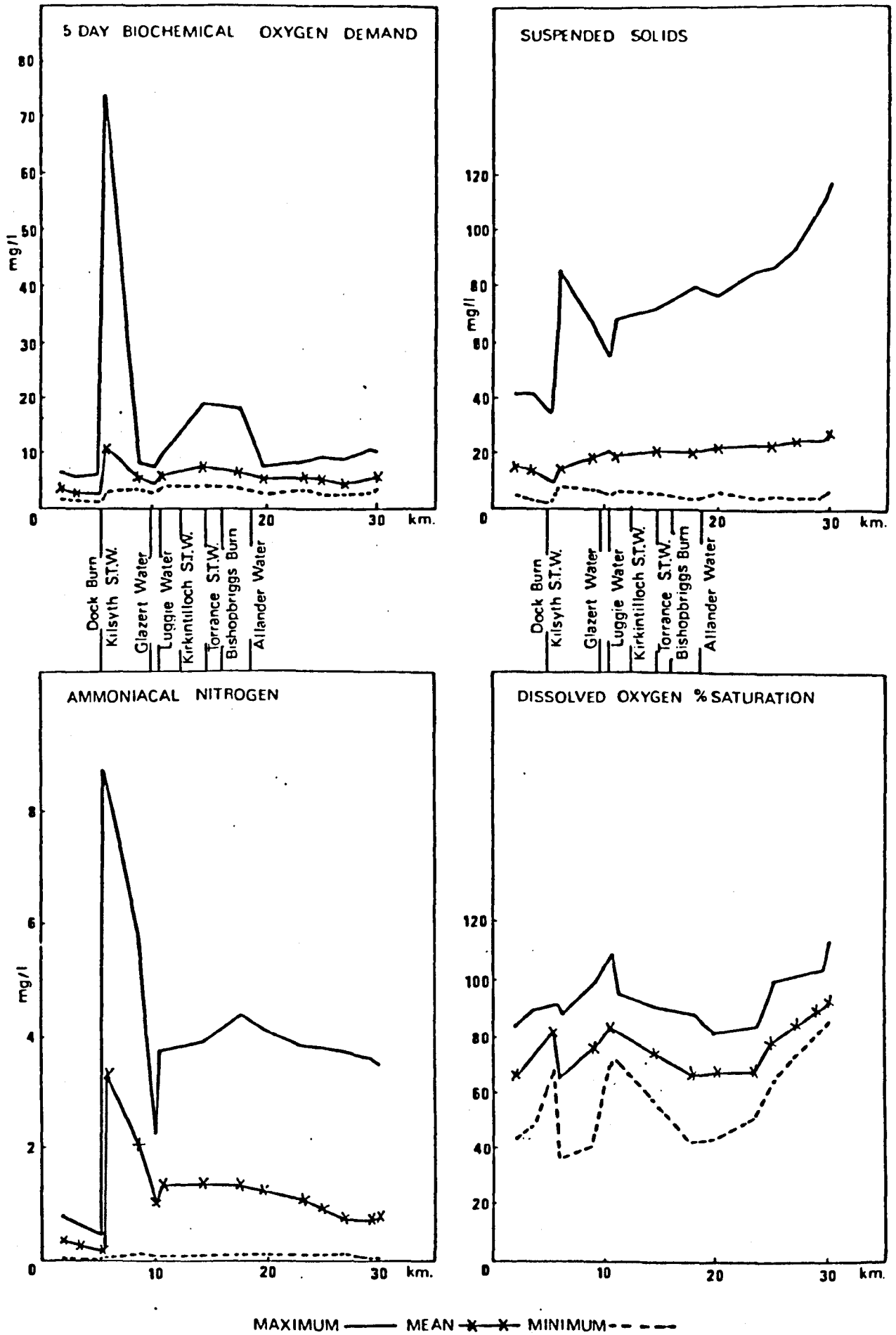


Figure 16. Summarised results of River Kelvin pollution survey 1977.

(from CRPB, 1977).

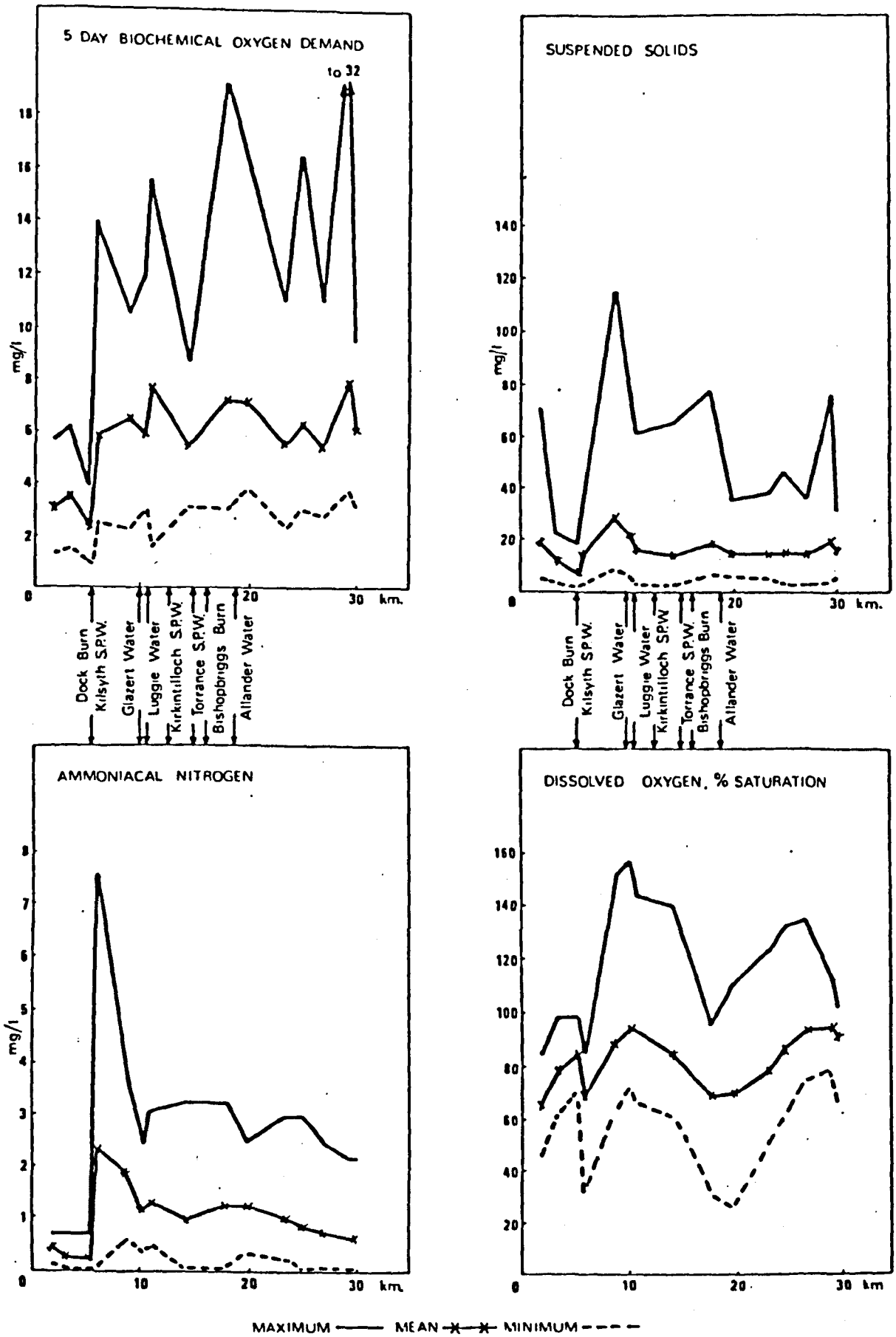


Figure 17. Summarised results of River Kelvin pollution survey 1978. (from CRPB, 1978).

(SRC) about various schemes to improve the sewage treatment facilities in the Kelvin Valley. There should be some improvement in the quality of the Bishopbriggs Burn if a re-sewering scheme, aimed at reducing the quality of sewage discharging to the burn during storms, is implemented.

In the Glazert Water there has been no development in the plan to connect the sewer from the Lennox Castle Hospital sewage treatment works to the public sewer. With the exception of the stretch of water immediately downstream of the hospital, the Glazert remained in good condition.

The Luggie Water continued to be polluted by the Cameron Burn which receives spasmodic discharges of contaminated drainage from two opencast sites. Elsewhere there was no significant change in the water quality of the river. However, the Bothlin Burn was adversely affected by discharges of acid waste from the B.S.C. Gartcosh Works and, for a short time, by the poor quality effluent discharged from Auchengeich sewage treatment works when the works were vandalised (CRPB, 1979).

The summarised results of monthly sampling, for BOD_5, suspended solids, ammoniacal nitrogen and dissolved oxygen, over the river Kelvin during the year 1979 are shown in Figure 18.

River Flow Gauging Stations

There are three gauging stations along the river Kelvin:

1. Bridgend, located just upstream of station 7.
2. Dryfield, located between stations 11 and 12.
3. Killermont, located upstream of station 17.

These gauging stations are maintained by the CRPB, and measure the water flow of the river in the catchment area.

The water flow within the river Kelvin valley for the years 1977-1979 at each gauging station is shown in Table 6.

Collection of River-Water Samples

Samples were collected monthly from 16 sampling stations along

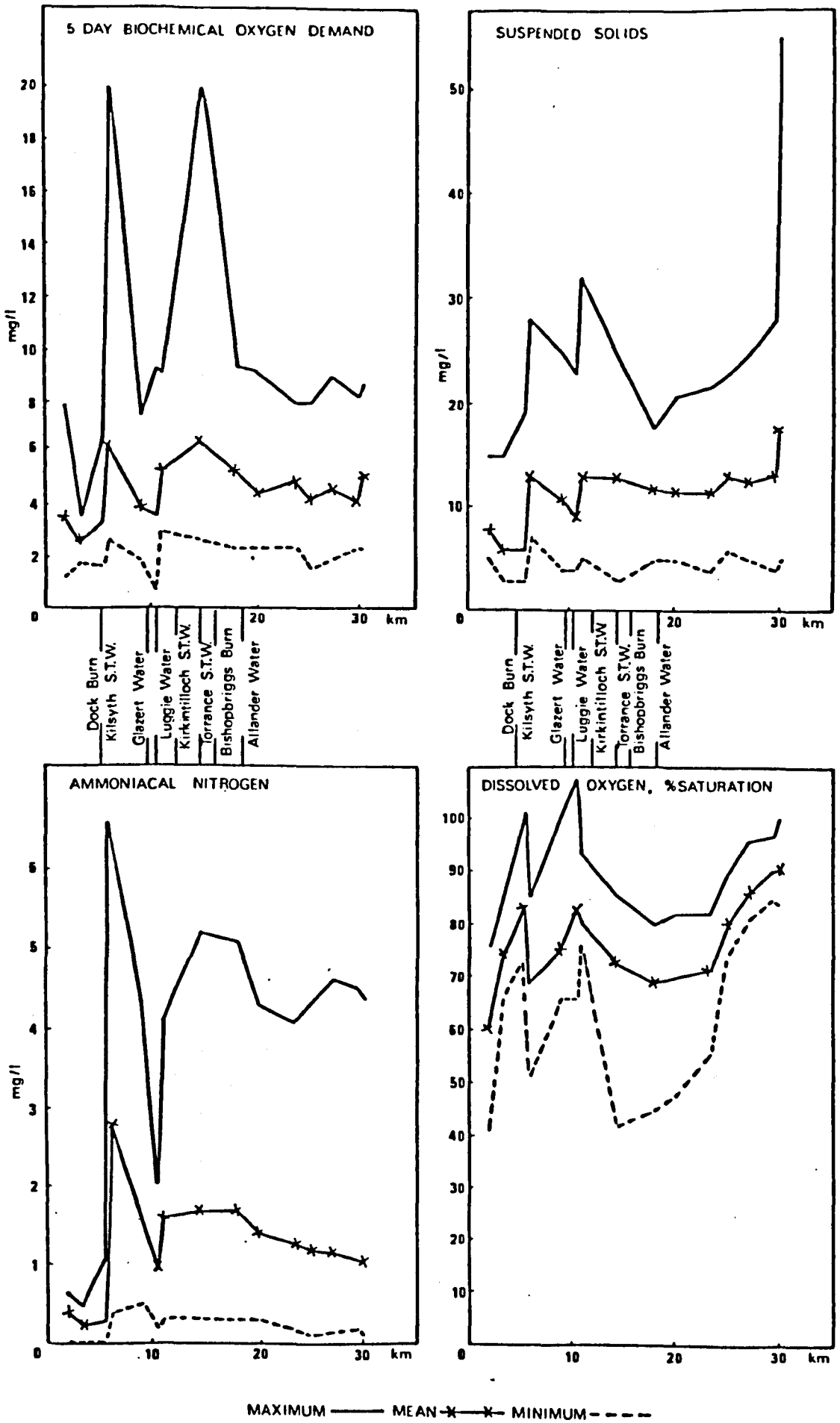


Figure 18. Summarised results of River Kelvin pollution survey 1979. (from CRPB, 1979).

Year	Station	National Grid Reference	Catchment (km^2)	Mean Flow m^3/s	Long Term Average m^3/s
	Bridgend	26/672749	63.7	1.417	1.550
1977	Dryfield	26/638739	235.4	6.070	6.369
	Killermont	26/558705	334.1	8.182	8.008
	Bridgend	26/672749	63.7	2.174	1.550
1978	Dryfield	26/638739	235.4	8.356	6.369
	Killermont	26/558705	334.1	8.417	8.008
	Bridgend	26/672749	63.7	2.476	1.550
1979	Dryfield	26/638739	235.4	8.458	6.369
	Killermont	26/558705	335.1	9.677	8.008

Table 6. Water flow in three gauging stations on the River Kelvin during the years 1977, 1978 and 1979. (from CRPB, 1977, 1978 and 1979).

the river Kelvin, at the same time as the CRPB was collecting samples. The samples of river water were collected during the period August 1978 to January 1980.

Water samples were collected just below the surface, from the middle of the river with a bucket tied to a rope. Glass-stoppered bottles (500 ml) were used to collect the samples which were brought back to the laboratory as soon as possible.

The temperature of the river water was measured at the time of sampling. The weather, turbidity of the river water, the time of sampling, and the river flow were also recorded (Appendix 3).

The pH of each sample was measured and BOD_5 tests were set up for the 16 samples on the day of sampling. The samples were stored at $4^{o}C$ overnight and used the following day for O_2 uptake measurement by the microbiological method.

BACTERIA

A. Pseudomonas cepacia (NCTC 10661)

It was obtained from the National Collection of Type Cultures (NCTC) (Central Public Health Laboratory, Colindale Avenue, London, NW9).

B. River-Water Bacteria

These bacteria were isolated from the River Kelvin:

i. Escherichia coli

ii. Klebsiella pneumoniae

iii. Enterobacter cloacae

iv. Serratia liquefaciens

v. Proteus mirabilis

Isolation

Five media were used for the primary isolation and cultivation of bacteria from river-water.

i. Nutrient Agar (code CM3) from Oxoid Ltd.

ii. MacConkey Agar No. 2 (code CM109) from Oxoid Ltd.

iii. Salmonella Shigella Agar (code CM99) from Oxoid Ltd.

iv. Tergitol 7 Agar, Difco Laboratories, Michigan, U.S.A.

v. Membrane Enriched Teepol Broth (code MM369), Oxoid Ltd.

(15 g of Purified Agar (code L28) Oxoid Ltd., as added to the membrane enriched Teepol Broth) (Department of Health and Social Security, H.M.S.O., 1969); 15g agar/litre teepol broth

The surface of the medium was inoculated with 0.2 ml of river water with a sterile glass spreader. The plates were incubated aerobically at 20°C and 37°C for 24-48 hr. Pure cultures were obtained from single colonies by standard bacteriological procedures. Single isolates were sub-cultured on nutrient agar (code CM3, Oxoid) and stored at 4°C.

Identification

Initially each isolate was stained by Gram's method.

Enterotubes: Enterotube Roche (Roche Diagnostica, London) detailed in Table 10, p.131, was used for the identification of Gram-negative bacteria. An isolated colony on MacConkey Agar was picked up with the tip of the Enterotube inoculating needle. The Enterotube was inoculated by first twisting the wire, then withdrawing it through each of the eight media compartments with a turning motion, reinserting it with a turning motion through the Dextrose, Lysine and Ornithine compartments, and breaking it at the notch by bending it. The caps were replaced on both ends of the tube. The blue tape on the side of the tube was stripped off and the inoculated tube incubated at 37°C for 24 hr.

The reactions were read and recorded for each test directly, except for the indole test. Here 3 or 4 drops of Kovacs reagent were added to the H_2S/Indole compartment of the incubated Enterotube with a sterilized (1 ml) syringe through the plastic film of the compartment. A positive test was indicated by the development of a red colour.

The positive reactions in each compartment were recorded by comparing the inoculated tubes with a non-inoculated tube. Bacteria were identified with the aid of the Coding book (Computer Coding and Identification System for Enterotube Roche).

Pseudomonas cepacia and river-water bacteria were used both as pure and mixed cultures. Mixed cultures: (prepared by mixing 1.0 ml from each bacterial suspension 3 x 10^{11}/ml) for O_2 uptake measurement in the polarograph.

i. E. coli + Ps. cepacia

ii. " + " + Kl. pneumoniae

iii. " + " + " + Ent. clocae

iv. " + " + " + " + Serr.liquefaciens

v. " + " + " + " + " + "

+ Proteus mirabilis.

CULTURE AND MAINTENANCE

A. Freeze-Drying of Organisms

The organisms were freeze-dried in ampoules and stored at 4°C for sub-culturing (Busby, 1967). Nutrient broth containing peptone was used (25 g Nutrient broth powder + 10 g Oxoid peptone/litre). An overnight culture of the organism in this medium was centrifuged with a bench centrifuge at 7,000 rpm (4032 x g) for 10 min. The pellet was resuspended in a smaller amount (10 ml) of the same medium to concentrate the organisms and 1.0 ml was pipetted into the sterile ampoules.

The ampoules were placed in the -70°C deep freeze for 15 min to freeze the suspension. They were transferred to an Edwards Freeze dryer (Edwards High Vacuum Ltd., Sussex, England) and dried overnight under high vacuum. The cotton-wool plugs were removed and the tubes were connected to the secondary drying head and further dried for 24 hr. The ampoules were sealed by flame with a blow-torch on the drying head and checked for a vacuum with a Spark tester (Edward Spark tester).

B. Measurement of Bacterial Growth

The Snyder & Koch + glucose medium (see Appendix 1) (500 ml) in a 2.0 litre dimpled Ehrlenmeyer flask was inoculated with 10 ml of an overnight broth culture of E. coli. The flask was incubated at 37°C with shaking (100 rpm). Samples (5.0 ml) were withdrawn from the flask at 1 hr intervals for 8 hr. The E^{1cm}_{650} was measured in an SP600 spectrophotometer (Pye Unicam, Cambridge, England). The inoculated medium before incubation was used as blank. The pH of the medium was 7.

PREPARATION OF THE SEED FOR OXYGEN POLAROGRAPH

1. Culture Grown in Nutrient Agar + Glucose

Roux bottles each one containing 200 ml of melted nutrient agar + glucose medium (see appendix I), were laid on their sides to give a large surface area for growth. The solid medium was inoculated by spreading 1.0 ml of an overnight broth culture over the surface with a sterilized glass spreader, and incubated at 37°C for 24 hr. The cells were harvested by adding 10 ml of 0.85% saline, emulsified and collected. The cells were washed twice with distilled water, harvested by centrifugation (MSE High speed 25) for 10 min at 7,000 rpm (8,500 x g), resuspended in distilled water to provide a thick suspension and stored on ice before use.

2. Culture Grown in Snyder and Koch Medium

i. Small—scale batch culture:

Aliquots of culture medium (Snyder & Koch; 500 ml) (see appendix I) were dispensed in sterilized dimpled 2.0 litre Erlenmeyer flasks. The medium was inoculated with 20 ml/l of an overnight nutrient broth starter culture. Cultures were incubated overnight at 37°C on a shaker (L.H. Engineering Co. Ltd.) at 100 rpm.

The cells were harvested, washed twice in distilled water

to provide a thick suspension, mixed well with a Rotamixer (Hook & Tucker Ltd., England). The cells were stored on ice before standardization and assay.

ii. Large-scale batch culture:

Cultures were grown in a fermenter (L.H. Engineering Co. Ltd., Stoke Poges, Bucks.). The fermenter vessel, containing 15 litres of culture medium (Snyder & Koch; see appendix 1), was sterilized by autoclaving at 121°C for 45 min. Glucose solution (10% w/v, 300 ml) sterilized by membrane filtration was added to the vessel through the inlet tube. Sterility was checked by leaving uninoculated medium for 18 hr. An overnight broth culture (500 ml) of E. coli 0142KH25 was used to inoculate the medium. Mixing of the culture medium was done with an impeller blade, revolving at 500 rpm. Sterile air was sparged through the culture at a rate of 10 l/min. Antifoam was added (antifoam C emulsion) with an automatic antifoam controller at the rate of 20% dose/3 min. The temperature was maintained at 37°C by thermostatically-controlled, water-filled submerged heating elements. The incubation period was 10 hr.

The culture medium was pumped out into a sterilized large aspirator, and the cells were harvested, washed twice in distilled water and deposited by centrifugation in an MSE High speed 25 centrifuge at 0°C (7,000 rpm; 8,500 x g) for 10 min (Measuring & Scientific Equipment Ltd., London). The cells were resuspended in distilled water to provide a thick suspension, mixed well with a Rotamixer (Hook & Tucker Ltd) and stored on ice before standardization and assay.

Attachment of River Organisms to Polarograph Magnets

In an attempt to obtain a bacterial seed for the polarographic oxygen uptake measurement, several polarograph magnets were suspended in the River Kelvin at Garscube Estate for 1 to 2 weeks, to find out if the river water organisms would attach to the magnets. Each magnet was placed in a plastic tea-urn-dolly (obtained from Toby Tea-Boy Ltd., Aldridge, Staffs, England). The magnets after removal from the river

were used in the polarograph test. Substrate solutions (1M glucose solution, 10 mM sodium pyruvate, 10 mM sodium succinate; 0.3 ml/test) were added to both standard dilution water and river water of known BOD_5. In each case the uptake of oxygen by the river-water organisms, adhering to the magnets, during the oxidation of the substrates was measured.

4. Standardisation of the Seed

Four methods were used to standardise the bacterial suspensions obtained by batch cultivation.

(i) Optical density: The optical density of the bacterial suspension was measured in the SP500 Spectrophotometer (Pye Unicam Ltd., England) at 650 nm against a distilled water blank. The washed cells were resuspended in distilled water to give $E^{650}_{1cm} = 1.02$ - $E^{650}_{1cm} = 1.2$ for a 1/100 dilution of the suspension.

(ii) Opacity tubes: Brown 's opacity tubes for the standardisation of bacterial suspension (Burroughs Wellcome and Co., London) were used. A sample of the bacterial suspension was taken, a suitable dilution made for the sample with a known ratio (1/100). This was transferred to one of the empty tubes (opacity tube) to give a column 2-3 inches high and the tube was laid upon a clearly printed page in a good light. The tube, containing the suspension, was compared with the opacity tubes to find the best matching tube.

The number of organisms counted in millions/ml was obtained by multiplying the tube number (Tube No. 6; the ten opacity tubes are numbered from 1-10) by the number of dilution X the equivalent of tube no. to the numerical table (which is 750 millions/ml for the organism E. coli). Dilutions were made from the suspension to obtain a seed with 3×10^{11}/ml.

(iii) Dry weight measurement: The suspension (1.0 ml), used to determine the dry weight of the organisms, was put in 3 small crucibles. These crucibles were weighed before and after drying in a thermostatically

controlled vacuum oven at 140°C (Townson and Mercer Ltd., Croydon, England). The dry weight of the organisms was obtained by subtraction. A dilution was made from the suspension to obtain a seed with 4.4 mg/0.1 ml of suspension.

(iv) Oxygen uptake measurement: Endogenous O_2 uptake for bacterial suspensions (0.1 ml) was measured with the oxygen polarograph (YSI Model 53) (see page 113 for the procedure). The seed suspension was diluted to give an endogenous O_2 uptake of 3.0-4.5 µl/min/mg dry weight in 0.1 ml of the seed suspension or 62-68 QO_2 value for the oxidation of 0.3 ml 1M glucose solution.

5. Seed Storage

Several methods were examined for the retention of maximum activity in the stored seed. i.e. maximum O_2 uptake in the polarograph.

I. Freeze-drying technique:

a. Freeze-drying on small filter paper discs: The seed (0.1 ml) was placed on small filter paper discs (1.0 cm diam),(obtained from Millipore S.A.). The discs were put in petri-dishes, frozen at -70°C for 15 min and freeze-dried. The dried discs were kept in a desiccator.

b. Freeze-drying in ampoules: The bacterial suspension (0.1 ml) was also freeze-dried in glass ampoules. (suspended in distilled water).

II. Storing the Seed at low temperature:

The E. coli cells grown in the fermenter (large-scale batch culture) were used in this experiment. The standardized suspension was stored at different temperatures.

(1) Storage at -25°C. The suspension (2.0 ml) in bijou bottles was stored at -25°C.

(2) Seed storage at -70°C. The suspension (2.0 ml) was stored at -70°C in bijou bottles.

(3) Seed storage in liquid nitrogen (-195.8°C). The seed was put into

2.0 ml capacity sterilized plastic vials and heat-sealed with hot forceps. The plastic vials were put on metal racks and stored in liquid nitrogen (-195.8°C). One plastic vial was taken out every two weeks to test the activity of the seed against glucose in the polarograph.

Endogenous and exogenous oxygen uptake were compared before and after the storage of the seed at various temperatures or as freeze-dried pellets, for the period of 8½ months, by oxygen polarography.

6. Oxidation Activity Measurement for the Seed

The activity of the stored seed, after thawing, was measured with the polarograph. The E. coli seed (stored at -70°C) was used in this experiment with 1M glucose solution (0.3 ml/test). 3.0 ml of distilled water was placed into the polarograph cell and 0.1 ml of the seed thawed quickly by rotating the container between hands, was added to measure the endogenous oxygen uptake for the seed. Exogenous oxygen uptake was measured in a cell containing 2.7 ml distilled water, 0.3 ml 1M glucose solution and 0.1 ml seed. The oxygen uptake for the substrate was measured by subtracting the endogenous from the total oxygen uptake.

The oxidation activity test for the seed was repeated at 1 hr intervals for 28 hr.

POLAROGRAPHIC OXYGEN ELECTRODE

The biological oxygen monitor (YSI model 53, Yellow Spring Instrument Co., Yellow Spring, Ohio, U.S.A.) (Plate 12) was used to measure the rate of O_2 uptake.

The biological oxygen monitor provides a means of measuring oxygen uptake by biological systems. This instrument produces in 5-15 min similar O_2 uptake curves to those obtained in 1-2 hours with the Warburg apparatus. It contains a polarographic electrode known as the probe which is responsible for the extremely sensitive results obtained. Figure 19 shows a diagram of the probe, plunger and sample chamber. A

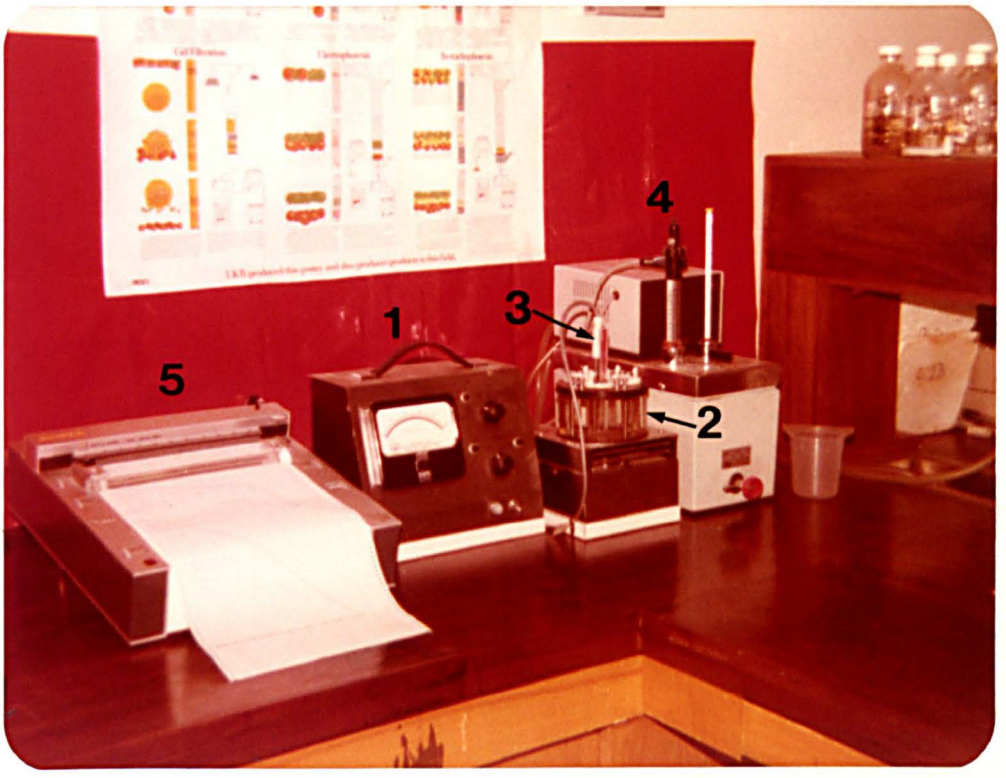


Plate 12. The Polarographic Oxygen monitor system.

(1) YSI model 53 electronic unit

(2) Standard bath assembly

(3) Oxygen Probes

(4) Thermostat

(5) Flat bed chart recorder

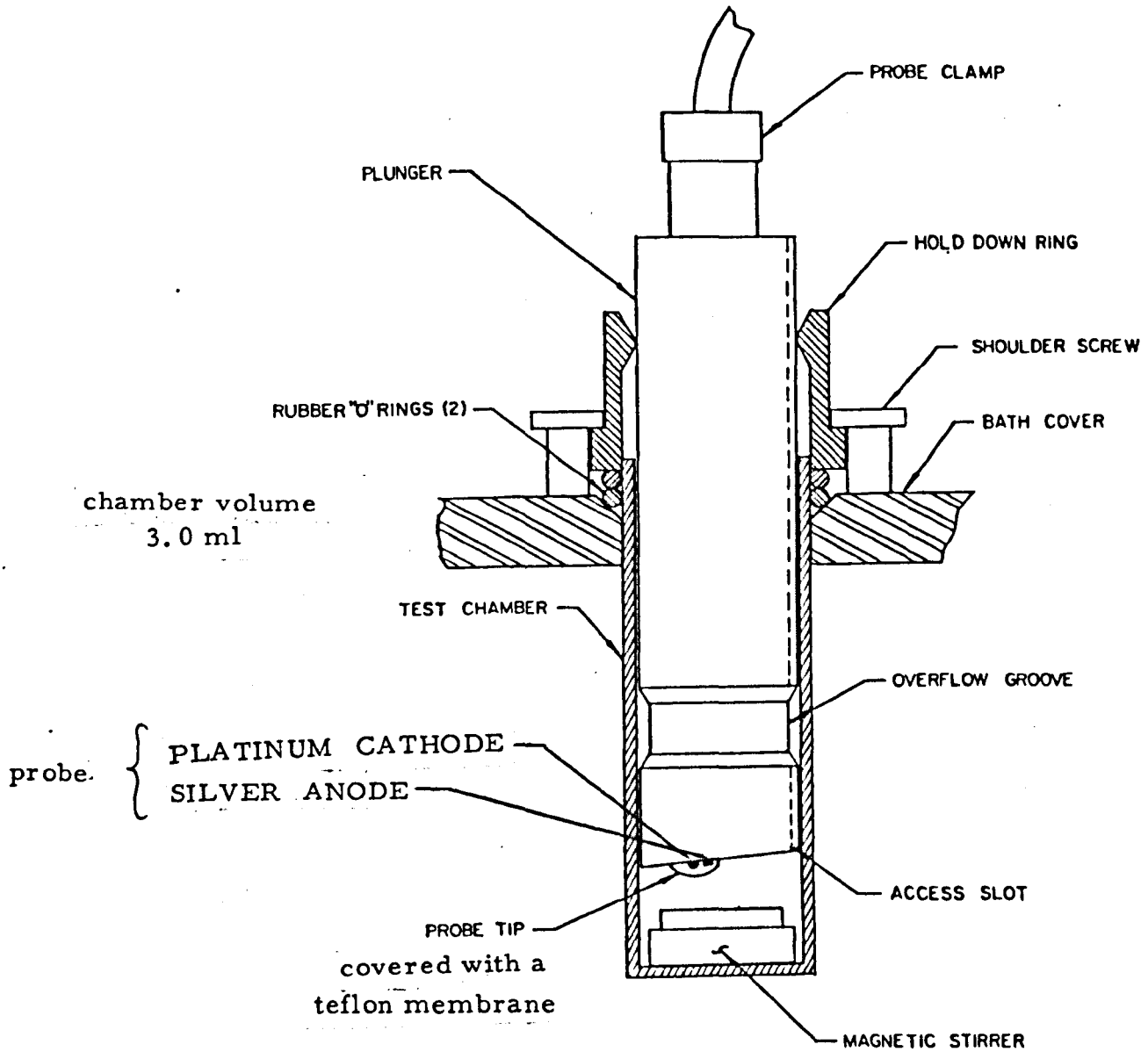


Figure 19. PROBE, PLUNGER AND SAMPLE CHAMBER.

The oxygen electrode is a special form of electrochemical cell, in which a current is generated that is proportional to the activity of oxygen present in a solution. The platinum and reference electrodes are shielded from the solution by a thin membrane which is permeable to oxygen. To maintain a stable oxygen gradient across the membrane it is necessary to stir the solution at 500-600 rpm.

thin Teflon membrane (0.001 in thickness) stretched over the end of the probe isolates the sensor elements from the environment, but is permeable to gases and allows them to enter the interior of the probe. When a suitable polarising voltage (0.1 V) is applied across the cell, oxygen reacts at the cathode causing a current to flow through the cell.

$$O_2 + 2e^- + 2H^+ \longrightarrow H_2O_2$$

$$H_2O_2 + 2e^- + 2H^+ \longrightarrow 2H_2O$$

The amount of current which flows is proportional to the amount of oxygen to which the membrane is exposed. The membrane diffusion is directly proportional to $\uparrow^{\text{oxygen}}_{\text{pressure}}$ and the oxygen-cell current relationship is stoichiometric, thus a linear relationship exists between external oxygen pressure and cell current. The current flowing at any instant can be observed on the meter as % saturation of O_2 and changes in current with time can be seen as a continuous trace on the potentiometric recorder. From which µl of O_2 uptake per minute can be calculated.

The rate of O_2 utilization by the seed is a function of both the rate of entry of substrate to the cell and its utilization within the cell.

The actual oxygen consumption rate was determined as follows. Distilled water (2.7 ml) was placed into the sample chamber with 0.3 ml of 1M glucose solution and the temperature was equilibrated for 1-2 min. Subsequently 0.1 ml of the standardized seed suspension was added, and the rate of oxygen uptake was measured. A trace of oxygen uptake on the recorder over a period of 3-15 minutes can be used to determine the actual amount of oyxgen consumed (µl/min), for example:

Oxygen uptake measured for sample (2.7 ml distilled water + 0.3 ml 1M glucose solution + 0.1 ml seed) in five minutes is shown in Figure 20. From the slope of oxygen uptake (Figure 20)

O_2 % at the time 1½ min = 75%

Note that all polarograph results are presented as traces of the recorder charts. All the polarograph results were measured at the working temperature of 37°C.

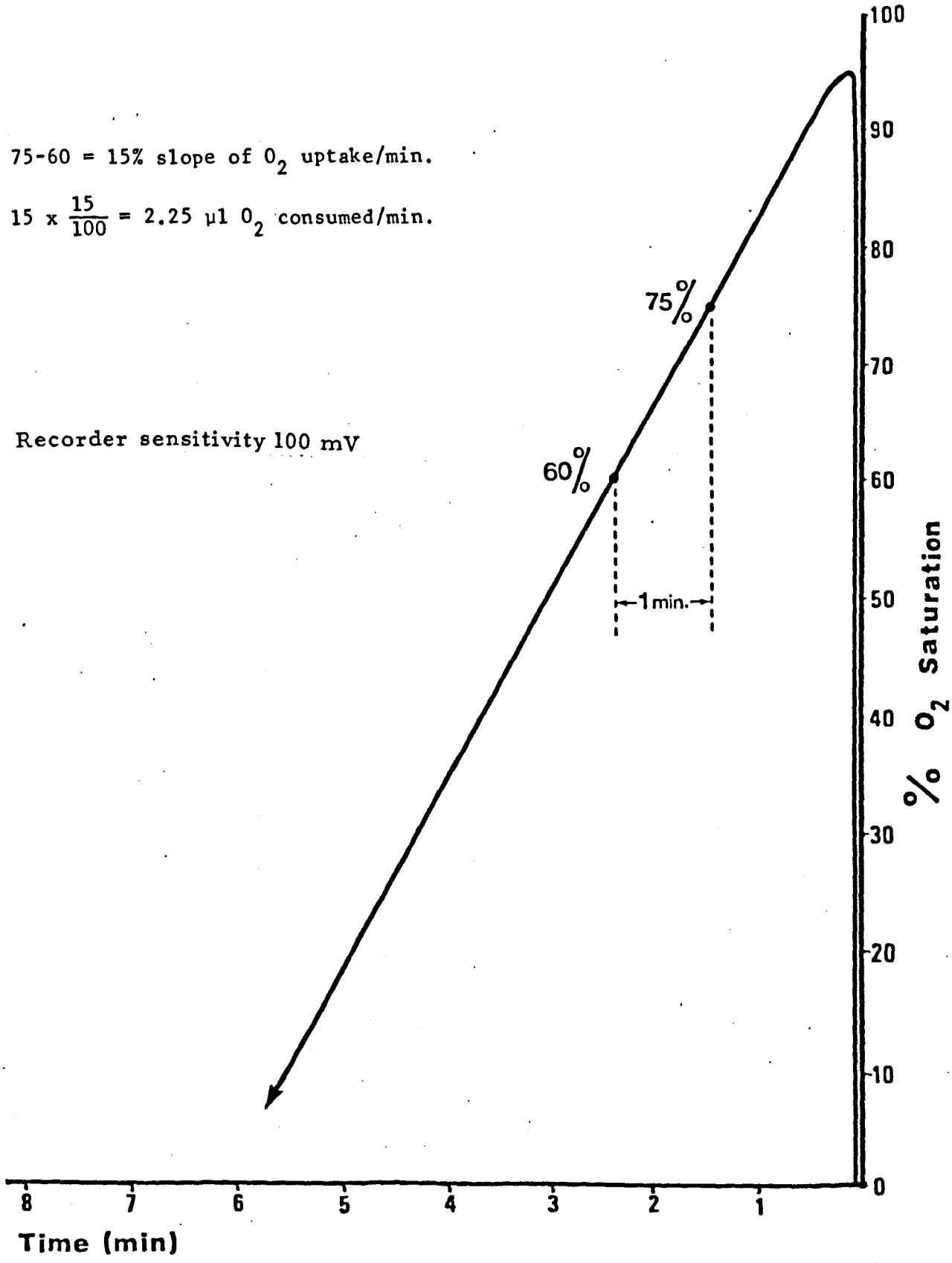


Figure 20 Oxygen uptake measurement from the slope of oxygen uptake obtained on the chart-recorder of the polarograph.

O_2 % at the time 2½ min = 60%

75-60 = 15% of the oxygen in the sample was used up within one minute. (Ringer's solution saturated with air at 37°C contains 5 µl O_2/ml of solution.)

Thus a 3 ml sample contained: 3 x 5 = 15 µl O_2 (when saturated)

A change of 15% in saturation means 15 x 15% = 2.25 µl O_2 was consumed in 1 min.

The oxygen uptake determined in µl/min was converted to QO_2 value, that is the amount of oxygen (µl) consumed in a time (hr.) per dry weight of the seed (mg).

QO_2 = µl/hr./mg dry weight of seed.

Endogenous O_2 Uptake Measurement of the Seed

Endogenous oxygen uptake is the amount of oxygen consumed by microorganism without the presence of added substrate as the cells aerobically metabolize their stored contents.

Endogenous oxygen uptake for a bacterial seed suspension was measured with the polarograph. Distilled water (3.0 ml) was put into a sample chamber of the polarograph with a magnetic stirrer, 0.1 ml of the seed was added, one to two minutes were allowed for sample temperature equilibrium and oxygen uptake was measured in 3-5 minutes. This oxygen uptake in the absence of substrate is due to the endogenous oxygen uptake by the cells.

Oxygen Uptake Measurement for Known Substrate Solutions

The rate of oxygen uptake by the seed for different substrates was studied with:

i. Solution I (0.1M glucose solution) prepared by dissolving 1.8016 g of glucose in 100 ml distilled water.

ii. Solution II (1M glucose solution) prepared by dissolving 18.016 g of glucose in 100 ml distilled water.

iii. Solution III (10 mM sodium pyruvate) prepared by dissolving 110 mg of sodium pyruvate in 100 ml distilled water.

iv. Solution IV (10 mM sodium succinate) prepared by dissolving 0.270 mg of sodium succinate in 100 ml distilled water.

Different volumes of each substrate solution were used for O_2 uptake measurements (Table 7). The total volume of each sample in the polarograph cell was 3.0 ml plus 0.1 ml seed.

FACTORS AFFECTING O_2 UPTAKE MEASUREMENT IN THE POLAROGRAPH

A. Effect of Seed Concentration

The standardized E. coli seed, stored at -70°C was used in this experiment with 1M glucose solution as the substrate (0.3 ml/test). Several dilutions were made of the seed suspension in distilled water to give the following concentrations:

i. The seed concentration (S_1) = 375×10^9 /ml
ii. 3/4 dilution of S_1 = 280×10^9 "
iii. 1/2 " " " = 180×10^9 "
iv. 1/4 " " " = 80×10^9 "
v. 1/8 " " " = 40×10^9 "

The oxygen uptake for 0.3 ml of the glucose solution was measured with all the different seed concentrations. The endogenous O_2 uptake for the seed was measured by adding 0.1 ml seed to 2.7 ml distilled water. The glucose (0.3 ml) was added and the O_2 uptake for the substrate in the polarograph was measured.

B. Effect of Temperature on O_2 Uptake

The E. coli seed, from small-scale batch cultures, and 0.1M glucose solution were used in this test. The polarograph chamber temperature was controlled with the Lauda Kontakt thermometer. Endogenous O_2 uptake for the seed was measured (see page 113). Oxygen

Solution	substrate (ml)	dist.water (ml)	total volume (ml)	seed (ml)
	0.1	2.9	3.0	0.1
	0.25	2.75	-	-
Glucose	0.5	2.5	-	-
(0.1M)	0.75	2.25	-	-
Glucose (1M)	0.3	2.7	3.0	0.1
	0.1	2.9	3.0	0.1
Sodium	0.25	2.75	-	-
pyruvate	0.35	2.65	-	-
(10 mM)	0.5	2.5	-	-
	0.1	2.9	3.0	0.1
Sodium	0.25	2.75	-	-
succinate	0.5	2.5	-	-
(10 mM)	0.75	2.25	-	-

Table 7. Amount of substrate solutions used for O_2 uptake measurement with the polarograph.

uptake for 0.3 ml of the glucose solution was measured in the polarograph.

This test was repeated at temperatures of 10, 20, 30 and 37°C, to show the effect of temperature on the O_2 uptake in the polarograph.

C. Effect of pH on O_2 Uptake

The standardized E. coli seed, stored at -70°C was used in this experiment with glucose solution (1M) as the substrate (0.3 ml/ test). Five solutions (0.1M buffer) were prepared with different pH values by mixing different volumes of the acid and base solutions. (pH 5.1, 6.4, 7.0, 8.5, 9.5). Endogenous O_2 uptake for the seed was measured (see page 113). Oxygen uptake was measured for the glucose solution by adding 0.1 ml of the seed and 0.3 ml of the glucose solution to 2.7 ml of each one of the buffer solutions in the polarograph, to show the effect of pH of the sample solution on the O_2 uptake.

D. Effect of Heavy Metals and other Toxic Materials

Twelve different heavy metal ions and KCN^- were examined for their effect on O_2 uptake in the polarograph (Table 8) for 1M glucose and the standardized seed. The concentration of these metals ranged between 0-10 ppm for mercury (Hg^{++}), from 0-20 ppm for silver (Ag^+), and from 0-40 ppm for the remainder in Table 12. The starting solution for each metal compound was prepared by dissolving 40 mg of the compound in 1 litre of distilled water. Several dilutions were made of the solution to give the following concentrations:

i	The starting dilution (S_1)	=	40 ppm
ii	3/4 dilution of S_1	=	30 ppm
iii	1/2 " " "	=	20 ppm
iv	1/4 " " "	=	10 ppm
v	3/4 " " solution iv	=	7.5 ppm
vi	1/8 " " S_1	=	5 ppm
vii	1/16 " " "	=	2.5 ppm

Metal	Compound	ionic form	concentration (ppm)
Mercury	$HgCl_2$	Hg^{++}	0-10
Silver	Ag_2SO_4	Ag^{+}	0-20
Copper	$CuSO_4.5H_2O$	Cu^{++}	0-40
Aluminium	$Al_2(SO_4)_3.16H_2O$	Al^{+++}	"
Iron	$FeCl_3.6H_2O$	Fe^{+++}	"
Lead	PbO_2 & $(CH_3COO)_2Pb.3H_2O$	Pb^{+}	"
Zinc	$ZnSO_4.7H_2O$	Zn^{++}	"
Nickel	$Ni(NO_3)_2.6H_2O$	Ni^{++}	"
Cadmium	$CdSO_4.8H_2O$	Cd^{++}	"
Chromium	$CrCl_3.6H_2O$	Cr^{+++}	"
Cobalt	$CoCl_2.6H_2O$	Co^{++}	"
Tin	$SnCl_2.2H_2O$	Sn^{++}	"
Potassium cyanide	KCN	CN^{-}	0-40

Table 8. Toxic metal compounds and the concentrations used in the polarograph.

Distilled water (2.7 ml) containing the required concentration of heavy metal was placed in the polarograph cell and 0.3 ml of the 1M glucose solution with 0.1 ml of the seed were added. The uptake of oxygen for the substrate in the presence of different heavy metal concentrations was measured over a period of 3 min. The uptake of oxygen for the substrate in the presence of no heavy metal concentrations was measured for the control.

Oxygen uptake by the control solution was subtracted from the oxygen uptake values for the tests containing substrate and different concentrations of heavy metals to determine the effect of the latter.

The same procedure was used with the cyanide solutions.

E. Effect of Concentrating River-Water on its O_2 Uptake

River-water samples were collected from the river Kelvin at Garscube Estate. Two different methods were used to concentrate the samples.

(i) Membrane filter and vacuum.

Samples (30 ml) were put into Sartorius membrane tubes (obtained from Sartorius-Membranfilter GmbH, 34 Gottingen, Germany) and put under vacuum for 3-4 hours to give a 10-fold concentration (3 ml were left from the sample).

(ii) Concentration by boiling.

Samples (200 ml) were put in a beaker and heated slowly on a bench gas heater until 20 ml of the sample was left (a 10-fold concentration was obtained).

River water samples concentrated ten-fold by both methods were used for the following tests: a) BOD_5 determination by the standard dilution method, b) COD determination and c) O_2 uptake measurement in the polarograph. In polarograph tests either 0.1 ml of E. coli standardized seed or 0.1 ml of E. coli seed plus 0.3 ml of 0.1M glucose solution were added to the concentrated water sample.

The effect of concentrating the water-samples was determined by repeating these tests with the unconcentrated river-water.

F. Effect of Heating (Autoclaving) on O_2 Uptake

1. For river water samples

Eleven water samples were collected from the River Kelvin (stations 2, 4, 5, 6, 7, 8, 10, 13, 14, 15 and 17) including i, clean river water, ii, polluted river water, and iii, sewage effluent. The BOD_5 was measured for the samples by the standard dilution method (see page 120). Samples were autoclaved for 15 min at 121°C, care was taken to ensure that the volume and pH of the liquid was the same before and after autoclaving. The pH of each sample was adjusted to 7.0 after autoclaving. Oxygen uptake was measured in the polarograph for the samples before and after autoclaving by adding 0.1 ml of the E. coli standardized seed (stored at -70°C) to 3.0 ml of each sample.

2. Cellulose and starch solutions

The effect of autoclaving on the oxygen uptake for cellulose and starch solutions was examined in the polarograph. Cellulose (18 g/l) and starch (18 g/l) solutions were prepared and autoclaved for 15 min at 121°C. Care was taken to ensure that the volume and pH were the same before and after autoclaving. Oxygen uptake for the cellulose and the starch solutions was measured before and after autoclaving by adding 0.1 ml E. coli seed (stored at -70°C) to 3.0 ml of each solution in the polarograph cell.

CHEMICAL AND BIOCHEMICAL MEASUREMENTS

A. Dissolved Oxygen

Dissolved oxygen content of a water sample was determined by the Rideal-Stewart modification of Winkler method (Rideal and Stewart, 1901). (For the reagents see appendix 1).

Samples for DO measurement were collected in 250 ml glass-

stoppered bottles. The bottle containing the sample was kept stoppered and the excess water was allowed to overflow. except when reagents were added. ↑ Concentrated sulphuric acid (0.7 ml) was added, followed by 2.0 ml of potassium fluoride solution, to facilitate permanganate decolorization in the presence of appreciable amounts of iron salts in the sample. One or two drops of N/8 permanganate were added. The quantity of permanganate solution added should be sufficient to give a pale violet colour that persisted for 5 min. Sodium oxalate solution (1.0 ml) was added to remove excess permanganate so that complete decolorization was obtained after about 10 min.

Manganous sulphate solution (1.0 ml) and 4.0 ml of alkaline iodide solution were added, mixed for a minute, and 1.5 ml of concentrated sulphuric acid was added to dissolve the precipitate completely. One hundred millilitres of this solution were pipetted into a 250 ml flask and the liberated iodine was titrated with N/80sodium thiosulphate solution. Starch solution (0.5 ml) was added as an indicator of iodine; each ml of N/80 thiosulphate required corresponds to 1.0 ppm of oxygen.

B. Biochemical Oxygen Demand

1. The standard dilution method

Samples for BOD determination were diluted with standard dilution water (see appendix 1 for the preparation of dilution water) according to the nature of the sample, eg. 1:1 to 1:2 (v/v) for river water samples, 1:5 (v/v) for sewage effluent samples, 1:30 (v/v) for primary settlement tank effluent samples, and 1:50 (v/v) for crude sewage samples. The diluted sample was mixed well, and the temperature was adjusted to 20°C in a shaking water-bath.

Two BOD bottles (250 ml, brown, narrow-necked glass-stoppered bottles) were completely filled with the sample, after dilution, and after 15 min the bottles were tapped gently to remove air-bubbles. The same mixing and transfer techniques were used for all samples. The

dissolved oxygen content of one bottle was determined immediately by the Rideal-Stewart modification of the Winkler method. The second bottle was incubated at room temperature (about 20°C) and the dissolved oxygen content was determined after 5 days. The dissolved oxygen content of the sample before incubation was kept at a level of about 9 ppm or less at 20°C. Biochemical oxygen demand was calculated as follows: BOD (ppm O_2) = (Initial DO (ppm) - Final DO (ppm)) x dilution factor.

2. Biochemical oxygen demand for the standard substrate

The standard substrate solution was prepared according to HMSO specifications (Department of Environment, 1972).

Glucose (150 mg) and glutamic acid (150 mg) both dried at 103°C for one hour in an oven were dissolved in 1.0 litre water. This solution was freshly prepared before each experiment and diluted 1:50 with the standard dilution water (see Appendix 1), to give a BOD_5 value of 220-240 mg/l. River water was used to seed the dilution water. 2 ml/l. Doubling dilutions were made of the substrate solution and the BOD_5 was determined for each solution.

3. Polarographic O_2 uptake for the standard substrate solution

Oxygen uptake was measured in the polarograph for each of the substrate solutions (see page 113). The results of the BOD_5 and O_2 uptake were compared for the substrate solutions.

C. Determination of Chemical Oxygen Demand

(See appendix 1 for the preparation of reagents).

Potassium dichromate (5.0 ml N/8) and 10 ml concentrated H_2SO_4 were pipetted into a 500 ml conical flask, cooled with running water, and 1.0 ml of silver sulphate solution was added. Subsequently 5.0 ml of the sample was added, mixed well and a few anti-bumping granules were added. The flask was fitted to a condenser and refluxed for 2.0 hr. After cooling 45 ml water was added. One drop of 1:10 phenanthroline indicator was added, and the residual dichromate titrated with N/8 ferrous sulphate

from a 5 ml micro-burette. The COD was calculated according to the formula:

$$COD = \frac{(\text{blank titration} - \text{sample titration}) \times 1000}{\text{volume of sample taken (ml)}} \quad \text{mg/l.}$$

The blank titration was obtained by repeating the test with 5.0 ml distilled water.

