THE ROLE OF BACTERIA

IN THE

SOLUBILISATION OF DIATOM FRUSTULES

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I declare that this thesis has been composed by myself and that the results described are my own.

ABSTRACT & SUMMARY

The primary objective of this study was to determine the role of bacteria in the solubilisation of the silicon in diatom frustules. The dissolution of diatom frustules and the internal cycling of silicon within a freshwater lake, where input from external sources is not significant, appears to be a major factor controlling the growth of the important primary producers; the diatoms. A review of the literature showed that there was very little information available on the relationship between frustule dissolution and microbial activity.

The following seven aspects were considered:

A. *The Effect of Natural Water Bacterial Populations on Silica Release from Diatom Cultures*

1. Natural populations of Loch Leven and Lough Neagh water bacteria enhanced the release of soluble silica from an axenic *Cyclotella meneghiniana* culture, compared with sterile autolysis, at 25°C. In the inoculated cultures 50% to 60% of the silica was released over a period of 30 days.

2. Natural populations of Loch Leven water bacteria also enhanced the release of soluble silica from xenic (contaminated with bacteria) cultures of *Asterionella formosa, Tabellaria flocculosa, Navicula pelliculosa* and *C. meneghiniana* compared with control cultures sterilised with $HgCl_2$ (lg./l.). Similar results were obtained with Lough Neagh populations incubated with *A. formosa*. Incubation was at 25°C. The amount of silica released varied with the diatom type. The maximum percentage silica released was 75% to 80%, after 45 days incubation.

3. The rate of silica release from xenic cultures of *A. formosa* and *T. flocculosa* in the presence of Loch Leven water bacteria, incubated at 4°C, was greater than a $HgCl_2$ sterilised control, but considerably less than at 25°C.

B. The Effect of Nutrient Availability on Natural Water Bacterial Populations and the Bacteria Contaminating the Diatom Cultures

1. The addition of nutrient supplements of glucose and Cas-amino acids to xenic suspensions of *N. pelliculosa* in Loch Leven water, at 25°C, did not influence the silica release. The addition of glucose (1g./l.) reduced the net amount of dissolved silica released. Similar results were obtained for xenic *C. meneghiniana* and Loch water with glucose (1g./l.).

2. Silica release in the presence of the bacteria contaminating the diatom cultures was greatest in the presence of all the nutrients available in heat sterilised Loch Leven water. Suspension in only the salt solution of the algal growth medium reduced the percentage of soluble silica released.

3. Natural water populations and diatom culture contaminants enhanced the release of silica to different extents.

C. The Effect of Agitated Incubation and Inoculation of Broken Diatoms on Silica Release

1. Increased agitation of suspensions of xenic *C. meneghiniana* did not influence the silica release, which suggests that oxygen is not limiting under these conditions.

2. The breaking of diatom cells by ultra-sonication did not enhance the release of silica to any greater extent than that obtained for unbroken cells in the presence of the water bacterial populations.

D. The Effect of Pure Bacterial Cultures on Silica Release

1. Pure cultures of Loch Leven bacteria enhanced the release of silica from xenic *A. formosa* and axenic *C. meneghiniana* compared with sterile control treatments. Cultures varied in the extent to which the silica was released.

2. Silica release in the presence of the one bacterium investigated was related to the disappearance of the Ruthenium Red staining component of the wall.

3. The extent of enhanced silica solubilisation could be related to the range of hydrolases produced by the bacteria. These were tentatively identified as *Erwinia* sp., *Flavobacterium* sp. and a *Pseudomonas* sp. The latter was least effective in silica release. The growth of these bacteria was not restricted to diatoms. Growth was also observed on the cyanobacterium, *Oscillatoria redekei*.

E. The Formation of Clumps of Diatoms

1. Natural populations of Loch Leven and Lough Neagh water bacteria caused the diatoms to form clumps.

2. This phenomenon was related to specific members of the bacterial population, but clumping did not appear to influence the release of silica.

F. *Preliminary Survey of the Natural Bacterial Population of Loch Leven*

A preliminary survey of the dominant bacteria in Loch Leven water, and associated with diatom cells, showed that they were predominantly Gram negative rods. An initial assessment of their physiological properties, possibly related to silica solubilisation, showed hydrolysis of tributyrin but not of starch or gelatin.

G. *The Relevance to Silicon Cycling in Loch Leven and Lough Neagh*

The results of this study have been discussed in relation to other factors influencing diatom dissolution which are apparent in the natural environments of Loch Leven and Lough Neagh. The interaction of temperature and sediment invertebrate activity with microbial activity are considered to be important.

