

STUDIES ON THE BIOLOGICAL
EXTRACTION OF PLANT MACRONUTRIENTS
FROM SEWAGE EFFLUENTS

by

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SUMMARY

Eutrophication of water supplies has necessitated the development of methods to prevent discharge of excessive quantities of nitrogen and phosphorus. Operation of a generally applicable process for removing these elements from sewage effluents has not yet been reported. The investigations described here were carried out to determine the feasibility of using the nutritional requirements of controlled ecological systems for this purpose. The results are presented as an alternative approach to the chemical methods that have received consideration.

It was demonstrated experimentally that the assumption, as expressed by Wuhrmann (1957), that particulate organic material in sewage cannot be utilized by activated sludge organisms during normal contact periods is not correct. Inclusion of solid material normally removed by sewage settlement was shown to increase the synthesis of new cell material. This increase resulted in greater assimilation of N and P from the sewage to the extent that the overall removal of these nutrients was increased from the usual level of 25-35 per cent to approximately 75 per cent. The highest removals were obtained at short retention times, but under these conditions a change in bacterial floc characteristics resulted in poor separation by gravity settlement. Since good floc settlement is of great practical importance, it was concluded that the omission of primary sewage settlement in sewage treatment would not, by itself, achieve satisfactory nutrient removal.

Denitrification of oxidized sewage requires the provision of hydrogen donor compounds containing a minimum of unoxidized nitrogen and a process

was demonstrated in which suitable nitrogen-free compounds were derived from sewage sludge by use of the acid phase of anaerobic sludge digestion followed by pH adjustment and ammonia stripping. Continuous-flow packed column experiments using these compounds showed that 93 per cent or more of the oxidized nitrogen in the effluent could be removed at a retention time of 24 minutes. The results compare favorably with those of others who have attempted to achieve denitrification with waste material available at a sewage treatment plant (64.7 per cent removal in 180 minutes, Johnson and Schroepfer, 1964; 72 per cent in 20 minutes, Bringmann and Kuhn, 1964).

Use of autotrophic algal nutrition was also investigated, since nutrient assimilation by heterotrophic organisms is normally incomplete due to lack of organic carbon in relation to the N and P present in sewage. By using a shallow stream instead of the usual sewage pond system, it was shown that a photosynthetically induced pH increase high enough to precipitate phosphate and cause a major loss of ammonia nitrogen to the atmosphere could be obtained. Phosphate precipitation caused settlement of planktonic algae as a granular sediment that could be removed from the stream. If not removed, the phosphate in the sediment redissolved during periods of lowered pH. A mechanical modification of the stream system was developed to reduce land requirements and heat loss and to increase light utilization. In this apparatus the removal of precipitated phosphate and excess algae was facilitated and nitrogen loss as ammonia was increased. Removal of N and P in excess of 90 per cent during daylight was obtained, indicating that the use of the pH increase to remove nutrients represents a new and more effective method of using

algal activity than the removal by assimilation described by previous investigators. The velocity of air movement across the surface of the algae-covered discs used in the experiments was found to have an important effect on ammonia loss. Experiments with P^{32} -phosphate demonstrated a linear relation between current speed (disc rotation) and metabolic phosphate uptake that was limited to the surface layer of the algal film. It was concluded that the increase in phosphate uptake that would be obtained by increased disc rotation would be insignificant compared to the relatively high concentration of phosphate in sewage. The results confirm, by a different experimental method, those obtained by Whitford and Schumacher (1965) with filamentous algal species.

The possibility of removing N and P by harvesting the net production of an aquatic community, as suggested by Odum (1961), was investigated using the ostracod population that colonized the algal stream. The nutrient content of the maximum population recorded was only 1.61 per cent of the daily N and 0.56 per cent of the daily P input to the stream. It was also shown that the irregular distribution of the population along the stream was due to an ostracod preference for water pH values not exceeding 8.0. This precluded the possibility of increasing the ostracod biomass in the underpopulated sections without major alteration in the method of stream operation. It was concluded that harvesting at any level above the primary producers in an aquatic community would be neither efficient nor reliable as a method for continuous nutrient extraction.

A modification of the conventional sewage treatment process is suggested in which both heterotrophic and autotrophic stages would be employed. Part of the effluent produced would contain relatively low concentrations of N and P, and N would be almost entirely removed from the remainder.

STUDIES ON THE BIOLOGICAL EXTRACTION OF PLANT
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1. INTRODUCTION

The term 'eutrophication' has been used by limnologists for many years to describe the change in biological productivity which all impounded waters undergo during their life history. The life-span of lakes is normally reckoned in thousands of years, during which major changes occur in both surface and bottom water due to continual inflow of dissolved and suspended materials derived from the catchment area. The lake passes from the initial oligotrophic phase through the mesotrophic and finally into the eutrophic phase. It continues in this phase until deposits from biological activity, both organic and inorganic, and materials transported by the catchment drainage eventually fill the lake to the extent that rooted plants can colonize the basin and gradually convert the area to marshland. Due to the activities of man the life-span of lakes in many regions of the world is being drastically reduced from thousands to hundreds, and even to tens of years, in some cases.

These changes are accelerated by agricultural malpractice, causing soil erosion, and by surface drainage containing artificial plant fertilizers. Another very important source of nutrients is the large volume of sewage and industrial wastes discharged into rivers from urbanized and industrial areas.

In many highly populated and industrialized centers the local river receives not only the normal nutrient load from the catchment area but also material brought into the area in the form of food and raw materials

from agricultural areas outside the catchment and much of it is eventually directed into one river in the form of waste products of various kinds.

Although the reduction in the life-span of lakes is in itself undesirable, the effects of eutrophication on the quality of surface waters are also causing increasing concern as there is an increasing demand for useable water in virtually every country in the world due to expanding populations and agricultural and industrial activities, but the total water resources remain unchanged. It is, therefore, vital that during its passage from the watershed to the sea a particle of water should be used many times over.

1.2 Undesirable Consequences of Eutrophication

For most purposes a moderate increase in inorganic ions, such as chloride or sulphate, caused by passage through an urban or industrialized area, will not be detrimental to re-use of the water. This is not so in the case of the plant macronutrients, nitrogen and phosphorus, as it has been established that it is these two elements that are the major cause of increased biological productivity in surface waters (Sawyer, 1944, 1952, 1965; Lackey and Sawyer, 1945; Welch, 1952; Gerloff and Skoog, 1957; Lackey, 1958; Bartsch, 1961; Shapiro and Ribeiro, 1965).

The effect of these nutrients is to produce excessive plant growth, usually in the form of phytoplankton "blooms". Due to their physical characteristics, these algae may cause

serious reduction in the efficiency of water-supply filtration systems, and some species impart objectionable tastes and odors to the water (Rohlich and Sarles, 1949). Subsequent decomposition of small filter-passing algae in pipe reticulation systems may cause heterotrophic bacterial growth within the pipes, causing blockages, and can produce objectionable tastes and odors. Death and decomposition of excessive biomass may cause oxygen depletion in the hypolimnion of impoundments (Sawyer, 1966), with extensive mortality amongst the aquatic fauna and flora, and the release of poisons, such as hydrogen sulphide, through anaerobic bacterial reduction of sulphate. Such conditions may also cause the resolution of iron and manganese from oxidized sediments, both of which must be removed before domestic or industrial use.

In addition to the chemical effects of eutrophication, the effect of excessive plant growth materially detracts from the aesthetic and recreational value of rivers and lakes. Among instances that have been described are the Madison Lakes in Wisconsin (Sarles, 1961), Lake Zoar in Connecticut (Curry and Wilson, 1955), Lake Washington (Anderson, 1961), the Great Lakes (Beeton, 1965) and the Potomac estuary (Shapiro and Ribeiro, 1965). Under the influence of wind and wave action, the algae may collect to form floating mats. This is particularly true of the blue-green species which tend to float and are, therefore,

more susceptible to wind concentration and deposition on shorelines, with subsequent decomposition. Certain species, such as Microcystis aeruginosa, are toxic to mammals and have been responsible for stock fatalities (Gorham, 1960).

Apart from these undesirable consequences, the loss of large quantities of nutrients to the sea as a result of effluent discharge is a deplorable waste of food-producing nutrients.

For these reasons, it is now becoming generally accepted by those concerned with problems of water supply that the conventional sewage treatment plant is no longer adequate, and that treatment must go further than the mere oxidation of carbonaceous material--the plant nutrient content must also be removed or reduced (Rohlich, 1964).

2. ELEMENTS OF MAJOR IMPORTANCE IN EUTROPHICATION

Although it has been pointed out by several authors that components of sewage effluent, such as trace elements and growth factors (Lackey, 1958; Provasoli, 1961) and other organic substances (Saunders, 1957; Pipes and Gotaas, 1960; Wiedeman and Bold, 1965; Wright and Hobbie, 1966), may have an effect upon phytoplankton growth, it is generally agreed that eutrophication of surface waters is primarily due to the presence of abnormal concentrations of nitrogen and phosphorus.

At the beginning of this century Chick (1903) isolated Chlorella pyrenoidosa from sewage and observed the ability of this organism to assimilate ammonia. She suggested that algae might play an important role in the purification of sewage.

Apart from these early observations very little was subsequently published in connection with sewage and algae for many years. More recently, mainly within the last 15 years, an increasing number of reports connected with these topics has been published. Most of these have been concerned with the use of open pond systems, variously termed stabilization ponds, oxidation ponds, bio-oxidation ponds, sewage lagoons, and maturation ponds, for the stabilization of raw sewage or sewage plant effluent. Among the many aspects of these pond systems that have been studied, the algal component has usually been considered as a convenient source of supplementary dissolved oxygen for the respiratory requirements of the bacterial flora but, in some instances, the ability of algae to remove nitrogen and phosphorus from solution has been observed and discussed. Studies on treatment ponds have shown that satisfactory stabilization of sewage or effluent can usually be achieved under suitable conditions (Caldwell, 1946; Gotaas, et al. 1951, 1954a, 1954b; Steel and Gloyna, 1955; Oswald, et al. 1953a, 1953b; Oswald and Gotaas, 1955; Neel, et al. 1961; Mackenthun and McNabb, 1961; Pipes, 1962; Parker, 1962; Oswald, 1963; Levin and Barnes, 1964; Golueke and Oswald, 1965; Clarke, 1965; Kott and Ingerman, 1966; Schulze, 1966).

In those studies in which algal assimilation of nutrients has received attention, it has generally been concluded that continuous efficient removal could not be achieved due to seasonal changes, particularly in temperature and light intensity (Curry and Wilson, 1955; Kaneshige, 1959; Bogan, 1960; Fitzgerald, 1961; Beck, 1965; Cooper, 1962; Oswald, et al. 1963; Vinberg, 1965; Weiss, et al. 1964; Assenzo and Reid, 1966).

2.1 Micronutrients

Apart from nitrogen and phosphorus and, of course, carbon, there is a number of other elements that have been found necessary for algal growth. The number and quantity of elements required varies with species, but K, Mg, Ca, S, Fe, Mn, Co, Mo and Zn are usually necessary (Weissner, 1962). The amounts of these elements that are present in natural waters vary according to geological, physical and chemical conditions, and attempts have been made to relate these variations to algal species distribution (Gessner, 1958; Kalle, 1958). There seems little doubt that, in some cases, lack of one or more of these elements in an available form may adversely affect phytoplankton growth in natural waters, particularly in the case of iron, since it is a constituent of many enzymes and of cytochromes and certain other porphyrins, and its concentration is normally low in oxygenated waters (Cooper, 1948; Hewitt, 1958). Talling (1962), in summarizing the literature on nutrient requirements of fresh-water algae, concludes that silicon, nitrogen and phosphorus are among the elements most likely to be significantly depleted by algal growth in surface waters.

2.2 Carbon Sources

There is much evidence to suggest that the "dark metabolism" of algae is essentially similar to that of non-photosynthetic organisms. It might therefore be expected that most intermediates in the major biochemical pathways of energy metabolism might be utilized by algae. This expectation is only partially fulfilled, and to varying degrees in various species.

The subject of heterotrophy in algae metabolism is very complex, and the reasons why certain substrates function as carbon and energy sources for some species, but not for others, are not entirely understood. The fact that a substrate will support the growth of a species, while a very closely related compound will not, is also largely unexplained. Algal heterotrophy has been reviewed by Krauss (1958), Pringsheim (1959) and by Danforth (1962).

In natural waters the amounts of organic carbon compounds present are likely to be small in relation to the mineral carbon present as carbon dioxide, bicarbonate and carbonate. According to Harvey (1945), carbon is not likely to be limiting in such waters, where other factors are usually controlling. However, in waters highly enriched with other nutrients, carbon may become limiting during periods of intense growth (Kaneshige, 1959; Aikins-Afful, 1961; Vinberg, 1965), with consequent effects upon bicarbonate-carbonate equilibria and pH value. The latter may reach levels above pH 10.0 (Dunn, 1967) or even pH 12.5 (Schutte and Elsworth, 1954), which are levels at which algal growth and survival are endangered (Steeman Nielsen, 1955; Gerloff, et al. 1950, 1952).

In general, it appears justifiable to conclude that, although other factors may be limiting in certain cases, most instances of man-induced eutrophication can be attributed to the presence of nitrogen and phosphorus. The question is, therefore, what can be done to prevent eutrophication or, if it has already occurred, what can be done to reduce its effects?

3. CONTROL OF EUTROPHICATION

Odum (1961), in a discussion of eutrophication from an ecological standpoint, has used the basic theory of ecosystem function to suggest a number of possible approaches to control the imbalance between production and consumption which is inherent in the algal bloom situation. These possibilities include:

- i. poisoning of the production processes
- ii. reduction of the effective light
- iii. converting nutrients to unavailable forms
- iv. increasing food intake by herbivors
- v. harvesting net production (and with it some of the nutrients) either as primary or secondary production
- vi. reducing the inflow of nutrients

Some of these possibilities have been investigated with varying degrees of success but none have so far proved entirely satisfactory.

3.1 Poisoning of Production Processes

In some cases a temporary solution to the problem of excessive plant growth may be achieved by periodic use of algicides. Copper sulphate is frequently used but has the disadvantages that its effectiveness decreases with increasing hardness of the water (Allen, 1966), and that susceptibility varies among different algal species and at different seasons (Crance, 1963; Chancellor, et al. 1958). At the concentrations used it may be toxic to the invertebrate population, thus affecting the natural supply of fish food, particularly since the oxidized compounds of copper are insoluble and accumulate in the bottom sediments.

Organic algicides of various kinds have been used in some instances, but suffer the disadvantage of relatively high cost and some uncertainty as to the long-term effects on the aquatic fauna. As with copper sulphate, there are wide differences in susceptibility (Maloney and Palmer, 1956; Funk and Gaufin, 1956; Ludmann and Kayser, 1966).

3.2 Reduction of Effective Light

Temporary control of algal growth has been achieved by excluding light from the water using substances such as carbon black. However, the difficulty in maintaining a film on the surface under any appreciable wind action makes this approach possible only on small, protected bodies of water (Stumm and Morgan, 1962).

Both algicide application and light exclusion are methods of temporarily alleviating the effects of eutrophication and are only possible in a limited number of cases. As such, they offer no generally applicable answer to the problem.

3.3 Conversion of Nutrients to Unavailable Forms

This method is unlikely to prove possible unless the continued input of nutrients has been greatly reduced. If this can be achieved, then reversal of eutrophication may be enhanced by preventing re-solution of nitrogen and phosphorus from the bottom sediments, either by dredging to remove the material, or by bottom sealing (Sylvester and Seabloom, 1965).

Artificial aeration of small lakes to prevent anaerobiosis in the hypolimnion may also help to keep certain nutrients such as phosphorus and iron unavailable by maintaining them in the insoluble oxidized form (Pomeroy, et al. 1965). Another method that has met with some success is the withdrawal of water from the hypolimnion of an impoundment; in this way water containing a higher concentration of nutrients derived from the sediments is removed (Thomas, 1965).

3.4 Increased Food Intake by Herbivores

The possibility of increasing the amount of plant material used in primary consumption by the herbivor population is unlikely to have a beneficial effect unless the herbivores are themselves removed. The energy obtained from the plant material at this level will be that derived from photosynthesis of carbon compounds. Oxidation of these materials will have no effect on the total amount of nutrients, other than carbon, unless nutrients incorporated in herbivor biomass are removed from the ecosystem in some way.

3.5 Harvesting Net Production, Either as Primary or Secondary Production

Odum (1961) is of the opinion that harvesting of large organisms having long individual growth periods, such as fish, is likely to be the most practical way of removing nutrient materials. He considers that the harvesting of algae or smaller herbivores would require frequent or continuous operations and present practical problems, whereas fish harvesting could be periodic.

While it is true that fish production would present fewer harvesting problems, and suitable fish, such as some Tilapia species and other herbivorous forms can be produced at rates of 1000 lb/acre/year or more, the amounts of nitrogen and phosphorus assimilated in fish tissue will be relatively minor, compared with the total content of the enriched ecosystem. Fish tissue contains an average of about 3.0 per cent N and 0.2 per cent P on a wet weight basis (Borgstrom, 1961; McGauhey, et al. 1963; Sylvester and Anderson, 1964). There is also evidence that the standing crop of fish may not always be related to the net primary productivity of the water body (Rupp and DeRoche, 1965).

3.6 Reduction of Nutrient Input

There are three principal methods by which nutrient concentrations in inland waters may be controlled:

- i. diversion of nutrient-rich waters from the receiving body
- ii. dilution of the nutrients by addition of water with low nutrient content
- iii. removal of nitrogen and phosphorus compounds at their source

3.6.1 Diversion

A frequent cause of rapid eutrophication has been the discharge of large volumes of treated sewage and industrial wastes. In certain cases it may be possible to prevent further enrichment by diverting the waste flow away from the receiving water body. For obvious

reasons this is usually only possible in coastal areas where discharge to the sea can be arranged.

Lake Washington in the N. E. United States is probably the best documented example and, since the diversion in 1965, some decrease in primary productivity has been observed (Edmondson, 1961; Oglesby and Edmondson, 1966).

3.6.2 Dilution

In cases where a copious supply of water with very low concentrations of nutrients is available, it may be possible to reduce the degree of enrichment of a lake by controlled addition of this water. Instances where this might be possible are limited. Sketelj and Rejic (1966) have proposed the diversion of the River Radovna through Lake Bled in Yugoslavia for this purpose, and in certain areas of North America it may also be possible. The only recorded instance in which dilution has been attempted is the case of Green Lake in Seattle (Sylvester and Anderson, 1964) in which some improvement has been noted after sufficient low-nutrient water had been added to flush out the lake about three times.

3.6.3 Removal

Neither diversion nor dilution can be considered as generally applicable methods for the control of eutrophication, and it is for this reason that most attention has been paid to the possibility of preventing the discharge of nitrogen

and phosphorus by removing these elements before the discharge of waste waters.

The basic problem is to devise methods by which relatively low concentrations of these elements can be removed from large volumes of water at acceptable cost.

4. METHODS FOR REMOVING NITROGEN AND PHOSPHORUS

4.1 Chemical Removal

Chemical precipitation of phosphorus appears to offer a method of removing phosphorus with a high degree of efficiency under suitable circumstances. The principle involved is to adjust the pH value of the waste water to a level at which phosphates are precipitated and can be removed by the addition of coagulants such as lime, aluminum hydroxide, aluminum sulphate, iron salts, or a combination of two or more of these materials (Lea, et al. 1954; Malhotra, 1963; Klotter, 1964; Malhotra, et al. 1964; Jegge, 1965; Wuhrmann, 1965; Thomas, 1966). The electrolytic purification of sewage-seawater mixtures, as demonstrated by Foyn (1964), also produces efficient precipitation of phosphate.

In a few cases the process has been operated on a pilot scale with satisfactory results, and it seems probable that, provided costs of plant and coagulants are acceptable, the method should prove efficient in removing phosphorus from sewage plant effluent in a full-scale system.

The chemical extraction of nitrogen presents a much greater problem in view of the solubility of nitrogen compounds in general. The only methods that have been considered to warrant investigation

are ion exchange and air-stripping of ammonia. However, the use of ion-exchange resins on sewage plant effluent is usually considered to be prohibitively expensive, although high removals can be obtained (Wuhrmann, 1964; Slechta and Culp, 1967).

Air-stripping of ammonia by passage through packed columns is also efficient, but according to Bayley (1967) the cost of power for air blowers alone would be unlikely to be less than R250/million gallons treated. However, a recent report by Slechta and Culp (1967) suggests that with suitable stripping-tower design the costs may be substantially less. In general, it appears that biological methods of nitrogen removal have been considered as more likely to provide an answer to the problem.

4.2 Biological Removal

All living organisms depend on phosphorus for energy transfer in their metabolic activities and it is believed that inorganic orthophosphate is the only form in which it can be accepted from the environment (Katchman, 1961). Orthophosphate, per se, plays no part in the metabolism, but during the attendant or precursory cell reactions orthophosphate ions enter the organic complex to form ATP, the energy-rich terminal phosphate which permits substrate phosphorylation and assimilation, primarily through a combination of the Embden-Meyerhof pathway and the Krebs cycle. A supply of phosphorus is, therefore, a prerequisite for biological growth and the amount required, other things being equal, will be related to the rate of biosynthesis.

Nitrogen is also an essential macronutrient and is required for the production of a variety of nitrogen compounds in cellular metabolism and synthesis. The nitrogen requirement of living organisms is normally several times greater than that for phosphorus, and the rate of assimilation will also be related to the rate of biosynthesis.

Since both these elements are required for the growth of organisms, it is evident that, given suitable conditions, the synthesis of living material offers a means of removing these elements from aqueous solution and concentrating them within the cell wall. If these organisms can then be removed, or if their activities cause the loss of the element from the aqueous phase, it may be possible to exploit their activity as a means of nutrient removal.

4.2.1 Heterotrophic removal

Carbon-heterotrophic organisms require organic compounds as their source of carbon for energy-releasing reactions and synthesis. By providing conditions for maximum production of new biological material, the rate of assimilation of nitrogen and phosphorus would also be at a maximum. Among the requirements necessary to achieve this condition is the provision of an environment containing all the necessary elements in an available form and in the correct proportions. If this requirement is not met, then lack of one of the nutrients will eventually limit growth of further new organisms.

The use of biological life in this way is, of course, the basis of conventional sewage purification methods. The objective of conventional treatment is not, however, the removal of nitrogen and phosphorus, but the removal of putrescible organic materials, the production of a fully oxidized effluent that will exert a minimum oxygen demand in the receiving water, and the destruction of pathogenic bacteria. A properly designed and operated system achieves this aim with a high degree of efficiency--but it does not remove nitrogen and phosphorus to any great extent. The reasons for this failure are evident if the biochemical reactions occurring within the treatment system are considered.

Sewage contains virtually all the nutrient elements required for heterotrophic bacterial growth. In the course of treatment, complex organic carbon compounds are broken down to simpler molecules by enzymatic action, which are then assimilated by the aerobic bacterial flora of the bio-oxidation stage. In this stage organic carbon is either oxidized to carbon dioxide or synthesized into new bacterial cells. The bacterial matter in excess of that required is removed by settlement and subjected to anaerobic methane fermentation. Thus, all organic carbon that has not been oxidized to carbon dioxide eventually reaches the anaerobic treatment stage. In this stage the aerobic bacterial matter that has been produced is broken down to simple compounds by a specialized population of

faculative and obligate anaerobes. The organic carbon compounds present are either oxidized to carbon dioxide or reduced to methane by the intermolecular oxidation-reduction reactions carried out by the bacteria. Both of these gases leave the aqueous phase and are released to the atmosphere. In this way all the organic carbon entering the treatment plant is either lost to the atmosphere as gas, or leaves the plant in solution in an oxidized form as inorganic carbon, either as dissolved carbon dioxide, or bicarbonate and carbonate ions.

The nitrogen and phosphorus content of the sewage, on the other hand, remains virtually unchanged. Although the elements may have been taken up in aerobic biosynthesis, they are subsequently released by fermentation and returned to solution in the anaerobic stage, where only a small amount is re-assimilated. The liquid phase in this stage thus contains high concentrations of nutrients other than organic carbon. This "anaerobic digester supernatant" is usually returned to the input to be recycled with the incoming sewage. The only way in which nitrogen and phosphorus can be lost from the cycle is in the small amount of excess anaerobic bacterial material that is removed from time to time. This material is drained of its water content, which contains most of the nitrogen and phosphate, and the residue is used for agricultural purposes. The actual amount of nitrogen and phosphorus removed in this

way is small in relation to the total quantities entering the plant. In the case of nitrogen, it is possible that a small amount may be lost to the atmosphere as volatile nitrogen compounds, such as ammonia or nitrogen gas. Any loss of phosphorus in this way is not possible. It is evident, therefore, that in a conventional sewage treatment plant the major part of the incoming nitrogen and phosphorus, although it may take part in the biological cycle of synthesis and decomposition several times, eventually leaves the system in the plant effluent, either in the unoxidized form of ammonia, or in the oxidized states of nitrite and nitrate, and as phosphate.

If removal of nutrients other than organic carbon is the objective, then some modification of the conventional system is obviously required.

4.2.2 Autotrophic removal

Since it is the nutrients responsibility for excessive growth of algae in surface water that must be removed from effluent discharges, the use of algae to extract these nutrients during their growth under controlled conditions, followed by their removal from the effluent, would offer a possible method. This approach would have the advantage that macro- and micro-nutrients essential for phytoplankton production would be selectively removed. There are, however, a number of problems associated with provision of the

environmental conditions necessary for controlled algal growth followed by their removal on the scale required to remove nutrients from large volumes of water. It is due to these problems that a satisfactory system employing photosynthetic autotrophs for nitrogen and phosphorous removal has not yet been developed.

5. RESEARCH OBJECTIVES

From critical consideration of the literature available on the many aspects of ecology and biochemistry that have bearing on the possibility of using biological activity to combat the effects of eutrophication, several variations on the basic theme of biological nutrition are suggested in this thesis as possible avenues of approach to the problem. These suggestions follow those of Odum (1961) for correcting the ecological imbalance brought about by eutrophication, in that the possibility of harvesting net production, together with incorporated nutrients, either as primary production of microphytes (Section C) or as secondary heterotroph production (Sections A and F) has been investigated to a degree sufficient to determine the feasibility in practice. An alternative method in which the physico-chemical changes in the water environment resulting from metabolic activity of organisms are exploited has also been investigated (Sections D and E). Some observations on the use of bacterial nitrate respiration to remove nitrogen from solution have been made, and particular attention given to the problem of providing a low-cost source of the hydrogen donor compounds required for this process (Section B).

Although none of the suggestions put forward in this thesis are considered to have been exhaustively investigated, it is hoped that sufficient data is presented to enable other workers in the field to judge whether further research on the lines suggested would be worthwhile.

Section A

REMOVAL OF NITROGEN AND PHOSPHORUS BY HETEROTROPHIC ORGANISMS

6. COMPOSITION OF MICRO-ORGANISMS

The relative amounts of N and P required for cell synthesis vary to some extent among different organisms but, for both mixed algal communities found in natural waters and for bacterial populations found in biological waste treatment processes, the stoichiometric relation between carbon, nitrogen and phosphorus in the organism is approximately 106:16:1. (Eckenfelder, 1956; Redfield, 1958).

From these relations an approximate indication of the nutritional requirements can be obtained, since new organisms must be built from identical materials. If one of these elements is present in sub-optimum amounts then the net synthesis of biomass that can be obtained is likely to be limited by that element.

In the conventional sewage treatment process a portion of the organic carbon present is converted to carbon dioxide by oxidative respiration, the remainder is stored or used to produce new growth of the heterotrophic bacterial population present. Nitrogen and phosphorus are also assimilated during synthesis but the amounts taken up in relation to the carbon utilized can vary appreciably, depending upon the environmental conditions. When these elements are present in excess the organisms may exhibit what has been termed "luxury consumption", i.e., increased uptake without equivalent increase in growth. This phenomenon has frequently been observed during culture of both autotrophic and heterotrophic

organisms in media of different composition. In the heterogeneous bacterial populations developed in aerobic waste treatment systems it has been shown by Hattingh (1963) that increased nitrogen concentration results in a higher nitrogen concentration in the organisms, and Levin and Shapiro (1965) have shown that by providing highly aerobic conditions, using air or oxygen, the same organisms show greatly increased uptake of phosphate. This extra phosphate is, however, rapidly released by the organisms if the dissolved oxygen is allowed to become depleted.

6.1 Nutrient Deficiencies in Sewage

In the effluent leaving a sewage treatment plant there is normally a residual of nitrogen and phosphorus which has not been removed by conventional treatment. Wuhmann (1957) has observed that in Zurich the C/N ratio in the settled sewage does not usually exceed 2 to 2.5, whereas the C/N ratio in the cells of the micro-organisms in the treatment process have a C/N ratio of between 5 and 6. Due to the relatively high phosphate concentration in sewage today, resulting from the almost universal use of phosphate-containing domestic detergent formulations (Sawyer, 1965, 1966), the difference in C/P ratios is even greater.

That nitrogen and phosphorus can be extracted by heterotrophic biosynthesis has been shown in the biological treatment of carbonaceous industrial wastes in which these elements are lacking. By adding the nutrients to wastes from cotton kivering, rope pulping and breweries, Helmers, et al. (1951, 1952) have shown that the rate of growth

of organisms is proportional to the amount of assimilable organic carbon utilized, and that the N and P requirement is directly related to the amount of bacterial growth. Similar results with other nutrient-deficient wastes have been obtained by Laughlin (1964) and by Bess and Conway (1966).

If it is possible to remove organic carbon, when present in excess, by the addition of N and P in suitable amounts, it should be possible to achieve the reverse in the case where organic carbon is the deficient nutrient, as in settled sewage. Thus, by addition of extra assimilable organic carbon to the sewage the amount of N and P incorporated into cellular growth should be increased. However, unless a source of carbon is available at low cost, the expense of carbon addition is likely to be economically unacceptable due to the large quantities of effluent to be treated.

6.2 Effects of Sewage Settlement

Preliminary settlement of the raw sewage arriving at a treatment plant removes a substantial proportion of the biologically available organic compounds in the form of settleable particulate material. The carbon contained in these solids is subsequently disposed of as gas in the carbon dioxide and methane produced by anaerobic methane fermentation. Because of the simplicity and efficiency of primary sewage settlement in removing a considerable proportion of the organic load, the method is used in virtually all conventional sewage treatment plants of any size.

It is perhaps because of this efficiency that the effect of primary settlement on the assimilation of nitrogen and phosphorus in the process appears to have been given very little consideration, since the process achieves its object of producing a fully oxidized effluent with great success.

In a recent survey of some 20 sewage works in South Africa (CSIR, unpublished) it was found that 33-44 per cent of the biologically available organic carbon in the sewage, as measured by the 5-day biochemical oxygen demand test (BOD), was removed by primary settlement of the suspended solids. The results of a similar survey in Great Britain for purely domestic sewage (Water Pollution Research Laboratory, 1967), showed a weighted average BOD removal of 48.1 per cent. From these figures it is evident that nearly half of the available carbon in the raw sewage is removed by primary settlement.

6.2.1 Effect of settlement on nutrient ratios

In contrast to the removal of carbon, the amount of ammonia nitrogen removed in this way is nil, due to its solubility, and Hanson and Flynn (1964) have shown that ammonia nitrogen accounts for approximately 85 per cent of the total nitrogen in sewage. Based on the British values for BOD and ammonia nitrogen given for 9 towns, and allowing for the 15 per cent non-ammonia nitrogen present, the BOD/N ratio in raw sewage is calculated to be 6.69; after primary settlement the ratio is decreased to 3.8.

Using the annual average values for 1963 to 1965, obtained at the Pretoria Sewage Works in South Africa, which treats an average flow of 7.35 million gallons per day, the BOD of the raw sewage is approximately 450 mg/l. After settlement it is reduced to approximately 250 mg/l. The ammonia nitrogen content remains unchanged by settlement at 40 mg N/l. Again allowing for 15 per cent non-ammonia nitrogen the BOD/N ratio of the raw sewage is calculated to be 9.78, and after settlement, 5.44. These values appear to be somewhat higher than the values obtained in Britain, possibly as a result of discharge of carbohydrate-containing industrial wastes from the local flour mills and similar industries.

The proportion of the available sewage carbon that is used for oxidative metabolism by a heterotrophic population will depend on a number of factors, but it seems reasonable to suppose that synthesis and storage, as well as oxidative metabolism, will increase if an increased amount of carbon becomes available. It has been shown by Hattingh (1963), in a study of the effect of BOD:N and BOD:P ratios on the biological oxidation of a synthetic substrate, that the N and P content of the organisms remains constant provided the BOD/N and BOD/P ratio is closer than 19 and 81 respectively. Since the ratios for both raw and settled sewage are well within this range, it would be expected that the composition

of excess material produced from added carbon would contain the same proportion of nutrients, i.e., increased synthesis would result in an equivalent increase in nutrient assimilation.

Wuhrmann (1957) has stated that most of the organic carbon in the particulate fraction of sewage is not available to activated-sludge organisms during the aeration time normally provided. That this might not be so was suggested by the fact that observations made during pilot operation of a new design of activated sludge system at the Pretoria Sewage Works showed that the N and P concentration in the effluent was unusually low when unsettled sewage was applied. Since conditions were otherwise normal, it was tentatively concluded that increased synthesis was occurring due to bacterial utilization of the increased particulate input.

If this conclusion could be shown to be correct, then it might be possible, without the need for specialized nutrient extraction stages, to significantly increase the amount of N and P removed in the conventional sewage treatment process by omitting the usual primary settlement stage. In this way the amount of heterotrophic biomass produced per volume of sewage treated would be increased and, together with the extra incorporated nutrients, could be removed from the water phase by the usual method of gravity settlement.

Because of the extra carbon entering the system, the size of the bio-oxidation stage employed would have to be increased. No other alteration would be necessary since the hydraulic load would remain unchanged. The increased synthesis would result in the production of increased amounts of biomass for disposal. This could be anaerobically digested to methane and carbon dioxide in the normal way, but the liquid resulting from digestion, which contains a minimum of soluble carbon compounds but very high concentrations of ammonia and phosphate, could not be returned and recycled with the incoming sewage as is the general practice.

6.2.2 Anaerobic digester supernatant

It does not appear to be generally realized that the practice of returning digester supernatant for recycling completely reverses any nutrient concentration and extraction by bacterial assimilation achieved in the aerobic stage. Since the liquid contains very little available carbon the C/N and C/P ratios of the incoming sewage are further increased over their already unfavorable levels. The treatment system acts only to delay the passage of N and P and almost all that entered the system eventually leaves in the effluent.

At the Pretoria Sewage works the records for 1963-65 show digester supernatant liquor concentrations varying between 500 and 1000 mg N/l, depending on the method of withdrawal from the anaerobic digesters. The volume of

this liquid is estimated to be between 0.5 and 1.0 per cent of the total daily flow. Based on a flow of 7.35 m.g.d., and using the higher values, the weight of nitrogen returned to the system in this way amounts to approximately 333 Kgs/day, almost all of which is eventually discharged in the plant effluent.

Although the extraction of N and P by chemical engineering methods from the full daily sewage flow would be prohibitively expensive in most cases, the treatment of a liquid some 20 times more concentrated than sewage with only 1 per cent of the volume, should present a far less formidable problem. In such cases, air stripping of ammonia should be feasible and, combined with phosphate precipitation with lime, might allow the reclamation of the nutrients for agricultural use.

7. EXPERIMENTAL APPROACH

To assess the practical potential of omitting primary settlement as a means of reducing the discharge of macronutrients, a series of experiments was carried out with both raw and settled sewage. The objective was to determine whether an increase in nutrient removal due to the presence of extra particulate carbonaceous material would occur.

Although synthetic sewage formulations have been widely used for investigation of this kind to reduce the practical problems involved, it was considered that in this case the use of synthetic sewage would not be suitable. The main reason for this decision was the fact that

it is not possible to produce a medium containing both colloidal and suspended particulate solids in which the macronutrient distribution is the same as in natural sewage. Since this distribution was considered to be the factor of major importance in the study, the use of natural sewage was considered to be essential.

Before the main experiments were carried out a series of short-term batch tests were conducted to obtain information on the magnitude of the factors involved, such as nutrient concentrations, growth rates and aeration times. These tests were followed by the main experiments in which a series of identical laboratory-scale activated-sludge units were used to compare the nutrient removal obtained using settled and unsettled sewage.

7.1 Short-Term Batch-Operated Experiments

These experiments were carried out in small glass aeration vessels into which samples of activated-sludge organisms and nutrient media were introduced.

7.1.1 Apparatus

A series of vertical glass cylinders (capacity 350 ml), fitted with glass air diffusers were used as aeration vessels. Each cylinder was calibrated for a working volume of 250 ml and an excess of air (500 ml/min.) was supplied to each during the experiments. To prevent loss of material due to foam formation, a small amount of silicone antifoam grease was applied to the rim of each cylinder.

7.1.2 Activated-sludge supply

To obtain a convenient source of activated-sludge organisms, an apparatus consisting of an aerated glass column, 1.7 m in height with a capacity of 15 l, was set up. An activated-sludge was developed and maintained on an input of settled domestic sewage. A system of solenoid valves and time switches was incorporated to provide a cycle of 7 hours aeration followed by a 1-hour settlement interval with no aeration. At the end of this settlement period part of the supernatant was automatically drained to waste (50 per cent of the total volume), and the system refilled with a fresh supply of sewage before aeration automatically recommenced. The sewage supply was renewed three times per week and glucose was added at a rate of 0.5 g/l to promote sludge synthesis.

7.2 Experiment 1

This experiment was carried out as a preliminary test to determine whether an increase in the ratio of available carbon to nutrients would result in increased removal of nutrients from solution.

7.2.1 Method

A portion of the prepared activated-sludge suspension was centrifuged to remove most of the soluble nutrients in the discarded centrifugate. The residual material was then resuspended in 0.1 M "tris amine" buffer at

pH 7.2 (2-amino-2(hydroxymethyl)-1,3-propane diol). The suspension was then vigorously stirred with a magnetic stirrer and 50 ml aliquots transferred with a wide-mouthed pipette to a series of 15 of the 350 ml glass aeration cylinders. The cylinders were divided into five groups of three, designated Series 1 to 5.

A solution of glucose was then dispensed to the cylinders in amounts sufficient to add 100 mg glucose carbon to Series 1, 200 mg to Series 2, and 300 mg to Series 3. Series 4 and 5 received extra carbon in the form of 5 and 10 ml of homogenized raw sewage sludge of known BOD content respectively. Each tube was then made up to the 250 ml working volume with settled sewage of known BOD content and continuous aeration applied.

Using values for the BOD of glucose solutions obtained by Sawyer (1952), the total BOD in the tubes of Series 1 to 5 was calculated to be 1178, 1922, 2675, 720 and 997 mg/l respectively.

The initial concentration of activated-sludge organisms was 340 mg/l suspended solids obtained by analysis of two 50 ml aliquots of the inoculum suspension.

The contents of one tube from each series was removed at intervals of 24, 48 and 72 hours and filtered under suction to obtain a clear filtrate.

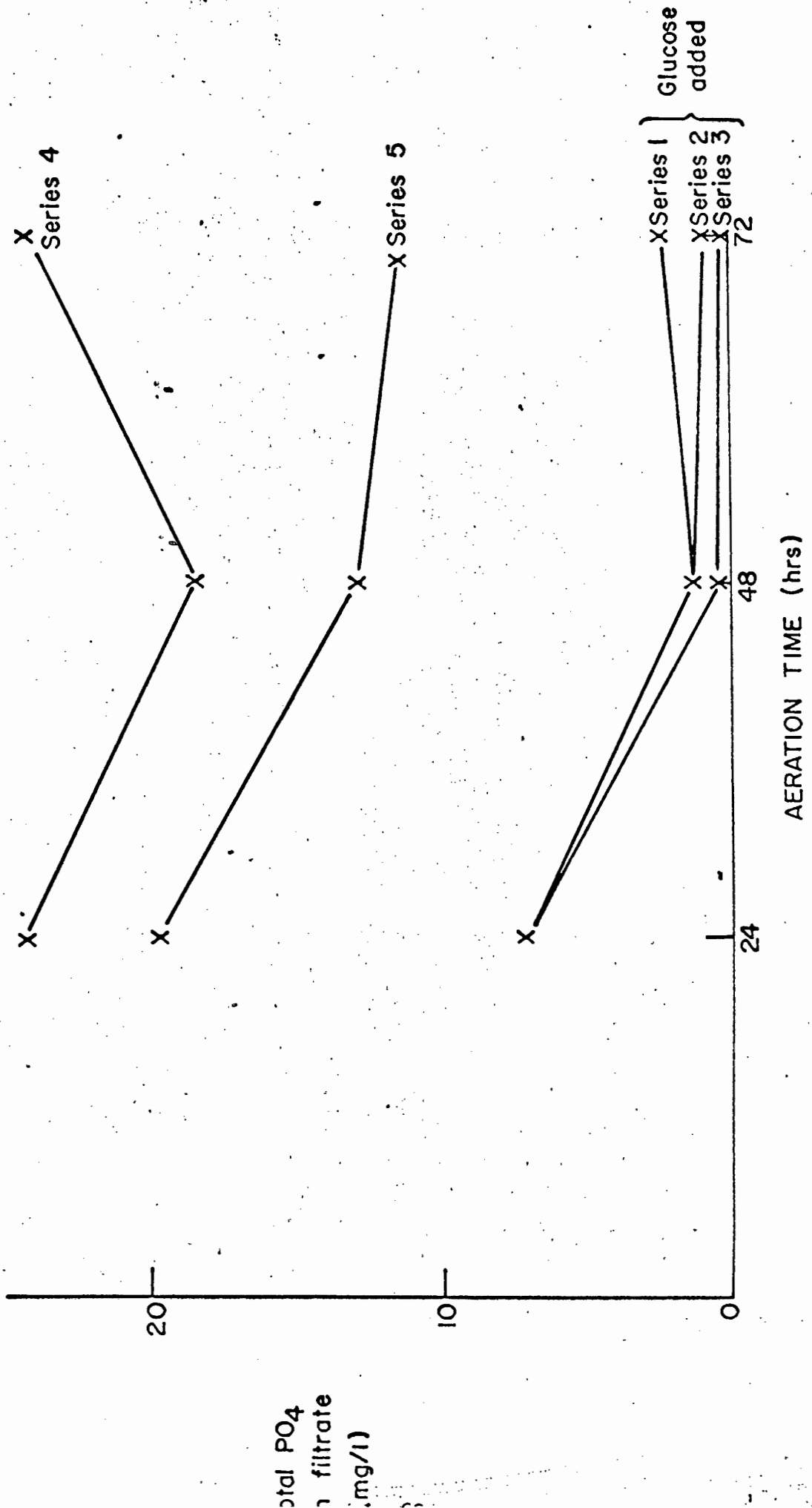


FIGURE 1.

Effect of aeration time on the uptake of phosphate

The filtrates were then analyzed for soluble phosphate to determine the reduction in phosphate concentration. Determination of nitrogen removal was not possible due to the presence of nitrogen in the tris amine buffer used. Unless otherwise specified, all analyses were carried out by accepted procedures (Standard Methods for the Examination of Water Sewage and Industrial Wastes, 1965).

7.2.2 Results and conclusions

The change in phosphate content of the filtrates after various periods of aeration are shown in Fig. 1. The results indicate that the increased content of available carbon in Series 1 to 3 resulted in a greater incorporation of phosphate into new cell material. In Series 4 and 5 there is evidence that insufficient carbon was present to allow complete phosphate assimilation. The results also suggest that aeration after the available carbon is exhausted may result in re-solution of phosphate, probably as a result of auto-oxidation of cell material.

In this experiment between 24 and 48 hours were required to allow assimilation of phosphate in Series 1 to 3. This relatively long period was evidently due to the low concentration of aerobic organisms present, compared to the concentration used in the conventional activated-sludge process, in which retention times of 6-8 hours are usual.

7.3 Experiment 2

This experiment was to determine the effect of activated-sludge concentration on the rate of phosphate assimilation and re-solution.

7.3.1 Method

Activated-sludge from the culture apparatus was centrifuged at 2500 g for 10 minutes and the supernatant discarded. The residue was suspended in 0.1 M tris buffer and centrifuged again. The supernatant was discarded and the residue resuspended in buffer. Portions of this suspension were dispensed to five pairs of the aeration cylinders to give a range of increasing sludge concentrations. One tube of each pair was used to determine the concentration of suspended solids. The suspensions in the remaining 5 tubes were made up to 50 ml with tap water and then 200 ml of glucose-enriched settled sewage with known BOD were added to each tube and aeration started. Small samples were withdrawn at intervals for determination of soluble phosphate concentration after removal of suspended material by centrifugation.

7.3.2 Results

The initial BOD concentration in each tube was 605 mg/l and the activated-sludge concentration ranged between 490 and 3240 mg suspended solids/l.

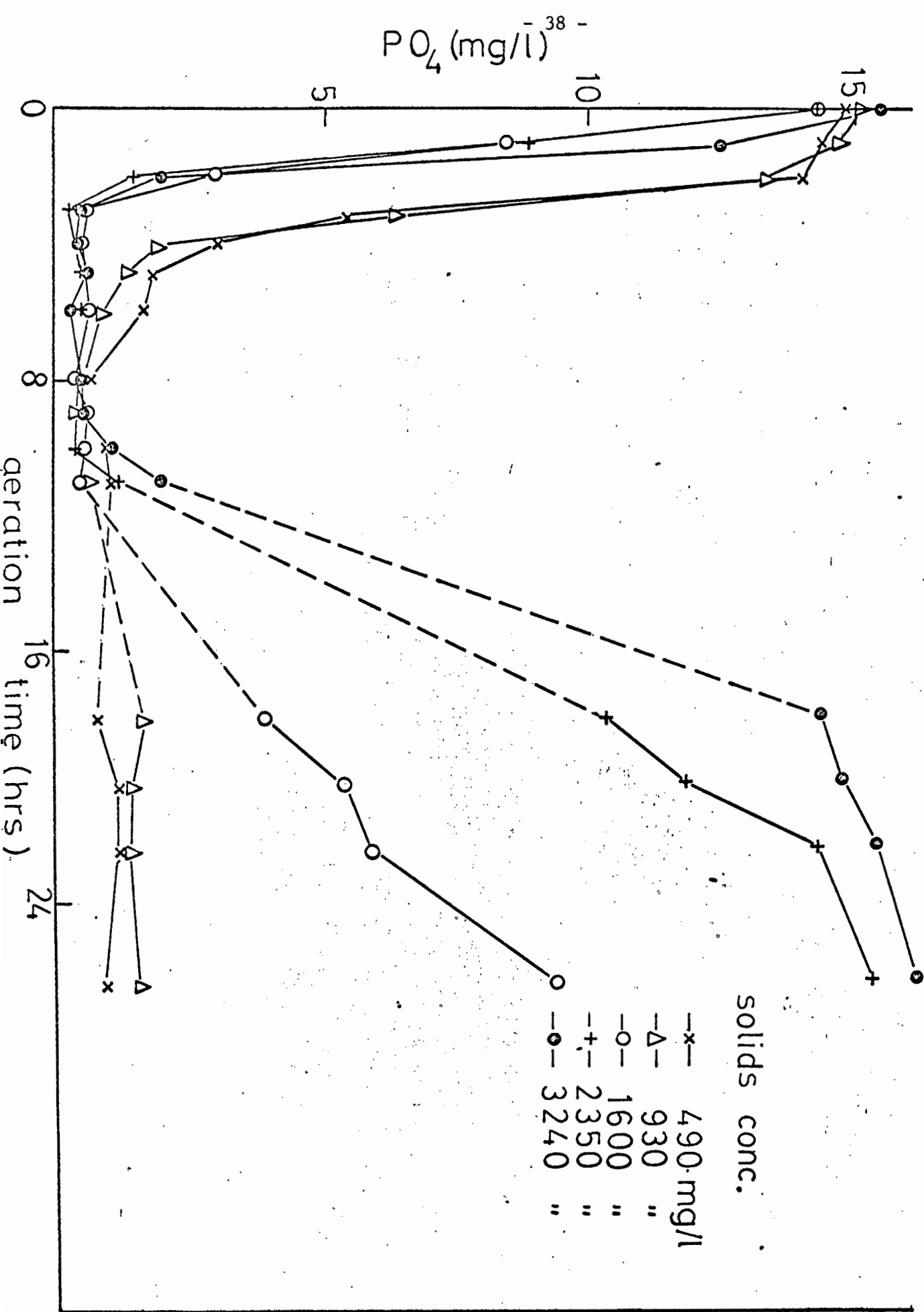


FIG.2. Effect of solids conc. on PO₄ uptake

From Fig. 2 it can be seen that the uptake of soluble phosphate was more rapid than in Experiment 1, and that very little phosphate remained in solution in the three higher sludge concentrations after 2-3 hours aeration. Phosphate uptake in the first 2 hours was less rapid in the two lower sludge concentrations, but had reached a maximum after 5 hours aeration. During the subsequent 4 hours the concentration of phosphate remained low, but after a total of 10 hours aeration a definite increase in phosphate concentration was observed in the two highest sludge concentrations. After 18 hours aeration an increased concentration had occurred in all the suspensions. Phosphate concentration after 26 hours aeration had increased to a level slightly above the initial concentration in the two highest sludge concentrations and to approximately 66 per cent of the initial value in the third highest concentration.

7.3.3 Conclusions

These results are of interest in that they clearly demonstrate the importance of aeration time on phosphate uptake by the aerobic micro-organisms. It is evident that as long as organic carbon is available, either in solution or as stored material within the cell, the assimilated phosphorus remains within the cell membrane. As the available organic carbon becomes exhausted auto-oxidation of the cellular material begins, and is accompanied by the release of nutrients that are not

required by the decreasing mass of viable cells.

The amount of nutrient released to solution would be expected to increase with increasing density of cell suspension and this is evident from the results. In the two highest sludge concentrations the eventual phosphate concentration exceeded the original values, indicating that sludge auto-oxidation had reached the stage where phosphorus contained within the original inoculum cell material was being released to solution.

The initial BOD concentration in each unit was 490 mg/l and the initial concentration of soluble phosphorus was approximately 5 mg P/l. This corresponds to a BOD:P ratio of 98:1 which was evidently sufficient to allow almost complete assimilation of phosphorus during cell synthesis. This ratio is in agreement with the optimum BOD:P value of 100:1 determined by Helmers, et al. (1951, 1952), and 81:1 by Hattingh (1963) during studies on the nutritional requirements of activated-sludge systems treating industrial wastes and synthetic sewage.

Activated-sludge concentrations used in conventional treatment plants normally range between 2500 and 3500 mg/l. At these concentrations it appears from results in Fig. 2 that, provided the BOD:P ratio is adequate, assimilation of soluble phosphate is complete after 3-5 hours of aeration and that longer retention times may reduce the removal of phosphate obtained.

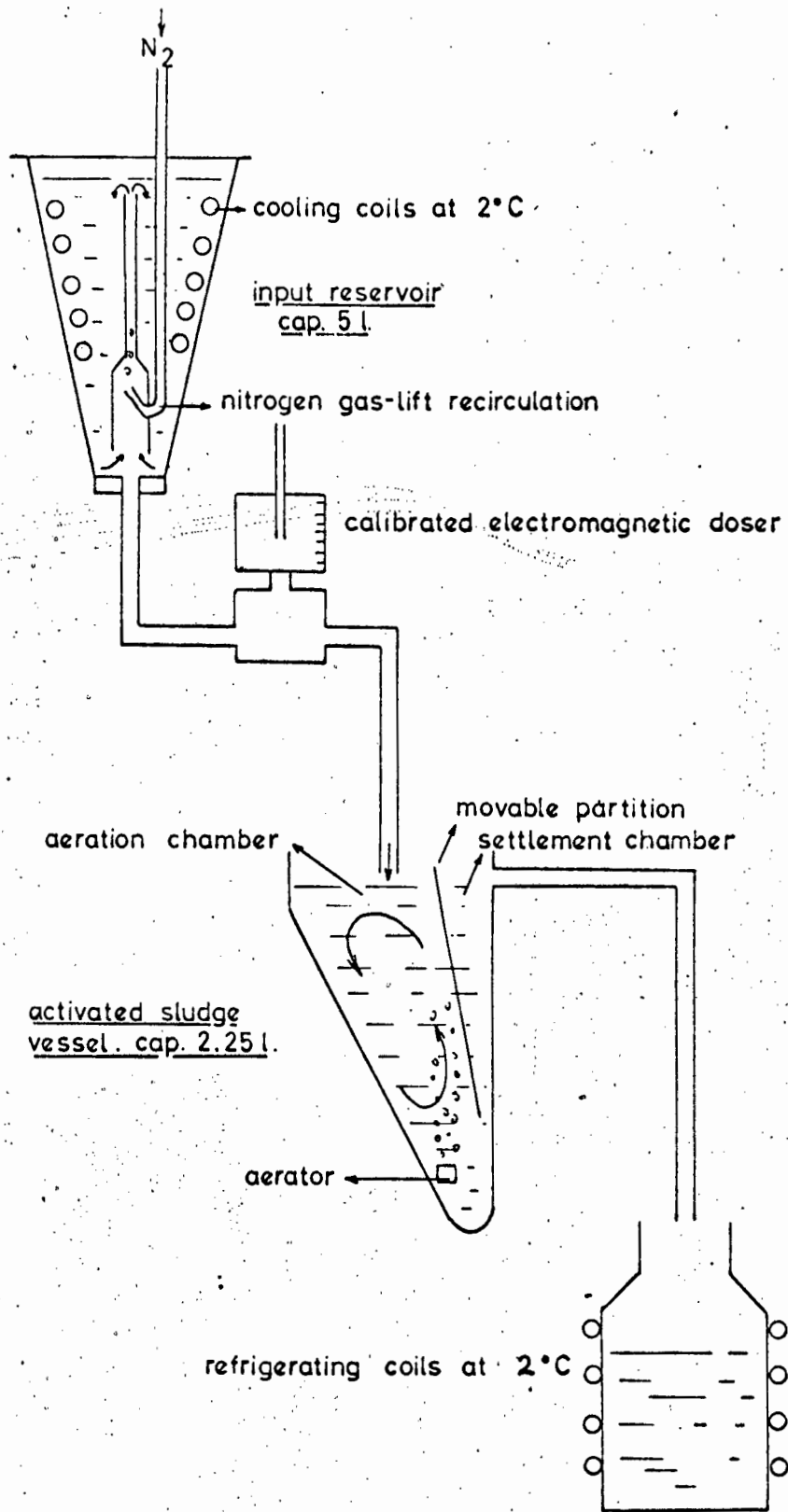


FIG. 3 :- LABORATORY-SCALE ACTIVATED SLUDGE UNIT.