MEMBRANE FILTRATION OF RIVER-WATER

To reduce the time of the BOD_5 test, various quantities of river-water (100 ml, 250 ml and 500 ml) were membrane filtered. The membrane, containing bacteria and insoluble particles of organic matter, was added to the BOD bottles (containing diluted river water sample) in the standard BOD_5 determinations.

The river-water sample(5-12 litres) was collected from the River Kelvin at Garscube Estate. The usual BOD_5 test was set up for the river-water sample. In addition, BOD_1 to BOD_5 tests were set up for the river-water sample with 1:1 (v/v) dilution and one of the membrane filters was added to each bottle as the seed. A clean membrane filter was added to one of the bottles as a control. Tests for BOD_1 to BOD_5 were also set up with standard dilution water and a membrane filter was added to each bottle. These served as the controls for the river-water samples.

Each BOD value for standard dilution water + membrane filter was subtracted from the corresponding BOD value for the river-water plus membrane filter to determine the actual BOD due to oxidation of the organic matter by river-water microorganisms, present in the river-water plus the river-water microorganisms on the membrane filter.

An attempt was made by this procedure to obtain a comparative BOD value in a shorter time. These tests were done with river water and various substrate solutions (glucose 1M, sodium pyruvate 10 mM, sodium succinate 10 mM, and glutamic acid 1M).

Increasing quantities of river-water (100 ml to 1.0 litre) were

membrane filtered and the membranes were cut into small pieces and placed in 2.7 ml standard dilution water. After vigorous shaking to remove microorganisms from membrane, the liquid was placed into the polarograph chamber with 0.3 ml of a suitable substrate and the O_2 uptake was measured. This test was repeated with 2.7 ml river-water instead of dilution water and the O_2 uptake was measured before and after the addition of substrate.

CELLULOSE BIODEGRADATION IN RIVER WATER

A. Cellulose Decomposition by River-Water Microorganisms

Cellulose films (cellophane) stained with Remazol Blue by the method of Moore, Basset and Swift (1979) were obtained from Dr. M.J. Swift, Department of Plant Biology and Microbiology, London University, Queen Mary College, London. The Remazol Blue dye can only be released from the cellulose by hot alkali extraction or by the enzymatic hydrolysis of the cellulose itself. The release of dye thus provides a means of estimating cellulose degradation.

Two cellulose films (2 x 5 cm) were put into each tea-dolly (Plastic tea-urn-dolly, obtained from Toby Tea-Boy Ltd., Staffs, England). Six of these prepared tea-dollies were suspended in the River Kelvin, at Garscube Estate, just below the surface of the water during a three month period. One tea-dolly, containing two cellulose films, was removed from the river every week. One of the films was used to estimate the extent of cellulose breakdown by extracting the residual dye from it. The film was washed gently in distilled water to remove the river water and any soluble dye and placed in 60 ml of 0.06M KOH solution in a 100 ml flask. The flask was placed in a pressure cooker and autoclaved for 10 min at 121°C. The volume of the extract was adjusted to 100 ml with distilled water in a volumetric flask. The optical density was measured at 595 nm in a spectrophotometer against a distilled water blank. An undigested cellulose film was used to measure the 100% control value.

The percentage weight of cellulose digested was calculated according to the formula:

$$\text{Percentage weight of cellulose digested} = \frac{\text{OD control - OD exposed}}{\text{OD control}} \times 100$$

where:

OD control = the optical density of the extract from an undigested cellulose film.

OD exposed = the optical density of the extract from a cellulose film suspended in the river.

The amount of cellulose digested was measured weekly for a period of five weeks to determine the cellulose decomposition versus time (days).

B. Isolation of Cellulose Decomposing Microorganisms

Three different media were used for the primary isolation and cultivation of cellulose decomposing microorganisms in river-water (Media C, D and E, appendix 1). Cellulose films suspended in the river for three weeks were used to inoculate the media. The films were washed gently in distilled water. One film was placed on the surface of Omeliansky's cellulose agar (see appendix 1). The plates were incubated at 20°C and 30°C for 24-48 hr. Pure cultures were obtained from single colonies growing on the cellulose agar. The pure cultures were sub-cultured finally on to Nutrient Agar.

Dubos and Omeliansky's liquid media (see appendix 1) were inoculated with a piece of cellulose film and the tubes were incubated at 20°C and 30°C for 24-48 hr. Subsequently, the liquid medium was spread over the surface of Omeliansky's cellulose agar and nutrient agar plates with sterilized glass spreaders. The plates were incubated at 20°C and 30°C for 24-48 hr. Pure cultures were obtained by streaking out single colonies from both media.

THE KELVIN VALLEY SEWAGE WORKS

The history of pollution in the river Kelvin dates back to the nineteenth century when Frankland (1873) in the Fourth Report of the Commission of River Pollution of 1868, reported that the River Kelvin carried the sewage of about one-eighth of the population of Glasgow including the effluent of printing-works, paper-works and distilleries, together with much liquid refuse from chemical and printing-works on the Glazert Water. The River Kelvin was the most filthy tributary of the River Clyde one hundred years ago.

Today in the Kelvin valley there are 15 sewage treatment works (STW) which discharge their effluents into the River Kelvin either directly, or indirectly into one of its tributaries, such as the Dock Water, the Board Burn, the Glazert Water, the Bothlin Burn, the Luggie Water, the Bishopbriggs Burn and the Allander Water (see Figure 15, page 80). These sewage works are shown in Table 9. Most of these receive their waste-water from domestic sources, in the surrounding towns and from nearby industries, and hospitals in the area.

These biological sewage treatment works are of two types, a) trickling filter and b) floating aerator systems, for example Plate 13a and b shows the primary settlement tank and the trickling filters at the Kirkintilloch (Dryfield) sewage treatment works and Plate 14a and b shows the primary settlement tanks and the floating aerators of the Bishopbriggs (Jellyhill) sewage treatment works.

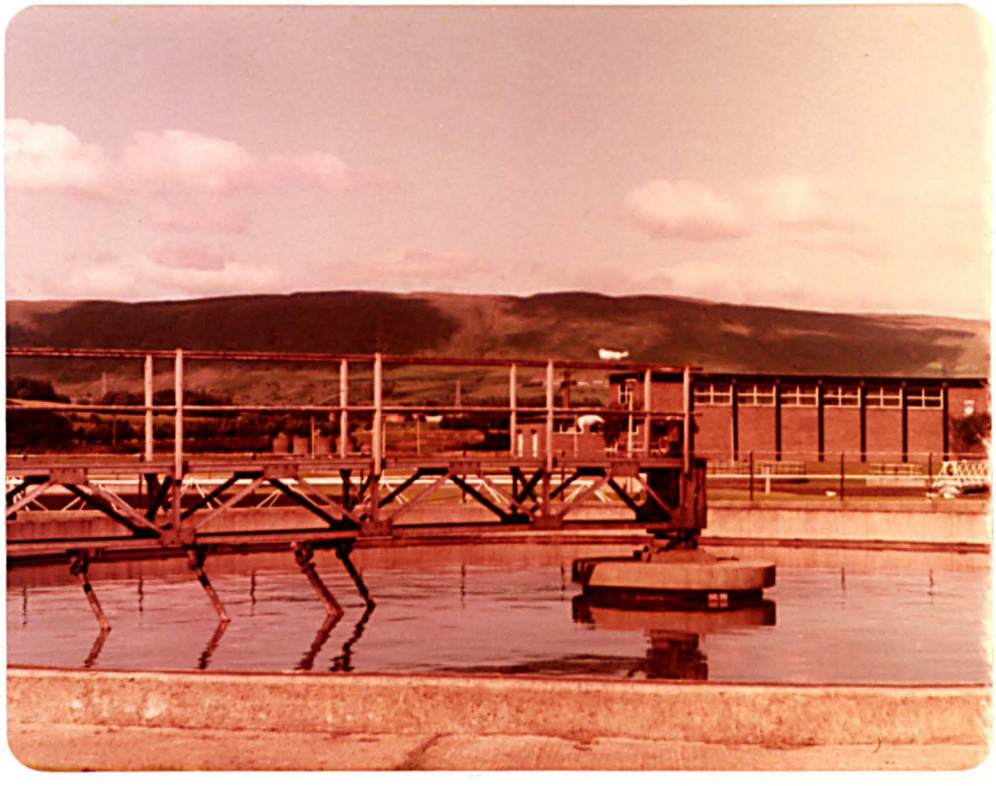
Sewage Sampling

Seven sewage works in the Dumbarton division of Strathclyde Regional Council were visited.

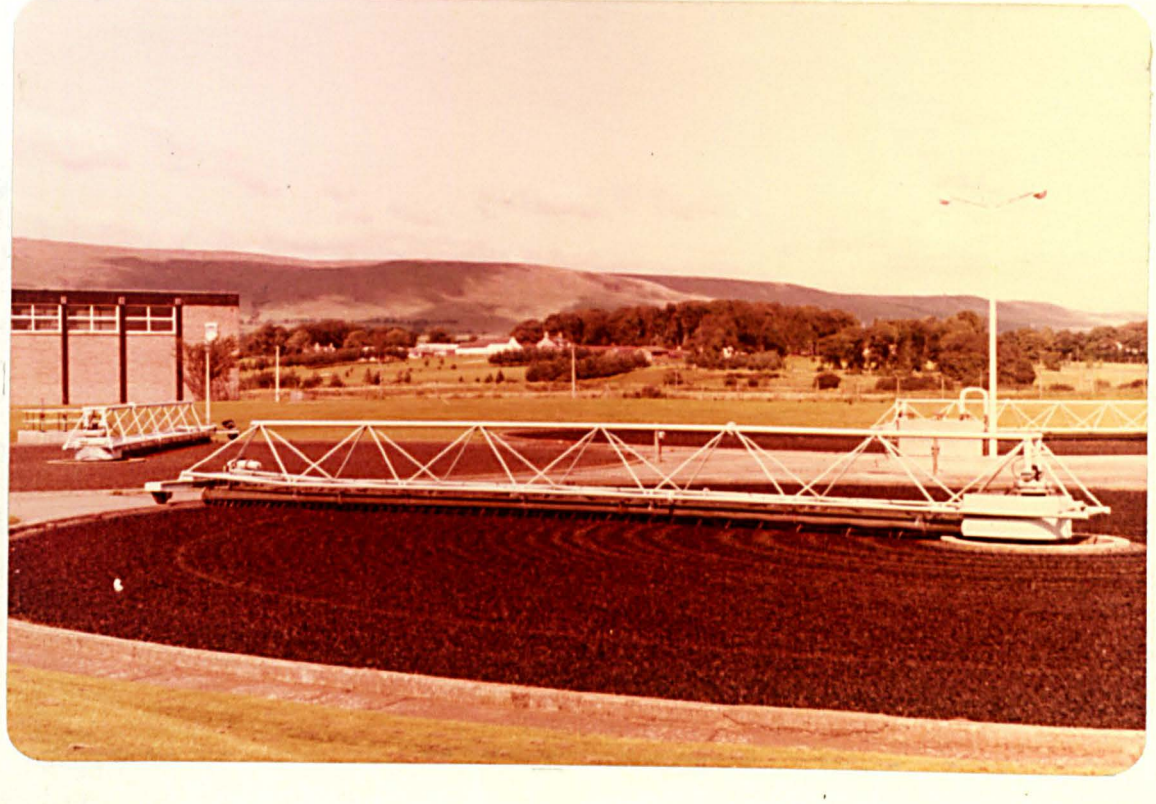
1. Kirkintilloch (Dryfield) STW.
2. Bishopbriggs (Jellyhill) STW.
3. Kilsyth STW.

Name of Works	Receiving Watercourse	NGR of point of discharge	Population served
Dullatur	Trib. of R. Kelvin	NS 744 775	180
Kilsyth	Dock Water	NS 709 772	10,500
Queenzieburn	R. Kelvin	NS 695 761	840
Croy	Board Burn	NS 721 754	1,625
Twechar	R. Kelvin	NS 695 754	2,600
Milton of Campsie (Birdston)	Glazert Water	NS 658 757	6,500
Greengairs	Cameron Burn	NS 784 705	1,300
Cumbernauld (Deerdykes)	Luggie Water	NS 714 719	17,000
Glenboig	Trib. of Bothlin Burn	NS 716 681	600
Auchengeich	Bothlin Burn	NS 685 716	18,500
Waterside	Luggie Water	NS 673 733	1,200
Kirkintilloch (Dryfield)	R. Kelvin	NS 637 739	36,000
Torrance	R. Kelvin	NS 614 734	2,500
Milngavie	Allander Water	NS 563 732	11,000
Bishopbriggs (Jellyhill)	Bishopbriggs Burn	NS 613 721	22,500

Table 9. Kelvin Valley sewage treatment works.

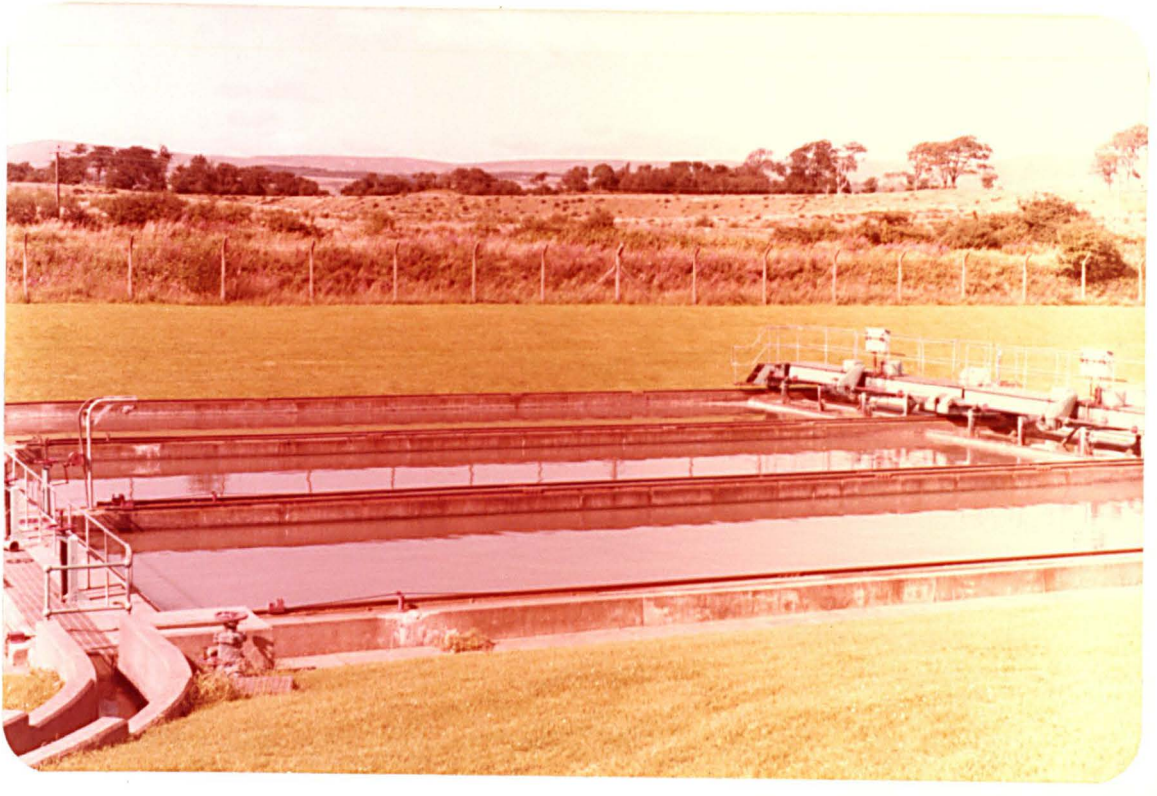


a. Primary settlement tank



b. Trickling filters

Plate 13. Kirkintilloch (Dryfield) STW.



a. Primary Settlement Tanks

b. Floating Aerators

Plate 14. Bishopbriggs (Jellyhill) STW.

4. Milton of Campsie (Birdston) STW.

5. Torrance STW.

6. Twechar STW.

7. Queenzieburn STW.

Samples were collected twice a week during a six month period (summer-winter). Glass-stoppered, narrow-necked bottles (1 litre) were filled and brought back to the laboratory for BOD_5 determination, pH measurements and O_2 uptake measurement with the polarograph.

Three different samples were collected from each sewage works, namely (i) crude sewage (for high BOD_5 values), (ii) primary settlement tank effluent (to give samples of an intermediate quality in the treatment process) and (iii) the final effluent (for low BOD_5 values).

The weather and time of sampling were recorded, and the temperature of the sample was measured. The storm condition in the sewage work was also recorded.

Polarographic Oxygen Uptake Measurement on Sewage

The *E. coli* seed stored at -70°C was added to 3.0 ml of sewage sample in the polarograph cell, and oxygen uptake was measured for 3-5 min. The endogenous oxygen uptake was measured for the seed (see page 113) and the sewage samples were tested for oxygen uptake without the addition of seed.

The actual oxygen uptake for the sewage samples was calculated by subtracting the endogenous oxygen uptake value for the seed from the oxygen uptake value for the sewage sample plus seed.

RESULTS

* Serotyped by the Central Public Health Laboratory, Colindale Avenue, London.

THE BACTERIAL INOCULUM (SEED)

A. Isolation of River-Water Organisms

It was necessary to obtain a stable bacterial seed, for the polarographic measurements, which could be standardized and retained for long periods. A variety of organisms were isolated from the River Kelvin (Table 10) and used both as pure and mixed strain cultures during the preliminary experiments. Pseudomonas cepacia NCTC 10661, was used both as a pure culture and also mixed with other river-water strains of bacteria in polarograph tests.

A river-water isolate, Escherichia coli (0142 K? H25)*, gave reproducible results with a range of both known substrate solutions (see Figures 27, 28 and 29) and effluent samples. This organism was used subsequently for all the polarographic experiments, each test was repeated at least 3 times.

B. Activity of the Seed

In order to obtain a standardized bacterial seed which could be stored and yet retain its activity, E. coli organisms obtained from a batch culture, were stored under the following conditions:-

1. Freeze-dried on filter paper discs (FPD)
2. Freeze-dried in ampoules
3. Stored under low temperature (a) $-25^{\circ}C$, (b) $-70^{\circ}C$, (c) $-195.8^{\circ}C$ (liquid nitrogen).

The uptake of oxygen by the bacterial cells, during the utilisation of soluble organic substrate was measured at weekly intervals in the polarograph, before (control) and after storage.

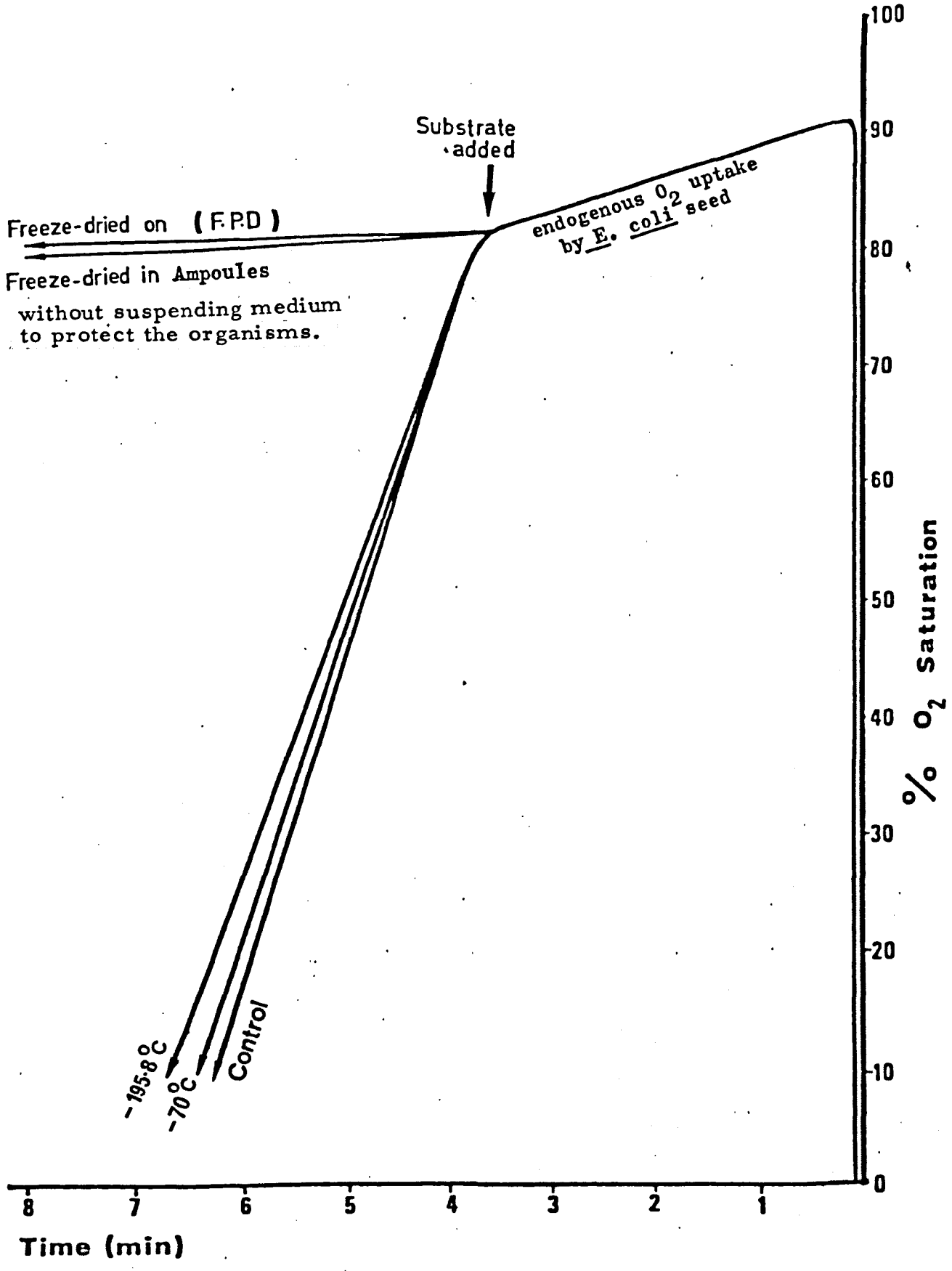
The activity of the seed stored under different conditions for 8½ months is shown in Figure 21. The samples stored at $-70^{\circ}C$ or in liquid nitrogen ($-195.8^{\circ}C$) retained activity comparable to that of the

Organism	Dextrose	Gas	Lysin	Ornithine	H_2S	Indole	Lactose	Dulcitol	Phenyl-alanine	Urea	Citrate	Confirmatory tests
E. coli	+	+	+	-	-	+	+	-	-	-	-	
Enterobacter cloacae	+	+	-	+	-	-	+	-	-	+	+	
Klebsiella pneumoniae	+	+	-	-	-	-	+	-	-	+	+	
Proteus mirabilis	+	-	-	+	-	-	+	-	+	+	-	
Serratia liquefaciens	+	+	+	+	-	-	+	-	+	-	-	
Enterobacter agglomerans*	+	-	-	-	-	-	+	-	-	-	-	SHA(-) ADO(-)
Klebsiella ozaenae*	+	-	-	-	-	-	+	-	-	-	-	SHA(-) ADO(+)
" rhinoschleromatis*	+	-	-	-	-	-	-	-	-	-	-	SHA(-) ADO(+)
Citrobacter freundii*	+	-	-	-	-	-	-	-	-	-	+	ADO(-) JTR(+)
Serratia rubidea*	+	-	+	-	-	+	+	-	-	-	+	VP(+) RHA(-)
Shigella boydii*	+	-	-	-	-	-	+	-	-	-	-	SHA(+)
" flexneri 1-5*	+	-	-	-	-	-	-	-	-	-	-	SHA(+)
" dysenteriae*	+	-	-	-	-	-	-	-	-	-	-	SHA(+)
Yersinia pseudotuberculosis*	+	-	-	-	-	-	-	-	-	+	+	MOT(-) JTR(v)
Staphylococcus spp												

Table 10. Isolated river-water organisms and the Enterotube results.

ADO = Adonitol test, MOT = Motility test, JTR = Jordans tartrate test, RHA = Rhamnose test, SHA = Shigella antisera test, VP = Voges-Proskawer test.

* These were identified at the first level only - confirmatory tests not done.



(Control)

Figure 21. Oxygen uptake for the E. coli seed before and after 8½ months of storage.

fresh seed (control), while the samples stored at -25°C and the freeze-dried samples had a much lower activity. The activity of the stored seed during 8½ months is shown in Figure 22.

Aliquots of the E. coli seed suspension, sufficient to do 3,000 tests, were obtained from one 15.0 litre batch culture grown in the fermenter. The standard seed was stored in sterilized plastic vials at -195.8°C in 2.0 ml amounts and in bijou bottles at -70°C in 2.0 ml amounts so that twenty tests could be done with each container.

C. Attachment of River-Water Organisms to the Polarograph Magnets

Since bacteria are known to adhere to solid surfaces, polarograph magnets were put in tea-urn-dollies and suspended in the River Kelvin for 1-2 weeks. The suspended magnets were carefully removed from the dolly and tested in the polarograph against substrate solutions (0.3 ml of 1M glucose solution, 10 mM sodium succinate, and 10 mM sodium pyruvate). There was no oxygen uptake, possibly due to the small number of organisms attached to the magnets in the film of slime.

D. Membrane Filtration of Increasing Volumes of River-Water to Provide Seed

1. The use of loaded membrane filters as the seed in BOD_5 tests

In an attempt to obtain BOD_5 results in a shorter time, increasing volumes of river-water (100, 250 and 500 ml) were membrane filtered. The loaded membranes were added to the BOD bottles in the five day determinations for river-water samples or standard dilution water. A clean membrane was added to one bottle as a control. The BOD_{1-5} were measured for river-water samples after the addition of the membrane loaded with river-water organisms and undissolved organic matter particles. The normal BOD_5 was also measured for each sample. The BOD curve in Figure 23 shows the amount of oxygen consumed (ppm) against time (days) for BOD determination in the presence of a membrane filter of 500 ml of river-water. The normal BOD_5 value was 4.6 ppm but after the addition of the membrane

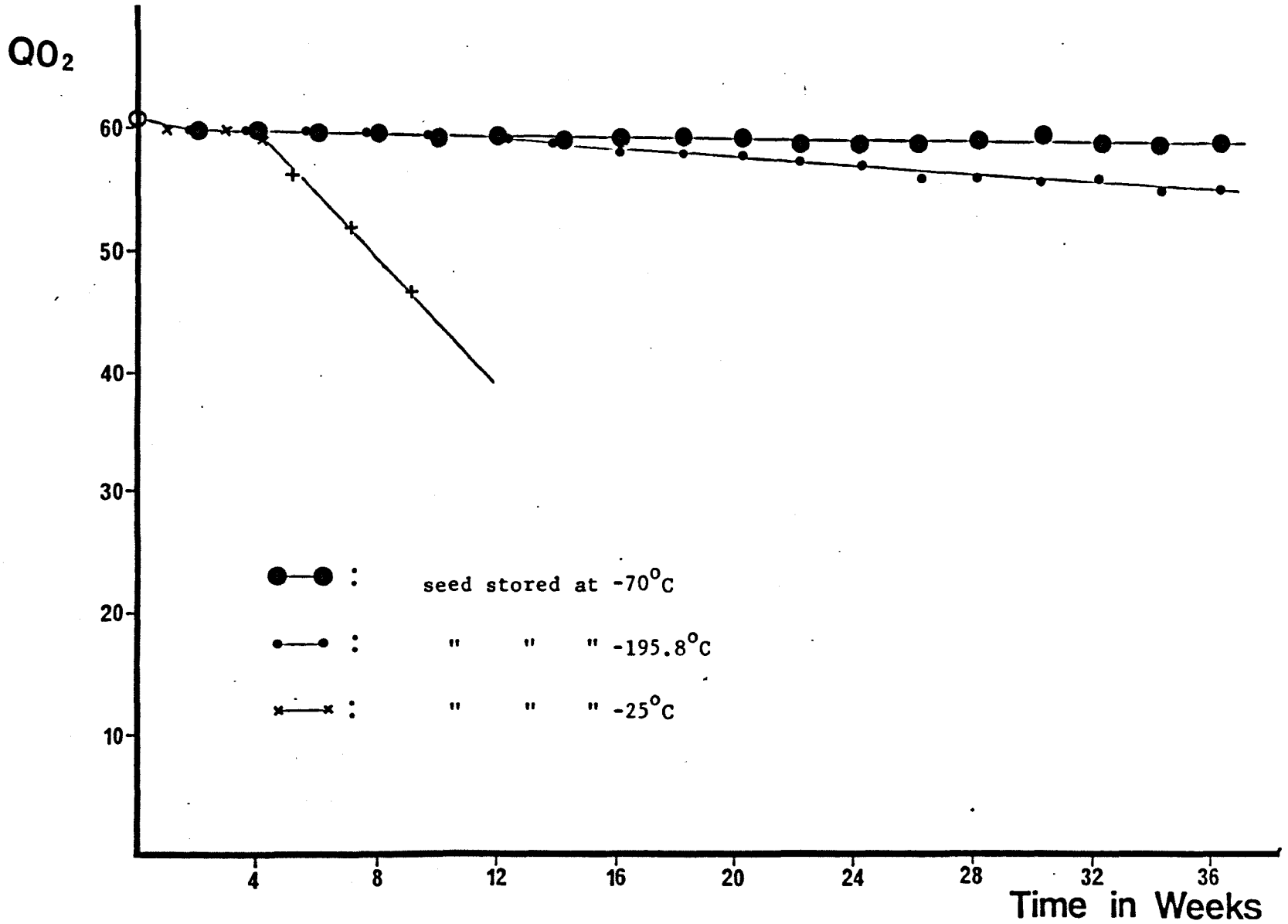


Figure 22. Oxygen uptake for the E. coli seed during 8½ months of storage at various low temperatures.

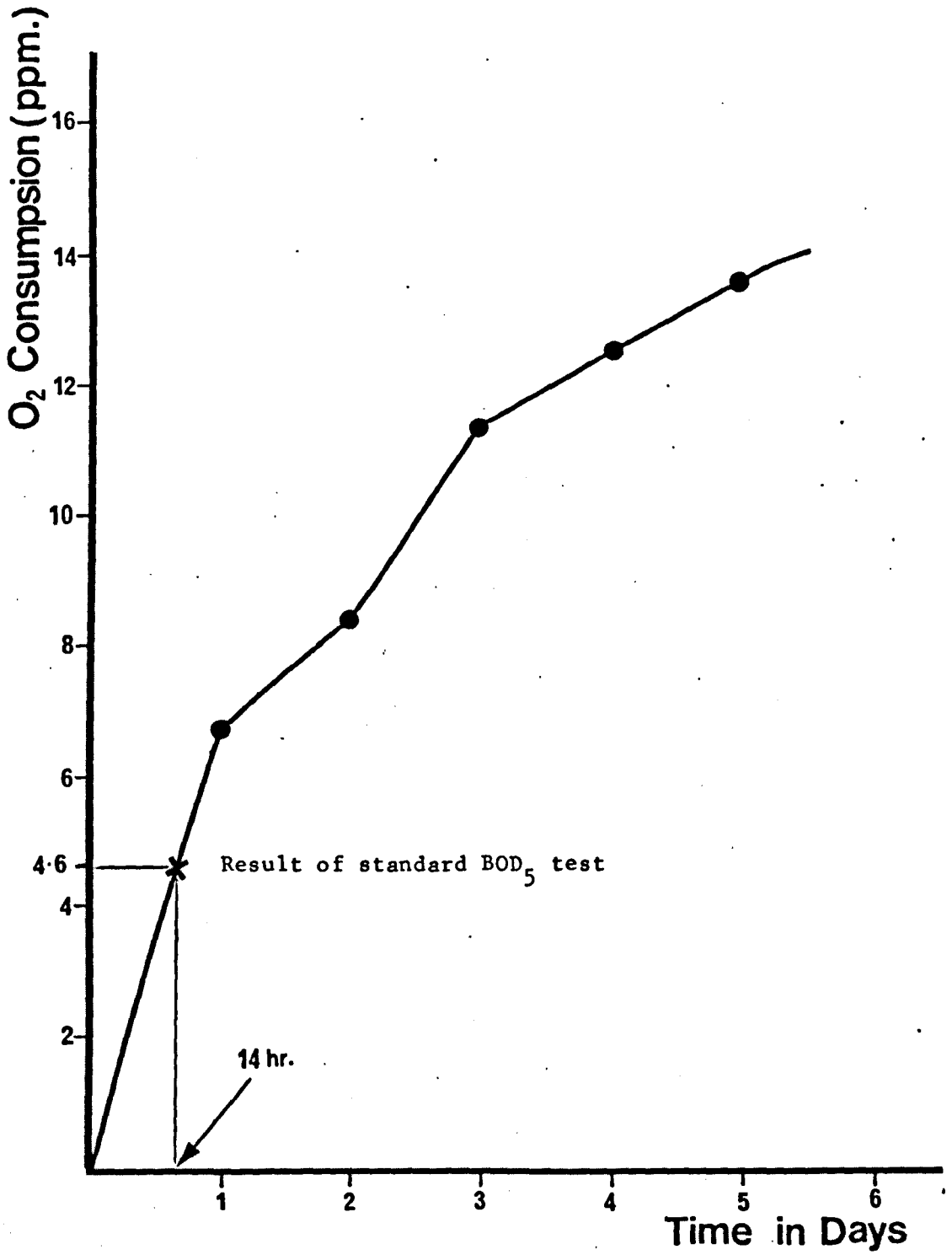


Figure 23. BOD curve for river-water + membrane filter seed of river-water.

✕ = BOD_5 for the RW sample obtained after 14 hr with adding membrane filter of 500 ml RW to the BOD bottle. (BOD_5 for the sample = 4.6 ppm.).

This experiment shows that the BOD_5 test could be reduced to BOD of <1.0 day by concentration of the bacterial population. However, in practice it would be impractical because of the need to still determine BOD_5 value.

filter this result (4.6 ppm) was obtained in 14 hours.

2. Seeding of the polarograph cell with the loaded membrane filter for oxygen uptake measurements

The loaded membrane filters were also used as the seed in the polarographic measurement of O_2 uptake. Membrane filters (MF) were cut into small pieces in 3 ml of river-water or distilled water and shaken vigorously. The liquor was put into the polarograph cell and O_2 uptake was measured with and without substrate (0.3 ml of 0.1M glucose). The results of experiments, in which a membrane filter of 100 ml of river-water was used, showed that O_2 uptake for river-water (RW) with added substrate was slightly higher than river-water without added substrate. Comparable results of O_2 uptake were obtained for both distilled water (DW) + membrane filter (MF) or DW + MF + substrate and for both RW + MF and RW + MF + substrate. With the addition of E. coli seed higher O_2 uptake levels were obtained for DW + MF + seed than RW + seed. Oxygen uptake was also higher for RW + MF + seed than DW + MF + seed. After the addition of substrate, O_2 uptake levels were RW + seed MF + DW + seed RW + MF + seed (Figure 24). Essentially similar results of O_2 uptake were obtained with the membrane filter of 500 ml of river water (Figure 25).

E. Cellulose Decomposition in River-Water

Degradation of cellulose in river-water was studied to show the time required for the in situ degradation of higher molecular weight organic matter in a river. Cellulose films were suspended in the river for 1-4 weeks. At each stage the % weight of cellulose degraded was measured for one film and the other film was used to isolate cellulose decomposers. The results of duplicate experiments (Table 11) showed a progressive decomposition of cellulose during the four week period. The amount of cellulose degradation against time is shown in Figure 26. Several

Note the beneficial effect of adding E. coli seed even in tests with the natural population of bacteria concentrated by membrane filtration.

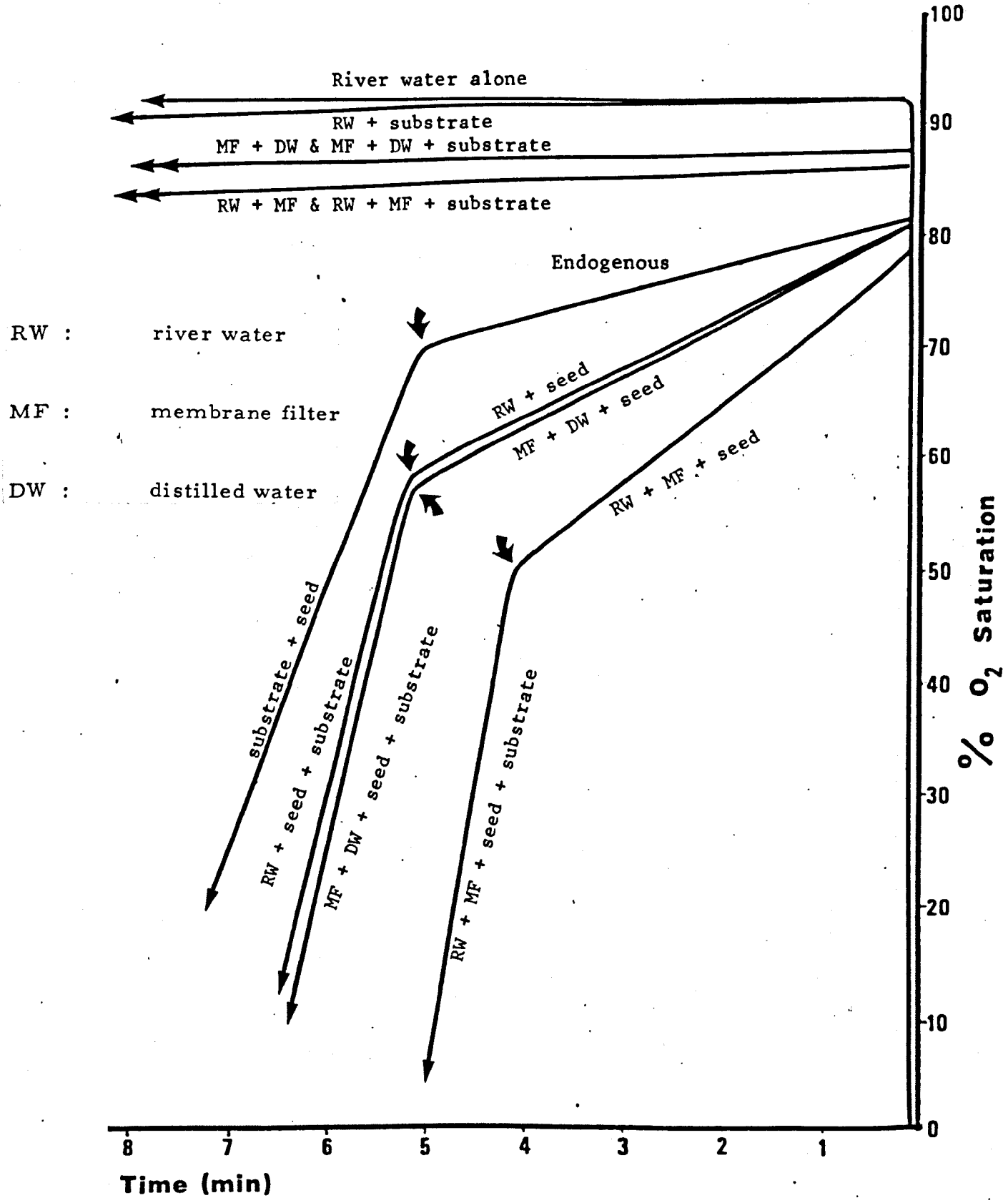


Figure 24. The use of membrane filters of river-water (100 ml) as the seed in the polarograph experiments.

↘ : substrate added. (0.3 ml 1M glucose)

This result indicates that the population of bacteria in the water sample can be concentrated to a level where O_2 uptake can be measured with river water alone (5). Compare these results with 500 ml concentrated water sample with those of 100 ml in Figure 24.

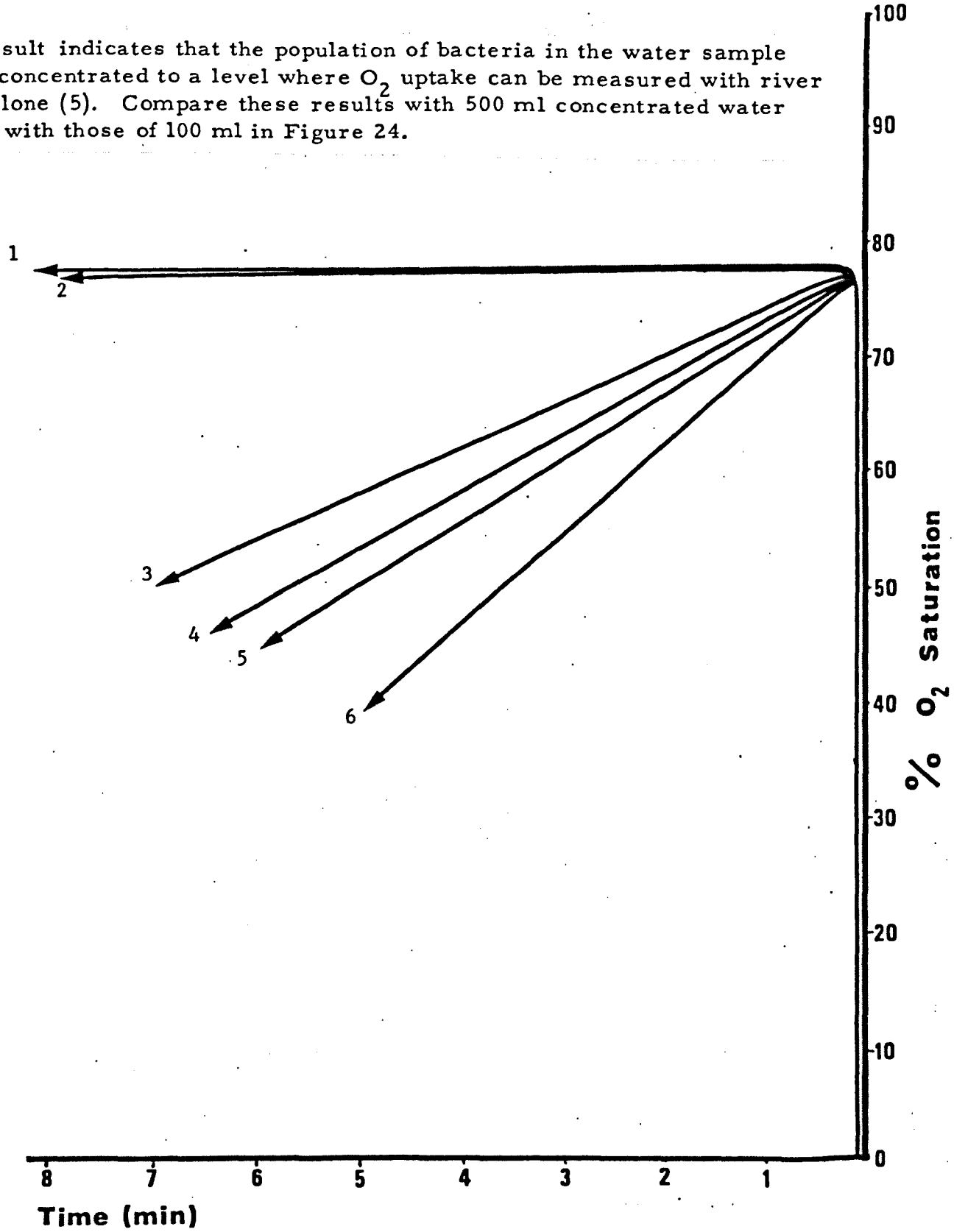


Figure 25. Polarographic O_2 uptake measurements for river-water with the membrane filter (500 ml) of river-water as the seed.

1. River water (RW) alone
2. RW + substrate (0.1M glucose)
3. Membrane filter (MF) + distilled water (DW).
4. MF + DW + substrate
5. RW + MF.
6. RW + MF + substrate.

Expt. 1		Expt. 2	
Week	% cellulose lost	Week	% cellulose lost
1	13.4	1	9.5
2	30.0	2	28.3
3	69.7	3	66.5
4	99.2	4	98.4

Table 11. Cellulose decomposition in the River Kelvin.

Cellulose strips stained with Remazol Blue were suspended in perforated plastic containers in the river (Tea-urn-dolly). The percentage loss of cellulose was measured at weekly intervals by extracting the residual dye from the residual cellulose.

$$\frac{\text{OD control - OD exposed}}{\text{OD control}} \times 100 = \text{Percentage loss of cellulose}$$

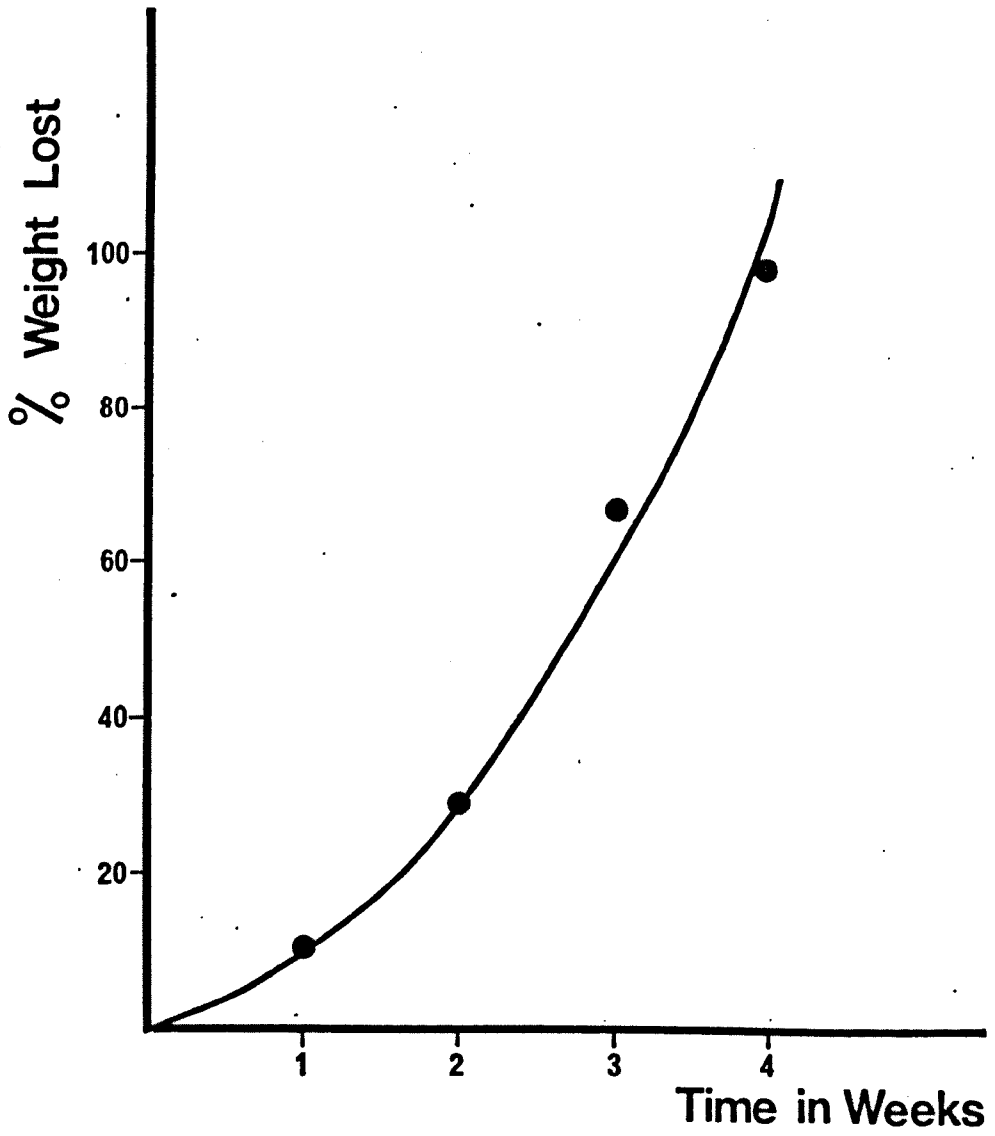


Figure 26. Cellulose decomposition in river-water

Note the small percentage amount of cellulose decomposed during the five day period equivalent to the BOD_5 test.

strains of bacteria and fungi were isolated from the suspended cellulose films. No attempts were made to continue the identification of these organisms for use in a seed inoculum, because it was concluded that the amount of degradation of complex polysaccharides occurring in the period of the BOD_5 test would be insignificant.

OXYGEN UPTAKE MEASUREMENTS FOR DEFINED SUBSTRATE SOLUTIONS

A. Known Substrate Solutions

To investigate the O_2 uptake for known substrate solutions and to show the relationship between concentration of substrate and O_2 uptake, various quantities of different substrate solutions (0.1-0.75 ml of 0.1M & 1.0M glucose, 10 mM sodium succinate, and 10 mM sodium pyruvate; see Table 7) were tested.

An increase in O_2 uptake was obtained as the concentration of substrate in the sample also increased; Figure 27 shows a typical result of O_2 uptake by E. coli in a glucose solution. Similar results of O_2 uptake by E. coli for sodium succinate and sodium pyruvate are shown in Figure 28 and 29.

B. The Standard Substrate Solution for BOD_5 and Polarograph Tests

The standard substrate solution (150 mg glucose and 150 mg glutamic acid/l) was used to compare the results of O_2 uptake by E. coli with BOD_5 results. Dilutions (¾, ½, ¼, 1/8, 1/16 and 1/32) were prepared from the 1/50 dilution of the standard substrate solution and oxygen uptake by E. coli was measured in the polarograph and the BOD_5 was measured for each dilution. The dilution water for BOD_5 tests was seeded with either river water (10 ml/l) or the standardized E. coli seed (0.05 ml/l).

As the substrate level increased the levels of O_2 uptake and BOD_5 values increased (Table 12). A linear relationship was found between the results of O_2 uptake plotted against BOD_5 results (Figure 30). The best

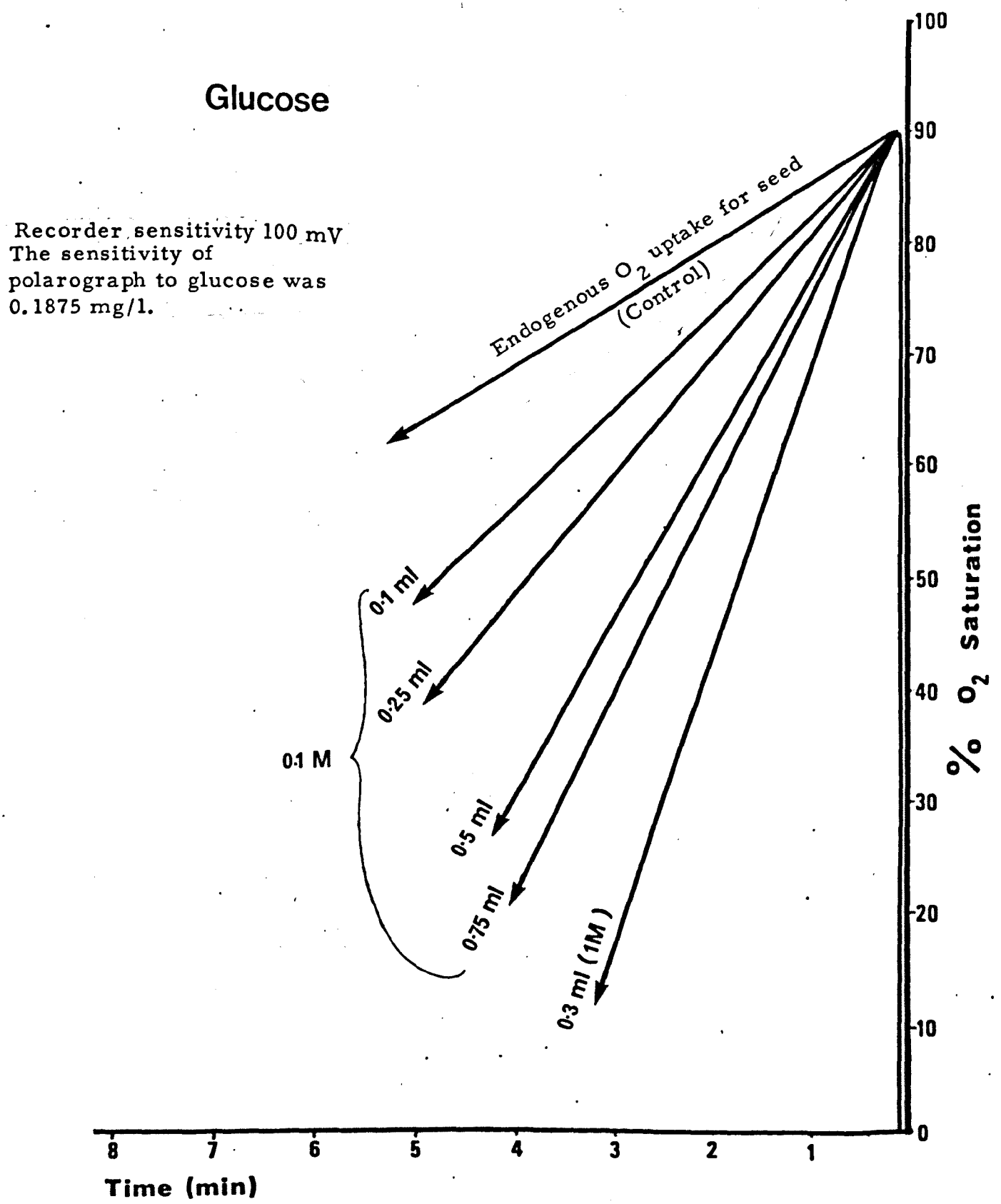


Figure 27. Oxygen uptake by E. coli with different quantities of glucose.