I. INTRODUCTION AND LITERATURE REVIEW

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1. THE IMPORTANCE OF DIATOMS IN WORLD PRIMARY PRODUCTIVITY

Diatom communities are found in all habitats where moisture and light are available; some even being capable of withstanding periods of desiccation. Within these habitats they exist either as plankton, or associated with surfaces e.g. as epiphytes or in the benthos of lakes and streams, (Patrick, 1977).

Their importance lies not only in their ubiquitous nature, but also in their contribution to primary production. It is estimated that they are responsible for 20 to 25% of world net primary production, mostly arising from the growth of planktonic species in the oceans. This is similar to the contribution made by the Poacae (grasses) present in the savannas, grasslands and cultivated areas, (Werner, 1977).

2. ALGAL PRODUCTIVITY AND EUTROPHICATION

Algal productivity in a freshwater lake is determined by a number of factors including temperature, light, the morphometry of the individual lake and the availability of nutrients. The two essential plant nutrients, nitrogen and phosphorus, are particularly important in relation to the phenomenon of eutrophication. Eutrophication occurs naturally with a progressive increase in plant nutrients from nutrient poor, or oligotrophic, to a eutrophic stage of high algal productivity. The deposition of algal cells leads to silting up and eventual disappearance of the lake. In the absence of man's increasing presence and activities this process occurs on a time scale measured in geological ages, rather than the life-span of men, (Warren, 1971).

The acceleration of eutrophication occurs where increases in the human population, often coincidental with increased industrial or agricultural activity, result in the rise in the concentration of nitrogen and phosphorus compounds, the production of dense planktonic algal populations or 'blooms' and changes in the species composition of lakes. This is often referred to as 'cultural eutrophication'. The algal flora changes from species adapted to low nutrient concentrations, to those which grow at high nutrient concentrations. In general blooms are composed of diatoms or cyanobacteria , their frequency and magnitude increasing over the years, and in the final stages, the flora is dominated by cyanobacteria. Lake Erie, North America and Lake Zurich, Switzerland are two notable examples of lakes which have reached this stage of cultural eutrophication, (Warren, 1971). The discharge of domestic effluent containing nitrogen rich human waste and phosphate based detergents along with run-off from agricultural land to which nitrate containing fertilisers have been applied can be two major sources of these nutrients (McCaull & Crossland, 1974). The presence of dense algal blooms limits the aesthetic and recreational value of a lake, the water being no longer suitable for swimming or fishing. The growth of particular species of algae which produce unpleasant odours and tastes (Palmer, 1977) may render the water unpotable, and the clogging of filters in water treatment plants may increase costs.

The loss of a bathing or fishing lake and an increase in the cost of water filtration, where a lake is used as a domestic water supply, may appear a small problem when compared to the massive scale of water management required in areas such as the Sudan, Saudi Arabia and India. In such areas, although water is perhaps not lacking in total quantity, it may not be present in 'the right place at the right time', leading

to widespread poverty and famine, (Overman, 1976). However a lack of concern about water supply in temperate regions, where water is generally taken for granted, has led to these problems which have even occurred in some of the largest bodies of freshwater in the world; the Great Lakes of North America. Only if appropriate action is taken now will the availability of potable water be prevented from becoming as serious as that of regions where water supply has always been more unpredictable.

In the British Isles, the two shallow lakes Loch Leven, Kinross, Scotland and Lough Neagh, Co. Antrim, Northern Ireland have been subject to cultural eutrophication. A major part of the algal productivity is attributable to diatoms and factors affecting diatom growth may be particularly important for the whole ecology of the lakes.

3. A GENERAL DESCRIPTION OF THE TWO LAKES

a. LOCH LEVEN

Loch Leven lies in the fertile plain of Kinross, Scotland. Apart from the north and south deeps, dead-ice hollows left behind at the end of the last glaciation, the loch is shallow with a mean depth of 3.9m., (see map Fig. 1). The brown trout of Loch Leven are world famous and were appreciated locally as long ago as 1633 when an act of Parliament was passed to protect the spawning grounds in the inflowing streams.