7.4 Small-Scale Continuous-Flow Experiments

To further investigate the effect of aeration time and organic carbon load rate on the removal of nitrogen and phosphorus by cellular assimilation, an apparatus was constructed consisting of 6 identical small-scale activated-sludge units.

7.4.1 Apparatus

The design of individual units is shown in Fig. 3 and the complete apparatus is shown in Plate 1.

The input reservoir of each unit was maintained at 20°C to reduce changes in composition due to bacterial action. A nitrogen-operated glass gas-lift pump was inserted in the reservoir to exclude atmospheric oxygen and to prevent variation in load rates due to sedimentation of solids. A calibrated electromagnetic doser allowed dosing of predetermined volumes (± 3 per cent) at 5 minute intervals. The aeration vessel was a modification of a design incorporating an internal settlement compartment recommended by Ludzack (1962) for investigations of this type. Effluent from the aeration vessel was collected in bottles contained in a waterbath at 20°C to reduce any further bacterial activity before analysis. The aeration vessels were immersed in a constant temperature water bath at 20°C and aeration was provided by glass diffusers receiving compressed air through individual flow-meters fitted with needle valves. Air was supplied at a rate of 2 l/minute to each unit, a value somewhat higher than that usually provided in activated-sludge aeration tanks.

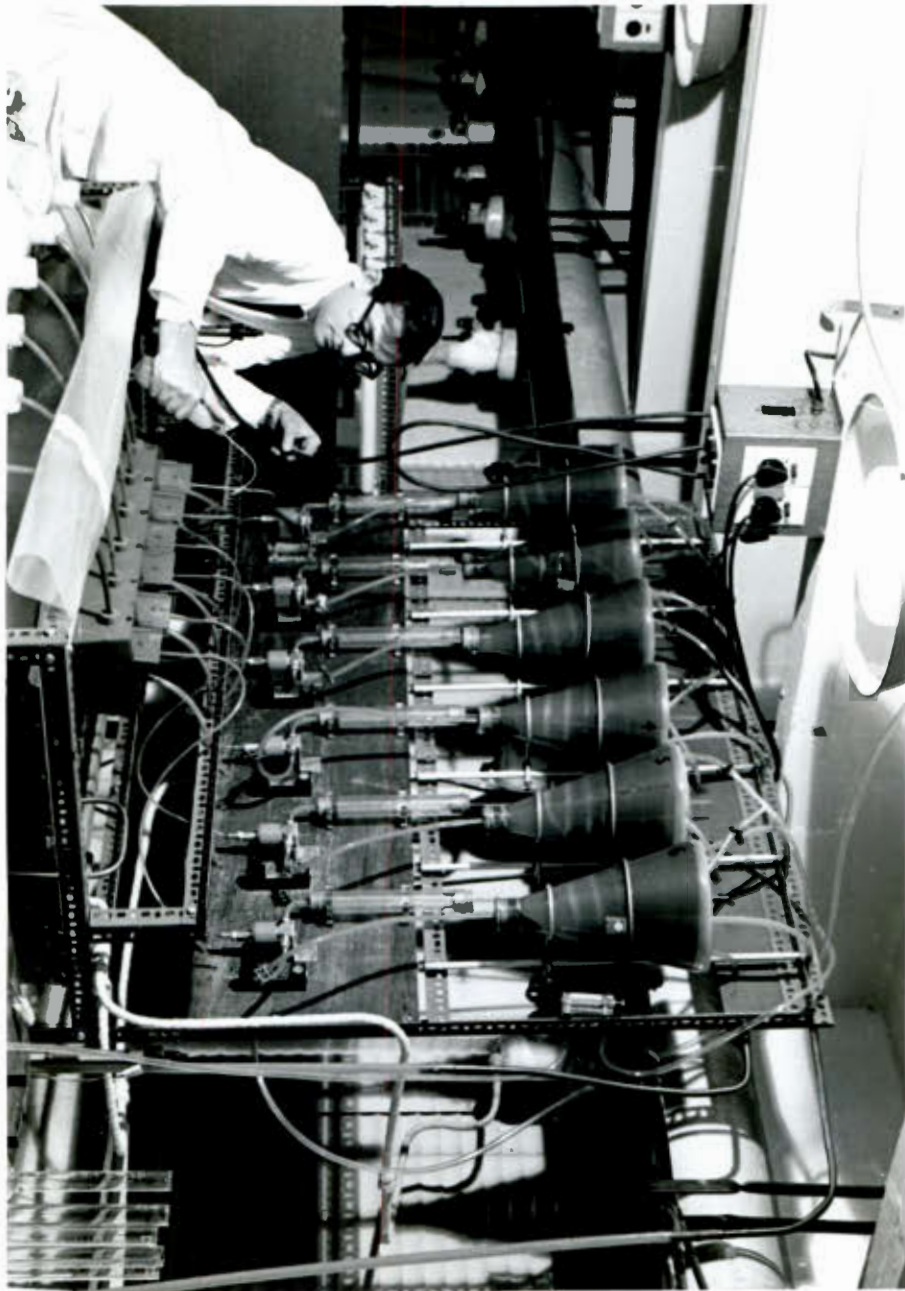


Plate 1. Laboratory-scale continuous-flow activated sludge units.

7.4.2 Method

A supply of raw or settled sewage was collected at intervals from the local sewage treatment plant and stored at 4°C. Before BOD determination, the Chemical Oxygen Demand (C.O.D.) was used as a relatively rapid test to confirm that the strength of each batch did not differ too widely from the required strength. If necessary, the sewage was diluted to the required strength with tap water. When unsettled sewage was used the gross particulate material was removed with a 1 cm wire mesh and the remaining larger solids were disintegrated with a high-speed propeller.

Samples were taken at 24-hour intervals during the experiments. Any sediment present in the effluent collection bottles was syphoned off once each day and returned to the aeration vessel. The whole content of the aeration vessel was then syphoned into a 5 l bottle and, after thorough mixing, duplicate samples for determination of suspended solids concentration were removed. The remaining volume was returned to the aeration chamber until this analysis had been completed. The removal procedure was then repeated, and a calculated volume, based on the analysis of the suspension, was removed to regain the required solids concentration on return of the suspension to the aeration chamber. The removed solids and the accumulated

effluent were retained for analysis.

On occasions when sludge settlement in the aeration chamber was poor and the accumulated effluent contained suspended material, the whole effluent production was centrifuged for 20 minutes at 2500 g in 6 litre batches. The excess solid material and the centrifugate were then analyzed separately.

When a high volumetric input was required the influent reservoir was replenished at intervals from 50 l drums stored at 4°C. The accumulated effluent was also removed at intervals and stored as a composite effluent at 4°C until analyzed.

The relatively large volumes of sewage required in some of the experiments made the use of several batches of fresh sewage necessary. For this reason, some variation in the BOD concentration between batches was unavoidable and could only be detected after the 5 days required for the BOD determination. However, by obtaining new supplies at the same time of day and by use of the preliminary COD test, it was possible to keep the variation to within 12 per cent of the required value. By adjustment of the dosing rate, it was possible to compensate for the variation and to keep the BOD load rate approximately constant during each run.

During the experiments the required conditions as regards loading and retention time were maintained for 2 days before detailed analysis was carried out. This period was allowed to permit stabilization of the system. Comprehensive analysis was started on the third day to obtain a nitrogen and phosphorous balance between influent and effluent during the subsequent 4-day period. Only 5 of the 6 units constructed were used in order to allow analysis to be completed in the time available.

7.5 Experiment 3

The procedure described above was employed to determine the effect of variation in aeration time on the rate of production of new organisms and removal of nutrients. Settled sewage was used as substrate in this experiment and the aeration time was fixed at 2, 4, 6, 8, and 10 hours. The five experiments were conducted simultaneously and the same batches of fresh sewage were fed to the five units. The average values for the sewage used were 235 mg BOD/l, total nitrogen 45 mg N/l, and total phosphorus 10.4 mg P/l. In computing the nutrient balances the actual concentrations and volumes used of each batch of sewage were employed. The nutrient content of the solids present in the aeration chambers at the beginning and end of the experiments were also taken into account. Total phosphorus was determined by the procedure described in Standard Methods (1965) after acid digestion to convert all phosphorus present to orthophosphate (see Appendix 1). In units in which solids production was rapid

Nutrient Balance Over 4 Days. All Weights in mg.	Aeration Time (Hours)				
	2	4	6	8	10
Total BOD in influent	26200	13550	9520	7250	5120
Total N in influent	4950	2590	1840	1210	1060
Total P in influent	1170	629	409	325	238
Total N in effluent	2337	1370	1222	870	751
Total P in effluent	207	227	228	205	165
Total suspended solids produced	25650	12060	6860	4640	2960
Total N in solids	2276	1088	566	411	268
Total P in solids	844	371	190	102	62
Total N in solids (per cent)	8.86	9.02	8.25	8.85	9.04
Total P in solids (per cent)	3.29	3.08	2.77	2.19	2.11
Sludge Growth Index*	0.98	0.89	0.72	0.64	0.58
Total N in effluent (mg/l)	20.33	23.45	31.31	30.60	32.14
Total P in effluent (mg/l)	1.92	3.54	5.36	7.34	7.18

*Sludge Growth Index = Unit weight of suspended solids produced per unit weight of BOD added.

Table 1. The effect of aeration time on the production of activated sludge and the removal of N and P from settled sewage.

the solids concentration was difficult to control at the required 3000 mg/l. The method employed was to reduce the concentration once each day to a level at which the average concentration over the subsequent 24 hours would be approximately 3000 mg/l.

7.5.1 Results

The data obtained using settled sewage as the substrate input are summarized in Table 1, from which it can be seen that the ratio of new organisms produced to amount of nutrient material added as BOD, termed the Sludge Growth Index, increased with decreasing retention time. The maximum value of 0.98 was obtained at a retention of 2 hours. The phosphorus content of the organisms produced showed a similar inverse relation to retention time in that the content increased from 2.11 per cent of the dry weight at 10 hours to 3.29 per cent at 2 hours retention. The nitrogen content of the solids did not show any evidence of a similar change with retention time.

The effect of increased incorporation of nutrients in the extra solids produced is reflected in the decreased concentration of total nitrogen and total phosphorus in the effluent. Calculations based on the total nutrients entering and leaving the system given in Table 1 show that the removal

obtained may represent those that would be obtained on a larger scale.

The substantial increase in the removal of nutrients at low retention times is quite marked and was obviously due to the increased production of solids. The unexpected increase in the phosphorus content at low retention times also contributed to the reduction in effluent phosphorus. However, the conditions under which maximum nutrient removal was achieved were also those associated with a deterioration in the settlement characteristics of the solids.

The latter observation is of importance, since the production of excess biological solids in a form that can be easily removed from the effluent is essential for the operation of a conventional treatment plant employing normal gravity separation of solids. If operation of the system at short retention times will result in loss of solids in the effluent, then any advantage gained in increased nutrient assimilation will probably be lost.

For this reason the next experiment, using raw sewage as the input, was designed to resolve the question of whether the deterioration in solids settlement was due to the decreased retention time or to the associated increase in BOD loading.

7.6 Experiment 4

This experiment was carried out with all the units operated at a retention time of 6 hours, since this period had been found to produce solids with satisfactory settlement characteristics in the previous experiment. By using unsettled raw sewage instead of settled sewage as the input, it was possible to determine the effect of variation in BOD load rate at constant retention time on the settleability of the solids produced and, at the same time, to detect any increase in nutrient assimilation as predicted to occur due to the increased BOD:N and BOD:P ratio in the raw sewage.

The concentration of suspended solids was maintained at approximately 3000 mg/l by the same method as in the previous experiment. Variation in BOD load was achieved by using raw sewage diluted with tap water to the required concentration. Differences in BOD concentration between batches of the sewage used were somewhat greater than before since use of the preliminary COD determination did not allow estimation of the BOD value with as much precision as was found with settled sewage. This difference was considered to be partly due to BOD sample dilution inaccuracies caused by the greater solids concentration in the sewage, and also to the greater variation in the ratio of COD and BOD, also probably as a result of the solid material present. The mean BOD value of the raw sewage used was 460 mg/l, with variation between batches from 415-535 mg/l, a maximum variation of 16.3 per cent from the mean.

Nutrient Balance Over 4 Days. All weights in mg. Retention Time - 6 Hours	Mean influent BOD concentration (mg/l)				
	468	395	315	196	109
Total BOD in influent	17980	15600	12110	7520	4180
Total N in influent	2160	1760	1450	914	509
Total P in influent	470	406	302	205	122
Total N in effluent	361	250	338	361	270
Total P in effluent	40	34	74	76	72
Total suspended solids produced	21580	18650	11370	6620	2930
Total N in solids	1719	1425	1012	584	291
Total P in solids	403	382	206	128	59
N in solids (per cent)	7.98	7.63	8.94	8.82	9.94
P in solids (per cent)	1.98	2.05	1.82	1.93	2.02
Sludge Growth Index	1.20	1.23	0.94	0.88	0.70
Total N in effluent (mg/l)	9.40	6.51	8.80	9.40	7.04
Total P in effluent (mg/l)	1.04	0.88	1.93	1.98	1.88

Table 2. The effect of BOD concentration on the production of activated sludge and the removal of N and P from raw sewage.

7.6.1 Results

The results obtained from this experiment are summarized in Table 2 and show a number of differences to those from the previous experiment. The reduction in nitrogen concentration was consistently greater, with a maximum removal of 85.7 per cent at the highest BOD loading and a minimum removal of 47.0 per cent at the lowest load rate. The phosphorus removal rate was also higher, with a maximum removal of 91.5 per cent at the two highest loadings and a minimum removal of 41.0 per cent at the lowest loading. The Sludge Growth Index was also somewhat higher than before, although it is probable that some of the increase was due to the increased amount of solid material present in the raw sewage, some of which would be inert but could become bound into the solid material produced. The increased assimilation of nutrients is again reflected in the reduced concentration present in the effluent but in this case the nitrogen, and to a lesser extent, the phosphorus concentrations were lower than before.

Both the nitrogen and phosphorus content of the solids produced were lower than in the previous experiment, probably as a result of incorporation of incoming solid material containing no nutrients. The percentage increase in phosphorus content associated with short retention time in the previous experiment was not observed in this

case, implying that the increase was in some way associated with retention time rather than loading rate.

Perhaps the most important observation from the practical standpoint was the decline in solids settleability occurring at the two higher loading rates. The settlement was not entirely satisfactory at the intermediate rate, but was excellent at the two lower rates.

7.6.2 Conclusions

The anticipated improvement in nutrient removal due to the increased amount of available organic carbon in the raw sewage was observed at all loading rates. The increase was greater at the higher loadings, due to the increase in Sludge Growth Index, but even at the lowest loading the 47.0 per cent removal of nitrogen achieved was almost as great as the maximum of 52.7 per cent obtained using settled sewage as the input.

If the results obtained at 6 hours retention in Experiment 3 are compared with those obtained at similar load rates in Experiment 4, also at 6 hours retention, the effect of using raw sewage, instead of settled sewage, on the nutrient removal is evident. In Experiment 3 the total BOD applied at 6 hours was 9,520 mg. From Table 1 it can be seen that of the 1,840 mg

nitrogen entering the system 1,222 mg were accounted for in the effluent, a removal of 33.6 per cent. In Experiment 4 the nearest BOD loads applied were either slightly higher (12,110 mg BOD) or lower (7,520 mg BOD) than the 6-hour retention load in Experiment 3. At the higher loading, the nitrogen removal was 76.8 per cent, and 60.5 per cent at the lower loading. For phosphorus the equivalent removals were 75.5 and 62.9 per cent. From these results it is evident that, even at the lower loading, the amount of nitrogen removed by assimilation when treating raw sewage was nearly twice as great as when settled sewage was used. The removal of phosphorus was also higher but the difference was not as large as in the case of nitrogen.

It is, therefore, evident that a considerable increase in nutrient removal can be obtained by omitting presettlement of the raw sewage in the biological oxidation process, however, as in the previous experiment, the marked deterioration in settlement characteristics observed at BOD loadings at which maximum nutrient removal was achieved indicates that practical operating difficulties are likely to be encountered in the full-scale treatment of raw sewage at high loading rates.

8. DISCUSSION OF RESULTS

The experimental work described in this section was based on the premise that the activated-sludge process is an ecological system in which nutrient limitation, in the form of carbon deficiency, is one of the main controlling factors.

On this basis the results can be explained in terms of changes in food-to-micro-organism ratio under the various experimental conditions imposed on the system, i.e., the governing effect of the amount of assimilable carbon available on the respiratory and synthetic activity of the population present.

Under the conditions imposed in Experiment 4 the same population density was provided with progressively less food supply during the same period of time. In these circumstances the food required for respiratory purposes would be the same in each system but the amount available for synthesis would be progressively less. The expected result would be a decrease in the synthesis of new material as the food supply was decreased. The Sludge Growth Index value, which is a measure of unit synthesis per unit of food supplied, has been calculated in Table 2 and demonstrates that the expected decrease in synthesis rate did occur as the food supply was reduced.

The conditions in Experiment 3 were somewhat different in that progressively less food was provided to support the same population for increasing time periods. In these circumstances the proportion of the available food used for respiration would be expected to be greater at the longer retention times, since the population density was the same in all cases, with proportionately less available for synthesis. This being so, it would be expected that the difference

between maximum and minimum Sludge Growth Index values would be greater in Experiment 3 than Experiment 4. The results indicate that, in practice, the differences in Sludge Growth Index obtained were similar in both cases. The reason for this deviation from predicted results can be explained on the basis of retention times in that, in Experiment 3, the retention times of 2 and 4 hours were too short for complete assimilation of the organic carbon provided, and the unutilized remainder passed out of the system with the effluent. Since BOD determinations were not carried out on the effluent there is no experimental evidence to confirm this explanation, nevertheless, visual observation of the rather turbid effluent produced under these conditions suggests that this explanation is probably correct.

In Experiment 2 a similar situation existed in that the same amount of food was provided in the same time period in each case, but the population density of micro-organisms was varied. The food required for respiratory purposes would be expected to increase with increasing population density and, as the supply was used up, the amount used for synthesis would decrease to the point where all the available food was required for respiration only. With complete exhaustion of the added food supply the population would begin to decline in numbers with the surviving individuals utilizing the carbonaceous material released by cell decomposition for endogenous respiration, but having no requirement for the macronutrients released at the same time. The concentration of macronutrients in solution would therefore be expected to increase with time.

The experimental results shown in Fig. 2 indicate that the course of the reactions was similar to that outlined above, in that there was

an initial demand for phosphorus for synthesis when added food was available which was greatest at the highest population densities. After about 10 hours the available food supply was exhausted and cellular decomposition began to occur at a rate directly related to the initial population density. At the higher population densities the rate of decomposition was more rapid with consequent increase in the re-solution of nutrients not utilized in respiratory activity.

These results emphasize the importance of maintaining a concentration of available carbon that is sufficient to support the synthesis of new material, as well as supporting the respiratory requirements of the population, if the removal of macronutrients by assimilation is the objective.

The purpose of this investigation was to determine whether suspended organic solids would be utilized during normal aeration periods and whether a significant decrease in the macronutrient concentration of a sewage treatment plant effluent would result if the primary settlement of sewage solids was omitted from the process. The results obtained provide evidence that this procedure would have the desired result in that as much as 76.8 per cent of the nitrogen and 75.5 per cent of the phosphorus was removed from the sewage by increased synthesis of micro-organisms.

Although these removal rates are considerably greater than those obtained during conventional sewage treatment, the amounts of residual macronutrients in the effluent are still substantial when the liquid volumes involved are considered. Operational difficulties due to poor solids settlement are also likely to be encountered, since Experiments 3 and 4 demonstrate that the

deterioration in settlement characteristics is related to the increase in BOD loading rate and not to the shorter retention times. Additional evidence for this has been reported by Anderson (1963), who showed that flocculation of activated-sludge particles results from the production of a sticky polysaccharide material to which micro-organisms adhere. The formation of the material does not appear to occur during the logarithmic growth phase and a relatively low food-to-micro-organism ratio is required. It does not, therefore, seem likely that high loading rates can be applied without resulting loss of nutrients in the effluent in the form of unsettled solids. If this is so, it appears that omission of primary settlement would necessitate the use of flocculating agents to prevent loss of solids. Although the effluent nutrient content would be materially decreased by the process, the economic aspects of flocculant addition and increased sludge disposal would require detailed evaluation.

Section B

DENITRIFICATION OF SEWAGE EFFLUENT

9. REMOVAL OF NITROGEN BY BACTERIAL RESPIRATION

The physico-chemical removal of phosphorus from sewage by precipitation of phosphate with suitable additives does not present as great a problem as does the removal of nitrogen. For this reason, the possibility of removing nitrogen from sewage by using micro-organisms to utilize oxidized nitrogen for respiratory purposes has been considered. The work so far reported suggests that the method may have practical application, although no full-scale use of a system of this type has yet been demonstrated.

The experiments described here were carried out to investigate a possible solution to the major practical problem of providing an inexpensive source of the hydrogen donor compounds required by denitrifying bacteria when oxidized nitrogen compounds are used as hydrogen acceptors.

9.1 Fundamental Aspects

Many micro-organisms are able to utilize nitrate-nitrogen and the overall process whereby nitrate is reduced to ammonia and assimilated for the synthesis of nitrogenous cell constituents is termed "nitrate assimilation". There are, however, several micro-organisms that can use nitrate, or some of its reduction products, as the terminal hydrogen acceptor in place of oxygen under certain conditions. This process has been termed 'dissimilatory nitrate reduction' by Verhoeven (1956) and "nitrate respiration" by Sato (1956). A third term, "denitrification",

Oxidation-reduction state of N atom	Formula	Compound
+ 5	NO_3	nitrate
+ 4	NO_2	nitrogen dioxide
+ 3	NO_2	nitrite
+ 2	NO N_2O_3	nitric oxide nitrohydroxylamate
+ 1	(NOH) N_2O N_2O_2 NO_2NH_2 $\text{NH}(\text{OH})_2$	nitroxyl nitrous oxide hyponitrite nitramide dihydroxyammonia
0	N_2 $(\text{NH})_2(\text{OH})_2$	nitrogen dihydroxylhydrazine
- 1	NH_2OH	hydroxylamine
- 2	$(\text{NH}_2)_2$	hydrazine
- 3	NH_4^+	ammonia

Table 3. Suggested intermediate compounds in the bacterial reduction of nitrate to ammonia.

9.1.2 Nitrite reduction

The nitrite reductases are widely distributed in micro-organisms and form part of the pathways for reduction of nitrite to ammonia in the assimilatory process as well as in the dissimilatory pathway to nitrogen gas. A nitrite reductase isolated from Pseudomonas stutzeri by Chung and Najjar (1956a) is a flavoprotein, requiring NADH as an electron donor and containing iron (cytochromes) and copper. The purified enzyme catalyses the production of nitric oxide from nitrite.

9.1.3 Nitric oxide reduction

Nitric oxide is converted to nitrogen gas by several denitrifying organisms and has been identified as an immediate reduction product in Ps. stutzeri (Chung and Najjar, 1956a,b). These workers claimed that the enzyme was a flavoprotein containing both copper and iron, but Fewson and Nicholas (1960) consider that the enzyme contains iron but no copper.

9.1.4 Formation of nitrogen

It has been suggested by several workers that nitrate reduction involves an intermediate at the +1 level of oxidation, such as hyponitrite, nitroxyl or nitrous acid, but the experimental evidence is inconclusive. Kluyver and Verhoeven (1954) found that denitrifying bacteria do not possess hyponitrite

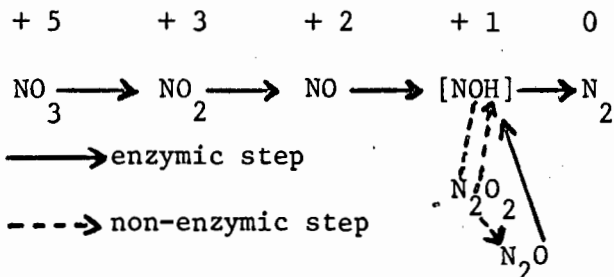
reductase activity, in contrast to Escherichia coli which can utilize hyponitrite as the sole source of nitrogen.

It has been stated that nitrous oxide can be utilized by bacteria (Kluyver and Verhoeven, 1954; McNall and Atkinson, 1957) but it seems unlikely that this is the case with denitrifying bacteria, since levels of cyanide, azide or 2:4-dinitrophenol which inhibit the production of nitrogen from nitrous oxide have no effect on the rate of production of nitrogen gas from nitrite (Sacks and Barker, 1952).

There is also some evidence that nitroxyl is an intermediate and is in equilibrium with hyponitrite, which can decompose to give nitrous oxide. Supporting evidence is given by Fewson and Nicholas (1960) in that nitric oxide is the immediate reduction product of nitrite in a number of denitrifying bacteria and may be produced during nitrate assimilation and, also, that nitric oxide reductase under anaerobic conditions is found in a number of organisms, whether assimilating or dissimilating nitrate.

Nicholas (1963) has summarized the available data in the form of a diagram which presents the most probable path of denitrification:

Oxidation-Reduction State of Nitrogen



9.1.5 Effect of oxygen on denitrification

The effect of oxygen tension on the denitrifying activity of micro-organisms has been investigated by a number of workers. Sacks and Barker (1949), using Pseudomonas denitrificans, found that oxygen suppressed the formation of both nitrate and nitrite reductases. When these enzymes were already present, the presence of oxygen reduced their activity, but the inhibition was reversible over short periods of time. They also found that aerobic growth over extended periods did not affect the ability of the organism to produce the reductases under anaerobic conditions, and concluded that nitrate reduction could occur at oxygen tensions of lower than 5 per cent by volume (approximately 2 mg $\text{O}_2/1$ at 25°C). Skerman, et al. (1951) suggested that oxygen did not directly inhibit nitratase and nitritase activity, and that the observed decrease in nitrate reduction was due to competition between molecular oxygen and nitrate as hydrogen acceptors. Collins (1955) demonstrated that the apparent occurrence of denitrification in aerobic solutions could be due to

inefficient aeration and mixing which permitted localized oxygen-free conditions in growing cultures.

Skerman and MacRae (1957a,b; 1961), working with Ps. denitrificans, showed that no nitrate reduction occurred at very low concentrations of dissolved oxygen; that nitrate reduction only occurs when the oxygen present is insufficient to satisfy the demand for electron acceptors; and that dissolved oxygen is only present when the demand is met. They also concluded that nitratase production ceases when an adequate supply of oxygen is available and that, probably, once formed, the enzyme is unaffected by the presence of oxygen. Evidence was also obtained to suggest that nitratase is only produced in growing cultures in which the oxygen supplied is insufficient for the respiratory requirements and that it is the partial pressure of oxygen in solution, and not merely its availability, which determines whether nitratase is produced.

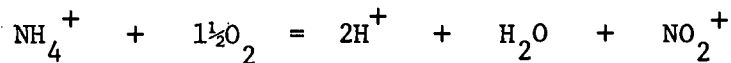
9.1.6 Requirements for denitrification

The requirements for microbial denitrification are not very complex. All that is required is a suitable temperature and pH value, a sufficient supply of mineral nutrients, a suitable hydrogen donor, and nitrate or an

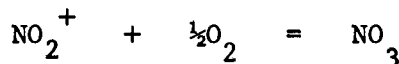
oxidized intermediate nitrogen compound. The final requirement is the absence of dissolved oxygen in quantities sufficient to effect the production or activity of the enzyme systems involved. If these conditions are provided in a sewage treatment system, then denitrification can be expected to occur to some degree if the necessary organisms are present. However, for denitrification to occur the presence of oxidized nitrogen is a prerequisite.

9.1.7 Requirements for the bacterial production of oxidized nitrogen

The organisms capable of oxidizing ammonia to nitrite are believed to be autotrophic forms, and of the five known genera, Nitrosomonas is the most commonly found. According to Delwiche (1956) the overall energy-yielding reaction of Nitrosomonas is:



According to Frobisher (1957) the oxidation of nitrite to nitrate can only be carried out by the autotrophic Nitrobacter, but certain heterotrophs are apparently capable of carrying out the complete oxidation of ammonia to nitrate (Schmidt, 1954).



Thus, four atoms of oxygen are required to oxidize biochemically one atom of ammonia nitrogen to nitrate. This requirement implies that to produce a sewage effluent containing nitrate, the amount of molecular oxygen supplied to the bacterial system must be increased to a level above that required for oxidation of the carbonaceous content only. Jeffrey and Morgan (1959) have shown that the actual oxygen demand for nitrification of an ammoniacal effluent agrees closely with the theoretical quantity, and Johnson and Schroepfer (1964) have stated that for nitrification of a typical domestic sewage the amount of oxygen required is approximately 50 per cent greater than that required for carbon oxidation alone.

This increase in oxygen requirement could represent a substantial increase in treatment costs where large volumes of sewage are involved, and illustrates the obvious disadvantage of a process in which nitrogen must first be completely oxidized and subsequently reduced to nitrogen gas. However, the problem of nitrogen removal from sewage effluent is sufficiently important to warrant increased treatment costs if a satisfactory method can be devised.

9.2 Denitrification in Sewage Treatment Processes

When nitrate and nitrite are detected in the effluent from a sewage bio-oxidation process it is likely that denitrification is occurring somewhere in the system. That denitrification can occur in an aerobic oxidation system, such as an activated-sludge plant, is considered to be due to oxygen deficiency developing within the particles of bacterial floc and to the presence of denitrifying organisms in large numbers (Wuhrmann, 1960; Bringmann, et al. 1959). The occurrence of denitrification in activated-sludge settlement tanks to a degree sufficient to impair settlement of solids has also been observed (Sawyer and Bradney, 1945).

The function of nitrate in the stabilization of waste waters, with subsequent release of gaseous nitrogen, has been discussed by McKinney (1956) and by McKinney and Conway (1957) and also by Symons (1957) and Symons and McKinney (1958). More recently a survey of five treatment plants in the United States has shown that significant loss of nitrogen by denitrification can occur, but the degree to which it is removed is very variable (Barth, et al. 1966). The same authors conclude that the recycling of oxidized nitrogen from the final effluent to undergo denitrification in an oxygen depleted zone, as investigated by Ludzack and Ettinger (1962), is probably not the most efficient method of removal as oxidized nitrogen would still be discharged.

Bringmann and Kuhn (1962, 1964, 1965) and Johnson and Schroepfer (1964) have sought to overcome the problem of the lack of oxidizable organic carbon in an oxidized effluent by adding untreated sewage to provide a source of hydrogen donors for the heterotrophic denitrifiers. However, since the unoxidized nitrogen in the added sewage cannot be removed by denitrification, there is a consequent loss of nitrogen in the effluent from the denitrification stage, usually as ammonia nitrogen.

Wuhrmann (1964) has described the use of a different system in which the aerobic bacterial solids from the activated-sludge aeration tank were passed to a stirred but unaerated tank. In this tank the bacterial respiration reduced the dissolved oxygen to a level at which nitrate respiration could take place and, with a retention time in the unaerated tank of a little under 3 hours, consistent reduction of nitrate from values of 15-20 mg N/l to less than 5 mg N/l were obtained for several consecutive weeks. No mention of a need to add extra hydrogen donors in the form of untreated sewage is made, in contrast to the method used by Bringmann and Kuhn (1962, 1964, 1965), and Johnson and Schroepfer (1964).

Of the various experimental approaches to the problem of denitrification on a practical scale that have been investigated it appears that the results obtained by Wuhrmann were the most favorable. However, the procedure involves the growth of denitrifying organisms in the aerobic conditions of the aeration

tank and the results of Sacks and Barker (1949) and of Skerman and MacRae (1957a,b; 1961) indicate that the production of nitratase in a denitrifying flora is adversely affected by growth in an aerobic medium. The development of a system in which a denitrifying flora is propagated under oxygen deficient conditions should result in the production of organisms with a higher level of nitritase activity. This higher level of activity should result in a decrease in the retention time required for the reduction of specific amounts of nitrate to gaseous nitrogen.

In a system of this kind the denitrifying stage would have to be maintained in an oxygen-free state and a supply of hydrogen donor and hydrogen acceptor (nitrate) provided. The latter would be present in the nitrified effluent entering the denitrification stage but unoxidized hydrogen donors would be present in very small amounts. For this reason, some external source of hydrogen donor compounds would have to be provided.

An investigation on these lines was carried out by Finsen (1959) using sucrose, ethyl alcohol, and also molasses to provide the hydrogen donor required. Although satisfactory denitrification was obtained, he concluded that the method was unlikely to prove satisfactory on a large scale due to the high cost of the materials used as hydrogen donor. Nevertheless, the results obtained did indicate that if a suitable and

inexpensive source of nitrogen-free hydrogen donor was available the method would have practical application.

Waste carbonaceous materials from industrial processes, such as the fermentation industry, are obvious possible sources of organic compounds with relatively low nitrogen content. However, such materials may not be locally available in the amounts required, and haulage costs from the industrial source may be economically unacceptable. For this reason, consideration was given to ways in which a suitable hydrogen donor source might be produced from materials available at the sewage treatment plant. It was concluded that the sewage solids obtained by settlement of the incoming raw sewage might be utilized for the purpose after treatment.

10. SEWAGE SOLIDS AS A SOURCE OF HYDROGEN DONORS

Organic compounds in sewage are normally in dilute solution or suspension and would be unsuitable in this form due to the excessive volumes of liquid that would be required to obtain the quantity of hydrogen donor needed. A further disadvantage would be the reduced nitrogen compounds contained in the sewage, such as ammonia and urea, which would pass through the denitrification system without being converted to nitrogen gas.

Raw sewage sludge, obtained by gravity settlement of the incoming sewage, is very much more concentrated than sewage itself and contains a wide variety of simple and complex organic compounds. Painter and Viney (1959) found the major components of the gross particulate material that

settled in 1 hour to be soluble and insoluble higher fatty acids, esters, carbohydrates and amino acids. This fraction contained between 30 and 40 per cent of the total organic carbon in the raw sewage. Hunter and Heukelekian (1965) found that 24 per cent of the settled solids consisted of carbohydrates, 19 per cent amino acids and 17 per cent esterified fatty acids and unsaponifiable matter. Both investigations showed that the proportion of organic nitrogen to organic carbon decreased with increasing particle size, the proportion of organic nitrogen being appreciably higher in the solids removed by centrifugation and candle filtration.

According to these reports it appears that raw sewage sludge contains substantial amounts of organic material that could serve as hydrogen donors for the nitrate respiration of denitrifying organisms.

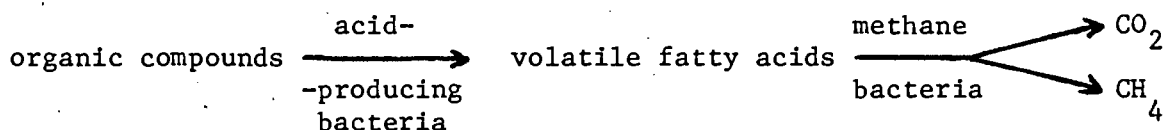
In the process of gravity separation of these solids, it is likely that a considerable reduction in the amount of reduced nitrogen compounds present would occur, since up to 80 per cent of the total nitrogen in raw sewage is in the form of ammonia and urea, which would remain in solution (Hanson and Flynn, 1964), and the unsettled colloidal fraction would retain a further fraction of the total nitrogen. This reduction in nitrogenous compounds would be advantageous since, apart from that which might be taken up for cell synthesis, this nitrogen would be unavailable for respiratory purposes and would appear in the effluent. It is likely, however, that despite the reduction caused by settlement, some excess nitrogen would be present and suitable treatment of the raw sludge would be necessary to remove this excess.

Direct removal of nitrogen from the raw sewage solids would be difficult, since most of the nitrogen is incorporated in amino acids and

other organic nitrogen compounds. If this nitrogen could be released in the form of ammonium compounds, however, it might then be possible to remove it from solution in some way, such as exposure to the air as a thin film at high pH. Under these circumstances substantial removals can be obtained as reported by Kreft, et al.(1958) and Bayley (1967) and confirmed elsewhere in this thesis. (Sections C and D)

A possible means of achieving this objective would be to subject the sewage solids to anaerobic fermentation. This process is normally used in sewage treatment to remove fermentable materials from the solids to produce a stable material that can be dried and used as an agricultural soil conditioner. In this process conditions are provided under which a population of methane-producing bacteria is maintained, with the result that all biologically available carbon compounds are decomposed to carbon dioxide and methane, which leave the liquid phase as gases. By this process the organic hydrogen donor compounds are reduced to low concentrations, which would be contrary to the objective in this case.

Anaerobic sludge digestion proceeds via two stages; an acid-producing stage followed by the methane-producing stage, which is usually considered to be the rate-limiting step;



In the first stage substrate breakdown by beta-oxidation of long chain fatty acids, de-amination of proteins, and disruption of carbohydrates occurs by enzymatic reactions, with the production of simple aliphatic compounds which are mainly acetic, propionic and butyric acids. Under the

highly reducing anaerobic conditions in the fermentation vessel the nitrogen released is almost quantitatively reduced to ammonium compounds.

The fatty acids are subsequently utilized as energy sources by the methane bacteria with the production of carbon dioxide and methane as end products.

If, for any reason, the activity of the methane-producing population is impaired, it has frequently been observed that there is a very rapid build-up of the unmetabolized acids as a result of the continued activities of the acid-producing population. The oxidation-reduction changes involved in the breakdown of complex organic compounds to simple acids are relatively small, compared to those in the second stage, and if the process were to be deliberately arrested at this point a source of simple carbon compounds containing very little bound nitrogen would be available. Almost all the original nitrogen present would be in the form of ammonium compounds, and, by suitable pH adjustment and thin-film exposure, it should be possible to remove most of the nitrogen to leave a nitrogen-free solution of simple organic carbon compounds to supply the requirement for a source of hydrogen donors.

10.1 Production of Volatile Fatty Acids

It has been observed in numerous instances that the production of volatile fatty acids during the anaerobic digestion of sewage sludges or industrial wastes is a rapid process, and that conversion of these acids into gaseous products ceases if the methane-producing bacterial population is inactivated. Under these conditions, the acid accumulation may reach concentrations of several

grams per litre (Kaplowsky, 1951; Hinden and Dunstan, 1960; McCarty and McKinney, 1961; McCarty, et al. 1962).

Inactivation of the methane-producing population can result from a number of causes, one of the most common of which is organic overloading of the digester. When this occurs the rapid production of volatile acids from the organic overload exceeds the buffer capacity of the digester liquor and the pH value falls rapidly to levels at which the methane-producing bacteria are inhibited. The tolerance of the acid-forming bacteria to low pH is much greater and acid production continues until all the organic load has been decomposed. In this way a high concentration of mixed acids is eventually produced, with acetic acid forming 70 to 80 per cent of the total.

The production of large quantities of volatile acids from sewage solids should therefore present no great difficulty, since deliberate overloading of a sludge digester will produce the conditions required for maximum acid production.

10.2 Use of Volatile Acids as Hydrogen Donors

According to Nason (1962), straight-chain hydrocarbon molecules cannot be utilized in biochemical reactions, whereas the homologous oxygen-containing straight-chain carbon compounds can serve as hydrogen donors in these reactions. The simple aliphatic acids, such as acetic and propionic acids are typical examples of such compounds and should therefore be suitable as hydrogen donors in the denitrification process.

11. EXPERIMENTAL OBJECTIVES

The experimental work described in this section was designed with the following objectives in view:

- i. To confirm that volatile fatty acids can be utilized as hydrogen donors in the denitrification process, and to compare their suitability with other organic compounds;
- ii. To determine the ratio of hydrogen donor to oxidized nitrogen required for denitrification;
- iii. To demonstrate the use of the proposed process as a continuous operation for the removal of nitrogen from oxidized sewage effluent.

11.1 Isolation of a Denitrifying Organism in Pure Culture

To avoid variation in experimental results due to the use of mixed bacterial cultures, it was decided that a pure culture of a denitrifying organism should be used for comparison of the suitability of volatile acids and other compounds as hydrogen donors, and for determination of the hydrogen donor : nitrate-nitrogen ratio required for denitrification.

An organism for this purpose was obtained from a sample of sewage-grown activated sludge. The sample was allowed to become anaerobic and 0.1 N KNO_3 was added as a nitrate source. After 2 days anaerobic incubation at 20°C a small sample was transferred to a nitrate medium containing 1 g/l KNO_3 , 3 g/l Bacto-Beef Extract and 5 g/l Bacto-Peptide at pH 7.0. Using standard methods of isolation and sub-culture a pure culture of an actively denitrifying bacterium was

isolated. The organism was gram-negative and appeared as short straight rods under microscopic examination. Detailed examination of the biochemical reactions of the organism resulted in its tentative identification as Pseudomonas stutzeri. However, subsequent expert examination demonstrated a number of differences to the reactions of Ps. stutzeri and complete identification was not obtained (see Appendix 2).

11.2 Respirometric Measurement of Denitrification

In the early stages of this section of the investigation an attempt was made to use a standard Model V85 Warburg respirometer for studies on the comparative suitability of different compounds as hydrogen donors. It was the intention to equate the volume of gas produced during denitrification with the change in nitrogen concentration in the substrate. Although a satisfactory modification of the standard respirometric technique and the reagents required was developed, the results obtained were not considered to be reliable for two reasons. The first of these was the fact that unexpectedly large amounts of hydrogen donor were apparently derived from the dense bacterial suspension used. Because of this release of donor, it was not possible to detect any marked difference in nitrate respiration between suspensions to which no extra donor had been added in the form of glucose, and those that contained up to 30 mg/l glucose. The second cause of inaccuracy was the fact that not all the oxidized nitrogen that was reduced was evolved

as nitrogen gas and no means was available for assessing quantitatively the form of the fraction of reduced nitrogen that did not appear as gaseous nitrogen.

For this reason an alternative method, that would permit the use of larger reaction volumes, was developed and used for the initial experiments.

11.3 Chemical Measurement of Denitrification

The analytical procedures employed for the determination of nitrate-nitrogen, nitrite-nitrogen, ammonia-nitrogen and Kjeldahl nitrogen were those described in Standard Methods (1965).

11.3.1 Apparatus

To permit use of larger volumes of reaction mixtures required for this approach, a number of 250 ml conical flasks were fitted with rubber stoppers through which were passed short sections of glass tube. A serum cap fitted to the end of the glass tube gave a gas-tight vessel into which samples could be introduced or removed via a hypodermic needle. The flasks were washed and sterilized in an autoclave before use.

11.3.2 Method

In all experiments an initial working volume of 200 ml of solution was used. To each flask was added 10 ml of pH 7.0 phosphate buffer solution to give a 0.05M concentration when made up to 200 ml. A concentrated inorganic nutrient medium used for the preparation of dilution water in the standard 5-day BOD test (Ministry of Housing and Local

Government 1956) was then added in quantities calculated to give a final volume of 200 ml of the required concentration after addition of the remaining reagents.

The flasks were then vigorously boiled for 1 minute to remove oxygen with a continuous stream of argon passing through the liquid via hypodermic needles. The flow of argon was continued while the solutions were rapidly cooled in ice. At this stage calculated volumes of standard KNO_3 solution and hydrogen donor, i.e., glucose, acetic acid, etc., were added to give the required concentrations and the solutions bubbled with argon for a further 15 minutes, after which the needles were withdrawn as the stoppers were fitted firmly into place. Finally, the bacterial suspensions were added with a syringe via the serum cap. After shaking to mix, a volume of argon was injected, usually about 30 ml, to maintain a slight positive pressure in the flask as samples were withdrawn during the course of the experiment.

11.3.3 Preparation of bacterial suspensions

A pure culture of the isolated denitrifying organism was subcultured at 25°C as required in the nitrate broth previously described.

For each experiment a culture of the organism was separated by centrifugation at 3000 g for 20 minutes. The pellet obtained was washed free of hydrogen donor contaminants from the culture medium by twice resuspending in phosphate buffer and recentrifuging. During these

operations air was excluded by maintaining an atmosphere of argon above the suspension.

11.4 Experiments 1 and 2

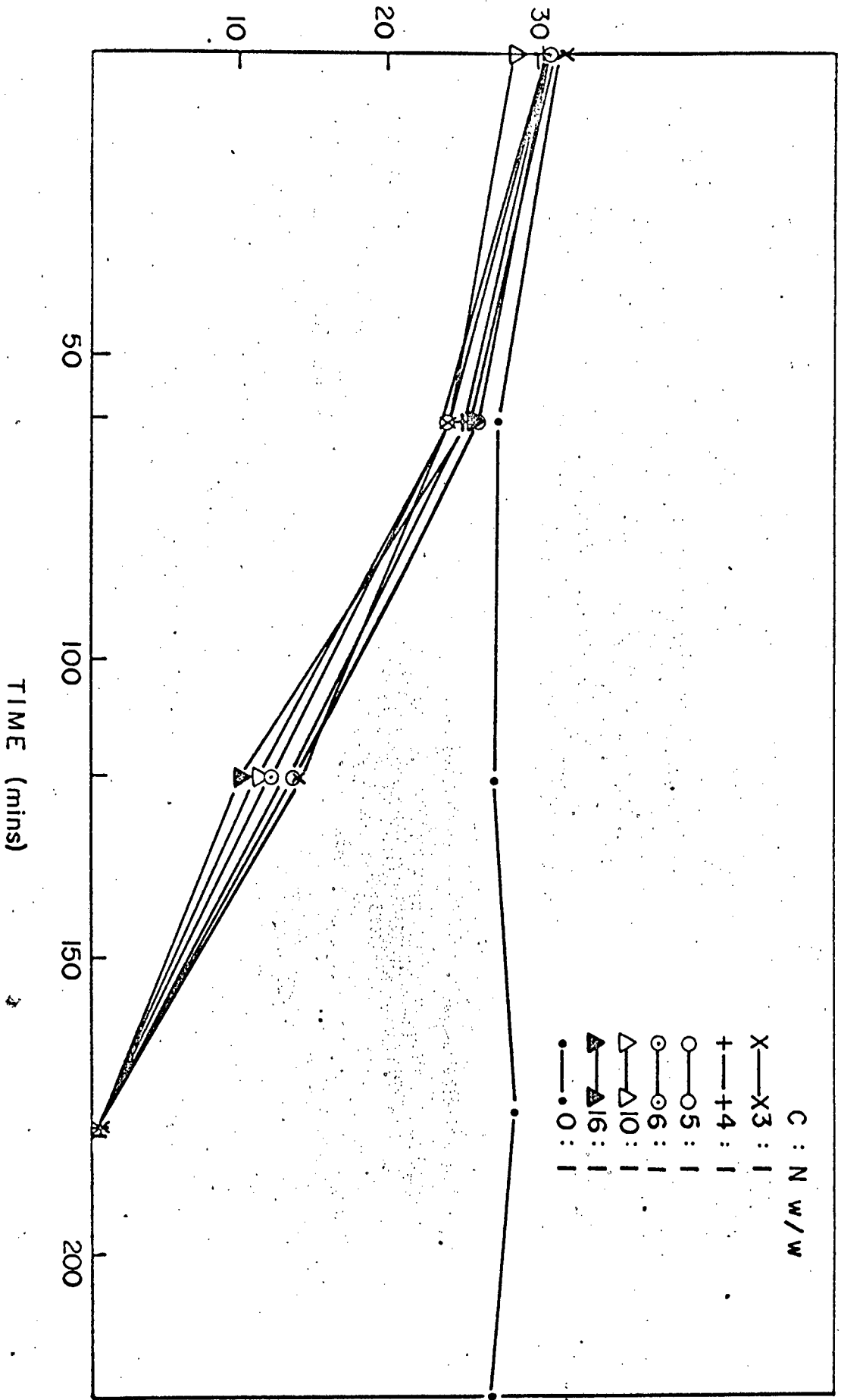
These experiments were carried out to determine the glucose-carbon:NO₃-N ratio on a weight basis required to obtain complete denitrification.

For each experiment a series of the flasks previously described was used and KNO₃ solution sufficient to give a final concentration of 30 mg NO₃-N/l was added to each. In Experiment 1, glucose solution was added in amounts sufficient to give a glucose-C:NO₃-N ratio of 3.0, 4.0, 5.0, 6.0, 10.0 and 16.0. In Experiment 2, glucose was added to give ratios of 0.25, 0.5, 1.0, 1.5, 2.0, 3.0 and 4.0. In each series an additional flask without glucose added was included as a control.

Equal volumes of bacterial suspension were added to each flask of each series. After mixing and removal of the first sample, the flasks were placed in a 25°C water bath. Small samples were removed at intervals with a syringe and the total nitrogen in solution was determined.

Since nitrogen in all its forms was determined at the beginning and end of the experiment; it was possible to demonstrate conclusively whether or not nitrogen has been lost from the aqueous phase. At a pH of 7.0 ammonia would not be lost from solution and any loss detected could only be due to the evolution of nitrogen gas, nitrous oxide or nitric oxide.

NO₃-N
mg/l



C : N w/w

- X—X3 : |
- +—+4 : |
- 5 : |
- ⊙—⊙6 : |
- △—△10 : |
- ▲—▲16 : |
- 0 : |

FIG. 4

Effect of C : N ratio on nitrate reduction

11.4.1 Results of Experiments 1 and 2

The changes in nitrogen concentration that occurred during the experiments are shown in Tables 4 and 5 and Figs. 4 and 5.

The quantity of glucose carbon added in Experiment 1 was evidently sufficient for the reduction of 30 mg $\text{NO}_3\text{-N}/\text{l}$ in all cases and the addition of glucose in excess of a 3:1 C: $\text{NO}_3\text{-N}$ ratio did not effect the rate of reduction since the curves shown in Fig. 4 are almost identical. In Experiment 2 the amount of glucose added was decreased and the results obtained showed that the amount of glucose present had a very marked effect on the reduction of nitrate. After 150 minutes neither nitrate nor nitrite were detectable in solutions containing C: $\text{NO}_3\text{-N}$ ratios of 2:1 or more. Results for the Kjeldahl nitrogen and oxidized nitrogen values at the beginning and end showed a loss of 27.4, 30.2 and 23.2 mg/N from the solutions in flasks 5, 6 and 7, compared with a loss of only 5.9 mg/N from the control.

In those solutions with C: $\text{NO}_3\text{-N}$ ratios of less than 2:1 the removal of nitrate was progressively less with decreasing amounts of glucose. However, even in the control flask 4.5 mg $\text{NO}_3\text{-N}$ were lost and not accounted for by an increase in Kjeldahl-N. This suggests that hydrogen donors in the form of materials derived from

the inoculum were released during the experiment, as was thought to be the case in the respirometric studies, but the effect was very much less pronounced due to the relatively lower concentration of organisms.

It was evident from the results that nitrite was an intermediate reduction product of the bacterial respiration but that its appearance was transitory; at no time did concentrations exceed 0.55 mg NO₂-N, indicating that the nitrite and nitrate reductase systems were of approximately equal activity.

The variation in ammonia and Kjeldahl nitrogen between flasks was unexpectedly high in Experiment 2, in view of the precautions taken in washing and dispensing the bacterial suspension, but in both experiments an increase in final ammonia nitrogen was observed in most cases.

In Experiment 1 the initial and final Kjeldahl-N and ammonia-N figures showed that in flasks 1, 2, 4, 5, and 6, the increase in Kjeldahl-N was largely due to formation of ammonia without decrease in organic nitrogen (cell material), whereas in the control there was a loss of organic nitrogen and an equivalent gain in ammonia. (Results for flask 3 are anomalous and attributed to analytical error). These results suggest that reduction of nitrate to ammonia occurred in those systems with added hydrogen donor but not in that in which it was in short supply, i.e., the controls.

11.4.2 Conclusions from Experiments 1 and 2

These initial experiments showed clearly that the presence of adequate hydrogen donor is an essential requirement for denitrification and that a glucose-carbon: $\text{NO}_3\text{-N}$ of 2 or more is required for complete reduction of nitrate. The longer period of time required for complete denitrification to occur when adequate hydrogen donor was present in the first experiment is explained by the fact that the concentration of organisms added was noticeably less than in the second experiment.

Since some nitrate reduction occurred in the controls, despite the precautions taken to remove all residual culture medium, it is probable that cell decomposition was responsible for the release of materials that could serve as hydrogen donors.

The reason for the production of ammonia from nitrate reduction in most of the flasks is not evident from the results obtained, but might have practical significance if it were to occur in a full-scale denitrification system.