Note that the maximum uptake of oxygen in the polarograph test volume of 3.0 ml was obtained with 0.3 ml 1M glucose solution and 0.1 ml of E. coli standardized seed.

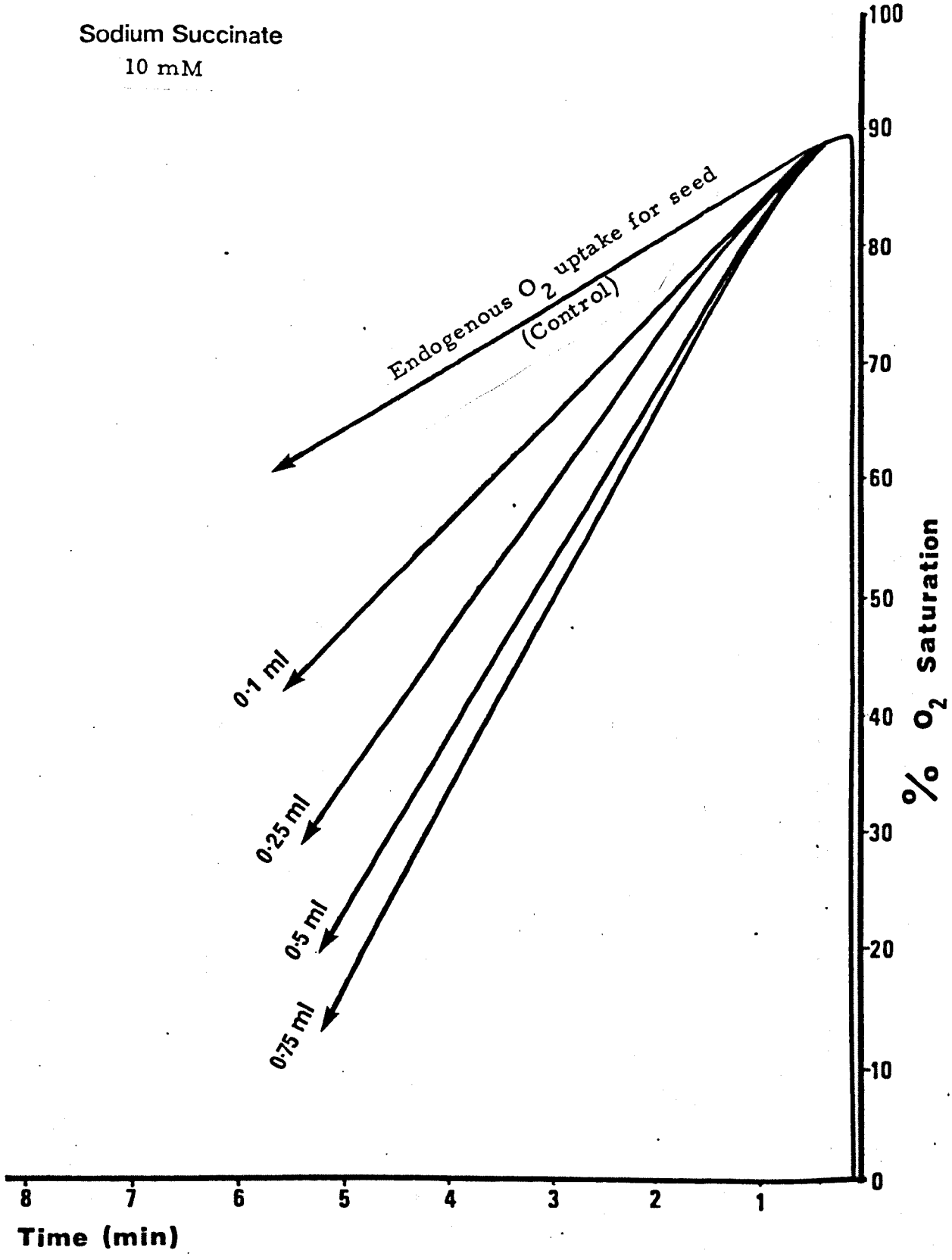


Figure 28. Oxygen uptake by E. coli with different quantites of 10 mM sodium succinate (0.1 - 0.75 ml).

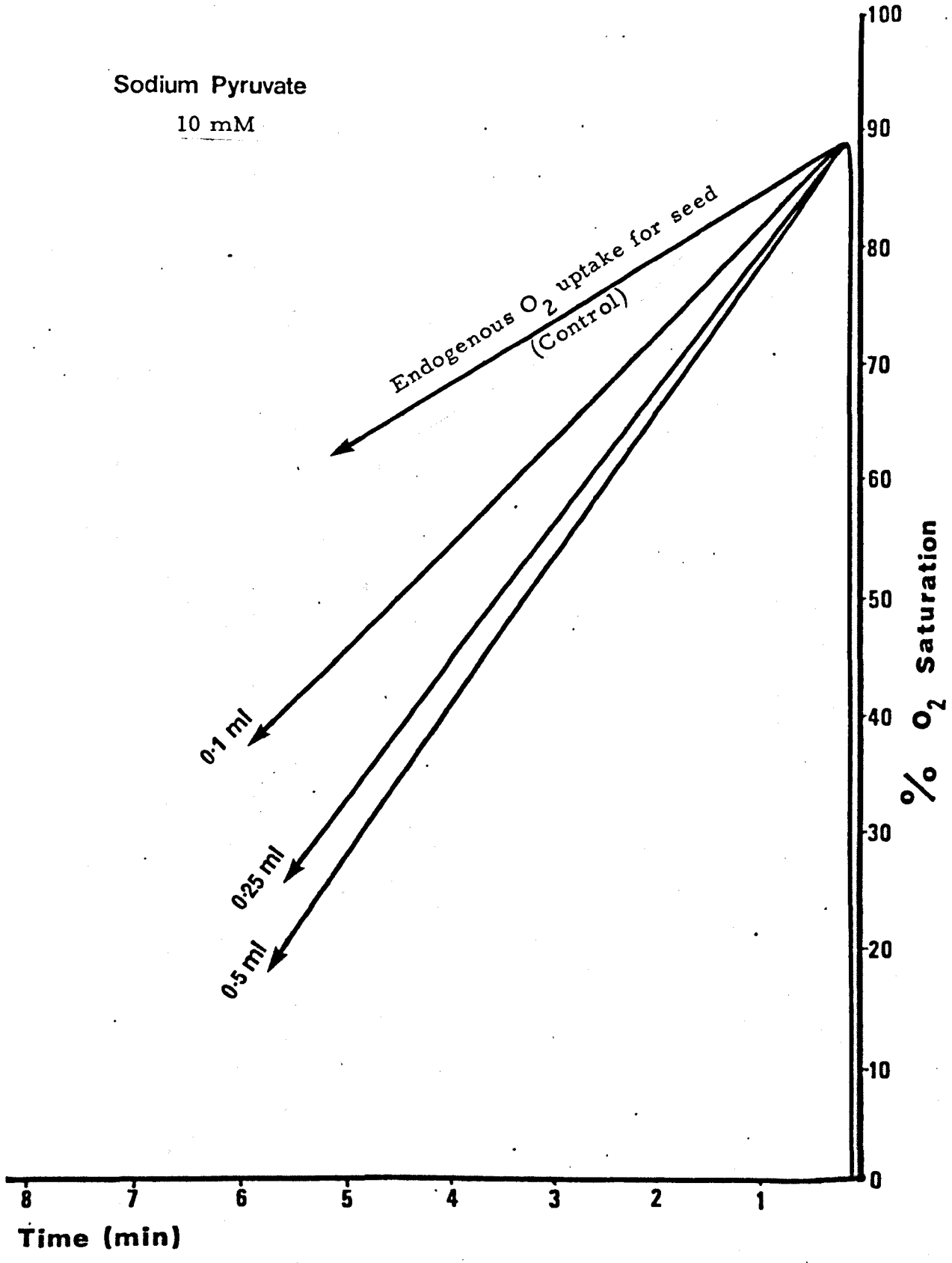


Figure 29. Oxygen uptake by E. coli with different quantities of 10 mM sodium pyruvate (0.1 - 0.5 ml).

Substrate conc. mg/ml	BOD_5 (ppm.)	Polarograph tests						
		Test 1		Test 2		Test 3		Mean
		O_2 uptake (µl/min)	*Q02	O_2 uptake (µl/min)	*Q02	O_2 uptake (µl/min)	*Q02	*Q02
6.0	208.5	0.620	8.52	0.625	8.58	0.6268	8.61	8.57
4.5	116	0.357	4.97	0.36	4.90	0.363	4.98	4.95
3.0	67.5	0.166	2.28	0.170	2.34	0.170	2.34	2.32
1.5	27.5	0.087	1.195	0.0876	1.203	0.088	1.211	1.203
0.75	12.5	0.0565	0.78	0.0576	0.79	0.0587	0.81	0.79
0.375	5.67	0.0426	0.585	0.0426	0.585	0.0426	0.585	0.585
0.1875	3.13	0.030	0.412	0.030	0.412	0.030	0.412	0.412

Table 12. Oxygen uptake by E. coli measured in the polarograph and by the standard BOD_5 method for varying concentrations of the standard substrate (150 mg glucose + 150 mg glutamic acid/litre).
*QO_2: amount of oxygen (µl) consumed/mg dry weight of E. coli/hr.

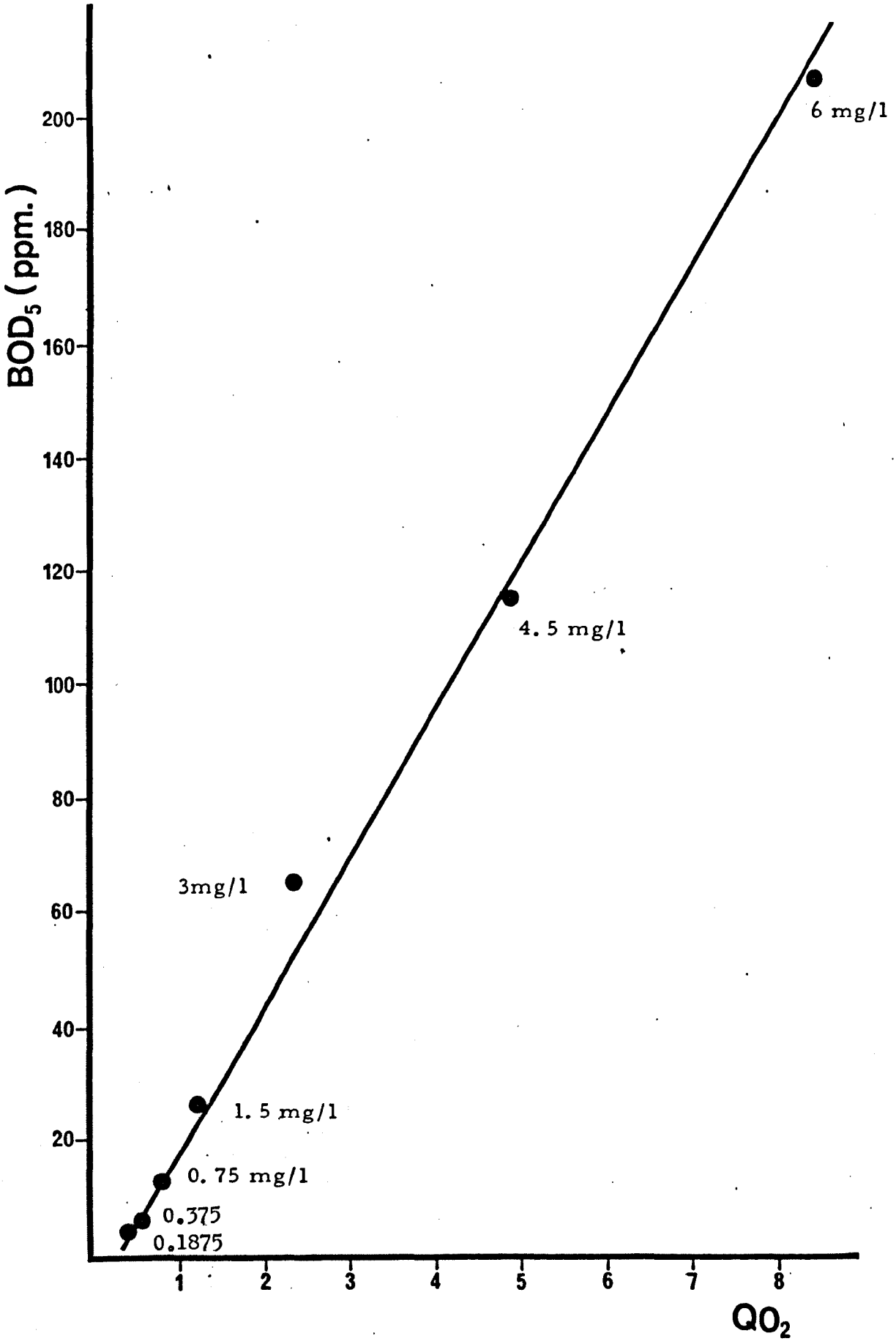


Figure 30. Comparison of BOD_5 and QO_2 for the standard substrate solution. (150mg glucose+150mg glutamic acid/l.)

Varying amounts of the standard substrate were tested in the BOD_5 test and in the polarograph test with E. coli.

line of fit through the points was determined statistically by the linear regression method with the SR-51-II Texas calculator. Statistical analyses of these results showed a highly significant relationship between O_2 uptake and BOD_5 (see Table 14, p. 179). The correlation coefficient obtained was + 0.9965 with method B and + 1.0 with method A (Kendall's coefficient of rank correlation) (see appendix 5 for the statistical analysis methods).

FACTORS AFFECTING O_2 UPTAKE MEASUREMENT WITH THE POLAROGRAPH

A. Seed Concentration

To determine whether O_2 uptake was affected by the variation in the concentration of organisms in the seed, varying concentrations of *E. coli* were used in the seed ($c 375 \times 10^9$, $c 280 \times 10^9$, $c 180 \times 10^9$, $c 80 \times 10^9$ and $c 40 \times 10^9$/ml). In each polarograph test 0.3 ml of 1M glucose solution was used as the substrate with 0.1 ml seed.

The uptake of oxygen was maximal with a concentration of $c 3.7 \times 10^{11}$ *E. coli* per ml (Figure 31). Oxygen uptake increased linearly with the corresponding increase in the seed concentration (Figure 32).

B. Temperature

Different temperatures (10, 20, 30 and 37°C) were used to show the effect of temperature on the uptake of oxygen by *E. coli* in polarograph experiments.

Maximum activity was obtained at a working temperature of 37°C; oxygen uptake increased with an increase in temperature (Figure 33 and 34). The temperature of 37°C was selected for all the polarographic tests throughout this study, even though it was substantially higher than the average river-water temperature.

C. The pH of the Water Sample

It was realized that the pH of different effluent samples might vary so the effect of pH on oxygen uptake was studied by preparing

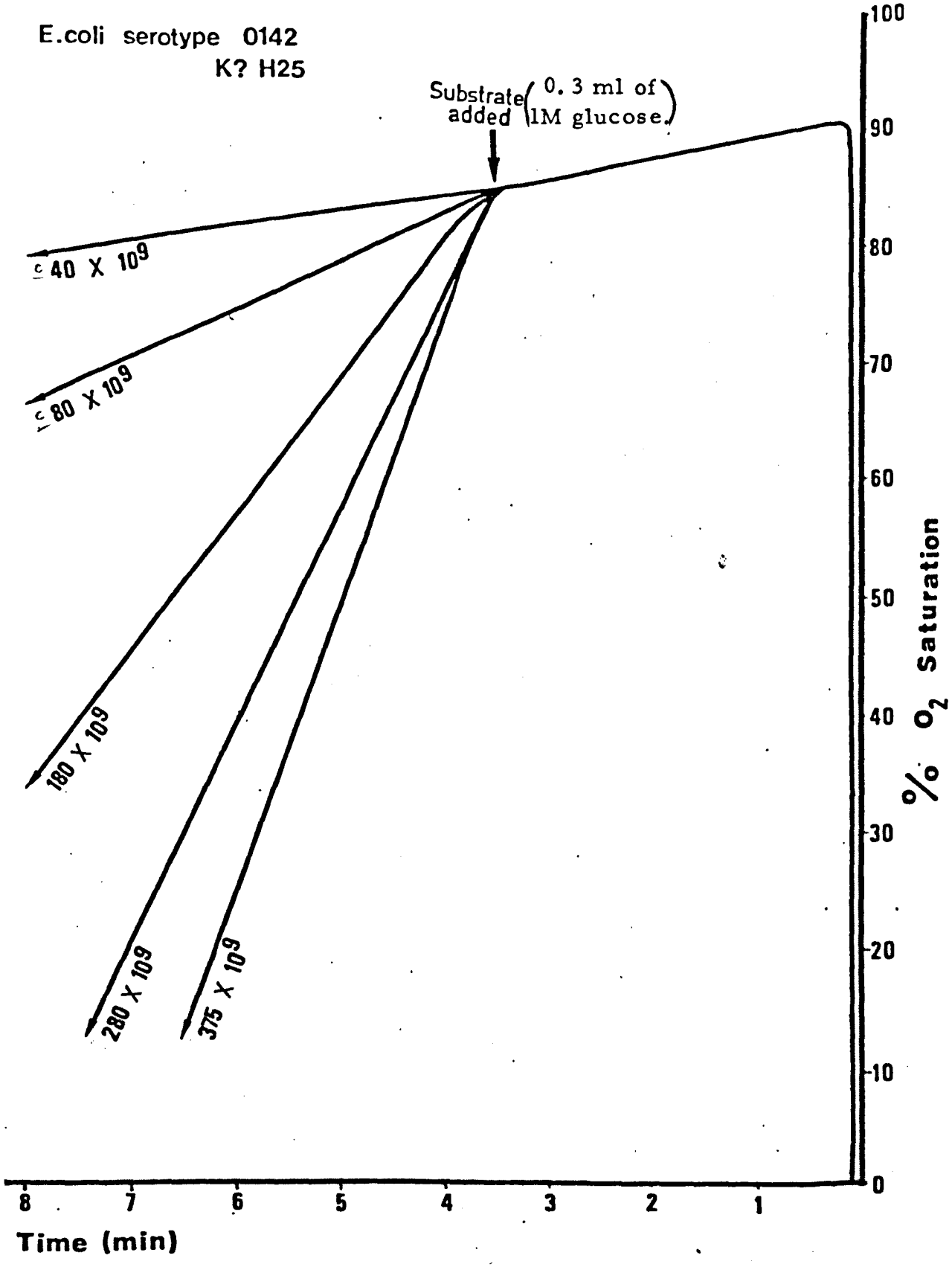


Figure 31. Effect of the seed concentration on the 0_2 uptake measurement in the polarograph test with glucose as substrate.

Note the maximum uptake of oxygen was obtained with c 375×10^9 E. coli cells.

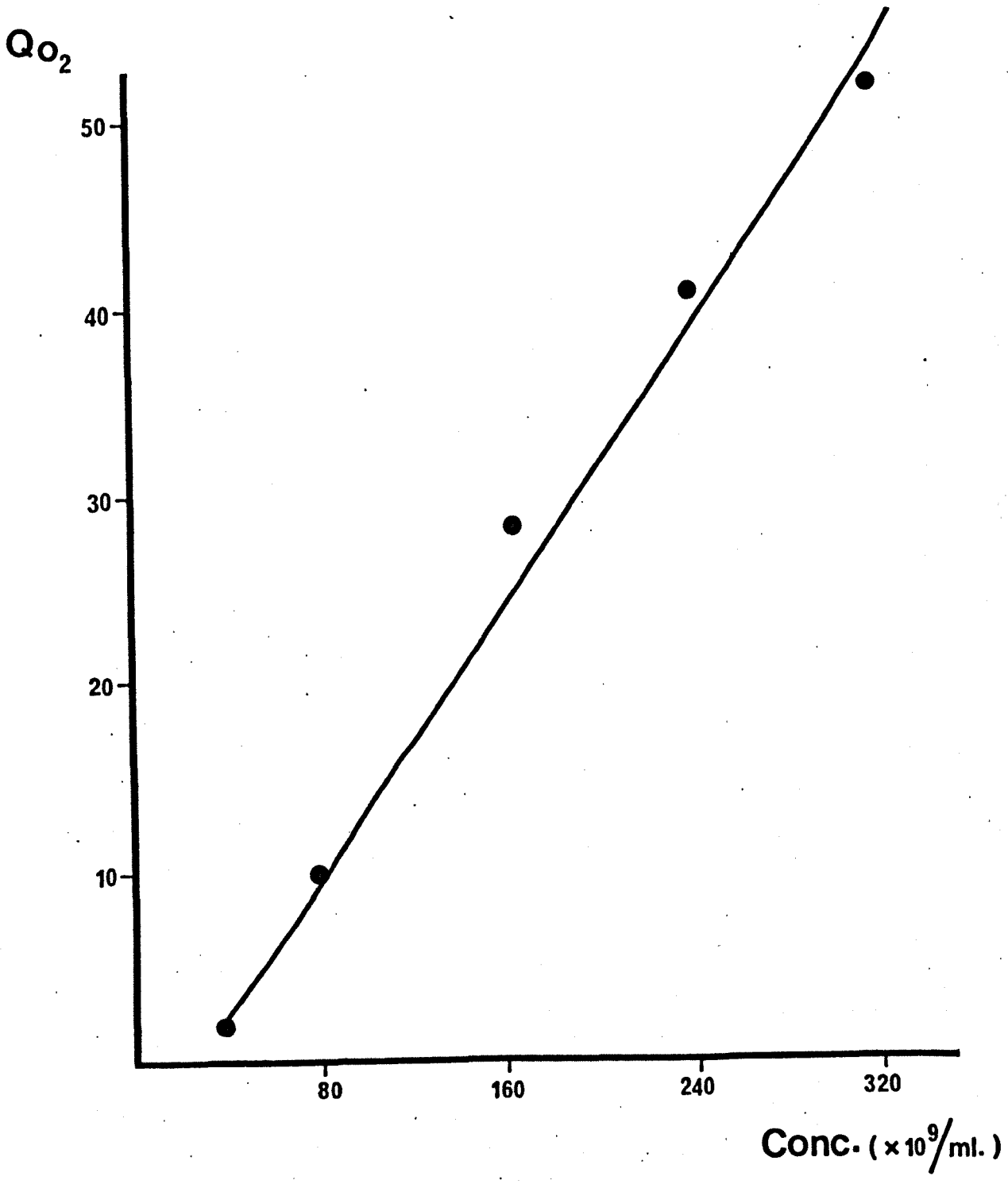


Figure 32. Relationship between seed concentration and O_2 uptake by E. coli in the polarograph test.

The tests were done at 37°C in the presence of 0.3 ml of 1M glucose solution and varying concentrations of E. coli in the seed.

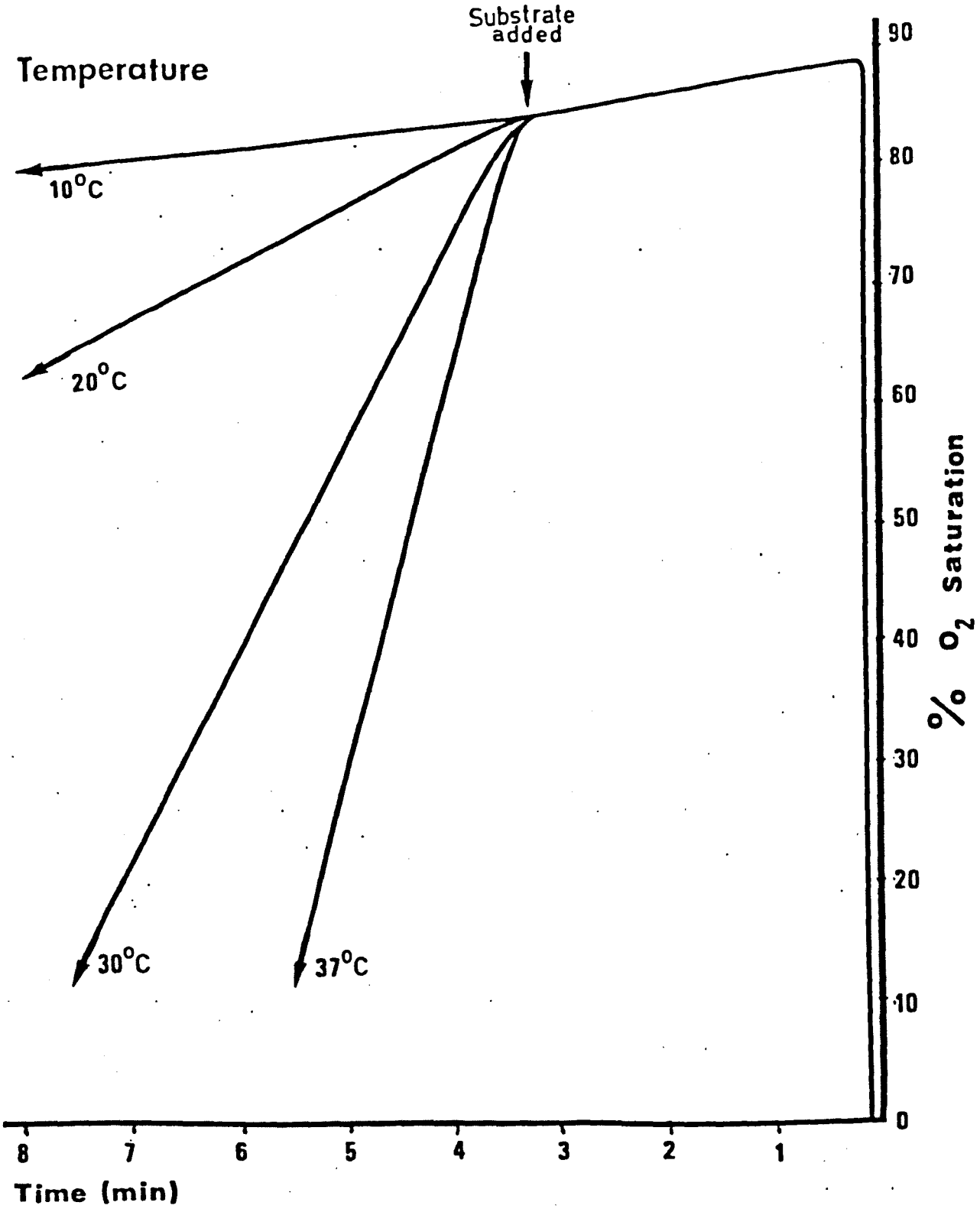


Figure 33. Effect of temperature on the O_2 uptake measurement in the polarograph test with E. coli as seed and 0, 3 ml of 1M glucose as substrate.

Note the maximum effect was obtained at 37°C. Although the average temperature of river - water was 5 - 12°C, the laboratory tests were all done at the optimum for O_2 uptake, i.e. 37°C.

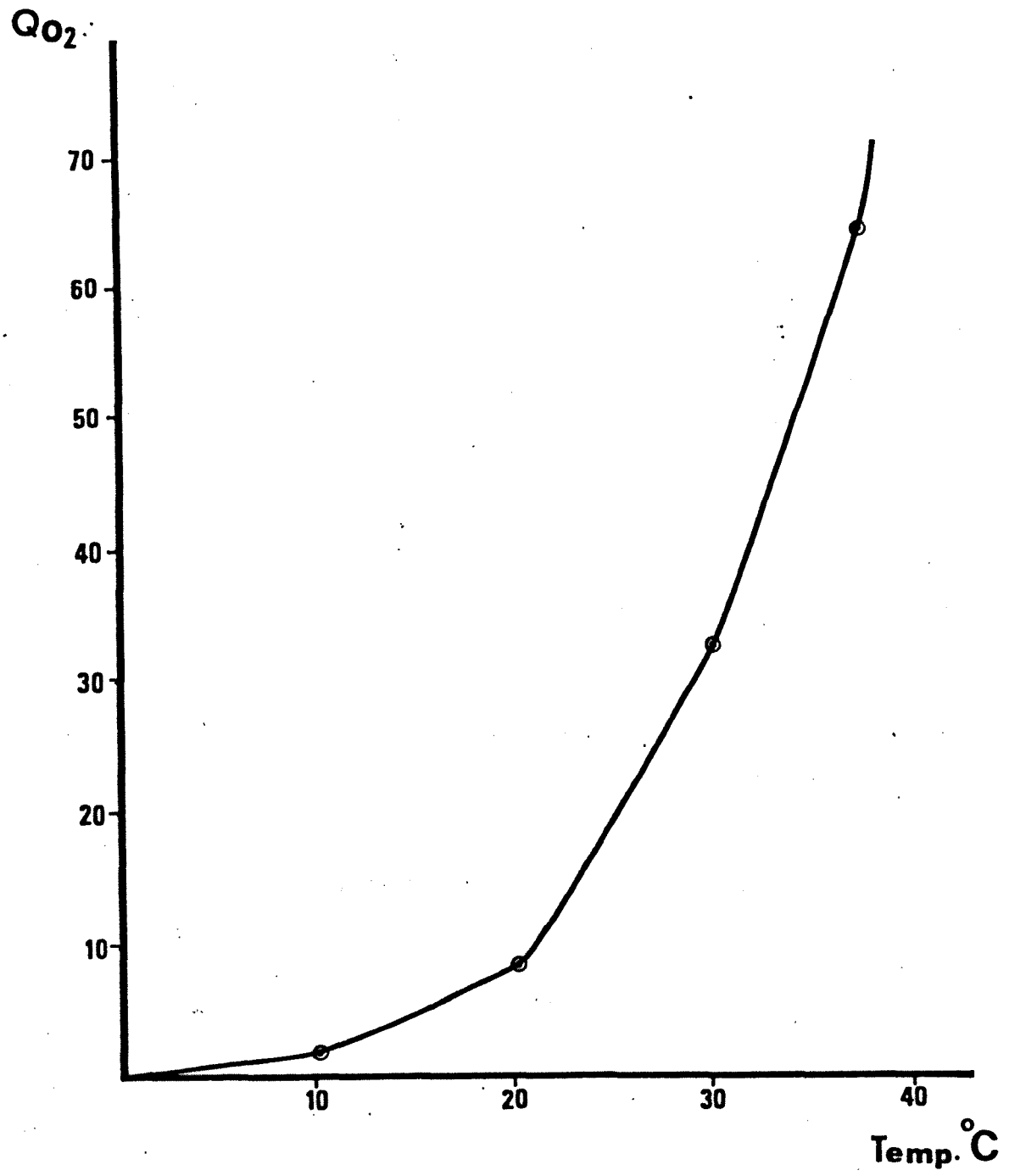


Figure 34. The relationship between temperature and 0_2 uptake in the polarograph for glucose by E.coli seed.

The uptake of oxygen by E. coli in the presence of glucose (0. 3 ml of 1M) increased as the temperature was raised. For routine testing 37°C was subsequently used.

solutions of 1.0M glucose of different pH (5.1 to 9.5) and the uptake of oxygen by E. coli was measured for each solution (0.3 ml/test).

Similar levels of O_2 uptake were found at pH values of 6.5, 7.0, 7.2 and 8.5 (Figure 35) and lower O_2 uptake levels were found for the pH values 5.1 and 9.5. The curve of O_2 uptake versus pH (Figure 36) shows the independence of O_2 uptake over the pH range of 6.5 - 8.5.

EXPERIMENTAL STUDIES WITH THE POLAROGRAPH

A. The Use of Concentrated River-Water

In an attempt to concentrate the number of organisms in a river-water sample two methods were used, a) by membrane filtration and b) heat evaporation at 80-90°C. Water samples from the River Kelvin were concentrated ten-fold and the BOD_5, COD and O_2 uptake were determined before and after concentration. In the polarograph the water samples were tested with and without seed and substrate, and with both seed and substrate together.

The results showed that both BOD_5 and COD were higher for the concentrated than for the unconcentrated samples (Table 13). The BOD_5 and COD results were higher for the samples concentrated by heat than by membrane filtration. Similar results were obtained for O_2 uptake, which indicated that heat might have some effect on the sample. After concentration of river-water both the number of organisms and substrate particles were increased.

B. The Effect of Autoclaving River-Water and Sewage Effluent

As the above experiment indicated that concentration by heating river-water affected the O_2 uptake by E. coli, experiments were done to show whether the heating process affected substrates in the river-water and sewage effluent samples. River-water samples were heated for 5, 10, 15 and 20 minutes in a pressure cooker at 121°C. Care was taken to prevent concentration of the samples by adjusting the volumes with sterile

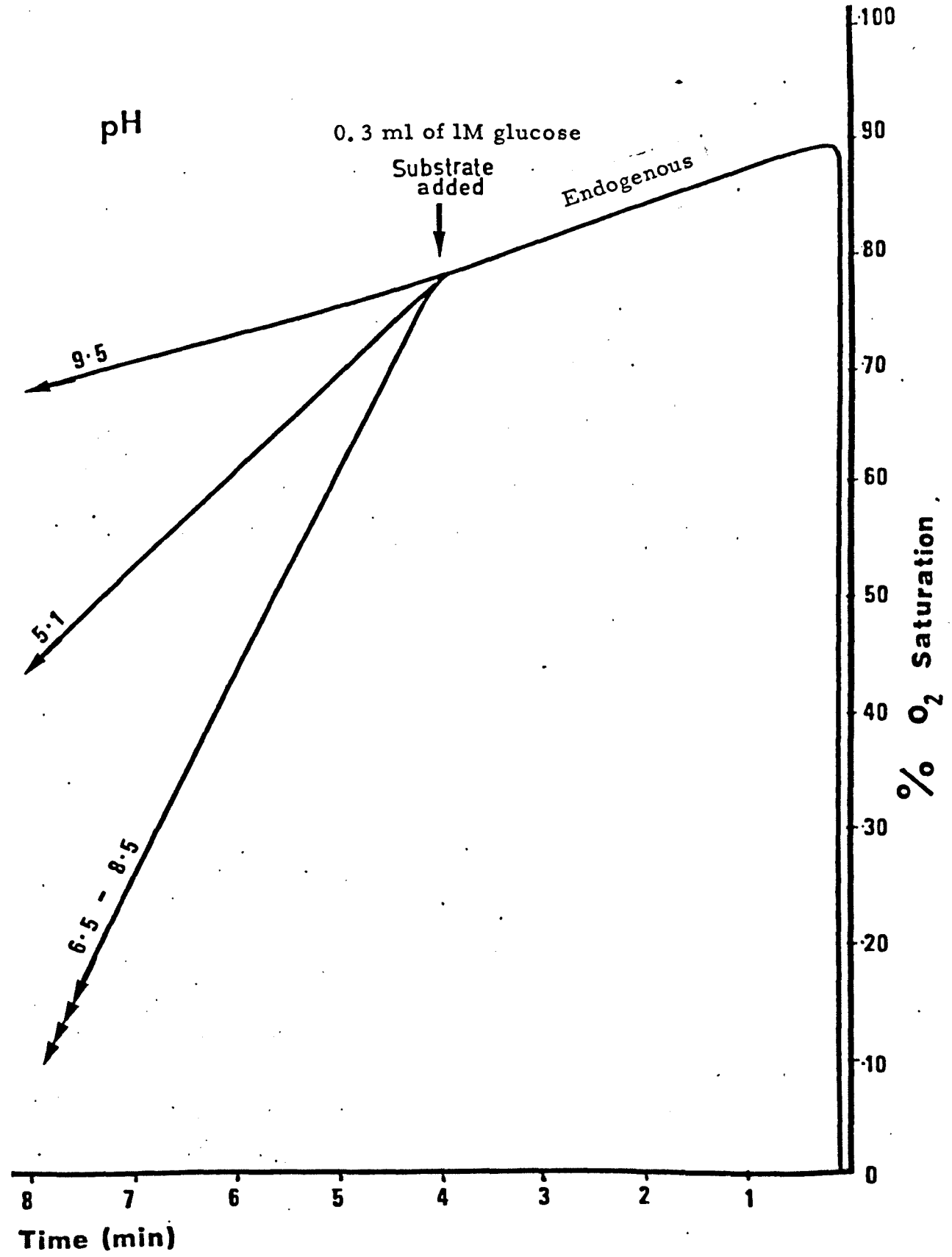


Figure 35. Effect of pH on the O_2 uptake measurement in the polarograph.

These polarograph traces indicate that O_2 uptake by E. coli in the presence of glucose is affected by acidic and alkaline pH.

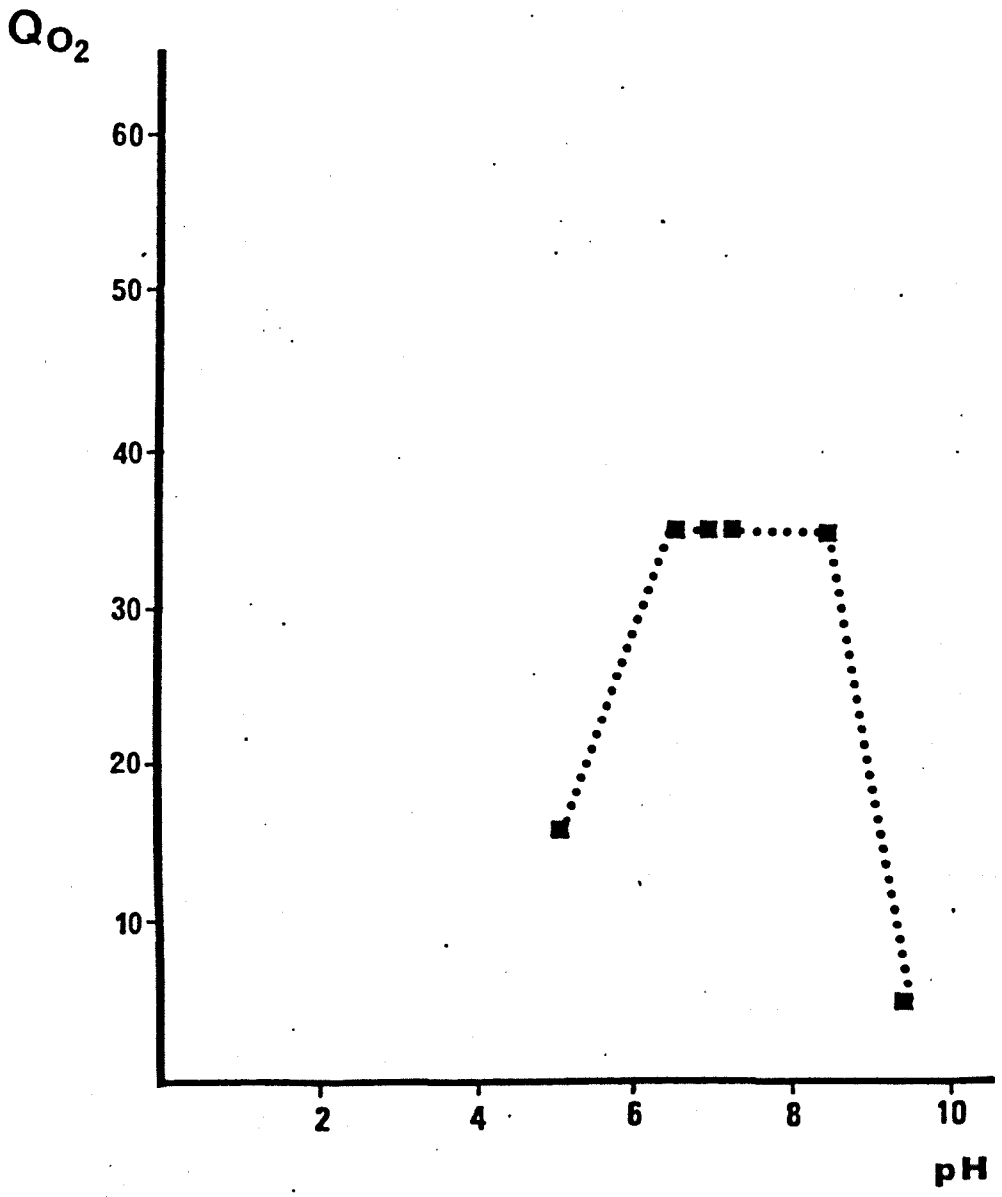


Figure 36. The relationship between pH and O_2 uptake

The uptake of oxygen by E. coli was measured in the polarograph in 0.01 M buffer solution at different pH values.

The substrate was 1M glucose. The polarograph test is unaffected by pH over the range 6.2 - 8.6.

Note that higher values of BOD_5, COD and O_2 uptake in the polarograph were obtained for river-water samples concentrated 10-fold by both membrane filteration and heat evaporation. The results were higher for the samples concentrated by heat than by MF, which indicates that heat might have some effect on the samples.

a. Results of BOD_5 and COD

Sample	BOD_5 (ppm)	COD (ppm)
River Water (RW) alone	6.0	13.6
10-fold conc. RW (by Sartorius MF)	140	15
10-fold conc. RW (by heat evaporation)	232	76.4

b. Results of oxygen uptake in the polarograph

Sample	O_2 uptake (µl/min)	QO_2
RW alone	0.0075	0.103
" + seed	0.195	2.68
" + substrate	0.010	0.144
" + seed + substrate	1.25	17.17
10-fold concentrated RW:		
A - by Sartorius MF		
1 - alone	0.0131	0.180
2 - with seed	0.285	3.9
3 - with substrate	0.025	0.343
4 - " " + seed	1.55	21.29
B - by heat evaporation (80-90^{0}C)		
1 - alone	0.0212	0.291
2 - with seed	1.2	16.48
3 - with substrate	0.025	0.343
4 - " " + seed	3.23	44.37

Table 13. Results of concentrated river-water

distilled water. In the polarograph higher O_2 uptake was obtained for the heated samples. This increase in oxygen uptake was related to the length of time of heating up to 15 min (Figure 37).

As maximum O_2 uptake was obtained for samples heated at 121°C for 15 min, river-water samples were collected from three different sites:-

(1) a clean river-water (BOD_5=2.1) from station 8

(2) a polluted river-water (BOD_5=4.8) from station 17

(3) sewage effluent (BOD_5=28.4) from the Dock Water.

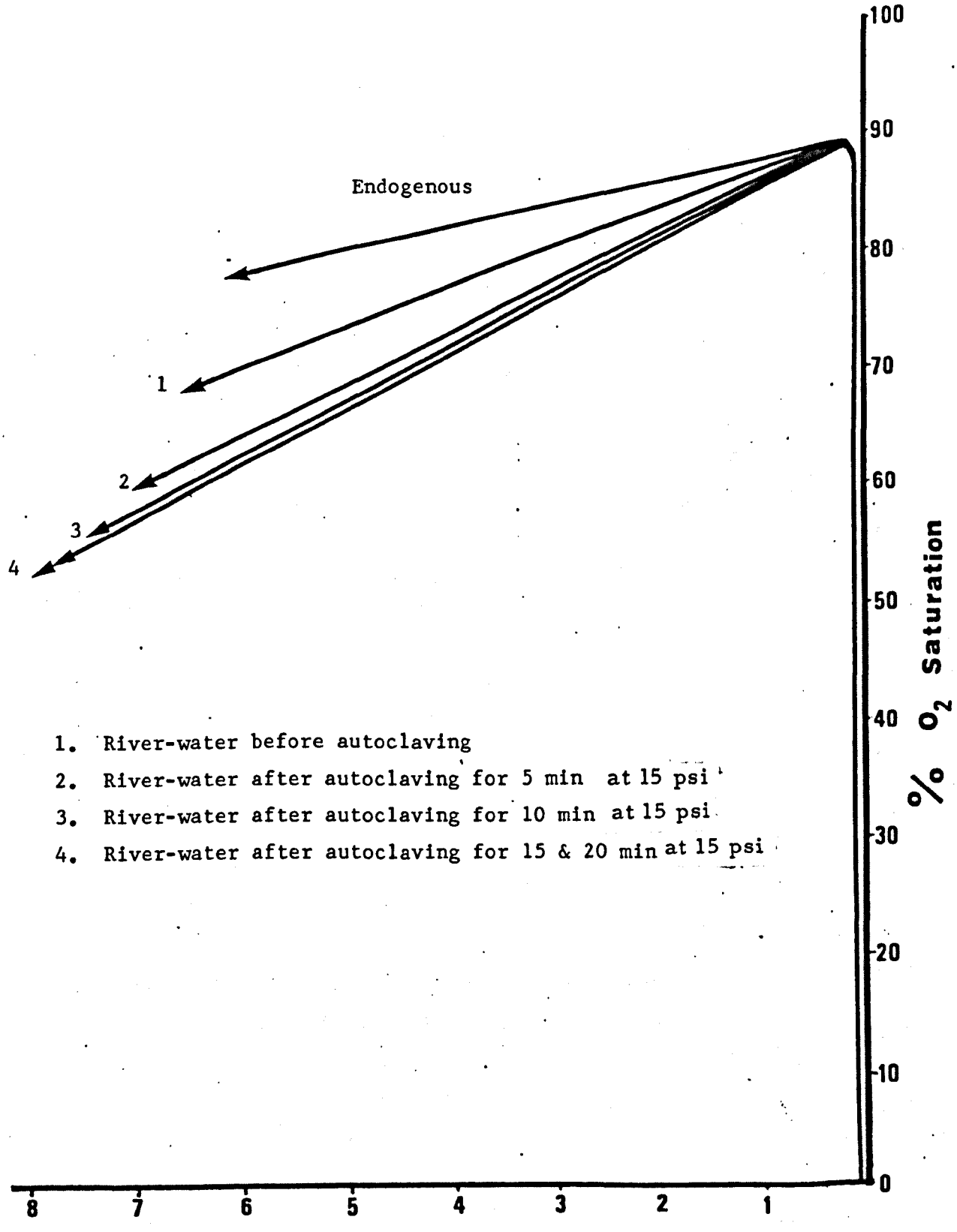
The BOD_5 was measured for the three samples without the addition of seed. Oxygen uptake was measured for the samples before and after heating at 121°C for 15 min. The uptake of oxygen for sample 1 was similar before and after heating, but higher levels were obtained for sample 2 and 3 after autoclaving (Figure 38).

C. The Effect of Autoclaving Cellulose and Starch Suspensions

To show the effect of heat on complex carbohydrates, both cellulose and starch suspensions were autoclaved at 121°C for 15 min. The O_2 uptake by E. coli was measured for the two polysaccharides before and after autoclaving. There was no O_2 uptake by E. coli for both cellulose and starch before autoclaving (Figure 39) but higher than zero O_2 uptake levels were obtained after autoclaving.

EFFECT OF HEAVY METALS AND OTHER TOXIC MATERIALS ON O_2 UPTAKE BY THE SEED INOCULUM

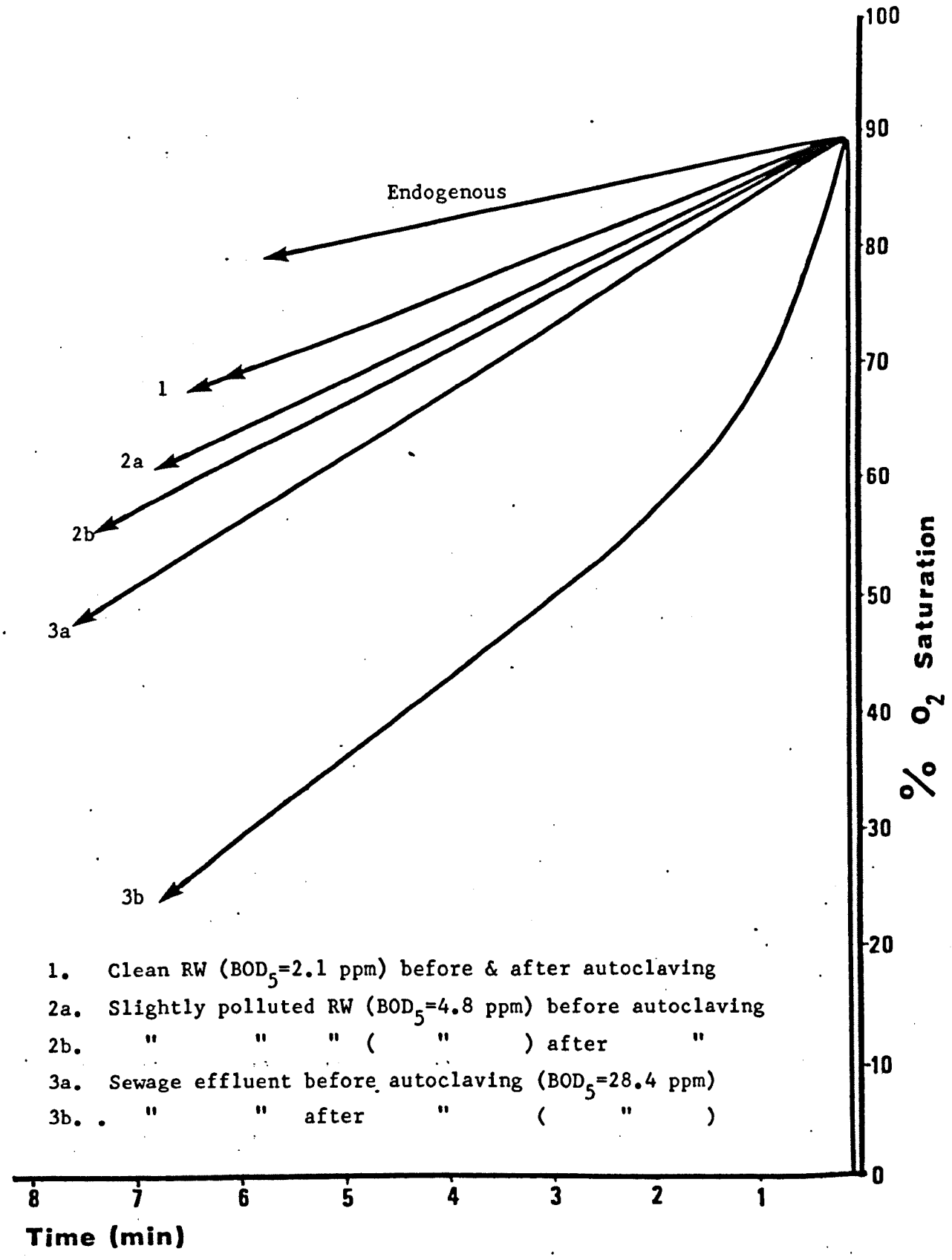
To investigate the toxic effect of heavy metals and other toxic materials on the O_2 uptake by the E. coli seed in the polarograph, 12 metals that commonly occur in sewage and cyanide were studied. A range of concentrations from 0-15 ppm for mercury and silver and from 0-40 ppm for the rest was used. Similar oxygen uptake measurements in the polarograph were measured in the presence of different concentrations of each metal in 1M



1. River-water before autoclaving
2. River-water after autoclaving for 5 min at 15 psi
3. River-water after autoclaving for 10 min at 15 psi
4. River-water after autoclaving for 15 & 20 min at 15 psi

Figure 37. Effect of autoclaving river-water at 121°C on the O_2 uptake measurement in the polarograph

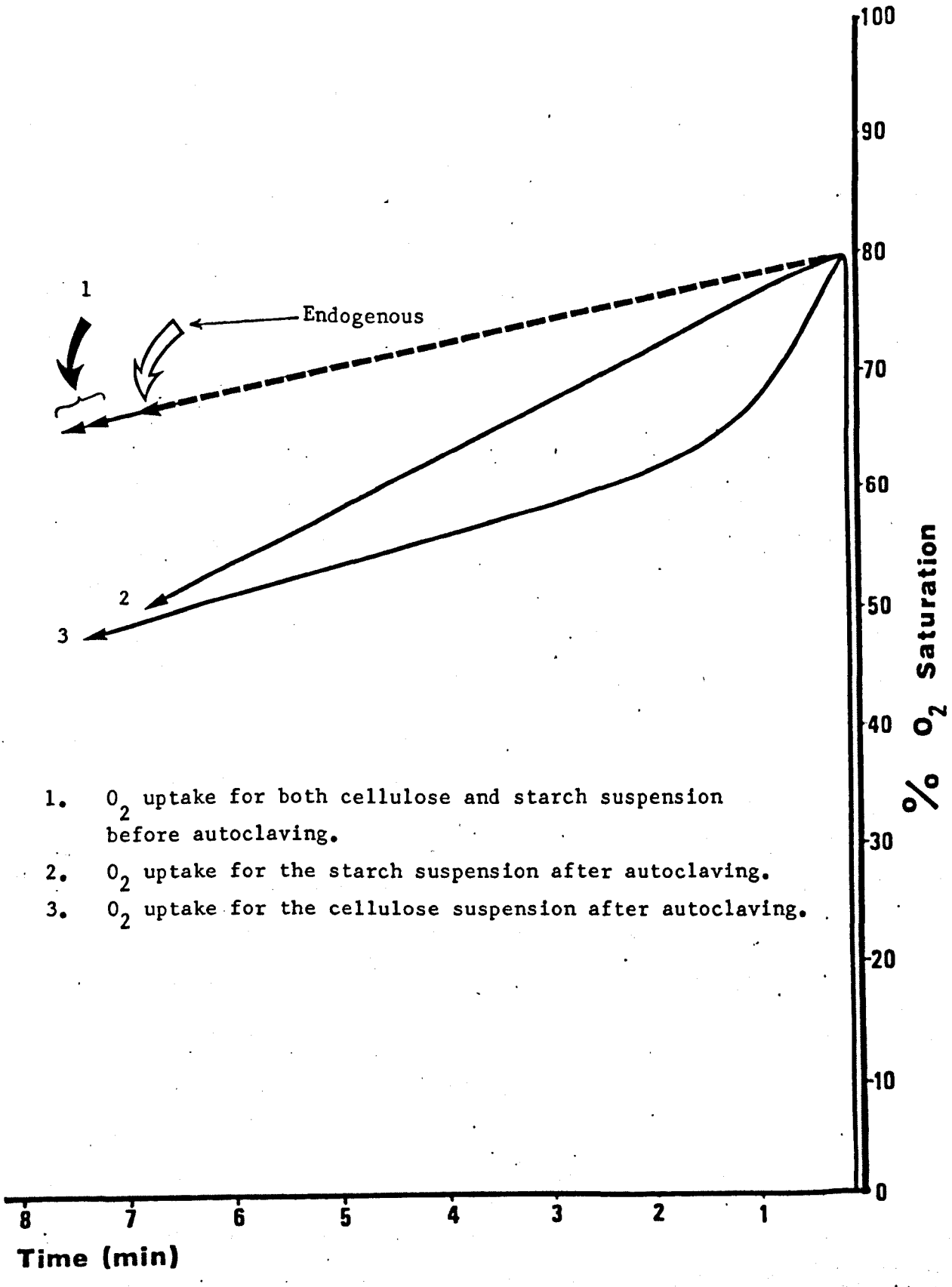
It would appear that autoclaving the water sample might provide an indication of the presence of complex carbohydrate concentration.