Since the beginning of this century occasional blue-green algal blooms have been recorded, but since 1963 algal blooms have occurred each year. This seems to be related to:

a. an increase in the discharge of domestic effluent from the small towns of Kinross and Milnathort, and from a woollen mill, and
b. the run-off of nitrogen, originating from fertiliser application, as 70% of the catchment area is agricultural land. Thus in recent

Fig. 1 Map of Loch Leven, Kinross (Depth in metres)
- Northern Deep area
- Harbour

years the loch has moved from a moderately rich nutrient level, to one of extreme eutrophication, (Morgan, 1974). The shallowness and exposed position of the loch results in a thorough mixing of the water and a thermocline is rarely formed, except for short periods of time in the deeps, if the weather is warm and calm during the summer. The thorough mixing and rich nutrient supply all contribute to the high biomass and rates of phytoplankton production which are observed in the loch (Bindloss, 1974). Macrophytes are relatively sparse, (Jupp, Spence & Britton, 1974) phytoplankton making the largest contribution to primary production, (Bailey-Watts, 1976a). Diatoms contribute more to the high biomass than any other algal group, predominating in the benthos as well as in the water column. However cyanobacteria and occasionally green algae can dominate for some periods, (Bailey-Watts, 1974).

A comprehensive report of most of the aspects of Loch Leven which have been studied can be found in the Proceedings of the Royal Society Edinburgh, (1974). Table 1 summarises some of the relevant details.

b. LOUGH NEAGH

Lough Neagh is the largest freshwater lake in the British Isles, however despite covering approximately 30 times the area of Loch Leven, the morphometry and gross chemistry of the two lakes are very similar, (Gibson *et al.*, 1971), (see Table 1). The deepest point of the Lough is a 33m. trench in the northwest corner, (see map, Fig. 2), the average depth of the lough being about 8m. The water is continually mixed vertically and horizontally as local winds are strong and occur frequently. Thermal stratification is rare, and only occurs for brief periods, (Battarbee, 1978a).

Table 1. Some of the Bathymetric and Chemical Characteristics of Loch Leven and Lough Neagh

	Loch Leven*	Lough Neagh**	Units
Surface Area	13.3	367	Km^2
Max. Depth	25.51	34	m.
Mean Depth	3.9	8.6	m.
Total volume	52.4×10^6	3.15×10^9	m^3
pH	7.2 - 9.3	7.9 - 9.4	
Soluble Phosphate	0.002 - 0.040	0.002 - 0.065	mgl^{-1}
Nitrate Nitrogen	0.1 - 1.9	0.2 - 0.9	mgl^{-1}
Silica (SiO_2)	0.1 - 11	0.1 - 7	mgl^{-1}
Chlorophyll 'a'	10 - 260	10 - 80	μgl^{-1}
Period during which chemical data obtained	1968 - 1971	1968 - 1970	

*Proc. of the Royal Soc. Edinb. (1974).