11.5 Experiment 3

The previous experiments established that the critical glucose-carbon: $\text{NO}_3\text{-N}$ ratio below which denitrification was incomplete was between the values of 1.0 and 2.0. The next experiment was designed to obtain a more precise estimate of this value for glucose and, at the same time, to compare the denitrifying activity of the organism isolated with a pure culture of the denitrifying bacterium Pseudomonas stutzeri

made available by the Department of Bacteriology of the University of Pretoria.

11.5.1 Method

The freeze-dried culture of Ps. stutzeri was reconstituted in the same nitrate broth as used for the isolated bacterium and, after one sub-culture in fresh medium, was prepared in the same way as in the previous experiments.

The same procedure and nitrate concentrations as before were used, with glucose-C:NO₃-N ratios of 1.2, 1.5, 1.7, 1.9 and a control with no glucose. In this experiment the Kjeldahl nitrogen was determined in each dilution at the beginning of the incubation and as soon as the nitrate concentration was observed to have disappeared. Nitrite and ammonia analyses were not carried out.

11.5.2 Results

The results given in Table 6 and in Fig. 6 indicate that in the case of the isolated organism a glucose-C:NO₃-N ratio of 1.5 or greater was sufficient to allow complete denitrification in 215 minutes but a ratio of 1.2 was not adequate.

For the P. stutzeri cultures the results are similar except that complete denitrification was obtained with a ratio of 1.3. The result for the

1.9:1 dilution appears to be atypical since, by the time nitrate had disappeared from the flasks, this dilution had reduced no more nitrate than had the control. It seems probable that this result was due to the unrealized omission of the inoculum in preparation.

11.6 Use of Organic Compounds from Sewage as Hydrogen Donors

Although it has been shown by other workers that sewage can be utilized as a source of hydrogen donors for the bacterial reduction of oxidized nitrogen (Johnson and Schroepfer, 1964), it was considered of interest to ascertain which of the major groups of compounds present in sewage, i.e., fatty acids and esters, carbohydrates and amino acids, were capable of carrying out this function.

11.6.1 Method

As representatives of the various groups, glucose and fructose were used as monosaccharides, lactose as a disaccharide and soluble starch as a polysaccharide. Glycine and glutamic acid were used as amino acids and acetic acid as a simple fatty acid. A nitrate-nitrogen concentration of 30 mg/l was employed as before and C:NO₃-N ratios of 2:1 and 3:1 to ensure that hydrogen donor was not likely to be limiting. The isolated organism was used as the denitrifier. Kjeldahl and ammonia nitrogen was determined at the beginning and end of the experiment, or as soon as nitrate was exhausted.

11.6.2 Results

The experimental results are given in Table 7 and Fig. 7.

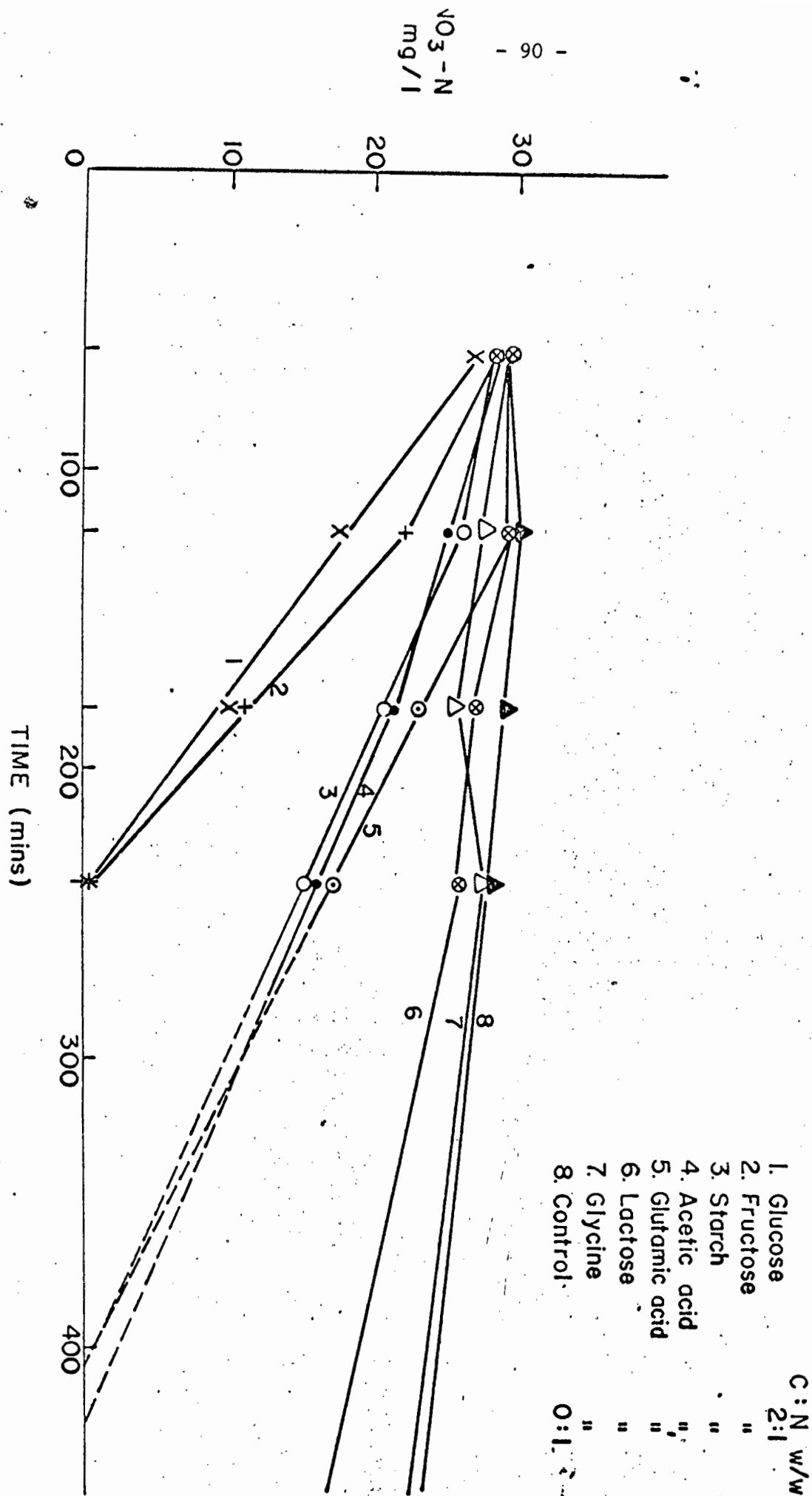


FIG. 7
Comparison of H⁺ donors

The results for carbon:NO₃-N ratios of 2 and 3 were almost identical and only those for the 2:1 ratio are presented graphically.

As might be expected, the results for glucose and fructose were very similar. Rapid denitrification began approximately 60 minutes after addition of the bacteria and denitrification was complete in less than 240 minutes. With starch, glutamic acid and acetic acid the delay was greater and rapid nitrate reduction did not begin until between 120 and 180 minutes after inoculation and was complete at between 400-500 minutes (by extrapolation from the curves).

As judged by the slope of the curves it appears that, once rapid reduction had started, the denitrification rates for glucose and fructose were slightly greater than for starch, glutamic acid and acetic acid.

In the case of glycine and lactose the reduction of nitrate was very slow and substantially the same as in the control, from which it is evident that glycine and lactose were not utilized as a hydrogen donor in the same way as the other compounds.

The results obtained from Kjeldahl and ammonia analysis are difficult to interpret. The concentration of initial and final ammonia-N present was not detectable in almost all cases, in contrast to previous experiments. The higher

total nitrogen values for glycine and glutamic acid would be expected in view of their nitrogenous nature. However, with these acids and with several of the other compounds there appears to have been a net loss of Kjeldahl nitrogen during the experiment which is difficult to account for, since oxidation to nitrite or nitrate followed by denitrification to gas could not occur under anaerobic conditions. It appears that experiments specifically designed for the purpose would be needed to investigate the total nitrogen balance in view of the uncertain accuracy of the results.

11.6.3 Conclusions

The results obtained are of interest in that they show that not all organic compounds could be utilized as hydrogen donors by the isolated denitrifying bacterium. The reason for this is not evident from the results, and it cannot be presumed that lactose and glycine would not be utilized by a mixed denitrifying flora. An observation of significance to the objective of this investigation was the fact that acetic acid was utilized almost as readily as glucose and fructose. This was of importance in view of the fact that acetic acid predominates in the mixture of acids produced in the anaerobic digestion of sewage solids.

C:N w/w

1	Glucose	2:1
2	Acetic	2:1
3	Propionic	2:1
4	Butyric	2:1
5	Control	0:1

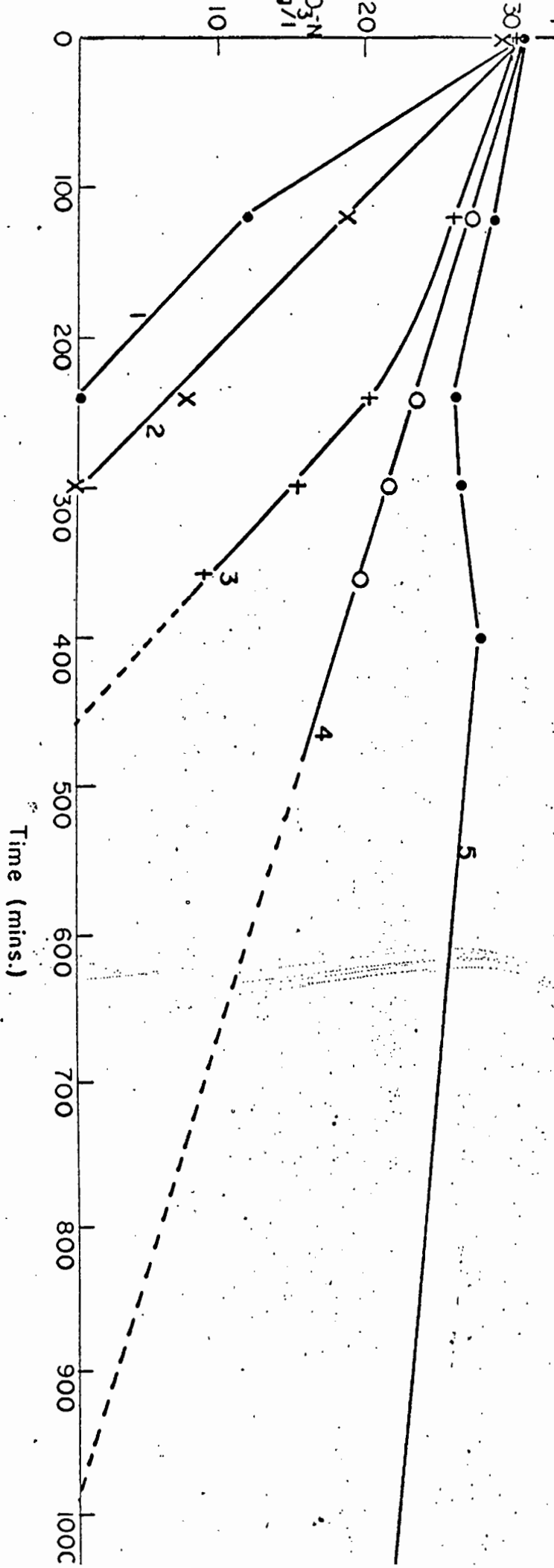


FIG. 8

Volatile acids as H^+ donors

11.7 Volatile Fatty Acids as Hydrogen Donors

In the acid phase of the anaerobic digestion of raw sewage solids, the main fermentation products are acetic and propionic acids with lesser amounts of butyric and valeric acids. To investigate the suitability of these compounds as hydrogen donors the first three acids were employed.

11.7.1 Method

The same organism and the same nitrate concentration and carbon:NO₃-N ratios as in the previous experiment were used.

11.7.2 Results

From Table 8 and Fig. 8 it can be seen that initial reduction of nitrate proceeded at a more rapid rate with glucose and acetic acid than with propionic and butyric acids. However, after an initial delay, the rate of reduction with propionic acid was very similar to the rate with glucose and acetic. The oxidation of butyric acid occurred at a much slower rate than was the case with the two other acids.

11.7.3 Conclusions

The two lowest members of the volatile fatty acid series were utilized as hydrogen donors by the isolated bacterium to produce a rate of denitrification close to that with glucose. This implies that acid sludge-digester liquor should also prove a suitable source of donors since these two acids are the predominant compounds present.

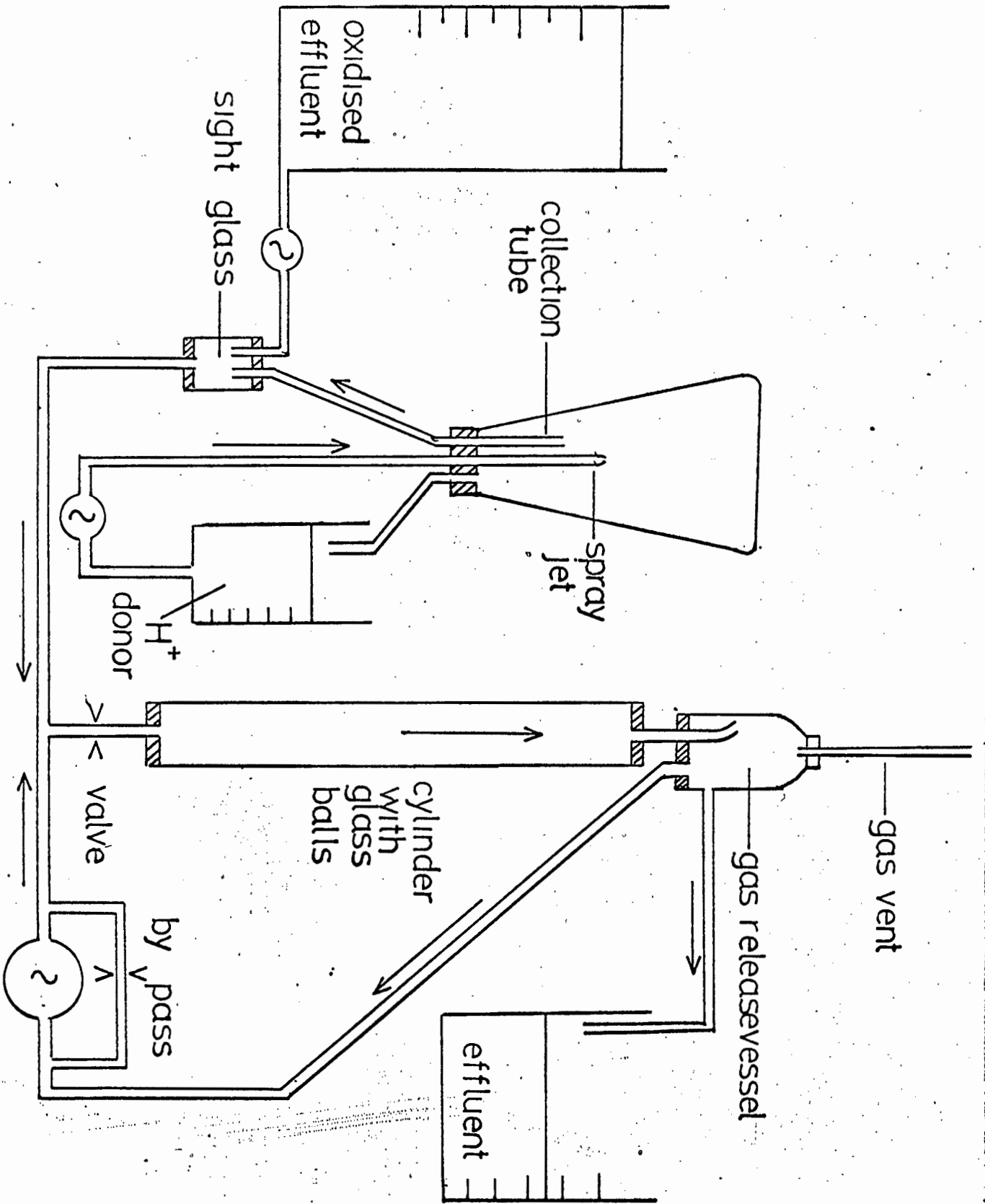


FIG. 9 Continuous-flow denitrification apparatus

12. DENITRIFICATION OF OXIDIZED SEWAGE EFFLUENT IN A CONTINUOUS-FLOW APPARATUS

In a previous experiment it was shown that both acetic and propionic acids could function as hydrogen donors during denitrification of nitrate medium by the isolated organism. It therefore remained to be shown that the acids produced during the acid-producing phase of anaerobic sludge digestion could serve the same function with a mixed culture of denitrifying organisms, such as would be present in practice, and that the operation could be carried out as a continuous process. For this purpose a small continuous-flow apparatus was constructed to simulate a treatment plant in which an oxidized sewage effluent and a hydrogen donor solution derived from raw sewage solids would be passed through an anaerobic vessel containing a mixed population of denitrifying micro-organisms.

12.1 Continuous-Flow Apparatus

The apparatus shown in Fig. 9 consisted of a vertical glass cylinder 65 cm in length with an internal diameter of 5 cm and a total volume of 1150 ml. The cylinder was closed at both ends with rubber stoppers through which passed the lower inlet and the upper outlet tubes. A small 350 ml plastic bottle was fitted to the outlet tube as a gas-release chamber and was vented by a 50 cm narrow-bore glass tube to reduce the ingress of atmospheric oxygen. Liquid passing from the glass cylinder into the gas-release chamber was returned to the positive displacement recirculation pump via a 1/2" bore P.V.C. tube and hence to the base of the cylinder for recirculation. An overflow pipe in the side of the gas-release chamber carried the overflow to a calibrated collection drum.

A supply of nitrified effluent and hydrogen donor was fed via separate small variable speed pumps to the base of the cylinder. A sight glass was interposed in the feed pipes to allow a visual check on the individual flows.

To provide an increased surface area for the development of a bacterial film and to help to retain the organisms within the vessel, the glass cylinder was packed with 275 selected glass marbles with a diameter of 1.6 ± 0.05 cm. A perforated polyethylene disc was placed at the base of the column of marbles to disperse the incoming liquid flow. The calculated combined surface area of the marbles and the inside of the cylinder was 2537 cm^2 and the measured liquid volume was 540 ml.

Variation in recirculation rate was obtained by fitting a by-pass tube with a valve to the recirculation pump and operating this in conjunction with a valve at the inlet to the cylinder.

In the early stages of operation some difficulty was encountered with overheating of the hydrogen donor dosing pump due to the very slow speed necessary to deliver the required dose. This problem was overcome by operating the pump at a speed sufficient to prevent overheating and passing the increased flow through a plastic jet to produce a spray inside an inverted 5 l conical flask. A tube exposed to the spray inside the flask collected a small portion of the falling liquid and passed it to the cylinder inlet tube with the nitrified sewage input. By inserting fan-shaped pieces of flat plastic of different sizes in the mouth of the collection tube, it was possible to

vary the proportion of the spray collected with satisfactory accuracy. The device gave continuous satisfactory service during the experiment described.

The apparatus was operated in an air-conditioned room without other means of temperature control. For this reason, temperatures varied over the range 23-27°C during the period of the experiments. This variation is not considered to have materially effected the validity of the results.

12.1.1 Method

The experiments described were carried out in the laboratories of the Gulf South Research Institute, New Iberia, Louisiana, U. S. A., using sewage of almost entirely domestic origin from the local sewage treatment plant. The per capita use of water in the United States is higher than that in South Africa and in most other countries. This was reflected in the total nitrogen content of the sewage effluent, which was found to be about half the concentration usual in South African sewage. This difference is evident when the nitrogen content of the effluent used is compared with that reported in other sections of this thesis. The difference does not, however, affect the validity of the results on the possible application of the process to stronger sewages, since the difference is one of dilution only.

12.1.2 Preparation of hydrogen donor solution

Approximately 45 l of raw sewage sludge were collected

from the local treatment plant in a steel drum. Ten litres of actively digesting anaerobic digester sludge were added as an inoculum of anaerobic acid-producing organisms. The mouth of the container was sealed with a rubber Bunsen valve to exclude air and the contents were allowed to ferment at room temperature for 5 days with daily shaking to mix the contents.

At the end of this period a suspension of calcium hydroxide was added with gentle stirring until a pH value of 10.5 was obtained. This treatment resulted in the precipitation of phosphate and flocculation of the solid material. After settlement for some hours the supernatant liquor was siphoned off as a pale brown, highly turbid liquid. Further settlement and syphoning produced 20.5 litres of opalescent liquid with a pungent odor containing approximately 340 mg $\text{NH}_3\text{-N/l}$ and 27 mg organic-N/l. The liquid was then recirculated as a thin film over an inclined rectangular plastic sheet (150 x 30 cm) for 45 minutes in an air current produced by an electric fan. Due to the high pH value, the loss of ammonia was rapid. Further settlement of solid material was also caused by this treatment, further reducing the organic nitrogen content. The final product contained 61.2 mg $\text{NH}_3\text{-N/l}$ and 7.0 mg organic N/l.

The solution was then analyzed for volatile fatty acids content by a chromatographic method using a silicic acid column (Standard Methods, 1965), and was found to contain 2850 mg acetic acid/litre. This estimation is not an exact determination since some propionic, and traces of butyric and valeric acids, are normally present in digester liquors. Due to the relatively small proportion of these acids present, and to the practical difficulty of estimating their separate concentrations, it is customary in sanitary sewage analysis to express their combined concentration in terms of acetic acid. The solution obtained in this way was used as the hydrogen donor source in all the experiments described in this section.

12.1.3 Preparation of nitrified sewage effluent

To produce a supply of completely oxidized sewage effluent for the experiments it was necessary to aerate bulk samples of the final effluent from the local sewage plant for 24 hours after addition of a small amount of an activated sludge suspension. After settlement and decantation a clear effluent containing no $\text{NH}_3\text{-N}$ or $\text{NO}_2\text{-N}$ and about 14.0 mg $\text{NO}_3\text{-N}/\text{l}$ was obtained.

12.1.4 Development of denitrifying flora

The continuous flow unit was started up with an input ratio of hydrogen donor solution : nitrified

effluent of 1:10. This ratio was intended to provide an excess of volatile acids as judged by the results previously obtained with a pure denitrifying culture and acetic acid as the hydrogen donor. The combined flow rate was adjusted to give a retention time in the marble-filled denitrifying vessel of approximately 90 minutes. The recirculation pump was initially operated at maximum recirculation rate, but it was soon evident that the rapid flow through the denitrifying vessel prevented the development of a satisfactory bacterial film. The flow rate was then progressively decreased until a stable film began to develop at a recirculation rate of 1.3 l/min. It was observed that much of the bacterial growth took the form of light brown clumps of filaments up to 1.5 cm in length, and that growth over individual marbles was irregular. Relatively little growth occurred on the inside of the cylinder wall.

12.2 Results

When the recirculation rate had been adjusted to a level that permitted establishment of a bacterial population, the volumetric ratio of hydrogen donor to nitrified effluent was progressively reduced from 1:10 to 1:39. At this ratio denitrification was complete, with no detectable quantities of oxidized nitrogen present in the effluent. The retention time was then progressively reduced over a 10-day period from 90

minutes to 14 minutes. At the latter retention denitrification was not complete; of the 14 mg $\text{NO}_3\text{-N}/1$ entering the system approximately 4 mg N/1 appeared in the effluent as nitrite and nitrate. The retention time was then increased by 5 minutes at 24-hour intervals until no oxidized nitrogen was detectable in the effluent. In this way it was established that for complete denitrification of a fully oxidized sewage effluent containing 14.0 mg $\text{NO}_3\text{-N}/1$, 25.7 ml of the hydrogen donor solution, equivalent to 73.2 mg acetic acid, was required for each litre of effluent treated under the experimental conditions. Under these conditions a minimum retention time of 24 minutes was required for the bacterial population present to completely denitrify the nitrate input.

12.2.1 Effect of recirculation

To obtain some indication of the effect of recirculation on the denitrification rate the unit was operated for 24 hours under identical conditions but with the recirculation system closed off. Under these conditions denitrification was incomplete, with both nitrite and nitrate present in the effluent. The nitrogen removal rate calculated from the influent and effluent values was 0.054 mg N removed/minute. With recirculation the equivalent value was 0.315 mg N removed/minute. Thus, by use of recirculation, the weight of oxidized nitrogen reduced in 24 minutes by the organisms present was increased 5.8 times.

NO₂&NO₃
in effluent
(mgN/l)

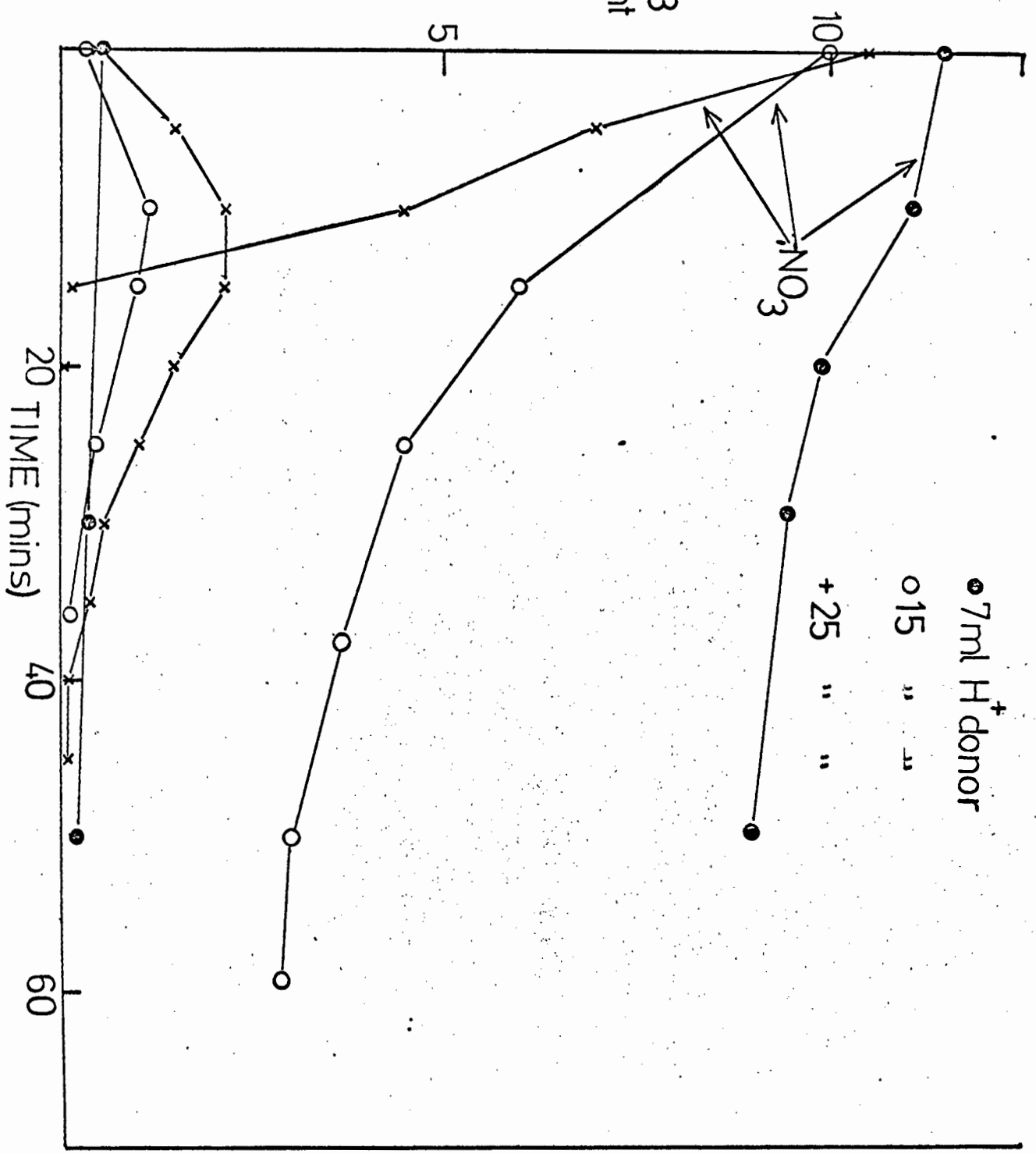


FIG.10. Volume of H donor for denitrification

12.2.2 Short-term confirmatory tests

Before determining the dry weight of organisms present in the column and thus bringing the experiment to an end, a series of short experiments were carried out to confirm the values obtained for the minimum hydrogen donor requirement and retention time. In these experiments the denitrifying column was drained under an atmosphere of nitrogen, to prevent introduction of air, and refilled with a mixture of hydrogen donor and nitrified effluent. The proportions of hydrogen donor used were 25 ml, 15 ml, and 7 ml per litre of influent. The recirculation pump was then started and samples were removed from the gas-release chamber at short intervals for determination of nitrite and nitrate nitrogen. The experimental results are shown in Fig. 10.

The results obtained by this method are in reasonably close agreement with those obtained during normal operation. It can be seen from Fig. 10 that reduction of nitrate was not complete after 60 minutes when 7 and 15 ml of hydrogen donor per litre of mixture were present. With the addition of 25 ml of hydrogen donor solution only a trace of nitrate was present after 15 minutes, and all nitrite was reduced within 40 minutes. Although the concentration of nitrite did not exceed 2.2 mg N/l at any time, complete removal took appreciably longer than the removal of nitrate. However,

after 30 minutes recirculation the total oxidized nitrogen present was reduced to 0.6 mg N/l, which is close to the retention time of 24 minutes that was required during continuous flow operation.

The denitrifying vessel was then emptied and the bacterial film washed off the marbles by agitation with distilled water in a glass bottle. The remaining film on the inside surface of the glass cylinder was also removed and, after centrifugal separation, 2.558 g dry weight of the material was recovered.

12.2.3 Denitrification rates

Since calculations based on the surface area provided for bacterial growth were not justified, due to the irregular growth observed on the surface of the marbles and on the inside walls of the denitrifying vessel, the dry weight of bacteria present was used to express the denitrifying activity of the system on a quantitative basis.

At an influent concentration of 14.0 mg $\text{NO}_3\text{-N/l}$ and a retention time of 24 minutes, the denitrifying vessel (capacity 540 ml) received 1.350 l effluent per hour containing 18.90 mg $\text{NO}_3\text{-N}$. Based on the dry weight of organisms recovered, this corresponds to a nitrate reduction rate of 0.007 mg $\text{NO}_3\text{-N/mg dry weight/hour}$.

This value represents a conservative estimate of the actual activity of the population, since it was evident from the texture of the material and its appearance under the microscope that a considerable amount of suspended debris derived from the input had become entrapped in the bacterial film. The actual weight of active organisms present must have been appreciably less than the dry weight recorded, since care was taken to ensure that any extra growth of denitrifiers in the recirculation system and gas-release bottle were regularly removed.

13. DISCUSSION OF RESULTS

The purpose of the work described in this section of the thesis was to examine the possibility of using sewage solids as a source of low-nitrogen hydrogen donor. In preliminary experiments with a pure isolate of a denitrifying organism derived from activated-sludge, the weight of donor required per unit weight of nitrate nitrogen reduced was determined for a number of pure compounds of the type found in sewage water, including the volatile fatty acids. From these experiments the amount of the compounds required, in terms of C:NO₃ ratio, was obtained.

For glucose the minimum ratio was found to be 1.5, and a similar value for acetic acid was also obtained. The equivalent value obtained using volatile acids derived from sewage solids as hydrogen donors was 2.04. The agreement between these values is considered to be satisfactory in view of the use of a mixed bacterial flora in the continuous flow experiments and the expression of the total mixed acids present in terms of their acetic acid equivalent.

At the required addition rate of 25 ml hydrogen donor solution per litre of effluent that was established, the concentration of ammonia and organic nitrogen in the effluent that was derived from the donor and remained unchanged by denitrification would be expected to be 1.6 mg N/l. In practice the actual amount of nitrogen detected by analysis of the filtered effluent did not exceed 1.0 mg N/l at any time, and was usually only detectable in trace amounts. The reason for this low value is not certain, but it seems possible that the ammonia nitrogen was utilized for the synthesis of nitrogenous cell material, since oxidized nitrogen must first be reduced to ammonia before it can be used for this purpose.

The very low residual nitrogen values recorded compare favorably with results obtained by others in this field in that the minimum values previously reported have been in the region of 5 mg N/l (Wuhrmann, 1964). It therefore appears that the use of sewage solids to produce a low nitrogen source of hydrogen donors offers a means of obtaining lower residual nitrogen concentrations than has been obtained by other means. The method of producing the material is relatively simple and, on a practical scale, should not prove unacceptable on a cost basis.

One important aspect that requires further investigation is the relation between the quantity of volatile acids that can be obtained from the settleable solid fraction of the sewage and the nitrate content of the settled sewage after oxidation. Unless the solids present are sufficient to produce enough hydrogen donor to meet the requirement for reduction of the nitrate to nitrogen gas, then either the denitrification of the entire effluent will not be complete, or only part of the total effluent can be completely denitrified.

The volume of sludge removed by settlement of the sewage used in the experiments described was not measured, but according to Escritt (1965), between 0.25 and 0.5 gallons of sludge are produced per head of population per day in Britain, where the average consumption of water is usually taken to be about 40-50 gallons per head per day. From these values an estimate for the volume of solids obtained from sewage settlement would be about 1 per cent of the sewage flow.

The yield of hydrogen donor solution from the sewage sludge used in the preparation of the donor was about 50 per cent of the original sludge volume. If it is assumed that in practice a similar volume would be produced, with a similar concentration of volatile acids, then the yield of donor solution would be 0.5 per cent of the original sewage volume. Since the experimentally determined donor requirement was 25 ml/l, or 2.5 per cent, it is evident that only enough donor would be obtained to reduce 20 per cent of the nitrate present to nitrogen gas. The production would actually be greater than this in practice, since about 60 per cent of the available carbon in the sewage is not removed by settlement and is used by the aerobic organisms in the treatment process for respiration and synthesis. Under normal operating conditions the Sludge Growth Index in an activated-sludge plant is about 0.5, which means that about half of the carbon is utilized for the synthesis of new material. This excess material would therefore contain about 30 per cent of the original carbon content of the sewage and would be added to the settled solids for conversion to volatile acids. In this way the amount of carbonaceous solids available for production of hydrogen donor would be increased from 40 to about 70 per cent of the original carbon content of the raw sewage. Under more

carefully controlled conditions of acid fermentation, and with more complete separation of the hydrogen donor solution from the residual solids, it would probably be possible to produce more donor from the sludge than was experimentally obtained. Nevertheless, it seems probable that the amount of available carbon derived from the sewage for conversion into donor materials would not be enough to remove more than about half of the nitrogen content of the sewage, or, alternatively, to completely denitrify half of the effluent flow.

Under these circumstances the method would not provide a complete answer to the problem of eutrophication due to the nitrogen content of sewage effluents. It would, however, offer a solution to the problem of nitrogen removal associated with the re-use of sewage effluent for domestic water supply in arid regions (Van Vuuren, 1965) and would also provide a source of nitrogen-free water for industrial use.

If it were possible to increase the concentration of suspended carbonaceous material in the raw sewage by some means, without material increase in the load of soluble material that would require biological oxidation, then denitrification of the entire sewage flow might be possible. One way in which this might be achieved would be to encourage the use of the sewer reticulation system for the disposal of comminuted municipal garbage and solid organic wastes from industry. The materials available would depend upon local conditions, but in all cases the material would need to be in a form that was removed from suspension with the normal sewage solids during primary settlement. The quantity of hydrogen donor subsequently produced by acid fermentation

of the solids would probably be sufficient to permit complete denitrification since Lough (1956) has estimated that a complete changeover to municipal garbage grinding and sewer disposal would increase the suspended solids content by 50-100 per cent, the grease content (which could also be utilized) by 60-150 per cent and the BOD by 30-50 per cent. Production of hydrogen donor solution by the method described should present no major problem, since it has been shown that garbage mixed with sewage solids digests at the same rate as sewage solids alone (Babbitt, 1944).

In this way a dual purpose would be served in that decomposable municipal garbage, itself a frequent source of environmental pollution, could be used to prevent pollution from another source, with consequent prevention of pollution from both sources.

If only half the effluent of a normal sewage plant can be denitrified with hydrogen donors derived from the normal settleable solids content, some alternative means is required to treat the remaining portion of the flow. Since autotrophic organisms do not require organic carbon compounds as a carbon source, and since sewage effluent contains an abundance of inorganic carbon in the form of carbon dioxide, carbonates and bicarbonates, the possibility of employing these organisms to remove nitrogen from sewage was investigated.

Section C

REMOVAL OF NITROGEN AND PHOSPHORUS BY AUTOTROPHIC ORGANISMS

14. INTRODUCTION

Conventional methods of sewage purification are based on the use of carbon heterotrophic micro-organisms to oxidize and assimilate decomposable organic compounds. In Section A it was shown that sewage is deficient in both carbon and nitrogen in relation to the amount of phosphorus present. In consequence, the amount of nitrogen and phosphorus that can be extracted by heterotrophic bacterial synthesis is limited and the excess appears in the oxidized effluent. Since the organic carbon content of this liquid is low, further removal of nitrogen or phosphorus by carbon heterotrophs is not possible unless further organic carbon is added. For this reason attempts have been made to use algae to achieve more complete stabilization of sewage and the extraction of nutrients, since most of these organisms, being photosynthetic autotrophs, do not require organic compounds as their source of carbon.

In recent years a number of reports have appeared describing the use of algal activity for the oxidation of raw and settled sewage on both laboratory and pilot-scale (MacKenthun and McNabb, 1961; Neel, et al., 1961; Parker, 1962; Oswald, 1963; Eppley and Macias, 1963; Weideman and Bold, 1965; Goleuke and Oswald, 1965; Assenzo and Reid, 1966; Schulze, 1966), with the commercial production of algae from

sewage as an animal food supplement (Gotaas and Golueke, 1958; Oswald, 1963; Golueke and Oswald, 1965), and with the removal of mineral nutrients from sewage and oxidized sewage effluents (Moeller, 1957; Kaneshige, 1959; Fitzgerald, 1961; Witt and Borchardt, 1960; Gates and Borchardt, 1964; Neos, et al., 1966; Schulze, 1966). All of these reports refer to work carried out using ponds or lagoons of various sizes and designs in which the environmental conditions and the characteristics of the communities developing in the ponds were observed and related to changes occurring in the chemical quality of the water.

From these studies much useful information has been accumulated on the effects of environmental variables on the growth and metabolism of those species of algae which are able to flourish in highly eutrophic waters, such as sewage and sewage effluents. A number of problems have been encountered which have so far prevented the development of an entirely satisfactory algal system for the removal of mineral nutrients. By summarizing an extensive literature on the subject the major problems may be described under the following headings:

14.1 Nutritional Requirements

Due to the rapid growth of algae in sewage effluent the carbon available in the form of alkalinity becomes exhausted before complete assimilation of other mineral nutrients can occur. This exhaustion causes an increase in pH value resulting in the precipitation of calcium phosphate which may settle to the pond bottom and subsequently return to solution during periods of low photosynthetic activity. By adding extra mineral carbon in

the form of carbon dioxide it has been shown that this precipitation effect can be avoided and increased algal growth obtained (Kaneshige, 1959; Aikins-Afful, 1961).

14.2 Environmental Requirements

Changes in both temperature and insolation rates have a marked effect on algal growth rates and, under winter conditions in temperate climates, nutrient assimilation is much less than in warmer months (Fitzgerald, 1961; Parker, 1962; Gates and Borchardt, 1964).

A substantial increase in the cellular dry weight of an algal culture can only occur when photosynthesis is in progress and therefore depends on the reception of light energy of sufficient intensity. An algal cell, however, can only utilize a limited amount of light energy at a time. There is an upper limit of intensity, or saturation value, at which light is utilized with full efficiency. Above this intensity all the incident energy is not used and some is wasted as heat. At intensities of about 1000 f.c. or more above the saturation value, inhibition of photosynthesis may also occur (Ryther, 1956). Tamiya (1953) has shown that intensities above 1000 f.c. slow the growth of Chlorella ellipsoidea and Krauss (1956) has shown that intensities above this level decrease photosynthesis after 15-30 minutes exposure.

14.3 Use of Available Light

The saturation effect imposes a major limitation on the efficiency with which solar energy can be utilized by an algal culture. It is known that in an algal suspension the light

intensity decreases with depth and concentration according to the Beer-Lambert law of light absorption. There will thus be a depth at which light intensity is just equal to the saturation intensity. All light penetrating to a greater depth will be utilized with maximum efficiency but at lesser depths only a fraction will be utilized. It has been shown that the fraction f of the energy of light that is utilized in photosynthesis by an algal culture is given by the equation;

$$f = \frac{I_s}{I_o} \left(\log_e \frac{I_o}{I_s} + 1 \right) \dots\dots\dots 1$$

where I_o is the incident intensity, I_s is the saturation intensity.

In temperate latitudes in summer at midday I_o is at least 8000 f.c.; on the South African Highveld the equivalent value is about 12000 f.c. For Chlorella pyrenoidosa Myers (1953) has estimated I_s to be 400 f.c. and similar values for common freshwater species have been found by other workers. Substituting values in equation 1 of 12000 and 400, the value of f is 0.145. This means that only 14.5 per cent of the visible light energy having an incident intensity of 12000 f.c. is utilized for photosynthesis. This utilization is 4.35 times as great as it would be if the light intensity were just at the saturation value of 400 f.c. Thus a 30 times increase in incident energy will only increase the amount of energy utilized by the algae by a factor of 4.35, the remaining energy being wasted as heat. It is therefore evident that some means of supplying light to individual algal

cells at about the light saturation value needs to be found if efficient use of available solar energy in terms of algal production is to be achieved.

14.4 Unialgal Culture

It has been suggested that certain algal species are more desirable than others, either because of their more rapid growth rates, or higher temperature tolerances, or because they have slightly higher light saturation values, or have higher contents of protein or other economically valuable compounds (Milner, 1951). In general, however, it has been found that, unless stringent precautions are taken as regards constant environmental conditions and prevention of contamination, this aim cannot be achieved. Such conditions cannot be maintained in open lagoons containing sewage or sewage effluent and it has been observed that, although almost unialgal growth may occur for short periods, it is the chemical composition of the water and the current environmental conditions, such as the development of stratification, that will determine which species will become dominant (Allen, 1955; Witt and Borchardt, 1960; MacKenthun and McNabb, 1961; Fitzgerald and Rohlich, 1964; Marais, 1966).

14.5 Mechanical Problems

The incorporation of plant nutrients into algal cells does not materially alter the actual quantity of nutrient present in the water-body. Subsequent death and decay and the decomposition of algal sediments results in the re-resolution of assimilated nutrients (Mackenthun, et al., 1961; Parker,

1962), plus the oxidizable organic carbon compounds produced during photosynthesis. Algal assimilation can only be used to remove nutrients if the algal cells are separated from the water-phase in an intact condition.

14.6 Removal of Algae

Due to their small size and physical characteristics, the removal of algal cells by gravity settlement has been found not to be practicable. The use of filters and fine screens has also been found unsatisfactory as rapid blockage of the material results in low separation rates. Periodic emptying and manual cleaning of lagoons has been suggested as a possible method by Bush (1961), and Pipes (1962) has shown that the preferential cultivation of filamentous forms, that can be removed by settlement or screening, is possible on a laboratory scale. The only methods which appear to offer a reliable means of algae removal are centrifugal separation as described by Golueke and Oswald (1965) for the pilot-scale production of algae from sewage as an animal feed supplement, and the flotation process described by Van Vuuren, et al. (1965). However, the cost of separation by the former method would be unacceptably high if used as part of a waste disposal system. A satisfactory and economically acceptable method of removing algae from large volumes of water, under all conditions, does not yet appear to have been demonstrated on a full scale plant, although flotation appears to offer this possibility.

Material analyzed	Per cent of dry weight	
	N	P
Microcystis sp. ¹	9.27	0.52
Anabaena sp. ¹	8.27	0.53
Microcystis aeruginosa ²	6.83	0.69
Aphanizomenon sp. ³	10.05	-
Aphanizomenon sp. ⁴	-	0.6
Anabaena cylindrica ⁵	6.95 - 9.0	-
Oscillatoria sp. ⁵	5.65 - 7.42	-
Oscillatoria brevis	7.45	1.28
Anabaena sp.	4.04	1.20
Lyngbya hemensii	7.46	1.07
Spirogyra sp. ³	3.81	-
Chlorella sp. ⁶	1.4 - 11.0	-
Chlorella vulgaris ⁷	8.0	1.1
Chlorella vulgaris ⁵	1.89 - 4.34	-
Chlorella pyrenoidosa ⁸	-	0.94 - 1.5
Scenedesmus bijugatus	4.2 - 6.1	0.53 - 1.8
Stigeoclonium stagnatile ⁹	6.52	1.89
Calothrix sp.	4.39	1.18
Tribonema aequale ⁵	1.74 - 2.95	-
Monodus subterraneus ⁵	1.22 - 2.94	-
Stream periphyton ¹⁰	3.29	-

- 1 Birge and Juday (1922)
 2 Gerloff and Skoog (1954)
 3 Prescott (1960)
 4 Phinney and Peek (1961)
 5 Fogg and Collyer (1953)

- 6 Spoehr and Milner (1949)
 7 Geoghegan (1953)
 8 Scott (1943)
 9 Bogan et al (1960)
 10 Kevern and Ball (1965)

Table 9. Nitrogen and phosphorus content of various algae.

As a widely experienced worker in this field, Wuhrmann (1964) has expressed the opinion that the upper limit for nitrogen removal by incorporation into autotrophic microphytes appears to lie between 65 and 85 per cent, and concludes that pond treatment does not offer a complete solution to the problem, except perhaps in regions where high light intensities and temperatures are experienced throughout the year.

15. THEORETICAL CONSIDERATIONS AND EXPERIMENTAL APPROACH

In a biological system designed for the removal of N and P the factors affecting the uptake of nutrients by growth and metabolism of the organisms, and also to the amounts of nutrients that have to be removed, must be considered.

15.1 Chemical Composition of Algae

Data on the nutrient content of various algal species obtained during this investigation and collected from the literature are presented in Table 9. It has been shown by Gerloff and Skoog (1954) and by Taub and Dollar (1965) and others, that the mineral composition of algal species can vary over a relatively wide range, depending upon the composition of the medium and other factors. This variation is evident from Table 9 but, in general, it can be seen that phosphorus accounts for about 1 per cent of the dry weight and nitrogen about 7.5 per cent in the cyanophytes and a lower value of about 4.2 per cent in the chlorophytes, giving an N/P ratio of 7.5 and 4.2 respectively.

15.2 Nutrient Content of Sewage

The work described in this section of the investigation was carried out using the final effluent discharged from the Pretoria Sewage Works in the Republic of South Africa. Analysis of 33 weekly samples of this effluent gave an average total nitrogen content of 24.0 mg N/l and 8.31 mg P/l. The average N/P ratio was therefore 2.88, which is substantially different to the N/P ratio for algae. This suggests that nitrogen concentration would be likely to limit the biological uptake of phosphorus from Pretoria sewage effluent.

15.3 Detergent Phosphates

Phosphate concentrations of the order observed are now commonly encountered in sewage effluents and are largely due to the increasing use of domestic synthetic detergents during the last decade. These materials contain complex phosphates to enhance their detergent effects and their use has increased the phosphorus content of sewage to 3 to 4 times the level before the advent of detergents (Stumm and Morgan, 1962).

The complex phosphates most frequently used are sodium tripolyphosphate, tetrasodium pyrophosphate, the sodium metaphosphates, and some organic phosphates. Certain algae appear to be able to utilize tripolyphosphate, probably by extracellular enzymatic hydrolysis to a less condensed form (Maloney, 1966), but most algae cannot, and depend on the phosphatase activity of aquatic bacteria to hydrolyze them to

the available ortho-form. (Overbeck, 1961). This hydrolysis is partly completed in the bio-oxidation stage of sewage purification, but it has been shown that from several days to several weeks may be required for complete conversion to the generally available orthophosphate form, depending on temperature and other environmental factors (Engelbrecht and Morgan, 1959; Sawyer, 1965; Finstein and Hunter, 1967). It is therefore evident that all of the phosphorus in sewage effluent may not be immediately available for algal synthesis and growth.

The amount of algal material that can be produced from 1 mg of nitrogen has been variously estimated at 10.5 mg (Stumm, 1964), 20 mg for Microcystis aeruginosa (Gerloff and Skoog, 1957) and 12 mg for lake phytoplankton (Sawyer, 1966). From the figures given in Table 9 the algal weight would vary between 14.6 mg and 24 mg. Using these values it appears that to remove 24.0 mg N/l from Pretoria sewage effluent between 320 and 570 mg of algae would have to be grown and removed from each litre of effluent. To remove 8.31 mg P/l in algae containing 1 per cent P dry weight about 830 mg of algae per litre would have to be produced..

15.4 Light Attenuation

When algal suspensions of this concentration are produced the problem of efficient light utilization is encountered due to the rapid attenuation of light within the medium. With large scale outdoor ponds this problem is likely to be of major importance as the light absorption characteristics of an algal

culture follow the Beer-Lambert law;

$$I_t = I_0 e^{-Ecd} \dots\dots\dots 2$$

where I_0 is the incident light intensity, I_t is the intensity of the transmitted light at any depth d , d is the depth in centimeters, c is the algal concentration in mg/l and E is the extinction coefficient in cm^2/mg . Determinations of E have been made by Tamiya, et al. (1953) for Chlorella ellipsoidea, who obtained a value of 3.8×10^{-3} , values of 1.0×10^{-3} to 2.0×10^{-3} were obtained by Oswald and Gotaas (1955), and Bogan, et al. (1960) obtained values of 2.0×10^{-3} to $3.9 \times 10^{-3} \text{ cm}^2/\text{mg}$ for Stigeoclonium stagnatile. By use of these values in equation 2, it is possible to calculate the intensity of illumination at any depth in an algal culture.

It has been found by Myers (1951, 1953, 1957) and by Krauss (1956) and others that the light saturation values for common freshwater algal species are in the region of 200-400 f.c. The minimum light necessary to maintain the basic metabolic reactions of Chlorella pyrenoidosa cultures has been determined as 24 f.c. (Krauss, 1956; Myers, 1957). The minimum intensity required for adequate photosynthetic activity in a lagoon is difficult to define but is probably not much less than 100 f.c. according to the results of Bogan, et al. (1960) for Stigeoclonium stagnatile. By adopting this value of 100 f.c. as the minimum intensity for effective nutrient assimilation in a lagoon and using a value for the extinction coefficient of $E = 2.0 \times 10^{-3} \text{ cm}^2/\text{mg}$, the depth

DEPTH FOR
 $I_t = 100$ f.c. (cms)

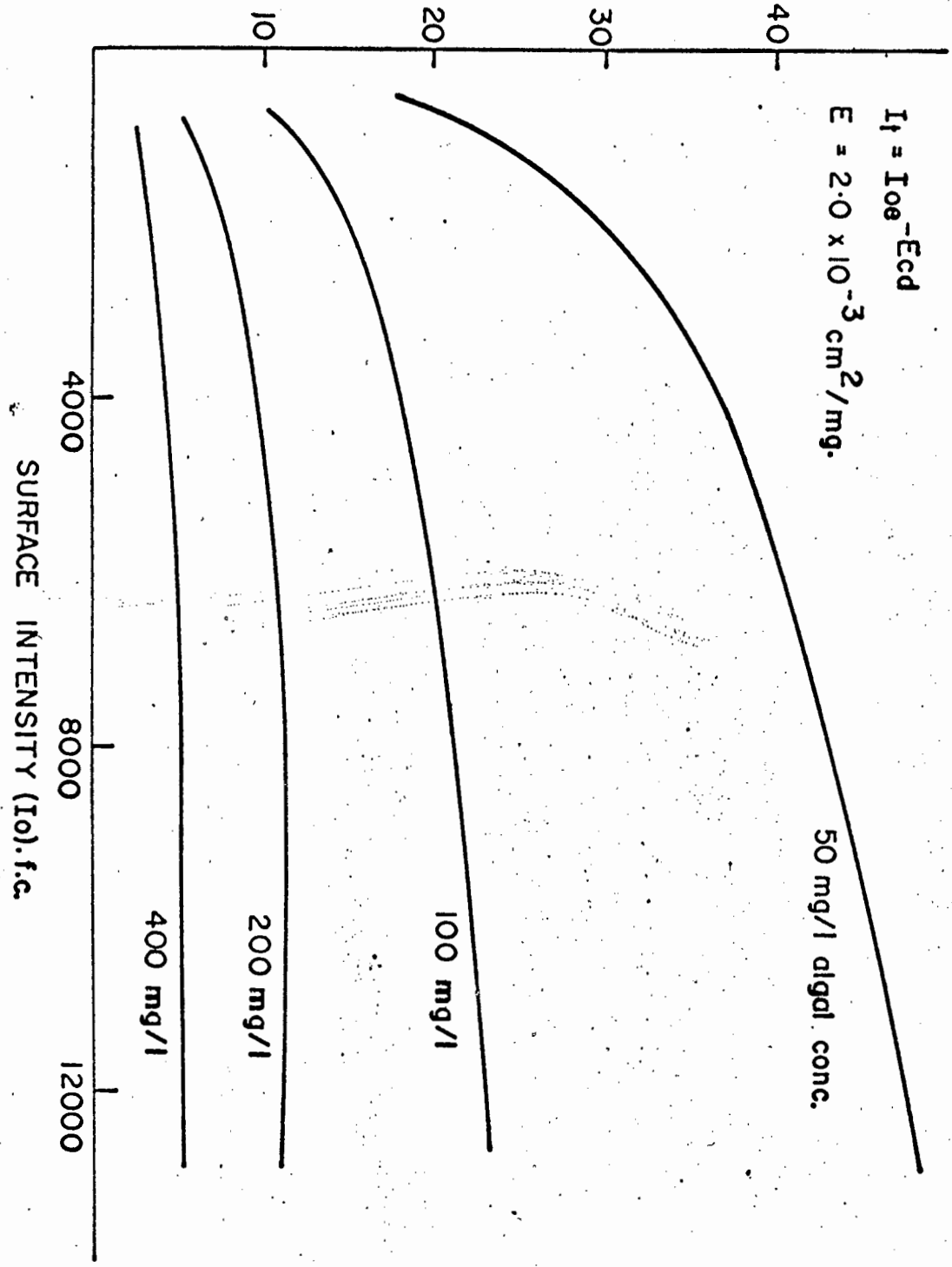


FIGURE 11.