1. Clean RW (BOD_5=2.1 ppm) before & after autoclaving
2a. Slightly polluted RW (BOD_5=4.8 ppm) before autoclaving
2b. " " " (") after "
3a. Sewage effluent before autoclaving (BOD_5=28.4 ppm)
3b. " " after " (")

Figure 38. Effect of autoclaving river-water and sewage effluent on the O_2 uptake by E. coli in the polarograph.

The difference between a and b is an indication of the presence of complex organic matter in the water and sewage samples.



1. O_2 uptake for both cellulose and starch suspension before autoclaving.
2. O_2 uptake for the starch suspension after autoclaving.
3. O_2 uptake for the cellulose suspension after autoclaving.

Figure 39. Effect of autoclaving cellulose and starch suspension on the O_2 uptake by *E. coli* in the polarograph.

glucose solution, eg. Figure 40 shows the decrease in the uptake of oxygen by E. coli for increasing mercury concentrations.

The results obtained with the 12 metals and cyanide are shown in Figures 41-53). The toxicity of silver and mercury was found to be higher than the other metals and cyanide. In terms of ppm, the order of toxicity was as follows: $Ag^{+} > Hg^{++} > Fe^{+++} > Cu^{++} > CN^{-} > Al^{+++} > Zn^{++} > Cr^{+++} > \begin{matrix} Pb^{++} \\ Sn^{++} \end{matrix} > Ni^{++} > Cd^{++} > Co^{++} > Pb^{++}$ (insoluble).

Silver. The most toxic metal (in terms of ppm) was silver (Figure 41) at a concentration of 0-2.5 ppm no reduction in O_2 uptake was observed, at 5 ppm. O_2 uptake was reduced by approximately 70%, and at 15 ppm O_2 uptake by E. coli in the presence of glucose was zero.

Mercury. Mercury was the next most toxic metal (Figure 42). At a concentration of 0-2.5 ppm no reduction in O_2 uptake was observed, at a concentration of 5 ppm 50% reduction in O_2 uptake was observed and at 10 ppm O_2 uptake was almost completely inhibited.

Iron. Ferric iron (Figure 43) up to 20 ppm gave no reduction in O_2 uptake, a reduction of 42% at the 20 ppm concentration, and a reduction of 82% at the concentration of 40 ppm was found.

Copper. No significant reduction in O_2 uptake was found up to 20 ppm at which concentration it showed an 18% reduction; at the higher concentration of 40 ppm copper caused a 68% reduction in O_2 uptake by E. coli (Figure 44).

Cyanide. Although cyanide is not a metal it was included because of problems arising from the discharge of cyanide in effluent from the electroplating industry. As shown in Figure 45 no reduction in O_2 uptake was observed up to 20 ppm where a 24% reduction was found; at concentration of 40 ppm the O_2 uptake was reduced by nearly 50%.

Aluminium. No reduction in O_2 uptake was observed up to 20 ppm at which concentration a 20% reduction was found. This increased to about

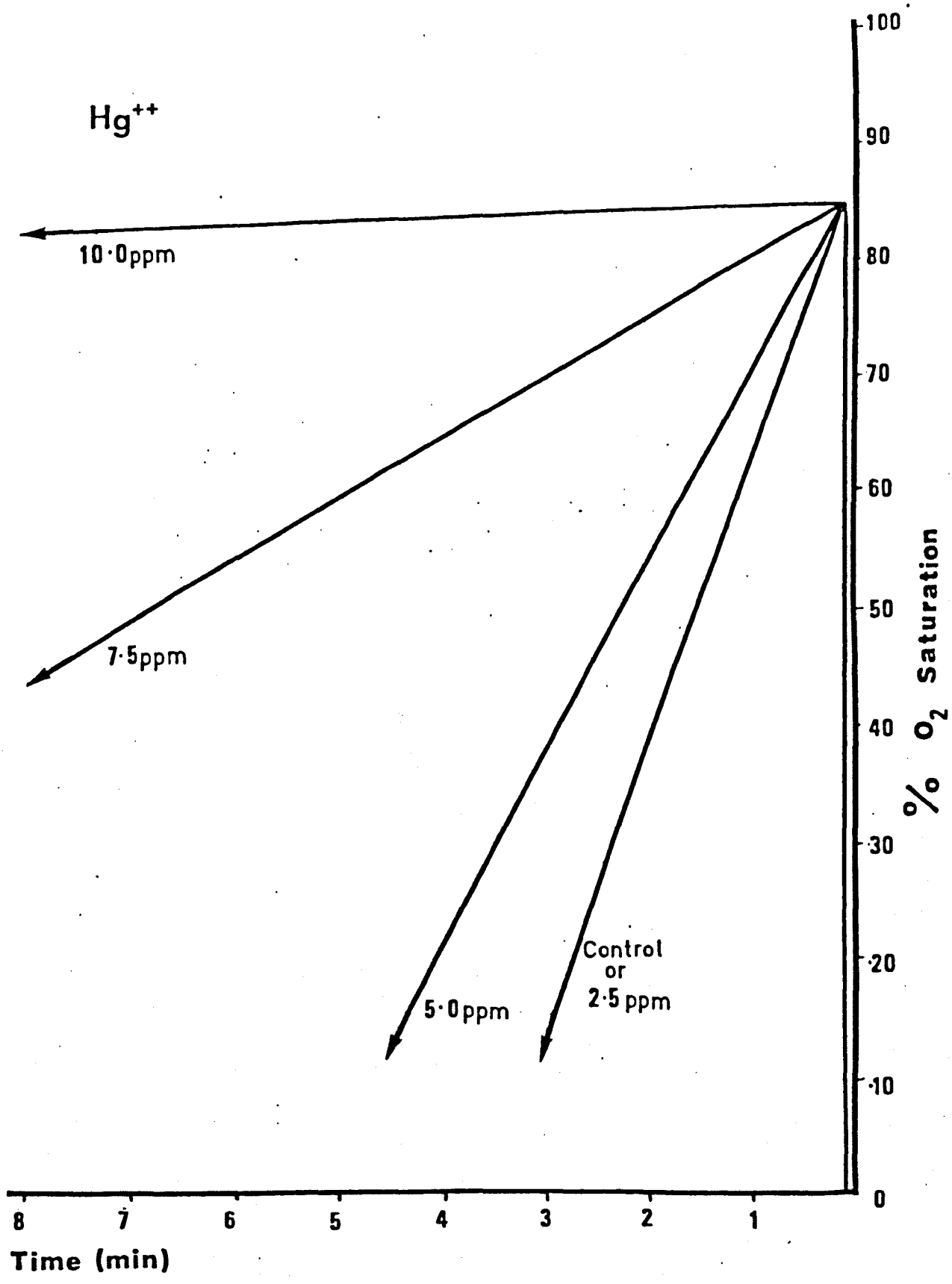


Figure 40. The inhibitory effect of mercury on the uptake of oxygen by the E. coli seed inoculum.

These polarograph recordings are included as an example of the inhibition of oxygen uptake by E. coli with a heavy metal salt. The substrate was 1M glucose.

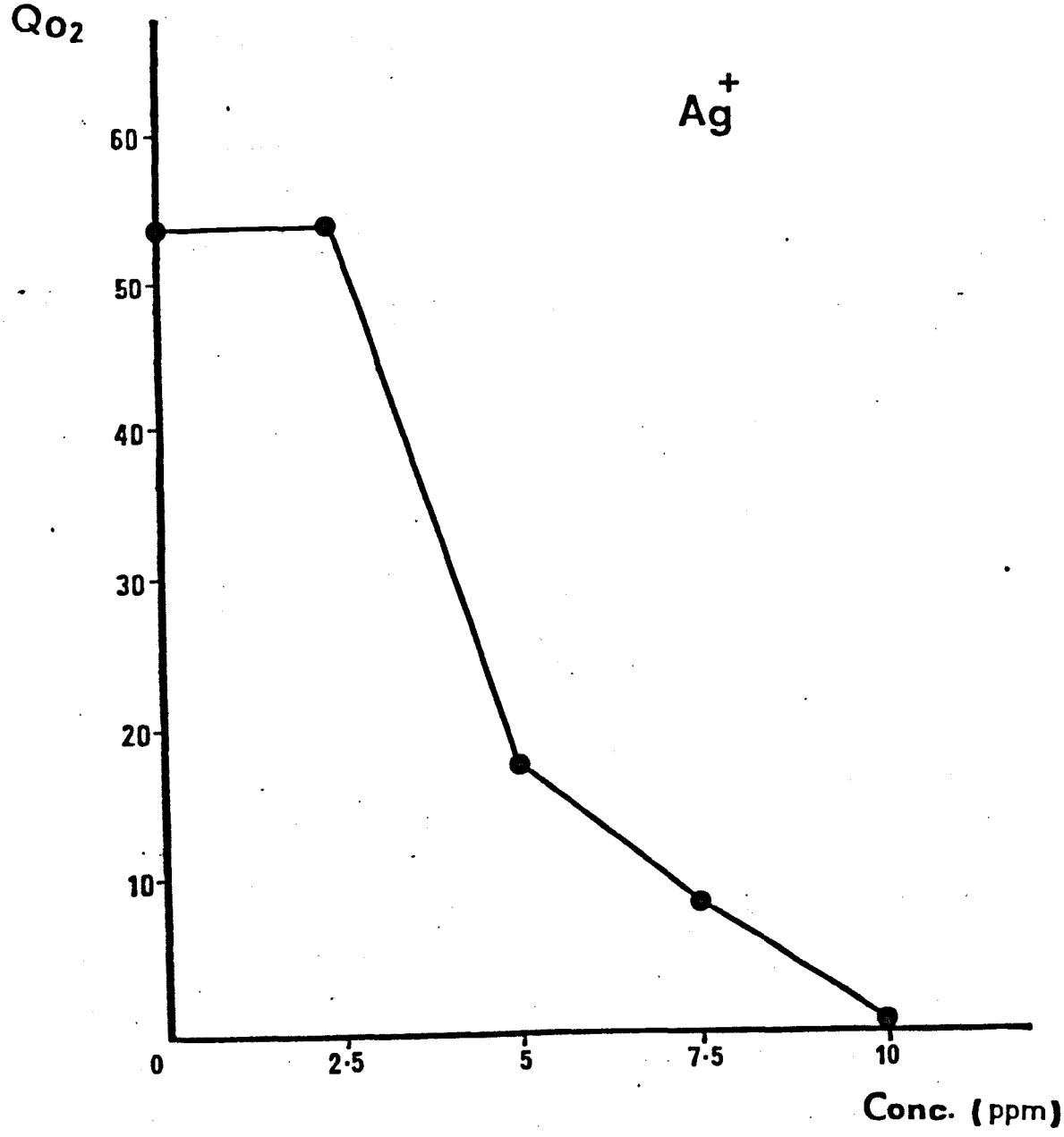


Figure 41. The effect of silver on the uptake of oxygen by E. coli.

Increasing concentrations of the silver salt in solution were used to examine the inhibitory effect on the oxygen uptake by E. coli seed in the presence of 0.3 ml of 1M glucose.

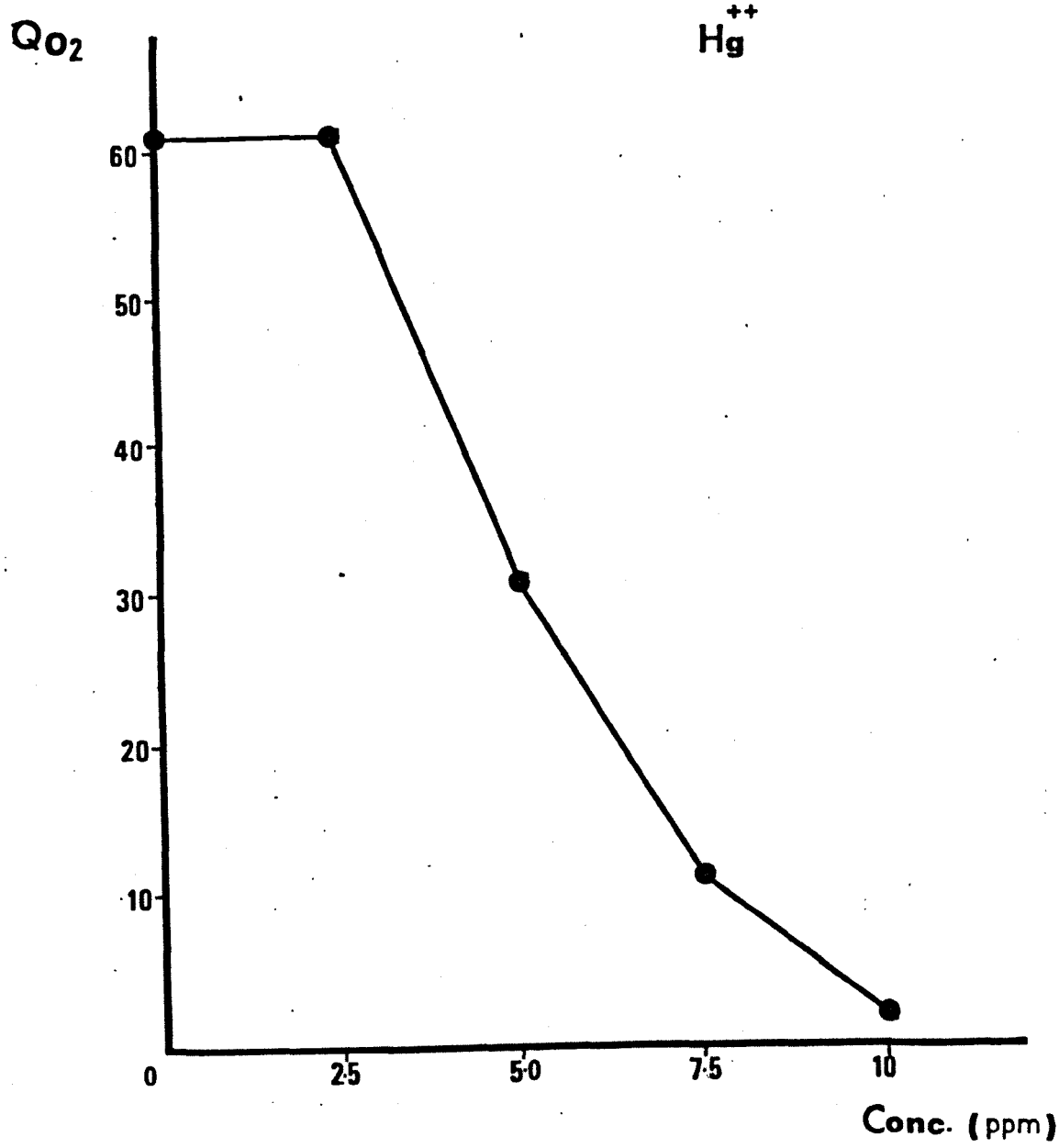


Figure 42. Typical results for mercury inhibition of E. coli utilization of glucose.

Increasing concentrations of the mercury ions in solution were used to examine the inhibitory effect on the O_2 uptake by E. coli seed in the presence of 0.3 ml of 1M glucose.

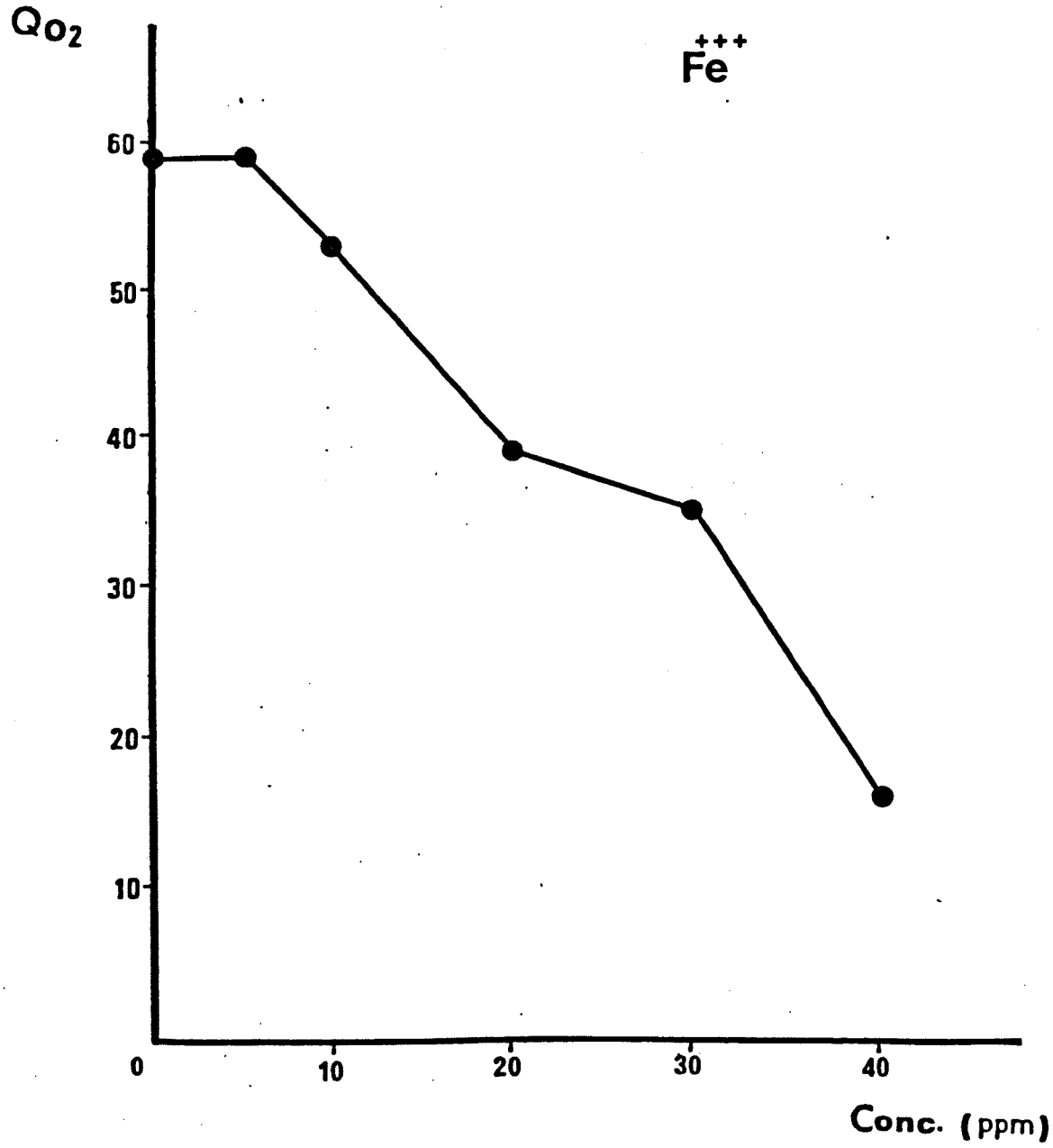


Table 43. Typical results for ferric iron inhibition of E. coli utilization of glucose.

Increasing concentrations of the ferric ions in solution were used to examine the inhibitory effect on the O_2 uptake by E. coli seed in the presence of 0.3 ml of 1M glucose.

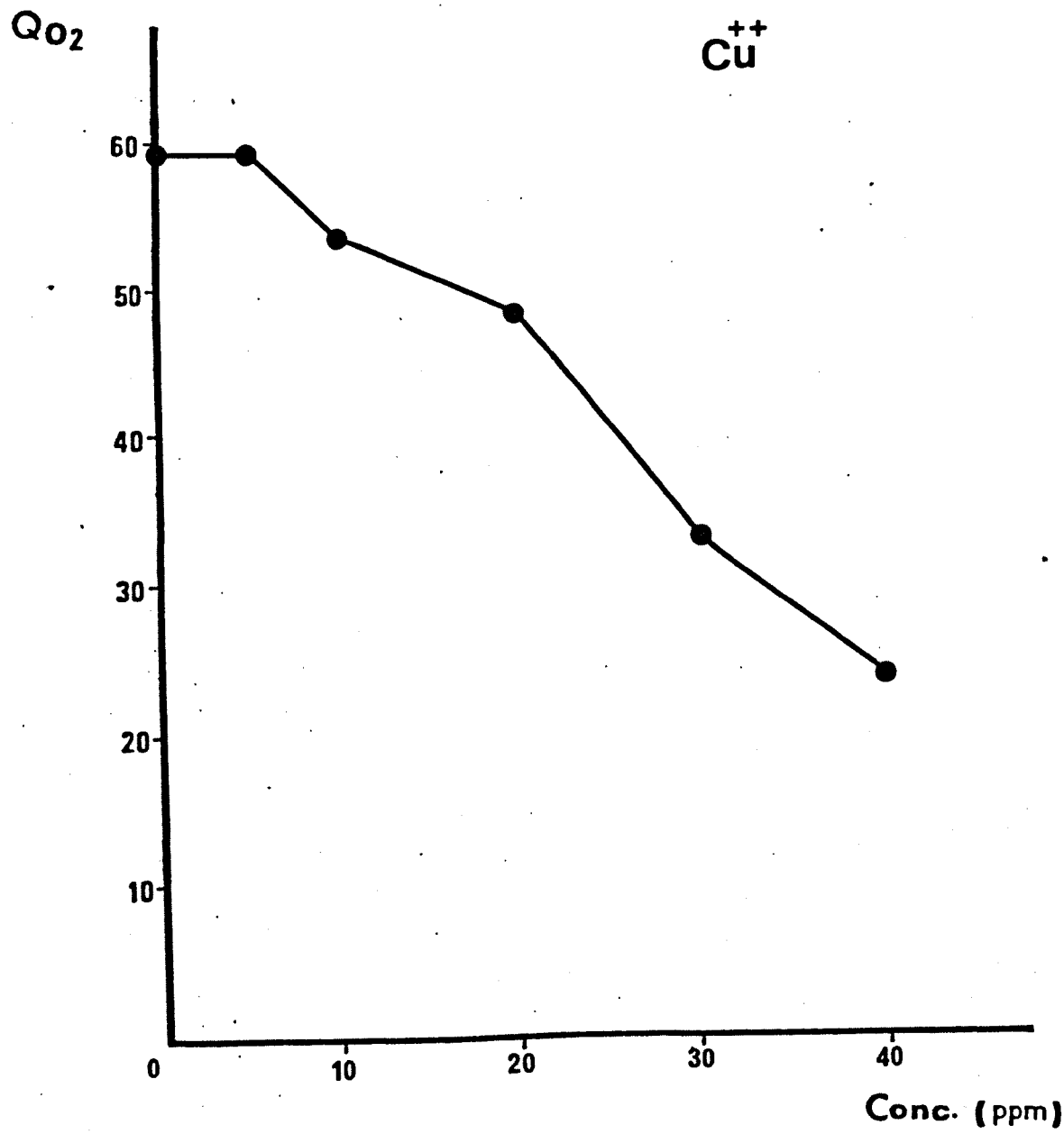


Figure 44. Typical results for copper inhibition of E. coli utilization of glucose.

Increasing concentrations of the copper ions in solution were used to examine the inhibitory effect on the O_2 uptake by E. coli seed in the presence of 0.3 ml of 1M glucose.

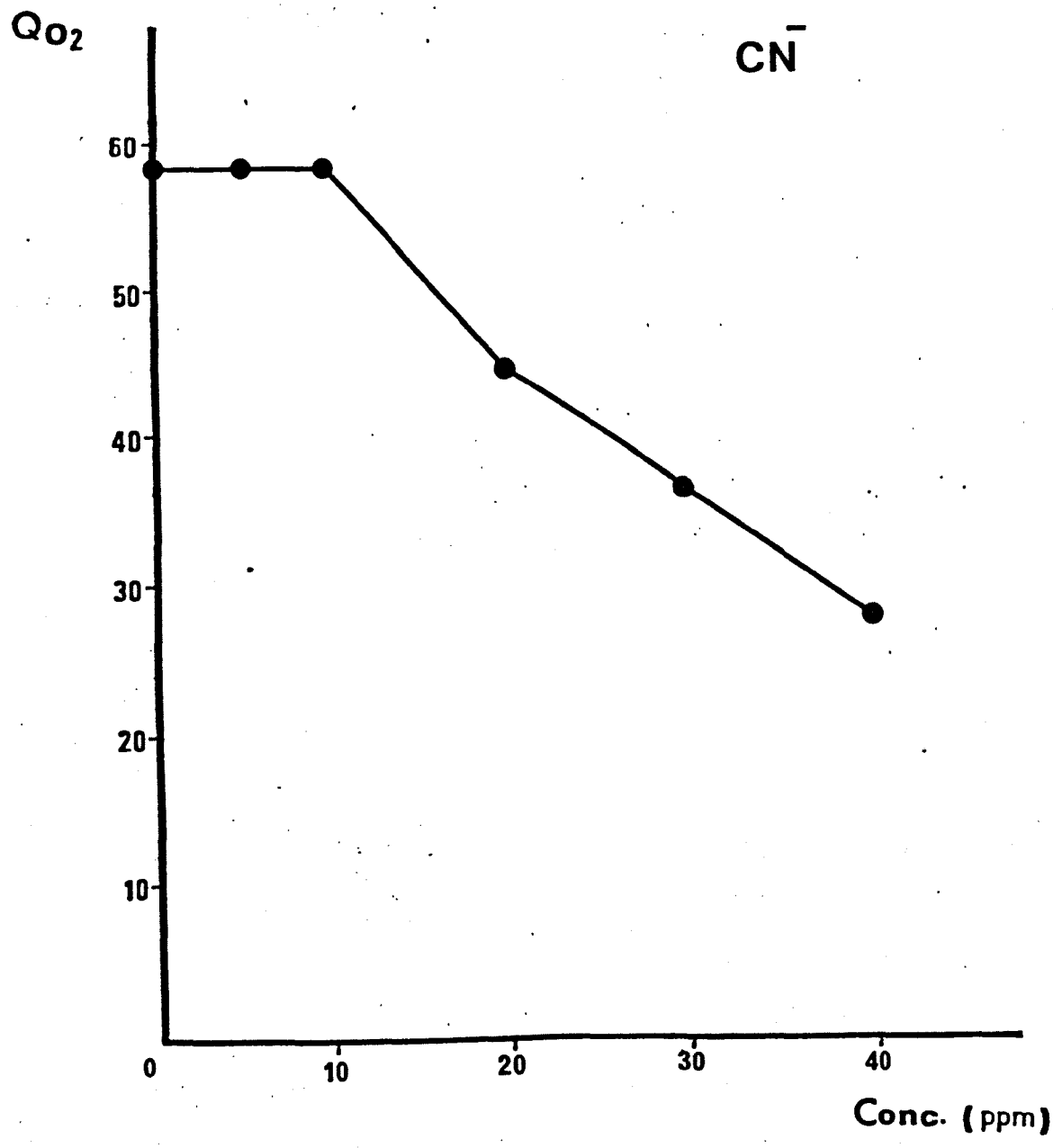


Figure 45. Typical results for cyanide inhibition of E. coli utilization of glucose.

Increasing concentrations of cyanide in solution were used to examine the inhibitory effect on the O_2 uptake by E. coli seed in the presence of 0.3 ml of 1M glucose.

40% at 40 ppm (Figure 46).

Zinc. Zinc (Figure 47) showed a slight effect at 10 ppm, a 24% reduction in O_2 uptake was found at 20 ppm and a 51% reduction in O_2 uptake was found at 40 ppm.

Chromium. Trivalent chromium (Figure 48) caused a 20% reduction in O_2 uptake at a concentration of 20 ppm and a 56% reduction at a concentration of 40 ppm.

Tin. The toxicity of tin was low up to a concentration of 20 ppm where it produced about 19% reduction in O_2 uptake; at 40 ppm the reduction in O_2 uptake was approximately 43% (Figure 49).

Nickel. Nickel (Figure 50) caused an 18% reduction in O_2 uptake at 10 ppm and the curve of O_2 uptake showed that the reduction of oxygen uptake increased slowly to a maximum of 35% at a concentration of 40 ppm.

Cadmium. Cadmium (Figure 51) produced a similar result to nickel and the maximum reduction of 31% in O_2 uptake was found at a concentration of 40 ppm.

Cobalt. Cobalt was even less toxic than cadmium (Figure 52). No considerable reduction in the O_2 uptake was found up to a concentration of 30 ppm and the maximum value of 29% was obtained at a concentration of 40 ppm.

Lead. Lead dioxide showed less toxicity than the other metals and cyanide in that no significant reduction in O_2 uptake was observed within the range of concentrations 0-40 ppm (Figure 53), whereas lead acetate produced an inhibition equivalent to chromium.

APPLICATION OF THE RAPID MICROBIOLOGICAL METHOD FOR RIVER-WATER SAMPLES

A. Oxygen Uptake Measurement for River-Water

To investigate the application of the rapid microbiological method for the measurement of oxygen uptake in river-water, samples were collected monthly from 16 stations on the River Kelvin over a period of two years. These were tested in the polarograph with *E. coli* seed and the results were compared with

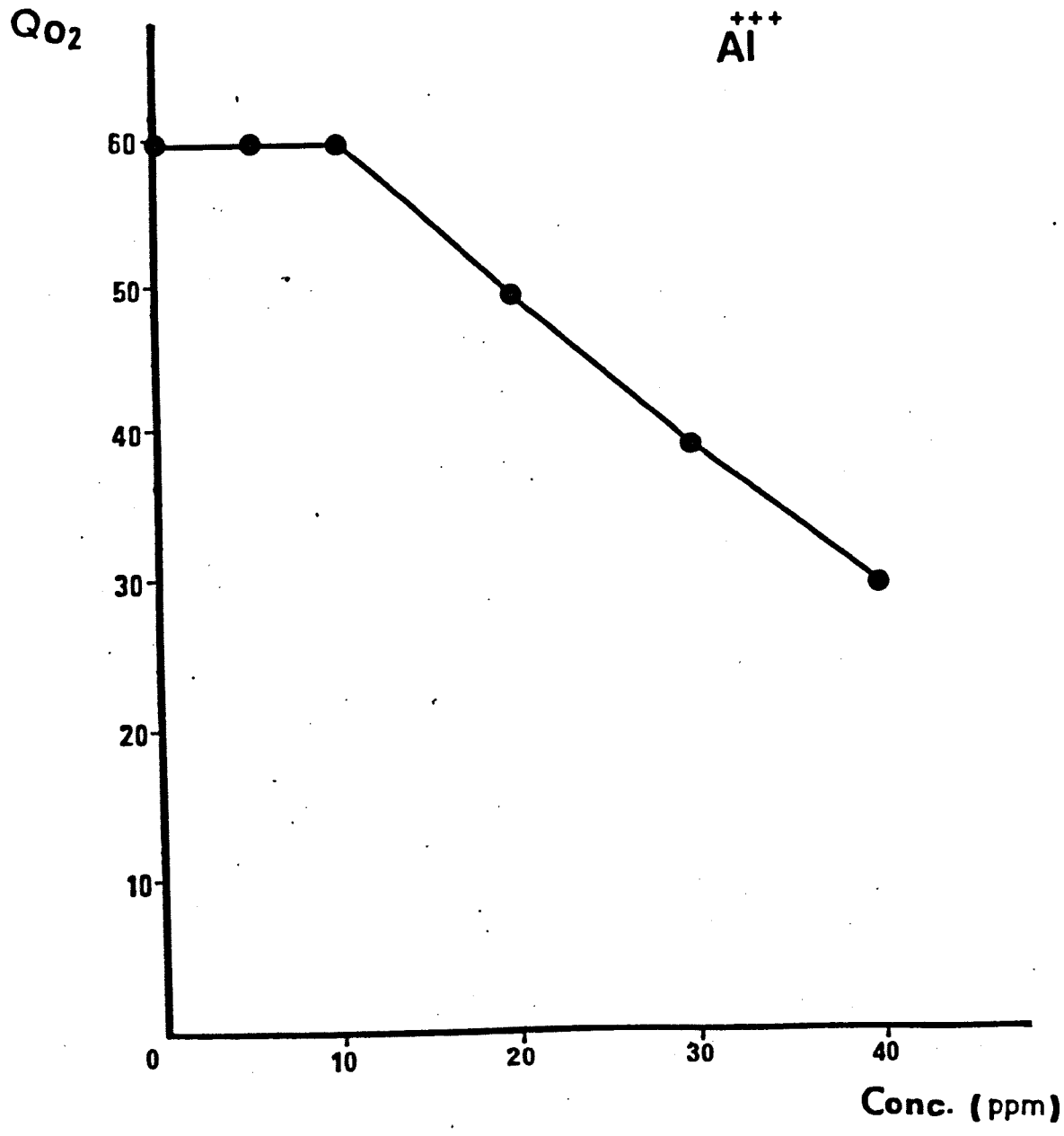


Figure 46. Typical results for aluminium inhibition of E. coli utilization of glucose.

Increasing concentrations of aluminium ions in solution were used to examine the inhibitory effect on the O_2 uptake by E. coli seed in the presence of 0.3 ml of 1M glucose.

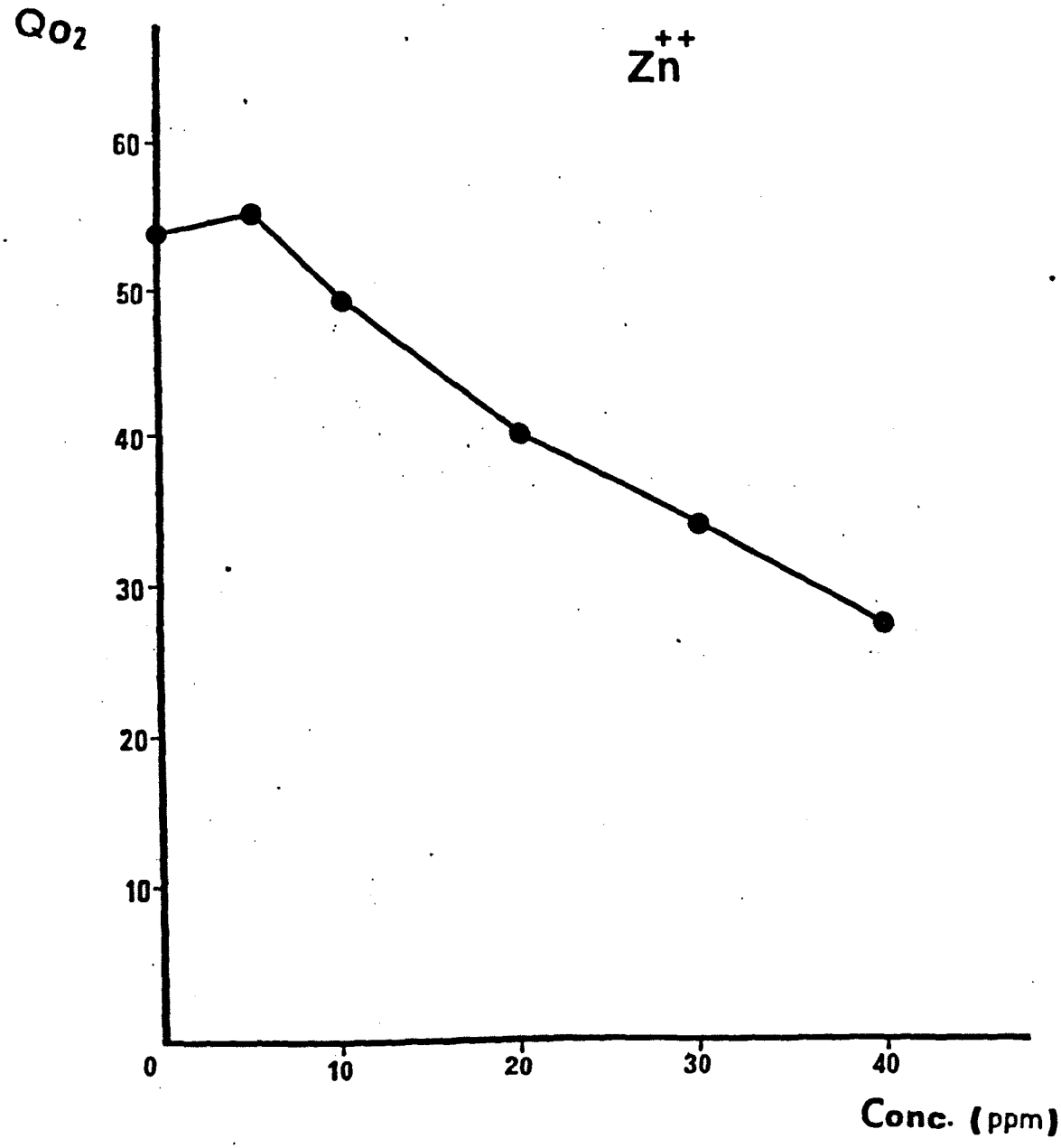


Figure 47. Typical results for zinc inhibition of E. coli utilization of glucose.

Increasing concentrations of zinc ions in solution were used to examine the inhibitory effect on the O_2 uptake by E. coli seed in the presence of 0.3 ml of 1M glucose.

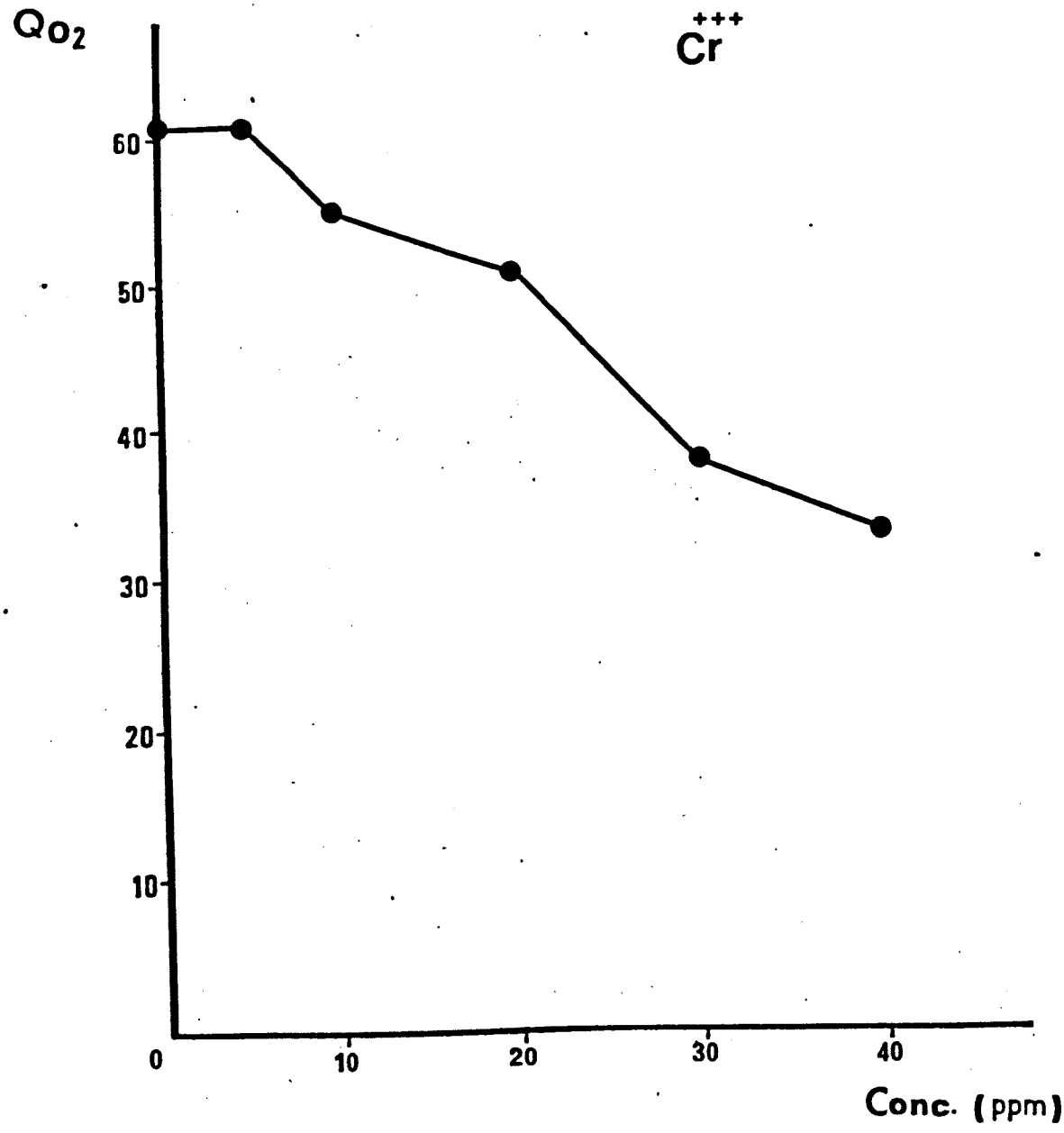


Figure 48. Typical results for trivalent chromium inhibition of E. coli utilization of glucose.

Increasing concentrations of chromium ions in solution were used to examine the inhibitory effect on the O_2 uptake by E. coli seed in the presence of 0.3 ml of 1M glucose.

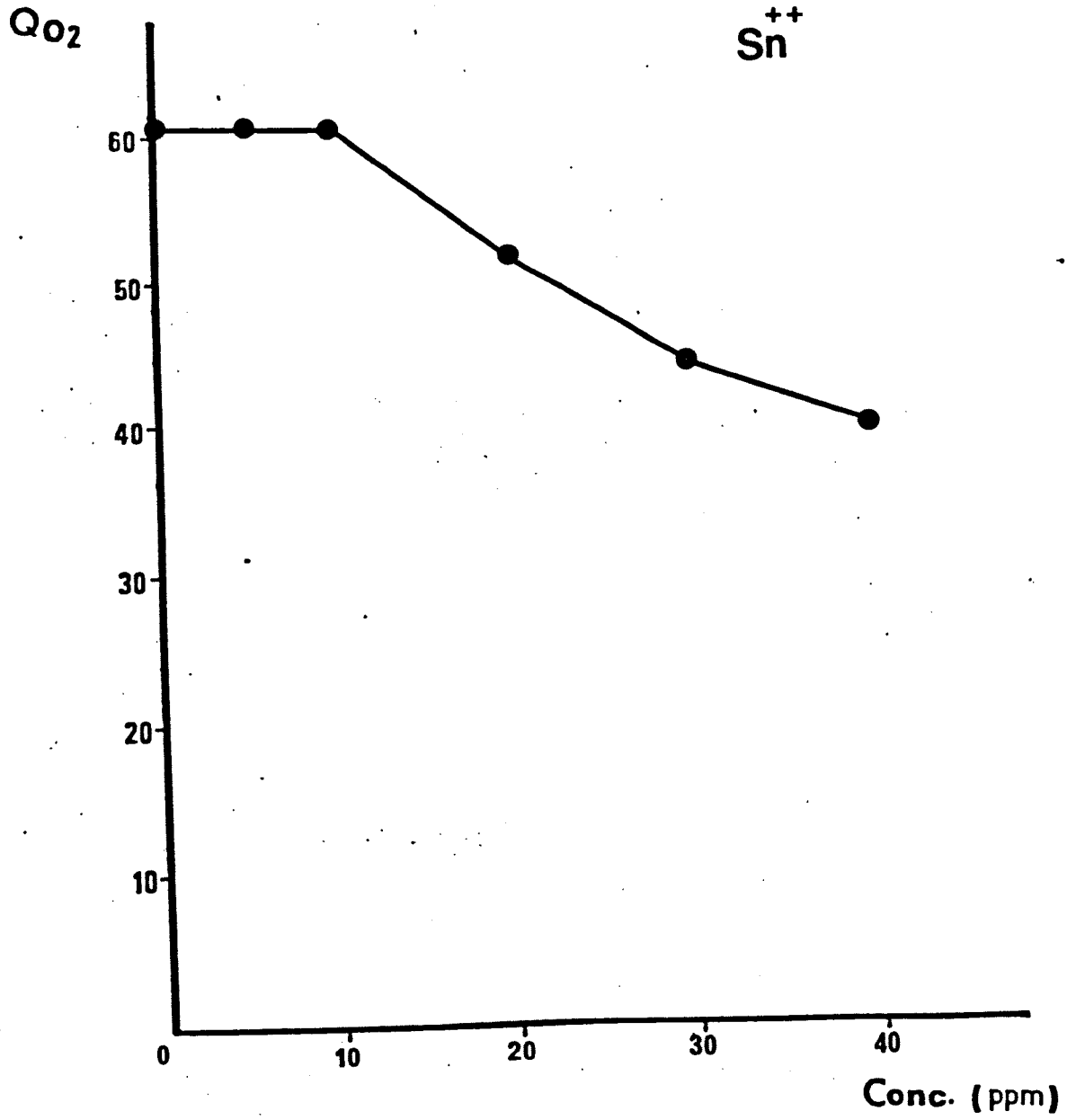


Figure 49. Typical results for tin inhibition of E. coli utilization of glucose.

Increasing concentrations of tin ions in solution were used to examine the inhibitory effect on the O_2 uptake by E. coli seed in the presence of 0.3 ml of 1M glucose.

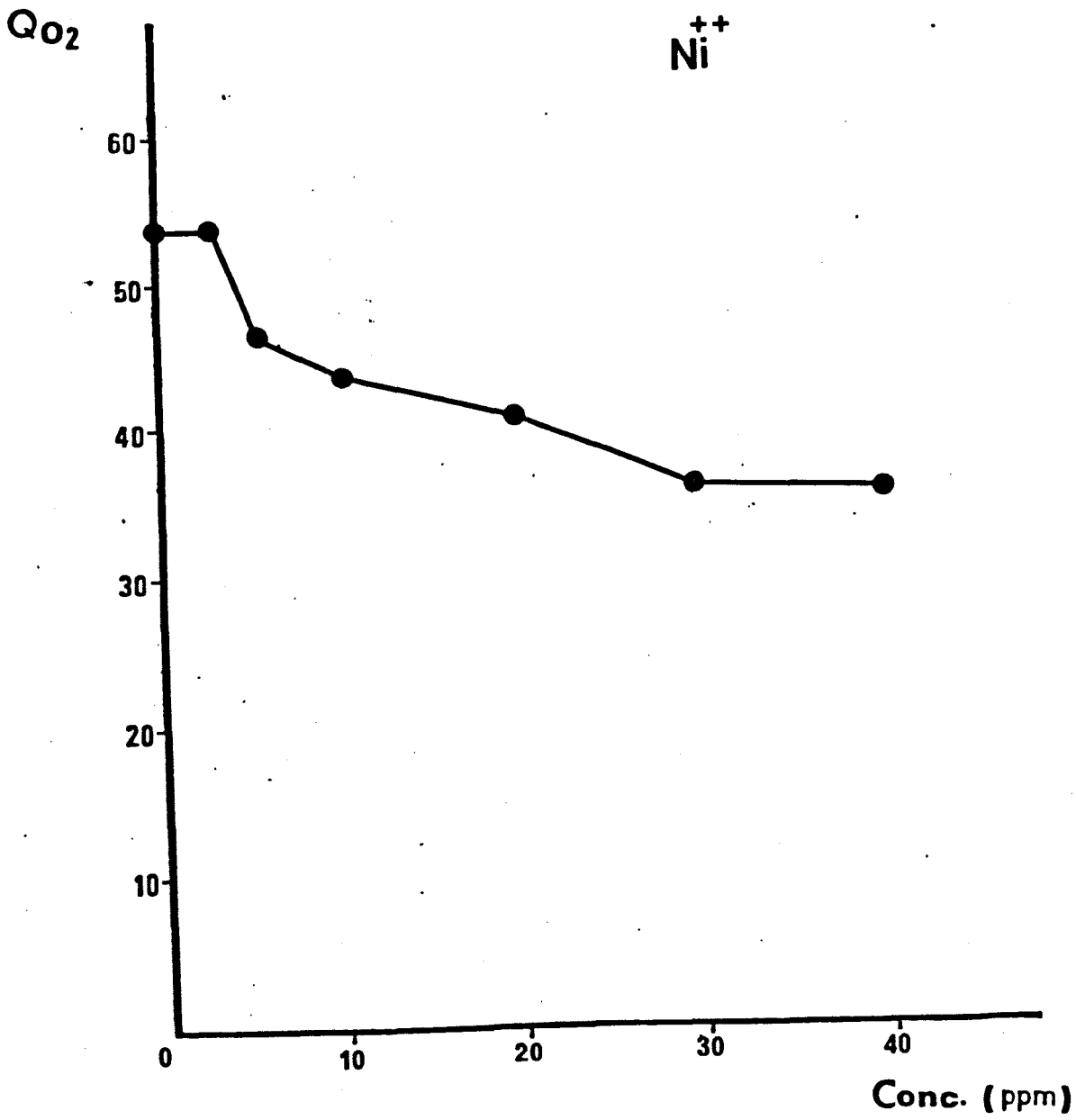


Figure 50. Typical results for nickel inhibition of E. coli utilization of glucose.

Increasing concentrations of nickel ions in solution were used to examine the inhibitory effect on the O_2 uptake by E. coli seed in the presence of 0.3 ml of 1M glucose.

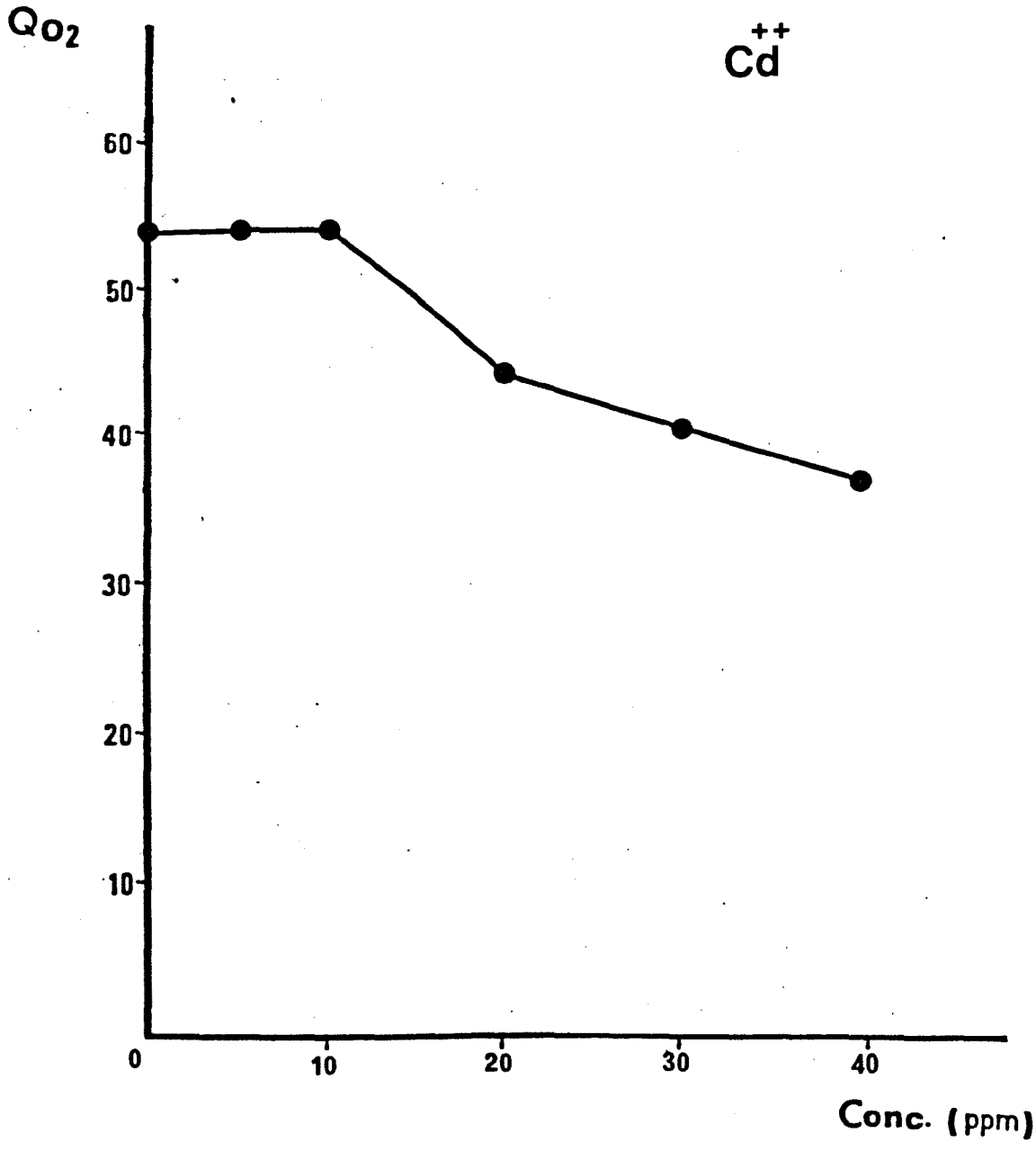


Figure 51. Typical results for cadmium inhibition of E. coli utilization of glucose.