**Gibson et al., (1971).

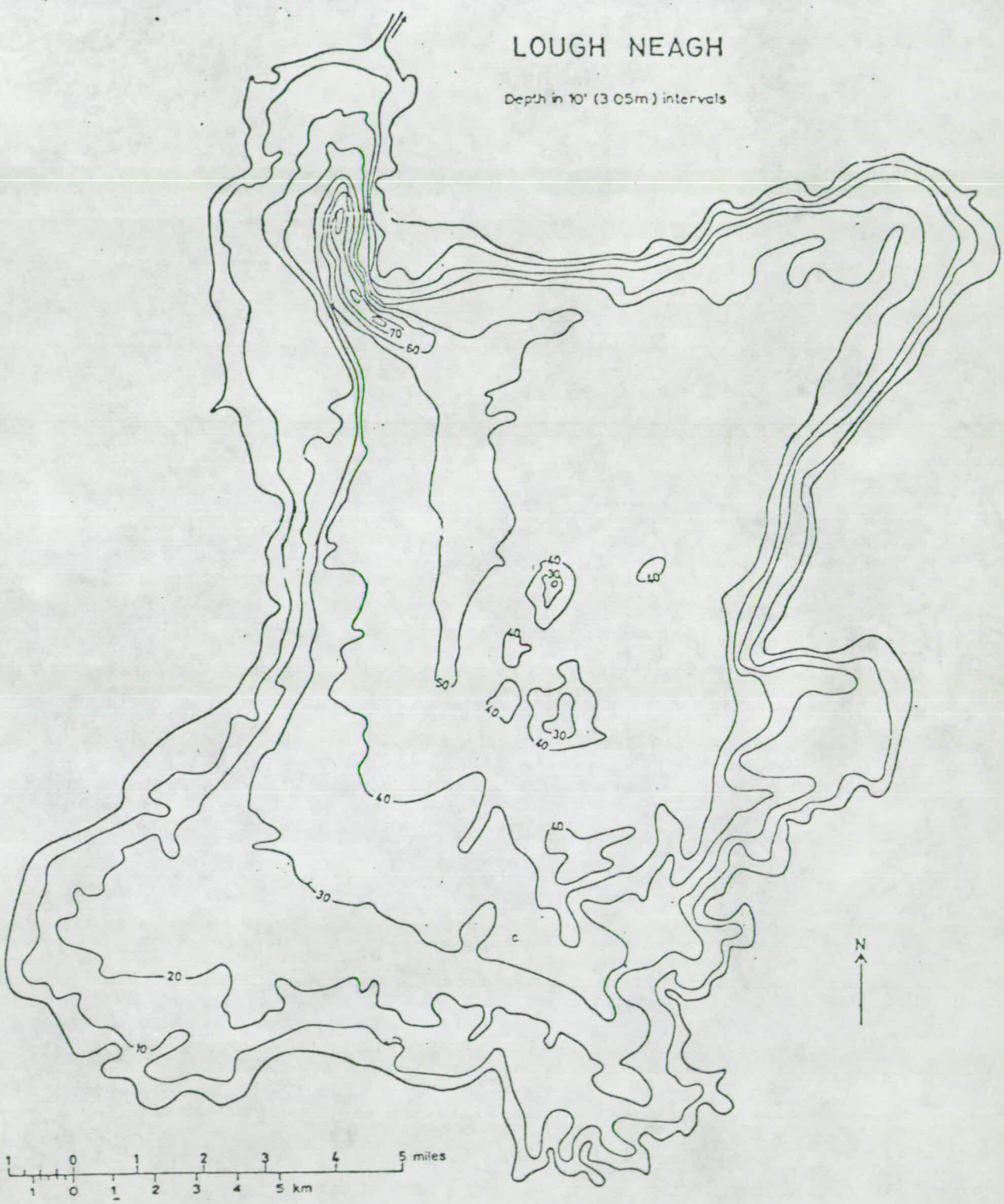


Fig. 2 Map of Lough Neagh, Northern Ireland

The catchment area of Lough Neagh is largely agricultural land, although the increase in algal productivity of recent years may also be related to an increase in the domestic sewage input as a result of the installation of mains sewage services and the construction of local authority housing estates, together with the introduction of phosphate rich detergents, (Battarbee, 1978a).

Diatoms and cyanobacteria are the major contributors to primary production. Although the actual species contributing to the crops usually varies between the two lakes, it has been noted that *Oscillatoria redekei* was the dominant species in both lakes during the period from January to June, 1969.

In general Loch Leven supports an appreciably higher crop of phytoplankton, for example in 1969 chlorophyll 'a' varied between 10 and 260µg/l in Loch Leven and 10 and 60µg/l in Lough Neagh. Phosphate-P concentrations tend to be similar, however Nitrate-N is higher in Loch Leven, the maximum being approximately double that of Lough Neagh (Gibson et al., 1971). Details of this can be found in Table 1.

4. THE ROLE OF SILICA IN DIATOM GROWTH IN THE TWO LAKES

Diatoms have an absolute requirement for silica (further details can be found on p.22) and in general the rise and fall of the soluble silica concentration in these two lakes forms an inverse relationship with the diatom crop volume, the diatom maxima coinciding with the lowest levels of dissolved silica. However, there have been instances in Loch Leven when both diatom numbers and silica concentration were increasing or decreasing. Therefore the relationship between planktonic diatom populations and the dissolved silica concentration may at times be complicated by zooplankton grazing or the uptake of dissolved silica by benthic diatoms, (Bailey-Watts, 1976a).

The replenishment of dissolved silica after a diatom maximum has been shown to be the result of re-cycling within the lakes, as opposed to input from external sources. During periods of low diatom activity in Loch Leven the dissolved silica concentration can build up at a rate of 0.11 - 0.16mg $SiO_2 l^{-1} d^{-1}$, where the highest rate of replenishment from the inflows is less than 0.092mg $SiO_2 l^{-1} d^{-1}$. A silica budget for Loch Leven over a 27 day period in October 1972 was carried out by Bailey-Watts (1976b). The release of soluble silica from sediment core samples was examined, and from this it was calculated that the silica input from the sediments was 0.126mg $SiO_2 l^{-1} d^{-1}$. At this time the inflows only accounted for 0.02mg $SiO_2 l^{-1} d^{-1}$. From these observations, both in the field and the laboratory, it can be seen that sediment input can account for as much as six times more silica than the streams.

In Lough Neagh during the period from June to October, 1970 the dissolved silica concentration rose from 0.67mg l^{-1} to 5.04 mg l^{-1}. Over the same period the input from the major rivers was only sufficient to raise the concentration by 0.82mg l^{-1}, the sediment input being about five times greater, (Battarbee, 1978a).