Light attenuation in algal suspensions

at which the intensity will be 100 f.c. for various algal concentrations and incident light intensities has been calculated and is shown diagrammatically in Fig. 11.

15.5 Pond Depth and Light Utilization

From Fig. 11 it can be seen that at algal concentrations required to remove the nitrogen from Pretoria sewage and at local light intensities (about 12000 f.c.), the intensity in a uniformly mixed culture would be less than 100 f.c. at depths greater than about 25 cm. That uniform mixing does not occur at all times and is strongly influenced by temperature and wind action has been shown by Marais (1966) in studies on sewage ponds in Zambia. Development of stratified conditions due to both daily and seasonal variations was associated with a decrease in the algal concentration, particularly in the non-motile forms.

The use of mixing devices to prevent stratification and, at the same time, to exploit the 'intermittent light effect' investigated by Rieke and Gaffron (1942) and Burk, et al. (1951), in which a very brief exposure to light is followed by a period in darkness during which light independent reactions are completed within the cell, has been considered. It was proposed that by suitable engineering design it would be possible to use controlled turbulence to briefly expose individual cells to the incident light, after which they would return to the relative darkness of the interior of the culture. However, the necessary period of

exposure has been found to be less than 1 millisecond for maximum efficiency and reports by Ippen (1953) and Powell, et al. (1965) indicate that a favorable light-dark sequence achieved by turbulence is unlikely. Subsequent attempts to exploit this effect have not been successful (Pyper, et al. 1966).

A partial solution to the problem of light attenuation was demonstrated by Myers and Graham (1961) using illuminated cones of glass or transparent plastic immersed base upward in the medium. In this way the algal yield was approximately doubled. The use of such a system for large scale treatment of sewage effluent does not seem likely due to the high cost and practical difficulties that would be involved.

The use of algal strains having slightly higher saturation values and higher temperature tolerances has been suggested as a means of achieving higher light utilization but, apart from the difficulty that would be involved in attempting to maintain unialgal conditions with sewage, Myers and Graham (1961), using the high temperature strain of *Chlorella* Tx71105, have shown that the small increase in efficiency achieved does not warrant the use of such specialized material.

16. DESIGN OF AN ALGAL NUTRIENT EXTRACTION SYSTEM

It has been observed that both self-purification and algal growth are more rapid in shallow rivers than in lakes and Cholnoky (1960) considers that algal deamination and assimilation of organic nitrogen is of major importance in the process of self-purification, and that pollution should be expressed

in terms of its nitrogen content.

Although discharge of effluents containing nitrogen is undesirable, Shapiro and Ribiero (1965) consider that in most cases the removal of P rather than N is more important in preventing eutrophication of rivers and lakes, particularly since certain blue-green algae are capable of fixing atmospheric nitrogen (Goering and Neess, 1964; Fogg and Stewart, 1965). Since complete assimilation of sewage phosphorus would require the production of very dense algal cultures, the possibility of combining algal assimilation and chemical precipitation of phosphate was considered.

16.1 Phosphate Removal

It has been shown by Owen (1953) and by Lea, et al. (1954) that it is possible to remove all forms of phosphate from sewage by precipitation with various reagents, including calcium hydroxide, and this was found to be the case with Pretoria effluent. It has also been reported by several investigators that algal photosynthesis can reduce the carbon dioxide and bicarbonate present to the extent that the resulting pH increase is sufficient to cause precipitation of calcium phosphate (Kaneshige, 1959; Fitzgerald, 1961; Aikins-Afful, 1961; Fitzgerald and Rohlich, 1964).

Since sewage normally contains considerable quantities of calcium and magnesium derived from excretory and domestic waste materials in addition to that originally present in the water supply, which may vary from very low values up to 300-400 mg/l CaCO_3 in limestone regions, it seems probable

that at elevated pH values sufficient is present to precipitate residual phosphate without need for the addition of extra calcium.

16.2 Removal of Algae and Precipitated Phosphate

The problem of removing algae from sewage effluents has been encountered by previous workers and methods such as centrifugation (Golueke and Oswald, 1965) and flotation (Van Vuuren, 1965) have been used with some success. Other observations of interest in this context were made by Bogan, et al. (1960), who observed that the precipitation of phosphate in a pond at high pH increased the rate of settling of algal suspensions, and by Bush, et al. (1961), who found that recirculation of effluent in a shallow pond favored the development of attached filamentous algae which might be removed by periodic draining and scraping.

16.3 Effect of Current Speed

Measurements of respiration rate and phosphate uptake in both lotic and lenitic species of filamentous algae have indicated that the metabolic rate of these algae is increased by the sweeping effect of a water current, probably due to the production of steeper nutrient gradients at the algal surface (Whitford and Schumacher, 1964 a,b; Schumacher and Whitford, 1965). These observations suggest that the algal phosphate assimilation rate might be greater in a rapidly

flowing system than under the comparatively slow moving conditions in a recirculated pond.

16.4 Use of a Stream System

In view of these observations by previous investigators it was concluded that a shallow flowing stream might offer advantages over a pond system in that;

- i. Turbulence would prevent undesirable stratification.
- ii. The algae present would receive adequate illumination during all the hours of daylight.
- iii. The photosynthetically induced pH increase would cause precipitation of phosphate and would promote settlement of the algal growth.
- iv. The development of more easily removable filamentous species would be favored.
- v. The rate of algal uptake of phosphate would be increased.

For these reasons the use of a shallow stream was investigated experimentally.

17. STREAM SYSTEM EXPERIMENTS

17.1 Preliminary Tests on a Shallow Stream System

Preliminary tests with a 150 ft. long plastic-lined shallow trench were carried out using a slow flow of water about 5 cm deep from an effluent pond containing a heavy growth of

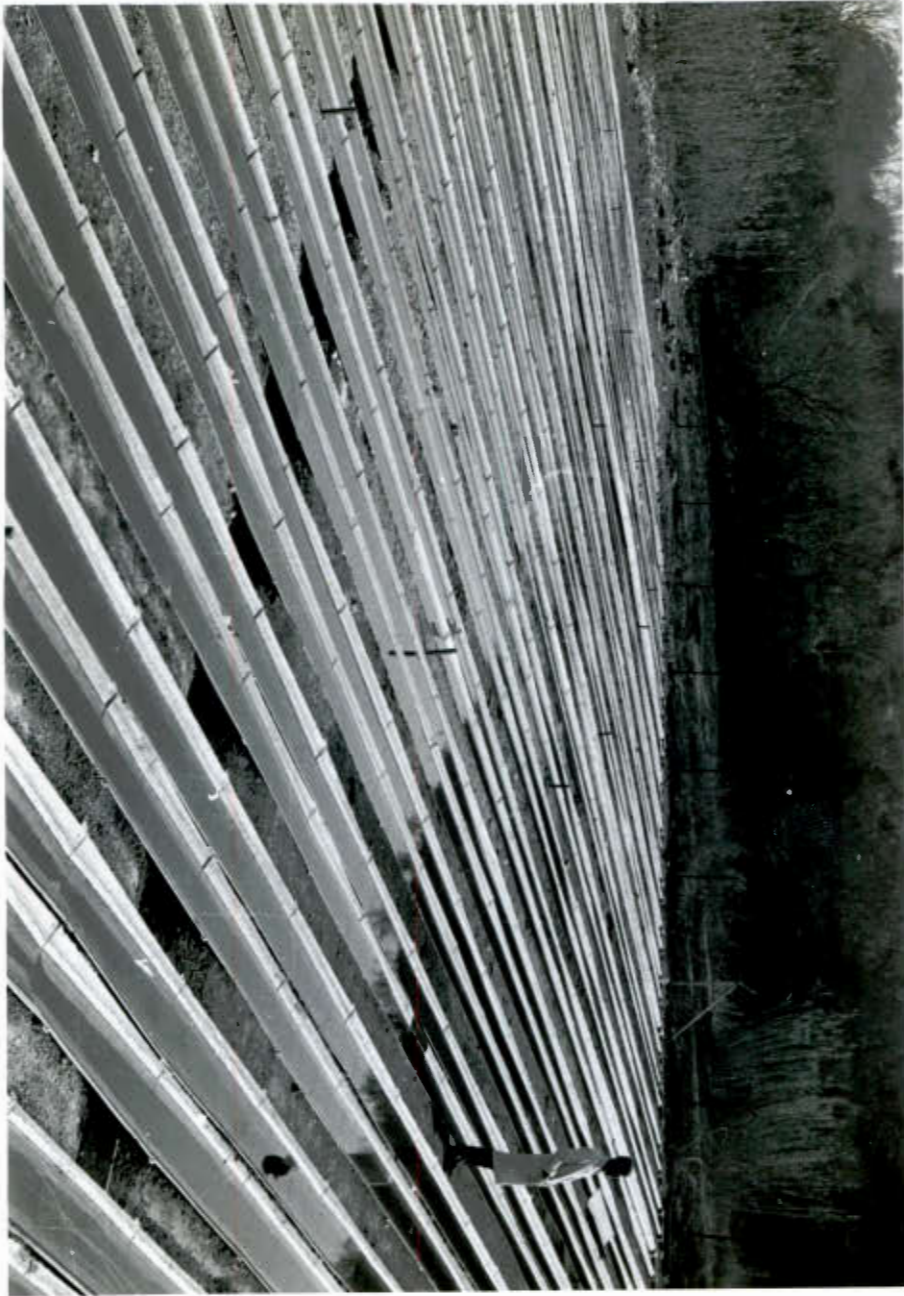


Plate 2. Experimental stream system. The algal sediment can be seen in the right foreground.

phytoplankton, mainly Scenedesmus bijugatus. After several days operation, it was observed that, although algal growth on the plastic lining was minimal, the previously planktonic Scenedesmus began to form a granular layer on the stream bottom and the effluent was almost devoid of suspended algal cells. Measurement showed pH values of 10.0 or more and analysis of the algal deposit showed an abnormally high phosphorus content of between 4 and 6 per cent of the dry weight. It was thus evident that exposure of the algal suspension in a shallow stream caused more rapid uptake of mineral carbon than in the pond and consequent precipitation of phosphate due to high pH. It also appeared that the precipitated material became attached to algal cells, causing them to settle to the bottom as a sediment.

As a result of these encouraging preliminary results a larger and longer stream system was constructed.

17.1.1 Experimental stream system

The stream consisted of 48 parallel asbestos cement channels, each approximately 200 ft. in length and connected in series with a slope of approximately 1:40. The general layout of the stream is shown in Plate 2.

It was originally intended that a collecting channel should pass along the ends of the channels at one side and lead to an algae settlement basin. Each channel would be fitted with a penstock to allow algal growth in the

channels to be scraped or brushed into the collecting channel at intervals. For various reasons this could not be done and, as an alternative, three collection sumps 2.5 x 2.5 x 4 ft. deep were constructed at the end of channels at 2808, 5094 and 9155 ft. distances from the inlet. The total length of the stream was 10300 ft. (3139 m).

In mid February 1966 the clean dry channel began to receive a continuous flow of sewage effluent from the adjacent treatment plant at a rate of 15 gpm. At this rate the retention time was approximately 24 hours, as determined by conductivity measurements at the outlet after a slug addition of strong salt solution at the input.

17.1.2 Inoculation of stream system

A variety of algae was collected from stones and other surfaces in an adjacent heavily polluted river and, together with 30 litres of mud and gravel from the bottom, was added to the stream input as an algal inoculum. About 50 litres of water from an eutrophic pond with a heavy phytoplankton population and 15 litres of mud from the pond bottom were also added.

During the first week of operation a strong growth of Spirogyra sp. developed in the first 1000 ft. of the stream. This algae disappeared within 4 days of its maximum growth and did not reappear in bulk at

any time, although small isolated patches occurred from time to time in the first half of the stream.

After 4 weeks of operation a bank of loose granular algal material from 2 to 5 cm. deep had developed along the whole channel bottom and regular analysis of the effluent passing along the stream and of the algal sediments was commenced.

17.2 Sampling Methods and Chemical Analysis

Samples of water from the stream were taken at weekly intervals between 8 a.m. and 10 a.m. at the inlet and at 4 points along the length at distances of 2600, 5342, 7712 and 10,000 ft. from the inlet. These points were designated Stations A1 to A5.

17.2.1 Water analysis

To determine the weight of phosphorus and nitrogen entering and leaving the system, and to follow the main chemical changes occurring along the stream length, the samples were passed through a loose plug of glass wool to remove gross detritus and analyzed for $\text{NH}_3\text{-N}$, $\text{NO}_3\text{-N}$, Kjeldahl-N, $\text{PO}_4\text{-P}$ and chemical oxygen demand (COD). The pH value and temperature were also measured at each point. The chemical analyses were carried out according to methods described in Standard Methods (1965).

17.2.2 Analysis of algal material

In the first month of operation it became evident that the three collection sumps provided were inadequate to cope with the amounts of algal sediment produced. The sumps became filled with sediment at a greater rate than could be removed and measured by manual methods in the time available. Since no means of continuous metered removal could be provided, it was decided to discontinue periodic algal removal and to study the performance of the stream without removal of the material produced during operation. This decision was unfortunate in that it was subsequently found that re-solution of the sediment components materially decreased the nutrient removal achieved.

An estimate of the quantity of algal biomass in the system and the quantity of nutrients contained in the material was required to account for the changes observed in water quality. Visual observation showed that the algal deposits were rather unevenly distributed along the stream bottom, due to irregularities in the grading of the stream bed. To obtain an estimate of the total material present the stream length was divided into 33 equal sections of 312 ft. and marked with posts designated Stations 1 to 33. Algal samples for analysis were taken at some or all of the Stations at intervals.

17.2.3 Algal sampling procedure

Standard samples of the granular algal deposit were taken by temporarily blocking the stream with a board and carefully removing a complete cross-section of the deposit at the sampling point. For this purpose a net 9 cm wide with 32 mesh/cm was used. The sample was placed in a bucket, diluted with a water jet, and allowed to settle for approximately 5 minutes. The supernatant was then decanted into a second bucket leaving behind any gravel and sand. The process was repeated once more and the algal supernatant decanted through a 32 mesh/cm net, to remove the faunal content, into 2 litre measuring cylinders. Settlement was rapid (less than 30 minutes) and a dense algal residue overlain by a moderately turbid green supernatant was obtained. The supernatants were carefully poured to waste and the sediments transferred to a series of weighed 1 litre beakers and dried at 105°C. After re-weighing to obtain the total dry weight, weighed portions of dry material were ashed at 650°C to obtain the organic weight and further portions were acid-digested to obtain the total nitrogen and phosphorus content (Standard Methods 1965).

17.3 Water Analysis Results

The monthly mean analytical values obtained are given in Table 10 and show that seasonal changes had a marked effect on water temperature in the stream, with minimum values recorded in June and July. The influent temperature did not fall below 17°C.

1966	Station	T ^o	pH	Kjeldahl N	NH ₃ -N	NO ₃ -N	PO ₄	COD
March	A ₁	25.0	7.3	5.0	3.7	14.3	23.2	74.0
	A ₂	21.2	8.5	6.1	4.5	14.2	20.9	71.2
	A ₃	20.2	8.6	7.3	5.8	14.5	26.2	58.2
	A ₄	21.0	8.9	6.3	5.0	16.2	16.3	55.2
	A ₅	24.0	9.3	2.3	1.2	17.7	4.5	45.5
April	A ₁	21.5	7.0	5.8	3.8	16.7	24.9	85.5
	A ₂	16.7	8.2	6.5	4.7	15.2	24.5	54.1
	A ₃	15.7	8.5	7.4	6.0	16.5	30.5	59.0
	A ₄	16.0	8.8	6.1	4.3	18.3	19.9	52.2
	A ₅	15.7	9.2	2.0	0.8	21.3	6.8	42.2
May	A ₁	20.5	7.2	7.3	5.5	15.9	25.3	89.5
	A ₂	13.2	8.0	8.7	7.6	14.6	26.2	67.2
	A ₃	10.5	8.1	9.5	8.2	14.8	34.6	64.0
	A ₄	10.5	8.5	8.7	7.1	15.8	31.7	60.2
	A ₅	10.4	8.7	3.8	2.2	20.4	18.6	40.2
June	A ₁	19.0	7.2	8.9	6.7	18.6	26.4	84.8
	A ₂	11.6	8.0	12.3	9.6	16.1	29.1	80.9
	A ₃	8.4	8.1	13.7	11.0	16.0	34.3	70.3
	A ₄	7.2	8.5	13.2	10.5	16.6	34.2	63.9
	A ₅	7.0	8.7	5.1	3.4	18.0	21.9	56.2
July	A ₁	17.7	7.5	9.3	8.2	17.1	24.2	88.0
	A ₂	9.3	8.0	12.9	10.6	16.2	27.1	89.3
	A ₃	7.3	8.1	14.3	12.3	15.7	32.7	65.3
	A ₄	7.0	8.3	12.4	10.7	16.1	32.0	65.3
	A ₅	6.7	8.5	5.8	4.0	17.9	21.0	50.0
August	A ₁	18.8	7.4	10.7	9.4	15.7	26.6	83.2
	A ₂	11.2	8.3	13.3	11.6	14.2	28.4	77.5
	A ₃	9.2	8.2	15.2	13.5	13.4	34.8	74.7
	A ₄	9.4	8.5	14.8	12.9	13.7	33.3	67.5
	A ₅	8.9	8.9	6.7	5.1	13.6	18.4	54.7
September	A ₁	22.0	7.6	10.9	9.0	12.3	25.8	68.5
	A ₂	20.0	8.7	10.4	9.2	11.4	20.2	70.5
	A ₃	19.5	8.8	13.2	11.9	10.3	23.5	74.5
	A ₄	19.5	8.7	14.5	12.4	9.8	27.3	72.5
	A ₅	19.0	9.0	10.4	8.8	9.2	19.2	62.2

Table 10 (continued)

Table 10 continued

1966	Station	T°	pH	Kjeldahl N	NH ₃ -N	NO ₃ -N	PO ₄	COD
October	A ₁	22.3	7.4	8.5	6.6	14.1	29.9	96.0
	A ₂	22.6	9.4	9.2	5.8	12.3	14.2	92.0
	A ₃	23.0	9.6	10.2	6.3	10.7	11.2	80.0
	A ₄	23.0	9.3	11.3	6.7	9.3	7.1	96.0
	A ₅	23.0	9.6	8.8	3.9	4.9	9.7	56.0
November	A ₁	24.0	7.8	15.6	10.8	7.6	22.4	-
	A ₂	29.0	10.2	8.7	6.6	8.6	6.3	-
	A ₃	29.0	10.2	7.6	5.0	8.1	6.5	-
	A ₄	30.0	10.2	6.8	4.6	7.4	6.8	-
	A ₅	30.5	10.3	4.2	2.4	3.2	4.8	-
December	A ₁	24.5	7.5	11.0	9.4	14.1	27.3	-
	A ₂	30.0	10.3	8.4	6.3	10.8	6.0	-
	A ₃	30.5	10.4	5.1	3.3	8.0	3.9	-
	A ₄	31.0	10.5	3.9	1.5	5.5	2.7	-
	A ₅	31.0	10.5	2.7	0.75	3.1	2.6	-

Table 10. Chemical analysis of stream water. Monthly mean values of Station 1 to 5. Results in mg/l.

at any time during the colder months, but the lower reaches of the stream were occasionally covered with ice after particularly cold nights. The maximum temperature range was recorded at Station A5 in the last sections of the stream, varying from 2°C in June to 33.5°C in November. The major change from influent temperature occurred in the section from the inlet to Station A2 in all cases, with lesser increases or decreases in the remaining sections.

17.3.1 Changes in pH value

The monthly mean pH values shown in Table 10 demonstrate that photosynthetic activity at all seasons was sufficient to raise the pH to 8.0 or above by the time the water had reached Station A2. The pH continued to increase at a fairly constant rate with distance downstream from March to August, with final pH values of not less than 8.5 at Station A3 at all times. From August to December the rate of pH increase was greater, with smaller increases between Stations A2 and A5, and final pH values were higher than in the previous months.

The rate of pH increase at comparable temperatures (March and October) was greater in October and was evidently due to the greater amount of algae in the stream, but even in March, when the minimum quantity of algae was present, sufficient carbon dioxide was removed by photosynthesis to raise the pH above 9.0 in the lower section of the stream.

17.3.2 Phosphate concentration

The mean influent phosphate concentration remained at values between 22.4 and 29.9 mg/l PO_4 during the period of operation.

In March and April the phosphate concentration was higher at Stations A2 and A3 than it was in the influent at Station A1. From May until September the phosphate concentrations were higher than in the influent at Stations A2, A3 and A4 but not at Station 5. In October to December concentrations were substantially less than at A1 at all Stations (Table 10).

From these results it is evident that, apart from the lower reaches of the stream at A5, the phosphate concentration exceeded that of the influent in all parts of the stream except in March and April and October to December. However, due to the reduction in concentration in the final section of the stream there was an overall removal of phosphate by the stream throughout the experimental period. This reduction varied from a mean minimum of 13.4 per cent in July to a mean maximum of 90.5 per cent in December, as can be seen from Fig. 12 prepared from data in Table 10.

The reasons for this increase in phosphate concentration in the upper sections of the stream can

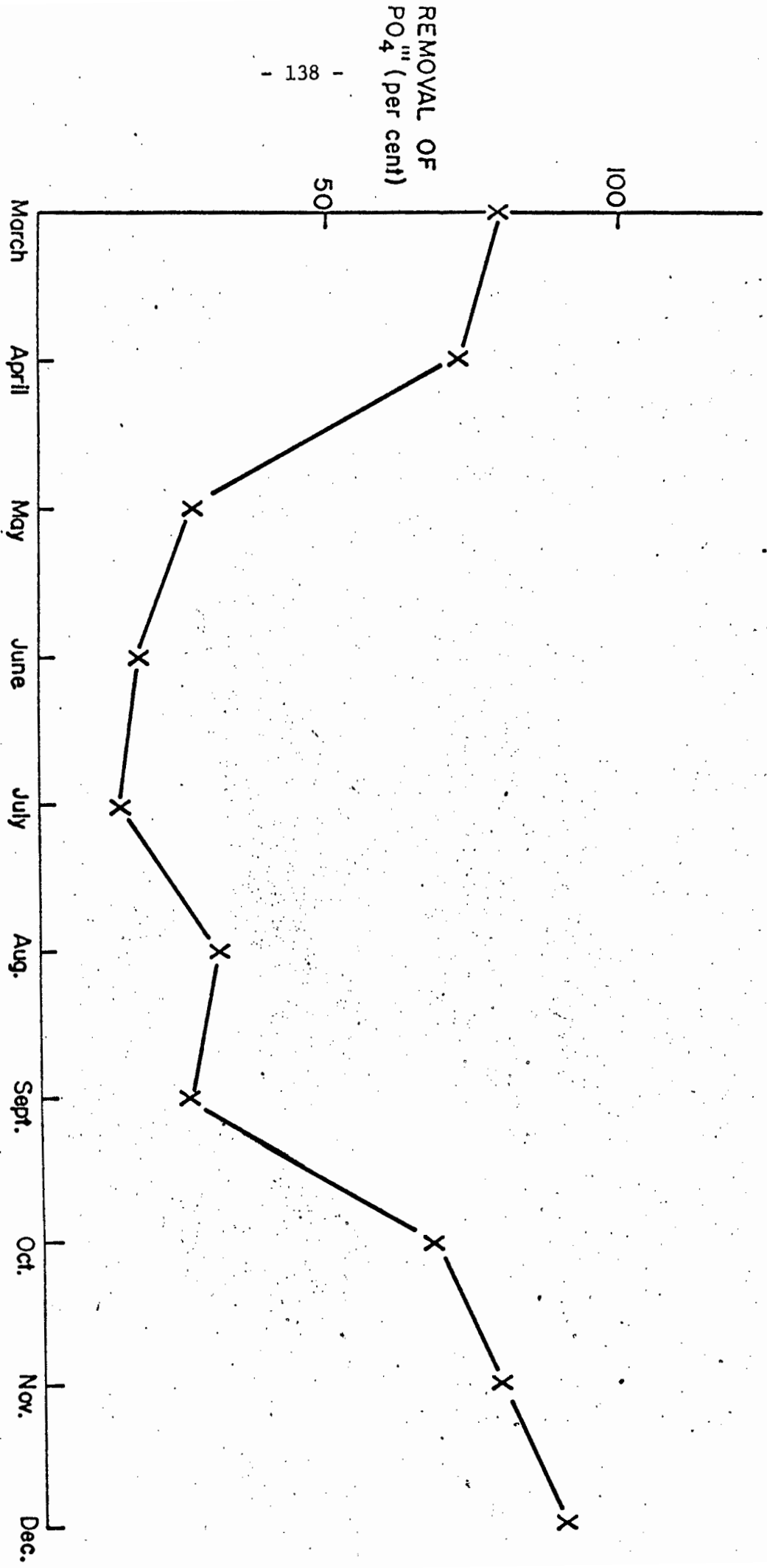


FIGURE 12.

Phosphate removal by algal stream. Monthly mean values

be explained in terms of retention times and pH changes as follows:

In March and April the phosphate concentration at Station A3 was about 5 mg/l higher than at the inlet. Station A3 was 5342 ft. below the inlet (A1 = 0 ft.) and approximately half-way down the stream. At a retention time of 24 hours this means that water sampled at A3 at 10 a.m. entered the stream some 12 hours earlier at 8 p.m. the previous night. During the night hours photosynthesis would cease and algal respiration would return carbon dioxide to the water, thus depressing the pH value. It has been shown by Fitzgerald and Rohlich (1964) that precipitated calcium phosphate is returned to solution at a pH value of 8.0 or below, and it is evident that during the night hours the pH of the upper section of stream fell to values low enough for previously precipitated phosphate to re-dissolve in water passing along the stream. This increased concentration would be maintained the following day until photosynthesis raised the pH to the level at which re-precipitation occurred. From Table 10 it can be seen that at A3 the pH value was 8.6 or less at 10 a.m., but at A4 the pH was 8.8 or more, and the phosphate concentration had decreased to approximately 10 mg/l less than at A3 due to re-precipitation.

At Station A2 the phosphate increase in these two months was low, due to the fact that water sampled at 10 a.m. at A2 had entered the stream about 5-6 hours earlier and had not been exposed to algal respiration and phosphate re-solution for more than an hour or two before dawn.

During the period May-August the pH value in the stream did not rise above 8.5 at any point above Station A5. This being so, a continuous re-solution of phosphate from previously precipitated material would be expected and was observed. During these months the concentration was higher than in the incoming water at all positions upstream of A5 but fell below A5 as the pH value rose to 8.5 or above.

In September the pH at all stations began to rise as a result of increased temperature and insolation, and the phosphate concentration decreased accordingly. During this month a temporary increase in phosphate at Station A4 was observed which was associated with a slightly lower pH at A4 than at A3. The reason for this temporary increase is not evident from the data but by October the pH at all stations downstream from A2 had risen to values above 9.0 and phosphate removal increased from 25 per cent in September to between 70 and 80 per cent in October and November. In November the pH

of the stream was consistently above 10.0 and concentrations of phosphate fell to less than 7 mg/l at Station A2 and decreased to less than 5 mg/l at A5, with lower values recorded in December.

17.3.3 Termination of water analysis

Structural changes at the Pretoria Sewage Works during December caused the flow of effluent to the stream to become intermittent and of variable quality, making analytical continuity difficult. Two sets of samples were taken during December after periods of at least 5 days continuous flow, after which no further analysis was carried out as it was considered that the most critical experimental period had been during the colder months.

17.4 Nitrogen Concentration

17.4.1 Kjeldahl nitrogen

This determination includes all forms of unoxidized nitrogen present. Results given in Table 10 show that almost all the nitrogen determined was due to ammonia-N and that other forms, collectively termed 'organic nitrogen', were never present in concentrations greater than 2 mg N/l, except in October and on the 8th of November when a maximum value of 7.4 mg/l was observed. On this occasion the nitrate concentration was below normal levels and the COD higher than usual, suggesting

that the bio-oxidation stage providing the stream influent was overloaded, possibly as a result of changes in the treatment plant.

Data in Table 10 also shows that the reduction in organic nitrogen compounds was less than 50 per cent, with monthly mean concentrations at A5 varying between 1.1 and 1.8 mg N/l, except in October when a value of 4.9 mg N/l was recorded.

17.4.2 Ammonia nitrogen

An increase in ammonia nitrogen from A1 downstream to A4 was observed throughout the experiment. Since this increase was observed at times when nitrate was also increasing, it could only have been derived from material present within the stream. Reduction of the original organic nitrogen entering the stream could not account for the increase in view of the small amounts present. Although it appears from Table 10 that the release of ammonia from the bottom deposits was greater during the colder months, in fact, when the concurrent increase in nitrate nitrogen that occurred in March-May is taken into account, it is evident that ammonia was released at much the same rate during March-October, but that in the period March-May, part of this released ammonia was being oxidized to nitrate.

The results therefore indicate that continual degradation of nitrogen compounds was occurring.

It has been shown by Saubert (1957) that Scenedesmus bijugatus is capable of deaminating organic nitrogen compounds to obtain the nitrogen required in the form of ammonia, and it is possible that algal deamination may have been responsible for part of the ammonia production. However, it seems probable that most of the ammonia resulted from bacterial reduction of organic nitrogen compounds in the anaerobic layers of the sediment. Due to the thickness of the sediment on the stream bottom, the algal cells several centimeters below the surface would receive no light and, in the lowest layers, no oxygen either. Despite this continued release of ammonia there was a continuous removal of nitrogen by the stream as described later.

17.4.3 Nitrate nitrogen

The increase in nitrate concentration in the stream observed during March-May could only have been caused by oxidation of ammonia by bacteria of the genus Nitrosomonas and Nitrobacter present in the stream community. The activity of these bacteria began to decline during May and, by June, nitrification had virtually ceased and no nitrate increase was subsequently observed during the experiment.

Nitrifying bacteria are known to be adversely affected by low temperature and a decrease in nitrate concentration in effluent from sewage oxidation plants is normally experienced during winter in colder climates. In this case it seems clear that the denitrifying flora originally derived from the biological sewage filter could not survive the low temperatures experienced during June and July and was eradicated. This eradication was evidently sustained, as shown by the decreased nitrate concentration along the stream in subsequent warmer months.

17.4.4 Chemical oxygen demand (COD)

One of the major detrimental effects of the discharge of effluents containing plant nutrients is that, although the effluent itself may exert a low oxygen demand on the stream, the organic compounds subsequently produced by photosynthesis due to the presence of nutrients, may exert a heavy oxygen demand at a later time. Decay of algal blooms has frequently caused fish mortalities from oxygen depletion due to this cause.

To assess the pollution potential of the effluent leaving the stream it was necessary to determine not only the actual nutrient concentrations, but also the amount of oxidizable material present either in solution or in algal cells. For this reason the COD was determined at Stations A1-A5 at weekly intervals.

Distance from inlet (ft.)	10th May		20th June		9th Aug.		8th Nov.		5th Jan.	
	Dry	Org.	Dry	Org.	Dry	Org.	Dry	Org.	Dry	Org.
0 - 936	19.6	8.9	20.8	10.2	16.1	8.3	37.6	12.1	35.3	16.8
936 - 2184	9.1	5.4	19.2	9.4	21.5	9.8	19.0	9.4	68.6	36.2
2184 - 3432	16.6	7.9	15.6	7.1	19.9	9.9	15.5	6.8	34.2	15.4
3432 - 4680	16.0	5.7	22.4	8.2	15.4	10.3	35.8	12.5	90.7	28.6
4680 - 5928	14.6	5.2	21.7	7.3	21.6	13.1	49.7	18.8	62.9	23.2
5928 - 7176	19.2	6.1	35.1	11.9	15.9	9.9	30.9	8.0	102.8	32.8
7176 - 8424	13.5	5.1	26.9	8.4	19.8	12.8	54.7	16.8	95.8	36.9
8424 - 9672	12.2	4.0	16.9	5.7	12.2	7.3	28.5	9.7	61.6	20.5
9672 - 10296	7.2	2.7	12.1	3.6	9.6	6.4	24.1	7.0	21.50	4.5

Table 11. Average dry and organic weight of algal sediment present at different seasons in nine approximately equal lengths of stream. Weights are given in gms. per 9 cm. section of sediment.

Date	Dry weight	Organic weight	Weight of N	Weight of P
10th May, 1966	15.0 ± 1.37	6.1 ± 0.70	0.39 ± 0.05	0.54 ± 0.07
20th June, "	21.7 ± 1.77	8.2 ± 0.66	0.50 ± 0.05	0.91 ± 0.11
9th Aug., "	17.6 ± 1.09	9.9 ± 0.63	0.51 ± 0.05	0.71 ± 0.07
8th Nov., "	33.3 ± 3.27	11.5 ± 1.14	0.67 ± 0.07	1.03 ± 0.15
5th Jan., 1967	66.5 ± 8.38	25.0 ± 2.91	1.55 ± 0.10	1.51 ± 0.23

Table 12. Average composition of algal sediment in the whole stream at different seasons. Weights are given in gms. per 9 cm. section of sediment.

The analytical results given in Table 10 show that a decrease in COD was consistently obtained during passage through the stream. This implies that the amount of algal material leaving the stream was very small, which was in agreement with the very clear water observed in the final section of the stream. Algal settlement and retention was therefore highly effective at the time of sampling.

17.5 Algal Sediment Analytical Results

Standard 9 cm section samples of the algal sediment in the stream were taken at some or all of Stations 1-33 on 10th May, 20th June, 9th of August, 8th November 1966, and 5th January 1967.

The individual analytical results obtained have been used to calculate the mean dry and organic weights for 9 approximately equal lengths of stream (Table 11). In Table 12 the mean composition of the algal sediment over the whole length of the stream (Stations 1-33) on the five sampling dates has been calculated for dry and organic weight and for the weights of nitrogen and phosphorus.

17.5.1 Chemical composition of algal sediment

Using the mean values for dry and organic weight (Table 12) the organic weight amounted to 40.7 per cent of the dry weight in May, 37.8 per cent in June, 56.4 per cent in August, 34.6 in November and 37.6 in January.

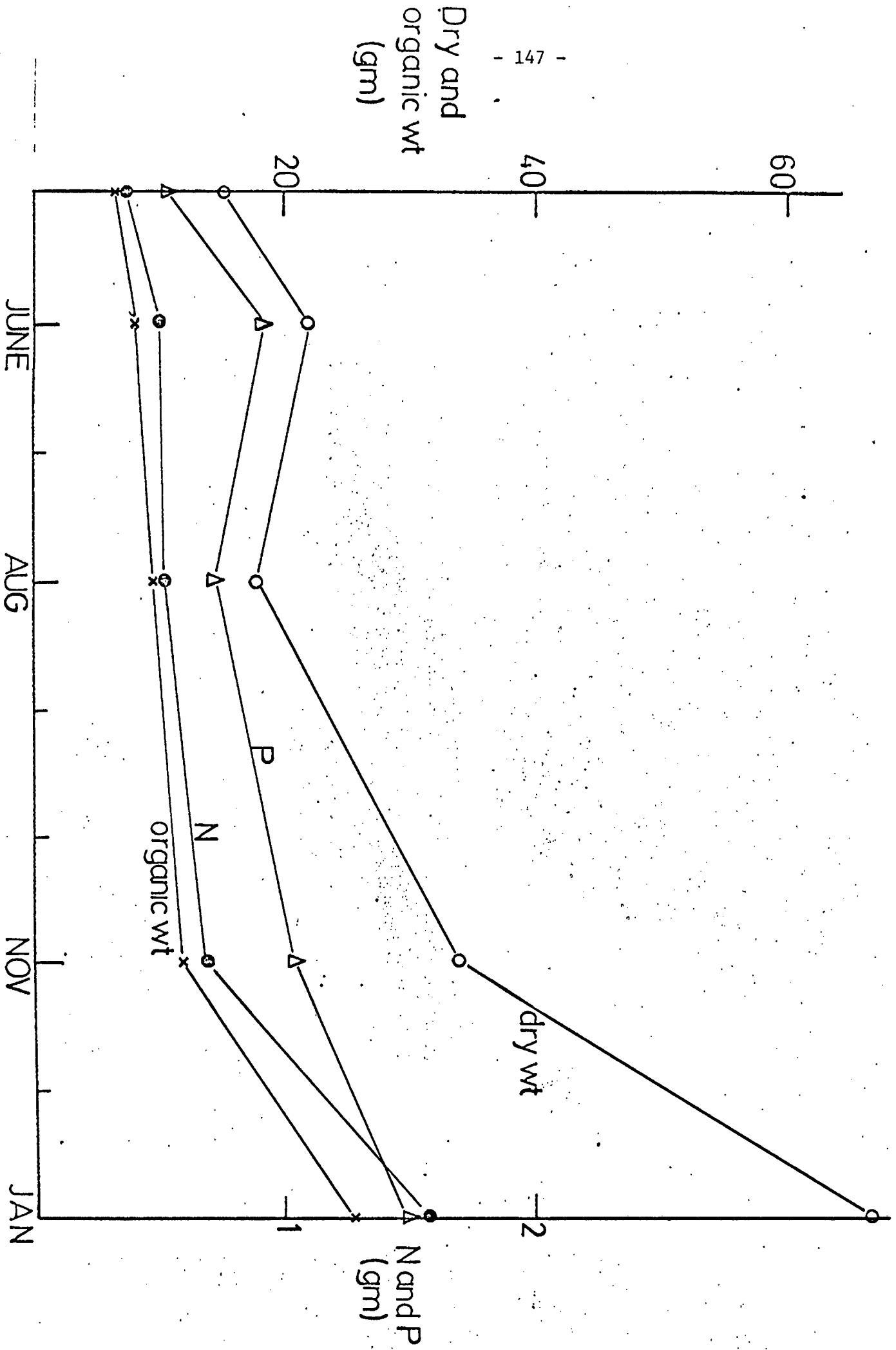


FIG 14. Seasonal changes in 9cm sections of algal sediment

These results show that the ratio of organic to dry weight was relatively constant except in August when the mineral content of the sediment decreased. This decrease is illustrated in Figure 14 in which the mean composition of the algal sediment in the stream is plotted against the sampling dates. It is evident that the nitrogen content closely followed the changes in organic weight, which showed a steady increase from May to November and a more rapid increase during December and January. Both dry weight and phosphorus changes followed the same course as each other, and differed from organic weight and nitrogen in that, after an initial increase from May to June, the values decreased during July and August. Since organic weight and nitrogen increased over the same period these results can only mean that soluble mineral material was lost from the sediment in the period June-August. Part of the mineral loss was evidently due to re-solution of precipitated phosphate during these colder months. The loss of phosphate is confirmed by the increase in phosphate concentration over the original concentration entering the stream during this period (Table 10), and the decrease in overall phosphate removal observed (Fig. 12).

The rate of increase in algal material, based on organic weight and nitrogen content, was slow from May to November. Using values from Table 12 the total dry algal material in the stream rose from 523.0 Kg on 10th May to

1161.1 Kg on 8th November, an increase of 2.2 times.

From November onward the increase was more rapid, rising to 2318.8 Kg on 5th January, a twofold increase over the value in November.

The oxidizable material, as measured by the COD, was always lower at all stations along the stream than in the influent. If the observed release of ammonia nitrogen in the upper section of the stream resulted from decomposition in the bottom layers of sediment, as suggested earlier, the amount of decomposition must have been small. Had decomposition occurred to a greater extent the organic compounds associated with production of ammonia would have been detected in the stream as an increase in COD value. Instead, a decrease was observed at all stations. The rate of release of organic decay products must, therefore, have been small and such compounds as were released were rapidly utilized by the heterotrophic population of the stream.

17.6 Growth Rates

If the rate of decomposition of the algal sediment was low, then the slow increase in biomass observed during the 6 month period May-November must have been due to the slow rate of algal growth during this time. In the following two month period from December to January the growth rate increased by a factor of three.

The reason for this difference in growth rates can be attributed to seasonal factors, since the influent water quality remained virtually unchanged during the experiment.

The two seasonal factors that would have affected the stream are temperature and light intensity, since both these factors have lower values in winter.

During the period of slow growth (May-October), the average water temperature in the stream at the time of sampling was below 20°C, and from May to August it was below 10°C. During the November-January period, on the other hand, the temperature was 30°C or a little higher.

17.7 Seasonal Changes in Light Intensity

According to records obtained from the local meteorological station the solar energy incident on a horizontal surface (such as the stream) at the experimental site in winter did not fall to less than 61 per cent of the mid-summer value. The midday light intensity in summer was measured as approximately 12,000 f.c. using a Weston Light Meter.

Light penetration into the algal sediment in the stream would be relatively small and incident light intensities below that required for high rates of photosynthesis at the algal sediment surface would not occur, even in mid-winter. It is therefore evident that the slow algal growth rate observed during the winter months was the direct result of decreased water temperature.

17.8 Overall Nutrient Removal

During the 303 days of operation from 8th March 1966 to 5th January 1967, the total amount of nutrients entering the system, based on a mean input of 15 gpm containing 24.0 mg N/l and 8.3 mg P/l, was 706 Kg of nitrogen and 245 Kg phosphorus.

17.8.1 Removal of phosphorus

From data in Table 10 the overall removal of phosphorus, based on monthly mean values, was 50 per cent. On this basis approximately half of the phosphorus entering the system should have been retained in the sediment, either as precipitated phosphate or in cell material, i.e. about 120 Kg P.

Data from Table 12 for the amount of phosphorus present in 9 cm sections on 5th January 1967, show that the total phosphorus present in the whole stream was 52.7 Kg P, which is approximately 44 per cent of the expected amount. This indicates that more than half of the phosphate precipitated during daylight hours returned to solution during the night and passed out of the system, and that samples taken between 8 a.m. and 10 a.m. did not detect this loss.

This result shows that over the whole period of operation the overall removal of phosphate was somewhat less than 25 per cent and not 50 per cent, as appeared from the water analysis results. The phosphate loss

was due to a combination of lowered pH value, due to cold weather, algal respiration at night, and to the fact that no facilities for removing a portion of the phosphate-containing sediment were provided.

17.8.2 Removal of nitrogen

During the same period the removal of nitrogen, based on monthly mean values (Table 10) was 24.3 per cent. Thus, of the 720 Kg N entering the system 175 Kg was removed. Nitrogen removed by algal assimilation would not be expected to be released due to the pH variation, as was the case with precipitated phosphate, and should therefore have been detected as an increase in the nitrogen content of the algal sediment. Using data from Table 12 the total amount of nitrogen present in the stream on 5th January was 54.0 Kg N. Thus, of the 175 Kg N removed by the stream only 31 per cent was accounted for by nitrogen retained in the sediment.

This discrepancy was not unexpected since some loss of ammonia nitrogen to the atmosphere had been anticipated (Saubert, 1957). This was due to the decrease in ionization of ammonia that occurs with increase in pH value according to the equation;

$$P_u = 100 \left[1 - \frac{1}{1 + \text{antilog} (\text{pH} - \text{pKa})} \right] \dots\dots\dots 3$$

where P_u is the percentage of the gas in the unionized form and pKa is a constant equal to the pH value at which 50 per cent of the gas is unionized (Fig. 13). From this relation approximately 5 per cent of the ammonia nitrogen is in the unionized form at 25°C at pH 8.0 and the percentage increases with increasing pH value.

The stream results indicate that significant loss of ammonia did not occur until Station A5, at which point the mean pH did not drop below 8.5 at any sampling time during the experiment (Table 10). From this table it is evident that at times of elevated pH values the loss of ammonia nitrogen was rapid, and the results indicate that about 69 per cent of the nitrogen removed by the system was not retained in the algal sediment but was lost to the atmosphere as ammonia gas.

17.9 Settlement Characteristics of the Algal Sediment

Throughout the period of operation of the stream the rapidity with which disturbed sediment resettled was very marked. The clarity of the water, especially in the lower sections, was also very noticeable.

As mentioned previously, the settlement sumps provided were very rapidly filled by the granular algal sediment and could easily

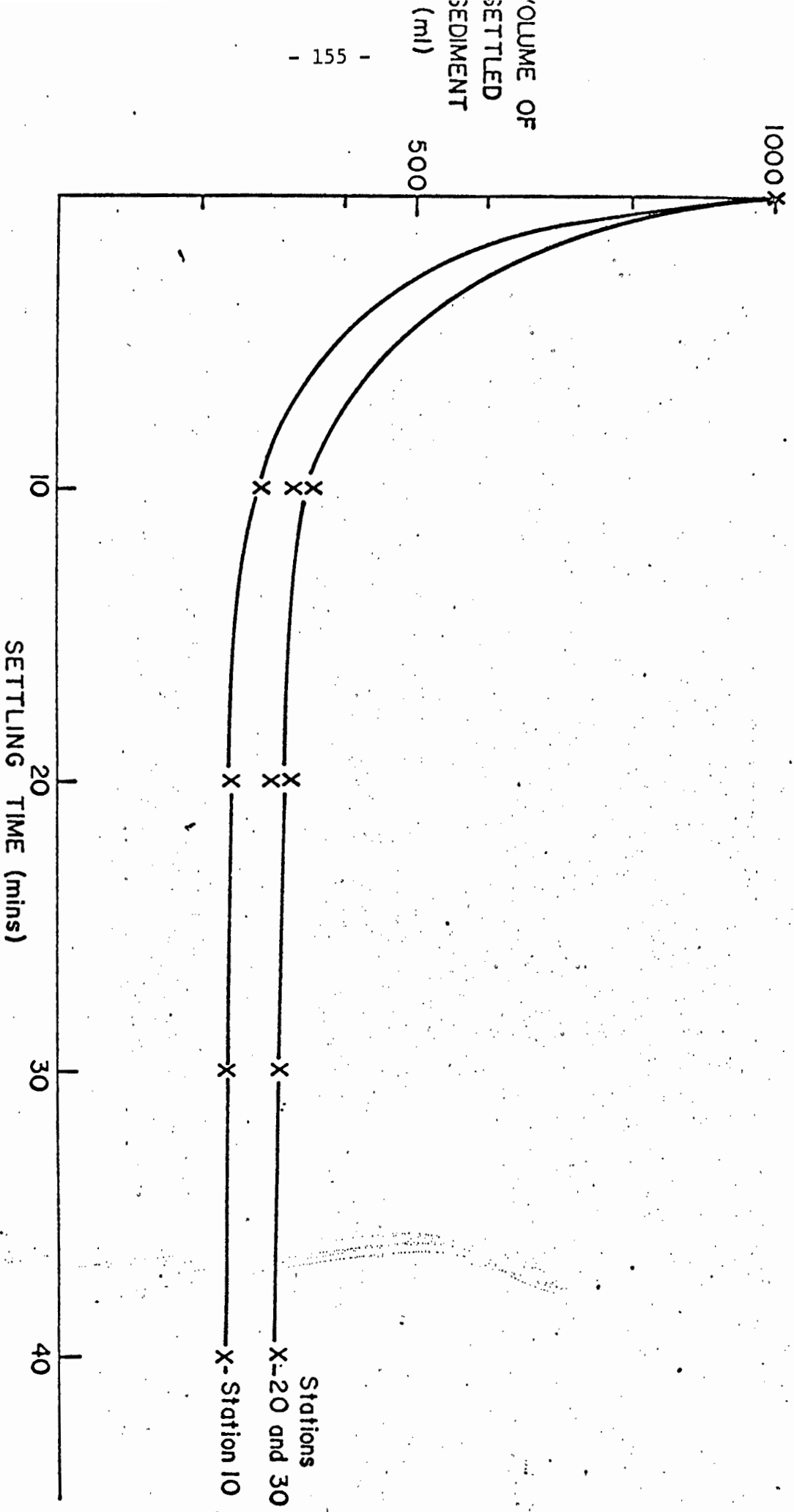


FIGURE 15.
Settlement of algal sediment

Station	Settle volume (ml) in settlement time (mins.)					Suspended solids (mg/l)	SVI*	SDI
	10 mins.	20	30	40	60			
Stn. 10	282 ml	248	238	233	231	2079	114.5	0.87
Stn. 20	335 ml	312	307	300	300	2633	116.5	0.86
Stn. 30	342 ml	320	313	303	305	2115	148.0	0.68

$$* \text{SVI} = \frac{\text{per cent settling by volume}}{\text{per cent suspended solids}}$$

$$\text{SDI} = \frac{100}{\text{SVI}}$$

Table 13. Settlement characteristics of algal sediment
from three positions in the algal stream.

be emptied with buckets if the stream was temporarily blocked. There is no doubt that, if a suitable settlement tank with hydraulic sediment removal had been provided, very efficient removal of the algal material could have been achieved.

To obtain an estimate of the rate of settlement a modification of the method used to determine settlement characteristics of activated sludge suspensions was employed. For sampling purposes the stream sediment was vigorously stirred with a broom and a sample of the resulting suspension collected with a bucket. The sediment was then allowed to settle and consolidate for 1 hour and the clear supernatant syphoned off. 300 ml volumes of the sediment were then resuspended with 700 ml of stream water in 1 litre cylinders and the volume of algae settling over a 30 minute period was noted. The cylinder was then well shaken and the dry weight of suspended algae determined.

The results obtained for sediment taken from different parts of the stream did not vary to any great extent. Typical results are shown in Table 13 and Fig. 15, from which it is evident that settlement was almost complete within 15 minutes, producing an algal sludge containing approximately 8.5 gms dry algal weight per litre. The values calculated for the volume occupied by 1 gm of solids after 30 minutes settlement, termed the Sludge Volume Index (S.V.I.) and also the Sludge Density Index (S.D.I.) confirm the favorable settlement characteristics of the sediment.

18. DISCUSSION OF RESULTS

The results obtained during 10 months operation of the stream showed that the principles upon which the use of an algal stream were founded were basically sound but, as a practical method for removing nutrients, the system as operated suffered from several defects.

18.1 Removal of Phosphate

Precipitation of calcium phosphate as a result of pH changes due to photosynthetic upset of the bicarbonate-carbonate equilibrium and reduction in alkalinity offers a practical method of removing phosphate from effluents, but unless the algal sediment produced is removed leaving sufficient biomass to raise the pH to the required degree, substantial quantities of precipitated phosphate return to solution when the pH value falls below about 8.5 and are lost from the system. Such conditions occur as a result of algal respiration during the hours of darkness. Removal of part of the algal sediment formed would reduce the production of respiratory carbon dioxide, possibly decreasing the pH variation, but some resolution and loss of phosphate would be likely to occur from the remaining sediment.

The slow growth rate observed during the winter months was due to lowered water temperature and, due to this decrease in algal metabolism, the pH value was not raised to values high enough to affect the solubility of calcium phosphate for a long enough period during the day to reprecipitate phosphate which re-dissolved during the previous night. As a result, a continuous loss of phosphate occurred during winter.

The cessation of photosynthesis during the hours of darkness and the low winter water temperatures were the main factors preventing efficient phosphate removal by the stream under the experimental conditions provided.

18.2 Removal of Organic Nitrogen

The organic nitrogen compounds in Pretoria sewage effluent were normally present in concentrations of less than 2 mg N/l, of which about 50 per cent was removed by the stream system. Due to the dilution factor when discharged to surface waters, the eutrophying effect of concentrations of this order is likely to be relatively small.

18.3 Removal of Nitrate Nitrogen

The negligible removal of nitrogen from March to May, when nitrification of ammonia to nitrate was almost complete, suggests that ammonia was the form in which nitrogen loss mainly occurred during winter, either by algal assimilation or, to a much greater extent, as ammonia lost to the atmosphere. In the warmer months, October to December, the reduction in nitrate concentration was greatly improved due to increased algal assimilation.

18.4 Removal of Ammonia Nitrogen

Loss of ammonia nitrogen to the atmosphere occurred at pH values above 9.0 and this loss appears to have been responsible for about 70 per cent of the nitrogen removed by the stream. This effect is obviously of considerable importance as a means of removing nitrogen from solution.

Since both $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ can serve as a nitrogen source for most microphytes, but $\text{NO}_3\text{-N}$ cannot be lost to the atmosphere as such, it would be advantageous if more of the nitrogen to be removed from the effluent was present in the form of ammonium salts, and not as nitrate. This requirement might be met by suitable operation of the sewage works bio-oxidation stage, since the normal practice is to underload the oxidation system to the extent that carbonaceous oxidation is complete and partial oxidation of ammonia is obtained.

18.5 Removal of Algae

It is evident that the precipitation of calcium phosphate in an algal suspension has the effect of flocculating normally planktonic species, producing heavy granular flocs with excellent settlement characteristics. The photosynthetically induced pH increase thus has an advantageous effect in two ways; it causes both precipitation of phosphate and the production of an easily removable floc containing phosphate, together with all the nutrients assimilated by the flocculated algal cells. The separation of algae achieved in this way is probably as good, or better, than that obtained by centrifugation and is very much less expensive in terms of equipment and power requirements.