Increasing concentrations of cadmium ions in solution were used to examine the inhibitory effect on the O_2 uptake by E. coli seed in the presence of 0.3 ml of 1M glucose.

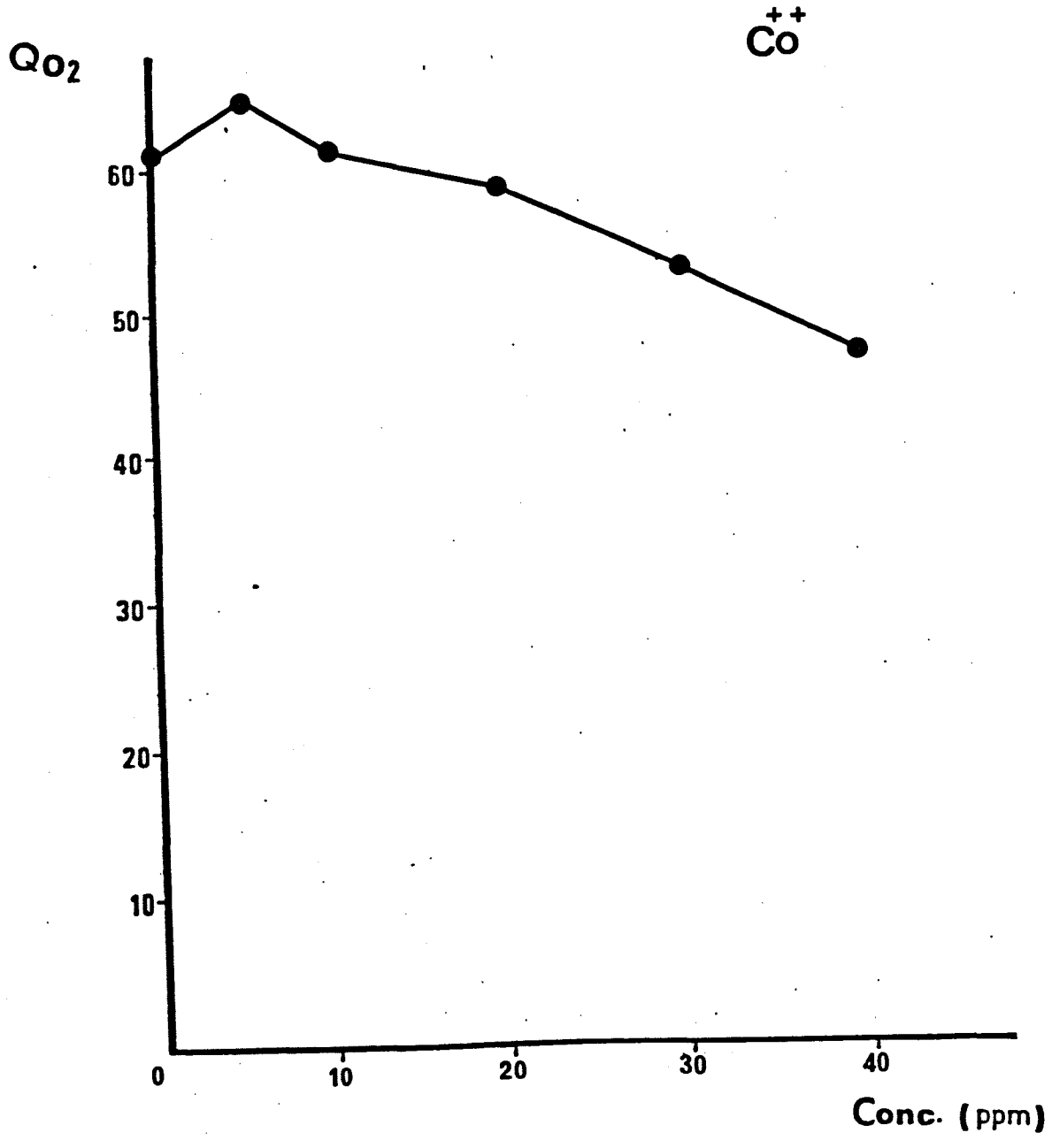


Figure 52. Typical results for cobalt inhibition of E. coli utilization of glucose.

Increasing concentrations of cobalt ions in solution were used to examine the inhibitory effect on the O_2 uptake by E. coli seed in the presence of 0.3 ml of 1M glucose.

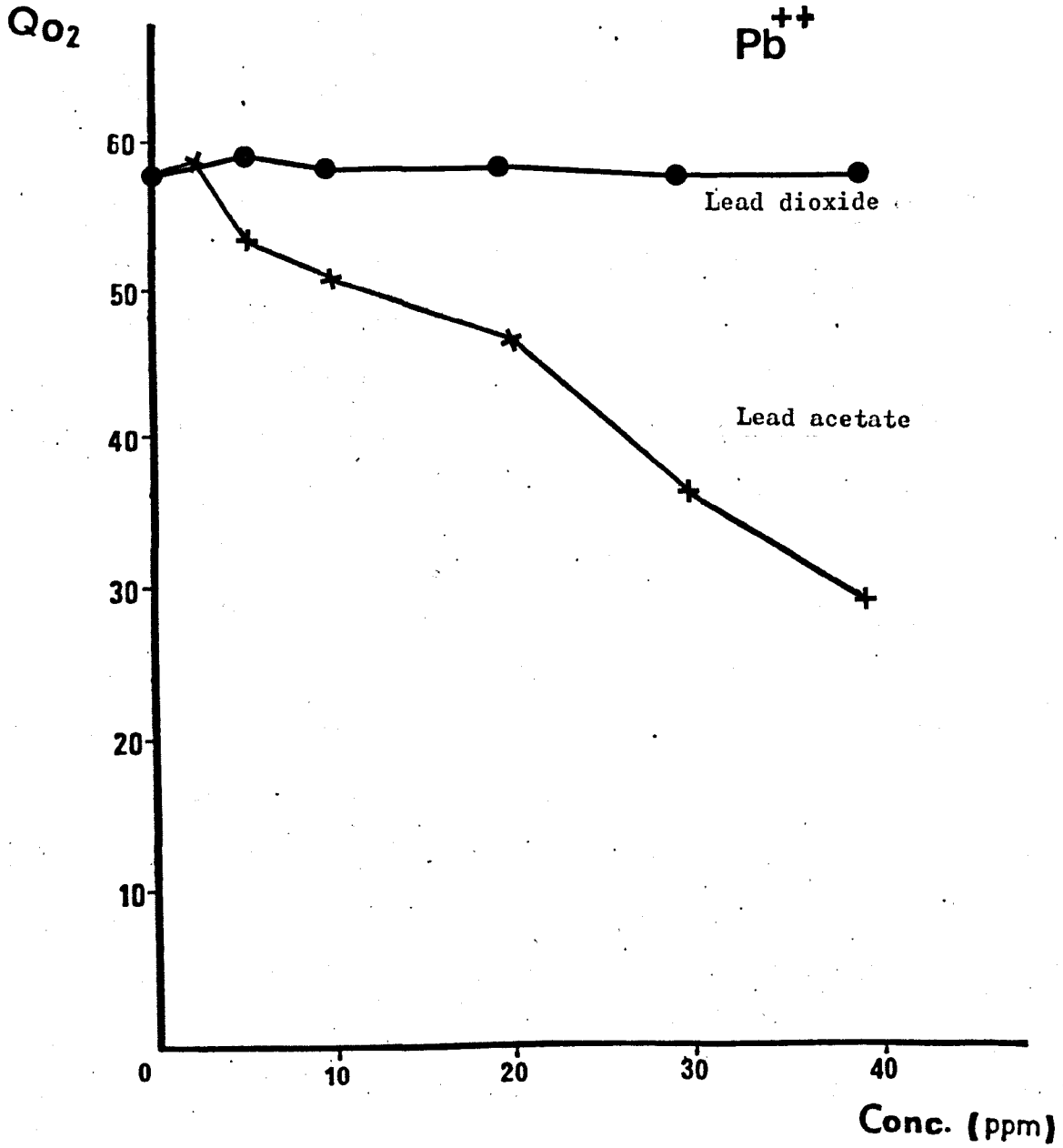


Figure 53. Typical results for lead inhibition of E. coli utilization of glucose.

Increasing concentrations of lead ions in solution were used to examine the inhibitory effect on the O_2 uptake by E. coli seed in the presence of 0.3 ml of 1M glucose. The lack of inhibition with lead dioxide was due to its insolubility in water.

the BOD_5 determinations. Figure 54 shows a typical result for low BOD_5 samples of river-water (BOD_5 = 0.6 - 20 ppm) which contain small amounts of readily utilisable organic matter. Higher levels of O_2 uptake were obtained for samples with higher BOD_5 values.

The tabulated results of O_2 uptake and BOD_5 for 254 river-water samples are shown in Appendix 3. In general, for river-water samples with low BOD_5 values the O_2 uptake values (in QO_2) were clustered around 0.66-6.0. This is more obvious in the scatter diagram of the results for 254 river-water samples (Figure 55). The QO_2 value of less than 6.0 indicated that a particular sample was unlikely to be heavily polluted and the BOD_5 would be expected to be less than 10 ppm.

A statistical analysis for the 254 river-water samples was done with the Kendall's coefficient of rank correlation (r) method and the linear regression method (see Appendix 5). The correlation coefficient (rs) was +0.189 (p = 4.45) by Kendall's method and 0.247 by line of regression method which indicated a significant relationship between O_2 uptake and BOD_5 values of $<$10.0 ppm.

Variation in the results of O_2 uptake in the different months and seasons could be seen from the data (see raw data, Appendix 3), therefore, the results were statistically analysed by seasons (spring, summer, autumn, and winter) (Table 14) and by the month results (Table 15).

The relationship between QO_2 and BOD_5 results of the winter months was significant (corr.coeff. 0.5501). The regression curve for BOD_5 v QO_2 (Figure 56) shows a linear relationship for the winter months results, while scattered points along the line of regression could be seen for the other seasons' results (Appendix 2 : 1 & 2). The regression curves for QO_2 v BOD_5 for the monthly results are shown in Appendix 2: 3-10.

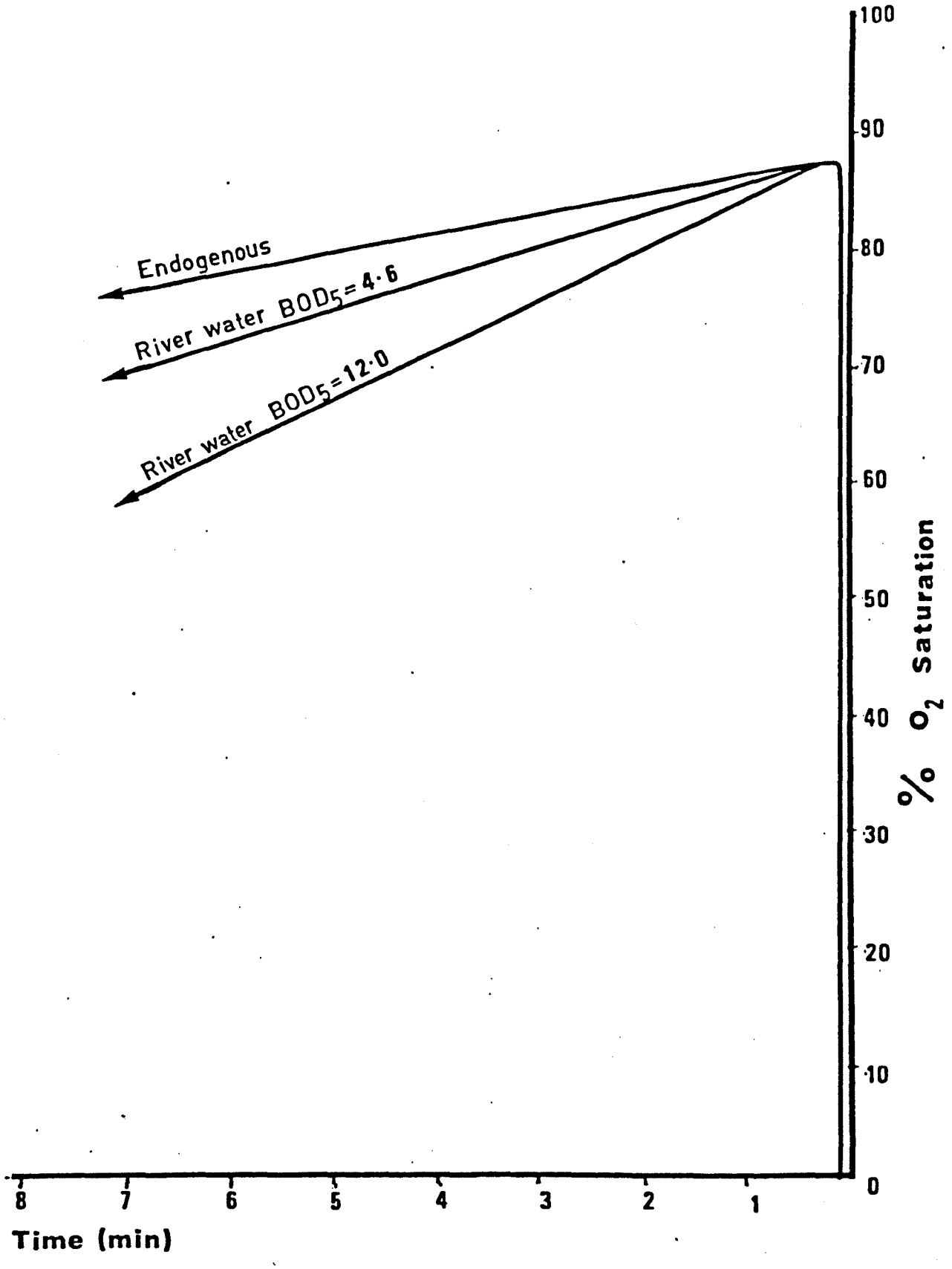


Figure 54. Typical results of O_2 uptake for low BOD_5 samples of river-water, in the presence of E. coli seed.

These results indicate that the river - water samples contained low levels of readily metabolizable material.

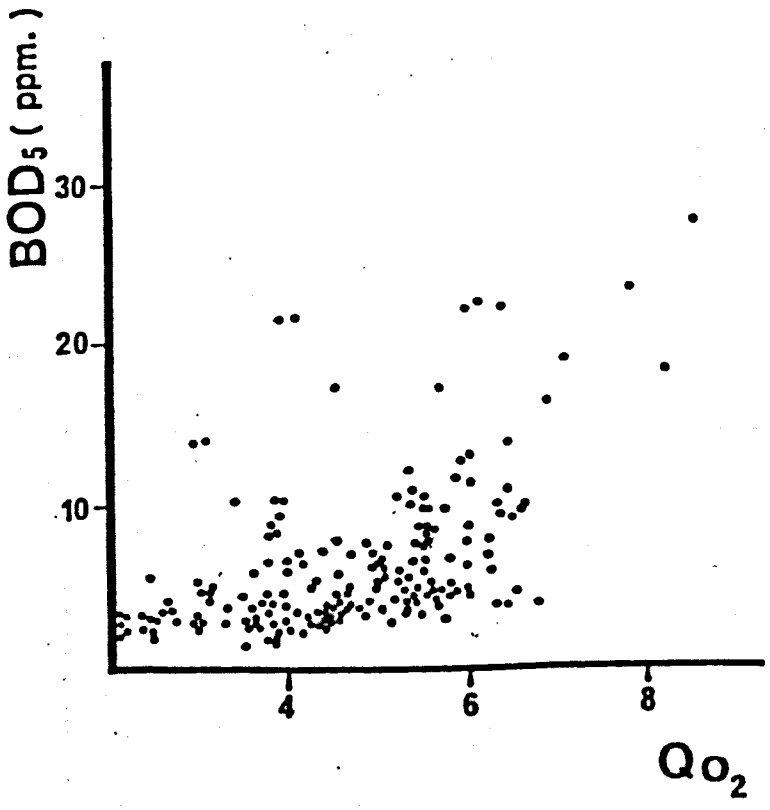


Figure 55. A scatter diagram for BOD_5 v QO_2 for 254 river-water samples.

This clearly shows that the relationship between BOD_5 and QO_2 breaks down when river - water samples with BOD_5 values $<$10.0 ppm are examine

Kind of Sample	No. of Samples	Normal distribution test*	• Method A			• Method B				
					correlation	correlation	$\bar{x}$		S.D.	
			r	p	coefficient	coefficient	BOD_5	QO_2	BOD_5	QO_2
river-water (spring)	48	(+)	+ 0.05	0.45	not significant	+ 0.09	9.88	4.48	7.96	1.19
river-water (summer)	46	(-)	+ 0.16	1.55	"	+ 0.40	6.95	5.85	5.576	1.55
river-water (autumn)	96	(-)	+ 0.13	1.87	"	+0.10	5.35	3.22	3.697	1.86
river-water (winter)	64	(+)	+ 0.55	6.34	highly significant	+ 0.52	5.19	4.59	4.47	1.08
river-water (all the seasons together)	254	---	+ 0.19	4.45	significant	+ 0.25	5.68	4.22	7.62	1.797
sewage	67	(+)	+ 0.84	9.94	highly significant	+ 0.94	96.22	7.07	66.20	2.70
standard substrate	7*	(+)	+1	---	highly significant	+ 0.997	62.97	2.69	76.03	3.04

* mean values of three experiments
* normal distribution test : see appendix 5.
• Method A and B: see Appendix 5.

Table 14. Statistical analyses of BOD_5 v QO_2 results with method A and B for river-water, sewage samples and standard substrate solutions.

Month	r_s	p	significance	level	No. of samples
August '78	0.39	1.92	not significant	-	14
September '78	0.13	0.68	" "	-	16
October '78	0.45	2.34	significant	5%	16
November '78	0.08	0.41	not significant	-	16
December '78	0.28	1.49	" "	-	16
January '79	0.75	3.96	significant	0.1%	16
February '79	0.79	4.23	"	0.1%	16
March '79	0.49	2.61	"	1%	16
April '79	0.02	0.09	not significant	-	16
May '79	0.35	1.89	" "	-	16
June '79	0.20	1.04	" "	-	16
August '79	0.28	1.44	" "	-	16
September '79	0.08	0.41	" "	-	16
October '79	0.33	1.76	not significant	-	16
November '79	0.42	2.21	significant	5%	16
December '79	0.69	3.56	significant	0.1%	16

Table 15. Statistical analysis results of monthly results of BOD_5 v QO_2 for river water determined with method A.

These results show the variable correlation between BOD_5 determinations and QO_2 for river-water samples with low BOD_5 values. On a monthly basis there appears to be a low level of correlation.

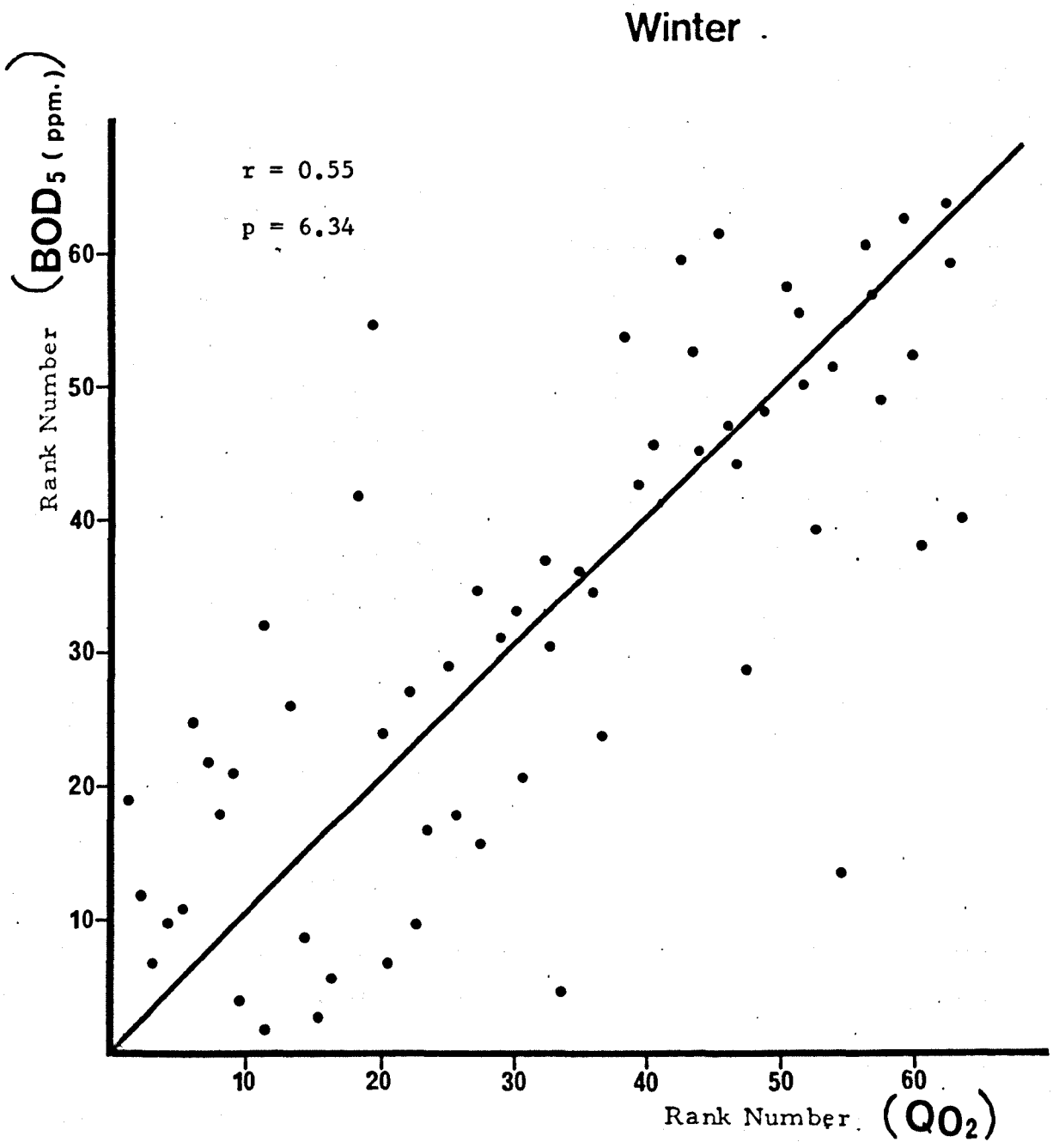


Figure 56. Regression curve of BOD_5 v QO_2 results of river-water, Winter months (December 1978, January, February and December, 1979).

the winter period some statistical significance was found but the of results indicates that the correlation between BOD_5 and QO_2 complete for samples with low BOD_5 values.

The results of QO_2 for the seasons were examined for their normality (normal distribution) by plotting a curve between the rank of QO_2 values and their rankit numbers. The line was plotted with the mean value ($\bar{x}$) of the QO_2 data, and $\pm$ standard deviation. A positive result was found for the results of spring and winter samples (Figure 57 & 58), while negative results were found for the summer results (Figure 59).

B. Comparison Between O_2 Uptake Results and Other Chemical Parameters

A comparative study was done between the results of O_2 uptake for the 254 river-water samples and the results of other chemical parameters:

1. Dissolved oxygen (DO)
2. Suspended solid (SS)
3. 4 hr permanganate value (PV)
4. Ammoniacal nitrogen (NH_3-N)
5. Phosphate (PO_4-P)
6. Nitrite ($N(NO_2)$)
7. Nitrate ($N(NO_3)$)

which were supplied by the CRPB. The results (raw data, Appendix 3) were analysed statistically for their correlation coefficient by season (spring, summer, autumn, winter) and all the 254 samples together. The results of the statistical analyses for QO_2 values with all the other experimental parameters are shown in Table 16. The differences between QO_2 and all the other results were significant for the winter months, while insignificant differences were found for the other seasons' results, with the exception of phosphate, nitrite and nitrate results of summer months and nitrite results of spring, in which a significant relationship was found with the QO_2 values.

APPLICATION OF THE RAPID MICROBIOLOGICAL METHOD FOR SEWAGE SAMPLES

A. Oxygen Uptake Measurement by Sewage Samples

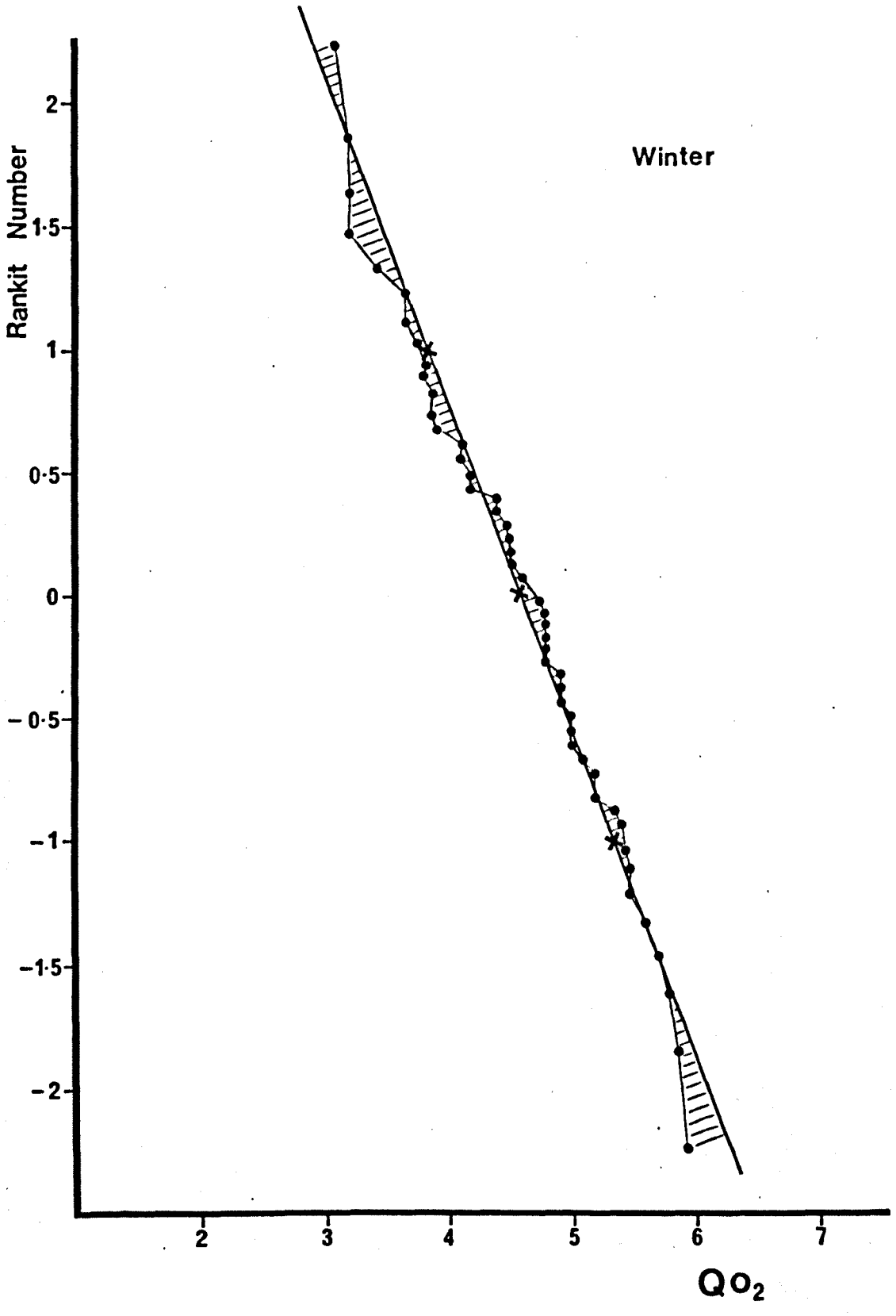


Figure 57. Rankit plot for the QO_2 results of the winter months, indicating that the values are normally distributed.

($\bar{x}$ = 4.6, SD = 0.76)

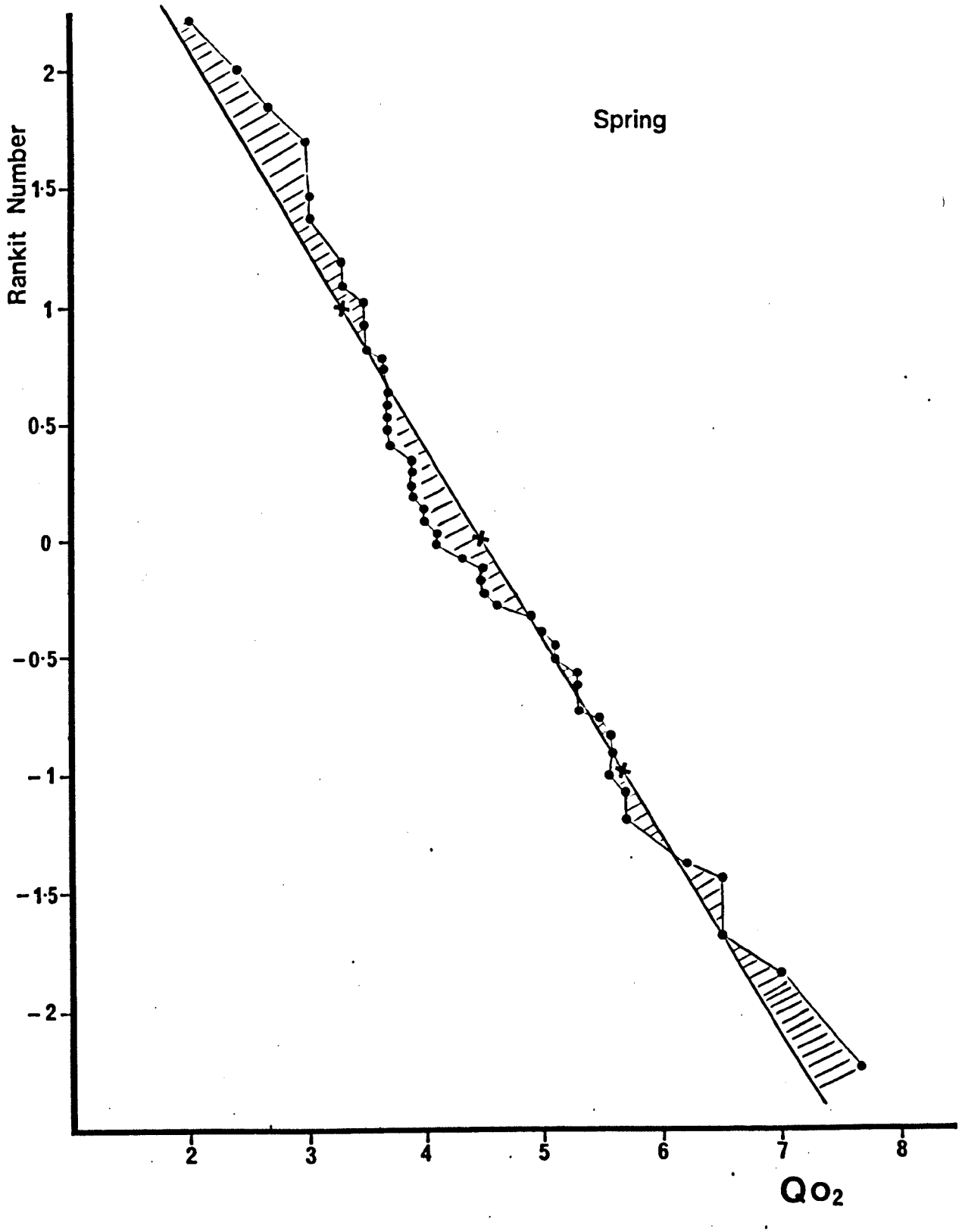


Figure 58. Rankit plot for the QO_2 results of the spring months, indicating that the values are normally distributed. ($\bar{x}$ = 4.48, SD = 1.19).

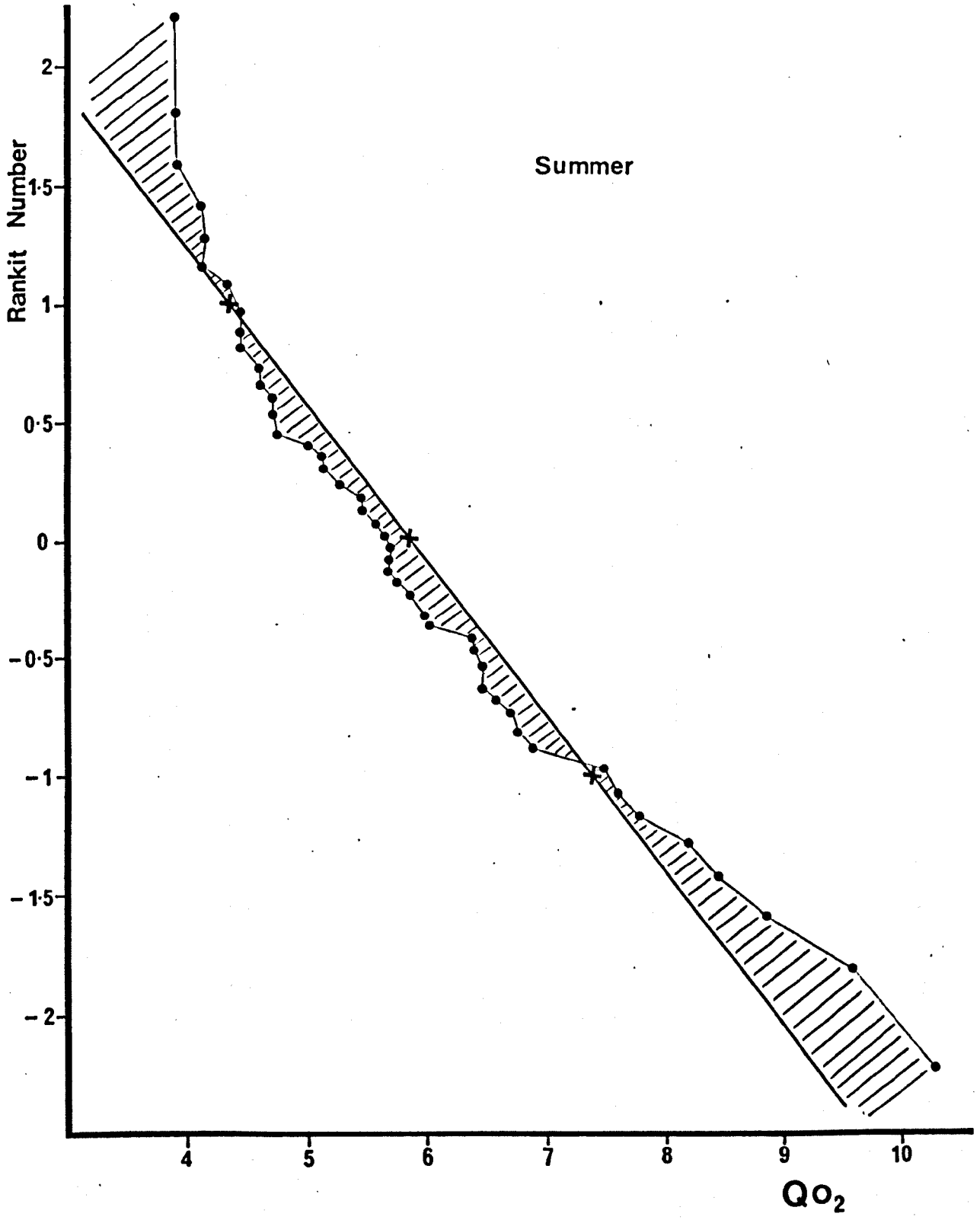


Figure 59. Rankit plot for the QO_2 results of the summer months, indicating that the values are not normally distributed. ($\bar{x}$ = 5.85, SD = 1.55).

Samples	*PV			*DO			*SS			*NH_3 - N			*PO_4 - P			*$N(NO_2)$			*$N(NO_3)$		
	$\bar{x}$	SD	corr coeff	$\bar{x}$	SD	corr coeff	$\bar{x}$	SD	corr coeff	$\bar{x}$	SD	corr coeff	$\bar{x}$	SD	corr coeff	$\bar{x}$	SD	corr coeff	$\bar{x}$	SD	corr coeff
spring	4.92	2.69	0.01	9.88	2.55	0.02	15.65	17.76	0.18	2.32	2.68	0.21	0.53	0.66	-0.05	0.08	0.09	-0.27 (+)	2.61	4.14	-0.05
summer	5.49	1.53	0.14	7.33	1.81	0.05	10.22	7.83	0.06	1.47	2.28	0.15	0.45	0.64	0.49 (+)	0.19	0.15	0.41 (+)	2.16	2.09	0.48 (+)
autumn	7.20	2.32	0.06	8.59	1.43	-0.10	25.81	25.64	-0.16	0.60	1.15	0.04	0.20	0.28	0.08	0.06	0.07	0.23	1.85	1.59	0.22
winter	4.01	1.43	0.38 (+)	10.18	1.60	-0.25 (+)	12.02	8.67	0.42 (+)	2.79	5.46	0.31 (+)	0.25	0.32	0.44 (+)	0.04	0.03	0.61 (+)	1.74	1.29	0.37 (+)
all seasons to-gether	5.66	2.45	-0.12	9.00	2.07	-0.10	17.59	19.47	-0.20	1.64	3.32	0.20	0.32	0.48	0.26 (+)	0.11	0.38	-0.05	2.02	2.41	0.17

Table 16. Statistical analysis results of QO_2 versus other chemical parameters for the river-water samples with method B. (linear regression method; see Appendix 5).

$\bar{x}$ = mean, SD = standard deviation, (+) shows a significant relationship.

* Chemical parameters obtained from the CRPB.

It would appear from these results that the microbiological polarograph test does not replace these particular chemical tests.

The uptake of oxygen in the polarograph was measured with sewage by adding 0.1 ml of E. coli seed.

samples. Samples (crude sewage, primary settlement tank effluent, and final effluent) were collected from seven sewage treatment plants in the Kelvin valley under different climatic conditions of dry weather, rain and heavy storm (see Appendix 4 : 1). Oxygen uptake and BOD_5 were measured for the samples. Appendix 4 shows the results for 67 sewage samples. The uptake of oxygen for the crude sewage samples, with high BOD_5 values, was greater than that for the primary settlement tank effluent samples, of lower BOD_5 values. Lower values of O_2 uptake were found for the final effluent samples, with lower BOD_5 values (Figure 60). The uptake of oxygen, without the addition of E. coli seed to the sewage samples, was much lower (Figure 61). The increased levels of O_2 uptake were reproducible in all the experiments with the addition of seed to the system.

B. Comparison of O_2 Uptake with BOD_5 for Sewage Samples

In order to compare the results of O_2 uptake for the sewage samples with the respective BOD_5 results, data was graphically presented. The line of best fit obtained from the statistical analysis of the data is shown in Figure 62. From the cumulative results, it was possible to identify by the polarographic method, the crude sewage liquot (BOD_5 : 160-265 ppm), the effluent from the primary settlement tanks (BOD_5 : 60-140 ppm), and the final effluent (BOD_5 : 5-40 ppm). The results of O_2 uptake paralleled those obtained by the BOD_5 test, ie. a linear relationship was found between O_2 uptake and BOD_5.

The data were statistically analysed to obtain Kendall's coefficient of rank correlation. A highly significant relationship was found between O_2 uptake and BOD_5. The correlation coefficient obtained was +0.8345. A value of +0.9398 was obtained with the linear regression method. The line of regression plotted for O_2 uptake and BOD_5

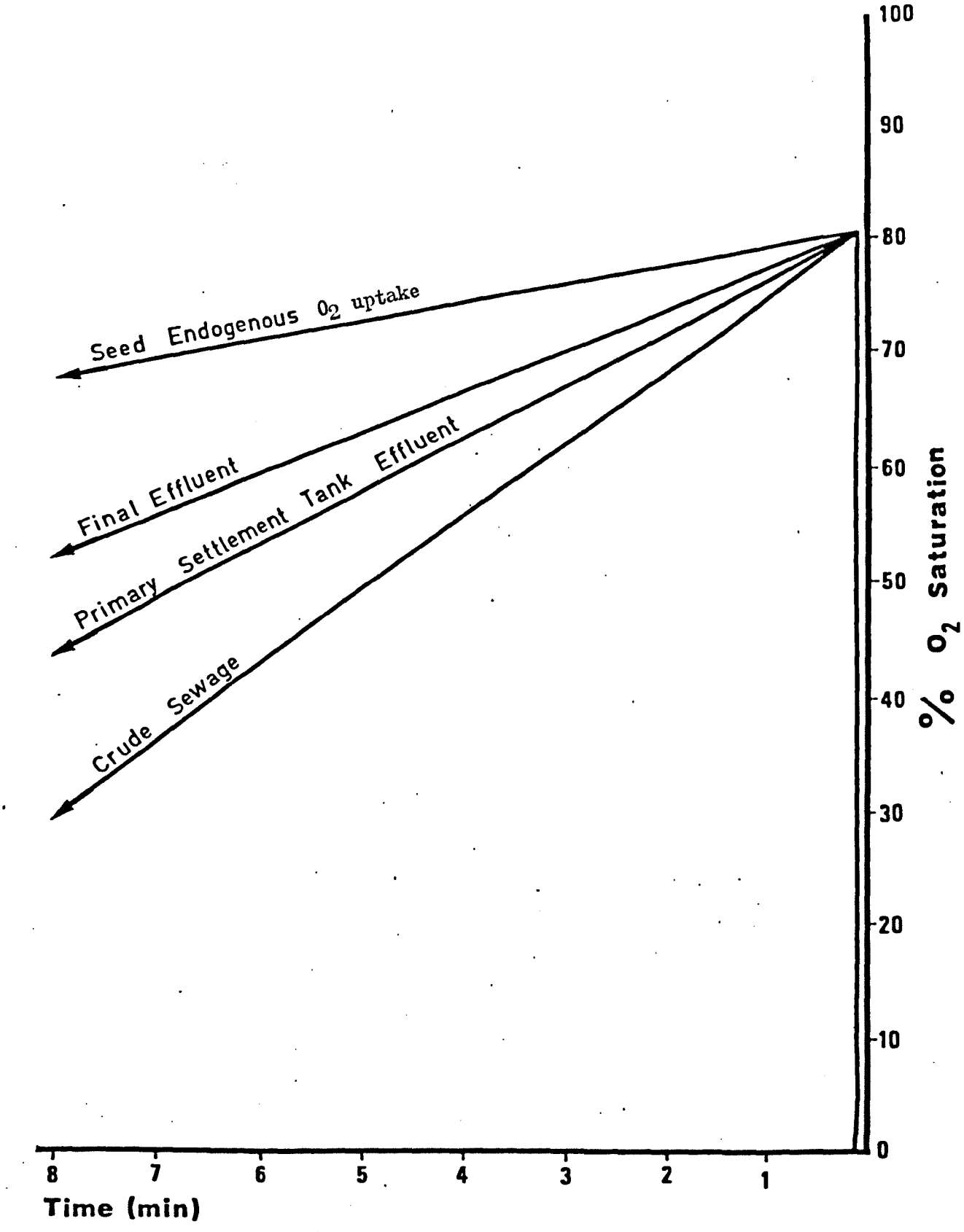
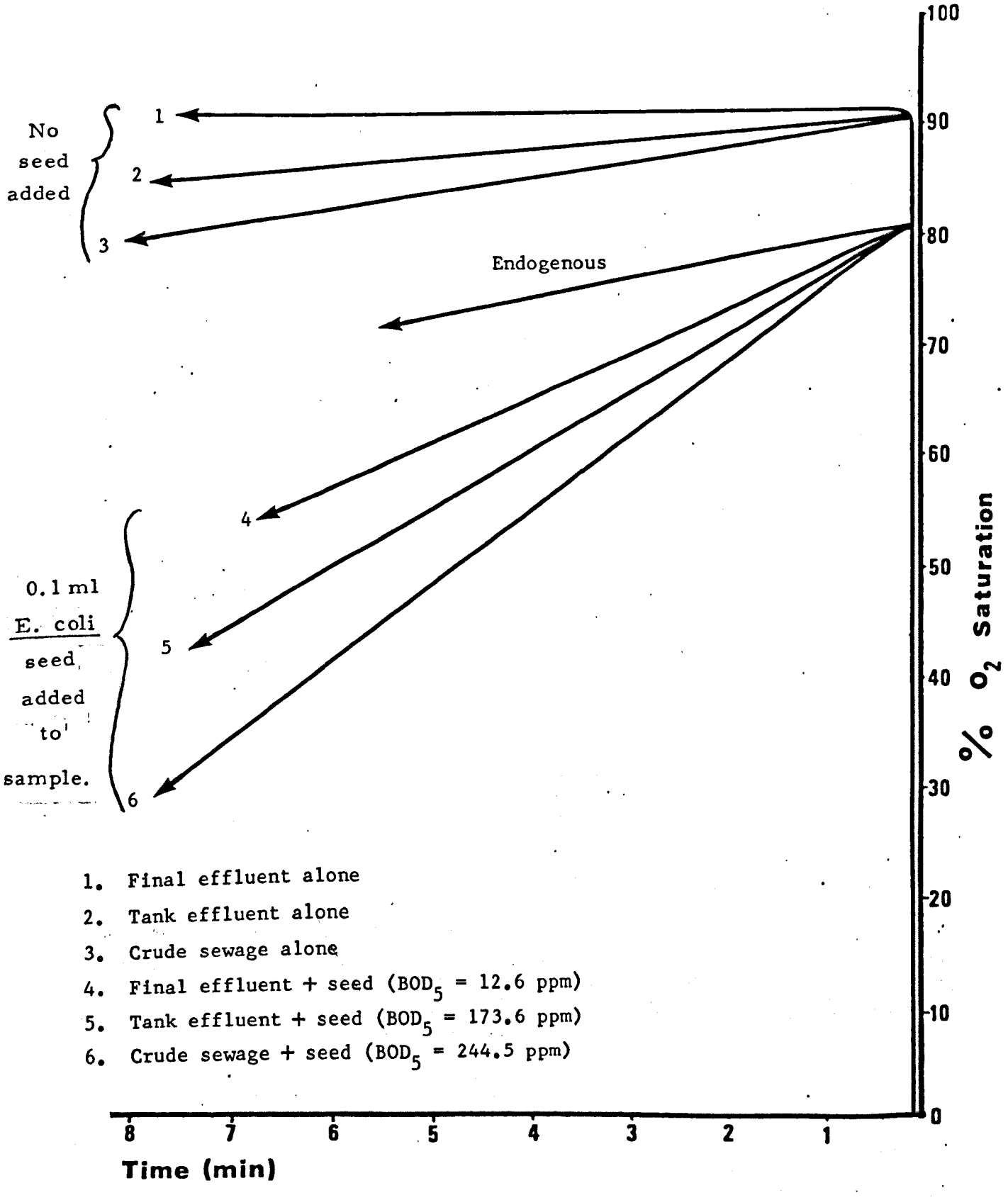


Figure 60. Oxygen uptake measurement of sewage samples with the addition of E. coli seed.

These examples of polarograph recordings clearly show the separation of polluted samples by the degree of oxygen uptake.



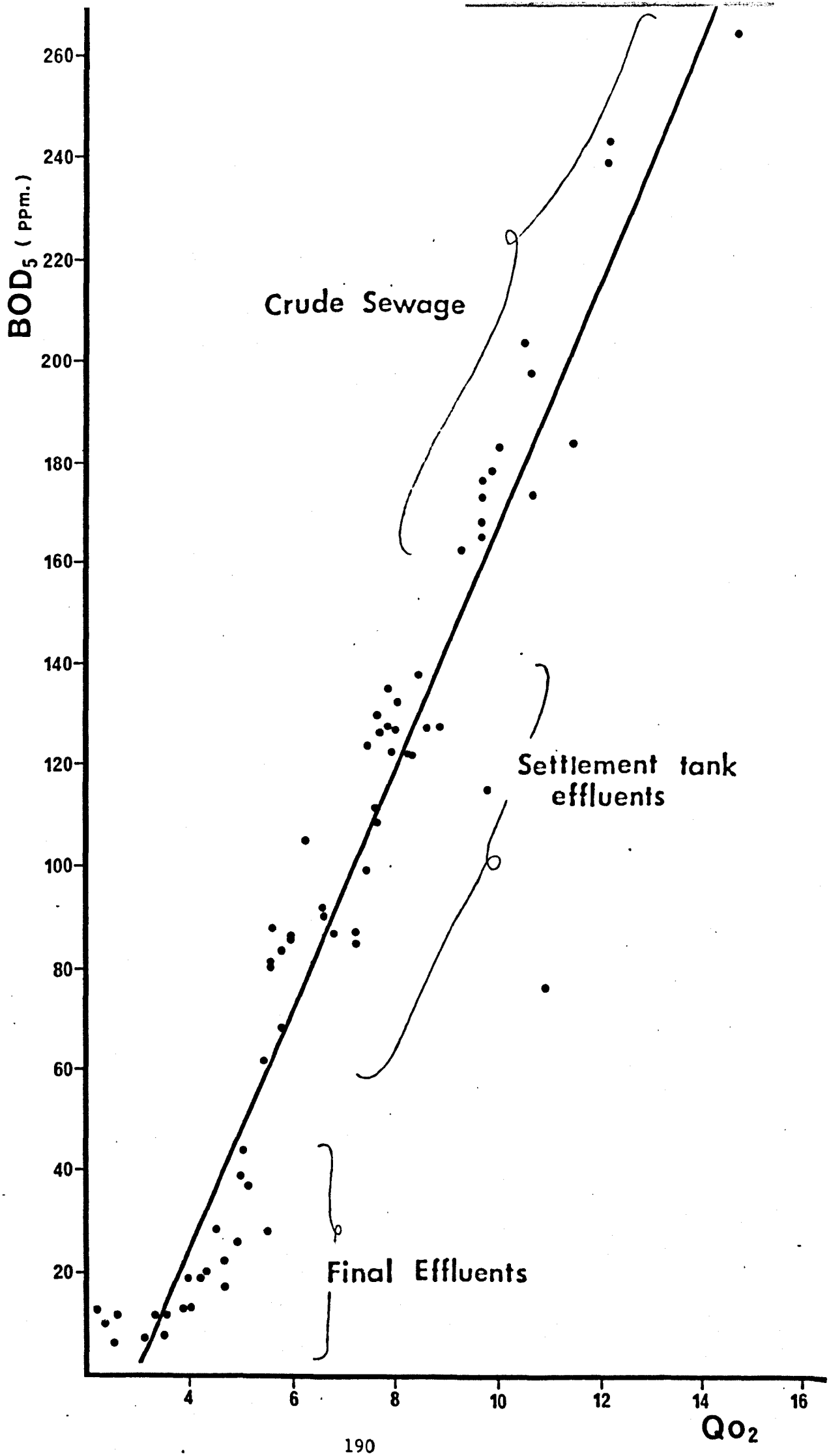
1. Final effluent alone
2. Tank effluent alone
3. Crude sewage alone
4. Final effluent + seed (BOD_5 = 12.6 ppm)
5. Tank effluent + seed (BOD_5 = 173.6 ppm)
6. Crude sewage + seed (BOD_5 = 244.5 ppm)

Figure 61. Oxygen uptake for sewage samples with and without adding seed.

results show that the use of a seed allows a greater uptake of n in the polarograph test even with sewage samples.

Figure 62. Curve of oxygen uptake (QO_2) with BOD_5 results of sewage samples

The relationship between BOD_5 determinations and the QO_2 values calculated from polarographic measurements. For polarograph tests E. coli seed (0.1 ml, c 315×10^9/ml) was added. It is noticeable that the polarograph test separates the crude sewage samples, settlement tank effluent samples and final effluent samples.


BOD5 (ppm.)
260
240
220
200
180
160
140
120
100
80
60
40
20
Crude Sewage
Settlement tank effluents
Final Effluents
4
6
8
10
12
14
16
Qo2

results (Figure 63) shows a linear relationship as the points clustered along the line of regression (slope = 1.0). The results of QO_2 were examined for their normality (normal distribution) by plotting a curve between the rank of QO_2 values and their rankit numbers. The line was plotted with the mean value ($\bar{x}$) of the QO_2 data, and $\bar{+}$ standard deviation. A positive result was found for the QO_2 values of the sewage samples (Figure 64).

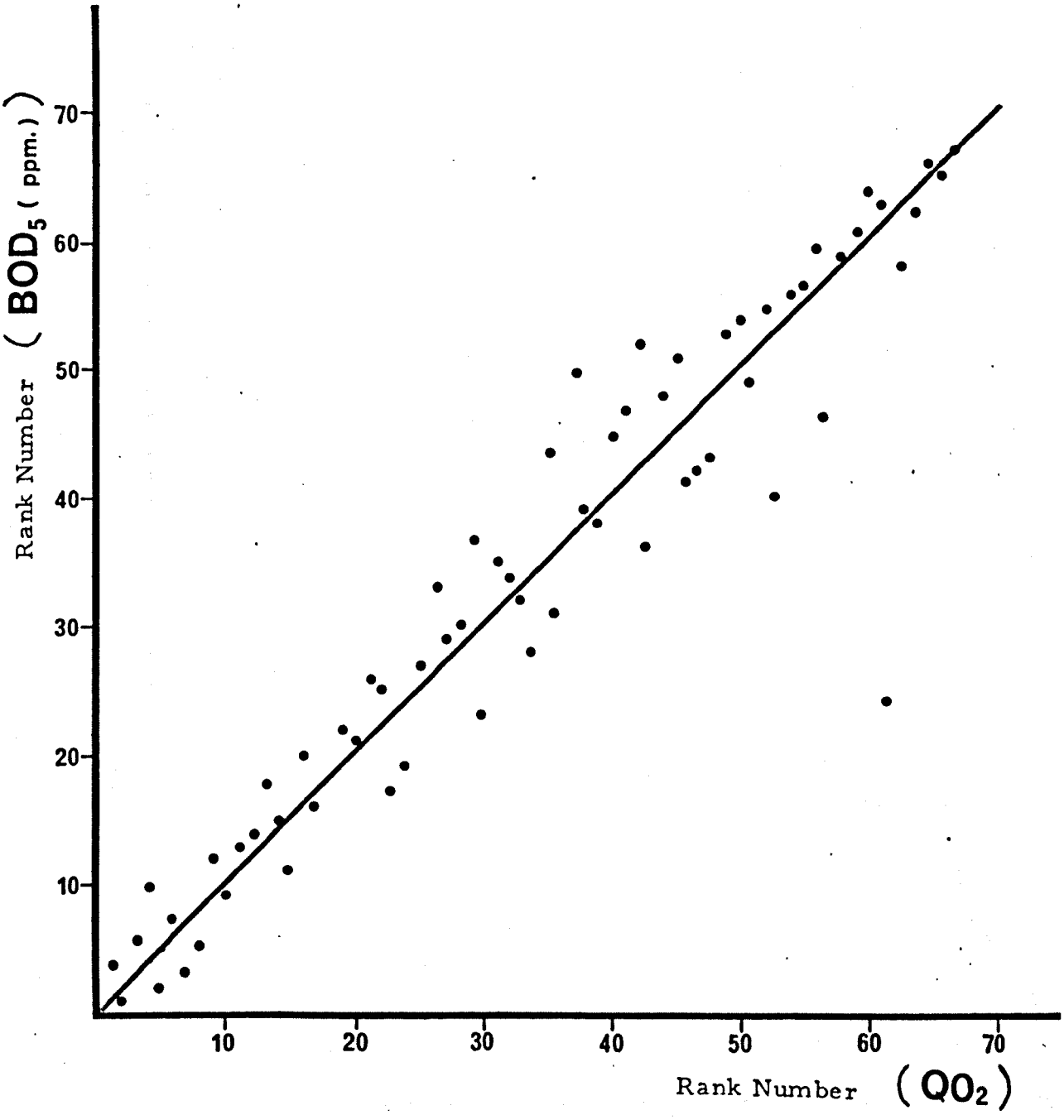


Figure 63. Regression curve for O_2 uptake and BOD_5 data for sewage samples

ation coefficient obtained for these samples was +0.9398
cates the value of this polarograph test for measuring BOD.

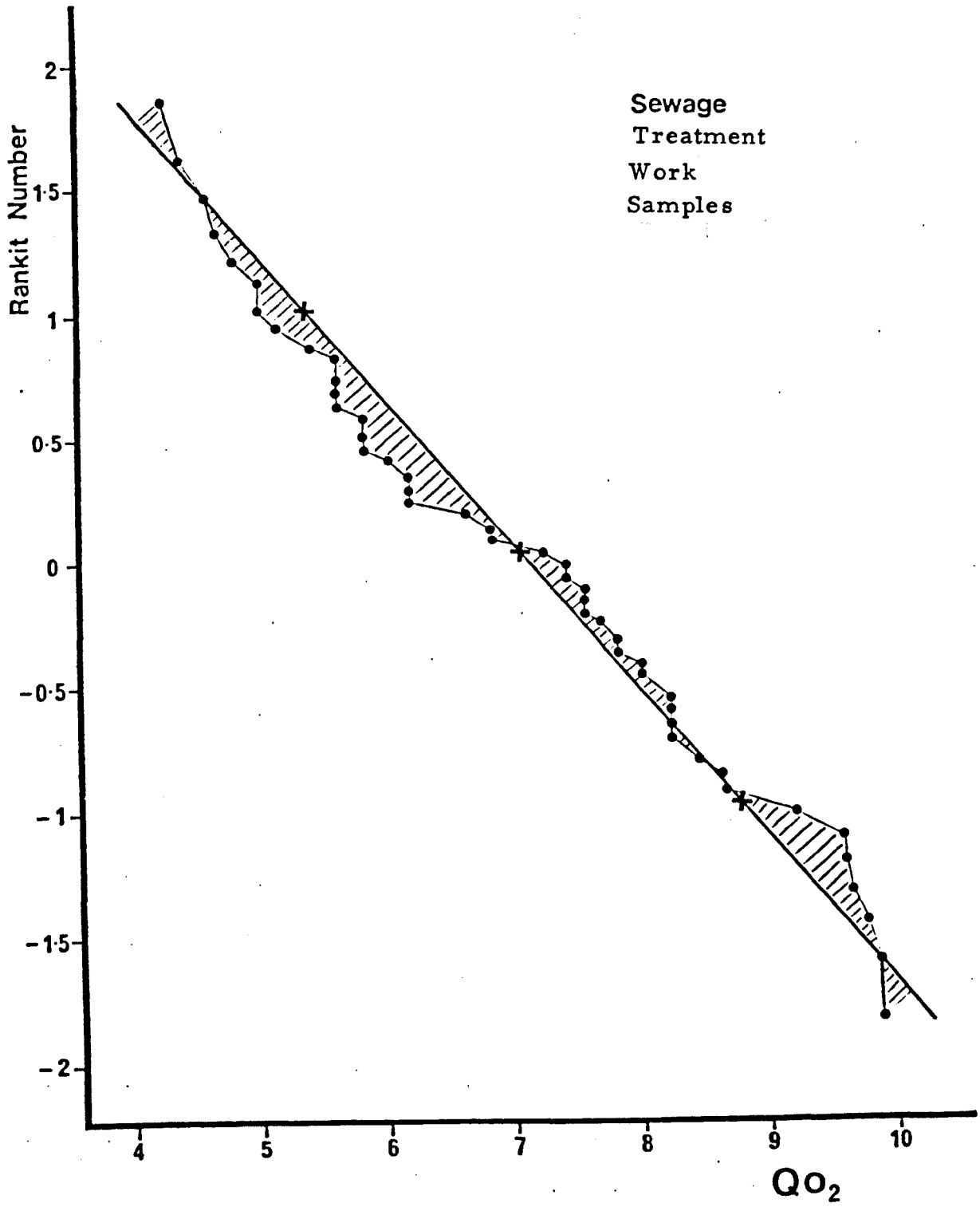


Figure 64. Rankit plot for the QO_2 results of the *sewage samples, showing normal distribution of values.

($\bar{x}$ = 7.04, SD = 1.76).

* sample numbers 10-60, Appendix 4.

DISCUSSION

THE SEARCH FOR A STANDARDIZED BACTERIAL SEED INOCULUM

The microbial population present in an effluent sample is one of the main factors which affect the biological decomposition of organic matter. In an effort to obtain a rapid BOD test it is necessary to accelerate the rate of decomposition of organic compounds by increasing the number of organisms in the sample (LeBlanc, 1974). Several workers attempted with limited success to prepare a bacterial seed inoculum for rapid biochemical oxygen demand determinations with the use of sewage organisms or a massive inoculum of mixed or pure cultures of bacteria as the seed (Heukelekian and Gellman, 1951; Zehnpfennig and Nichols, 1953; Tidwell and Sorells, 1956; Dillingham, Knuth and Wessman, 1958; Dillingham and Jose, 1960; Lee and Oswald, 1954, 1959a, 1959b), but none of those workers was able to obtain a seed which gave reproducible results. This was due to variation in oxidative capacity of the organisms, and Karube et al (1977) used microorganisms immobilized in polyacrylamide gel as the seed in a polarographic determination of BOD. The organisms remained active in the gel for a period of only 10 days, and they were not capable of oxidizing organic compounds of a waste-water, so a seed of this type would be unsuitable for a standardized microbiological method.

The preparation of a bacterial seed inoculum for this study involved the isolation of a variety of organisms (see Table 10) from the River Kelvin. During preliminary experiments, the activity of these organisms was measured in the polarograph against different substrate solutions, both as pure and mixed cultures. The organisms were grown on nutrient agar + glucose medium or in the Snyder and Koch medium. The cells were grown overnight at 37°C, harvested by centrifugation, washed twice in distilled water, and resuspended in a small amount of distilled water to give a thick suspension. The suspension was standardized and 0.1 ml was used per test. The organism Pseudomonas

cepacia known to degrade a wide variety of organic compounds (Stanier, Adelberg and Ingraham, 1977) was also tested as a pure or mixed suspension with the other organisms. Results showed that there was no great advantage of using mixed cultures.

A river-water isolate of E. coli (0142 K? H25) gave more reproducible results than all the other organisms, including the Pseudomonas. It showed a high level of activity against different subtrates (glucose, glutamic acid, sodium pyruvate and sodium succinate) with a reasonably low endogenous rate of oxygen uptake. This was confirmed by the work of Dietrich and Burris (1967). They found that both E. coli and A. aerogenes had low endogenous respiration rates compared with their respiration rates in the presence of added glucose. Similar results were also found by Clifton (1963b). One of the disadvantages of using Pseudomonas cepacia was that the endogenous oxygen uptake was too high with a considerably lower exogenous oxygen uptake, so it was concluded that the effect of exogenous substrates upon endogenous respiration differs among organisms from inhibition, to no effect, to enhancement (Dawes and Ribbons, 1962). Therefore it was decided to use E. coli as the seed for the microbiological method.

The use of pure cultures of bacteria (E. coli) as the seed for measuring BOD is in agreement with the views of LeBlanc (1974); better reproducibility was usually obtained with pure cultures of bacteria because organisms vary in their oxidative capacity for different organic substrates. Ruchhoft et al (1939) found that pure cultures of zoogloeal bacteria were more effective than activated sludge in purifying sewage. Dillingham, Knuth and Wessman (1958) in choosing an inoculum for their rapid manometric BOD test for pulp and paper mill wastes found that oxidation appeared to be more reproducible with the pure cultures of bacteria and reasoned that the composition of a heterogeneous seed would

affect the rate of oxidation. It was accepted that the oxidative capacity of such a pure culture acting over short intervals would not be expected to equal that of a heterogeneous microflora acting over a period of days (Montgomery, 1967).

The advantages of a pure culture inoculum are that oxygen uptake measurement would be more reproducible than an undefined inoculum or a mixed culture of known composition due to the variation of the oxidative capacity of different organisms (McKinney and Horwood, 1952; Zehnpfennig and Nichols, 1953; Tidwell and Sorrels, 1955), the rate of endogenous respiration would be comparatively low, less work and time would be involved in the preparation of pure cultures than mixed cultures of known organisms, and complications would not arise from the interference of protozoa, nitrifying bacteria, fungi, or photosynthesis (Montgomery, 1967).

In this study the river-water isolate of E. coli was grown in the Snyder and Koch medium in a large-scale batch culture fermenter, harvested by centrifugation, washed twice in distilled water, and the resuspended deposit was standardized.

The standardized seed was stored under different conditions in order to determine whether the optimum activity could be maintained for long periods. The freeze-dried seed gave lower levels of oxygen uptake (Figure 21) after a few weeks, possibly due to excessive drying of and the lack of a suspending medium
the cells during the freeze-drying process. ↑ This could decrease the number of surviving cells both immediately and after storage (Fry and Greaves, 1951; Ungar, Farmer and Muggleton, 1956). A minimum moisture content below which further drying of bacterial cells may become lethal was recognized by Fry (1966).

Storage of the standardized seed suspension at -25°C reduced its activity after 4 weeks (see Figure 22). However, when the seed was

stored in 2.0 ml amounts at -70°C or -195.8°C comparable oxygen uptake values to the fresh seed were obtained even after storage for 8½ months (see Figure 21 & 22). The activity of the stored seed at -70°C after the storage of 8½ months was slightly higher than the seed stored at -195.8°C, because of the lower temperature of -195.8°C used. These results agree with the studies on the freezing of stationary-phase E. coli cells, which are resistant to thermal shock below 0°C (Meynell, 1958). When cells were suspended in distilled water to minimize the effects of concentrating extracellular solutes, survival after freezing between -22 to -79°C and subsequent thawing approached or exceeded 90% (Harrison, 1956; Clement, 1961; Wagman and Weneck, 1963). This indicated that crystallization of the external medium did not kill E. coli. However, survival was less when the suspending medium was a solution of sodium chloride, nutrient broth or peptone (Major, McDougal and Harrison, 1955; Clement, 1961). When the suspending medium was sodium chloride, immediate survival appeared to be minimal and the storage death rate was highest at temperatures above the eutectic point of the medium (Clement, 1961; Lindeberg and Lode, 1963).

The retention of high activity in the stored seed at -70°C or -195.8°C was possibly due to another factor, that is the high concentration of cells in the seed, as it was found by Major, McDougal and Harrison (1955) that high concentrations of E. coli cells in a suspension was associated with a lower storage death rate as the cells were probably protected by compounds leaking from neighbouring cells.

The seed was frozen slowly because it was shown that the rate of freezing and thawing influenced the proportion of bacteria that survived (Kyes and Potter, 1939; Turner and B ayton, 1939; Weiser and Hargiss, 1946; Lovelock, 1953; Mazur, Rhian and Mahlandt, 1957). Sato (1954) studied the effect of freezing velocity, temperature, storage and warming

on the survival of E. coli. He found that rapid cooling was more damaging and that the survival of rapidly cooled cells tended to decrease as they were cooled to lower temperatures. This did not occur in slowly cooled cells because time was allowed for extracellular crystallization and gradual dehydration of the cell. Similar conclusions were reached by Araki and Nei (1962), Mazur (1961) and Mazur and Schmidt (1968) (Hagen,1971).

The activity of the standardized seed against known substrate solutions (1M glucose, 10 mM sodium pyruvate and sodium succinate) was tested. Increasing levels of oxygen uptake were found for increasing levels of substrate (see Figures 27, 28 and 29); this was in agreement with the findings of Krishnamurty (1964) and Karube et al (1977).

Several other attempts were made to obtain a bacterial seed inoculum for the rapid BOD measurement. No rapid oxygen uptake for the substrates was obtained by the river-water organisms, possibly attached to the polarograph magnets possibly because of the low number of adherent bacteria on the magnets. It would be too difficult to standardize the number of organisms attached to each magnet as this number will vary from one river to another and from one magnet to another. Although the addition of membrane filters, loaded with river-water organisms enabled BOD_5 values to be obtained in a shorter time (14-18 hr, see Figure 23), it was deemed too difficult to standardize the membrane, because of the variation in the number of organisms retained on each membrane. Similar problems occurred with the oxygen uptake measurement for the known substrate solutions when the membranes were used as seed (Figure 24 & 25). The use of cellulose decomposers was eliminated because cellulose decomposition in river-water occurred after 5 days. Consequently, the oxygen demand of cellulose decomposers would not be included in a BOD_5 test. The 5 day test was chosen because the

estimated time by the British Royal Commission on sewage disposal for the flow of water from source to estuary was 5 days (McGowan, Frye and Kershaw, 1913). Consequently, no further attempts were made to continue the identification of cellulose decomposers for use in a seed inoculum in the polarographic oxygen uptake measurements.

The major advances in the search for a stable bacterial seed, made in this study, were, first, the bacterial seed could be standardized by measuring the endogenous and exogenous oxygen uptake within 3 minutes in the oxygen polarograph. Secondly, the standardized seed could be stored for a period of 8½ months with the retention of 100% activity. Thirdly, one batch-culture of the E. coli produced enough seed for 3,000 rapid microbiological tests.

It should be stressed that for the first time, in the search for rapid BOD tests, it has been possible to obtain a large volume of standardized seed which could be stored at -70°C or -195.8°C for at least 8½ months with the retention of close to 100% activity.

It is concluded that the criteria required for a standardized seed are (i) a pure culture of E. coli (0142 K? H25) grown in Snyder and Koch (1966) medium in a fermenter for 10 hours at 37°C; (ii) harvested cells should be resuspended in a small amount of distilled water; (iii) the activity of the suspension should have a QO_2 for the endogenous oxygen uptake of 4.0-6.2 or a QO_2 for 0.3 ml of 1M glucose of 62-68; (iv) the seed should be stored in 2.0 ml amounts at -70°C or -195.8°C.

FACTORS AFFECTING THE MEASUREMENT OF BOD WITH THE OXYGEN POLAROGRAPH

A. Physical Effects

(a) pH values. The pH of organic wastes was reported by several workers to affect the rate of oxidation. Heukelekian and Gellman (1951) studied the effect of the initial pH value on the oxidation of 7

industrial wastes in respirometers. In general, oxidation was most rapid with an initial pH value between 6 and 8. Later studies confirmed that a pH range of 6-8 was satisfactory for many wastes (Sawyer, Frame and Wold, 1955; Anderson, 1964; Lamb et al, 1964; Karube et al, 1977). Therefore, it was decided to study the effect of pH on the rate of oxygen uptake in the present study.

The rate of oxygen uptake was independent at pH values over the range 6.5-8.5 and lower oxygen uptake levels were found at pH values below 6.5 and above 8.5 (see Figure 35). It was concluded that reproducible results in the rapid microbiological method could be obtained if the pH of the samples were between 6.5-8.5. Most samples of river-water or sewage effluent tested were within this optimum pH range of 6.5-8.5.

(b) Temperature. Temperature is known to accelerate the oxidation of organic wastes by microorganisms. In an effort to obtain a shorter test for BOD measurement with a given waste, it is desirable to accelerate the reaction by increasing the temperature (LeBlanc, 1974). Several workers in the field obtained a BOD test in a shorter time (eg. 2 days) by increasing the temperature of the BOD_5 test (20°C) (Zehmpfennig and Nichols, 1953; Tidwell and Sorrel, 1955, 1956; Orford and Matusky, 1959, see Figure 4). Tool (1967), using the Hach manometric apparatus for measuring the BOD of waste-water and glucose-glutamic acid solution, reported that by increasing the temperature to 35°C results comparable to the 5-day BOD at 20°C were obtained in 2.5 days.

Dillingham, Knuth and Wessman (1958) studied the effect of temperature on the oxidation of spent sulphite liquor by A. aerogenes in the respirometric method. Oxidation was markedly faster at 30°C than at 20°C or 25°C. Similar results were obtained by Young and Clark (1965a,b) using electrolytic respirometers for measuring the rate of oxygen uptake for sewage and glucose-glutamic acid solution; oxygen uptake was

approximately twice as fast at 35°C as at 20°C (Montgomery, 1967).

Karube et al (1977), using immobilized microorganisms in polyacrylamide gel in an oxygen electrode system, found an increase in the rate of oxygen uptake for organic substrates as the temperature was raised. In the rapid microbiological method the rate of oxygen uptake, for organic substrate by the standardized E. coli seed, was maximal with the working temperature of 37°C and lower levels of oxygen uptake were found with the temperatures of 30°C, 20°C and 10°C (see Figure 33). Therefore, it was decided to use the temperature of 37°C for all the polarographic tests, although this was considerably higher than the river temperature or the BOD_5 test temperature.

(c) Heating of samples. The effect of heat on river-water or sewage effluent sample's oxygen uptake was tested in the oxygen polarograph because, with the experiment of concentrating river-water, O_2 uptake was higher for the sample concentrated by heating than the sample which concentrated by membrane filtration (see Table 13). A polluted river-water sample, autoclaved at 121°C gave a slightly higher level of oxygen uptake. This increase in the oxygen uptake was maximal after 15 minutes heating and was possibly due to the release of simple compounds from the polymers available in the polluted river-water sample.

Oxygen uptake for a clean river-water sample (BOD_5 = 2.1 ppm) showed no increase in oxygen uptake, measured before and after autoclaving for 15 minutes. This result confirmed the above conclusion, because with the absence of organic pollutants in the clean river-water sample, no oxygen uptake increase was observed. Oxygen uptake for an autoclaved sewage effluent sample (BOD_5 = 28.4 ppm) showed a rapid increase during the first minute but immediately levelled off, which indicated a slight release of simple compounds from polymers available in the effluent sample (Figure 38).

The BOD_5 test does not include the oxidation of complex compounds and since there was only a slight increase of oxygen uptake after heating the samples, there seemed to be few advantages in making this a step in the rapid microbiological test.

B. Chemical Effects

(a) Effect of heavy metals and other toxic materials. A variety of heavy m etals may be present in waste-waters (Chen et al, 1974; Klein et al, 1974) which may also contain other toxic chemicals such as KCN. They may affect biological treatment processes, self-purification of streams, and BOD determinations (Scott, 1930; Kalabina et al, 1944; Heukelekian, 1947; Placak, Ruchhoft and Snapp, 1947; Krieger and Moore, 1949; Ingols, 1954, 1956; Heukelekian and Gellman, 1955; Mowat, 1976). The concentrations of metals at which inhibition occurs varied considerably from one metal to the other, for example mercury and silver were found to be more toxic than the other chemicals (Heukelekian, 1947; Ingols, 1954; Mowat, 1976). The toxicity of each metal is the function of the concentration of ionic form of that metal and not the total concentration of the metal (Allen, 1976). Similar conclusions were reached for lead (Davies et al, 1976) and zinc (Pagenkopf, 1976).

The toxic effects of 12 heavy metals and potassium cyanide were examined and all of them showed some inhibition of oxygen uptake by the bacterial seed (E. coli). On a ppm basis the toxicity of mercury and silver was higher than the other metals and KCN. Decreasing levels of oxygen uptake were found as the metal concentration increased, eg. Figure 40 shows decreasing levels of oxygen uptake with increasing the concentration of mercury. The concentration of metals which inhibit oxygen uptake in the rapid microbiological method was significantly higher than the concentrations which caused inhibition in the BOD_5 test.

For example, Mowat (1976) found that complete toxicity occurred at 1.0 ppm mercury in the BOD_5 test, while with the microbiological test no reduction in the oxygen uptake was observed for mercury until the concentration was 5.0 ppm, where 50% reduction was found (Figure 42). In general, for the rest of the metals and KCN, no considerable reduction in the oxygen uptake was observed up to the concentration of 20 ppm with the rapid microbiological test (Figures 43-53), while in the BOD_5 test toxicity was found at the concentration of 1-5 ppm. The order of toxicity for the metals and KCN, tested with the microbiological test, was roughly in the following order: $Ag^{+} > Hg^{++} > Fe^{+++} > Cu^{++} > CN^{-} > Al^{+++} > Zn^{++} > Cr^{+++} > Pb^{++} > Sn^{++} > Ni^{++} > Cd^{++} > Co^{++} > Pb^{++}$ (insoluble). No significant reduction in oxygen uptake was obtained with lead dioxide with the range of concentration used (0-40 ppm) due to the insolubility of lead dioxide in water, Figure 53; similar result was found by Mowat (1976).

The toxicity of heavy metals and potassium cyanide was lower in the rapid microbiological test than in the BOD_5 test, for the following reasons: first, the rapidity of the microbiological test (3-5 minutes) does not allow the metallic ions enough time to act on the bacterial cells of the seed. Secondly, the very high concentration of organisms in the seed reduces the number of metallic ions per bacterial cell. Thirdly, the high temperature of the test ($37^{\circ}C$) in the microbiological method decreases the toxicity of the chemicals on the organisms in the seed (E. coli). Van Eseltine and Rahn (1949) found that E. coli could grow in six times as much toxic chemical at $37^{\circ}C$ than at $15^{\circ}C$. This indicates the superiority of the rapid microbiological test over the other BOD methods.

(b) Concentration of utilizable substrate. River-water samples were concentrated ten-fold by either membrane filtration or by heat evaporation. The BOD_5, COD and oxygen uptake in the polarograph were determined before and after concentration. Both BOD_5 and COD results were higher for the

concentrated samples (Table 13a). Oxygen uptake for the concentrated samples, by both methods, was higher than the oxygen uptake for the unconcentrated sample (Table 13b). This result indicated that the level of organic matter in a polluted water directly affected the rate of oxygen uptake in the rapid microbiological method. As the level of organic substances in the unconcentrated sample was too low (low BOD_5 value = 6.0 ppm), a low level of oxygen uptake was obtained. After concentration (high BOD_5 value = 140 or 232 ppm), higher levels of oxygen uptake were obtained.

Subsequently, higher levels of oxygen uptake were obtained when, in this experiment, known substrate solution (0.3 ml of 1M glucose) was added to the river-water samples in the polarograph (Table 13b). This result also indicated that the level of organic substrate in a polluted water directly affected the rate of oxygen uptake in the rapid microbiological method.

C. Biological Effects

(a) Number of organisms in the sample. Oxygen uptake by river-water samples alone was measured in the polarograph. Although slight oxygen uptake was found in tests lasting 30 minutes, no considerable rapid oxygen uptake was observed because the number of active organisms present in the sample was too low (Figure 24 & 25). A slight increase in oxygen uptake was obtained after the addition of substrate, which confirmed the presence of low numbers of active organisms in the sample. The increased levels of oxygen uptake for a ten-fold concentrated river-water sample, with and without the addition of substrate also indicated that the number of active organisms in the sample affected oxygen uptake in the polarograph (Table 13b).

With the sewage samples, although considerable levels of oxygen uptake were obtained without the addition of seed (Figure 61) results were

variable, possibly due to the interference of toxic materials in some of the sewage samples (Rudolfs et al, 1950) or due to the stage of the growth of the organisms, as they passed through the logarithmic stage into the stationary phase and the oxygen uptake obtained was mainly the endogenous. Gaffney and Heukelekian (1961) found similar effects in the oxidation of acetate (10 g/l) in Warburg respirometers. When the organisms consisted of bacteria which had passed through the logarithmic phase into the stationary phase, the effect on the substrate was less although the number of acetate-oxidizing organisms per ml increased from 20 or 37 x 10^6 to 123 x 10^6.

The results showed that the number of active organisms in the sample also affected oxygen uptake measurement and consequently a standard seed was added in the rapid microbiological test.

(b) Concentration of seed. Variation in the oxygen uptake levels of organic substrate by different concentrations of the E. coli seed were obtained in the polarograph. Increasing levels of oxygen uptake were obtained with increasing seed concentration (Figure 31). Similar effects were found by Butterfield and Wattie (1938). They studied the rate of oxidation of a dextrose-peptone mixture in bottles by adding increasing concentrations of B. aerogenes (3.2 x 10^3, 3.2 x 10^4, 3.2 x 10^5 and 3.2 x 10^6/ml). There was a marked effect on the rate of oxygen uptake, the oxygen uptake consumed after 10 hours was 0.2, 0.34, 2.0 and 3.5 mg/l respectively. The oxidation of phthalic acid by a pure bacterial culture in a respirometer was studied by Wilson and Harrison (1960) who found that the rate of oxidation was increased by increasing the concentration of inoculum. Similar results were found by Bhatla and Gaudy (1966) and Karube et al (1977).

The uptake of oxygen in the polarograph was maximal with the concentration of 3.7 x 10^{11} E. coli organisms per ml. Oxygen uptake

increased linearly with the corresponding increase in the seed concentration (Figure 32).

Consequently, the concentration of the seed was standardized for the oxygen uptake measurement in the rapid microbiological method, and the highest concentration at which maximum levels of oxygen uptake were obtained, was monitored to maintain the maximum rate of oxidation.

From these investigations of the factors affecting oxygen uptake measurement in the polarograph, it was concluded that for the standard rapid microbiological test at 37°C, 0.1 ml of the E. coli seed (3.7×10^{11} per ml) must be used, and the pH of the sample should be within the pH range 6.5-8.5. The heavy metals (5.0 ppm) and other toxic chemicals (20 ppm) can be tolerated.

THE USE OF THE RAPID MICROBIOLOGICAL METHOD FOR THE EXAMINATION OF POLLUTED ENVIRONMENTS

A. River-Water Samples

There has been little published about the application of a rapid method for the BOD determination of river-water samples. The rapid methods were applied to the determination of BOD in waste-water samples from domestic or industrial sources. The test was later utilised in the design and control of sewage treatment processes.

Dillingham, Knuth and Wessman (1958) suggested a comparatively rapid manometric method for predicting the BOD_5 of pulp and paper wastes (see page 52). River-water samples were also included in the study. Samples were incubated in a Warburg respirometer at 37°C for 18 hours and also tested by the BOD_5 method. The correlation between the manometric and BOD_5 results was studied; an adequate correlation was obtained at the 95% confidence limits.

Similarly, Dillingham and Jose (1960) reported an evaluation of the 18-hour short-term pure culture manometric method for determining BOD

as a means of predicting long-term BOD (eg. the BOD_5 test). The correlation and precision of manometric and BOD_5 were determined for river-water and river-water to which various amounts of glucose were added. The correlation curves (Figure 8B) allowed the prediction of BOD_5 from 18-hours manometric BOD with about the same 95% confidence limits as when using the BOD_5 test alone.

In the present investigation, river-water samples were examined for their oxygen uptake measurement with the 5 min rapid microbiological test. The samples were collected monthly from 16 sites along the river Kelvin over a period of two years. Comparative BOD_5 determinations were done for the river-water samples (see Appendix 3). Higher levels of oxygen uptake in the polarograph were obtained for samples with comparatively higher BOD_5 values (Figure 54), which indicated that with the rapid microbiological test the BOD of a river-water sample could be estimated in 3-5 minutes.

It was concluded from the graphical presentation of the BOD_5 and QO_2 results for 254 river-water samples of low BOD_5 values (mean value 5.67 ppm), the QO_2 values were clustered between 0.66 and 6.0. Therefore, it would be possible, with the rapid microbiological test to determine that a particular water sample, with a QO_2 value of 6.0, would not be heavily polluted. The BOD_5 would be expected to be well below the government standard of 20 ppm.

The statistical analysis of the results of QO_2 and BOD_5 for the 254 river-water samples (Table 14) showed a significant relationship between the two variables at the level of 0.1%; the correlation coefficient (r_s) was +0.25, but see the comments on p.180.

Although the value of the correlation coefficient (r_s) seemed to be low for a significant correlation, the precise form of the relationship between BOD_5 and QO_2 data cannot be inferred from the value of r_s alone.

because this is calculated from ranks and not from the actual values of the variables with Kendall's coefficient of rank correlation method. Whilst the correlation coefficient (r_s) is a useful measure of closeness of association, it is not by itself a complete summary of the observations (Kendall, 1962). The same comment applies with even more force to the significance level of the coefficient. This level depends on the sample size (n) and the value of r_s; with large sample size (which is the case with the river-water samples; n = 254) quite small values of r_s can be significant, whereas with smaller samples larger values of r_s are needed.

When the results of QO_2 and BOD_5 for river-water samples were analysed statistically according to the seasonal or monthly results, some significant correlations were obtained (Table 14 and 15). A significant correlation was obtained between QO_2 and BOD_5 results in the winter months. There was less correlation found in the results for spring, summer and autumn. Similarly, the monthly results showed a significant correlation for the BOD_5 and QO_2 results for October-March but not for April-September.

The seasonal variation in the correlation between the results of BOD_5 and QO_2 for the river-water samples could be due to the statistical analyses methods, which rely on normally distributed data, because significant correlations were found for the normally distributed data obtained during the winter months. The normality test for the QO_2 data was determined by ranking the values of each season and plotting them against their corresponding rankit numbers. A line was drawn through the points with the mean value ($\bar{x}$) of the data and $\bar{x} \pm 1$ (Figure 57, 58 and 59); the results of this test are shown in Table 14.

The lack of correlation between the BOD_5 and QO_2 values for some of the river-water samples might also be attributed to other causes. First, slight differences in the amounts of soluble organic substrates in

the river-water samples might not be detected in the polarograph, eg. the differences between BOD_5 of 2.0 ppm and 3.0 ppm is too small to be detected in the 5 minutes microbiological test. This is also confirmed by the variations observed in the BOD_5 test, because the comparative BOD_5 values of this laboratory and the CRPB's for the river-water samples showed some considerable variations (see Appendix 3). Secondly, variation in the availability of soluble from non-soluble organic compounds during different seasons might cause differences between the two measurements. The variation in river temperature, from $-1^{o}C$ to $+6^{o}C$ for the winter months, and from 7 to $18^{o}C$ for the summer months, the amount of rainfall, and the quantity and quality of the sewage effluents discharged into the river, might affect the breakdown of non-soluble to soluble organic substrate over a period of 5 days but not 5 minutes. Thirdly, numerous environmental factors might interfere with the BOD_5 determination of the river-water samples, such as, 1) the abundant growth of filamentous algae and aquatic plants occurred during spring and summer in some parts of the river (mainly sites 13, 14 and 17) which may affect BOD_5 measurements (Fitzgerald, 1964); 2) differences in the microbial content of the river caused by the discharge of raw sewage from overloaded sewage works during periods of heavy storm, or the presence of toxic metals. Variation in the bacterial count of the river Kelvin during the different months of the year was reported in a survey of the pollution of the river Kelvin by Best and Stewart-Tull (1970, unpublished results), which supports this suggestion; 3) interference by protozoa was reported by Butterfield, Purdy and Theriault (1931), Zehnpfennig and Nichols (1953) and Busch (1958) (see page 26, 32 and 41 accordingly); 4) differences in the incubation temperature of the BOD_5 test from one laboratory to another. Fourthly, human errors may occur in the titration of the DO measurement in the BOD_5 determination or the dilution water used in the BOD_5 determination may be

contaminated with toxic substances, eg. copper in distilled water. The results obtained with such water are always low (APHA, AWWA, WPCF, 1965) because the copper has a toxic effect on the microorganisms in the sample.

Therefore, the author believes that the results obtained with the rapid microbiological test reflected a better picture of the level of pollution than the results of the BOD_5 test. The rapid microbiological test detects the oxidation rate of soluble organic substrates in a sample more accurately than the BOD_5 test which is affected by so many interfering factors.

B. Sewage Samples

As mentioned previously, most studies on BOD methods were applied to waste-water and sewage samples because the BOD test is an acceptable indicator of the quality of effluents and their load on the dissolved oxygen levels of the receiving water.

Zehnpfennig and Nichols (1953) showed a mean deviation of 2.2 per cent for the results of a special BOD_2 procedure as compared to 4.3 and 5.7 per cent for the normal BOD_2 and BOD_5 tests. No explicit explanation could be given by the authors for the improved reproducibility of the special BOD_2 test.

A comparison was done by Orford and Matusky (1959) between the results of a short-term BOD test in the dilution method with the BOD_5 test (see page 33). They concluded that for raw and settled domestic sewage the BOD_5 incubation period could be shortened to 2 days without seriously affecting the reproducibility of the results. Lee and Oswald (1954) compared the results from the Warburg and BOD_5 methods for fresh domestic and fresh synthetic sewage as well as for sterilized domestic and synthetic sewage. They cited several objections to the Warburg method (see page 52), including the fact that higher values were obtained with this procedure. Lamb et al (1964) attributed the limited application of

manometric techniques in the examination of industrial wastes to the amount of training and care required by staff in order to obtain meaningful results. Tool (1967) compared the results of a developed manometric method (2.5 days) (see page 51) with the BOD_5 results for wastewaters and found that the results agreed within $\pm$ 5 per cent. If the incubation temperature was increased to 35°C, comparable results ($\pm$ 5%) were obtained in 2.5 days for the BOD_5.

Several workers suggested the use of different respirometers for the BOD measurement of sewage (Montgomery and Gardiner, 1971; Tebbutt and Berkun, 1976) or a polarographic test (Krishnamurty, 1964; Eye and Ritchie, 1966). No correlation was found between the results of BOD_5 and the respirometric O_2 uptake of Montgomery and Gardiner for sewage seeded with settled sewage seed; Krishnamurty (1964) did not show any correlation for his rapid polarographic method results. However a good correlation was found between the results of the large-volume respirometer of Tebbutt and Berkun (1976) and the BOD_5 test for crude domestic sewage, and between the results of the membrane electrode system of Eye and Ritchie (1966) and BOD_5 for a standard substrate, no significant reduction in the time of the test was achieved by these methods. Therefore, it was concluded that a rapid method for measuring the BOD of effluent and sewage samples was still required and consequently, we decided to investigate the application of the rapid microbiological test to sewage samples. For this purpose, we examined crude sewage, primary settlement tank effluent and final effluent samples from sewage works.

The QO_2 and BOD_5 were measured for the sewage samples (see Appendix 4). The QO_2 values were higher for the crude sewage samples (high BOD_5 values) than for the primary settlement tank effluent samples (intermediate range BOD_5 values). Even lower QO_2 values were found for the final effluent samples (low BOD_5 values (Figure 60). The results

showed that the 5 min rapid microbiological method could be used to separate the crude sewage samples from the primary tank effluent samples and the latter from the final effluent samples. The results were highly reproducible for the 67 sewage samples due in part to the presence of a greater range of utilizable organic substrates.

It should be stressed that the QO_2 results for the sewage samples without the addition of seed were not reproducible with all the samples (Figure 61), due to the variation in the bacterial content of the samples and the possible heavy metals toxic effect towards the sewage organisms present in those samples. Similar findings in BOD tests were reported by several workers (Scott, 1930; Kalabina et al, 1944; Heukelekian, 1947; Placak, Ruchhoft and Snapp, 1947; Krieger and Moore, 1949; Ingols, 1954, 1956; Heukelekian and Gellman, 1955). But reproducible results were obtained after the addition of seed, because it was previously shown in this investigation (see page 202) that with the rapid microbiological test the toxicity of heavy metals was less interfering up to much higher levels than in the BOD_5 test.

A linear relationship was found between the results of the two tests (Figure 62) and it was also noted that there was clustering of QO_2 and BOD_5 results for untreated (crude sewage), partially treated (the effluent from the primary settlement tank), and final effluent samples.

The statistical analysis for the QO_2 and BOD_5 results of 67 sewage samples showed a highly significant correlation between the results of both tests at the level of $p = 0.1\%$. The correlation coefficient (r_s) was + 0.9398 with the linear regression method; the regression curve is shown in Figure 63.

The differences between BOD_5 of 20 ppm, 60-140 ppm and 160-265 ppm could be easily recognized within 3-5 minutes by the rapid microbiological test. On the other hand with the BOD_5 test it has been found by river inspectors that 7 days are required before the results are obtained.

It was concluded that if the QO_2 value is less than 5.0 the effluent is unlikely to be heavily polluted, and one would expect the BOD_5 to be below the government standard of 20 ppm, whereas, QO_2 values of 6-14 would indicate high levels of pollution with BOD_5 values of 40-265 ppm. This could be presented as a pollutional index for assessing the quality of sewage samples during any stage of the treatment process and the quality of the effluents (Figure 65).

GENERAL DISCUSSION

Over the past decade there have been numerous attempts to develop a rapid method for BOD measurement. Although a great variety of methods have been used, no acceptable procedure has emerged. The process of the BOD test is well documented so the continued development of the test has been attempted, either by accelerating the reaction with an increase in the temperature, or by increasing the number of organisms in the seed inoculum. Other workers have suggested the use of different manometric or respirometric methods. More recently the use of an oxygen electrode system, with the addition of a massive inoculum of pure or mixed culture of bacteria, was investigated. Unfortunately, the lack of reproducibility, economic cost, complexity, practicability for routine pollution control work, rapidity, and the lack of a significant relationship with the existing BOD_5 test, caused failure in the various attempts. However, the tremendous increase in the discharge of industrial and sewage effluents into rivers makes it essential to develop a new test for water pollution control.

Many workers (Caldwell and Langelier, 1948; Dawson and Jenkins, 1949; Heukelekian and Gellman, 1951; Hoover et al, 1951; Ludwig, Oswald and Gotaas, 1951) showed the difficulty of interpreting results obtained by manometric techniques because they were not comparable to those obtained by the BOD_5 method (Montgomery, 1967). Similar conclusions

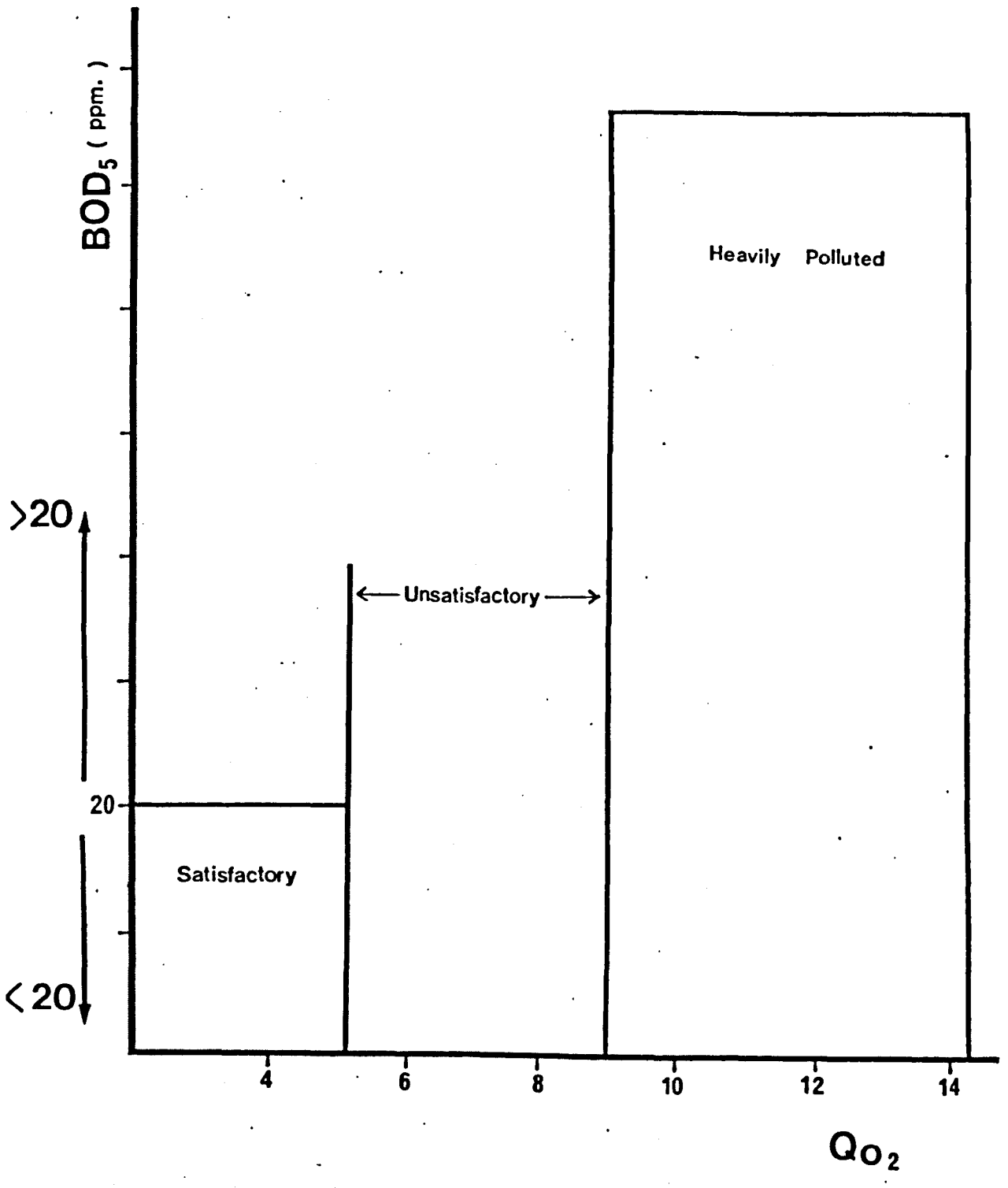


Figure 65. A pollutional index for the comparison of BOD_5 with the QO_2 results

were reached by Lee and Oswald (1954).

Montgomery (1967) reviewed the respirometric BOD method and believed it might be possible to develop a respirometric BOD test that involved the use of a pure culture inoculum as an extension of the work of Dillingham, Knuth and Wessman (1958). He also concluded that no single rapid respirometric test would satisfy all the requirements of the dilution method (Montgomery and Gardiner, 1971); Krishnamurty (1964) found the main difficulties were associated with the endogenous respiration and variations in potency of the seed.

A change in the seed concentration, the use of a pure culture or an increase in temperature are means of increasing the rate of biodegradation. Better reproducibility is usually obtained after the addition of a special inoculum, although bacteria are present in most wastes being analysed for BOD measurement (LeBlanc, 1974).

The initial aim in this study to prepare a standardized bacterial seed inoculum in large quantities was achieved. This could be stored for a long period with the retention of maximum activity.

During the course of this study the use of a polarographic oxygen electrode was also reported by Karube et al (1977). However, a very limited application of the method was shown as the seed, immobilized soil microorganisms in polyacrylamide gel, could only be stored for 10 days with retaining activity, and it was difficult to use this system to estimate the BOD of waste-waters which contain organic compounds undegraded by the seed microorganisms isolated from soil.

The rapid microbiological test developed in this study was superior to other BOD methods, because it fulfilled all the requirements of a rapid method:- 1. The method is theoretically valid. 2. The rapidity with which a result is obtained (3-5 min) is a great advantage in the field of pollution, and 3. The result is obtained immediately on

a chart recorder, which is by itself a means of preventing one source of human error. The advantages of the test will be apparent to river inspectors as it will enable them to determine within 3-5 minutes the quality of the effluent in a treatment plant, waste-waters or polluted waters. Such a facility will provide a scientific foundation for their decision to allow a discharge to be emptied into a river or to demand the close-down of a polluting operation. The test may also prove to be useful in the continuous monitoring of the quality of effluents at each stage of a manufacturing or treatment process.

The economic advantages of the method developed in this study are considerable. There would be considerable savings from the elimination of BOD_5 bottles and the storage space they require during a period of 5 days.

The oxygen polarograph apparatus used in this study could be made portable for direct field testing, for example by the connection of a small power generator to the instrument.

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APPENDICES

APPENDIX 1

MEDIA AND REAGENTS

A. Snyder & Koch Medium (1966)

It is a chemically defined medium (Snyder and Koch, 1966) based on the following constituents:

K_2HPO_4	2.3 g	
KH_2PO_4	0.78 g	
$(NH_4)_2SO_4$	1.0 g	
$MgSO_4.7H_2O$	0.1 g	
Sodium citrate	0.6 g	$C_3H_4(OH)(COONa)_3.2H_2O$
Distilled water	980 ml	

(sterilized by autoclaving at 121°C for 15 min.)

20 ml of glucose solution (10% w/v) were added. This solution was sterilized by membrane filtration. The pH was 7.

B. Nutrient Agar + Glucose

Nutrient agar, code CM3 (Oxoid, London; 28 g) was dissolved in 1.0 litre of distilled water by heating at 100°C and sterilized at 121°C for 15 min. The medium was cooled to 50°C and to each 200 ml of this medium 10 ml of glucose solution (20% w/v) was added. The glucose solution was sterilized by membrane filtration.

C. Dubos medium

Sodium Nitrate	0.5 g	
di-Potassium hydrogen phosphate	1.0 g	
Magnesium sulphate	0.5 g	($MgSO_4.7H_2O$)
Potassium chloride	0.5 g	
Ferric sulphate	traces	

dissolved in 1.0 litre distilled water.

The medium was dispensed in 10 ml amounts in 6 x 5/8" test-tubes. Filter paper strips were added to each tube as a source of cellulose, and sterilized by autoclaving at 121°C for 15 min.

D. Omeliansky medium

Peptone	1.0 g	
Di-potassium hydrogen phosphate	1.0 g	
Magnesium sulphate	0.5 g	($MgSO_4.7H_2O$)
Sodium chloride	traces	
Calcium carbonate	2.0 g	

added to 1.0 litre Distilled water.

The medium was dispensed in 10 ml amounts in 6 x 5/8" test-tubes, containing filter paper strips placed in as a source of cellulose, and sterilized by autoclaving at 121°C for 15 min.

E. Omeliansky Cellulose Agar

Cellulose powder (10 g/l) and agar (code L28, Oxoid; 15 g/l) were added to the liquid medium prior to sterilization.

F. Dilution Water. was prepared by adding the following reagents to freshly distilled water, according to the Analysis of Raw, Potable and Waste Waters, HMSO (Department of the Environment, 1972).

i - Ferric Chloride solution (0.125 g of $FeCl_3.6H_2O$ dissolved in 1.0 litre distilled water).

ii - Calcium chloride solution (27.5 g $CaCl_2$ dissolved in 1.0 litre distilled water).

iii - Magnesium sulphate solution (25 g $MgSO_4.7H_2O$ dissolved in 1.0 litre distilled water).

iv - Phosphate buffer stock solution (42.5 g KH_2PO_4 dissolved in 700 ml distilled water, 8.8 g sodium hydroxide was added, this solution gave pH 7.2, then 2 g ammonium sulphate added and diluted to 1.0 litre).

To each litre of distilled water, aerated by bubbling air through a filter at 20°C, one millilitre of each of the above stock solutions were added in sequence to yield the standard dilution water. Storage

vessels for dilution water were cleaned regularly with chromic-sulphuric acid mixtures.

G. Reagents for Dissolved Oxygen Determination: prepared according to the Approved Methods for the Physical and Chemical Examination of Water (Institution of Water Engineers, 1960).

i - Manganous sulphate solution (50 g of $MnSO_4.4H_2O$ dissolved in distilled water, and the volume adjusted to 100 ml).

ii - Alkaline iodide solution (70 g of KOH or 50 g of NaCl and 15 g potassium iodide (KI) were dissolved in distilled water, cooled and made up to 100 ml).

iii - Sodium thiosulphate solution (N/80 strength, accurately standardized 3.102 g/l).

iv - Sodium oxalate solution (2.0 g of $(COONa)_2$ dissolved in 100 ml distilled water).

v - Potassium permanganate solution (N/8 ; 19.755 g of $KMnO_4$ dissolved in 1.0 litre distilled water).

vi - Potassium fluoride solution (10 g of KF dissolved in 100 ml distilled water).

vii - Concentrated sulphuric acid (36N).

viii - Starch solution 10 g/l (used as indicator).

H. Reagents for Chemical Oxygen Demand Determination: prepared according to the Analysis of Raw, Potable and Waste Waters, HMSO (Department of the Environment, 1972).

i - Potassium dichromate (N/8) (6.129 g $K_2Cr_2O_7$ dissolved in distilled water and the volume adjusted to 1.0 litre).

ii - Ferrous sulphate (N/8) (34.75 g $FeSO_4.7H_2O$ dissolved in 100 ml of 25% (v/v) sulphuric acid, and diluted to 1.0 litre).

iii - Saturated silver sulphate sosution (1.0 g of Ag_2SO_4 added to 20 ml of 50% (v/v) H_2SO_4).