18.6 Disadvantages of Stream Operation

The unfavorable aspects of a stream system may be listed as follows:

- i. The high surface/volume ratio causes rapid loss of the intrinsic heat of the effluent when its temperature is above ambient. This loss results in unfavorably low temperatures during winter months.
- ii. Lowered water temperature decreases algal metabolism and day-time pH changes may not be sufficient to produce the required effects.
- iii. Re-solution of precipitated phosphate in the algal sediment required to produce the necessary pH change will occur during the hours of darkness and during periods of low water temperature.
- iv. Current speed in the stream as operated was approximately 5 cm/sec. This speed was probably too low to take advantage of the higher metabolic rate reported for algae in contact with rapidly moving water, and turbulence may have been too low to obtain the maximum possible loss of ammonia.
- v. Construction costs for a stream system to treat large volumes of sewage effluent would be relatively high, and a large land area would be required.

In view of the shortcomings listed above, the possibility of using a modified system, based on the same principles, in which these disadvantages could be reduced or overcome was investigated, and is described in Section D.

Section D

DEVELOPMENT OF A MODIFIED ALGAL STREAM SYSTEM

19. INTRODUCTION

The disadvantages of the experimental stream were listed at the end of the previous section and a means was sought whereby the advantages of the stream could be retained and the disadvantages overcome.

The four main problems to be solved were the rapid loss of intrinsic heat in cold weather, the presence of an inactive algal residue which acted as a reservoir of phosphate for re-solution, the need for a more rapid current speed to increase turbulence and loss of ammonia nitrogen and also to increase the algal metabolic rate and the high land area requirement.

19.1 Modification of the Stream System

A reduction in land area and the rate of heat loss would be possible by using a greater water depth, thus reducing the surface/volume ratio. For this purpose a tank sunk below ground level, or suitably lagged, could be used if no external heat source were to be applied. Use of a greater water depth would raise the problem of illuminating a sufficient area of active algal material to produce the required pH increase, and a further difficulty would be the fact that the current speed in a relatively deep tank would be very low. However, if the

algal surface could be passed through the water the effect would be the same as water passing over the algal surface, with the difference that the effective current speed would then be independent of the hydraulic retention time and would only depend on the rate of movement of the algal surface.

19.1.1 Design of apparatus

With these requirements in mind a system was designed in which the stream bottom would be replaced by a series of vertical parallel discs upon which an algal community would be developed. The discs would be contained in a tank with a relatively low surface/volume ratio and would rotate while half submerged in the water. The effective current speed would then depend upon the disc rotation rate and not upon hydraulic loading. The area of algal surface exposed would depend upon the number of discs which, in turn, would depend upon the distance between discs required to allow sufficient light for algal growth to occur on the disc surfaces.

Although an autotrophic algal unit of this kind has not previously been described, the use of rotating discs is not new in the field of sewage treatment. Pöpel (1959) and Hartmann (1960) have described the use of such discs to both support and aerate a

heterotrophic bacterial film in a sewage oxidation unit. A small commercial unit of this type is now available in Germany. Similar investigations have also been described by Kolbe (1965).

19.1.2 Light intensity on disc surfaces

To determine whether the necessary distance between discs would be too great for the method to have practical possibilities, an estimate of the separation was obtained with a simulated algal disc system.

A series of pairs of semi-circular hardboard sections were prepared with radii varying from 6" to 40". A slotted baseboard was constructed in which the sections were held vertically at various distances apart. One board of each pair was drilled with holes at distances along the vertical radius into which a small selenium barrier cell fitted with an opal glass cover could be inserted, so that the opal glass lay flush with the inside surface of the disc.

The smooth surfaces of the discs were painted with gloss-finish green paint, of the same color as the algal growth observed in previous experiments, to approximate the reflection characteristics of a film of wet algae.

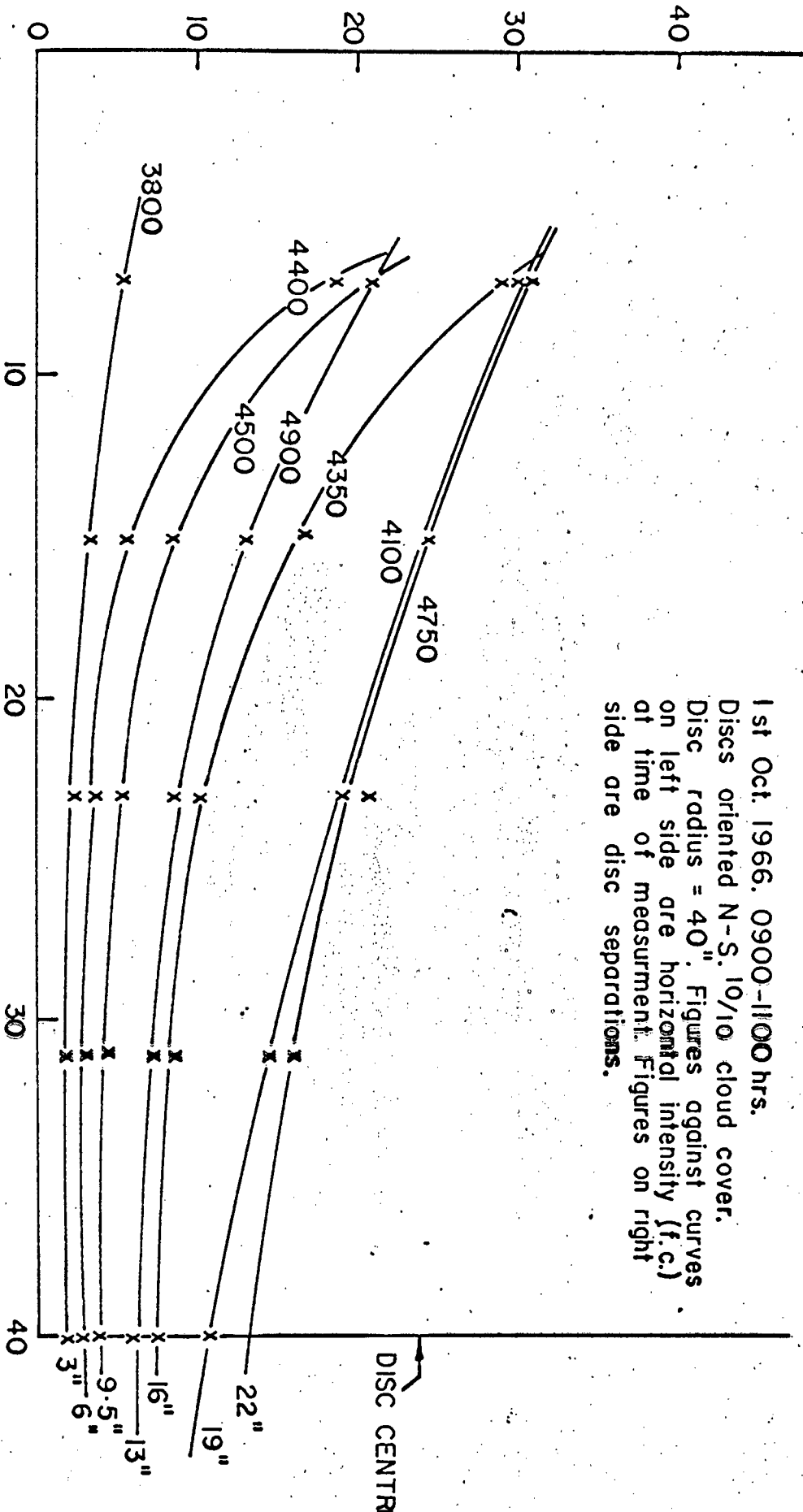
The apparatus was placed in the open with each pair of discs, painted surface inward, in the plane of the meridian so that at noon the sun's rays fell parallel to the disc surface. This position was selected since, with the disc oriented E-W, no direct light would reach the southern surfaces at any time, as the laboratory site lay south of the equator.

The selenium barrier cell used was calibrated against a Weston Light Meter and a smooth curve relating the two was obtained.

Measurements of light intensity falling on the opal glass on the inner surface of the discs were then made at various distances along the vertical radius with pairs of discs of different diameter, and at various distances apart. These measurements were made on four days at intervals of several weeks.

Prior to each series of measurements the incident light intensity was determined by holding the photocell parallel to the ground. In overcast weather this was particularly necessary due to intensity fluctuations resulting from changes in cloud cover.

The data obtained on 1st October 1966 were taken as those representing a day with complete cloud cover



DISTANCE FROM PERIPHERY (Inches)

FIGURE 16.

Incident light intensity on disc surfaces

on a date near to the spring equinox. The average annual light intensity would be approximated at the equinoxes and the reduction due to 10/10 cloud cover would represent the lowest intensity likely to be experienced for any length of time. On the South African Highveld cloud cover is normally absent in winter and incident solar energy is about 60 per cent of the mid-summer value, measured as approximately 12000 f.c. with a Weston Light Meter, and would thus be unlikely to be less than on the date of the measurements.

A typical result for the measurements obtained on this date is given in Figure 16, in which data for a disc of 40" in radius is shown. Due to the variation in incident intensity during measurements the results have been expressed as a percentage of the incident value at the time of measurement. It can be seen that the intensity at the disc surfaces falls off rapidly from the disc edge when the distance between the discs is small. At wider separations the decrease becomes more regular, and there is evidently a separation distance at which the light attenuation becomes approximately linear along most of the radius.

On the assumption that daylight intensity at the experimental site was unlikely to fall below 4000 f.c. for extended periods of time, it was possible to estimate

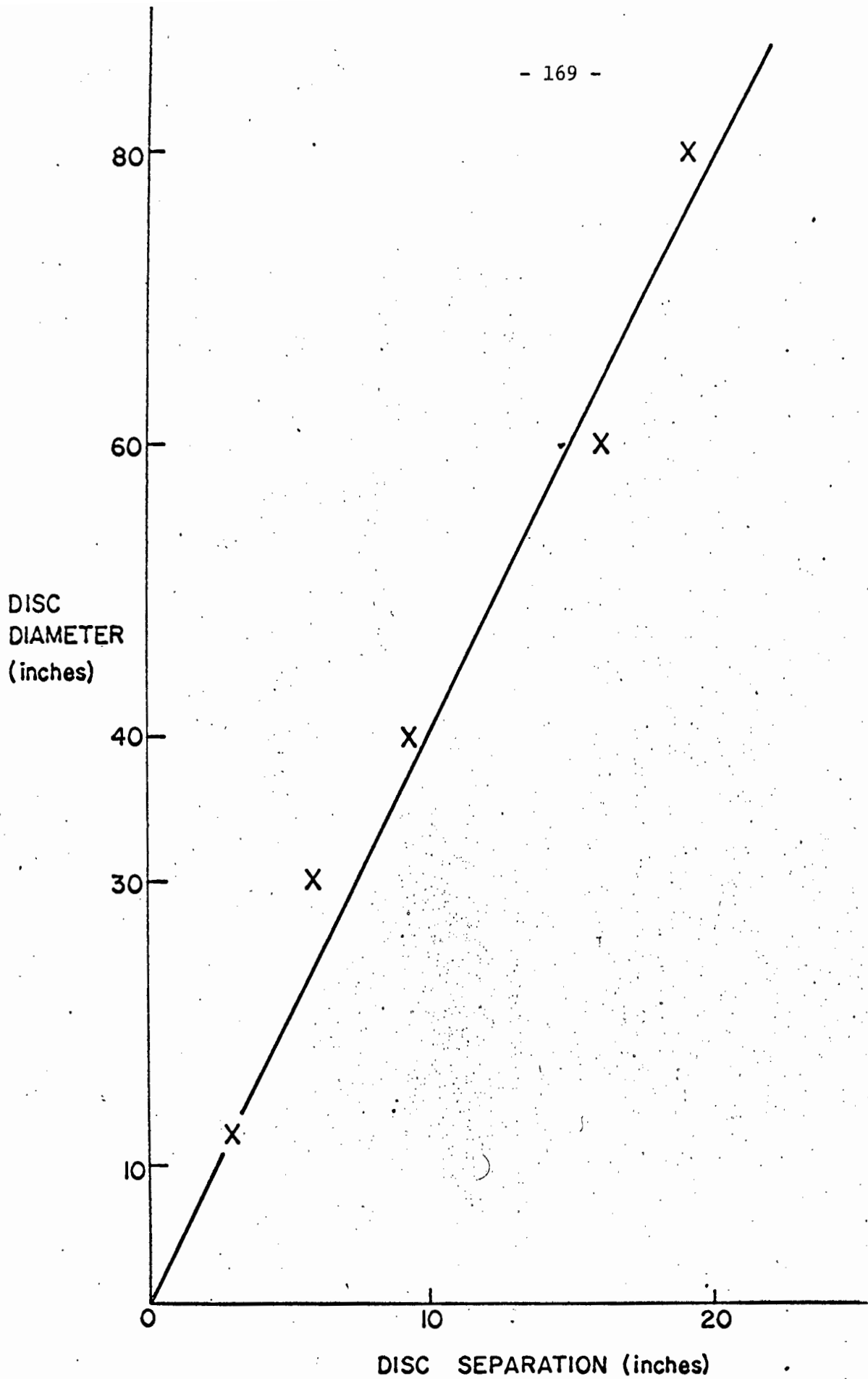


FIGURE 17.

Disc separation required to give 10 per cent of incident light intensity at the disc center

the disc separation necessary to allow any required intensity at the center of the disc for discs of different radii.

Using a value of 400 f.c. as the saturation intensity for common freshwater algae the separation required to give an intensity of this value at the disc center can be obtained by reference to Fig. 17, in which the separation required for 10 per cent of an incident intensity of 4000 f.c. to reach the disc center is shown.

From Fig. 17 the necessary distance between 80" diameter discs would be 20 inches, for 40" diameter it would be 10", etc. Using these values, it can be calculated that the ratio between disc surface and the enclosing tank or pond surface would have a minimum value of 6.28. By using a disc system, therefore, an algal surface would be produced more than six times greater than would be present as a thin layer in a tank or pond of equivalent dimensions. By overlapping parallel rows of discs a greater difference might be possible. A further advantage of this system would be that light inhibition, as a result of too high intensities, would only occur at the extreme edges of the discs, in contrast to a pond system where inhibition occurs in the surface layers.

19.1.3 Light quality at the surfaces

No attempt was made to measure the color or wavelength of the light reaching the inner surfaces of the discs; only the intensity of illumination was recorded. A variable fraction of the light received would be reflected between the inner surfaces of the discs and its spectral composition would be different from normal daylight, with a high proportion of energy in the region of 5000A in the green section of the spectrum.

No relevant information could be found in the literature on the probable effects on algal metabolism of light with this composition. It was therefore thought advisable to operate a prototype disc unit with disc diameter and separation according to values indicated in Fig. 17 to determine whether, in fact, the light would support growth. At the same time the question of whether a surface constantly leaving and entering the water would maintain a satisfactory algal film, would be resolved.

20. OPERATION OF A SMALL-SCALE ROTATING DISC APPARATUS

A small experimental unit consisting of 9 P.V.C. discs, 12 inches in diameter set 2.5 inches apart on a horizontal shaft, rotating in a 20 litre tank at 3 rpm was constructed. The tank was surrounded by

a constant temperature waterbath to prevent nighttime temperatures falling below 25°C and the unit was set up in an exposed position on the laboratory roof. Effluent from the local sewage works was automatically dispensed at 30 minute intervals at a rate of 20 litres/day. A selection of algae obtained from the algal stream and a local river were added as an inoculum.

After 25 days an algal film about 1.5 mm in thickness had grown on the disc surface from the periphery to the center. Microscopic examination showed the film to consist almost entirely of algal cells with a small amount of adhering organic debris. Segments of algae from the surfaces were removed and the dry weights per unit area determined. The results showed that growth was uniform on each disc, but there was some variation between discs. The nitrogen content of the algal film varied between 5.23 and 5.34 per cent of dry weight, the phosphorus content from 1.03 to 1.08 per cent.

20.1 Floral Composition

Microscopic examination of the film showed the presence of a mixed algal community consisting of:

Nitzschia-palea

Scenedesmus bijugatus

Chaetopeltis sp.

Two Lyngbya sp.

One of the Lyngbya sp. could not be identified and has now been described as a new species under the name of Lyngbya hemensii (Cholnoky-Pfannkuche, in press).

The diatom Nitzschia palea tended to dominate at the faster moving outer edge and the Lyngbya sp. were dominant nearer to the center. This distribution was probably associated with differences in light intensity and with effects of current speed.

These results showed that a community of algae could be grown on a partly submerged rotating disc and that the light reaching the inner surfaces between the discs was adequate for algal growth. The separation of 2.5 inches was actually less than the 3 inch separation indicated from the curve in Fig. 17, suggesting that closer spacing of the discs might be possible.

20.2 Nutrient Removal in the Disc Apparatus

The algal film developed on the unit was used for a number of preliminary short-term tests to determine the potential of the system for nutrient removal.

For these experiments the tank was emptied and refilled with 20 litres of sewage works effluent of normal quality. Disc rotation was continued at the previous rate of 3 rpm and samples for analysis were removed at intervals over a period of 24-48 hours. Distilled water to replace evaporation was added as required.

20.2.1 Removal of nitrogen

In Fig. 18 is shown the course of nitrogen loss over a period of 50 hours during the first experiment. The ammonia fraction of the Kjeldahl

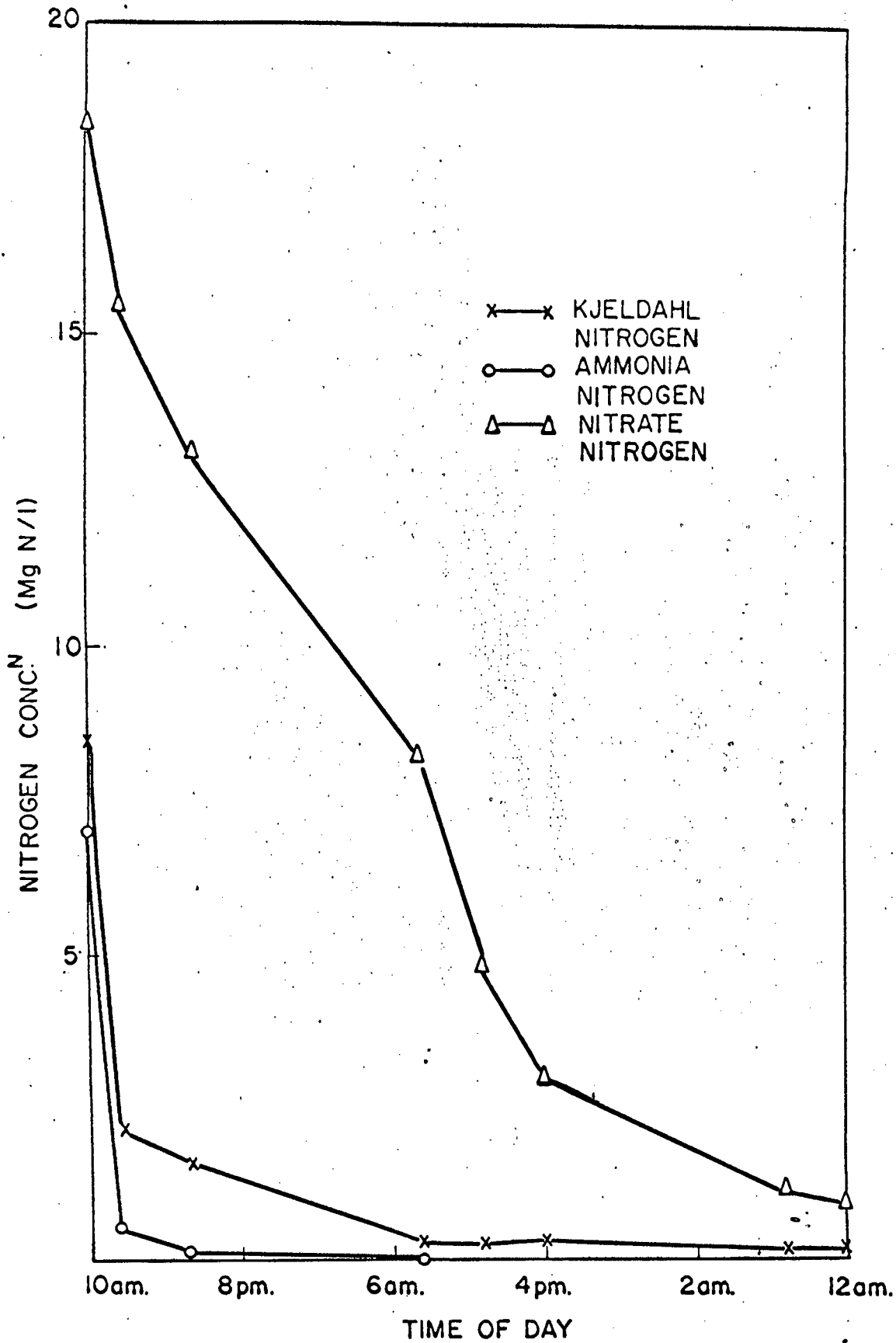


FIGURE 18.

Loss of nitrogen from the rotating disc prototype

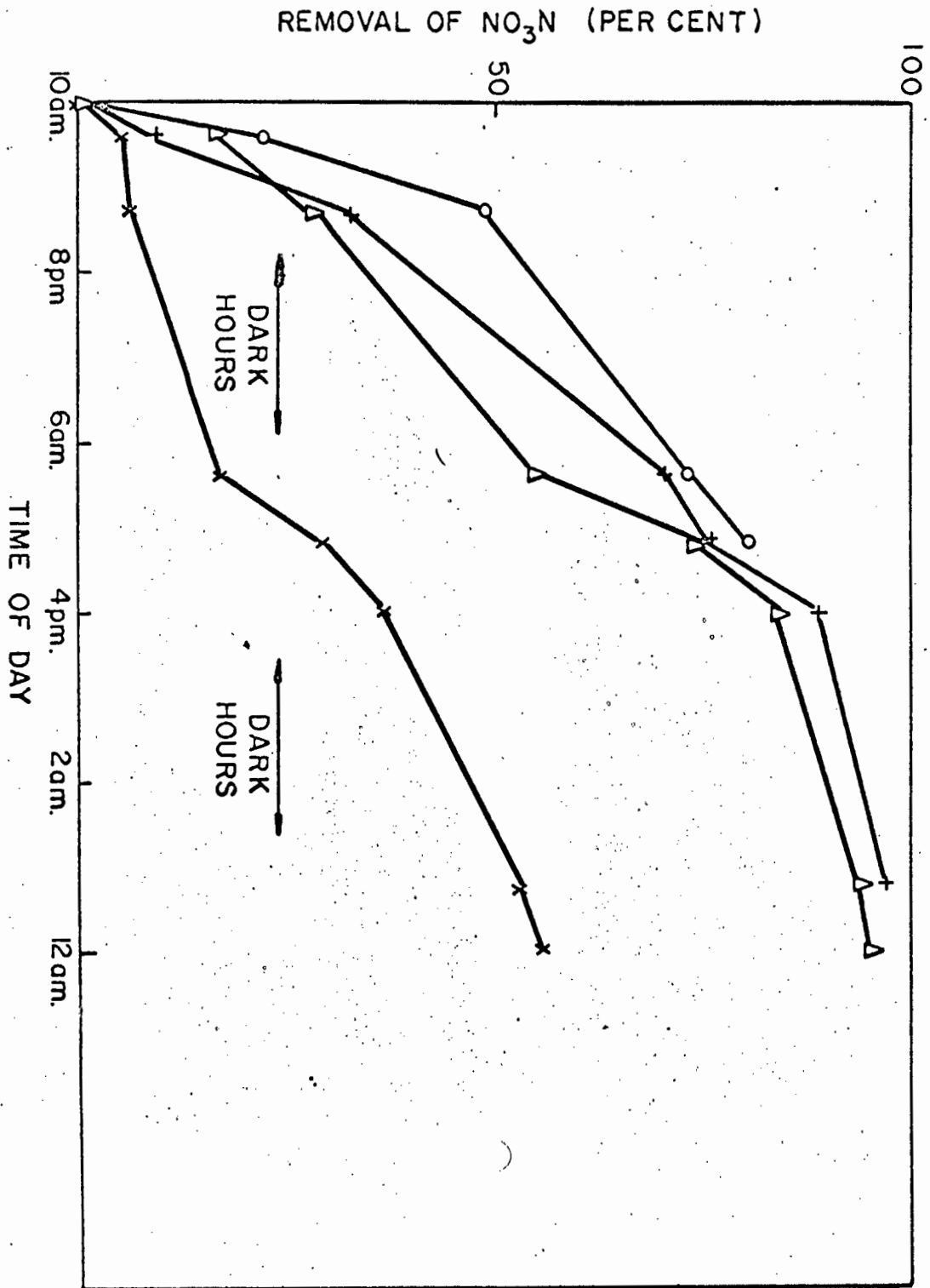


FIGURE 19.

Variation in nitrate removal during light and dark hours in 4 experiments (NO₃N as per cent of influent concⁿ)

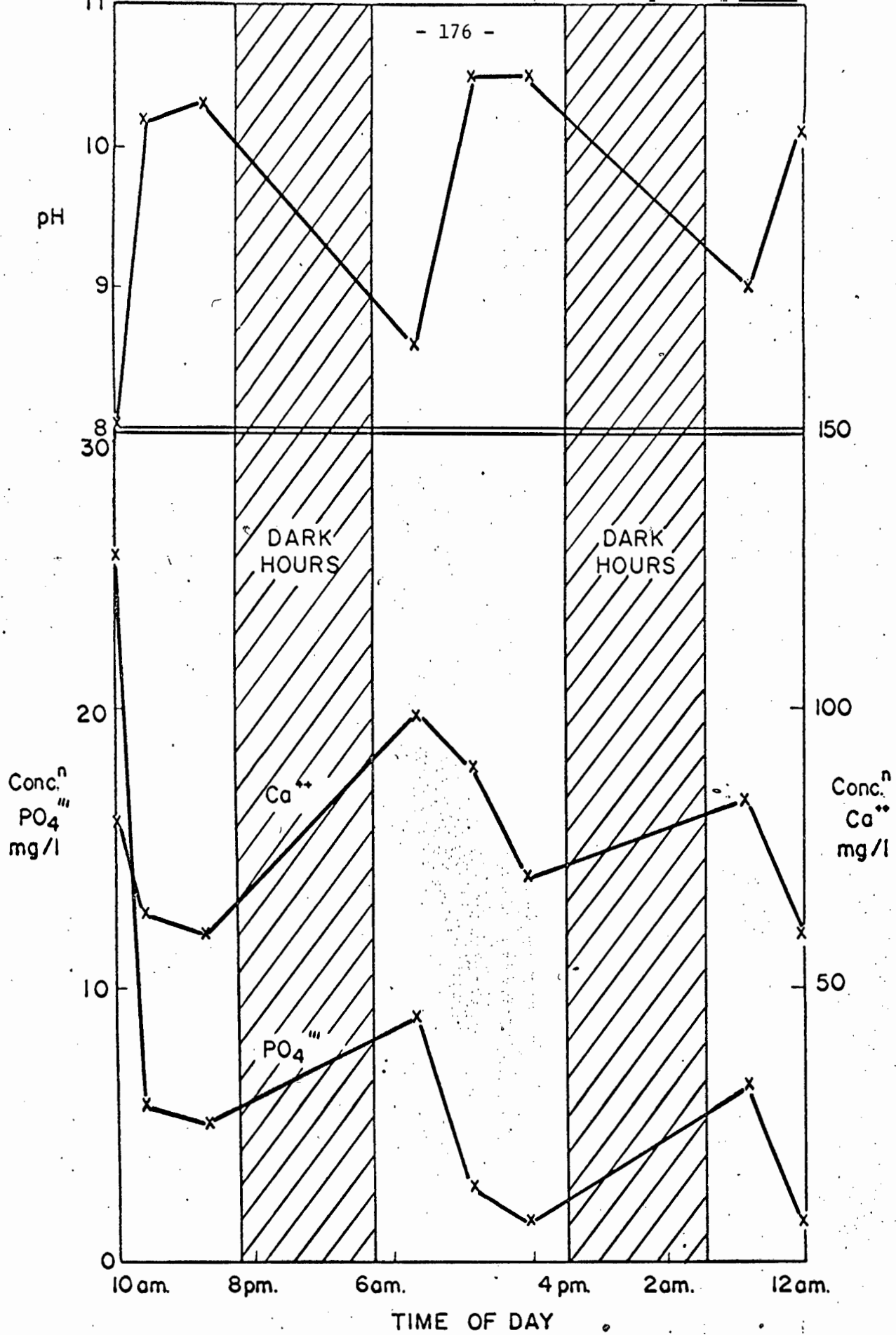


FIGURE 20

Effect of pH on PO_4^{3-} and Ca^{2+} concentration in the rotating disc prototype

nitrogen content was rapidly lost during the first two hours. The remaining organic fraction of the Kjeldahl nitrogen was removed at a very much slower rate which was approximately linear with time.

Nitrate removal did not follow the same pattern as for ammonia, the concentration declining in a more regular manner with two more steeply sloping sections to the curve. These periods of more rapid removal corresponded to hours of daylight in all four experiments, Fig. 19.

It was evident from the results that photosynthesis during light hours caused more rapid nitrate assimilation, but the process continued to a lesser extent during the hours of darkness. It is possible that assimilation of organic nitrogen followed a similar course but, due to the much lower concentrations present, the analytical results do not show the effect.

20.2.2 Phosphate removal

Reduction in phosphate concentration was closely correlated with changes in calcium concentration and both were dependent upon the pH value, as can be seen from Fig. 20, in which the results of the first experiment are shown. The rapid increase in pH value between 10 a.m. and midday to values above 10.0 was the result of the photosynthetic activity of the algal film. At this pH value the solubility of calcium phosphate is low and, by

midday, approximately 80 per cent of the phosphate present had been precipitated. During the night the pH fell due to algal respiration and partial re-solution of calcium phosphate occurred to give a phosphate concentration at 8 a.m. of less than 40 per cent of the original. The same effect was observed in all four of the short-term experiments that were carried out and the results, expressed as per cent removal of phosphate, show a marked diurnal cycle of precipitation and re-solution, in contrast to the continued removal of nitrate during both light and dark hours.

20.2.3 Loss of ammonia to the atmosphere

It was considered necessary to confirm that the rapid loss of ammonia observed was due to the escape of volatile unionized ammonia and not only to algal assimilation.

For this purpose the algal growth was cleaned from the prototype discs and the unit refilled with a glycine-sodium chloride-sodium hydroxide buffer solution. The pH was then adjusted to the required level and ammonium chloride solution added to give an $\text{NH}_4\text{-N}$ concentration of approximately 30 mg/l. Previous analysis had shown that the presence of glycine did not effect the ammonia analysis. Disc rotation was started and samples for analysis removed at intervals.

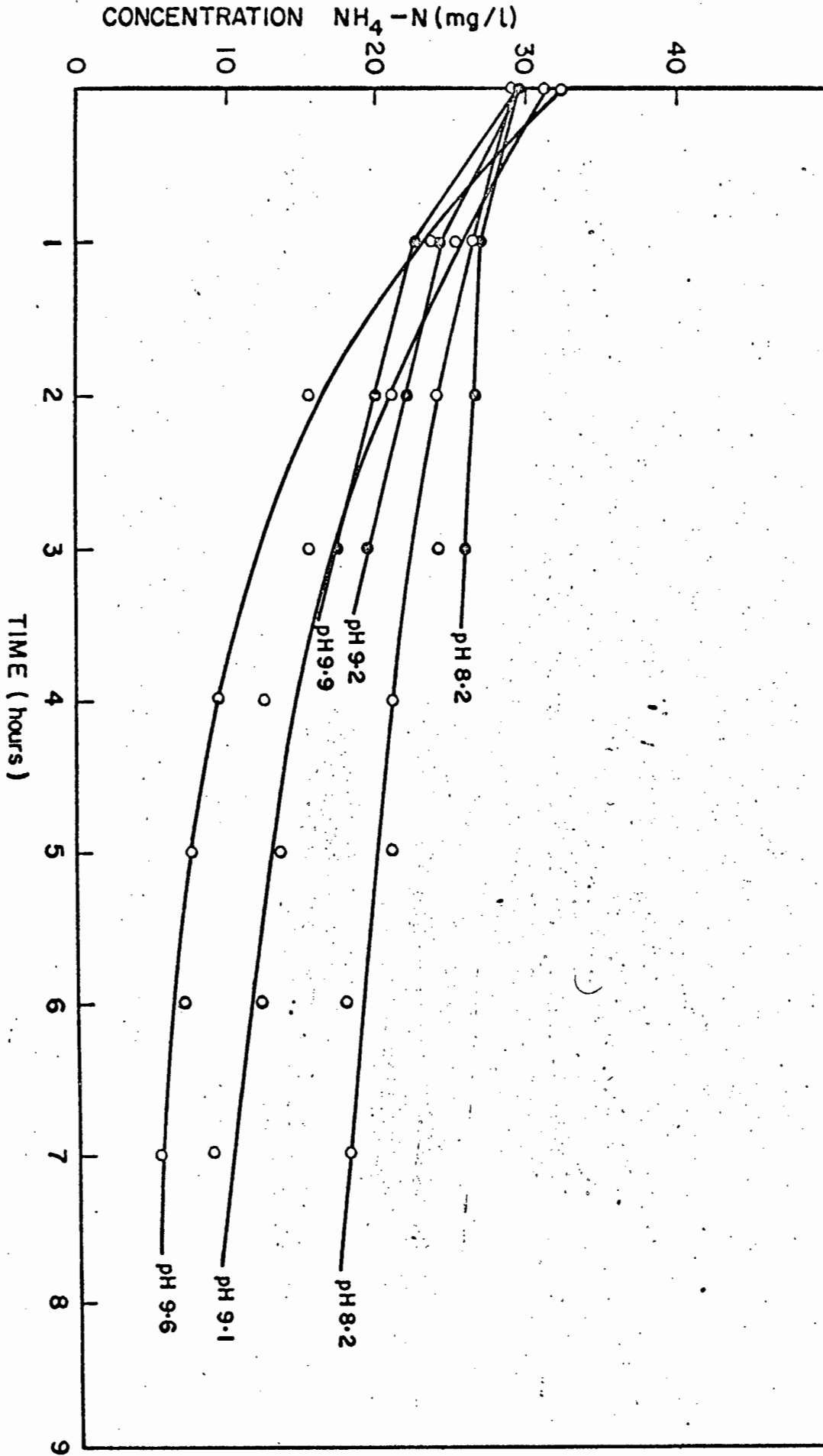


FIGURE 21.
Effect of pH on rate of loss of ammonia
from buffer solutions.

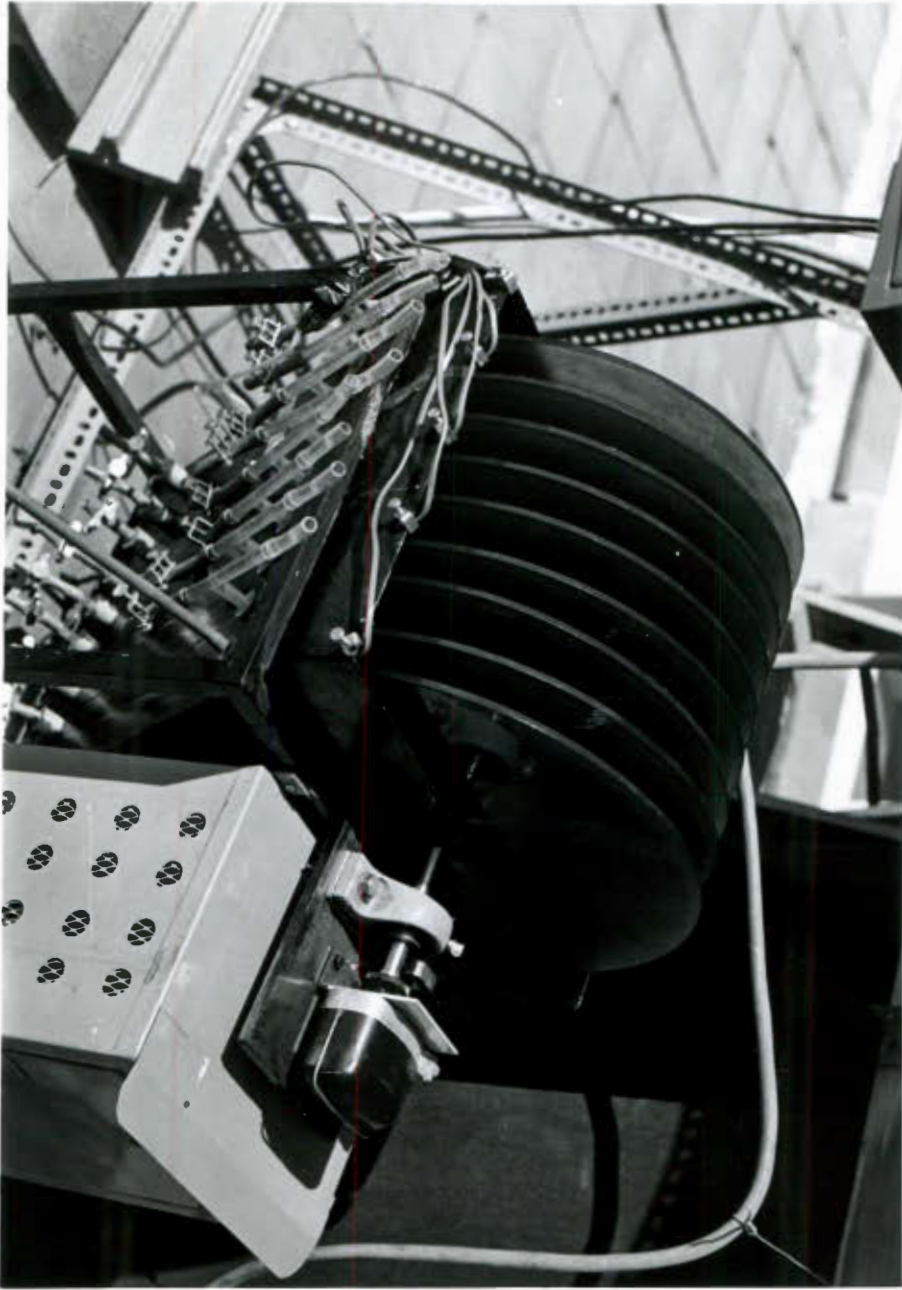


Plate 3. Experimental algal disc apparatus.

The analytical results shown in Fig. 21 confirmed that substantial quantities of ammonia were lost from the water film and that the rate of loss increased with pH value. The rate of loss observed was, however, noticeably less than that observed with algae-covered discs at similar pH values. This observation led to consideration of factors other than pH which might effect the rate of ammonia loss and to a series of experiments described later in this section.

20.2.4 Experiments with an improved disc apparatus

During operation of the disc unit some difficulty was encountered due to temporary power interruptions and subsequent failure of the motor to resume rotation when power was restored. This resulted in extended periods without rotation during which the algal film was damaged by partial or total desiccation. For this reason a second unit of similar design with larger discs (16 in. diameter) was constructed. (Plate 3). Rotation at 3 rpm was obtained by using a 6V geared windscreen wiper motor powered by a modified 230V operated battery charger. A relay system was incorporated so that a 6V car battery was switched into the motor circuit during power failures. Damage to the algal film due to power failure was thus avoided.

Ammonia-N
(mg/l)
- 182 -

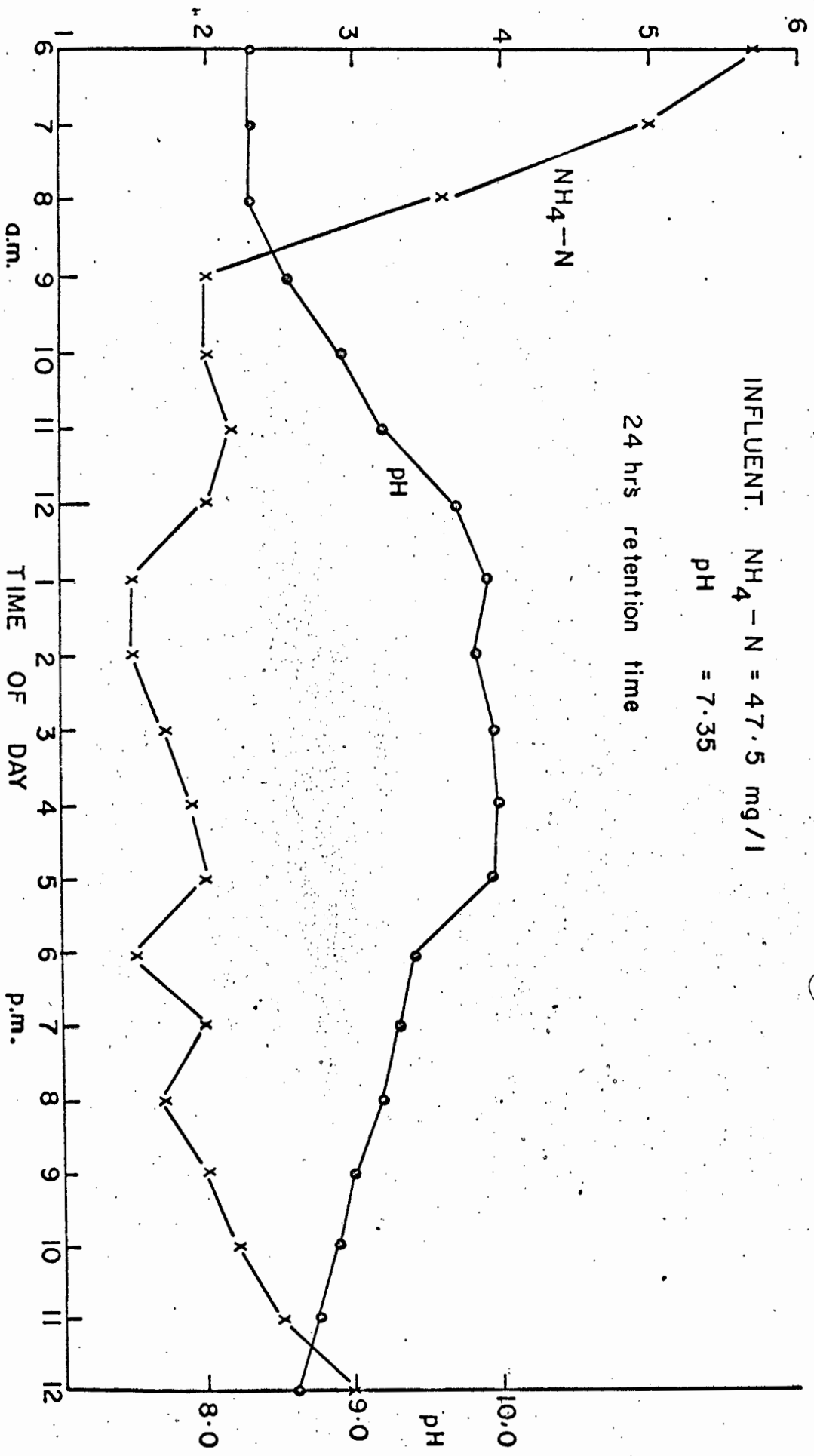


FIGURE 22.

Removal of ammonia nitrogen by algal disc apparatus

A small conical settlement tank with a capacity of 1.2 l was connected between the outlet of the disc apparatus and the effluent collection vessel to retain settleable material.

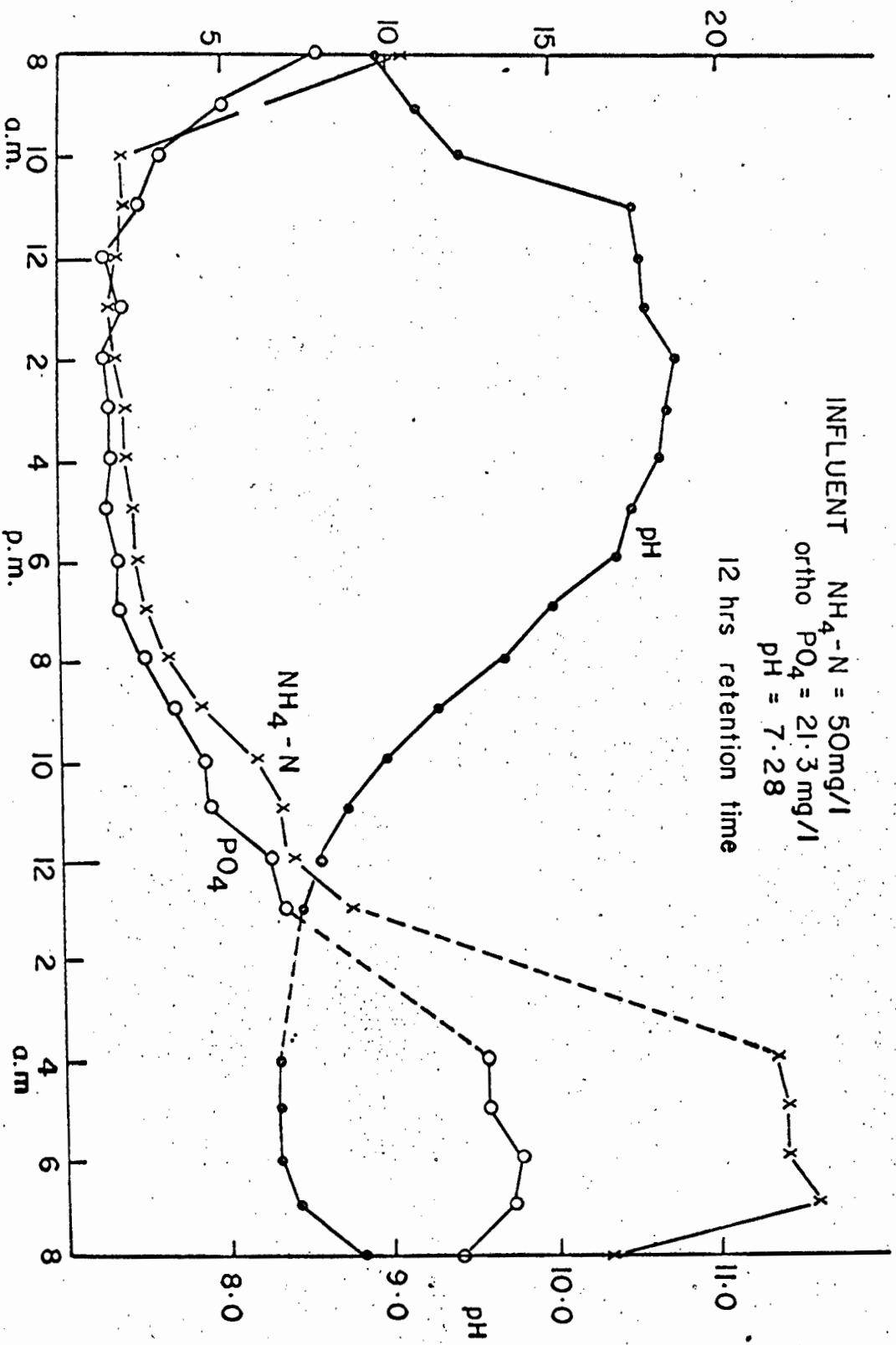
The apparatus was inoculated with the algal material removed from the original unit and after about 15 days operation a similar community had developed on the discs.

20.2.5 Results

To simulate an un-nitrified sewage effluent with a high ammonia nitrogen content sufficient ammonium chloride was added to the effluent supply to raise the ammonia nitrogen to approximately 50 mg N/l. The supply was then adjusted to give a retention time of 24 hours and, after 4 days under these conditions to allow the system to stabilize, hourly analysis on the effluent from the unit was carried out.

The results showed that almost complete removal of ammonia nitrogen occurred when the pH value was above 8.5. Although the pH began to decrease after sunset, the decrease in ammonia loss was not greatly affected until the early hours of the morning, as can be seen from Fig. 22. The alkalinity fell from an input value of 142.0 mg/l as CaCO_3 to a minimum of 35.5 mg/l, rising again at night to a maximum of 98.5 mg/l in the early morning.

ammonia-N
and
ortho - PO₄
(mg/l)



INFLUENT NH₄-N = 50mg/l
ortho PO₄ = 21.3 mg/l
pH = 7.28
12 hrs retention time

FIGURE 23.

Removal of ammonia nitrogen and ortho phosphate by algal disc apparatus

The removal of orthophosphate by precipitation was less efficient. The maximum reduction during daylight hours was from an input level of 22.0 mg $\text{PO}_4/1$ to 7.5 mg $\text{PO}_4/1$, but filtration through fine filter paper reduced the minimum value to 3.4 mg $\text{PO}_4/1$, indicating that settlement of precipitated phosphate was incomplete.

From samples of algae removed from the discs the total algal weight present during the experiment was calculated to be 24 gm dry weight, or approximately 10 gm dry weight per square meter of disc surface.

After time had been allowed for further algal growth the measurements were repeated with the hydraulic retention time reduced to 12 hours. The results showed a similar rapid loss of ammonia during the day but the rate of loss decreased after sunset as algal respiration caused the pH value to fall as shown in Fig. 23. Phosphate removal by precipitation and settlement was much greater during daylight hours than in the previous experiment and analysis of the settled material indicated that this was due to the precipitation of magnesium in solution as a hydroxide floc at pH values of 10.1 or above. This floc appeared to adsorb and remove from suspension the fine precipitate of phosphate that was present. Since pH values of 10.1 were not reached in the previous experiment this may

account for the less efficient phosphate removal observed. Both the experiments described were carried out on dates near to the September equinox on days with intermittent cloud cover during which the incident light intensity varied between 3500 and 10,100 f.c. Under these conditions it appears that an algal film containing between 24 and 39 gm algal dry weight, as in the second experiment, was required to raise the pH value above 10.0.

From Fig. 23 it can be seen that the influent concentration of 50.0 mg N/l was reduced to less than 5 mg N/l between the hours of 9 a.m. and 9 p.m. and that orthophosphate was reduced to a similar level. The relation between pH and nutrient removal is clearly evident.

During the night hours the removal rate decreased to about 50 per cent for both ammonia nitrogen and orthophosphate, evidently due to the greater production of carbon dioxide by the thicker film of algae and to the more rapid throughput.

21. FACTORS EFFECTING LOSS OF AMMONIA FROM SOLUTION

It was observed that the rate of loss of ammonia from buffer solutions in the disc apparatus without algal growth was not as rapid as obtained when sewage effluent was used in conjunction with an algal film. A reason for this difference was sought on the basis of gas solubility.

When a liquid containing a volatile solute is brought into contact with a gas phase, molecules of the solute gas are continuously exchanged between the liquid and gas phases and an equilibrium value is eventually reached which is related to the partial pressure of the gas in the gas phase according to Henry's Law, which states;

$$\frac{C}{p} = H \dots\dots\dots 4$$

where C is the concentration of the soluble gas in the liquid in mg/l, H is the solubility coefficient in mg/l atmosphere and p is the equilibrium partial pressure in atmospheres of the soluble gas in the gas phase. Where the solute gas ionizes in solution, as in the case of ammonia, the total concentration will usually be higher than the concentration of the unionized form, and will be related to the pH of the liquid.

According to equation 4 a decrease in the ammonia concentration C will occur if the partial pressure of ammonia in the air is less than 100 per cent of the equilibrium value p. For maximum loss of ammonia across the air/water interface the partial pressure of ammonia in the adjacent air must be low. To achieve this condition a continuous replacement of air at the interface would be required.

The experiments with buffer solutions previously described were carried out in a closed room without air circulation, whereas the higher ammonia removal rates were obtained in experiments in the open air. It was considered that under indoor conditions the partial pressure of ammonia in the air adjacent to the disc surfaces might have risen sufficiently to depress the rate of loss from solution. If this was the case, then adequate

$\text{NH}_3\text{-N}$
(mg/l)

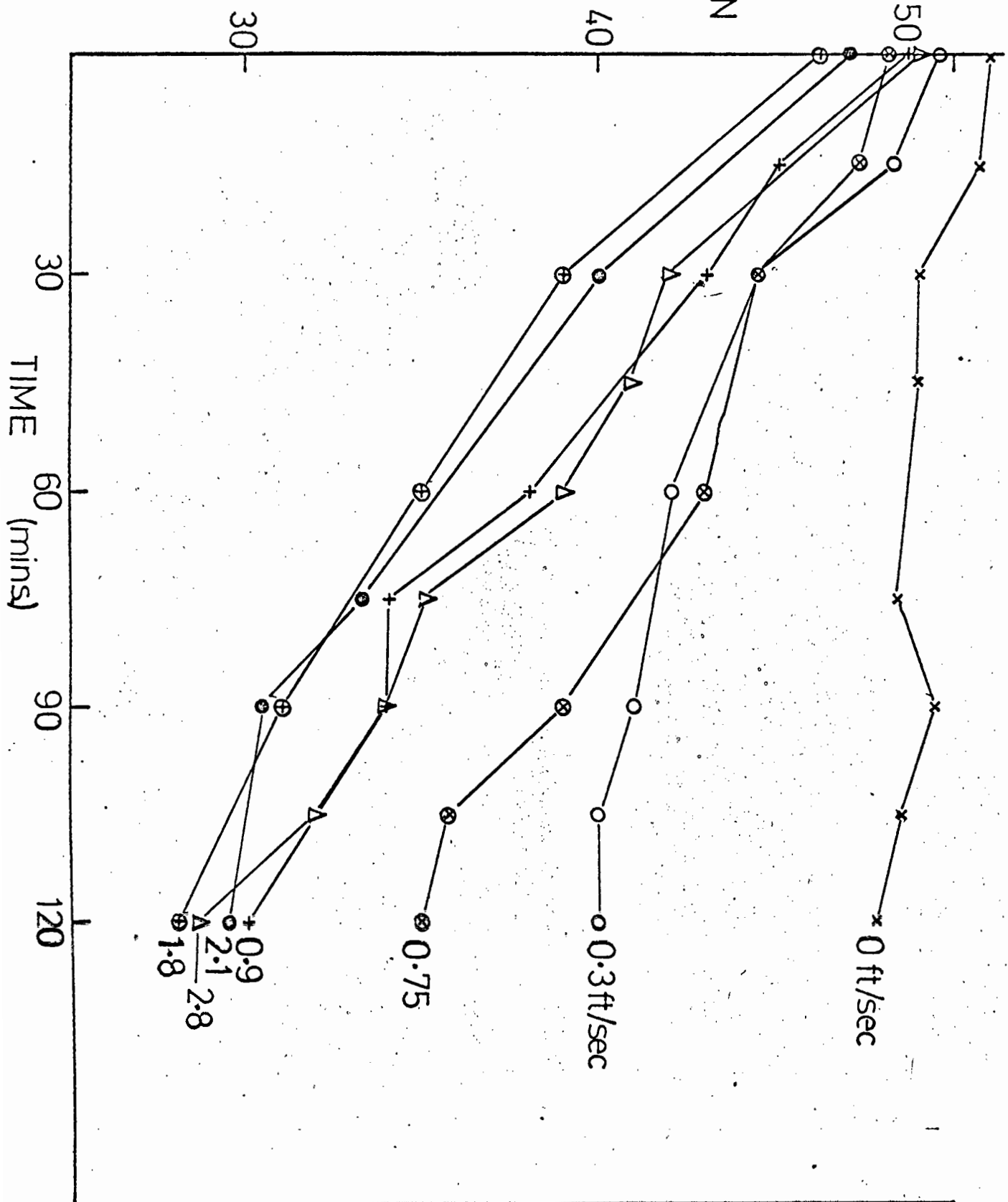


FIG. 24. Effect of windspeed on ammonia loss

A range of wind speeds from zero to 2.35 ft/sec. were employed, but velocities of less than 0.5 ft/sec. could not be measured accurately as the anemometer rotation became irregular.