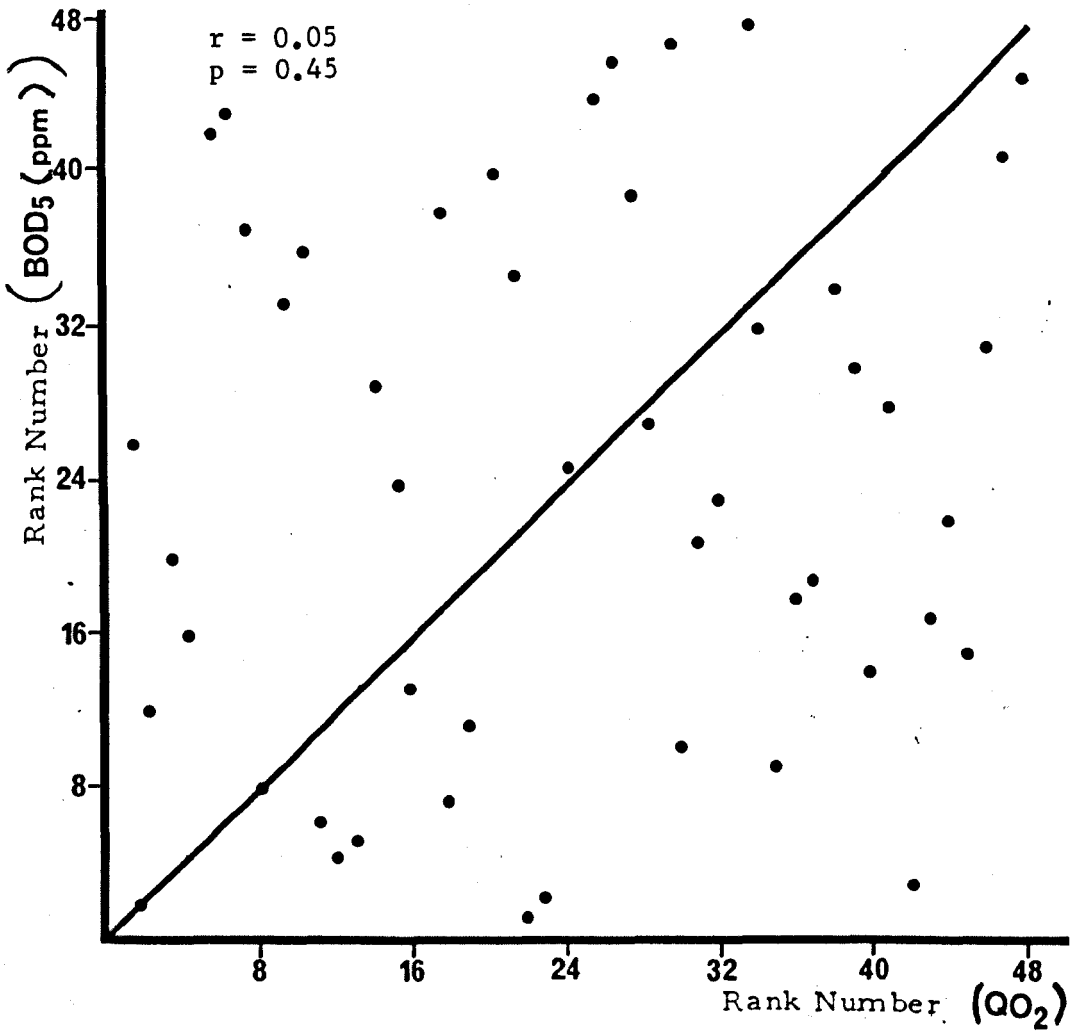
iv - Sulphuric acid (concentrated).

v - 1 : 10 - Phenanthroline-Ferrous sulphate indicator (obtained from BDH, England.)

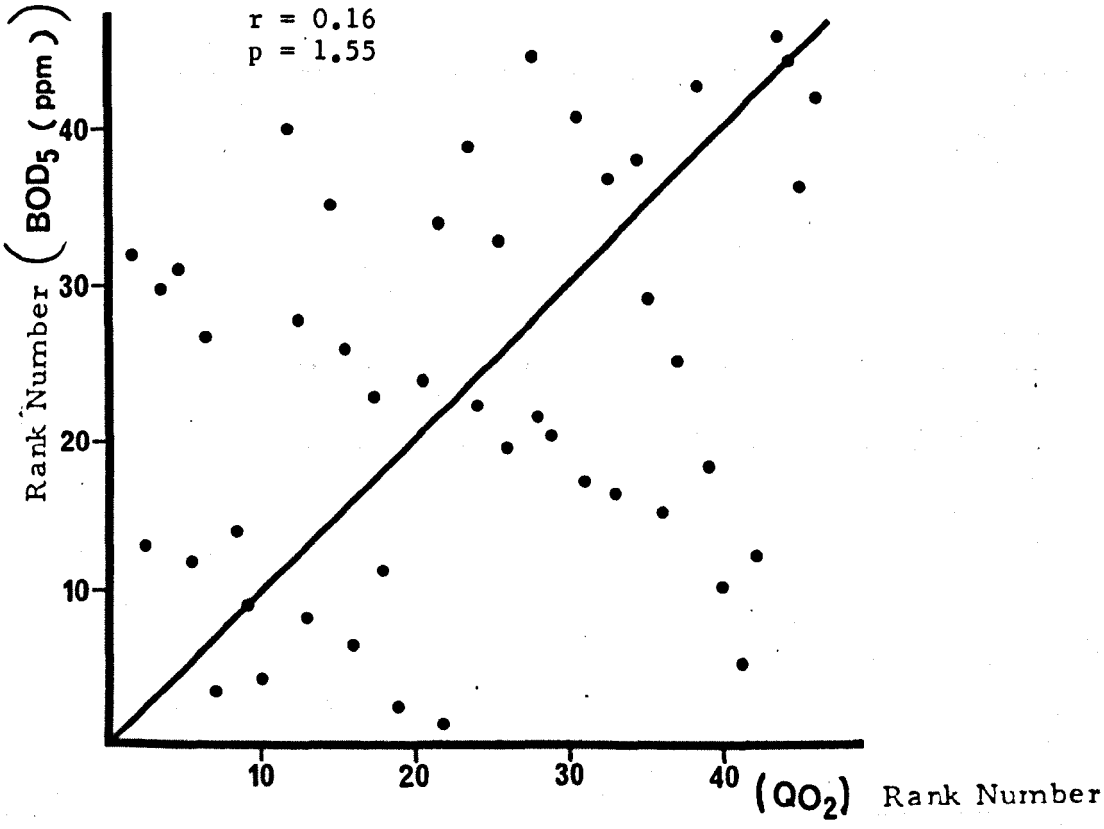
vi - Anti-bumping granules.

APPENDIX 2

Regression curves of BOD_5 v QO_2 for the river-water samples

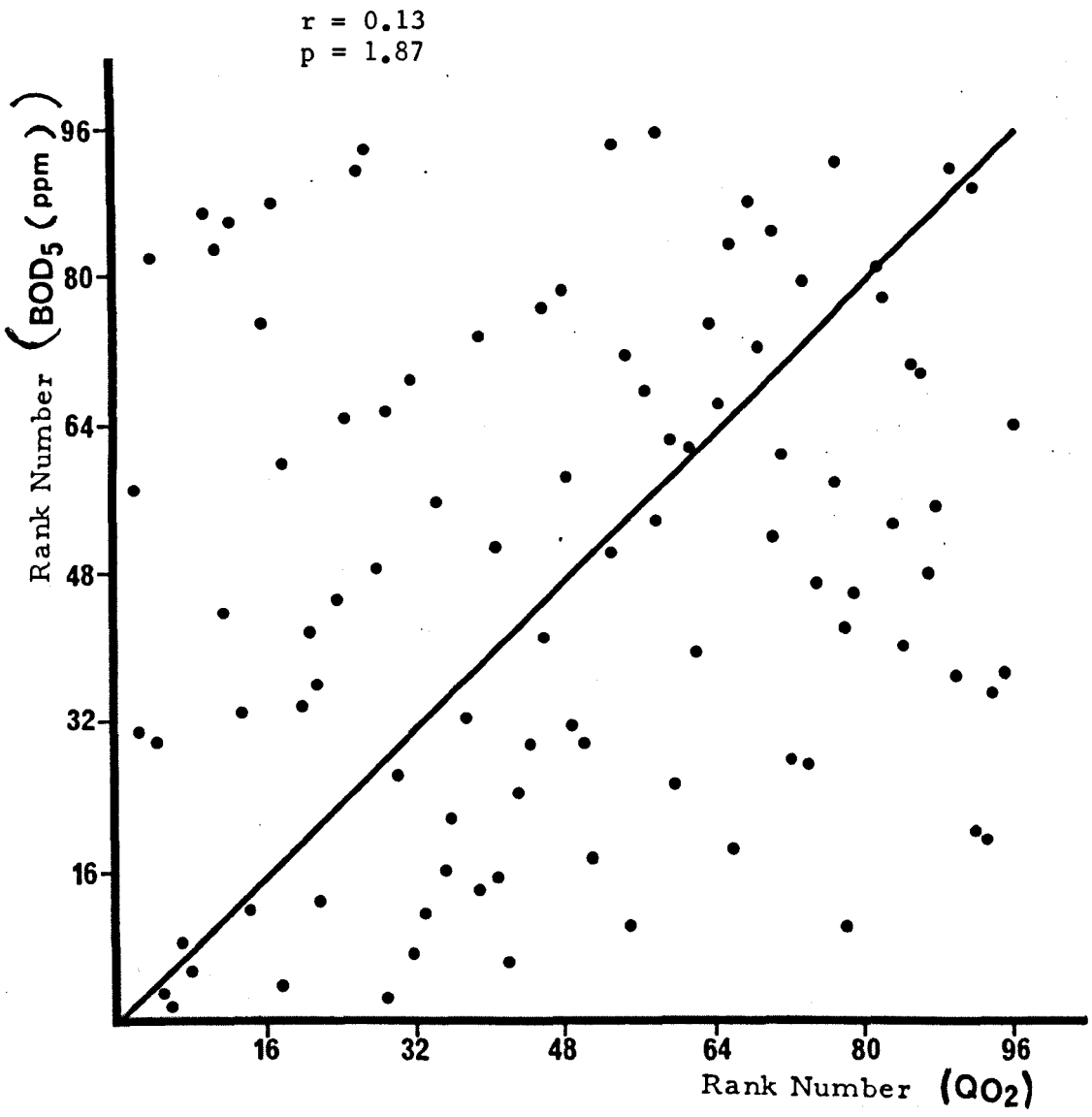


A. Spring months (March, April, May, 1979)

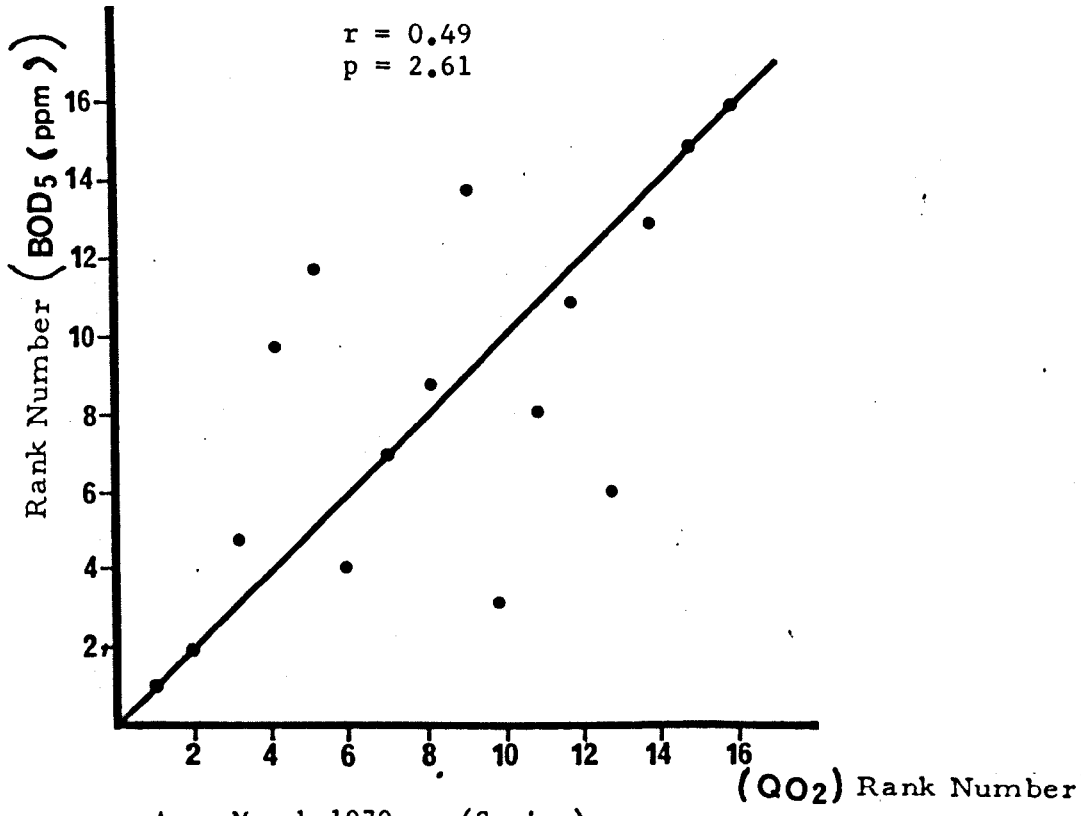


B. Summer months (August, 1978, June, July, August, 1979).

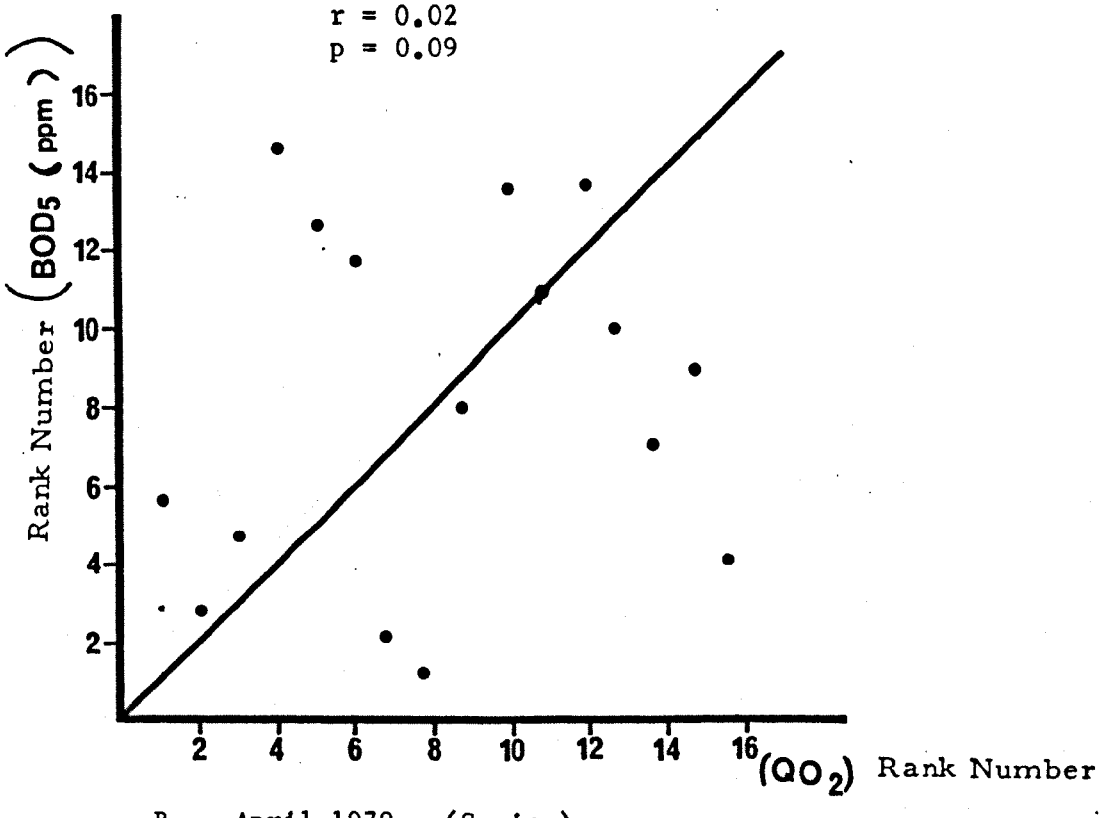
Appendix 2:1 Regression curve of BOD_5 v QO_2 for spring and summer months



Appendix 2:2 Regression curve of BOD_5 v QO_2 for autumn months (September, October, November 1978 and 1979)

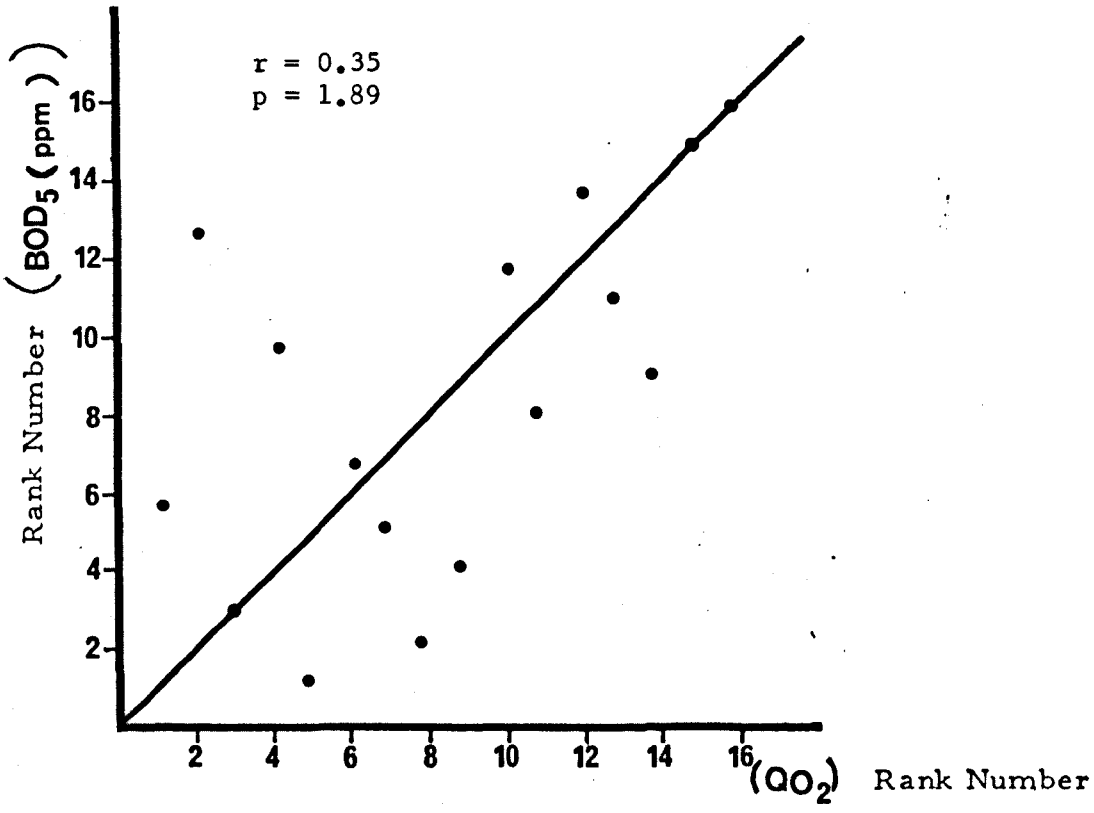


A. March 1979 (Spring)

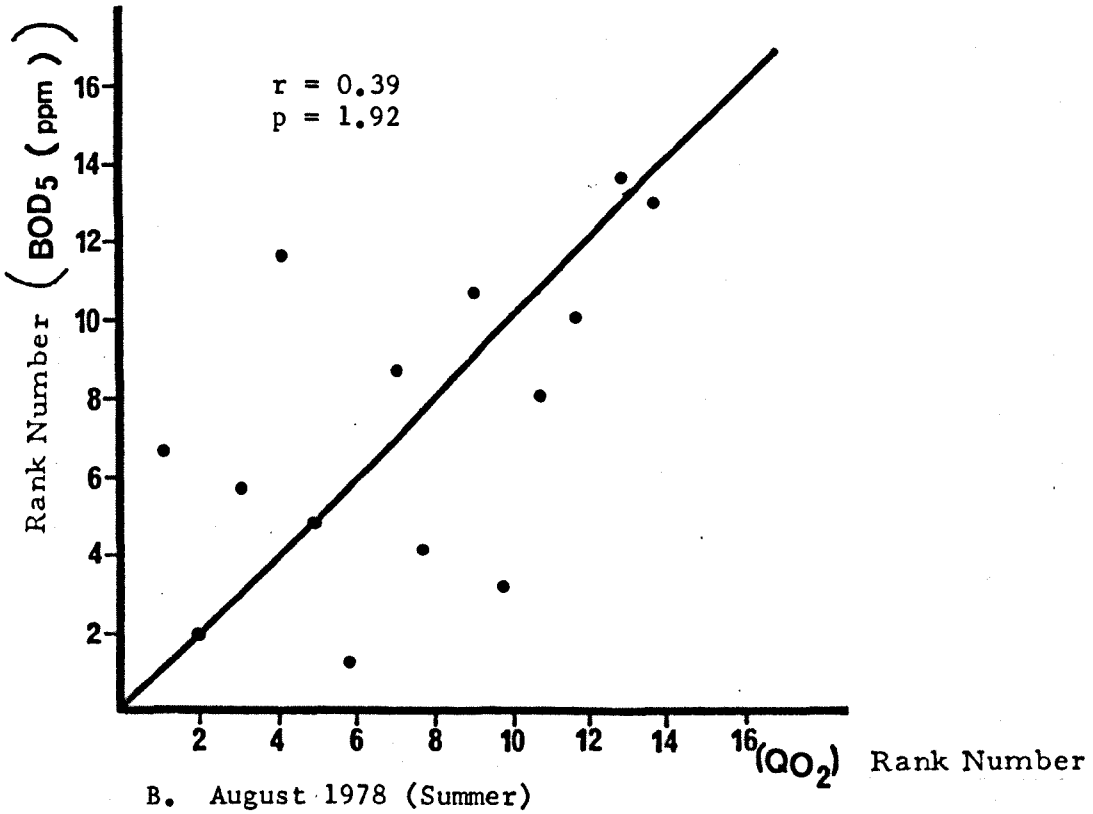


B. April 1979 (Spring)

Appendix 2:3 Regression curve of BOD_5 v QO_2 for March and April 1979 (Spring)

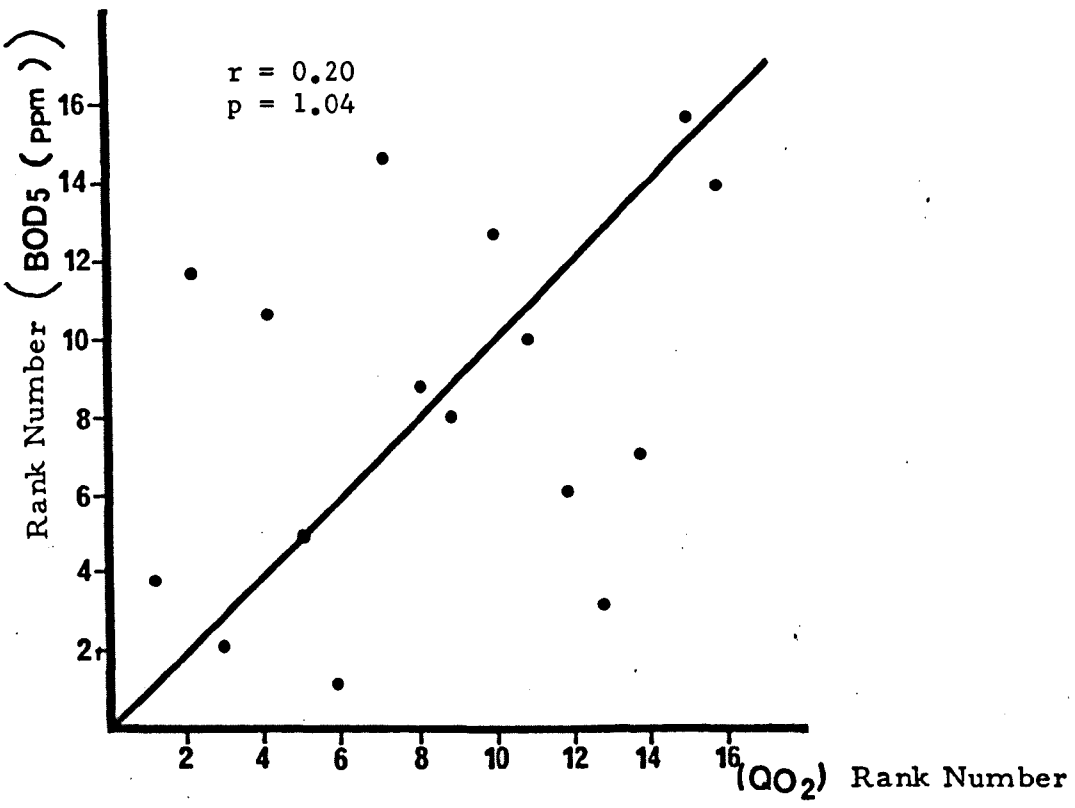


A. May 1979 (Spring)

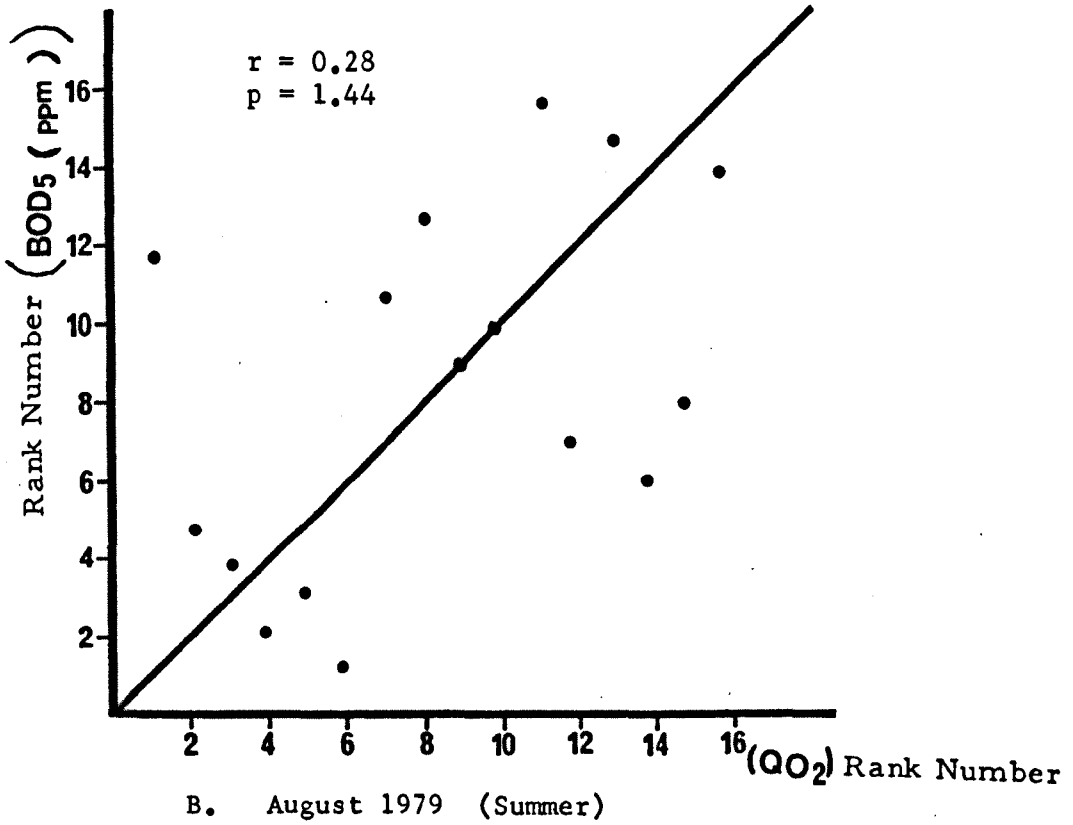


B. August 1978 (Summer)

Appendix 2:4 Regression curve of BOD_5 v QO_2 for May 1979 (spring) and August 1978 (summer)

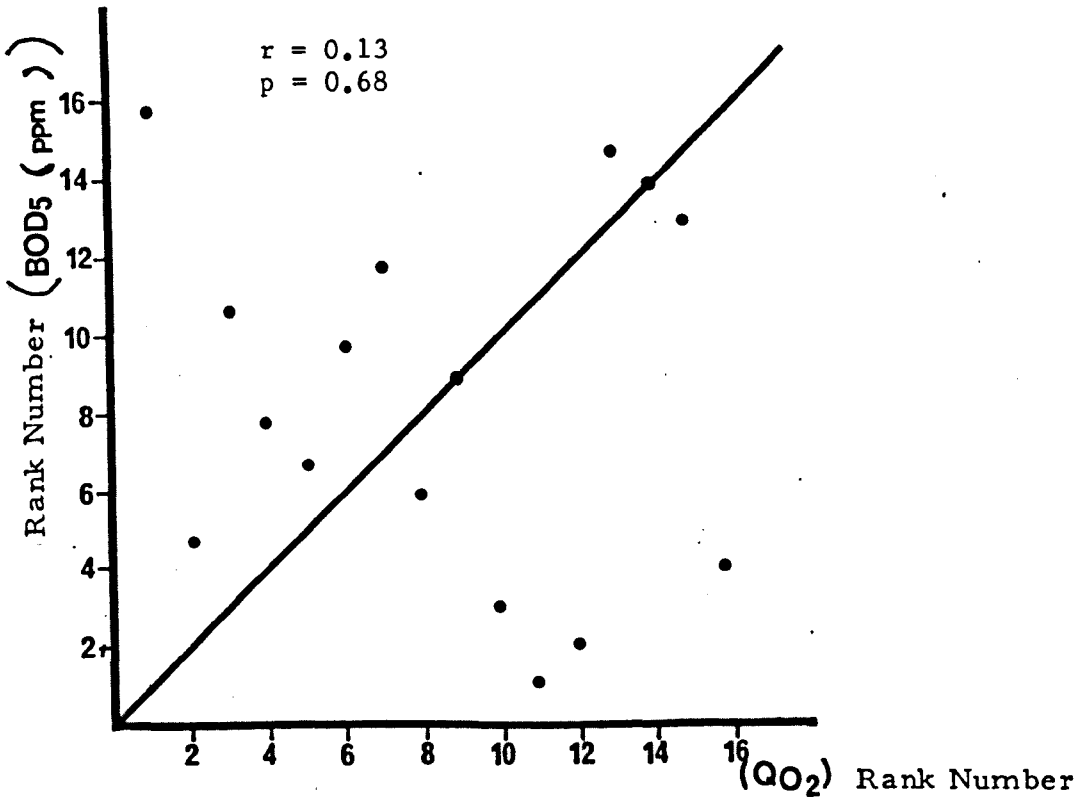


A. June 1979 (Summer)

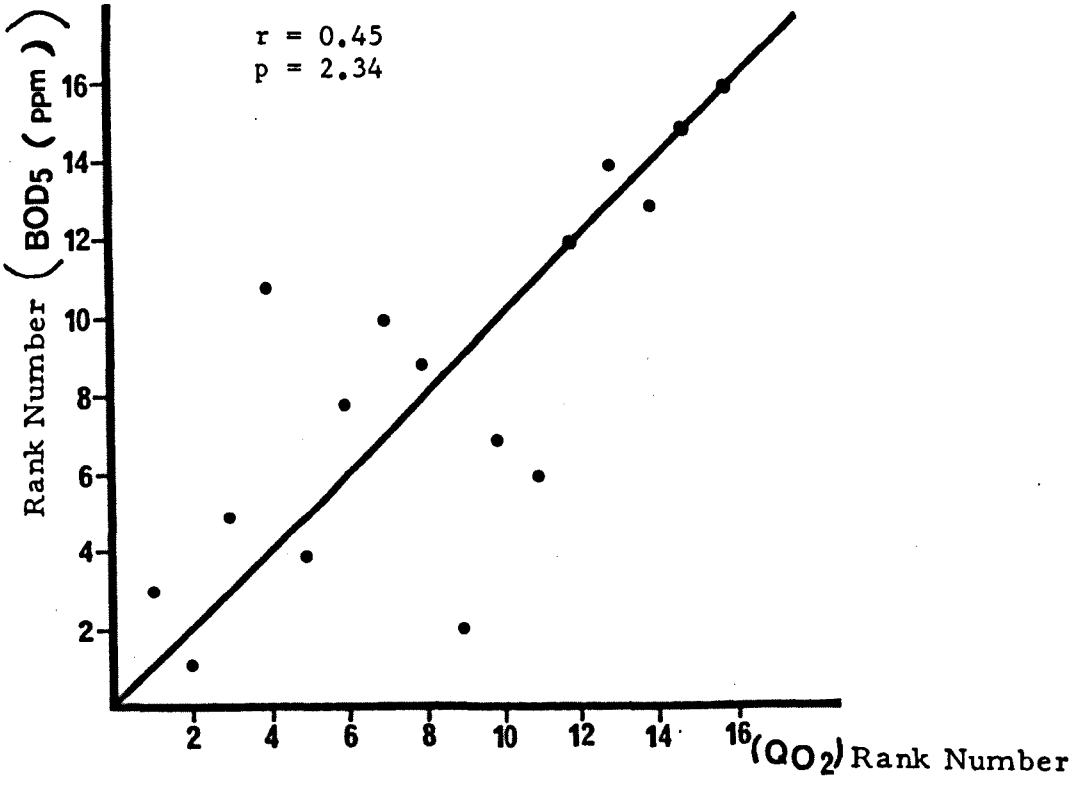


B. August 1979 (Summer)

Appendix 2:5 Regression curve of BOD_5 v QO_2 for June and August 1979 (Summer)

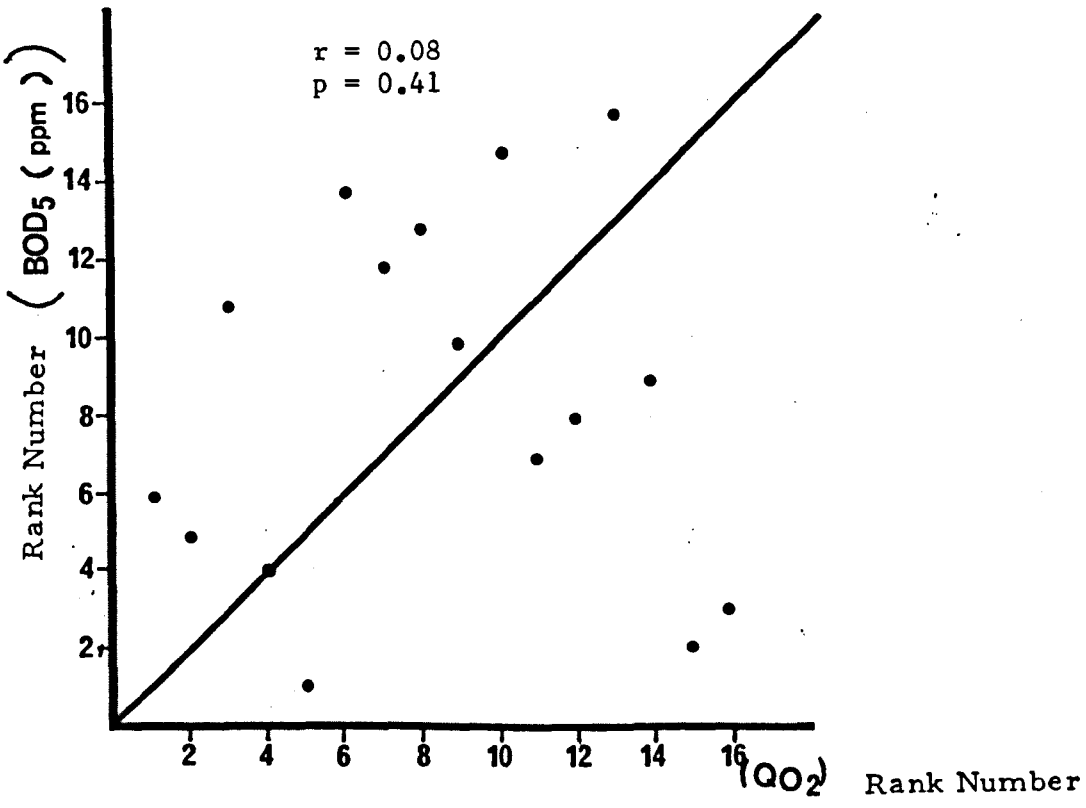


A. September 1978 (Autumn)

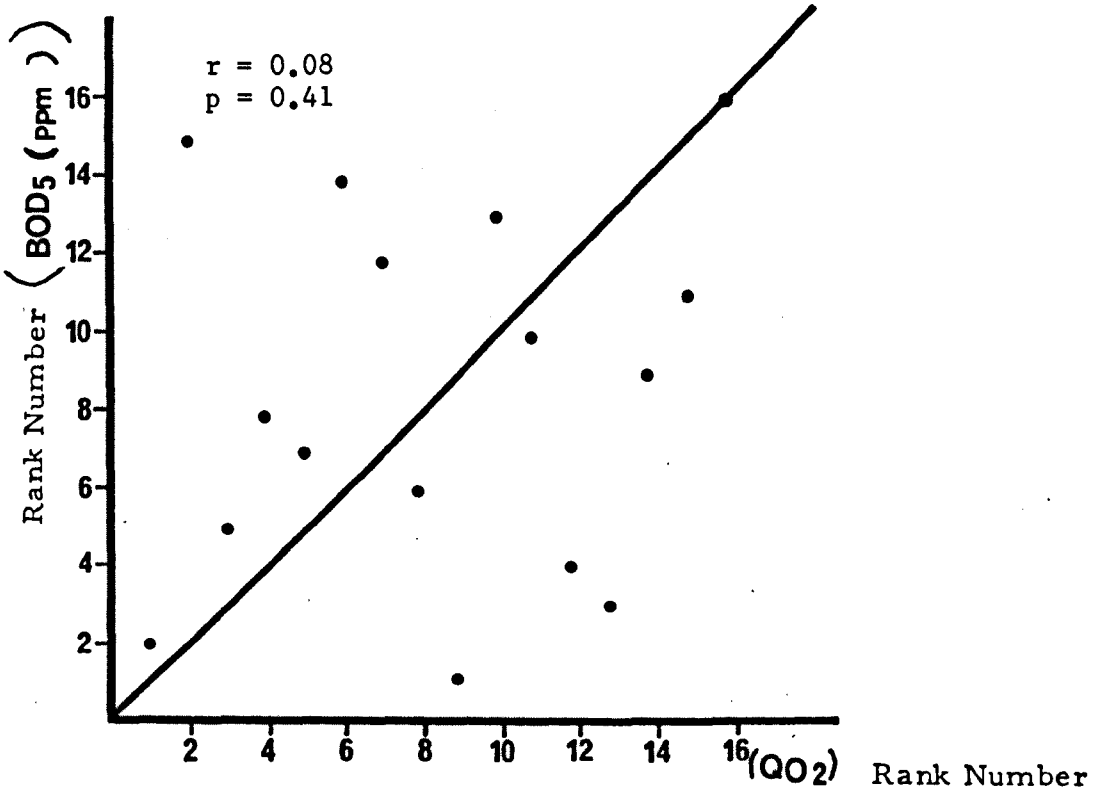


B. October 1978 (Autumn)

Appendix 2:6 Regression curve of BOD_5 v QO_2 for September and October 1978 (Autumn)

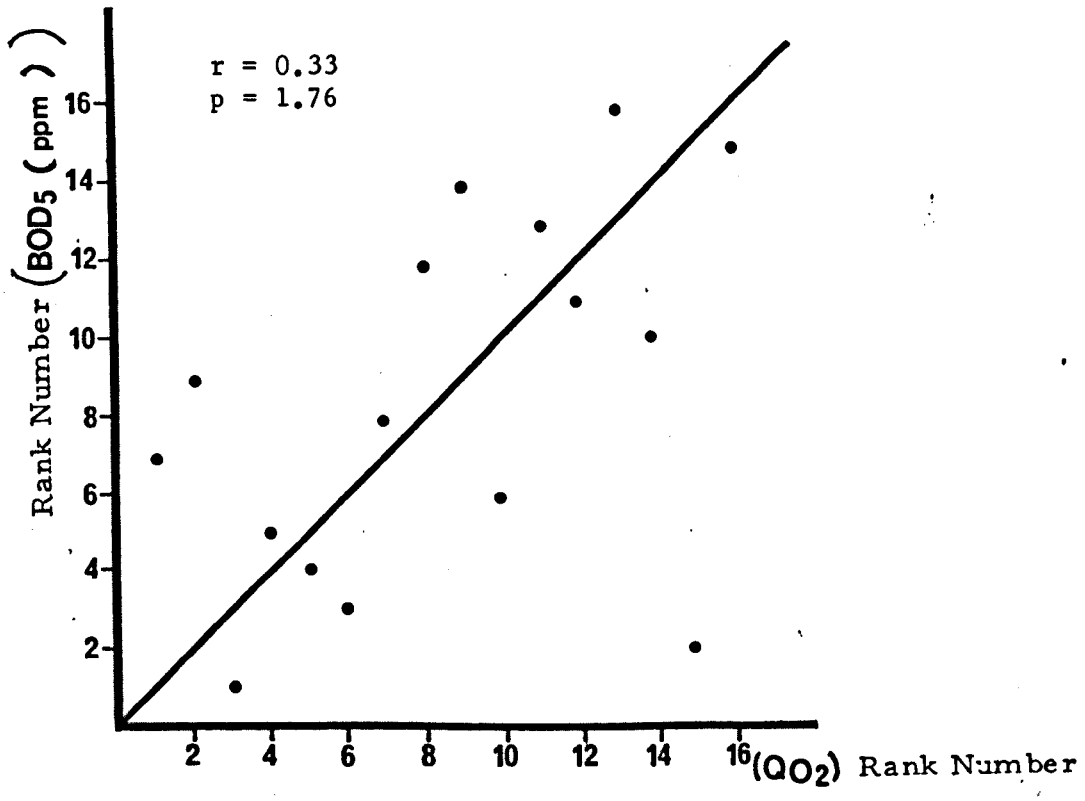


A. November 1978 (Autumn)

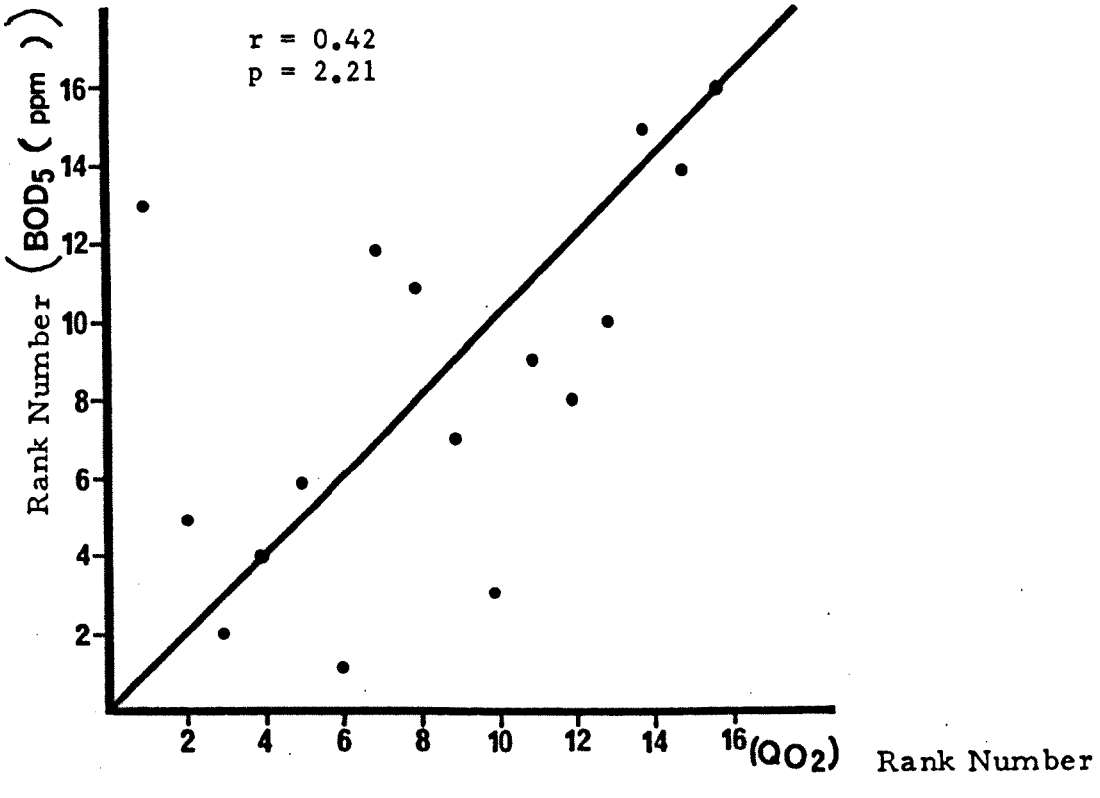


B. September 1979 (Autumn)

Appendix 2:7 Regression curve of BOD_5 v QO_2 for November 1978 and September 1979 (Autumn)

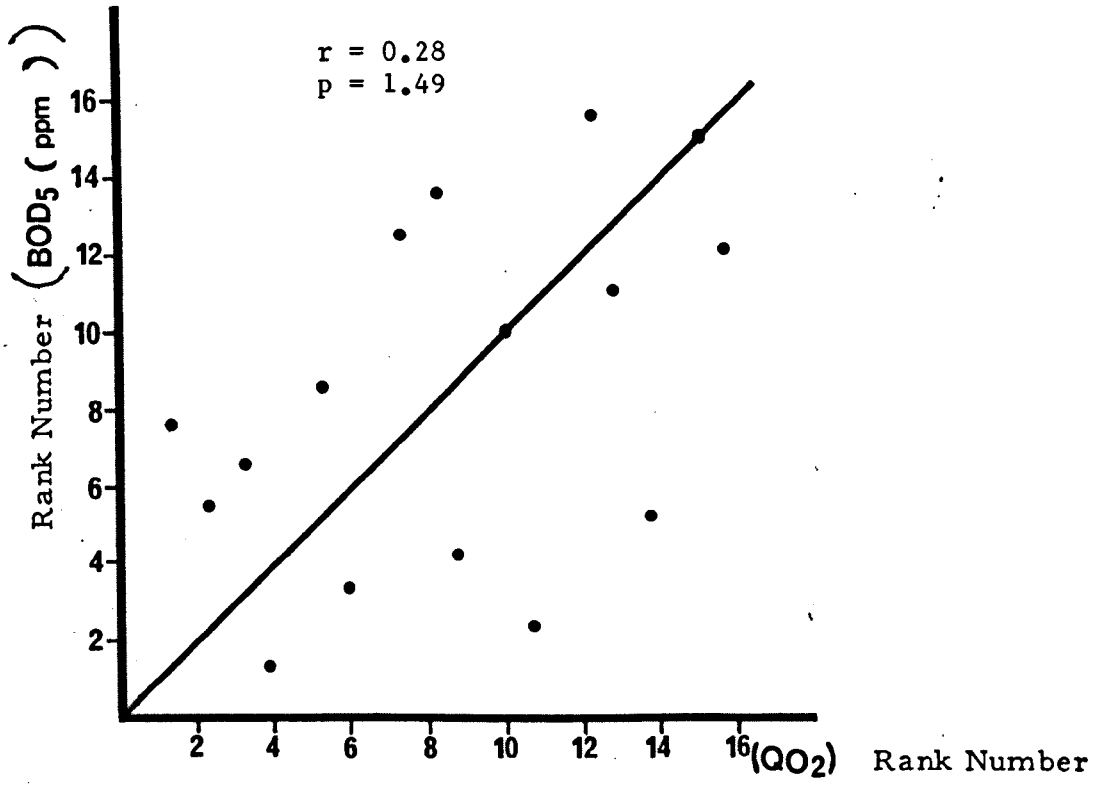


A. October 1979 (Autumn)

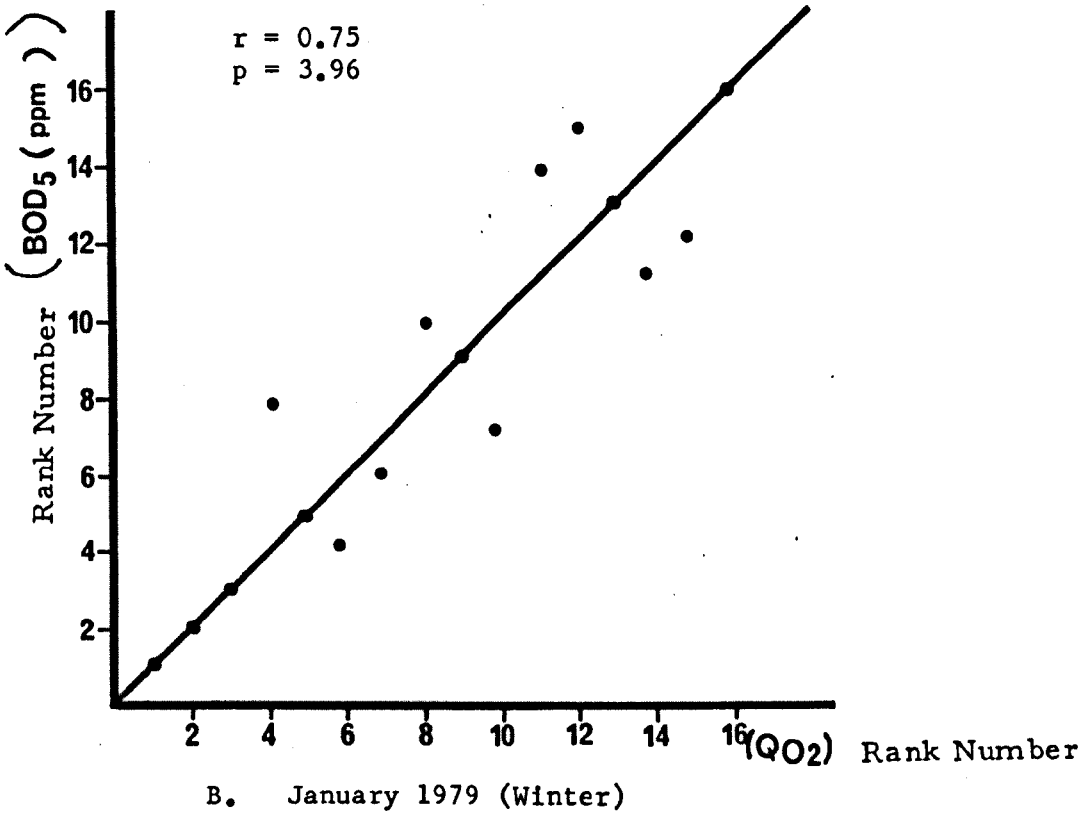


B. November 1979 (Autumn)

Appendix 2:8 Regression curve of BOD_5 v QO_2 for October and November 1979 (Autumn)

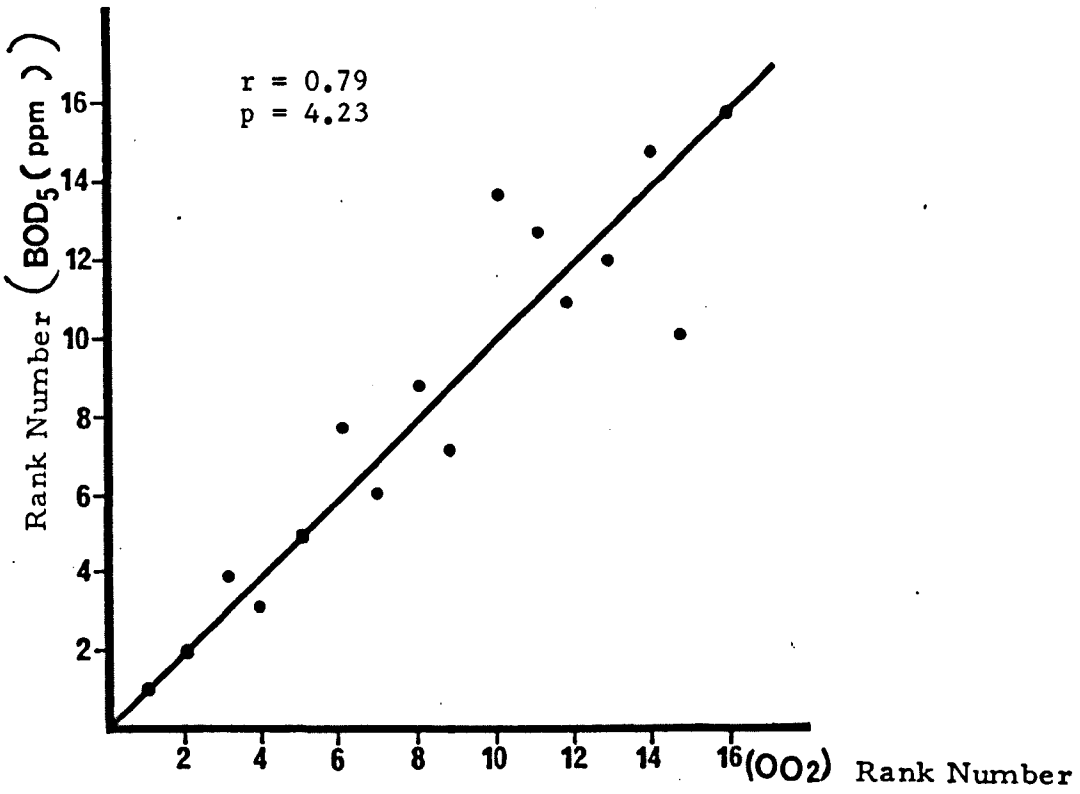


A. December 1978 (Winter)

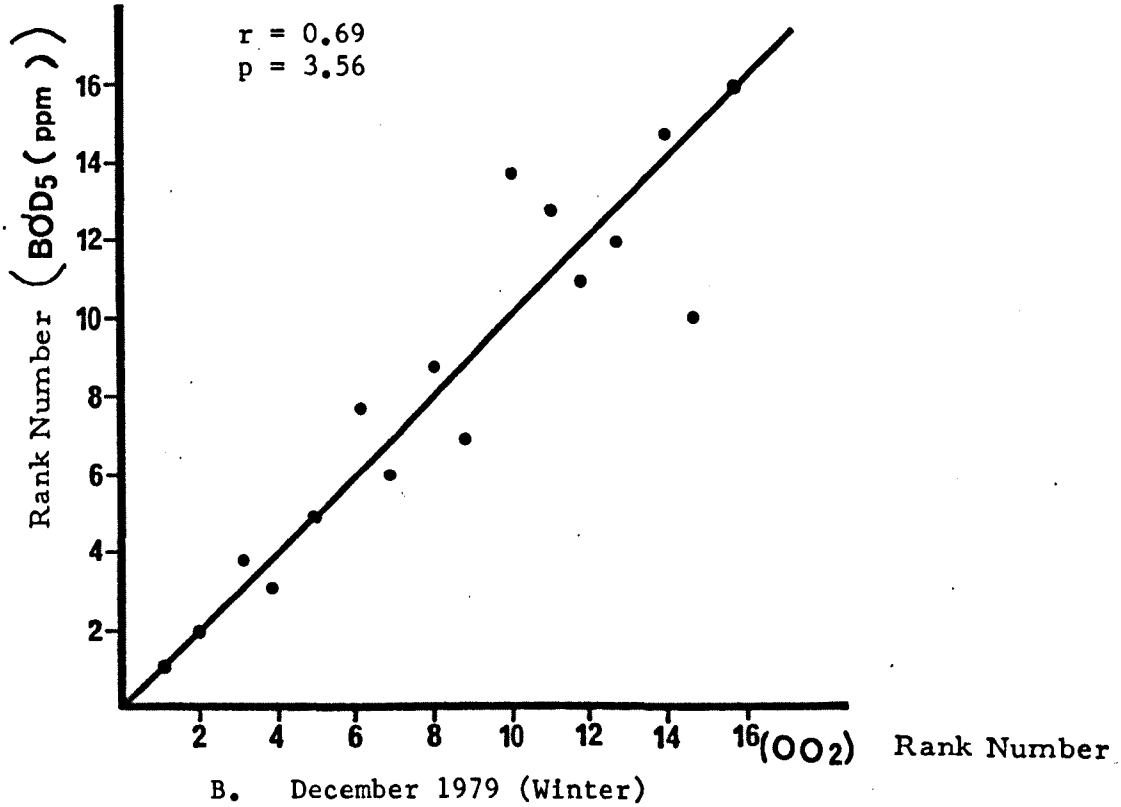


B. January 1979 (Winter)

Appendix 2:9 Regression curve of BOD_5 v QO_2 for December 1978 and January 1979 (Winter)



A. February 1979 (Winter)



B. December 1979 (Winter)

Appendix 2:10 Regression curve of BOD_5 v QO_2 for February and December 1979 (Winter)

APPENDIX 3

Raw data of chemical and biological results of river-water samples

River Kelvin

Date: 31/8/1978

Code	Site No.	BOD_5	•CRPB BOD_5	•S.S.	•PV	•DO	•NH_3-N	•PO_4-P	•$N(NO_2)$	•$N(NO_3)$	pH	O_2 uptake	QO_2	Temp. °C	Time	*Water Flow
1	S1	6.9	2.9	11	6.4	4.9	0.800	0.130	0.155	0.850	6.2	0.46	3.9	10	11.05	N
2	S2	3.9	1.8	3	5.4	5.6	0.050	0.080	0.080	0.450	6.4	0.46	3.9	10	11.20	"
3	S3	3.3	2.1	2	3.44	8	0.050	0.070	0.090	0.650	6.7	0.55	4.7	10	11.35	"
4	S4	12	8.5	17	5.6	3.4	6.300	0.745	0.125	0.200	6.6	0.54	4.6	12	11.35	"
5	S5	7.5	5.9	12	4.8	5.9	4.350	0.440	0.140	0.400	6.9	0.55	4.7	11	11.45	"
6	S6	17.6	7.1	11	6.6	7.1	1.900	0.185	0.410	2.350	7.1	0.81	6.9	10	11.50	"
7	S7	6	5.5	42	6.4	4.8	2.500	0.500	0.370	1.450	6.9	0.54	4.6	11	12	"
8	S8	4.5	4.4	7	1.8	10.4	0.100	0.255	0.055	1.650	6.8	0.67	5.7	10	12.05	"
9	S9	4.5	5.3	8	3.2	7.9	0.950	0.270	0.260	1.550	7	0.60	5.1	11	12.15	"
10	S10	6.9	6.1	29	6.4	8.5	0.100	0.640	0.290	5.600	7.2	0.67	5.7	12	12.20	H
11	S11	6	5.1	17	4.4	8.1	0.350	0.480	0.255	3.550	7.3	0.45	3.9	11	12.25	"
12	S12	8.4	18.5	12	5	6.9	0.700	0.670	0.305	4.050	6.8	0.75	6.4	11	12.45	"
13	S13	14.4	7.7	15	6	7.7	0.800	1.205	0.385	5.200	7.1	1.20	10.28	11	12.35	"
14	S14	11.7	17.6	13	4.8	5.5	1.050	0.860	0.380	3.850	7	0.66	5.66	12	1.0	"
15	S15	-	4.9	9	3.6	8.1	0.700	0.200	0.085	-	-	-	-	-	-	-
16	S17	-	6.9	6	3.8	5.9	1.000	0.610	0.475	3.850	-	-	-	-	-	-

*Water flow: L = low, N = normal, H = high, S = flooding.