Measurements at zero ft/sec. were made with the unit surrounded with a 2 ft. vertical wall of plastic film open at the top. The lowest wind speed applied was estimated to be about 0.3 ft/sec. by measuring the movement of soap bubbles. At higher speeds the anemometer readings were used in conjunction with the calibration curve provided.

21.1.1 Results and conclusions

The results obtained from experiments at 6 different wind speeds are shown in Fig. 24. It can be seen that there is some irregularity in the values obtained for ammonia nitrogen in any one experiment. The reason for this was shown to be due to incomplete mixing of the tank contents, due to the relatively slow rotation of the mixing propeller necessary to prevent undue turbulence, which could effect ammonia loss at low wind speeds. When the rate was increased to produce turbulent mixing there was much less variation between samples taken at different positions in the tank.

Despite this variation it is evident from Fig. 24 that the loss of ammonia nitrogen was influenced by the velocity of air movement across the disc surfaces. As the velocity increased from

zero to 0.9 ft/sec. the rate of loss increased from 0.5 to approximately 3.1 mg $\text{NH}_3\text{-N}$ /min. In contrast, the increase in rate of loss as the air speed increased by a factor of 3 from 0.9 to 2.85 ft/sec. was very much less, rising from 3.1 to 3.4 mg $\text{NH}_3\text{-N}$ /min.

The results indicate that, under the experimental conditions, the partial pressure of ammonia in the air passing the disc surfaces at velocities up to about 1.0 ft/sec. was sufficient to decrease the rate of loss from solution. Above this speed the rate of loss was much less influenced, due to the fact that the volume of air passing the surface was sufficient to prevent partial saturation with ammonia.

These observations suggest that the explanation put forward to account for the greater rate of ammonia loss from the disc apparatus, when operated outside the laboratory, is correct. If the critical wind speed of about 1 ft/sec. also applied to a larger scale disc apparatus, the possibility would arise of using the natural movement of the open air to remove ammonia nitrogen from solution on a large scale. In this way it might be possible to overcome the problem of high blower operating costs for air-stripping of effluent ammonia in towers (Bayley, 1967).

22. DISCUSSION OF RESULTS

The results described in this section show that a modification of the algal stream in the form of a system of rotating discs can materially improve the degree to which plant macronutrients can be removed from a sewage effluent. The system offers a means of exploiting the effects of pH increase due to the metabolic activity of a thin film of algae containing relatively little inactive material, and it avoids the problems of light utilization encountered in the use of dense cultures. The results indicate that the removal rates obtained are considerably greater than could be expected by algal assimilation alone.

The rate of ammonia loss from the air/water interface on the disc surface is influenced by the velocity of the air passing across the surface at low air speeds. For the size of apparatus used the critical wind speed required for maximum ammonia loss was found to be in the region of 1 ft/sec; a value usually exceeded under open air conditions. With larger disc diameters it is probable that higher velocities would be required for maximum ammonia loss. These velocities might still be within the range of normal wind movements, raising the possibility of large scale disc systems for removal of effluent ammonia at low cost. Since nitrogen removal by this method would depend on the presence of ammonia nitrogen, it would be advantageous to operate the sewage treatment plant so as to produce a minimum of oxidized nitrogen in the effluent.

Prevention of the accumulation of excessive quantities of algal film, with possible accumulation of precipitated phosphate, could be achieved by application of a simple scraper-collector arm to the sides of the rotating discs. Regular removal of the sediment in the settlement tank would also prevent re-resolution of phosphate.

Possibly the greatest difficulty to be overcome before such a system could be put to practical use would be the effect of algal respiration during the hours of darkness.

The decrease in nitrogen and phosphate removal during the night, such as that observed in the experiment with a 12 hour retention time, might be overcome by provision of fluorescent strip lights above or between the discs. The light supplied would allow photosynthesis to continue to a reduced extent during the night and lessen the pH decrease. Alternative methods for reducing algal respiration might also be devised. Direct pH control by the addition of alkali might be possible, particularly since it was observed that the reduction in alkalinity during daylight reduced the amount of lime required to reach a pH of 10.3 by as much as 75 per cent, when compared to that necessary to achieve the same increase in the untreated influent.

Section E

THE EFFECT OF CURRENT SPEED ON ALGAL PHOSPHATE UPTAKE

23. RESEARCH OBJECTIVE

The purpose of the algal disc system described in the previous section was to provide conditions for maximum metabolic activity in the algal film. One of the major variables in this system was the effective current speed over the algal film caused by rotation of the discs.

It has been reported by Whitford and Schumacher (1964_{a, b}) and Schumacher and Whitford (1965), in studies on several freshwater filamentous algal species, that an increase in both respiration and phosphate uptake occurs with increase in current speed. This effect was found in both lotic and lenitic species, and was considered to provide evidence of an increase in metabolic rate. Since the disc apparatus was capable of producing effective current speeds over a wide range, without change in hydraulic retention time, it was considered necessary to determine whether an increase in the metabolic rate of the algal film on the discs would result from an increase in rotation rate. By this means it might be possible to increase the desired changes in the effluent passing through the system and to obtain greater removal of macronutrients.

In the work reported by Schumacher and Whitford (1965), tufts of the filamentous algae were suspended on thin wires in magnetically stirred vessels containing radioactive phosphate solution. The current

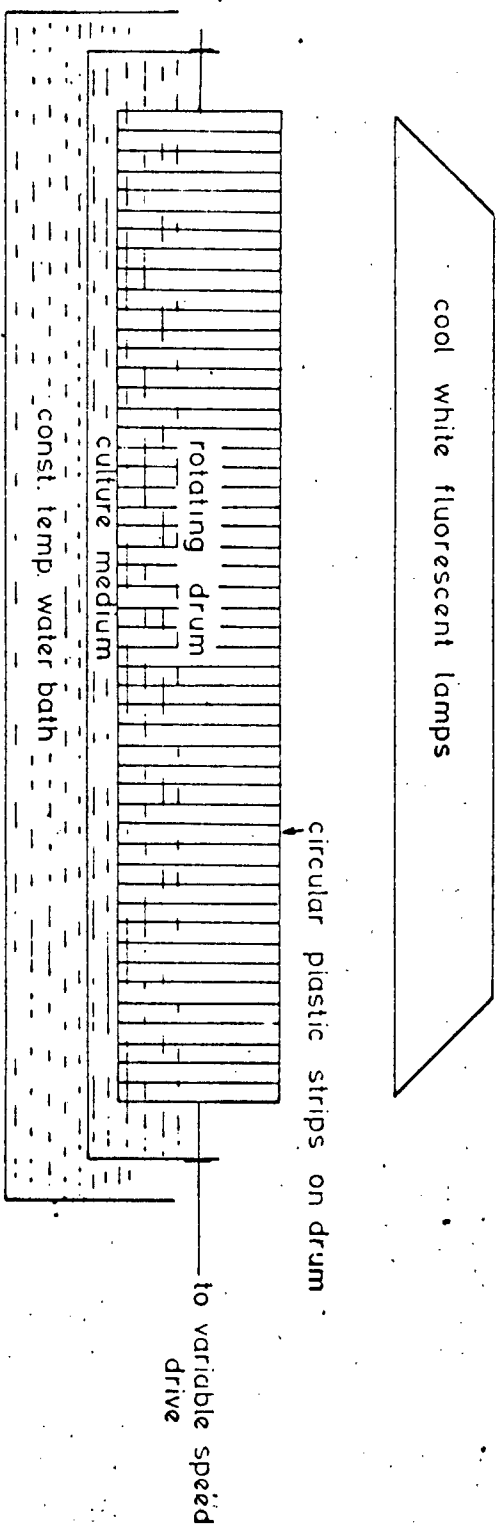


FIG 25 ROTATING DRUM ALGAL CULTURE UNIT.

speed was estimated with pitot tubes or with pieces of floating cork. This experimental method was not suitable for investigations on the disc system, and an apparatus was designed with which algal communities grown on a solid substratum could be moved through static water at known speeds. This apparatus consisted of two units, the first being used for culture of algae on a solid substratum and the second for immersion of the algae in radiophosphorus solutions for test purposes.

24. EXPERIMENTAL METHODS

24.1 Culture Unit

The unit consisted of a hollow cylinder (10" dia x 60") mounted on a shaft rotating at constant speed. (Fig. 25). Approximately half of the rotating cylinder circumference was immersed in algal culture medium contained in a tank surrounded by a temperature controlled water jacket. Onto this cylinder were clipped 1" wide circular plastic strips cut from the same material as the cylinder and divided transversely at one point. Four 80W "cool white" fluorescent tubes were mounted approximately 12" above the upper surface of the cylinder giving a maximum intensity of illumination on its surface of 400 f.c. A metal frame over the tank supported a thin sheet of transparent polythene film which served to exclude dust and insects attracted by the lights.

It was not possible to cover the apparatus in such a way as to prevent possible contamination of the culture in the tank and it was at first intended that mixed cultures

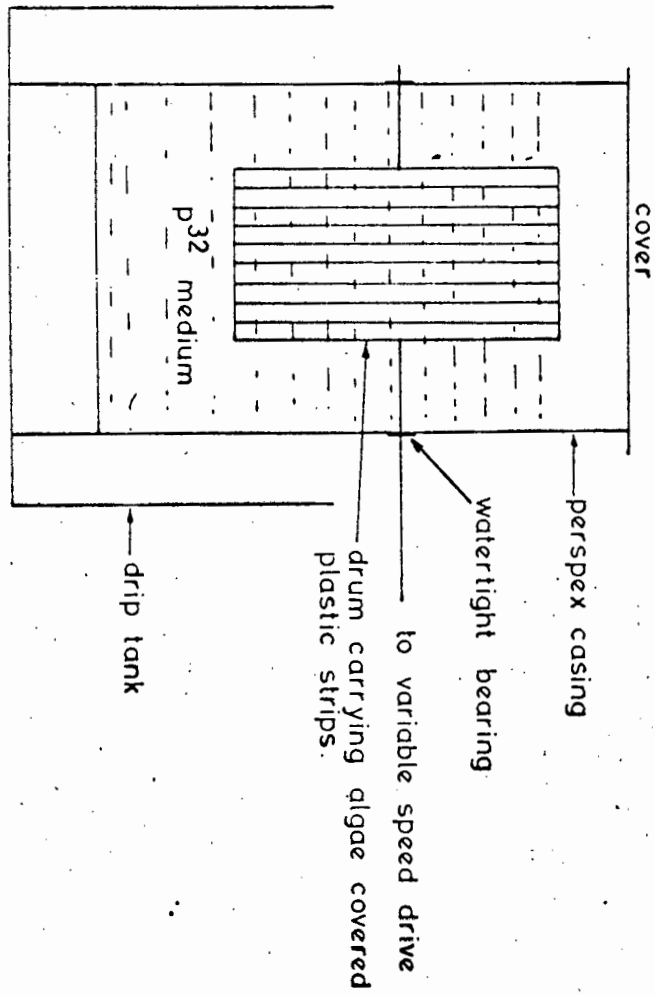


FIG. 26 ROTATING DRUM EXPERIMENTAL UNIT.

should be studied. It was subsequently found, however, that massive initial inoculation would allow a unialgal culture of Scenedesmus bijugatus to be maintained for several months at a time, during which several batches of algae could be grown on the plastic circular strips rotating in the medium.

24.2 Experimental Unit

This unit consisted of a Perspex tank with vertical sides and a curved bottom in which was fitted a 10" diameter water-tight Perspex drum mounted on a stainless steel shaft passing through a water-tight gland in the side of the tank. (Fig. 26). The shaft was driven via a sprocket and chain drive from an electric motor with a variable speed gear. A Kopp Variator gear was also fitted to provide fine speed adjustment.

The Perspex tank had a working capacity of 26 l and was temperature controlled by an aquarium thermostat and heater. A florescent lamp above the unit gave an intensity of 100 f.c. on the upper surface of the drum.

In operation the tank was filled with the required solution so that the drum was immersed to within 0.75 inches of its upper edge. In this way the tendency for the rotating drum to impart a rotary motion to the solution was greatly reduced, and observations with small floating particles indicated that, apart from turbulence in the region where the exposed area of the drum entered and left the water, the rotary effect was minimal.

From a knowledge of the rate of rotation and the circumference of the test strips on the drum the velocity of movement of the algal film through the water could be accurately calculated and expressed as a current equivalent.

A number of unialgal cultures of Scenedesmus bijugatus, obtained by isolation and subculture from material obtained from a sewage maturation pond, were grown in modified Algeus medium with urea substituted for glycine.

By sub-culturing in 5 litre flasks at room temperature in diffused daylight, a volume of 40 litres was obtained to provide a massive inoculation for the culture unit. After addition, the culture tank was filled to the working volume by addition of further Algeus medium made up in sterilized tap water.

The culture unit was maintained at 25°C and continuously illuminated while rotated at 7 rpm. Approximately one third of the culture medium was run to waste at 14 day intervals and replaced with an equal volume of fresh medium made up in distilled water. Under these conditions a film of S. bijugatus approaching 0.5 mm in thickness developed on the plastic strips within 15-20 days.

24.3 Sampling Methods

A number of preliminary experiments were carried out to develop a suitable method for sampling the P³²-containing algal film on the test strips. Initial tests showed that scraping

with a spatula, followed by passage through a fine stainless steel mesh in a syringe to obtain homogeneous suspension, caused cell rupture and release of activity into solution with consequent unsatisfactory agreement between replicates.

After further tests the procedure adopted was as follows:

After immersion of the test strip in the radiophosphorus solution in the experimental unit the strip was removed, the adhering liquid allowed to drain back into the tank, and the remaining droplets removed with filter paper.

The strip was then marked off into segments, usually 4 or 8, and the algal film adhering to the surface of the strip was gently removed with a soft squirrel-hair brush and transferred to 5 ml of distilled water in a conical 15 ml centrifuge tube. After most of the algae had been removed from the section of the strip and transferred to the tube, the brush was washed in distilled water and the procedure repeated with the remaining sections of the strip, using a separate tube for the algae from each area.

The tubes were then centrifuged for a short time (1-2 minutes) to pack the algal cells at the bottom of the tube and the clear supernatant decanted. The cells were then resuspended in 1 ml of distilled water and transferred to a weighted planchet. The tube

was then rinsed into the planchet with a further 0.5 ml of water and the planchet dried under an infrared lamp and transferred to a drying-oven at 105°C for 2 hours, after which it was cooled in a dessicator and the dry weight of algal material determined.

Tests on the supernatant during this procedure confirmed that under these conditions insignificant amounts of activity were lost from the cells into the distilled water.

After determination of dry weights the samples were fixed with acetone and mounting fluid, and the activity counted in a Phillips Automatic Counter using a conventional shielded G.M. tube working in anticoincidence with a G.M. guard tube.

Initial runs were made in phosphate-free Algeus medium with sufficient $P^{32}\text{-PO}_4$ to give approximately 140 cpm/ml. Under these conditions algal activities approaching 2×10^4 cpm/mg dry weight were obtained at higher current speeds. Due to the "dead time" of the counter these levels were too high for direct counting. By adding inactive phosphate to give a concentration of 0.002 m/l $\text{PO}_4\text{-P}$ the activities were reduced to acceptable levels and were subsequently further reduced by decreasing the exposure time in the active solution.

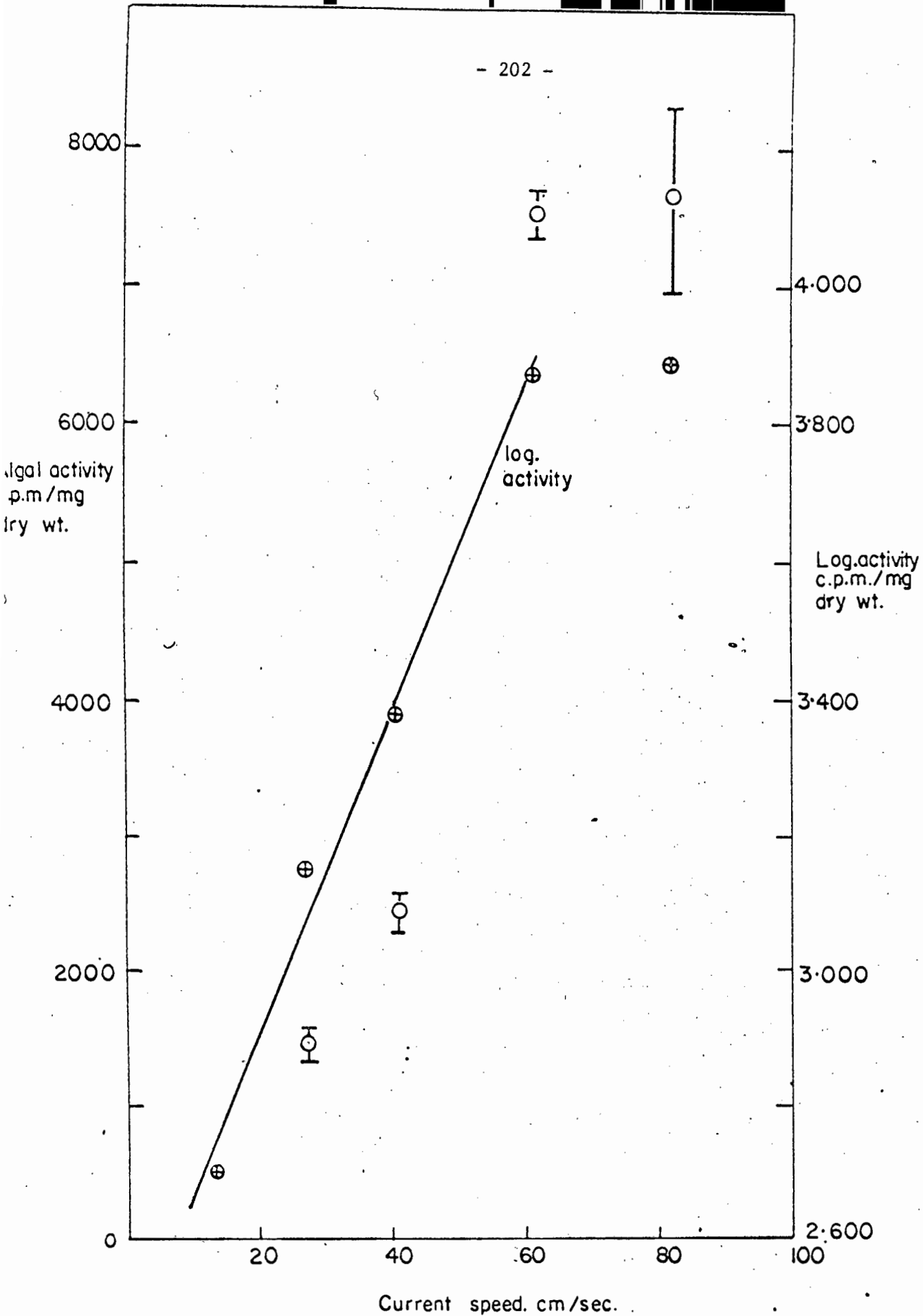


FIGURE 27

Expt. I. Total concentration $PO_4 - P = 0.002$ mg/l.
Activity of medium = 140 c.p.m./ml.
The 90% confidence limits for each value are indicated. ⊕ = log. values

24.4 Experiment 1

Algae-covered strips from the culture units were immersed overnight in aerated PO_4 -free Algeus medium to remove excess phosphate. The strips were then placed in the experimental unit containing Algeus medium with 0.002 mg/l PO_4 -P and radioactive phosphate added to give a solution activity of approximately 140 cpm/ml. Individual strips were then rotated in the solution for one hour. Five strips were used, each being rotated at one of 5 different speeds (10, 20, 30, 44, 60 rpm). At the end of each period, eight algal samples were removed from each strip and the activity in cpm/mg dry weight determined by the procedure previously described.

24.4.1 Results

Seven algal samples were obtained from the strip rotated at 27.3 cm/sec. and 8 samples in the other 4 experiments. The uptake of P^{32} phosphate as a function of current speed is shown in Fig. 27, in which the 90% confidence limits are shown. It can be seen from the figure that the rate of uptake appeared to be directly related to current speed up to 40 cm/sec. but beyond this speed there was an increase in the rate of uptake with no marked difference between 61.4 and 81.9 cm/sec. A plot of the log of the activity against current speed showed an approximately linear relation up to 60 cm/sec.

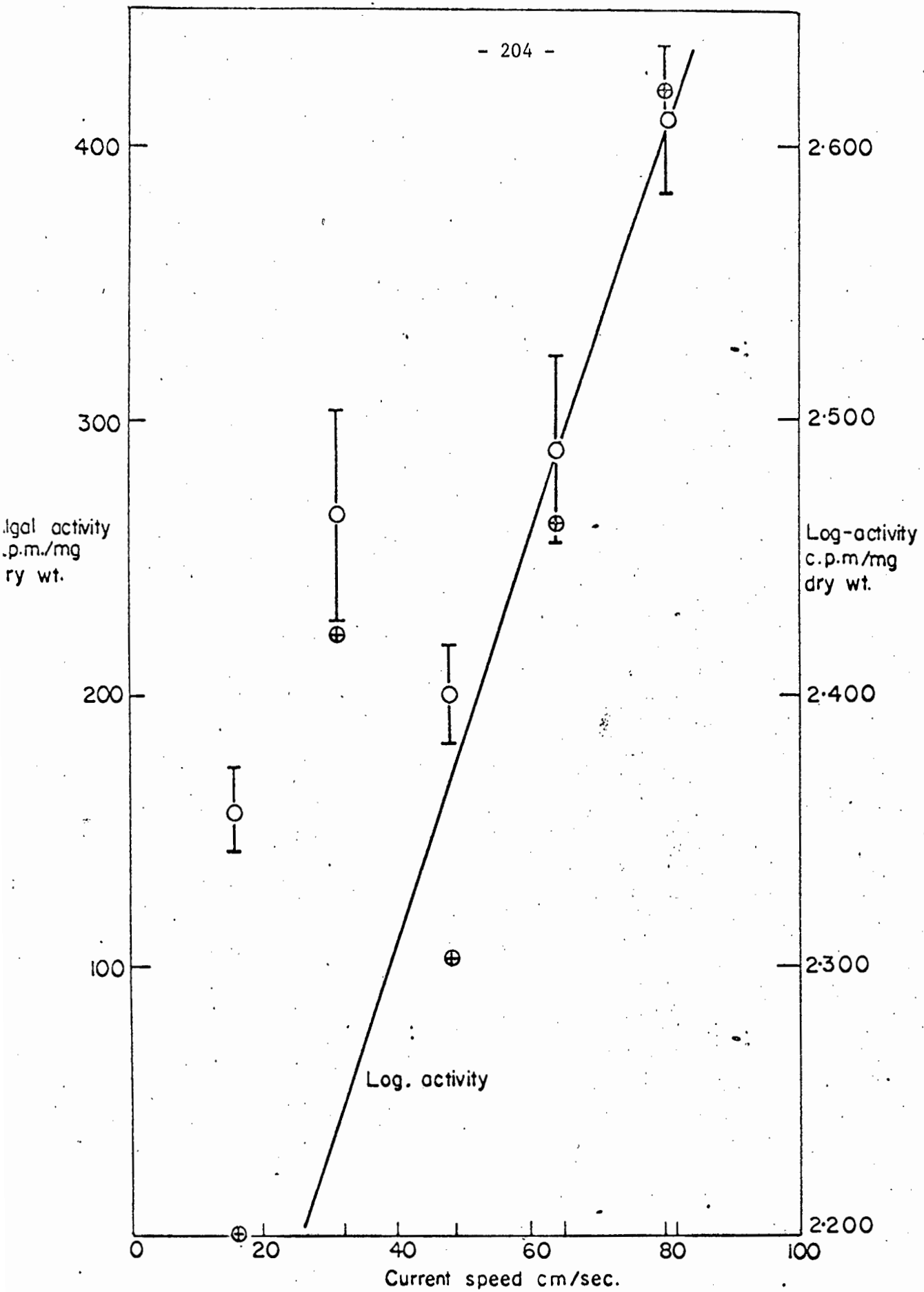


FIGURE 28.

Expt. 2 Total concⁿ PO₄-P = 0.002 mg/l
 Activity of medium = 140 cpm/ml.
 The 95% confidence limits for each

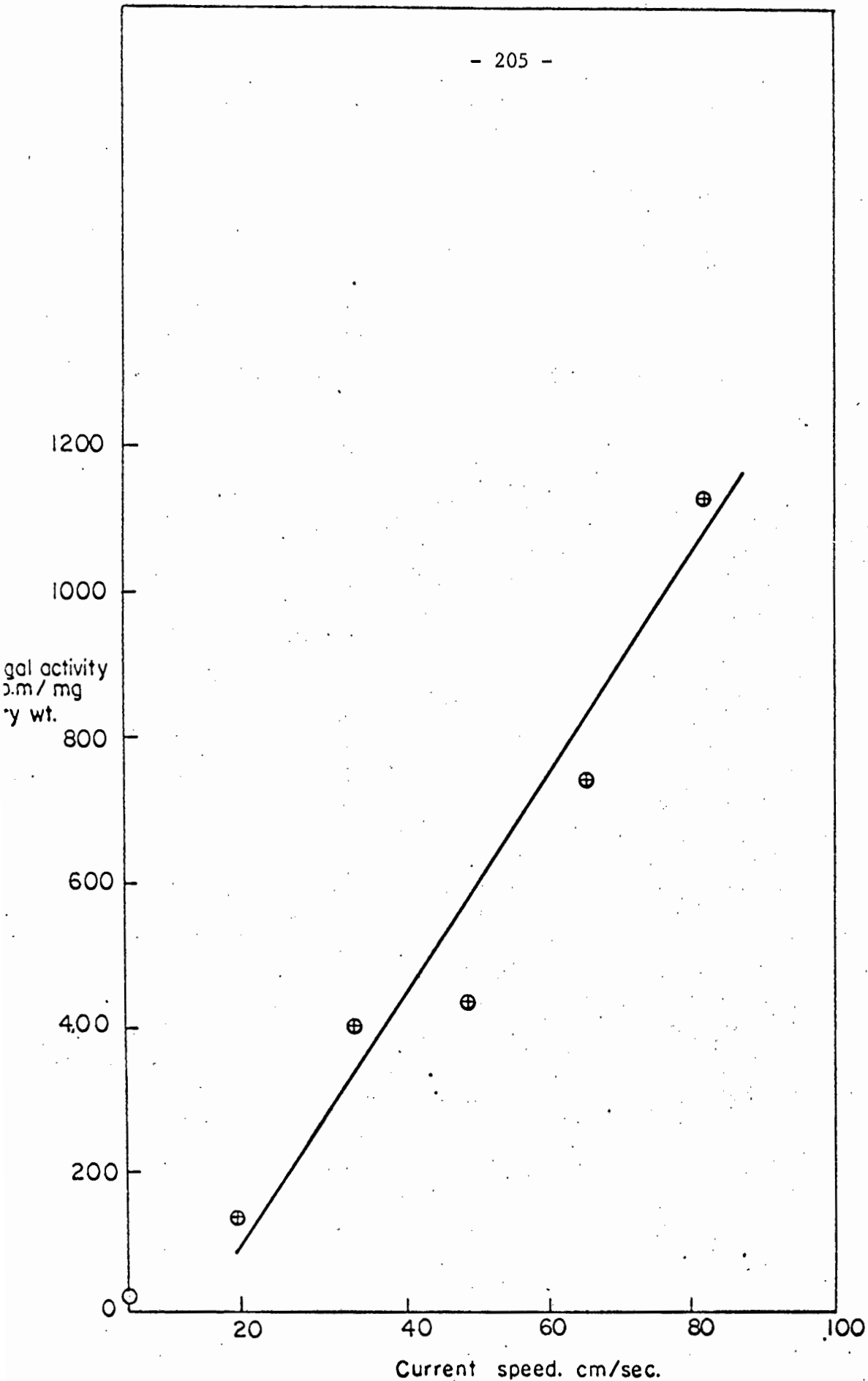


FIGURE 29

Expt. 3. Total concⁿ PO₄-P = 0.002 mg/l.
Activity of medium = 98.5 c.p.m./ml.

24.5 Experiment 2

24.5.1 Results

The experiment was then repeated under the same conditions at intermediate current speeds with 20 instead of 60 minutes exposure. The results obtained are shown in Fig. 28. In this case the results differed from Experiment 1 in that a plot of the logarithm of the activity gave a straight line for the three high current speeds instead of the lower speeds. There was also, as before, considerable variation in activity among samples taken at any one current speed.

24.6 Experiment 3

This experiment was a repeat of Experiment 2 under the same conditions with an extra run without rotation. Again there was considerable variation among samples taken from the same strip, but a plot of the mean values suggested that the relation between current speed and phosphate uptake might be linear (Fig. 29).

24.7 Conclusions

It was concluded from these experiments that although a relation between current speed and phosphate uptake was evident, the experimental method was not sufficiently accurate to determine the relation with confidence. The reason for this inaccuracy was then sought.

From inspection of the data obtained in Experiments 2 and 3, it appeared that an inverse relation between the specific activity of a sample and its dry weight existed. Theoretically the specific activity should be independent of the sample weight.

Calculation of the mean sample weight at each speed showed that the strips tested at the two lowest speeds had an appreciably thinner algal film on their surfaces and also had a higher specific activity per dry weight. Examination of individual results for each speed in Experiment 2 showed that, almost without exception, in cases where the sample weight removed was small, i.e., low algal density/unit area, the activity/dry weight was much greater.

These observations led to the conclusion that the uptake of phosphate by the algae-covered surface was strongly influenced by the thickness of the algal film, when measured in terms of phosphate uptake/weight of algae. This was unexpected since the algae films used were less than 0.5 mm in thickness. The results suggested that a low phosphate concentration gradient developed around algal cells shielded by other cells from contact with the culture medium, and that this gradient decreased the rate at which phosphate ions could be taken up by the underlying cells.

24.8 Experiment 4

To test this supposition several clean plastic strips were placed in the culture unit until a distinct green algal

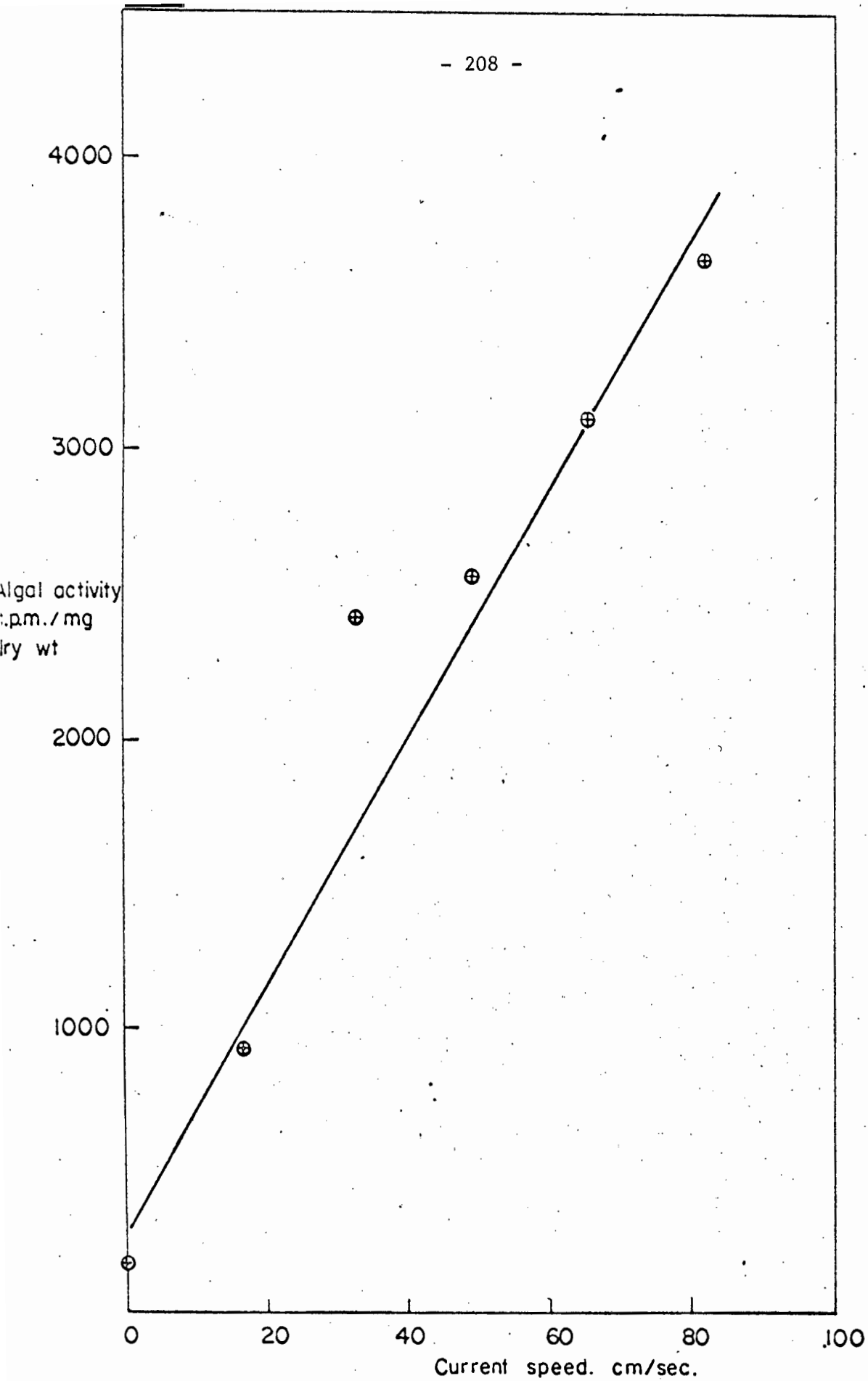


FIG. 30.

Expt. 4. Total concⁿ PO₄-P = 0.002 mg/l
Activity of medium = 70 c.p.m./ml.

film on the plastic surface no more than a few cells thick was produced. The strips were then treated in the same way as in Experiments 2 and 3 except that the activity of the medium was reduced to 70 cpm/ml and, also, due to the thin film, fewer samples were taken from each strip in order to allow accurate weighing of the material removed.

24.8.1 Results

The results are shown in Fig. 30 and it is evident that, for a very thin film of algal cells, the rate of uptake of phosphate is linearly related to the equivalent current speed up to at least 81.9 cm/sec.

It can also be inferred that although the film was very thin, the rate of phosphate uptake by the underlying cells was decreased to some extent since, in the samples used for tests at 32.7 cm/sec and 49.1 cm/sec., the algal sample weights indicate that the algal thickness was somewhat less and, as a result, the specific activity per dry weight of algae was noticeably greater. This is indicated in Fig. 30 by the two points lying somewhat above the straight line shown.

A further point of interest is the fact that although the activity of the culture medium was about half that in Experiment 2, the specific activity per dry weight of the algae after immersion was approximately 10 times greater than in Experiment 2, evidently as a result of the reduced film thickness.

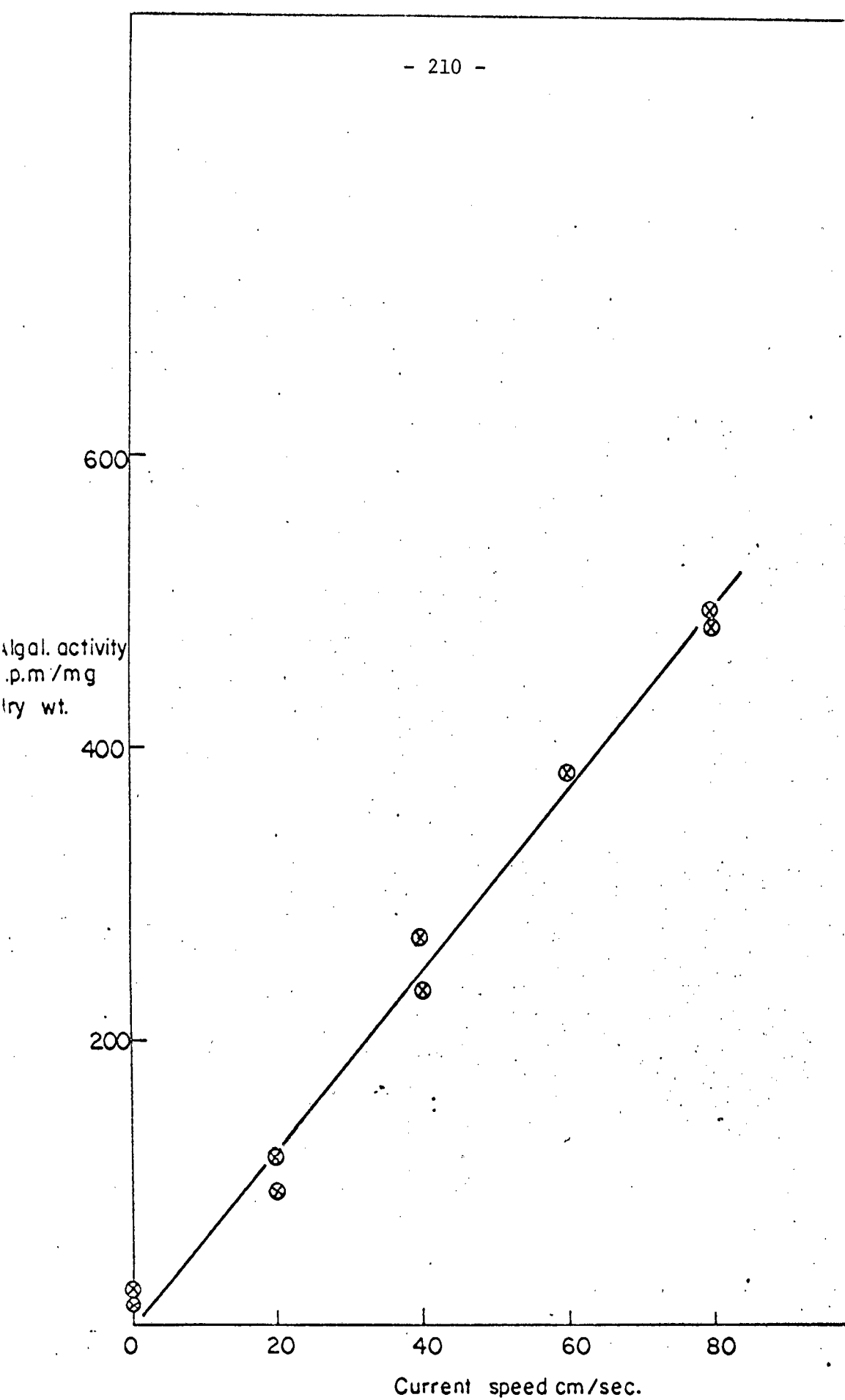


FIGURE 31.

*Expt. 5 Total concⁿ PO₄ - P = 0.002 mg/l.
Activity of medium = 47 c.p.m./ml.*

24.9 Experiment 5

From the results obtained it was obviously necessary to ensure uniform algal growth on the strips if the linearity of the relation between phosphate uptake and current speed was to be confirmed. For this purpose a thin film of algae was grown on 15 plastic strips. From these, 9 were selected as having uniform film thickness as judged by their appearance and depth of green coloration. These strips were prepared and sampled as in the previous experiment. The activity of the medium was lower than in Experiment 4, being 47 cpm/ml instead of 70 cpm/ml, and the immersion time 15 instead of 20 minutes.

24.9.1 Results

From Fig. 31 it is clearly evident that a linear relation existed between phosphate uptake by a thin film of Scenedesmus bijugatus and the equivalent current speed obtained by movement of the film. The quantitative uptake of phosphate was approximately doubled by a doubling in current speed under the conditions described, giving an increase in uptake of 6.2 per cent per cm/sec. increase in current speed.

25. THE EFFECT OF POISONS ON THE UPTAKE OF PHOSPHATE

The results of Experiments 4 and 5 demonstrated the relation between phosphate uptake and the current speed. That this increase in uptake was due to increased metabolic activity of the algal cells could not be

assumed unless it was shown that the observed increase was due to phosphate absorption, and not merely to adsorption on the cell surfaces.

From tests using cotton fibres and dead algal filaments, it was concluded by Whitford and Schumacher (1964^{a,b}) that adsorption of phosphate was not important after the first few minutes of exposure. It seemed possible that results obtained with these materials might not be entirely reliable. For this reason a series of experiments were carried out to measure the phosphate uptake of an algal film before and after exposure to conditions that would be expected to prevent further cellular absorption.

25.1 Method

The same apparatus and procedure was used as in the previous experiments to measure the phosphate uptake, with the difference that either ultra violet light or a chemical inhibitor was applied to the algal film after samples had been removed for activity counts during the first 2 hours. The chemical poisons used were potassium cyanide and mercuric chloride. The ultra violet irradiation of the film was achieved by placing two Phillips TUV6W ultra violet lamps approximately 1 cm above the portion of film exposed above the water surface. The intensity rating for the lamps at 1 meter distance was given as 0.85 micro watts/cm² at 2537A.

25.2 Results

The experimental conditions and the results obtained are shown in Figs. 32 and 33.

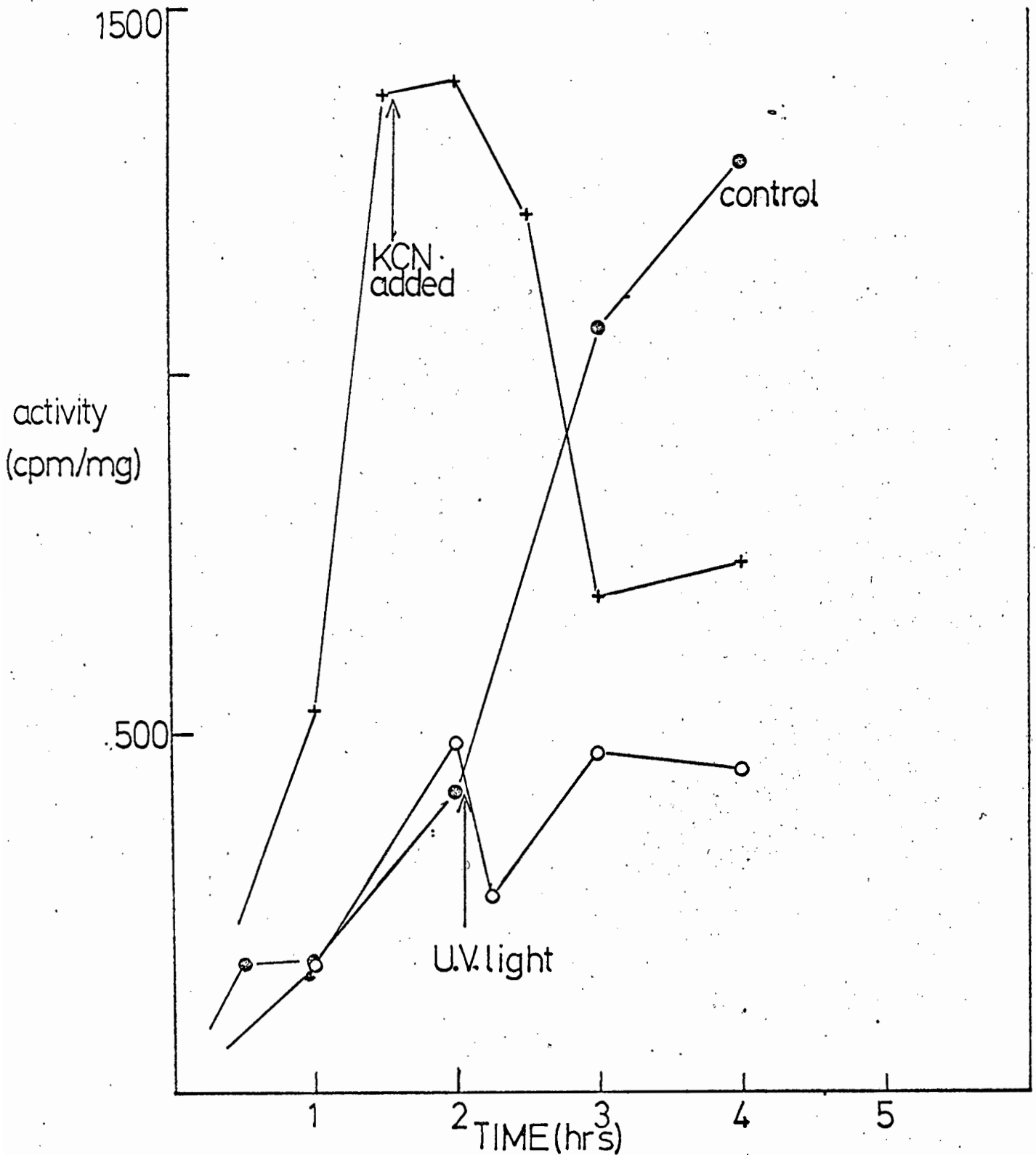


FIG. 32. Effect of poisons on PO_4 uptake

In Fig. 32 it can be seen that the phosphate uptake was again approximately linear with exposure time in the control experiment. When the same solution activity (249 cpm/ml) was used with a new algal film, the activity of the film was close to that of the control for the first 120 minutes. The sample taken 10 minutes after a 5 minute exposure to U.V. light showed a marked loss of activity in the film, which subsequently returned to the level attained before irradiation. No further increase in the activity occurred during the subsequent time of observation, compared to a continued increase in activity in the control algal film.

The subsequent experiment was carried out in fresh medium (activity 292 cpm/ml), and KCN solution sufficient to give a concentration of 0.0077 mg KCN/l was rapidly mixed with the medium after an algal sample had been taken 90 minutes from the start. In the subsequent 30 minutes very little phosphate was incorporated into the film. In the following 60 minutes there was a rapid decrease in the phosphate content of the film to a level of about 50 per cent of the value before addition of cyanide. Very similar results were obtained when mercuric chloride was added to the medium (Fig. 33). In the first of two experiments the concentration of mercuric chloride in the medium after the addition of 2.5 ml of a 35 g/l solution was 28 mg HgCl_2 /l. In the second case the concentration was doubled. In both cases the steady increase in phosphate content

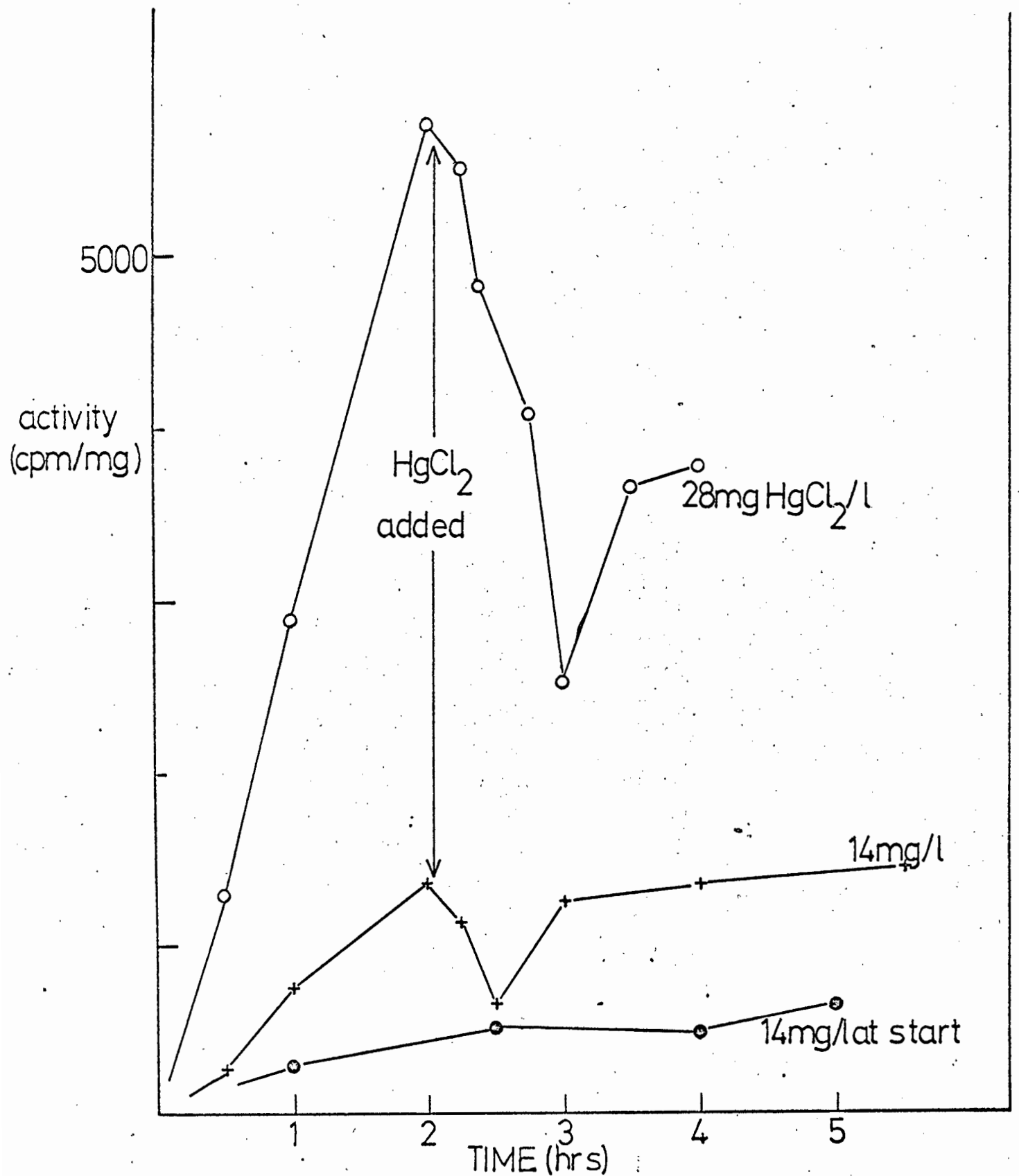


FIG. 33. Effect of poisons on PO₄ uptake

ceased upon addition of the reagent. This was followed by a period of 30-45 minutes during which phosphate was lost from the algal film. A subsequent increase in phosphate to a level somewhat below that observed before addition of mercuric chloride was observed in both cases.

At the conclusion of the experiment at the lower concentration, a fresh algal film was rotated in the same solution to compare the phosphate uptake when the poison was present from the start. From Fig. 33 it can be seen that the incorporation of phosphate was much less rapid and did not reach the same level in 5 hours as had been reached in 2 hours by the previous film.

25.3 Conclusions

The results obtained show quite clearly that exposure to ultra violet light or to solutions of cyanide or mercury had the effect of preventing further accumulation of radioactive phosphate by the exposed algal film. The gain in phosphate before exposure was more pronounced in the experiments with cyanide and at the higher mercury concentration due to the thinner algal film present. In these cases the subsequent loss of phosphate from the film was more marked, possibly due to more complete exposure of individual cells to the poison with a consequent increase in the toxic effects.

When 14 mg HgCl_2 /l was present from the start, there was an initial increase in the phosphate content of the film, suggesting that, although the cells were not active, some

phosphate was adsorbed. The amount adsorbed remained relatively unchanged during a total of 5.5 hours immersion. From this result it was clear that adsorption alone could not account for the steady accumulation of phosphate observed in all cases. It was therefore evident that the phosphate uptake was mainly the result of actual assimilation, and not adsorption, the effect of which was small after the first few minutes of exposure.

Although obtained by different experimental methods, these results support those of Whitford and Schumacher (1964a, b), who also concluded that adsorption was of little importance after the first few minutes. The same authors demonstrated that the increase in phosphate uptake was due to increased metabolic rate as a result of a water current.

26. DISCUSSION OF RESULTS

The results obtained indicate that current speed has a marked effect on the uptake of phosphate, and that the relation between rate of uptake and current speed is linear up to an equivalent current speed of at least 82 cm/sec. This change in rate is evidently not due to physical adsorption, but to change in the rate of cellular assimilation. The fact that a very thin algal film was required to demonstrate the relation between the two factors indicates that the change in rate was very probably associated with the phosphate concentration gradient that would develop around a cell under static conditions.

The practical significance of the results does not appear to be very great since the effect of current speed was shown not to extend below the surface layer of cells. Although an increase in metabolic rate might cause a significant decrease in the very low phosphate concentrations used in the experiments, it is doubtful if the increased phosphate assimilation would cause a detectable decrease in the relatively high concentrations present in sewage effluent. It is possible that changes in rotation rate might serve some purpose in that increased rotation during the day might increase CO_2 uptake and further raise the pH, while decreased rotation at night would reduce algal respiration and lessen the pH decrease occurring during the hours of darkness.

Section F

NUTRIENT EXTRACTION BY HERBIVOR REMOVAL

27. INTRODUCTION

In the early stages of operation of the algal stream it was observed that large numbers of Ostracods had colonized the algal sediment. At this stage of the investigation the good settlement characteristics of the sediment had not been confirmed, and possible difficulty in removing algal cells from the water was anticipated. It was considered that because of their greater size compared to algal cells, the ostracod population might offer a method of removing nutrients by their separation from the algal sediment, an approach similar to that suggested by Odum (1961).

From a summary of published data on ecological efficiency both MacFadyen (1963) and Slobodkin (1964) have concluded that efficiency at the invertebrate trophic level lies somewhere between 10 and 20 per cent. Harvesting at a higher trophic level than the primary producers would therefore be expected to be a less efficient method of nutrient removal. Nevertheless, because of the difficulties anticipated in removing algal cells, a limited study was carried out to determine the level of nutrient removal that could be expected.

28. EXPERIMENTAL APPROACH

For this assessment the faunal species composition, the macronutrient content of the dominant species, their biomass and the population density distribution along the stream was determined.

28.1 Faunal Sampling

The faunal content of the algal sediment samples described in Section C was obtained by sieving with a water jet. The fauna was almost entirely restricted to ostracods and chironomid larvae, presumably due to the highly specialized environment of the algal stream and the restricted possibility of colonization by non-flying animals during the experimental period. The original ostracod population probably reached the system in the form of eggs contained in the mud used as an algal inoculum.

Each sample was examined with a stereomicroscope while alive, as preservation made it difficult to distinguish between ostracods and granular masses of algae. When necessary, live samples were stored overnight at 2°C and counted the next day.

The number of ostracods in each sample was counted by spreading the material over a transparent dish marked with a graticule after excess water had been poured off to restrict movement of the animals. The whole sample was counted in each case, since use of the subsampling method described by Allanson and Kerrick (1959) gave variable and inaccurate results, evidently due to the use of living instead of preserved material.

28.2 Ostracod Species Composition

In all the samples examined only two species were found. The smaller species, Cypridopsis inaequalva (KLIE 1933, provisional), was by far the most common and, because of the mesh size used, only the adults were retained. Measurements

Station	Distance from inlet (ft.)	Number of Ostracods					
		10th May 1966	20th June 1966	9th Aug. 1966	8th Nov. 1966	14th Dec. 1966	5th Jan. 1967
1	312	0	0	1	1	0	0
2	624	42	7	1	1	-	-
3	936	36	47	0	0	-	0
4	1248	65	225	10	1	1	-
5	1560	169	143	25	50	-	1
6	1872	-	309	109	136	-	-
7	2184	29	324	257	456	4	13
8	2496	106	1424	3121	132	-	-
9	2808	30	138	49	12	-	18
10	3120	-	178	476	72	160	-
11	3432	96	559	1122	104	-	-
12	3744	102	868	476	131	-	-
13	4056	-	1114	1206	1361	145	1309
14	4368	38	987	2269	435	-	-
15	4680	20	125	399	79	-	186
16	4992	-	144	390	252	46	-
17	5304	65	552	172	50	-	1
18	5616	21	401	212	32	-	-
19	5928	-	143	73	90	6	3
20	6240	22	39	4721	2661	-	-
21	6552	-	28	66	37	-	3
22	6864	10	68	29	72	1	-
23	7176	-	37	46	91	-	6
24	7488	10	23	22	29	-	-
25	7800	19	49	15	13	0	1
26	8112	-	26	12	2	-	-
27	8424	7	31	10	7	-	1
28	8736	-	21	10	2	0	-
29	9048	0	3	83	3	-	0
30	9360	-	6	7	1	-	-
31	9672	0	7	7	0	0	0
32	9984	0	10	23	2	-	-
33	10296	0	8	9	0	-	4

Table 14. Distribution of ostracods on six sampling dates.

Numbers per 9 cm. section of sediment.

of length of several hundred individuals from different samples showed little variation, the mean length being 0.725 mm., with a standard deviation of 0.001.

The second species, Heterocypris congenera (VAVRA, 1897), was greater in length (1.54 mm) but few in number, comprising less than 1 per cent of the total numbers. Examination showed that a few juvenile H. congenera of similar length to the C. inaequalva were present in the samples, but their numbers were so small that no attempt was made to separate them in subsequent chemical estimation of the N and P content of the C. inaequalva.

28.3 Ostracod Population Density

Some variation in the number of ostracods in equidistant samples taken along the length of the stream was anticipated, but the variation observed was not distributed over the whole length of the stream as had been expected. It can be seen from Table 14 that the majority of the ostracods was found in the region between 1000 and 6500 ft. from the inlet and that few were present in the first 1000 ft. or in the last 4000 ft. These differences were found on all sampling dates but were more marked on some occasions.

The obvious possibility that the number of ostracods in each sample depended on the amount of algal material was considered, but a graphical plot of organic content of each sample against the number of ostracods showed no relation between the two quantities.

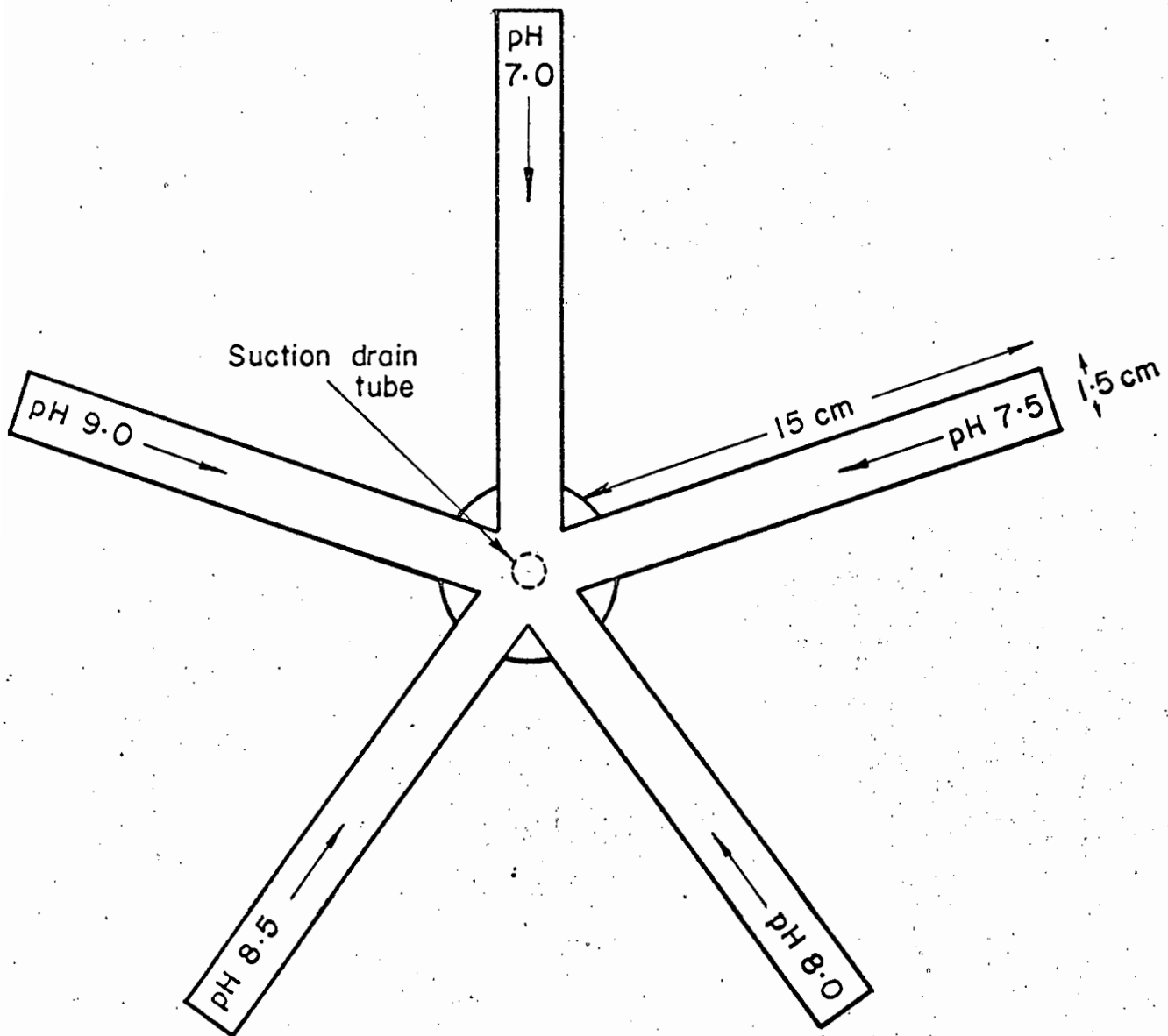


FIGURE 34.

Multi-choice pH preference unit with 5 equal radiating channels

Station Distance (ft.)	Calcium concentration (mg/l)					
	26th July	2nd Aug.	9th Aug.	16th Aug.	23rd Aug.	30th Aug.
A1 0	22.0	36.1	33.1	38.1	38.1	28.1
A2 2600	28.1	38.1	39.1	36.1	38.1	32.1
A3 5342	26.1	46.1	45.1	42.1	36.1	36.1
A4 7712	26.1	44.1	41.1	44.1	44.1	32.1
A5 10000	30.1	42.1	42.1	40.1	48.1	32.1

Table 15. Calcium Concentrations in the Stream Water

29. pH PREFERENCE TESTS

The influence of pH on ostracod distribution was investigated by a series of laboratory preference tests.

29.1 Multi-Choice Apparatus and Method

A test unit with 5 radial arms was constructed from Perspex (Fig. 34). Five 20 l aspirators of aerated tap water were adjusted exactly with H_2SO_4 or NaOH to pH values of 7.0, 7.5, 8.0, 8.5 and 9.0 and a controlled equal flow of water supplied to the distal end of each arm of the unit (approx. 30 ml/min.). A pH electrode was used to check that the pH in each channel was constant at the value required. The apparatus was set up in a darkened room with one 60 watt electric lamp placed 6 ft. vertically above the center of the apparatus to avoid phototactic effects. A small tube with a fine mesh was positioned 1 cm above the floor of the central chamber and connected to a water suction pump. Excess water was

pH value	Number of ostracods in each channel						
	Test No. 1	2	3	4	5	6	7
7.0	8	17	27	20	10	11	14
7.5	16	13	12	14	17	6	9
8.0	12	7	17	9	8	1	11
8.5	4	3	5	2	10	6	5
9.0	11	1	5	7	7	2	1

Table 16. Multi-choice pH preference tests.

pH value	7.5	8.0	8.5	9.0
7.0	P = 0.4	0.1	0.01	0.01
7.5	---	0.3	0.02	0.01
8.0	---	---	0.02	0.1
8.5	---	---	---	0.9

Table 17. Significance of differences in pH preference from data in Table 16.
Difference considered significant if
P = 0.05 or less.

Test No.	No's in each half of channel	
1	12	18
2	14	16
3	21	9
4	16	14
5	15	15
6	7	23
7	17	13

Table 18. Two-choice pH preference control test. P = 0.7.

removed in this way as it had been found that a centrally-placed gravity-drain tube did not work satisfactorily, due to surface tension effects, and prevented the release of test animals in the center of the test unit. It was also found necessary to roughen the floor of the channels to prevent the animals being displaced by the water flow as they moved up the channels from the central release point.

29.1.1 Results

The tests were carried out with C. inaequalva from the stream which had been separated from debris with minimum handling to avoid damage. In each experiment a group of animals was released at the center of the apparatus. After a period of 4 hours the numbers that had moved into each of the 5 channels were recorded (Table 16). Seven experiments were made and the difference in numbers of ostracods choosing each pH value was evaluated for statistical significance using the "t" test (Snedecor, 1956). This test, as with all parametric statistics, rests on certain assumptions, i.e., normal distribution and the null hypothesis that each population has the same mean and the same variance. Data interpretation is therefore subject to these limitations (Greig-Smith, 1964). The significance of the differences is shown in Table 17 from which it was concluded that both pH 7.0 and 7.5 were significantly preferred to pH values above 8.0.

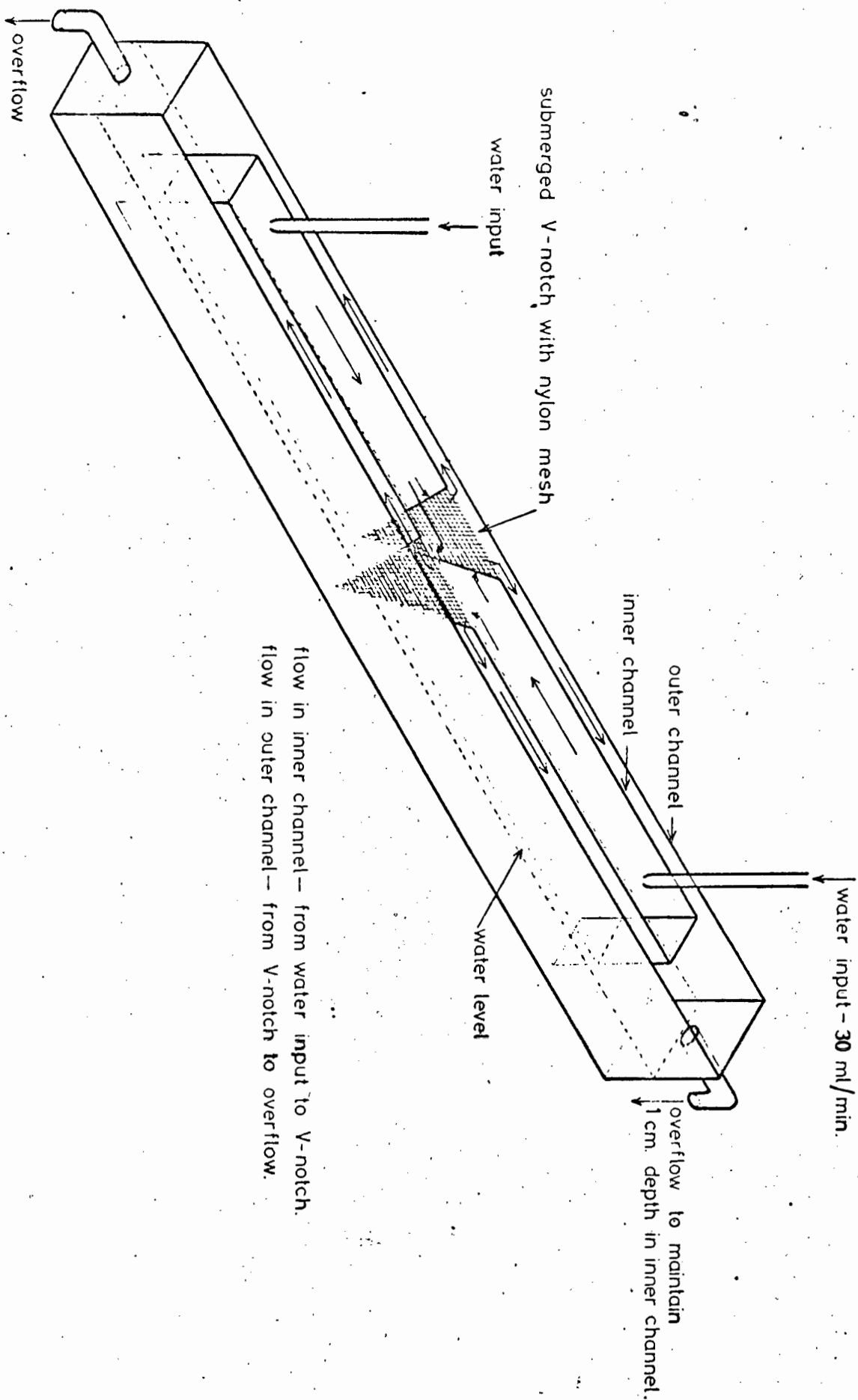


FIG. 35. TWO-CHOICE pH PREFERENCE UNIT CONSISTING OF INNER AND OUTER CHANNELS CONNECTED BY A MESH-COVERED V-NOTCH.

The reliability of the above experiments suffered from the fact that control experiments were not carried out to confirm that no preference was shown for any particular channel when all pH values were the same, that tap water was used, and that the method of suction drainage sometimes caused some of the experimental animals to be held against the outlet mesh and possibly damaged. For these reasons a second series of pH preference tests were carried out with a two-choice instead of a multi-choice system.

29.2 Two-Choice Apparatus and Method

The apparatus consisted of a square-section Perspex channel 30 cm in length with 2 cm walls and a width of 1 cm. At the mid-point along each side a 45° V-notch reaching to the floor of the channel was cut and the aperture covered by fine nylon mesh attached to the inner wall with adhesive (Fig. 35).

This channel was placed in a second channel wide enough to give a 0.3 cm clearance between its inner wall and the outer wall of the inner channel. When the inner channel was filled with water the liquid passed through the V-notches and into the outer channel, rising to an overflow tube positioned to maintain a depth of 1 cm in both the inner and outer channels. In this way both sides of the mesh covering the V-notch outlets from the inner channels were immersed and surface tension effects which had given trouble with the multi-choice unit were eliminated. The floor of the inner channel was roughened, as in the multi-choice

Test No.	pH Comparison	Numbers in each 1/2 of channel	
		Lower pH	Higher pH
1	7.5 - 8.0	12	18
2		14	16
3		17	13
4		15	15
5		18	12
6	7.5 - 8.5	17	13
7		16	14
8		23	7
9		14	16
10		16	14
11	7.5 - 9.0	28	2
12		18	12
13		21	9
14		19	11
15		23	7
16	8.0 - 8.5	17	13
17		17	13
18		20	10
19		16	14
20		14	16
21	8.0 - 9.0	20	10
22		21	9
23		17	13
24		15	15
25		16	14
26	8.5 - 9.0	16	14
27		12	18
28		11	19
29		17	13
30		16	14

Table 19. Two-choice pH preference tests.

pH value	8.0	8.5	9.0
7.5	P = 0.8	0.05	0.001
8.0	-	0.05	0.05
8.5	-	-	0.4

Table 20. Significance of differences in preference from data in Table 19.

unit, and test animals were placed at the center of the channel with a soft brush.

In these tests filtered water taken from the natural environment of the ostracods was used and, after pH adjustment with H_2SO_4 or NaOH, was passed from 20 litre aspirators at a controlled rate to the ends of the channels as before.

29.2.1 Results

A series of 7 control runs was first carried out to confirm that no preference was shown for either limb of the channel. Water at pH 7.5 was passed from the same aspirator to each end of the channel and the number of animals in each half of the channel counted after 3 hours exposure time. The results of these tests showed that there was no significant difference in preference ($P = 0.7$) between the two limbs at the same pH (Table 18).

A series of experiments was then carried out to check the results of the previous series at pH values of 7.5, 8.0, 8.5 and 9.0. Each experiment lasted for 3 hours and was repeated five times. The results are shown in Tables 19 and 20 from which it can be seen that the results are in close agreement with those obtained with the multi-choice unit (Table 17). The two-choice method did, however, detect a preference for the lower pH in the tests comparing pH 8.0 and 8.5 which was not shown by the multi-choice test.

29.3 Conclusions from pH Preference Experiments

From the results of both series of experiments, it would be predicted that the majority of the ostracod population would be found in regions with pH values of 8.0 or below, i.e. approximately between Stations A1 and A3 (0 - 5342 ft.).

This result is in quite good agreement with the sampling data except for the low ostracod density in the first 1000 ft. of the stream. In this section the pH value was between 7.0 and 7.5 and, from the pH preference results, would be expected to have had a high population density. In fact, the opposite was the case, which suggested that the water was not the only factor influencing the distribution.

30. FOOD REQUIREMENT TESTS

Since the pH preference results did not account for the low population density observed in the first 1000 ft. of the stream, the nature of the algal sediment was investigated as a possible influence on the distribution.

30.1 Algal Sediment as a Food Source

Five samples of sediment from regions of high and low population density were taken at Stations 3, 13, 18, 24 and 31, being respectively 936, 3744, 5616, 7488 and 9672 ft. from the inlet (cf. ostracod distribution, Table 14). Each sample was filtered through a fine net to remove detritus and any faunal content.

The samples were examined to determine the dominant algal species with the following results:

Station 3 : Diatoms dominant, mostly Nitzschia sp and some Naviculoid species

Station 12: Chlorella sp dominant

Station 18: Chlorella sp (as above) dominant and much Characium sp.

Station 24: Characium sp (as above) and Oocystis sp in about equal proportions

Station 31: Oocystis sp dominant

30.2 Dietary Suitability of the Algal Sediment

The possible physical or chemical unsuitability of the different algal species as a food source was first investigated.

Eighteen 10 ml beakers were set up containing 5 ml of matured, filtered aquarium water. The beakers were divided into groups of 3 into which was placed sufficient algae from each of the 5 prepared samples to cover the bottom of the beakers. The sixth group of 3 beakers contained water without algae as a control. Ten ostracod adults were placed in each beaker which was then maintained at room temperature near a window to provide adequate light for the algae. The beakers were examined after 4, 8 and 18 days to determine the number of individual surviving.

30.2.1 Results

Tables 21 and 22 show that after 8 days the survival rate in the controls was less than in all the beakers

Algal Sample Station No.	Number surviving after:								
	4 Days			8 Days			18 Days		
	A	B	C	A	B	C	A	B	C
3	9	7	9	5	5	7	0	0	0
12	10	7	8	2	2	4	0	0	0
18	6	4	5	3	3	4	0	1	1
24	10	7	7	9	7	7	6	6	6
31	10	10	10	10	9	9	3	5	4
Control	4	5	7	1	3	3	0	0	0

Table 21. Nutrition tests with algae samples from 5 Stations in the stream. Each sample fed to 3 groups of ostracods.

Algal Sample	Per cent survival		
	4 Days	8 Days	18 Days
3	83.3	56.7	0.0
12	83.3	26.7	0.0
18	50.0	33.3	6.7
24	80.0	76.7	60.0
31	100.0	93.3	40.0
Control	53.3	23.3	0.0

Table 22. Per cent survival from data in Table 21.

containing algae, with samples from Stations 24 and 31 having the highest survival rate. After 18 days all the test specimens had died in samples from Stations 3, 12 and the control, and very few survived with algae from Station 18, but with algae from Stations 24 and 31, the survival rate was 60 and 40 per cent, respectively.

30.2.2 Conclusions

These results are contrary to what would be expected if the sparsely populated regions of the stream were due to unsuitability of the algae as a food source. In fact, the highest survival rates were on algae from the lowest populated sections of the stream from which it was evident that algae from any point of the stream could act as a food source.

31. FOOD PREFERENCE TESTS

The experiment described above did not take into account the possibility that, although algae from any section of the stream would be accepted as food material if no alternative was presented, due to differences in physical or chemical qualities the ostracods might exhibit a preference for certain algal species. A series of preference tests were carried out to examine this possibility.

31.1 Apparatus and Method

An apparatus consisting of a 12" diameter flat-bottomed plastic cylinder fitted with a removable false floor 1" from the bottom was constructed. Five circular holes, spaced at

From these values it is evident that to remove a single day's input of phosphorus, it would be necessary to remove a quantity of ostracods approximately 178 times greater than the total population present on the 9th August. Even if it were possible to develop a uniform population density along the whole of the stream, the total population would be increased by a factor of less than 2 and would still contain an insignificant fraction of the daily nutrient input. The fact that the low density was shown to be due to high pH values in the lower half of the stream makes it very unlikely that the population density could be increased, since the pH increase was a direct result of biochemical activities occurring in a shallow stream system of the design employed. An attempt to lower the pH value to a more favorable level would immediately reduce the amount of phosphorus removed from the effluent by precipitation - a mechanism of considerably greater potential for phosphorus removal than the removal of herbivores.

DISCUSSION OF RESULTS

The results of this study demonstrated that use of the herbivore component in the trophic structure developed in a shallow highly eutrophic stream of the type employed would not provide a practical means of removing plant nutrients. The number of herbivores that would need to be removed each day would be far greater than the total adult population present at any time during the investigation. This would be the case even if it were possible to promote equal ostracod growth in the less densely populated regions of the stream.

33.2 Nutrient Content of the Ostracod Population

According to the data on stream population in Table 14, the highest number recorded was on 9th August. On this date the mean density for the 33 sampling stations was 467 per 9 cm section, with a standard error of ± 178.4 . Assuming the values to be approximately normally distributed, the 95 per cent confidence interval is 467 ± 363.9 individuals per 9 cm section. This confidence interval is high due to the wide range of values.

Using the upper limit of 831 as the average density for the whole length of the stream, the total population present on this date is calculated as close to 29×10^6 individuals.

Calculation of the 95 per cent confidence interval for the weight of nitrogen and phosphorus in the ostracods from Table 27 gives an upper limit of $1.30 \text{ mg N}/10^3$ individuals and $0.159 \text{ mg P}/10^3$ individuals.

Using these values the maximum weight of nitrogen contained in the whole population on 9th August is computed as 37.7 gm N. For phosphorus the equivalent figure is 4.61 gm P.

The daily input of nitrogen and phosphorus to the stream, based on mean concentrations of 24.0 mg N/litre and 8.31 mg P/litre at a flow of 15 g.p.m. was 2334 gm N/day and 807 gm P/day.

Comparing these daily input quantities with the weight contained in the ostracod population, it is evident that the total population contained only 1.61 per cent of the daily nitrogen input and 0.56 per cent of the daily phosphorus input.

The value for mean length of a number of individuals taken from the samples analyzed was 0.725 mm (standard error = \pm 0.011). In view of this very close agreement between mean lengths it was concluded that very few juveniles of the larger H. congenera of greater or lesser size were present in the samples prepared for analysis.

After the final sorting, the ostracods were placed in a shallow dish from which most of the water was removed to restrict movement. Batches of between 2000 and 4000 individuals were then counted out and placed in mesh-bottomed dishes full of algae from Station 12 in the algal stream. The animals were left in contact with the algae for 4 hours to allow them to feed. The algae was then removed by washing it through the mesh, and the clean ostracod samples were dried in dishes under an infrared lamp, followed by oven-drying at 105°C. After determination of the dry weight, the material was acid digested and analyzed for total nitrogen and total phosphorus.

33.1.2 Results

In Table 27 the results are given for 14 samples, each consisting of several thousand individuals. The agreement between samples is close, with mean values for nutrient content of 1.26 mg/N and 0.152 mg/P per 1000 ostracods.

Number of Individuals in Sample	Weight per 1000 individuals (mg)			Per cent of Dry Wt.	
	Dry Wt.	N	P	N	P
2000	15.4	1.14	0.136	7.40	0.88
3000	17.7	1.30	0.155	7.34	0.86
3000	17.3	1.17	0.145	6.76	0.84
3000	17.1	1.23	0.140	7.19	0.82
3000	18.5	1.27	0.152	6.86	0.82
4000	17.5	1.25	0.148	7.14	0.85
4000	18.8	1.36	0.179	7.23	0.95
4000	17.7	1.20	0.155	6.78	0.87
4000	18.2	1.28	0.140	7.03	0.78
4000	17.8	1.23	0.142	6.92	0.80
4000	17.9	1.29	0.156	7.20	0.87
4000	18.5	1.35	0.161	7.31	0.87
4000	18.0	1.26	0.160	6.99	0.89
4000	18.1	1.28	0.163	7.09	0.90
Mean Value and Standard Error	17.75 ± 0.218	1.26 ± 0.016	0.152 ± 0.0031	7.09 ± 0.055	0.86 ± 0.012

Table 27. Nitrogen and phosphorus content of algae-fed
adult Cypridopsis inaequalva.

of a microscope lamp focussed on the window. The animals collecting at the window were periodically syphoned off and more water added as necessary. In this way a mixture of ostracods and chironomid larvae free of other contaminants was obtained. The chironomid larvae were then removed with forceps, or, when time permitted, by releasing the mixed sample into a tank of small fish which fed selectively on the chironomids but ignored the ostracods.

To separate juvenile C. inaequalva from the required adults, a nylon mesh sieve was used. The average length of the short axis of the body of adult C. inaequalva was approximately 0.3 mm and a sieve of this mesh size was employed. The sample of mixed ostracod sizes was placed in the sieve and agitated in a basin of water while washed with a water jet. In this way a large proportion of the smaller specimens passed through the mesh while the larger specimens were retained. The remaining juveniles were then individually removed with a bulb pipette, leaving a sample consisting almost entirely of adult C. inaequalva.

Measurement of 270 of these individuals gave a mean length of 0.726 mm (standard error = \pm 0.003).

from the sample data already obtained, but for chemical analysis of nutrient composition, relatively large numbers of adult individuals, free of algae and detritus, were required for determination of a reliable mean value, in view of their very small individual weight.

33.1 Ostracod Samples for Analysis

To obtain the large numbers of individuals required, about 10 litres of algal sediment from regions of high population density were obtained. The faunal content was removed by washing the whole volume through a 32 mesh/cm net. The sample obtained contained much sand, gravel and organic debris and many chironomid larvae, in addition to the ostracods.

33.1.1 Preparation of clean ostracod samples

In order to separate the fauna from mineral and organic debris, advantage was taken of the photophylic reaction of the ostracods and chironomids.

A 5 litre conical flask was painted black on the outside and a small elongated window 1 cm wide and 2 cm high was made in the paint about 10 cm from the bottom. A glass syphon tube was arranged to withdraw water from the area close to the inside of the window. The sample was then washed into the flask, which was then filled with water and placed on the bench with the beam

unexplained. The possibility of unionized ammonia toxicity appears to be ruled out by the fact that pH values never exceeded 8.0 in this section and that both higher pH and higher ammonia concentrations were experienced in regions of high ostracod density.

It seems possible that the lack of ostracods in this section may have been due to changes in water quality at night, since sediment density was high in this region and a combination of bacterial and algal respiration may have raised the carbon dioxide concentration at the sediment surface to unacceptable levels. The suggestion is speculative only, since no determination of free carbon dioxide concentration changes between day and night were made.

33. NITROGEN AND PHOSPHORUS CONTENT OF OSTRACOD MATERIAL

During the development and growth of an ostracod individual there is continuous uptake and excretion of compounds from the environment, a proportion of which is incorporated into the body of the animal. From the practical aspect of nutrient removal, only that material which is present in the body of the animal at the time of harvesting can be physically removed from the water phase. This quantity would be at a maximum in the case of a fully-grown, sexually mature adult with the gut full of food material.

To assess the proportion of nutrient material in the stream system that was present in the form of adult ostracod biomass, it was necessary to determine both the number of animals present and their chemical composition. An estimate of the ostracod population could be derived

To prepare the test apparatus the clean algae from Station 3 was pipetted onto two of the mesh-bottomed dishes. The collected humus sediment was then spread with a pipette over the algae in one dish. Sufficient humus sediment was added in an even layer to approximate the sediment layer thickness found in the stream. A third dish was filled with a layer of humus alone and the fourth and fifth dishes received algae from Stations 7 and 15.

31.2.2 Results

The experiment was repeated 9 times with 24-hour exposures and the results are summarized in Tables 25 and 26. From these tables it is evident that no significant difference in preference for any of the five samples presented was shown, indicating that the organic humus material was not responsible for the low population densities.

32. DISCUSSION OF PREFERENCE TEST RESULTS

From the results obtained from tests comparing ostracod preference for pH values and for various food materials, it appears that the low population density observed downstream of Station 20 was primarily due to a preference for water with a pH value not exceeding about pH 8.0 during the day. The low population density in the first 1000 ft. of stream could not be accounted for by either unfavorable pH or food source and remains

Test No.	Number of individuals choosing each algal sample				
	A	B	C	D	E
	Humus	Diatoms	Diatoms Overlain by Humus	Algae from St. 7	Algae from St. 15
1	148	123	80	101	97
2	38	29	104	38	21
3	9	19	19	22	3
4	14	23	35	24	18
5	2	7	14	5	1
6	25	42	96	67	58
7	1	7	15	5	4
8	4	8	24	9	13
9	25	14	43	49	36

Table 25. Food preference tests with algae and overlying humus material.

Sample	B	C	D	E
A	P = 0.8	0.7	0.8	0.9
B	-	0.4	0.8	0.8
C	-	-	0.7	0.3
D	-	-	-	0.7

Table 26. Significance of differences in preference from data in Table 25.

filters tended to settle out in a blanket several millimeters thick over the diatom growth in the stream bed. In the preparation of samples from this region for the preference experiments, however, most of this humus material had been washed away, leaving relatively clean diatom material for the tests.

31.2 Effects of Organic Detritus

It was thought possible that this humus material might repel, or in some way prevent the ostracods from feeding on the underlying algal material. To test this hypothesis a further series of tests using the food preference apparatus was carried out to compare ostracod preferences for algae from Station 3, both clean and overlain by humus material, for humus material without algae, and for Chlorella sp. sediment taken from Stations 7 and 15 in regions of high ostracod density.

31.2.1 Method

A sample of humus material was collected from Station 3 by dislodging the humus with a wash-bottle jet without disturbing the algae beneath and collecting it in a beaker held downstream of the disturbed area. Clean algal material from Station 3 was then obtained from the disturbed area.

that algae from Station 3 (mainly diatoms) were significantly preferred to algae from the other four Stations and that there was no marked difference in preference between these other four Stations.

31.1.2 Conclusions

From these results it would be expected that the ostracod density in the stream would be highest in the region of Station 3 and at a lower and approximately constant level in the remaining sections, if food preference was the factor dominating the distribution.

This expectation did not agree with the observed distribution, since at Station 3 and downstream from Station 24 the density was much lower than in the section from Station 6 to 20 (Table 14). These results implied that high pH, and not food preference, was the factor responsible for the low density below Station 20 and that some other factor was responsible for the low density at Station 3, since both pH and algal species were favorable in this region.

After further examination of the sediments in the stream and consideration of the water analysis and preference test results, it was concluded that the only obvious difference between the first 1000 ft. of stream around Station 3 and the downstream section was the fact that organic humus material derived from the sewage

Test No.	Number of individuals choosing each algal sample				
	St. 3	St. 12	St. 18	St. 24	St. 31
1	20	1	6	3	8
2	24	46	13	10	15
3	19	3	16	6	21
4	85	48	60	46	24
5	26	18	21	32	9
6	25	17	11	12	28

Table 23. Food preference tests with algal sediment from regions of high and low ostracod density.

Station No.	12	18	24	31
3	P=0.001	0.001	0.001	0.001
12	--	0.4	0.1	0.2
18	--		0.2	0.1
24	--		--	0.7

Table 24. Significance of differences in preference from data in Table 23.

equidistant positions from the center of the floor, were fitted with shallow dishes with mesh bottoms. The tops of the dishes fitted flush with the false floor and were secured with rubber "O" rings (Fig. 36). The outer surface of the cylinder was covered with black plastic film to exclude all light and a black opaque lid with a central 4 inch diameter aperture was provided.

The cylinder was filled with stream water at pH 8.0 to a depth of 5 inches and algal samples from the five stations in the stream were carefully pipetted into the mesh-bottomed dishes. The opaque lid was then fitted and the apparatus placed in a dark room with one lamp placed approximately 2 ft. above the central aperture to avoid any phototactic effects. A number of C. inaequalva was then released at the center of the false floor with a pipette.

Twenty-four hours after release, the mesh-bottomed dishes were removed and the algae washed through the mesh with a water jet, leaving the ostracods retained on the mesh for counting. Six experiments were carried out using fresh material each time.

31.1.1 Results

The numbers of ostracods found in each sample in the series of tests are given in Table 23, and the significance of the differences found between samples ("t" test) has been calculated in Table 24. From these results, it was concluded

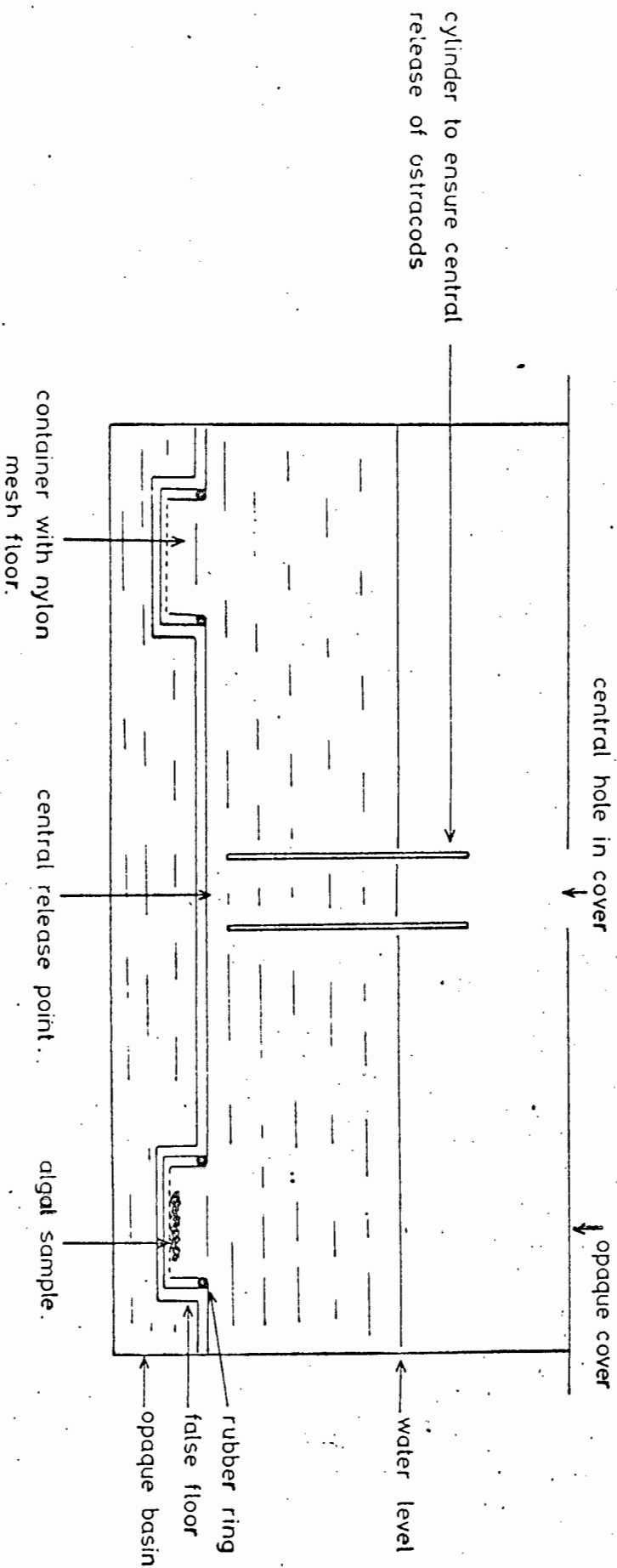


FIG. 36 APPARATUS FOR FOOD PREFERENCE TESTS.

Although the data was obtained from a specialized stream environment, the results indicate that the nutrient content of sewage is so high that even a system containing a herbivore population several hundred times greater in biomass could not withstand the disruption in population age structure caused by daily removal of the number of individuals that would be required. The method, therefore, is unlikely to offer a practical means of sewage nutrient extraction.

GENERAL DISCUSSION AND CONCLUSIONS

The eutrophication of water supplies due to the continuous discharge of domestic and industrial waste waters is a serious problem, and is likely to become more so as the rate of urban and industrial development increases. Although the problem is now recognized by those concerned with the maintenance of adequate water supplies, the methods by which this problem can be overcome have still to be developed. Although certain lines of approach appear to hold more promise than others there is, at present, no certainty as to which will prove to be the most generally applicable. It is therefore necessary that all the possible avenues of approach should be considered, and, where possible, some estimate of their practical feasibility obtained.

In this thesis an attempt has been made to use information available on the ecology and biochemistry of biological nutrition for the development of ecological systems, based on sewage effluent as the nutrient medium, in which conditions are such that nitrogen and phosphorus are lost from the system and not recycled, as is normally the case in nature.

The experiments described in Section A demonstrated that organic carbon present in particulate sewage material can be utilized by activated sludge organisms if the usual primary solids settlement treatment stage is omitted. By this means a more favorable C:N:P ratio for synthesis of new organisms can be provided and increased assimilation of N and P obtained. Although the method is simple, it does not appear to have been considered for this purpose, probably because the need for more complete nutrient removal has only been realized in recent years, and

because of the efficiency of primary sewage settlement as a simple method of removing a substantial proportion of the organic load in the raw sewage.

The changes in rate of organism production and nutrient assimilation observed during experimental demonstration of the method is explained by the governing effects of aeration time and the amount of assimilable carbon present on the respiratory and synthetic activity of the microbial population. At high organic load rates, these changes resulted in an approximately twofold increase in the removal of nitrogen and phosphorus from solution, raising the removal rate from the normal 25 to 35 per cent to about 75 per cent.

Although a decrease of this magnitude would reduce eutrophication rates, it would not achieve the desired objective of prevention. The operational difficulty that would probably be encountered in removing the increased production of cell material, as indicated by the experimental results, together with the problem of disposing of the material by methods which would ensure that the nutrient content would not return to the receiving watercourse, suggest that this method is not likely to offer an acceptable solution to the problem.

The second approach that was investigated involved the bacterial reduction of oxidized nitrogen to nitrogen gas. The problem of deficiency in available carbon in relation to the amount of nitrogen present was also encountered in this method, in that insufficient carbon is normally present to allow removal of nitrogen from the total sewage flow. However, an advantage over the first approach lies in

the fact that complete nitrogen removal from about half the total flow would apparently be possible, while the remainder would contain the usual amount present after conventional treatment. The ability to remove nitrogen from a portion of the total flow would offer a source of reusable water for a variety of domestic and industrial purposes. There is also the possibility that the reclaimable fraction could be increased by deliberate use of the sewer reticulation system for the disposal of domestic and industrial carbonaceous wastes.

Despite the fact that a waste product, in the form of sewage solids, was shown to provide a suitable source of hydrogen donors necessary for denitrification, it is not certain that this material would prove the most economic source in practice. There are a number of points that would require further investigation, since the use of a biological process to produce hydrogen donors from a variable material, such as sewage solids, might give rise to changes in acid composition. Changes of this type might require extra operational control to maintain the $C:NO_3-N$ requirement. Another possibility is the development of toxic conditions in the acid fermentation process. No information is available on continuous operation of an anaerobic digester for acid production under these conditions, and digester failure would cut off the supply of hydrogen donor.

The process as described would introduce three new operations into the denitrification sequence:

- (1) Acid fermentation
- (2) Ammonia stripping
- (3) Lime sludge disposal

These extra operations would increase the cost of donor production to an unknown extent. It would obviously be necessary to obtain some estimate of cost of production, compared with the use of an industrial chemical, since in some countries the cost of production of organic chemicals, such as methyl alcohol, has decreased considerably during the last decade due to the exploitation of natural gas resources.

Whatever the source of hydrogen donor that proves most generally suitable, the results obtained from the experiments described in Section B suggest that the use of bacterial nitrate respiration to remove oxidized nitrogen is technically feasible. The nitrogen content can apparently be reduced to very low levels, the engineering and operational requirements do not appear to be complicated, and no nitrogen-containing solid waste is produced that would require separate disposal.

The deficiency in available organic carbon in relation to the amounts of nitrogen and phosphorus present in sewage apparently precludes complete removal of the nutrients by heterotrophic assimilation or by bacterial denitrification. For this reason the use of autotrophic organisms was investigated, since sewage effluents normally contain relatively high concentrations of inorganic carbon in an available form.

The concept of a shallow algal stream for extracting nutrients was developed from a consideration of the ecological requirements of microphytes and the changes they produce in the water environment. The results obtained showed that the pH change associated with active

photosynthesis could be used to remove phosphate by precipitation and ammonia nitrogen by loss to the atmosphere. The design of the system used was not ideal in that the excess algal sediment produced could not be removed from the stream, although the good settlement characteristics would have permitted this to be done without difficulty. The accumulated sediment formed a reservoir of phosphate for re-resolution during periods of lowered pH value, which decreased the overall removal obtained. Had the excess been removed from the stream, the removal would have been greater, but it is likely that a general decrease in efficiency during winter would still have occurred due to lowered temperatures.

Oxidized nitrogen accounted for at least 50 per cent of the total nitrogen entering the stream throughout the experiment. This was disadvantageous in that, if a less nitrified effluent had been available, the loss of ammonia nitrogen to the atmosphere would have been considerably greater.

The information obtained from operation of the stream led to the development of a rotating algal disc design in which some of the shortcomings of the stream were overcome. The major advantages of a disc system would be decreased land requirement, decreased loss of intrinsic heat, improved light utilization, reduced presence of inactive solids to form a phosphate reservoir, variable current speed, increased loss of ammonia nitrogen and ease of removal of excess algal material.

At increased rotation speeds an increase in algal phosphate uptake by the algal film on the discs was shown to occur and was found to be

due to an increase in the metabolic rate and not to phosphate adsorption on the algal surfaces. However, the practical implications of these findings do not appear to be very great, since the effect was found to be confined to a thin surface layer of algal cells. Due to this limitation, it seems likely that any increase in phosphate uptake resulting from increased rotation rate would be insignificant compared to the amount of phosphate that must be removed from sewage effluent. It is possible, however, that variations in metabolic rate in the surface layer due to changes in rotation rate could be used to alter the pH changes obtained.

The rate of movement of air at the air/water interface was found to have an important influence on the rate of loss of ammonia. When the velocity of air movement across the discs fell below a specific level the loss of ammonia was reduced. This would be an important consideration in a full-scale apparatus of this type, and would also influence the use of the method when water re-use was intended, since the conditions for high ammonia loss would also be those promoting a high evaporation rate. This aspect was not investigated but would obviously require consideration if further development of methods involving exposure of thin water films was intended.

Periodic removal of the herbivore component of the trophic structure developing in an aquatic environment, as suggested by Odum (1961), does not appear to offer a means of removing nutrients to the degree required. Although the results described were obtained from the ostracod population of an artificial stream, it seems likely that

similar results would be obtained with other herbivorous populations, since both MacFayden (1963) and Slobodkin (1964) have concluded that ecological efficiency at the invertebrate level does not exceed 10 to 20 per cent. Even if the efficiency was much greater, it is still unlikely that the requirements for a stable and harvestable invertebrate population could be met, since the factors operating on aquatic populations are both numerous and subtle in their effects. Some may be obvious, such as the influence of pH value, but some may be difficult to detect, such as those responsible for the almost complete absence of ostracods in the first 1000 ft. of algal stream. Seasonal factors, such as changes in temperature and light intensity, have major effects on species and population composition and it seems certain that the cost of providing the conditions for a suitable population, or succession of populations, that would contain a significant amount of nitrogen and phosphorus would far exceed the cost of several alternative methods of nutrient removal.

The experimental work that has been described leads to the conclusion that it would be possible to devise a sewage treatment system based entirely on biological activity that would remove most, but not all, of the nitrogen and phosphorus in sewage. Part of the process would be carried out by heterotrophic organisms and their environmental requirements could be provided and maintained without great difficulty. The section of the process employing autotrophic organisms might prove more difficult to control due to seasonal changes but it should be possible to maintain a relatively steady temperature in a rotating disc unit by use of waste digester heat. By suitable choice of size and disc separation for local

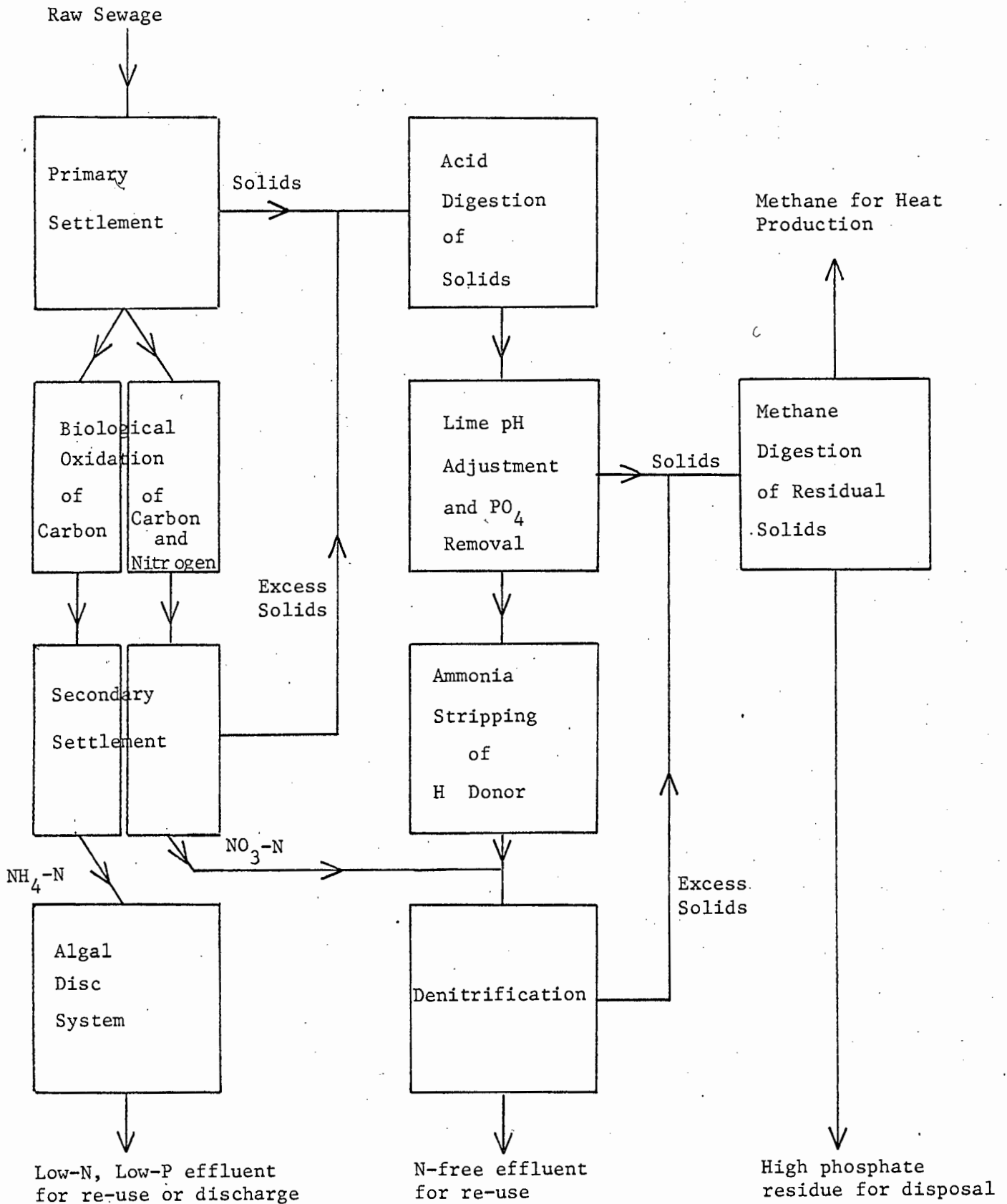


Figure 37. Schematic layout of a sewage treatment plant utilizing biological activity for nutrient removal.

conditions, it should also be possible to arrange that the light intensity on the discs would always be sufficient to support an algal community. Provision of artificial illumination or a means for alkali addition during periods of darkness would also probably be required.

A flow diagram of a system of this type might be as shown in Figure 37.

Before a system of this kind could be employed, it would be necessary to carry out further research and development on the four new stages that would be needed, i.e., acid fermentation, lime addition, ammonia stripping and the design of the denitrification stage. Information on the comparative merits of packed column and suspended growth reactors would be required and the settlement characteristics of the excess denitrifying sludge would also be of importance. Anaerobic digestion of all the residual sludges should be possible to provide methane as an energy source for part of the power required for heat and lighting. The digester supernatant would contain a high concentration of phosphate which could be used for agricultural purposes.

It is obvious from the foregoing paragraphs that many questions remain to be answered before economic comparisons between the biological methods for nutrient extraction suggested in this thesis and alternative chemical methods can be made. Perhaps the most important question that requires an answer, in a world whose population is steadily increasing, is whether we can afford to continue to lose the use of essential nutrient elements contained in waste materials derived from human activities. Even today

a considerable proportion of the world population is suffering from a deficiency in dietary protein and this situation will worsen unless transport facilities are improved, and the production of suitable food materials is increased.

In some parts of the world, particularly in the East, the return of excreted nutrients to the soil has been the basis of agriculture for many centuries. It seems that this method of nutrient conservation and recycling for food production must now be extended and improved if eventual widespread famine is to be averted in heavily populated areas lacking in natural resources.

The most productive method of utilizing reclaimed nutrients also requires consideration. Wheat is one of the most productive cereals and yields are normally in the region of 1 ton per acre per year. The production of animal protein is usually not more than 200 lbs. per acre per year, but production of vegetable protein, in the form of soybeans, may also approach 1 ton per acre per year. In contrast, production of algae in sewage ponds can be as high as 60 tons dry weight per acre per year, and algal protein production can reach 12 tons. This difference is mainly due to the much shorter growth period required to produce an algal "crop", usually less than 5 days, and the fact that unwanted herbivores can be efficiently controlled by chemical additives (Oswald, 1962). The potential rate of protein production by algal culture is thus some ten times greater than by conventional agriculture.