•: Test done by the CRPB. (SS: suspended solid; PV: parmanganate value; DO: dissolved oxygen).

River Kelvin

Date: 15/9/1978

Code	Site No.	BOD_5	•CRPB BOD_5	•S.S.	•PV	•DO	•NH_3-N	•PO_4-P	•$N(NO_2)$	•$N(NO_3)$	pH	O_2 uptake	QO_2	Temp. °C	Time	*Water Flow
17	S1	5.4	5.2	13	7.6	9.2	0.025	0.065	0.005	1.050	6.5	0.29	4.35	10	11	H
18	S2	5.7	3.1	42	8.2	9.4	0.050	0.050	0.005	0.600	7	0.26	3.9	11	11.10	"
19	S3	4.8	6.1	23	8.8	9.3	0.063	0.050	0.005	0.600	7	0.28	4.2	11	11.25	"
20	S4	6	4.1	22	6.2	7.5	0.450	0.130	0.030	0.750	7	0.40	6	11.5	"	"
21	S5	6	6.8	25	9.2	8.9	0.100	0.060	0.010	0.650	6.9	0.38	5.7	11	11.30	"
22	S6	5.6	4.1	4.9	8.6	8.9	0.250	0.105	0.035	1.250	6.9	0.12	1.8	11	11.35	"
23	S7	4.8	3.7	46	8.6	8.6	0.125	0.075	0.015	0.700	7	0.23	3.45	11	11.45	"
24	S8	4.2	4.9	21	10.8	9.7	0.050	0.045	0.010	0.700	6.9	0.35	5.25	11	11.55	"
25	S9	4.5	3.5	43	9.8	9.2	0.087	0.090	0.015	0.800	6.8	0.35	5.25	11	12	"
26	S10	6.9	5.6	69	10	9.1	0.225	0.125	0.045	1.900	7.1	0.36	5.4	11	12.05	"
27	S11	4.5	5.3	44	9.6	9.4	0.100	0.085	0.025	1.050	7	0.32	4.8	11	12.15	"
28	S12	4.5	4.1	42	10	9.2	0.113	0.100	0.025	1.050	7	0.32	4.8	11	12.15	"
29	S13	13.6	7.5	11	5.4	8.3	0.150	0.410	0.110	2.800	7.2	0.11	1.65	11.5	12.30	"
30	S14	4.8	4.1	48	11.4	9.1	0.150	0.105	0.030	0.050	7.1	0.125	1.88	11	1.20	"
31	S15	5.4	3.7	23	9.4	9.7	0.050	0.045	0.015	0.800	7	0.23	3.45	12	1.30	"
32	S17	4.5	4.9	62	10.2	9.1	0.125	0.100	0.025	0.950	7	0.11	1.65	11	1.55	"

River Kelvin

Date: 30/10/1978

Code	Site No.	BOD_5	•CRPB BOD_5	•S.S.	•PV	•DO	•NH_3-N	•PO_4-P	•$N(NO_2)$	•$N(NO_3)$	pH	O_2 uptake	QO_2	Temp. °C	Time	*Water Flow
33	S1	2	5	5	5.6	5	0.560	0.145	0.035	0.800	6.8	0.135	1.62	10	10.55	N
34	S2	1.6	3.4	4	5	6.8	0.270	0.070	0.025	0.650	6.9	0.105	1.25	"	11.10	"
35	S3	0.6	4.7	4	4.2	8.2	0.280	0.020	0.030	0.750	7.1	0.105	1.26	"	11.20	"
36	S4	2.7	6	16	4.2	2.4	8.800	0.710	0.035	0.350	7.1	0.165	1.98	"	"	"
37	S5	2	4.6	9	4	6.3	3.100	0.320	0.040	0.600	7.2	0.105	1.26	"	11.25	"
38	S6	4.6	7.5	12	7.2	8.9	0.450	0.200	0.110	3.650	7.2	0.18	2.16	"	11.30	"
39	S7	3.6	6.3	11	4.6	6.8	1.860	0.215	0.080	1.800	7.2	0.135	1.62	"	11.40	"
40	S8	1.2	3	5	4	10	0.060	0.250	0.045	1.950	7.2	0.15	1.8	"	11.50	"
41	S9	6.4	7.6	13	6	8	1.050	0.245	0.065	1.750	7.2	0.21	2.52	"	11.55	"
42	S10	5.2	6.5	11	5.8	8.2	0.380	0.530	0.265	5.050	7.6	0.21	2.52	"	12	"
43	S11	4.2	7	11	4.8	8.4	0.670	0.320	0.130	3.000	7.4	0.135	1.62	"	12.05	"
44	S12	3.6	7.4	8	4.6	7.6	0.720	0.490	0.140	3.750	7.4	0.135	1.62	"	12.25	"
45	S13	21	20	42	9.2	7.5	1.340	2.150	0.490	10.100	7.2	0.27	3.24	11	12.15	"
46	S14	4.2	6.6	7	4.6	7	0.680	0.490	0.175	3.950	7.2	0.105	1.26	10	12.35	"
47	S15	20.7	17	27	8.4	7.6	1.500	0.330	0.075	0.850	7	0.24	2.88	"	12.50	"
48	S17	3.1	5.3	7	4.8	6.4	0.570	0.360	0.140	3.200	7.2	0.15	1.8	"	1.15	"

River Kelvin

Date: 15/11/1978

Code	Site No.	BOD_5	•CRPB BOD_5	•S.S.	•PV	•DO	•NH_3-N	•PO_4-P	•$N(NO_2)$	•$N(NO_3)$	pH	O_2 uptake	QO_2	Temp. °C	Time	*Water Flow
49	S1	5.9	6.5	40	7.4	8.9	0.190	0.070	0.015	1.400	6.8	0.14	1.85	10	11.30	S
50	S2	2.6	1.9	37	6.8	9.8	0.100	0.060	0.010	0.800	7	0.17	2.25	9	11.40	"
51	S3	2.3	2.2	35	7.8	9.7	0.090	0.055	0.010	0.600	7	0.095	1.26	"	11.50	"
52	S4	3	3.2	25	5.8	9	0.280	0.110	0.015	1.000	7	0.185	2.44	"	"	"
53	S5	5.6	5.4	33	5.4	9.4	0.080	0.055	0.010	0.750	7	0.125	1.65	10	12	"
54	S6	8.6	7.1	137	12.6	9	0.340	0.215	0.050	1.950	7	0.095	1.26	9	12.10	"
55	S7	6.2	3.4	66	8.6	9.3	0.220	0.115	0.025	1.000	7	0.11	1.45	"	12.15	"
56	S8	3.5	2.7	44	8.6	10.1	0.100	0.080	0.010	0.750	6.8	0.08	1.06	"	12.20	"
57	S9	5	3.2	55	9.4	9.7	0.140	0.090	0.015	0.900	6.8	0.05	0.66	"	12.25	"
58	S10	10.1	4.4	124	14.2	8.9	0.230	0.205	0.040	1.950	7	0.11	1.45	"	12.30	"
59	S11	5.3	5.8	69	11	9.7	0.160	0.125	0.025	1.150	7	0.11	1.45	"	12.40	"
60	S12	7.4	4.1	72	10.6	9.5	0.190	0.135	0.025	1.150	6.9	0.095	1.26	"	12.55	"
61	S13	17	10	64	6.8	9	0.250	0.195	0.050	2.600	7	0.125	1.65	10	12.50	"
62	S14	7	4.7	80	11.4	9.6	0.150	0.140	0.025	1.200	6.9	0.065	0.86	8	1.10	"
63	S15	3.5	2.5	40	9	9.7	0.140	0.080	0.010	1.000	6.8	0.05	0.66	9	1.20	"
64	S17	8.3	6.1	86	11	9.2	0.190	0.140	0.025	1.150	6.9	0.095	1.26	9	1.40	"

River Kelvin

Date: 14/12/1978

Code	Site No.	BOD_5	•CRPB BOD_5	•S.S.	•PV	•DO	•NH_3-N	•PO_4-P	•$N(NO_2)$	•$N(NO_3)$	pH	O_2 uptake	QO_2	Temp °C	Time	*Water Flow
65	S1	1.5	1.4	7	3.8	8	0.080	0.065	0.015	1.050	6.7	0.51	4.8	4	11.15	N
66	S2	2	1.7	5	3.8	10.1	0.010	0.055	0.010	0.900	7	0.40	3.78	"	11.25	"
67	S3	2	1.9	5	4	10.4	0.020	0.060	0.010	0.800	7	0.45	4.2	"	11.30	"
68	S4	17.7	11	25	4.8	5.1	6.800	0.360	0.035	0.900	7	0.54	5.1	"	"	"
69	S5	4	4.2	8	3.8	9.4	1.170	0.115	0.015	0.800	7	0.48	4.5	"	11.40	"
70	S6	2.4	4.4	9	5.8	10.1	1.575	0.120	0.065	2.150	7.4	0.62	5.8	"	11.45	"
71	S7	3	3.4	7	3.8	9.1	1.095	0.115	0.025	1.050	7	0.34	3.2	"	11.50	"
72	S8	1.2	1.	2	3.6	10.3	0.020	0.080	0.015	1.050	7.2	0.34	3.2	"	11,55	"
73	S9	3	3.1	5	4	9.7	0.680	0.105	0.020	1.150	7	0.33	3.1	"	12	"
74	S10	4.5	5.6	18	7.4	9.9	1.340	0.130	0.080	2.100	7.4	0.75	7.09	"	12.05	"
75	S11	2.8	3.9	9	5	9.9	1.010	0.015	0.045	1.400	7.2	0.33	3.1	"	1.30	"
76	S12	4.4	3.8	8	4.8	9.8	0.960	0.155	0.050	2.150	7.3	0.60	5.6	"	1.50	"
77	S13	9.3	7.3	5	4.8	8.5	2.200	0.785	0.125	5.650	7.3	0.63	5.9	6	1.40	"
78	S14	5.1	3.9	3	4.8	9.4	0.940	0.200	0.045	1.950	7.2	0.42	3.9	4	2	"
79	S15	8.2	7.2	18	6.8	10.6	0.470	0.125	0.020	0.900	7.1	0.42	3.9	3	2.05	"
80	S17	3.4	3.2	7	5.4	9.5	0.850	0.175	0.040	1.850	7.2	0.34	3.2	4	2.25	"

River Kelvin

Date: 29/1/1979

Code	Site	BOD_5	•CRPB BOD_5	•S.S.	•PV	•DO	•NH_3-N	•PO_4-P	•$N(NO_2)$	•$N(NO_3)$	pH	O_2 uptake	QO_2	Temp °C	Time	*Water Flow
81	S1	3.4	3.0	6	3.4	8.7	0.24	0.065	0.010	1.100	6.9	0.33	4.42	1	11.40	N
82	S2	0.8	2.1	9	3.4	10.6	0.15	0.050	0.005	1.100	7.1	0.26	3.43	1	11.45	"
83	S3	1.2	2.0	8	2.4	11.9	0.19	0.030	0.005	1.00	7.2	0.29	3.83	1	12	"
84	S4	23.1	31.0	45	7.8	5.8	10.60	0.660	0.065	0.700	7.1	0.45	6.01	1	"	"
85	S5	11.1	9.2	15	3.4	10.2	2.70	0.155	0.015	0.950	7.2	0.38	5.02	1	12.05	"
86	S6	3.0	2.7	0	3.4	11.7	3.15	0.105	0.040	2.550	7.6	0.35	4.62	0	12.15	"
87	S7	4.2	4.6	15	3.4	9.9	1.80	0.145	0.015	1.400	7.2	0.36	4.82	1	12.20	"
88	S8	1.6	2.0	4	1.4	12.4	0.80	0.270	0.010	1.600	7.3	0.29	3.83	1	12.30	"
89	S9	2.8	3.1	14	2.8	10.5	1.29	0.160	0.015	1.550	7.2	0.33	4.42	1	12,35	"
90	S10	3.0	3.6	14	3.8	11.5	4.30	0.370	0.055	2.650	7.7	0.36	4.82	1	12.40	"
91	S11	2.6	3.1	18	3.4	10.7	2.55	0.240	0.030	2.150	7.4	0.34	4.52	1	12.45	"
92	S12	6.0	5.9	13	4.0	10.5	2.70	0.365	0.045	2.600	7.4	0.41	5.42	1	1.10	"
93	S13	7.8	7.6	9	4.2	9.5	3.85	1.650	0.105	6.950	7.3	0,38	5.02	3	12.55	"
94	S14	4.2	4.0	12	3.0	10.1	3.0	0.270	0.045	2.700	7.3	0.35	4.62	1	1.15	"
95	S15	7.8	9.1	22	5.8	11.5	1.22	0.110	0.010	1.050	7.1	0.36	4.82	1	1.20	"
96	S17	5.4	3.9	11	3.8	10.3	2.40	0.255	0.035	2.450	7.5	0.38	5.02	1	1.35	"

River Kelvin

Date: 16/2/1979

Code	Site No.	BOD_5	•CRPB BOD_5	•S.S.	•PV	•DO	•NH_3-N	•PO_4-P	•$N(NO_2)$	•$N(NO_3)$	pH	O_2 uptake	QO_2	Temp °C	Time	*Water Flow
97	S1	3.4	-	8	2.6	8.5	0.640	0.070	0.025	0.800	6.8	0.29	4.79	0.0	11.55	N
98	S2	2.8	-	5	1.8	9.9	0.490	0.035	0.020	0.800	7	0.25	4.21	0.0	12.05	"
99	S3	3.4	-	9	1.6	11.4	1.020	0.040	0.020	0.800	7.1	0.27	4.54	-1	12.20	"
100	S4	23.6	-	55	8	5.2	14.600	0.510	0.050	0.300	7	0.37	6.19	0	"	"
101	S5	8.8	-	21	3.2	9.6	5.800	0.260	0.030	0.550	7.2	-.33	5.48	0	12.25	"
102	S6	6	-	10	3	12	3.600	0.160	0.055	2.100	7.4	0.30	4.95	-1	12.35	"
103	S7	6.6	-	25	4.2	9.9	4.400	0.130	0.030	0.950	7.4	0.34	5.70	0	12.40	"
104	S8	3.6	-	9	0.8	13.2	0.710	0.185	0.045	1.950	7.4	0.27	4.54	0	12.55	"
105	S9	3.6	-	14	1.8	11.4	2.010	0.175	0.040	1.450	7.2	0.29	4.82	0	"	"
106	S10	10	-	14	4.2	11.6	6.800	0.690	0.060	3.350	7.5	0.33	5.45	0	1.0	"
107	S11	6	-	12	2.2	11.4	4.100	0.350	0.045	2.250	7.3	0.31	5.20	-1	1.05	"
108	S12	6.2	-	11	3.8	11	5.200	0.560	0.075	2.800	7.2	0.36	5.95	-1	1.20	"
109	S13	11.7	-	8	4.8	10.5	7.700	1.750	0.115	6.600	7.2	0.35	5.86	1	1.15	"
110	S14	6.3	-	10	3.4	10.5	5.100	0.535	0.060	3.100	7.3	0.33	5.48	-1	1.35	"
111	S15	5.4	-	12	5.8	12.3	2.160	0.330	0.020	0.800	7.1	0.31	5.20	"	1.40	"
112	S17	5	-	12	3.6	10.6	40.100	0.620	0.055	2.950	7.4	0.30	4.95	"	2.0	"

River Kelvin

Date: 19/3/1979

Code	Site No.	BOD_5	•CRPB BOD_5	•S.S.	•PV	•DO	•NH_3-N	•PO_4-P	•$N(NO_2)$	•$N(NO_3)$	pH	O_2 uptake	QO_2	Temp °C	Time	*Water Flow
113	S1	4.4	4.4	7	4	9.7	0.400	0.040	0.010	0.800	7.1	0.47	6.5	2	11.35	N
114	S2	2.4	2.1	4	2.6	12.1	0.100	0.002	0.005	0.850	7.3	0.41	5.7	1	11.45	"
115	S3	2.0	2.3	3	2.4	12.1	0.150	0.002	0.005	0.700	7.4	0.30	4.05	1	11.55	"
116	S4	23.4	20.0	35	6.8	5.8	10.400	0.580	0.045	0.950	7.3	0.56	7.7	2	"	"
117	S5	5.0	5.6	8	3	11	1.710	0.130	0.015	1.000	7.4	0.41	5.7	1	12	"
118	S6	6.3	6.7	9	3.6	13.1	1.440	0.060	0.050	2.600	7.7	0.45	6.2	1	12.05	"
119	S7	3.0	3.8	10	2.8	11.1	1.340	0.090	0.015	1.650	7.4	0.385	5.3	1	12.15	"
120	S8	2.0	2.3	5	1.2	12.5	0.330	0.150	0.020	2.400	7.5	0.30	4.05	1	12.25	"
121	S0	3.6	3.0	8	2.6	11.5	0.960	0.080	0.015	1.950	7.4	0.33	4.6	1	12.30	"
122	S10	9.8	6.9	21	5.6	11.7	2.230	0.210	0.060	2.800	7.9	0.47	6.5	1	12.35	"
123	S11	5.0	4.9	14	3.6	11.7	1.550	0.150	0.040	2.400	7.6	0.385	5.3	1	12.40	"
124	S12	5.4	5.4	17	3.8	11.3	1.560	0.220	0.050	2.750	7.7	0.385	5.3	1	12.55	"
125	S13	18.9	14.0	13	5.2	10.4	4.650	1.500	0.090	4.900	7.7	0.51	7.06	2	12.45	"
126	S14	6.4	6.6	17	3.8	11	1.760	0.250	0.055	2.750	7.6	0.37	5.04	1	1.55	"
127	S15	10.5	11.0	28	3.8	12.1	1.220	0.150	0.015	1.500	7.4	0.40	5.5	2	2.10	"
128	S17	5.8	4.7	12	4.2	11.1	1.660	0.210	0.045	2.650	7.7	0.356	4.9	2	2.30	"

River Kelvin

Date: 26/4/1979

Code	Site No.	BOD_5	•CRPB BOD_5	•S.S.	•PV	•DO	•NH_3-N	•PO_4-P	•$N(NO_2)$	•$N(NO_3)$	pH	O_2 uptake	QO_2	Temp °C	Time	*Water Flow
129	S1	7.9	7.9	8	4.6	8.7	0.550	0.150	0.015	1.000	7.1	0.40	5.56	8	11.30	N
130	S2	2.8	2.1	5	3.4	10	0.360	0.150	0.015	0.900	7.3	0.27	3.66	7	11.40	"
131	S3	2.6	2.4	3	3.6	12.5	0.180	0.125	0.015	0.700	7.5	0.27	3.66	7	11.50	"
132	S4	22.3	12.3	17	3.4	6.2	5.000	0.900	0.030	0.500	7.4	0.30	4.1	8	"	"
133	S5	8.6	4.1	10	3.2	10.5	2.500	0.400	0.020	0.650	7.6	0.40	5.56	7	11.55	"
134	S6	4.2	3.4	7	4	12.5	0.100	0.200	0.055	2.300	8	0.40	5.56	8	12	"
135	S7	8.4	4	9	3.8	12.3	1.430	0.300	0.040	1.400	7.6	0.27	3.70	7.5	12.10	"
136	S8	3.8	3.6	2	2.6	14.3	0.002	0.225	0.015	1.400	8.1	0.18	2.67	7	12.15	"
137	S9	4.6	2.9	4	3	13.1	0.630	0.225	0.030	1.300	7.8	0.22	3.09	7	12.20	"
138	S10	14.8	12	16	10	8.1	3.100	0.600	0.215	2.000	7.7	0.24	3.29	8	12.25	"
139	S11	10.6	8.5	8	4.2	11.3	1.780	0.450	0.100	1.600	7.7	0.28	3.91	8	12.30	"
140	S12	14.2	8.7	10	6.6	10.4	2.040	0.600	0.100	2.000	7.6	0.25	3.49	8	12.50	"
141	S13	21.8	11	15	5.8	10.9	2.500	1.750	0.220	8.050	7.6	0.22	3.09	9	12.40	"
142	S14	16.2	8.8	9	3.8	8.8	2.200	0.550	0.100	2.200	7.6	0.27	3.70	7	1.50	"
143	S15	7.2	5.7	4	4	11.4	1.240	0.300	0.015	0.750	7.9	0.15	2.05	7	1.55	"
144	S17	10.4	7.7	8	4.4	8.7	1.460	0.425	0.100	1.950	7.6	0.37	5.1	7	2.15	"

River Kelvin

Date: 16/5/1979

Code	Site No.	BOD_5	•CRPB BOD_5	•S.S.	•PV	•DO	•NH_3-N	•PO_4-P	•$N(NO_2)$	•$N(NO_3)$	pH	O_2 uptake	QO_2	Temp °C	Time	*Water Flow
145	S1	3	3.3	6	4.8	7.2	0.580	0.175	0.035	0.950	6.8	0.28	3.91	9	12	N
146	S2	2.7	2.5	5	4	8.4	0.290	0.110	0.030	0.850	7	0.27	3.70	"	12.05	"
147	S3	3	3.2	4	3.6	9.7	0.260	0.070	0.030	0.750	7	0.25	3.49	"	12.20	"
148	S4	27.6	22	47	10.8	1.6	12.800	2.650	0.040	0.150	6.9	0.31	4.32	"	"	"
149	S5	17.4	20	28	6.6	5.9	6.600	1.200	0.050	0.600	7	0.33	4.5	"	12.30	"
150	S6	7.2	6.5	16	6.4	9.6	0.310	0.325	0.125	30.000	7.4	0.30	4.1	"	12.35	"
151	S7	6.6	6.8	15	5.6	9.4	2.900	0.470	0.145	1.400	7.4	0.27	3.70	"	12.40	"
152	S8	3.6	3	9	3.8	13.2	0.100	0.520	0.065	1.950	8	0.28	3.91	"	12.45	"
153	S9	3.9	4.3	10	3.4	11	1.460	0.410	0.120	1.550	7.6	0.27	3.70	"	12.50	"
154	S10	18	18	11	6.6	7	5.450	0.915	0.415	3.650	7.6	0.28	3.91	10	12.52	"
155	S11	7.8	9.1	16	4.6	9.3	3.550	0.620	0.255	2.600	7.6	0.33	4.5	"	12.55	"
156	S12	21.9	20	22	6.6	7.3	4.500	1.250	0.305	3.250	7.4	0.24	3.29	"	1.10	"
157	S13	31	76	97	15.8	6.9	8.600	3.450	0.240	8.800	7.2	0.37	5.1	"	1.05	"
158	S14	10.5	9.4	18	5.6	6.1	3.500	0.790	0.240	3.450	7.3	0.25	3.49	"	1.20	"
159	S15	30.4	37	80	13	7.4	1.800	0.390	0.025	0.400	6.2	0.33	4.5	"	1.25	"
160	S17	5.4	8	22	5.6	6.2	2.220	0.825	0.245	3.400	7.2	0.22	3.09	11	1.40	"

River Kelvin

Date: 18/6/1979

Code	Site No.	BOD_5	•CRPB BOD_5	•S.S.	•PV	•DO	•NH_3-N	•PO_4-P	•$N(NO_2)$	•$N(NO_3)$	pH	O_2 uptake	QO_2	Temp °C	Time	*Water Flow	Cl
161	S1	4.5	3.4	8	5.2	5.4	0.530	0.030	0.045	0.650	7	0.43	5.97	14	11.55	L	16
162	S2	2.4	2.5	6	4.6	7.5	0.350	0.015	0.045	0.650	7	0.31	4.32	13	12	"	22
163	S3	3.6	3.7	5	2.4	8.8	0.290	0.010	0.050	0.650	7.3	0.55	7.62	15	12.10	"	26
164	S4	28.4	12.5	22	6.4	4.1	12.500	1.050	0.030	0.050	"	0.61	8.44	18	"	"	40
165	S5	22.5	6.4	13	4.2	6.3	5.800	0.640	0.055	0.400	7.2	0.42	5.76	17	12.15	"	32
166	S6	3.6	4.4	7	6	7.8	0.460	0.010	0.230	4.050	7.1	0.37	5.14	16	12.20	"	84
167	S7	3.6	7.6	12	4.6	7.6	2.500	0.400	0.130	1.500	7.2	0.60	8.23	15	12.25	"	36
168	S8	2.1	3.5	5	4	12.4	0.150	0.605	0.060	2.600	"	0.40	5.56	17	12.35	"	30
169	S9	3	9.3	8	3.8	10.5	1.400	0.455	0.110	1.900	7	0.30	4.1	16	12.40	"	34
170	S10	6	6	5	6	3.8	4.800	0.865	0.565	3,650	7.2	0.48	6.58	17	12.45	"	82
171	S11	2.7	7.4	7	4.6	8.2	2.300	0.500	0.250	2.550	7	0.57	7.82	"	12.50	"	50
172	S12	3.9	6.8	7	4.6	7.1	1.380	0.765	0.310	4.150	"	0.46	6.38	16	1.05	"	46
173	S13	18.4	18.4	11	7.6	7.6	0.770	4.000	0.740	11.250	7.5	0.64	8.85	15	1.0	"	54
174	S14	5.4	6.4	5	4.4	5.5	1.140	0.945	0.375	4.450	7	0.34	4.73	18	2.05	N	48
175	S15	5.4	6.5	5	4.4	8.2	1.720	0.445	0.030	0.450	"	0.30	4.1	17	2.10	N	26
176	S17	4.8	6.6	5	5.2	7	1.090	0.590	0.315	3.800	"	0.49	6.78	16	2.30	L	36

River Kelvin

Date: 22/8/1979

Code	Site No.	BOD_5	•CRPB BOD_5	•S.S.	•PV	•DO	•NH_3-N	•PO_4-P	•$N(NO_2)$	•$N(NO_3)$	pH	O_2 uptake	QO_2	Temp °C	Time	*Water Flow
177	S1	2.4	2.1	5	7.4	5.6	0.150	0.065	0.050	0.810	6.9	0.38	5.28	13	11.55	N
178	S2	3.0	2.7	4	6.6	7.2	0.040	0.040	0.045	0.700	6.9	0.37	5.09	"	12.05	"
179	S3	3.9	3.1	4	6	8.5	0.080	0.040	"	0.790	7.1	0.32	4.44	12	12.15	"
180	S4	6.8	7	16	4.4	4.5	4.600	0.270	0.165	0.970	7.2	0.3	4.1	13	"	"
181	S5	3.9	3.5	7	5.4	7.5	1.070	0.075	0.165	0.760	7.1	0.49	6.72	12	12.20	"
182	S6	4.5	4.5	10	6.4	9.2	0.200	0.070	0.115	1.580	7.5	0.43	5.88	13	12.25	"
183	S7	3.9	2.7	8	5.6	7.4	0.530	0.100	0.105	1.050	7.1	0.47	6.48	12	12.35	"
184	S8	3.3	2.1	3	5.2	9.9	0.080	0.050	0.070	1.290	7	0.32	4.44	13	12.40	"
185	S9	2.7	2.6	4	6.2	8.6	0.220	0.070	0.060	1.160	7	0.32	4.44	12	12.45	"
186	S10	10.8	10	31	10	8.2	0.330	0.070	0.300	2.800	7.3	0.47	6.48	13	12.50	"
187	S11	4.8	3.2	7	7	8.5	0.300	0.145	0.165	1.430	7.1	0.40	5.46	14	12.55	"
188	S12	6.9	5.1	11	6.8	8.2	0.360	0.080	0.205	1.390	7.1	0.40	5.46	13	1.20	"
189	S13	8.0	4.3	6	4.8	8	0.440	1.250	0.310	6.190	7.2	0.70	9.6	"	1.05	"
190	S14	3.9	5	6	6.6	8.1	0.340	0.325	0.205	1.590	7.2	0.55	7.5	14	2.30	"
191	S15	12.8	10	13	8.8	7.5	1.260	0.280	0.100	1.100	7	0.44	6.06	13	2.40	"
192	S17	4.2	2.4	5	7.2	7.7	0.340	0.100	0.190	1.210	7	0.41	5.7	"	3.0	"

River Kelvin

Date: 26/9/1979

Code	Site No.	BOD_5	•CRPB BOD_5	•S.S.	•PV	•DO	•NH_3-N	•PO_4-P	•$N(NO_2)$	•$N(NO_3)$	pH	O_2 uptake	QO_2	Temp °C	Time	*Water Flow
193	S1	2.4	3.8	5	6.8	6.5	0.280	0.140	0.035	1.160	6.9	0.38	5.34	10	11.25	H
194	S2	3	3.6	15	7.8	9	0.200	0.070	0.020	0.430	7.1	0.14	2.05	11	11.35	"
195	S3	3.6	6.6	19	7.2	9	0.240	0.080	0.025	0.920	7.1	0.14	2.05	10	11.40	"
196	S4	4	4	12	4.8	4.1	1.590	0.210	0.110	1.690	7.2	0.56	7.82	11	"	"
197	S5	4	4.5	18	7	8.2	0.390	0.100	0.025	1.020	7.2	0.26	3.7	10	11.50	"
198	S6	2.8	2.2	13	9.6	9.3	0.280	0.160	0.080	2.170	7.3	0.52	7.2	"	11.55	"
199	S7	3.6	3.7	10	7.4	7.6	0.460	0.100	0.050	1.350	7.3	0.55	7.62	"	12.05	"
200	S8	3.3	2.2	5	5.8	10.1	0.210	0.120	0.030	1.620	7.2	0.18	2.46	"	12.10	"
201	S9	2.4	3.7	8	6.8	8.7	0.410	0.100	0.040	1.510	7.2	0.10	1.44	"	12.15	"
202	S10	4	4.9	32	12	8.7	0.360	0.220	0.140	3.560	7.4	0.40	5.56	11	12.25	"
203	S11	3.2	3.6	15	8	8.8	0.480	0.120	0.085	2.210	7.4	0.33	4.53	10	12.30	"
204	S12	4.4	5.1	16	8.6	8.4	0.470	0.160	0.080	2.320	7.4	0.12	1.64	11	12.50	"
205	S13	5.5	7.3	6	4.4	8.1	0.960	0.650	0.260	5.990	7.5	0.56	7.82	12	12.40	N
206	S14	3.6	4.7	18	8.8	8.2	0.430	0.180	0.080	2.470	7.4	0.46	6.38	11	1.30	H
207	S15	2.8	3.7	7	6.6	9.5	0.036	0.140	0.045	1.450	7.3	0.48	6.58	"	1.40	"
208	S17	4	3.9	21	8.8	8.2	0.400	0.180	0.070	2.180	7.3	0.18	2.46	"	2.0	"

River Kelvin

Date: 29/10/1979

Code	Site No.	BOD_5	•CRPB BOD_5	•S.S.	•PV	•DO	•NH_3-N	•PO_4-P	•$N(NO_2)$	•$N(NO_3)$	pH	O_2 uptake	QO_2	Temp °C	Time	*Water Flow
209	S1	4.6	2.7	10	7.2	7.2	0.520	0.060	0.025	2.070	7	0.22	3.09	6	11.05	N
210	S2	5.4	2.8	6	5	8.1	0.120	0.050	0.015	1.530	7.1	0.27	3.70	7	11.20	"
211	S3	6.4	4.7	4	4.2	8.9	0.130	0.045	0.020	1.480	7.4	0.40	5.52	"	11.30	"
212	S4	6.8	5.5	16	4.4	4	6.000	0.490	0.050	1.600	7.3	0.34	4.73	8	"	"
213	S5	6	4.5	9	5	7.7	0.890	0.155	0.030	1.570	7.4	0.22	3.09	7	11.35	"
214	S6	4.8	1.9	8	6.6	10.7	0.120	0.110	0.050	3.350	7.7	0.40	5.52	"	11.40	"
215	S7	6	3.9	8	4.6	8.2	1.110	0.140	0.045	2.450	7.5	0.30	4.11	"	11.50	"
216	S8	6	3.4	2	5	11.2	0.060	0.230	0.020	1.930	7.5	0.15	2.05	"	11.55	"
217	S9	6.3	3.9	7	5.2	9.7	0.520	0.120	0.030	2.020	7.4	0.27	3.70	"	12	"
218	S10	12	9.5	75	10.6	9.1	0.820	0.290	0.190	5.310	7.6	0.48	6.54	"	12.05	"
219	S11	7.5	6.9	15	5.8	9.2	0.700	0.200	0.095	3.450	7.6	0.28	3.91	"	12.10	"
220	S12	6.3	5.8	9	6.2	8.8	0.800	0.325	0.105	4.240	7.6	0.18	2.46	"	12.35	"
221	S13	15.6	21	8	5.8	8	2.750	1.450	0.270	7.980	7.6	0.37	5.14	9	12.20	"
222	S14	7.8	6	7	6	8.5	0.970	0.325	0.115	4.330	7.5	0.30	4.11	8	1.20	"
223	S15	10.5	9.2	16	7.8	9.5	1.260	0.200	0.025	1.170	7.4	0.28	3.91	7	1.30	"
224	S17	5.7	5.3	8	6	8.5	0.750	0.210	0.100	3.800	7.4	0.22	3.09	"	1.45	"

River Kelvin

Date: 30/11/1979

Code	Site No.	BOD_5	•CRPB BOD_5	•S.S.	•PV	•DO	•NH_3-N	•PO_4-P	•$N(NO_2)$	•$N(NO_3)$	pH	O_2 uptake	QO_2	Temp °C	Time	*Water Flow
225	S1	2.5	2.3	9	5.7	7.6	0.030	0.025	0.015	1.000	6.8	0.12	1.64	9	11.15	H
226	S2	2.2	1.8	6	5.3	9.7	0.060	0.030	0.010	0.850	6.9	0.135	1.85	"	11.25	"
226	S3	3.6	1.7	7	6.8	9.6	0.030	0.020	0.010	0.750	7.1	0.105	1.44	10	11.30	"
228	S4	5.2	2.8	12	3.1	5.2	0.040	0.190	0.045	0.950	7.1	0.375	5.15	"	"	"
229	S5	3.3	2.7	7	4.5	8.8	0.560	0.035	0.015	0.750	7	0.195	2.68	9	11.35	"
230	S6	3.2	2.6	16	6.9	10.1	0.250	0.090	0.060	1.450	7.4	0.345	4.73	"	11.45	"
231	S7	2.4	1.9	8	4.6	8.7	0.480	0.055	0.025	0.950	7.2	0.225	3.09	"	11.50	"
232	S8	2.4	1.6	27	5	10.6	0.080	0.060	0.020	0.950	7.1	0.135	1.85	"	11.55	"
233	S9	2.1	0.7	23	5.2	8.4	0.300	0.050	0.020	;.000	7.1	0.165	2.26	"	12	"
234	S10	4.8	3.3	34	8.4	9.7	0.440	0.110	0.090	1.550	7.1	0.45	6.18	"	12.05	"
235	S11	2.7	3.1	32	6.5	9.3	0.390	0.060	0.050	1.150	7.5	0.21	2.88	"	12.15	"
236	S12	3	2.7	25	6.3	9.4	0.330	0.080	0.050	1.350	7.3	0.255	3.50	"	12.30	"
237	S13	14.4	18	67	11	8.9	1.380	0.445	0.230	3.650	7.5	0.465	6.38	10	12.25	"
238	S14	2.7	2.4	14	6.4	9.2	0.340	0.065	0.045	1.300	7.3	0.285	3.91	9	12.40	"
239	S15	2.5	2.2	8	6.1	10.2	0.030	0.030	0.015	0.950	7	0.15	2.06	"	12.50	"
240	S17	3.3	2.7	17	6.2	9.5	0.260	0.065	0.040	1.200	7.1	0.195	2.67	"	1.05	"

River Kelvin

Date: 19/12/1979

Code	Site No.	BOD_5	•CRPB BOD_5	•S.S.	•PV	•DO	•NH_3-N	•PO_4-P	•$N(NO_2)$	•$N(NO_3)$	pH	O_2 uptake	QO_2	Temp °C	Time	*Water Flow
241	S1	2.1	1.2	15	4.8	8.9	0.150	0.050	0.015	0.950	6.9	0.225	3.09	4	11.40	N
242	S2	2.1	3.3	9	4.5	10.4	0.070	0.045	0.015	0.900	6.9	0.225	3.09	3	11.45	"
243	S3	3.3	2.3	6	3.2	11	0.110	0.045	0.015	0.800	7.1	0.27	3.70	"	11.55	"
244	S4	6.8	4.7	3	3.4	5.7	5.7	0.440	0.045	0.850	7.2	0.45	6.18	"	11.55	"
245	S5	3	3.1	9	3.4	10	1. 80	0.110	0.015	0.800	7.2	0.18	2.47	"	12.05	"
246	S6	1.8	2.0	12	4.5	12	0.400	0.085	0.060	1.550	7.3	0.30	4.12	"	12.10	"
247	S7	2.4	2.1	7	4.2	10.2	0.730	0.085	0.020	1.000	7.2	0.27	3.70	"	12.15	"
248	S8	2.1	1.9	4	2.1	12	0.060	0.100	0.020	1.300	7.1	0.18	2.47	"	12.20	"
249	S9	1.8	1.6	7	2.4	10.8	0.440	0.080	0.020	1.150	7.1	0.18	2.47	"	12.25	"
250	S10	4.4	3.8	21	6.3	11.2	0.800	0.130	0.050	1.650	7.4	0.45	6.18	"	12.30	"
251	S11	3	3.0	12	4.3	11	0.650	0.100	0.035	1.350	7.5	0.285	3.91	"	12.35	"
252	S12	3.3	2.8	13	4.4	10.9	0.630	0.130	0.040	1.550	7.3	0.30	4.12	"	12.50	"
253	S13	10	11.6	8	5.2	10	1.430	0.455	0.180	3.700	7.4	0.49	6.79	5	12.45	"
254	S14	3.3	2.9	17	5	10.6	0.640	0.150	0.045	1.650	7.4	0.39	5.35	4	1.05	"
255	S15	5.1	4.7	13	4.8	11.6	0.440	0.100	0.020	0.950	7.2	0.36	4.94	"	1.15	"
256	S17	3	2.5	13	3.9	10.8	0.580	0.120	0.040	1.450	7.3	0.225	3.09	"	1.30	"

APPENDIX 4

Result analyses on 67 sewage samples

Sample No.	Nature of sample	Temp °C	pH	BOD_5 (ppm)	Climatic conditions and flow	QO_2	DO (ppm)	Time
1	crude sewage	15	7.1	173.4		10.71	0.8	2.20
2	" "	"	"	127.5		8.92	3.6	2.25
3	tank effluent	"	7.0	99.2	rain/N	7.42	2.0	2.30
4	final "	"	6.8	37		5.15	7.4	2.40
5	crude sewage	16	"	244.5		11.95	0.0	2.30
6	tank effluent	16	7.2	173.6	dry/AN	9.68	0.0	2.35
7	final "	14.5	7.2	12.6		3.92	6.1	2.40
8	crude sewage	16	6.9	239.7		11.95	0.9	11.10
9	tank effluent	16	7.1	176.7	rain/AN	9.68	0.2	11.15
10	final "	14	7.0	12.9		4.12	6.4	11.20
11	crude sewage	15	7.4	204		10.51	0.0	11.30
12	" "	16	7.6	168.3		9.68	0.0	11.30
13	tank effluent	15	7.0	89.9		6.59	0.2	11.35
14	final "	"	6.8	25.5	rain/N	4.95	6.1	11.40
15	crude sewage	"	7.1	83.6		10.09	0.2	12
16	tank effluent	"	7.0	124		7.42	0.0	12.05
17	final "	"	6.8	19.3		4.33	6.6	12.10
18	crude sewage	"	7.2	198.2		10.65	0.0	11.15
19	" "	16	7.1	165.7		9.68	0.0	11.15
20	tank effluent	15	7.0	91.4		6.59	0.3	11.20
21	final "	"	6.9	21.9	rain/N	4.66	6.2	11.25
22	crude sewage	"	7.0	178.5		9.82	0.1	12.05
23	tank effluent	"	6.0	127		7.69	0.0	12.10
24	final "	"	6.8	18.9		4.19	6.7	12.15
25	crude sewage	"	7.1	132.6		8.04	0.0	10.0
26	" "	"	"	137.7		8.45	0.0	10.05
27	tank effluent	"	6.9	83.7	heavy storm	5.77	0.2	10.10
28	final "	16	6.6	38.4	/H	4.95	4.5	10.15
29	crude sewage	15	6.9	122.4		7.93	3.6	10.40
30	tank effluent	"	7.0	86.8		5.98	0.5	10.45
31	final "	"	6.8	43.5		5.15	3.7	10.50
32	crude sewage	12	7.2	163.2		9.27	4.4	11.40
33	tank effluent	13	7.3	105.4	rain/N	6.18	0.0	11.43
34	final "	"	7.2	13.8		2.06	5.6	11.47

N = normal, AN = above normal, H = high

Appendix 4:1 Results of 67 sewage samples

Sample No.	Nature of sample	Temp °C	pH	BOD_5 (ppm)	Climatic conditions and flow	QO_2	DO (ppm)	Time
35	crude sewage	12	7.1	81.6		5.56	3.0	12.10
36	tank effluent	13	7.0	80.6	rain/N	5.56	0.3	12.14
37	final "	"	6.8	10.2		2.27	3.0	12.16
38	crude sewage	12	7.1	265.2		14.84	4.0	10.45
39	tank effluent	13	7.2	130.2		7.62	0.0	10.40
40	final "	12	7.0	6.6	rain/N	2.47	5.4	10.35
41	crude sewage	13	"	127.5		7.83	3.4	11.20
42	tank effluent	"	7.1	68.2		5.77	0.2	11.15
43	final "	12	6.9	12		2.47	3.8	11.10
44	crude sewage	10	7.0	183.6		11.54	3.9	11.20
45	tank effluent	11	7.1	111.6		7.62	0.0	11.15
46	final "	10	7.0	7.2	rain/N	3.09	4.8	11.10
47	crude sewage	11	6.9	86.7		5.98	2.9	12.15
48	tank effluent	11	"	62		5.36	-.4	12.10
49	final "	10	6.8	11.4		3.50	4.1	12.05
50	crude sewage	"	"	76.5		11.13	4.2	3.0
51	tank effluent	"	"	114.7	rain & wind	9.89	2.2	3.05
52	final "	"	7.0	27.6	/N	5.56	5.8	3.10
53	crude sewage	"	7.1	127.5		8.04	4.6	11.50
54	tank effluent	"	7.2	86.5		7.21	5.6	11.55
55	final "	"	6.9	7.2	rain & wind	3.50	8.9	12.0
56	crude sewage	8	7.1	135.1	/N	7.83	6.1	12.20
57	tank effluent	"	"	85.2		7.21	6.4	12.22
58	final "	9	7.0	12		3.29	7.9	12.25
59	crude sewage	10	6.7	122.4		8.24	5.1	2.50
60	tank effluent	"	6.8	86.8	rain/AN	6.80	2.2	2.55
61	final "	11	6.9	16.8		4.74	7.8	2.57
62	crude sewage	9	7.0	122.4		8.24	6.5	12.10
63	tank effluent	8	"	88.3		5.56	6.6	12.07
64	final "	"	"	18.2	cloud/N	3.92	9.3	12.05
65	crude sewage	9	6.9	127.5		8.65	6.0	12.35
66	tank effluent	"	6.7	108.5		7.62	5.2	12.33
67	final "	7	"	28.2		4.53	8.5	12.30

Appendix 4 : 1 continued

APPENDIX 5

Statistical analyses of BOD_5 v QO_2 results

Statistical analysis of BOD_5 v QO_2 results

Two different comparison methods were applied to the results of BOD_5 and QO_2.

A. Kendall's coefficient of rank correlation, r.

The formula used was:

i. $$r = \frac{2\,S}{n(n-1)} \qquad \text{for } n \geqq 10 \text{ or no ties}$$

ii. $$r = \frac{S}{\sqrt{\tfrac{1}{2}n(n-1) - x}\,\sqrt{\tfrac{1}{2}n(n-1) - y}} \qquad \text{for } n > 10.$$

for both formula

where

r = the rank correlation coefficient

S = is the $\sum$ p-q which calculated from the rank table

n = sample volume (number of pairs of observations)

x = number of ties in the first observation (x) which is calculated as follows:

$$x = \tfrac{1}{2}\left[\sum_{xi} (\sum_{xi}-1) + \sum_{x2} (x_2-1) + \ldots\ldots\right]$$

y = number of ties in (y) observation which is calculated as follows:

$$y = \tfrac{1}{2}\left[\sum_{y} (\sum_{y1}-1) + \sum_{y2} (y_2-1) + \ldots\ldots\right]$$

Then calculate

$$p = \frac{S\sqrt{18}}{\sqrt{n(n-1)(2n+5)}}$$

with this method:-

5% significant if $p > 1.960$

1% significant if $p > 2.576$

0.1% significant if $p > 3.291$

not significant if $p < 1.960$

In Tables 14 and 15, the results of this analysis are given.

B. Linear regression method.

The SR-51-II Texas calculator was used to determine the

regression curve for the BOD_5 and QO_2 results. The correlation coefficient was measured with mean ($\bar{x}$), and standard deviation (SD) for both BOD_5 and QO_2 data.

In Tables 15 and 16 the results of this analysis are given.

The Normal Distribution Test

The normality test was applied to the results of QO_2 to show the distribution of the results. The results were ranked and the corresponding rankit number for each QO_2 value was determined from the Rankit table. Mean and standard deviation were calculated with the Texas calculator, for the QO_2 data. Curves of normality between QO_2 values and rankit number were plotted. Positive results obtained when the points are reasonably clustered along the line drawn by $\bar{x}$ and $\bar{x} \pm$ S.D. Figures 57, 58, 59 and 64 show the results of this test.

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G15 *Measurement of Organic Pollution by a Rapid Microbiological Method.* T.A-G. Mulla-Ali and D.E.S. Stewart-Tull (Department of Microbiology, Alexander Stone Building, University of Glasgow, Garscube Estate, Bearsden, Glasgow G61 1QH, Scotland).

The Biochemical Oxygen Demand (BOD) test is widely used in the determination of organic pollution. As originally defined biochemical oxidation was measured after five days which was the time calculated for water to flow from its source to the sea. With the increase in the discharge of industrial effluents into rivers and legislation to control levels of pollution it has become necessary to develop rapid techniques to measure BOD (see review, ref. 1). However, there are few reports of microbial methods and it is doubtful whether these could be used in the field (2-4).

In the oxygen polarographic method to be described the BOD is determined from a standard calibration curve after five minutes. The test involves the use of a standardised, pure bacterial seed grown in large-scale batch culture and stored below $-70^{\circ}C$. Each batch produced sufficient seed for three thousand tests. The effects of temperature, pH, seed concentration and heavy metal inhibition on the reliability of the test were examined. The optimum values were $37^{\circ}C$ at pH 6–8 with standard seed of 68 QO_2. Mowat (5) showed a reduction in oxygen uptake in the BOD test in the presence of heavy metals. For example, a toxic effect was found with 0.5 ppm Hg^{2+} whereas in this microbiological test 50% reduction in O_2 uptake was found at 5 ppm. A good correlation was obtained between BOD_5 and kO_2 values with samples of river water, raw sewage and sewage effluents.

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