The production of algae in enriched natural waters can also be high. Lake Sebasticook in the N.E. United States, for example, is fertilized by agricultural run-off and some sewage discharge and produces a standing crop reaching 2260 lbs. wet weight per acre during the warm summer months (MacKenthun and Ingram, 1967). Nutrient enriched sea water provides another potential source of algal protein, and in this case production might not be limited to situations capable of sewage enrichment. Unproductive estuarine areas, salt marshes and lagoons exist in many parts of the world where adequate sunlight for continued year-round algal growth is available. Such regions might be artificially fertilized and used to produce not only food in the form of algae and selected edible herbivores, such as shrimps, but also to provide a competitive source of raw materials for chemical industries and perhaps for production of paper products using cellulose fibres obtained from filamentous algal species.

Despite the fact that comparatively little research has been carried out on the practical aspects of algae culture on a large scale, it has been reported from Japan by Tamiya, et al. (1953) that Chlorella for human consumption can be grown in defined mineral media at a cost of about 20 cents per pound. Oswald (1962) has reported that in California algae for livestock consumption can be produced from sewage ponds at a cost of 2 to 4 cents per pound if the cost of sewage treatment that would otherwise be necessary is taken into account.

A review of literature on the subject, published during the last fifteen years, indicates that it would now be technically feasible to

use liquid wastes from urban areas to produce unprecedented yields of protein per unit of surface area in either fresh or saline water. This appears to be a field of research in which intensified effort is urgently needed to develop new and improved methods of mass culture and harvesting, to evaluate methods of product preparation and nutrient values, to develop new applications for the products of algal culture and, perhaps most important, to advance and simplify the necessary technology to the stage at which underdeveloped nations having suitable climatic conditions and geographic situations can use algae culture to augment their food supplies.

Development of methods to overcome the more immediate problems of uncontrolled eutrophication must come from many branches of science, and it is hoped that the work described in this thesis may provide sufficient information for others to judge whether further research on the biological nutrition approach would be worthwhile.

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REFERENCES

- Aikins-Afful, E. P., 1961. Performance of laboratory oxidation ponds. Masters Thesis. University of Wisconsin.
- Allanson, B. R. and Kerrick, J. E., 1959. A statistical method for estimating the number of animals found in field samples drawn from polluted rivers. Int. Soc. Limn., Vienna, August.
- Allen, C. K., 1966. Use of copper sulphate in water treatment. Water and Sewage Works, J. Ref. No.
- Allen, M. B., 1955. General features of algal growth in sewage oxidation ponds. Publ. No. 13, California State Water Pollution Control Board.
- Anderson, G. C., 1961. Recent changes in the trophic nature of Lake Washington. In "Algae and Metropolitan Wastes". Tech. Report W61-3. U. S. Department of Health, Education and Welfare.
- Anderson, R., 1963. Ph.D. Thesis, Department Bact., University of Wisconsin.
- Assenzo, J. R., and Reid, G. W., 1966. Removing nitrogen and phosphorus by bio-oxidation ponds in Central Oklahoma. Water and Sewage Works, August, 294-299.
- Babbitt, J. E., 1944. Garbage disposal as a sewage problem. Sewage Works J., 19, 861-71.
- Barth, E. F., Mulbarger, M., Salotto, B. V., and Ettinger, M. B., 1966. Removal of nitrogen by municipal waste water treatment plants. Journal of Water Pollution Control Fed., 38, 7, 1208.
- Bartsch, A. F., 1961. Induced eutrophication, a growing water resource problem. In "Algae and Metropolitan Wastes". Tech. Report W61-3, U. S. Department of Health, Education and Welfare.
- Bayley, R. W., 1967. Desorption of waste water gases in air. Effluent and Water Treatment J., February, March.
- Beck, W. M., 1965. The biological removal of nitrogenous compounds from sewage treatment plant effluents. Robert A. Taft., San. Engng. Center, Pub. No. 999-WP-25, U. S. Department of Health, Education and Welfare.
- Beeton, A. M., 1965. Eutrophication of the St. Lawrence Great Lakes. Limnol. and Oceanog., 10, 240.
- Bess, F. D., and Conway, R. A., 1966. Aerated stabilization of synthetic organic chemical wastes. Journal of Water Pollution Control Fed., 38, 939.

- Birge, E. A., and Juday, C., 1922. The Inland Lakes of Wisconsin. The Plankton. 1. Its quality and chemical composition. Wisconsin Geological and Natural History Survey, Bulletin No. 64. Scientific Series No. 13, 1-222.
- Bogan, R. H., Albertson, O. E., and Pluntze, J. C., 1960. The use of algae in removing phosphorus from sewage. J. Amer. Soc. Civ. Engineers. Sanit. Engng. Div., September 1960, 1-20.
- Borgstrom, G., 1961. Fish as Food. Academic Press, New York.
- Bringmann, G., Kuhn, R., and Wagner, B., 1959. Laboratory experiments with biological removal of nitrogen from waste water in the form of nitrogen gas. Gesundh. Ing., 80, 364.
- Bringmann, G., and Kuhn, R., 1962. Ein Schnell-Denitrifikationsverfahren mit automatischer Redox Steuerung. Gesundh. Ing., 82, 11, 333.
- Bringmann, G., and Kuhn, R., 1964. Halbtechnische Stabilisierung und Intensivierung der Denitrifikation. Gesundh. Ing., 85, 1, 19.
- Bringmann, G., and Kuhn, R., 1965. Schnell Denitrifikation im Unterdruck Verfahren. Gesundh. Ing., 86, 1, 16.
- Burk, D., Cornfield, J., and Schwartz, M., 1951. The efficient transformation of light into chemical energy by photosynthesis. Sci. Monthly. 73, 213-223.
- Bush, A. F., Isherwood, J. D., and Shiva, Rodgi, 1961. Dissolved solids removal from waste water by algae. J. Amer. Soc. Civ. Engineers. Sanit. Engng. Div., May, 39-57.
- Caldwell, D. H., 1946. Sewage oxidation ponds - Performance, operation and design. Sew. Ind. Wastes, 18, 433.
- Chancellor, R. J., Coombs, A. V., and Foster, H. S., 1958. Control of aquatic weeds by copper sulphate. Proc. 4th Brit. Weed Cont. Conf. Soc. of Chem. Ind., London 1960, 80.
- Chick, H., 1903. A study of a unicellular green algae occurring in polluted water, with special reference to nitrogen metabolism. Proc. Roy. Soc., London, 74, 458.
- Cholnoky, B. J., 1960. Beitrage zur Kenntnis der Okologie der Diatomeen in dem Swartkops-Bache nahe Port Elizabeth (Sudost Kaapland). Hydrobiologia, 16, 229.
- Cholnoky, Pfannkuche K., 1968. Einige neue und seltene cyanophyceen aus südafrika. Nova Hedwigia (in press).

- Chung, C. W., and Najjar, V. R., 1956a. Cofactor requirements for enzymatic denitrification. I. Nitrite reductase. J. Biol. Chem., 218, 617-26.
- Chung, C. W., and Najjar, V. R., 1956b. Cofactor requirements for enzymatic denitrification. II. Nitric oxide reductase. J. Biol. Chem., 218, 627-32.
- Cillie, G. G., Van Vuuren, L. R. J., Stander, G. J. and Kolbe, F. F., 1966. The reclamation of sewage effluents for domestic use. 3rd Int. Conf. on Water Pollution Res., Munich, Section II, Paper I.
- Clarke, C. E., 1965. Hog waste disposal by lagooning. J. Sanit. Engng. Div. Am. Soc. Civ. Engrs., 91, SA6, 27. Paper No. 4567.
- Collins, F. M., 1955. Effect of aeration on the formation of nitrate-reducing enzymes by Ps. aeruginosa. Nature. London, 175, 173.
- Cooper, L. H. N., 1948. Some chemical considerations on the distribution of iron in sea water. J. Marine Biol. Assn., United Kingdom, 27, 314.
- Cooper, R. C., 1962. Some public health aspects of algal-bacterial nutrient recovery systems. Amer. J. Pub. Health, 52, 252.
- Crance, J. H., 1963. The effects of copper sulphate on Microcystis and zooplankton in ponds. Progr. Fish Cult., 25, 198.
- Curry, J. J., and Wilson, S. L., 1955. Effect of sewage-borne phosphorus on algae. Sew. Ind. Wastes., 27, 11.
- Danforth, W. F., 1962. Substrate assimilation and heterotrophy. In "Physiology and Biochemistry of Algae." R. A. Lewin, Ed. Academic Press, New York and London.
- Delwiche, C. C., 1956. Biological transformations of nitrogen compounds. Ind. and Eng. Chem., 48, 1421.
- Dunn, I. G., 1967. Diurnal fluctuations of physicochemical conditions in a shallow tropical pond. Limnol. and Oceanog., 12, 1, 151.
- Eckenfelder, W. W., 1956. In "Biological Treatment of Sewage and Industrial Wastes." Volume I., Reinhold, New York.
- Eckenfelder, W. W., 1961. Wastes Eng. May, June, July.
- Edmondson, W. T., 1961. Changes in Lake Washington following an increase in nutrient income. Proc. Intl. Assn. Theoret. and Appl. Limnol., 14, 167.

- Engelbrecht, R. S. and Morgan, J. J., 1959. Studies on the occurrence and degradation of condensed phosphates in surface waters. *Sew. Ind. Wastes.*, 31, 4, 458-478.
- Eppley, R. W. and Macias, F. M., 1963. Role of the alga Chlamydomonas mundana in anaerobic waste stabilization lagoons. *Limnol. and Oceanog.*, 8, 411-416.
- Escritt, L. B., 1965. Sewerage and sewage disposal: calculations, design and specification. 3rd Edition, C. R. Books Ltd., London.
- Fewson, C. A., and Nicholas, D. J. D., 1960. The utilization of nitric oxide by micro-organisms and higher plants. *Nature*, London, 188, 794-6.
- Fewson, C. A., and Nicholas, D. J. D., 1961a. Utilization of nitrate by micro-organisms. *Nature*, London, 190, 2-7.
- Fewson, C. A., and Nicholas, D. J. D., 1961b. Nitrate reductase from Pseudomonas aeruginosa. *Biochim. Biophys. Acta.*, 49, 335-49.
- Finsen, P. O., and Sampson, D., 1959. Denitrification of sewage effluents. *Water and Waste Treatment J.*, May, June.
- Finstein, M. S., and Hunter, J. V., 1967. Hydrolysis of condensed phosphates during aerobic biological sewage treatment. *Water Research*, 1, 247.
- Fitzgerald, G. P., 1961. Stripping effluents of nutrients by biological means. *Algae and Metropolitan Wastes*. U. S. Public Health Service, Sec. TRW61-3, 136-139.
- Fitzgerald, G. P., and Rohlich, G. A., 1964. Biological removal of nutrients from sewage; laboratory experiments. *Verh. Internat. Verein. Limnol.*, 15, 597-608.
- Fogg, G. E., and Collyer, D. M., 1953. Carnegie Inst. of Washington, Publication No. 600, 179.
- Fogg, G. E. and Stewart, W. D., 1965. Nitrogen fixation in blue-green algae. *Sci. Progr.*, 53, 191-201.
- Foyn, E., 1964. Removal of sewage nutrients by electrolytic treatment. *Verh. Internat. Verein. Limnol*, XV, 569.
- Frobisher, M., 1957. *Fundamentals of Microbiology*. 6th Edition, W. B. Saunders Company.
- Funk, W. H., and Gaufin, A. R., 1965. Control of taste-and-odor-producing algae in Deer Creek Reservoir. *Trans. Amer. Microscop. Soc.*, 84, 263.

- Gates, W. E., and Borchardt, J. A., 1964. Nitrogen and phosphorus extraction from domestic waste-water treatment plant effluents by controlled algal culture. *Journal of Water Pollution Control Fed.*, 36, 4, 443-462.
- Geoghegan, M. J., 1953. Algal culture from laboratory to pilot plant. *Carnegie Inst. of Washington, Publication 600*, 187.
- Gerloff, G. C., and Skoog, F., 1954. Cell content of nitrogen and phosphorus as a measure of their availability for growth of Microcystis aeruginosa. *Ecology*, 35, 3, 348-353.
- Gerloff, G. C., and Skoog, F., 1957. Nitrogen as a limiting factor for the growth of Microcystis aeruginosa in Southern Wisconsin Lakes. *Ecology*, 38, 4, 556-561.
- Gerloff, G. C., Fitzgerald, G. P., and Skoog, F., 1950. The mineral nutrition of Coccochloris peniocyctis. *Amer. J. Botany*, 37, 835.
- Gerloff, G. C., Fitzgerald, G. P., and Skoog, F., 1952. The mineral nutrition of Microcystis aeruginosa. *Amer. J. Botany*, 39, 26.
- Gessner, F., 1958. Die Binnengewasser. In "Handbuch der Pflanzenphysiologie" (W. Ruhland, ed.), Vol. IV, 179, Springer-Berlin.
- Goering, J. J., and Neess, J. C., 1964. Nitrogen fixation in two Wisconsin lakes. *Limnol. and Oceanog.*, 9, 4, 530-539.
- Golueke, C. G., and Oswald, W. J., 1965. Harvesting and processing sewage-grown planktonic algae. *Journal of Water Pollution Control Fed.*, 37, 4, 471-497.
- Gorham, P. R., 1960. Toxic waterblooms of blue-green algae. *Canadian Vet. Jour.* 1, 235.
- Gotaas, H. B., Oswald, W. J., Ludwig, H. F., and Lunch, V., 1951. Algae symbiosis in sewage oxidation ponds. 2nd Prog. Rep., University of California, Institute of Engineering Research.
- Gotaas, H. B., Oswald, W. J., and Ludwig, H. E., 1954a. Photosynthetic reclamation of organic wastes. *Scientific Monthly*, 79, 6, 368.
- Gotaas, H. B., Oswald, W. J., and Golueke, C.G., 1954b. Algal-bacterial symbiosis in sewage oxidation ponds. 5th Prog. Rep., University of California, Institute of Engineering Research.
- Gotaas, H. B., and Golueke, C. G., 1958. Recovery of algae from waste stabilization ponds. *University of California, IER Bulletin Issue No. 7 and 8, Series 44*, 1957, 1958.

- Greig-Smith, P., 1964. Quantitative Plant Ecology. 2nd Edition, 34, Butterworths, London.
- Hanson, A. M., and Flynn, T. F., 1964. Nitrogen compounds in sewage. Proc. 19th Int. Waste Conf., Purdue University Engineering Extension, Series No. 117, 32.
- Hartmann, H., 1960. Untersuchungen über die biologische Reinigung von Abwasser mit Hilfe von Tauchtropfkörpern. Doctorate Thesis at Technical High School, Stuttgart.
- Harvey, H. W., 1945. Recent advances in the chemistry and physics of sea water. Cambridge University Press.
- Hattingh, W. H. J., 1963. Activated sludge studies. I. The nitrogen and phosphorus requirements of the micro-organisms. Water and Waste Treatment, July, August.
- Helmers, R. N., Frame, J. D., Greenberg, A. E., and Sawyer, C. N., 1951. Nutritional requirements in the biological stabilization of industrial wastes. Sew. Ind. Wastes, 23, 7, 884.
- Helmers, R. N., Frame, J. D., Greenberg, A. E., and Sawyer, C. N., 1952. Nutritional requirements in the biological stabilization of industrial wastes. Sew. Ind. Wastes, 24, 4, 496.
- Hewitt, E. J., 1959. The metabolism of micro nutrient elements in plants. Biol. Rev., 33, 333.
- Hewitt, E. J., 1958. The role of mineral elements in the activity of plant enzyme systems. In "Handbuch der Pflanzenphysiologie" (W. Ruhland, Ed.), Volume IV, Springer, Berlin.
- Hinden, E., and Dunstan, G. H., 1960. Effect of detention time on anaerobic digestion. Journal of Water Pollution Control Fed., 32, 930-938.
- Hunter, J. V., and Heukelekian, H., 1965. The composition of domestic sewage fractions. Journal of Water Pollution Control Fed., 37, 8, 1142.
- Hutchinson, G. E., 1957. "A treatise on Limnology", Volume 1, Geography, Physics and Chemistry. Wiley, New York.
- Ippen, A. T., 1953. Carnegie Institute of Washington, Publication 600, Washington, D. C., 259-272.
- Jeffrey, E. A., and Morgan, P. F., 1959. Oxygen demand of digested sludge liquor. Sew. and Ind. Wastes, 31, 1, 20.
- Jegge, C., 1965. Precipitation of phosphates as a third stage of sewage purification. Chimia, 19, 484.

- Johnson, W. K., and Schroepfer, G. J., 1964. Nitrogen removal by nitrification and denitrification. *Journal of Water Pollution Control Fed.*, 36, 8, 1015.
- Kalle, K., 1958. Das Meerwasser als Mineralstoffquelle der Pflanze. In "Handbuch der Pflanzenphysiologie." (W. Ruhland, Ed.), Volume IV, 170. Springer, Berlin.
- Kaneshige, H. M., 1959. Stabilization ponds for the removal of algal nutrients from sewage and sewage effluents. Ph.D. Thesis, University of Wisconsin.
- Kaplovsky, A. J., 1951. Volatile acid production during digestion of seeded, unseeded and limed fresh solids. *Sew. and Ind. Wastes*, 23, 713-721.
- Katchman, B. J., 1961. Phosphates in life processes. In "Phosphorus and its Compounds." Volume II, Interscience Pub. Inc., New York.
- Kevern, N. R., and Ball, R. C., 1965. Primary productivity and energy relationships in artificial streams. *Limnol. and Oceanog.*, 10, 1, 74-87.
- Klein, L., 1959. River pollution. I. Chemical Analysis, Butterworths, London.
- Klotter, H. E., 1964. Investigations into the removal of orthophosphates and condensed phosphates from sewage, a contribution to the problem of the third stage of sewage purification. *Vom Wasser*, 31, 181.
- Kluyver, A. J., and Verhoeven, W., 1954. Studies on true dissimilatory nitrate reduction. II. The mechanism of denitrification. *Leewenhoek ned. Tijdschr.*, 20, 241-62.
- Kolbe, F. F., 1965. A promising new unit for sewage treatment. *The Civil Engineer in South Africa*, 7, 12, 327-333.
- Kott, Y., and Ingerman, R., 1966. The biochemical dynamics of waste stabilization ponds. *Int. J. Air Water Pollution*, 10, 603.
- Krauss, R. W., 1956. Photosynthesis in the algae. *Ind. and Engineering Chem.*, 48, 9.
- Krauss, R. W., 1958. Physiology of the freshwater algae. *Ann. Rev. Plant Physiol.*, 9, 207.
- Kreft, G., Van Eck, H., and Stander, G. J., 1958. A short note on the possibility of removing ammonia from sewage effluents by raising the pH. *Water and Waste Treatment Journal*, July, August.

- Lackey, J. B., and Sawyer, C. N., 1945. Plankton productivity of certain Southeastern Wisconsin lakes as related to fertilization. *Sew. Wks. Jour.*, 17, 3, 573.
- Lackey, J. B., 1958. Effect of fertilization of receiving waters. *Sew. Ind. Wastes*, 30, 1411.
- Laughlin, J. E., 1964. Waste treatment in a new beet-sugar plant. Prepr. 4th Ind. Water Waste Conf., Texas Water Pollution Cont. Assn., D2-D20.
- Lea, W. L., Rohlich, G. A., and Katz, W. J., 1954. Removal of phosphates from treated sewage. *Sew. and Ind. Wastes J.*, 26, 3.
- Levin, G. V. and Barnes, J. M., 1964. Froth flotation for harvesting algae and its possible application to sewage treatment. Proc. 10th Ind. Waste Conf., Purdue University of Engineering Extension, Series No. 117.
- Levin, G. V., and Shapiro, J., 1965. Metabolic uptake of phosphorus by wastewater organisms. *Journal of Water Pollution Control Fed.*, 37, 6, 800-821.
- Lough, R. C., 1956. Review of sewage treatment processes and practice overseas and in New Zealand. Pollution Advisory Council, Publication No. 2, Wellington, New Zealand.
- Lüdmann, D., and Kayser, H., 1966. Contributions on the toxicity of weed-killers to the biocoenose in waters. II. Fish-food organisms. *Gas-u, WassFach*, 107, 256.
- Ludzack, F. J., and Ettinger, M. B., 1962. Controlling operation to minimize activated sludge effluent nitrogen. *Journal of Water Pollution Control Fed.*, 34, 9.
- MacFadyen, A., 1963. *Animal Ecology*, 241, Pitman, London.
- MacKenthun, K. M., and Ingram, W. M., 1967. Biological associated problems in freshwater environments. U. S. Department of Interior. *Federal Water Pollution Control Administration*, 34.
- MacKenthun, K. M., and McNabb, C. D., 1961. Stabilization pond studies in Wisconsin. *Journal of Water Pollution Control Fed.*, 33, 12, 1234-1251.
- Malhotra, S. K., Lee, G. F., and Rohlich, G. A., 1964. Nutrient removal from secondary effluent by alum flocculation and lime precipitation. *Int. J. Air Water Pollution*, 8, 487.

- Malhotra, S. K., 1963. Nutrient removal from secondary effluent by alum flocculation and lime precipitation. J. Appl. Chem. Abstract 13.
- Maloney, T. E., and Palmer, C. M., 1956. Toxicity of six chemical compounds to thirty cultures of algae. Water and Sew. Wks., November.
- Maloney, T. E., 1966. Detergent phosphorus effect on algae. Journal of Water Pollution Control Fed., 38, 1, 38-45.
- Marais, G.V. R., 1966. New factors on the design, operation and performance of waste stabilization ponds. Bulletin W.H.O., 34, 737-763.
- McCarty, P. L., and McKinney, R. E., 1961. Volatile acid toxicity in anaerobic digestion. Journal of Water Pollution Control Fed., 33, 223-232.
- McCarty, P. L., Jeris, J. S., and Murdoch, W., 1962. Proc. 17th Ind. Waste Conf., Purdue University, Engineering Extension, Series No. 112.
- McGauhey, P. H., Elliassen, R., Rohlich, G., Ludwig, H. G., and Pearson, E. A., 1963. Comprehensive study on protection of water resources of Lake Tahoe Basin through controlled waste disposal. Lake Tahoe Area Council, Al Tahoe, California.
- McKinney, R. E., 1956. The role of chemically combined oxygen in biological systems. Proc. Amer. Soc. Civil Engr., 82, 4, 1053.
- McKinney, R. E., and Conway, R. A., 1957. Chemical oxygen in biological waste treatment. Sew. and Ind. Wastes, 29, 10, 1097.
- McNall, E. G., and Atkinson, D. E., 1957. Nitrate reduction. II. Utilization of possible intermediates as nitrogen source and as electron acceptors. J. Bact., 74, 60-6.
- Milner, H. W., 1951. Possibilities in photosynthetic methods for production of oils and proteins. J. Amer. Oil Chem. Soc., 28, 363-367.
- Moeller, D. H., 1957. Effect of temperature on plant nutrient removal from sewage effluent by algae. Masters Thesis, University of Wisconsin.
- Myers, J., 1951. Physiology of the algae. Ann. Rev. Microbiol., 5, 157-180.
- Myers, J., 1953. Carnegie Institute of Washington, Publication 600. Washington, D. C.
- Myers, J., and Graham, J., 1961. On the mass culture of algae. III. Light diffusers; High versus Low temperature Chlorellas. Plant Physiology, 36, 342-346.

- Myers, J., 1957. Algal cultures. Encyclopedia of Chemical Technology. First Supplement. Volume. The Interscience Encyclopedia, Inc., New York.
- Nason, A., and Takahashi, H., 1958. Inorganic nitrogen metabolism. Ann. Rev. Microbiol., 12, 203.
- Nason, A., 1962. Symposium on metabolism of inorganic compounds. II. Enzymatic pathways of nitrate, nitrite, and hydroxylamine metabolisms. Bact. Rev., 26, 16.
- Neel, J. K., McDermott, J. H., and Monday, C. A., 1961. Experimental lagooning of raw sewage at Fayette, Missouri. Journal of Water Pollution Control Fed., 33, 6, 603-641.
- Neos, C., and Varma, M. N., 1966. The removal of phosphate by algae. Water and Sewage Works, December, 456-460.
- Nicholas, D. J. D., 1963. Biochemistry of nitrogen fixation. In "Symbiotic associations, 13th Symposium Soc. Gen. Microbiol." Ed. P. S. Nutman and B. Mosse, 92-124, Cambridge University Press.
- Odum, E. P., 1961. Factors which regulate primary productivity and heterotrophic utilization in the ecosystem. In "Algae and Metropolitan Wastes." Tech. Report W61-3; U. S. Department of Health, Education and Welfare.
- Oglesby, R. T., and Edmondson, W. T., 1966. Control of eutrophication. Journal of Water Pollution Control Fed., 38, 9.
- Oswald, W. J., Gotaas, H. B., Ludwig, H. F., and Lynch, V., 1953a. Algae symbiosis in oxidation ponds. II. Growth characteristics of Chlorella pyrenoidosa cultured in sewage. Sew. Ind. Wastes, 25, 1, 26.
- Oswald, W. J., Gotaas, H. B., Ludwig, H. F., and Lynch, V., 1953b. Algae symbiosis in oxidation ponds. III. Photosynthetic oxygenation. Sew. Ind. Wastes, 25, 6, 692.
- Oswald, W. J. and Gotaas, H. B., 1955. Photosynthesis in sewage treatment. ASCE Proc. No. 686, September.
- Oswald, W. J., 1962. The coming industry of controlled photosynthesis. Amer. Jour. Pub. Health, 52, 2, 235-242.
- Oswald, W. J., Golueke, C. G., Cooper, R. C., Gee, H. K., and Bronson, J. C., 1963. Water reclamation, algae production and methane fermentation in waste ponds. Int. J. Air Water Pollution, 7, 627.

- Oswald, W. J., 1963. The high-rate pond in waste disposal. Developments in Industrial Microbiology. Am. Inst. Biol. Sci., Washington, D. C., Volume 4, 112-119.
- Overbeck, J., 1961. The phosphatases of Scenedesmus quadricauda and their ecological importance. Verh. Int. Limnol., 14, 226-231.
- Owen, R., 1953. Removal of phosphorus from sewage effluent with lime. Sew. and Ind. Wastes J., 26, 3, 261.
- Painter, H. A., and Viney, M., 1959. Composition of a domestic sewage. J. Biochem. and Microbiol. Technol. and Engng., 1, 2, 143.
- Parker, C. D., 1962. Microbiological aspects of lagoon treatment. Journal of Water Pollution Control Fed., 34, 149-161.
- Phinney, H. K., and Peek, C. A., 1961. Klamath Lake, an instance of natural enrichment. Algae and Metropolitan Wastes. U. S. Public Health Service. SECTR W61-3, 22-27.
- Pipes, W. O., 1962. Production of large concentrations of a filamentous alga on organic wastes. 17th Ind. Waste Conf., Purdue University, Engineering Extension, Series No. 112.
- Pipes, W. O., and Gotaas, H. B., 1960. Utilization of organic matter by Chlorella grown in sewage. Appl. Microbiol., 8.
- Pipes, W. O., 1962. pH variation and BOD removal in stabilization ponds. Journal of Water Pollution Control Fed., 34, 1140.
- Pomeroy, L. R., Smith, E. E., and Grant, C. M., 1965. The exchange of phosphate between estuarine water and sediments. Limnol. and Oceanog., 10, 2.
- Pöpel, F., 1959. Developments in Public Health Engineering in Germany. Proc. Inst. Civ. Engrs., December.
- Powell, C. K., Chaddock, J. B., and Dixon, J. R., 1965. The motion of algae in turbulent flow. Biotechnology and Bioengineering, 7, 295-308.
- Prescott, G. W., 1960. Biological disturbances resulting from algal populations in standing waters. The ecology of the algae, Spec. Pub. No. 2. Pymatuning Laboratory of Field Biology. University of Pittsburgh, 22-27.
- Pringsheim, E. G., 1959. Heterotrophie bei Algen und Flagellaten. In "Handbuch der Pflanzenphysiologie." (W. Ruhland, ed), Volume II, 303, Springer, Berlin.

- Provasoli, L., 1961. Micro-nutrients and heterotrophy as possible factors in bloom production in natural waters. In "Algae and Metropolitan Wastes." Tech Report W61-3. U. S. Department of Health, Education and Welfare.
- Pyper, G. R., Weber, W. J., and Borchardt, J. A., 1966. Mass culturing of algae in controlled light-dark detention systems. I. Effects of culture density. Proc. 21st Ind. Waste Conf., Purdue University, Engineering Extension, Series No. 121.
- Redfield, A. C., 1958. Amer. Scientist, 46, 205.
- Rieke, F. F., and Gaffron, H., 1942. Flash saturation and reaction periods in photosynthesis. Jour. Phys. Chem., 47, 299-308.
- Rohlich, G. A., and Sarles, W. B., 1949. Chemical composition of algae and its relationship to taste and odor. Taste and Odor Control Journal, 18, 1.
- Rohlich, G. A., 1964. Methods for the removal of phosphorus and nitrogen from sewage plant effluents. In "Advances in Water Pollution Research," Volume 2, Pergamon Press, 207-214.
- Rupp, R. S., and DeRoche, S. E., 1965. Standing crops of fishes in three small lakes compared with C¹⁴ estimates of net primary productivity. Trans. Amer. Fish Soc., 94, 9.
- Ryther, J. H., 1956. Limnol. and Oceanog., 1, 61.
- Sacks, L. E., and Barker, H. A., 1949. The influence of oxygen on nitrate and nitrite reduction. J. Bacteriol., 58, 11-22.
- Sacks, L. E., and Barker, H. A., 1952. Substrate oxidation and nitrous oxide utilization in denitrification. J. Bact., 64, 247-52.
- Sarles, W. B., 1961. Madison's lakes: must urbanization destroy their beauty and productivity. In "Algae and Metropolitan Wastes." Tech. Report W61-3. U. S. Department of Health, Education and Welfare.
- Sato, R., 1956. In 'Inorganic Nitrogen Metabolism.' Ed. W. D. McElroy and B. Glass, 167, John Hopkins Press, Baltimore.
- Saubert, S., 1957. Amino acid deamination by Nitzschia thermalis and Scenedesmus bijugatus. South African Journal of Science, August, 335-339.
- Saunders, G. W., 1957. Interrelations of dissolved organic matter and phytoplankton. Botanical Rev., 23, 6, 389.

- Sawyer, C. N., 1944. Fertilization of lakes by Agricultural and Urban drainage. *Journal N. E. Water Wks. Assn.*, 61, 925.
- Sawyer, C. N., 1952. Some new aspects of phosphates in relation to lake fertilization. *Sew. Ind. Wastes*, 24, 6, 768.
- Sawyer, C. N., 1965. Eutrophication of lakes by domestic drainage. *Jour. Am. Water Wks. Assn.*, 29, November.
- Sawyer, C. N., and Bradney, L., 1945. Rising of activated sludge in final settling tanks. *Sew. Wks. J.*, 17, 6, 1191.
- Sawyer, C. N., 1956. Biological treatment of sewage and industrial wastes. Reinhold Publishing Company, New York, 3-17.
- Sawyer, C. N., 1965. Problem of phosphorus in water supplies. *Am. Wat. Wks. J.*, November 1965, 1431-1439.
- Sawyer, C. N., 1966. Basic concepts of eutrophication. *Journal of Water Pollution Control Fed.*, 38, 5, 737-744.
- Schmidt, E. L., 1954. Nitrate formation by a soil fungus. *Science*, 119, 187.
- Schulze, K. L., 1966. Biological recovery of waste water. *Journal of Water Pollution Control Fed.*, 38, 12, 1944-1958.
- Schumacher, G. J., and Whitford, L. A., 1965. Respiration and P³² uptake in various species of freshwater algae as affected by a current. *J. Phycol.*, 1, 78-80.
- Schütte, K. H., and Elsworth, J. F., 1954. The significance of large pH fluctuations observed in some South African vleis. *J. Ecol.*, 42, 148.
- Shapiro, J., and Ribeiro, R., 1965. Algal growth and sewage effluent in the Potomac estuary. *Journal of Water Pollution Control Fed.*, 37, 7.
- Skerman, V. B. D., Lack, J., and Millis, N., 1951. Influence of oxygen concentration on the reduction of nitrate by a Pseudomonas sp. in the growing culture. *Aust. J. Scientific Res.*, 4, 512-25.
- Skerman, V. B. D., and MacRae, I. C., 1957a. The influence of oxygen on the reduction of nitrate by adapted cells of Pseudomonas denitrificans. *Can. J. Microbiol.*, 3, 215-30.
- Skerman, V. B. D., and MacRae, I. C., 1957b. The influence of oxygen availability on the degree of nitrate reduction by Pseudomonas denitrificans. *Can. J. Microbiol.*, 3, 505-530.

- Skerman, V. B. D., and MacRae, I. C., 1961. The influence of oxygen on the formation of nitratase in Pseudomonas denitrificans. Can. J. Microbiol. 7, 169-74.
- Sketelj, J., and Rejic, M., 1966. Pollutational phases of Lake Bled. In "Advances in Water Pollution Research." Proc. 2nd Intl. Conf. Water Pollution Res., Pergamon Press, London. Volume 1, 345.
- Slechta, A. F., and Culp, G. L., 1967. Water reclamation studies at the South Tahoe public utility district. Journal of Water Pollution Control Fed., 39, 5.
- Slobodkin, L. B., 1964. Growth and regulation of animal populations. 137. Holt, Rinehart and Winston.
- Snedecor, G. W., 1956. Statistical Methods. 5th Ed., Section 2,7. Iowa State University Press, Ames, Iowa.
- Standard Methods for the examination of water, sewage, and industrial wastes. 1965. 12th Ed. Amer. Pub. Health Association, New York.
- Steel, E. W., and Gloyna, E. G., 1955. Concentration of radioactivity in oxidation ponds. Sew. Ind. Wastes, 27, 8, 941.
- Steeman Nielson, E., 1955. The production of organic matter by the photoplankton in a Danish lake receiving extraordinarily great amounts of nutrient salts. Hydrobiologia, 7, 68.
- Stumm, W., and Morgan, J. J., 1962. Stream pollution by algal nutrients. 12th Annual Conf. Sa. Engng. Univ. Kansas, 16-26. Harvard University Sanitary Engng. Reprint No. 45.
- Stumm, W., 1964. Advances in water pollution research, Volume 2, 216-220, Pergamon Press, London.
- Sylvester, R. O., and Anderson, G. C., 1964. A lake's response to its environment. J. San. Engng., Div. Proc. Amer. Soc. Civ. Engr., 90, SA1, 1.
- Sylvester, R. O., and Seabloom, R. W., 1965. Quality of impounded water as influenced by site preparation. Dept. Civ. Engng., University of Washington, Seattle.
- Symons, J. M., 1957. The biochemistry of nitrogen in the synthesis of activated sludge. Ph.D. thesis, Mass. Inst. of Tech., Cambridge.
- Symons, J. M., and McKinney, R. E., 1958. The biochemistry of nitrogen in the synthesis of activated sludge. Sew. and Ind. Wastes, 30, 7, 874.

- Talling, J. F., 1962. Freshwater Algae. In "Physiology and Biochemistry of Algae." R. A. Lewin, Ed. Academic Press, New York and London.
- Tamiya, H., et al., 1953. Kinetics of growth of Chlorella, with special reference to its dependence on quantity of available light and on temperature. Carnegie Inst. of Washington, Publication 600, 204.
- Tamiya, H., et al., 1953. Correlation between photosynthesis and light-independent metabolism of the growth of Chlorella. Biochim. et biophys. acta, 12, 23-40.
- Taniguchi, S., and Itagaki, E., 1959. Solubilization and purification of particulate nitrate reductase of an aerobically grown Escherichia coli. Biochem. et Biophys. Acta 31; 294-295.
- Taub, F. B., and Dollar, A. M., 1965. Control of protein level of algae, Chlorella. J. of Food Science, 30, 2, 359-364.
- Thomas, E. A., 1965. The eutrophication of lakes and rivers, cause and prevention. Robert A. Taft San. Engng. Center, Publication No. 999-WP-25. U. S. Department of Health, Education and Welfare.
- Thomas, E. A., 1966. Phosphate elimination in the activated sludge plant at Mannendorf and phosphate fixation in lake and sewage sludge. Vjschr. naturf. Ges. Zürich., 110, 419.
- Van Vuuren, L. R. J., Meiring, P. G. J., Henzen, M. R., and Kolbe, F. F., 1965. The floatation of algae in water reclamation. Int. J. Air Wat. Poll., 9, 823-32.
- Van Vuuren, L. R. J., and Van Duuren, F. A., 1965. Removal of algae from waste water maturation pond effluent. Journal of Water Pollution Control Fed., 1256-1262.
- Verhoeven, W., 1956. Some remarks on nitrate metabolism in microorganisms. In 'Inorganic Nitrogen Metabolism.' Ed. W. D. McElroy and B. Glass, 61, John Hopkins Press, Baltimore.
- Vinberg, G. G., 1965. Cultivation of green planktonic algae in sewage. J. Sci. Fd. Agric., 16, 275.
- Water Pollution Research Laboratory, 1967. Domestic sewage - Load per person per day. Water Pollution Contr., 66, 2, 193.
- Weideman, V. E., and Bold, H. C., 1965. Heterotrophic growth of selected waste stabilization pond algae. J. Phycol., 1, 66-69.
- Weiss, C. M., Shannon, E. M., and Bruce, T. K., 1964. Pilot studies on the use of oxidation ponds for tertiary treatment of sewage effluents. Proc. 13th Munic. Ind. Waste Conf., 97.

- Weissner, W., 1962. Inorganic Micronutrients. In "Physiology and Biochemistry of Algae." R. A. Lewin, Ed. Academic Press, New York and London.
- Welch, P. S., 1952. Limnology. 2nd Ed. McGraw-Hill Book Company, Inc.
- Whitford, L. A., and Schumacher, G. J., 1964a. Effect of current on mineral uptake and respiration by fresh water algae. Limnol. and Oceanog., 6, 4.
- Whitford, L. A., and Schumacher, G. J., 1964b. Effect of a current on respiration and mineral uptake in Spirogyra and Oedogonium. Ecology, 45, 1.
- Witt, V., and Borchardt, J. A., 1960. The removal of nitrogen and phosphorus from sewage effluent through the use of algal culture. J. Biochem. and Microbiol. Technol. and Engng., 2, 2, 187-203.
- Wright, R. T., and Hobbie, J. E., 1966. Use of glucose and acetate by bacteria and algae in aquatic ecosystems. Ecology, 47, 3, 447.
- Wuhrmann, K., 1964. Nitrogen removal in sewage treatment processes. Verh. Internat. Verein. Limnol., 15, 580-596.
- Wuhrmann, K., 1965. Waste water treatment with particular reference to phosphate elimination. SVF Fachorg. Text. Veredl., 20, 432.
- Wuhrmann, K., 1957. Die dritte Reinigungsstufe: Wege and bisherige Erfolge in der Eliminierung eutrophierender Stoffe. Schwitz. Z. Hydrol., 19, 409.
- Wuhrmann, K., 1960. Effect of oxygen tension on biochemical reactions in biological treatment plants. Paper No. 3, Conf. on Biological Waste Treatment, Manhattan.
- Wuhrmann, K., 1964. Nitrogen removal in sewage treatment processes. Verh. Internat. Verein. Limnol., 15, 580.

APPENDIX 1 to 7.

APPENDIX 1

Determination of Total Phosphate

A mixture of nitric and sulphuric acids was used to convert all inorganic and organic phosphorus to ortho phosphate.

Method

A digestion mixture containing 100 ml concentrated sulphuric acid, 500 ml concentrated nitric acid and 400 ml distilled water was used.

For samples containing up to 1 mg PO_4 /1.5 ml of the acid mixture was used. For solid samples containing higher concentrations of phosphate larger amounts were added.

The solutions were evaporated to the evolution of sulphuric acid fumes and small additions of nitric acid were made, if necessary, to obtain a clear digestate for phosphate analysis.

APPENDIX 2

Identification of isolated denitrifying organism by
Dr. E. Billing, Department of Microbiology,
University of Reading.

Morphology Oxoid CM3, 3 days at 20°C. Short straight rods, sides parallel, ends rounded arranged singly and in pairs. Monomorphic. Gramnegative. Motile (CMI, 24 hours at 20°C) Single polar flagellum (CM3, 24 hours at 20°C, electron microscopic examination)

Colony CM3, 3 days at 20°C. Moderately good growth. Off-white, translucent, circular, convex, entire margin, smooth, shiny, 1.0 - 2.0 mm. diameter. In old cultures the margin of the colony becomes crenate.

Growth in liquid medium CMI, 3 days at 20°C. Good growth. Thin off-white pellicle, uniform turbidity, small viscid deposit.

<u>Antibiotic sensitivity</u>	Penicillin	-
	Chloramphenicol	-
	Terramycin	-
	Aureomycin	-
	Polymyxin B	+
	0/129 (2:4 - diamino - 6:7 - diisopropyl-pteridine)	-
Kovacs' oxidase	+ 24 hours at 20°	
Catalase	+ 24 hours at 20°	
Indol production	- 8 days at 20°	
Methyl red test	- 8 days at 20°	
Voges-Proskauer test	- 8 days at 20°	
Ammonia from tryptone	+ (weak) 8 days at 20°	
Nitrate reduction	+ 8 days at 20°	
	Gas observed at 1 day, NO ₃ ⁻ reduced	
	beyond NO ₂ ⁻ in 8 days	

Christiansen's Urea	Alkaline in 4 days at 20°
Thornley's Arginine	Alkaline in 2 days at 20°
Hugh & Leifson's medium (glucose)	Oxidative
Peptone water sugars:	
Glucose	-
Lactose	-
Sucrose	-
Maltose	-
Mannitol	-
Glycerol	weak acid (7 days)
Starch	-
Litmus Milk	Alkaline in 4 days at 20°C
Gelatin stab	No liquefaction in 14 days at 20°C
King's media	No diffusible pigments or fluorescence.

This organism is similar to Ps. stutzeri (as described by Stanier, et al.) in quite a number of features, but does not (under the conditions used here) liquefy gelatin or produce acid from starch. It is possible, however, that when using other methods, enzymes for both gelatin liquefaction and starch hydrolysis can be demonstrated.

APPENDIX 3

Nitrogen Concentration	Flask No.	C:N Ratio	Time from start (minutes)				
			0	60	120	180	255
NO ₃ -N mg/l	1	3:1	32.1	24.4	14.2	ND	ND
	2	4:1	30.3	25.3	13.3	ND	ND
	3	5:1	30.7	26.2	14.0	ND	ND
	4	6:1	30.3	24.6	12.4	ND	ND
	5	10:1	28.5	24.6	11.5	ND	ND
	6	16:1	31.2	26.2	10.2	ND	ND
	7	0:1	30.3	27.5	27.5	29.1	27.1
NO ₂ -N mg/l	1	as above	Trace	0.3	0.8	1.8	ND
	2		Trace	0.2	0.8	Trace	ND
	3		Trace	0.3	0.7	ND	ND
	4		Trace	0.3	0.9	ND	ND
	5		Trace	0.3	0.7	ND	ND
	6		Trace	0.3	0.8	ND	ND
	7		Trace	Trace	Trace	Trace	Trace
Kjeldahl-N mg/l	1	as above	16.2	-	-	-	22.4
	2		15.4	-	-	-	25.2
	3		12.6	-	-	-	36.4
	4		18.2	-	-	-	22.4
	5		13.2	-	-	-	25.2
	6		23.8	-	-	-	23.8
	7		19.6	-	-	-	19.6
NH ₄ -N mg/l	1	as above	ND	-	-	-	5.6
	2		ND	-	-	-	8.4
	3		7.0	-	-	-	5.6
	4		11.2	-	-	-	9.8
	5		ND	-	-	-	5.6
	6		9.8	-	-	-	2.8
	7		ND	-	-	-	9.8

Table 4. Effect of glucose-C:NO₃-N ratio on denitrification.

Temperature = 25°C. Initial pH = 7.05. Final pH = 7.15.

ND = Not Detectable.

APPENDIX 4

Nitrogen Concentration	Flask No.	C:N Ratio	Time from start (minutes)						
			0	30	120	150	180	210	240
NO ₃ -N mg/l	1	0.25	31.6	28.0	23.0	22.6	-	20.8	21.7
	2	0.5	31.6	23.5	17.6	17.2	-	14.0	13.1
	3	1.0	31.6	26.6	13.1	7.6	-	11.3	10.4
	4	1.5	31.6	26.6	9.5	6.8	-	4.1	4.1
	5	2.0	31.6	27.5	4.9	ND	-	ND	ND
	6	3.0	31.6	23.5	0.4	ND	-	ND	ND
	7	4.0	31.6	26.2	0.9	ND	-	ND	ND
	8	0.0	31.6	29.8	27.5	29.3	-	26.6	27.1
NO ₂ -N mg/l	1		ND	0.1	0.1	0.2	0.2	0.1	0.1
	2		ND	Trace	0.5	0.3	0.5	0.4	0.2
	3	as	ND	Trace	0.4	0.1	0.4	0.3	0.2
	4		ND	Trace	0.4	0.2	0.5	0.4	ND
	5	above	ND	Trace	0.4	0.2	ND	ND	ND
	6		ND	Trace	0.2	ND	ND	ND	ND
	7		ND	Trace	0.1	ND	ND	ND	ND
	8		ND	ND	ND	ND	ND	ND	ND
Kjeldahl-N mg/l	1		28.0	-	-	-	-	-	22.4
	2		29.4	-	-	-	-	-	33.6
	3	as	15.4	-	-	-	-	-	35.0
	4		18.8	-	-	-	-	-	23.8
	5	above	16.8	-	-	-	-	-	21.0
	6		22.4	-	-	-	-	-	23.8
	7		29.4	-	-	-	-	-	37.8
	8		25.2	-	-	-	-	-	23.8
NH ₄ -N mg/l	1		8.4	-	-	-	-	-	14.0
	2		7.0	-	-	-	-	-	15.4
	3	as	7.0	-	-	-	-	-	11.2
	4		1.4	-	-	-	-	-	11.2
	5	above	4.2	-	-	-	-	-	9.8
	6		7.0	-	-	-	-	-	5.6
	7		ND	-	-	-	-	-	5.6
	8		2.8	-	-	-	-	-	1.4

Table 5. Effect of glucose-C:NO₃-N ratio on denitrification.

Temperature = 25°C. Initial pH = 7.0. Final pH = 7.2.

Nitrogen Concentration and Organism	C:NO ₃ -N	Time from start (minutes)							
		0	60	120	155	215	275	335	1045
<u>Pseudomonas</u> isolate NO ₃ -N mg/l	1.2:1	29.6	23.5	13.3	9.0	8.4	7.4	3.4	Trace
	1.5:1	28.0	20.3	12.9	10.8	Trace	ND	ND	ND
	1.7:1	30.0	23.5	11.7	6.8	Trace	ND	ND	ND
	1.9:1	29.4	21.7	11.5	7.5	Trace	ND	ND	ND
	0:1	30.3	30.2	28.7	30.3	30.0	28.0	27.5	24.4
Kjeldahl-N mg/l	as	9.8	-	-	-	-	-	-	ND
	above	12.6	-	-	-	-	9.8	-	-
		2.8	-	-	-	-	16.8	-	-
		23.8	-	-	-	-	2.8	-	-
		26.6	-	-	-	-	-	-	2.8
<u>P. stutzeri</u> NO ₃ -N mg/l	as	32.1	24.4	17.6	12.4	6.7	ND	Trace	ND
	above	31.4	24.4	17.4	12.0	6.8	ND	Trace	ND
		29.8	22.8	15.8	9.9	5.4	ND	Trace	ND
		29.8	26.9	26.4	25.3	27.1	26.2	24.4	Trace
		31.4	28.2	27.5	27.5	27.1	26.2	22.8	17.8
Kjeldahl-N mg/l	as	4.2	-	-	-	-	-	4.2	-
	above	ND	-	-	-	-	-	7.0	-
		ND	-	-	-	-	-	2.8	-
		ND	-	-	-	-	-	-	5.6
		ND	-	-	-	-	-	-	2.8

Table 6.: Denitrification by Ps. stutzeri and the isolated Pseudomonas sp. glucose as carbon source. Temperature = 25°C.

Nitrogen Concentration	Carbon Source	C:N Ratio	Time from start (minutes)						
			0	60	120	180	240	300	1350
NO ₃ -N mg/l	Starch	2:1	32.1	28.5	26.6	21.2	15.8	-	ND
	Starch	3:1	28.0	28.7	27.5	25.2	21.2	-	ND
	Glucose	2:1	28.0	27.3	18.1	10.8	ND	ND	ND
	Glucose	3:1	30.3	27.5	19.9	6.3	ND	ND	ND
	Fructose	2:1	32.5	28.9	22.6	11.7	Trace	ND	ND
	Fructose	3:1	32.5	26.6	22.1	11.3	ND	ND	ND
	Lactose	2:1	29.4	29.6	29.8	27.3	26.2	-	ND
	Lactose	3:1	31.6	30.3	29.8	28.5	26.6	-	ND
	Glycine	2:1	31.4	29.4	28.0	26.2	28.0	-	20.3
	Glycine	3:1	31.4	29.1	27.4	26.2	26.6	-	19.4
	Glutamic acid	2:1	29.8	29.1	29.8	23.5	17.2	-	ND
	Glutamic acid	3:1	31.2	27.1	26.6	23.9	16.7	-	ND
	Acetic acid	2:1	32.1	28.7	25.7	21.7	16.7	-	ND
	Acetic acid	3:1	32.1	28.7	25.7	22.6	15.3	-	ND
Control	0:1	32.1	28.0	30.2	29.6	28.4	-	23.5	
Kjeldahl-N mg/l	Starch		23.8	-	-	-	-	-	16.8
	Starch		25.2	-	-	-	-	-	21.0
	Glucose		15.4	-	-	-	-	4.2	-
	Glucose		18.2	-	-	-	-	15.4	-
	Fructose		12.6	-	-	-	-	11.2	-
	Fructose		14.0	-	-	-	-	28.0	-
	Lactose		11.2	-	-	-	-	-	19.6
	Lactose	as above	11.2	-	-	-	-	-	15.4
	Glycine		56.0	-	-	-	-	-	42.0
	Glycine		56.0	-	-	-	-	-	61.6
	Glutamic acid		29.4	-	-	-	-	-	18.2
	Glutamic acid		35.0	-	-	-	-	-	23.8
	Acetic acid		15.4	-	-	-	-	-	4.2
	Acetic acid		-	-	-	-	-	-	9.8
Control		12.6	-	-	-	-	-	7.0	
NH ₄ -N mg/l	Starch		ND	-	-	-	-	-	ND
	Starch		ND	-	-	-	-	-	ND
	Glucose		ND	-	-	-	-	ND	-
	Glucose		ND	-	-	-	-	ND	-
	Fructose		ND	-	-	-	-	ND	-
	Fructose		ND	-	-	-	-	ND	-
	Lactose		ND	-	-	-	-	-	ND
	Lactose	as above	ND	-	-	-	-	-	ND
	Glycine		ND	-	-	-	-	-	ND
	Glycine		ND	-	-	-	-	-	ND
	Glutamic acid		14.0	-	-	-	-	-	ND
	"		7.0	-	-	-	-	-	5.6
	Acetic acid		1.4	-	-	-	-	-	ND
	"		ND	-	-	-	-	-	ND
Control		1.4	-	-	-	-	-	ND	

Table 7. Use of various carbon compounds as hydrogen donors.

Temperature = 25°C.

APPENDIX 7

Nitrogen Concentration	Carbon Source	C:N Ratio	Time from start (minutes)					
			0	120	240	300	360	1350
NO ₃ -N mg/l	Glucose	2:1	30.5	11.9	ND	ND	ND	ND
	Glucose	3:1	31.2	13.1	ND	ND	ND	ND
	Acetic acid	2:1	29.4	19.0	7.2	Trace	ND	ND
	Acetic acid	3:1	29.4	19.0	8.1	Trace	ND	ND
	Propionic acid	2:1	30.5	26.2	20.3	15.1	9.0	ND
	Propionic acid	3:1	32.1	25.3	19.0	11.5	8.4	ND
	Butyric acid	2:1	30.7	27.1	23.5	21.7	19.9	ND
	Butyric acid	3:1	30.5	26.2	20.8	19.2	17.4	ND
	Control	0:1	31.2	28.7	26.0	26.6	28.0	22.1
Kjeldahl-N mg/l	as	as	9.8	-	-	22.4	-	-
			5.6	-	-	2.8	-	-
			2.8	-	-	-	-	2.8
			4.2	-	-	-	-	9.8
	above	above	8.4	-	-	-	-	ND
			5.6	-	-	-	-	ND
			ND	-	-	-	-	ND
			ND	-	-	-	-	12.6
9.8	-	-	-	-	12.6			
NH ₄ -N mg/l	as	as	ND	-	-	2.8	-	-
			ND	-	-	ND	-	-
			ND	-	-	-	-	4.2
			ND	-	-	-	-	4.2
	above	above	ND	-	-	-	-	4.2
			ND	-	-	-	-	7.0
			ND	-	-	-	-	7.0
			ND	-	-	-	-	2.8
ND	-	-	-	-	4.2			

Table 8. Use of lower fatty acids as hydrogen donors.

Temperature = 25°C.