It has been suggested that the most likely source of the re-cycled silica is the dissolution of diatom shells or frustules, this being the 'most soluble' particulate silica present, (Bailey-Watts, 1976b; Dickson, 1975). The presence of large numbers of recognisable diatom frustules in the sediments could be accounted for if only partial dissolution of the frustule surface occurred, (Battarbee, 1978a).

5. SILICON CHEMISTRY IN AN AQUEOUS ENVIRONMENT

Depending on the pH and concentration, silica can exist in an aqueous environment at either end of the equilibrium

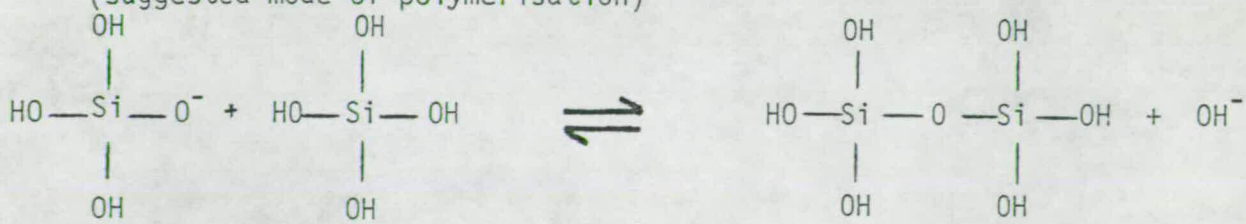
silicic acid (monomeric) $\rightleftharpoons$ amorphous silica (polymeric)

Fig. 3 illustrates the various steps in this equilibrium over the complete pH range and the relative concentration of ionic species in equilibrium with amorphous silica. In natural waters the dissolved silica is predominantly present as silicic acid (Stumm & Morgan, 1970). The concentration and pH range found in Lough Neagh and Loch Leven have been inserted on the graph. From this it would be expected that within the lakes amorphous silica would remain insoluble, without the influence of some factor other than the pH of the whole water body. It is interesting to note that the silica concentration in Lough Neagh in 1970 was increasing at its greatest rate when the pH was in the region of 7.8 to 8.4 and not in June when the pH was just above 9, i.e. it was impossible to identify a causal relationship between gross pH measurement and silica release, (Dickson, 1975).

The process of polymerisation and condensation which results in the formation of amorphous silica gel can be summarised as follows:

1. Monomeric silicic acid $\rightleftharpoons$ polysilicic acid particles

(suggested mode of polymerisation)



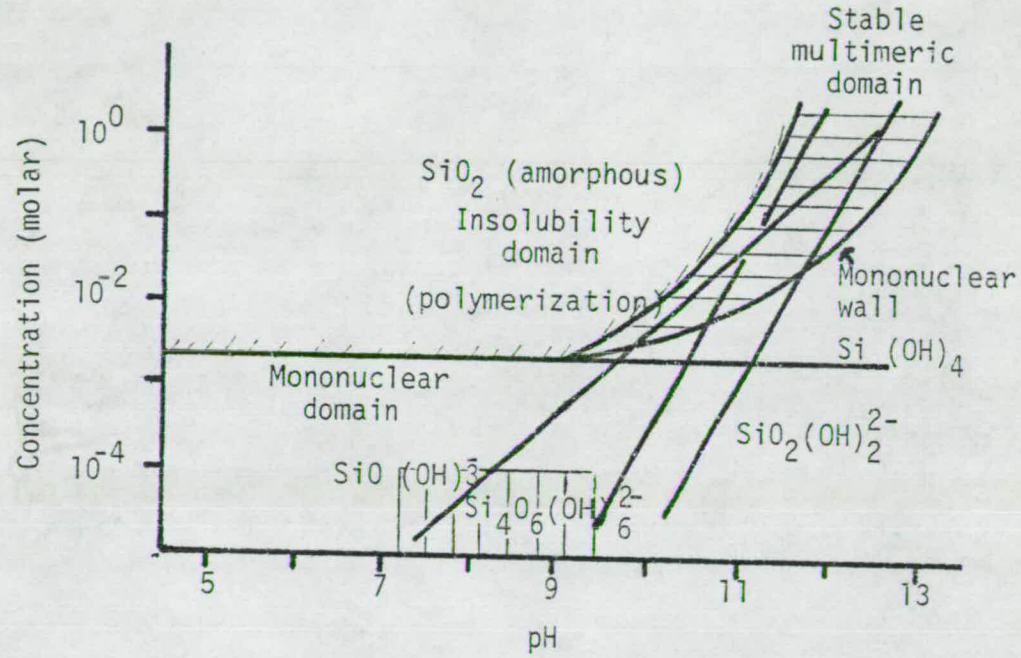
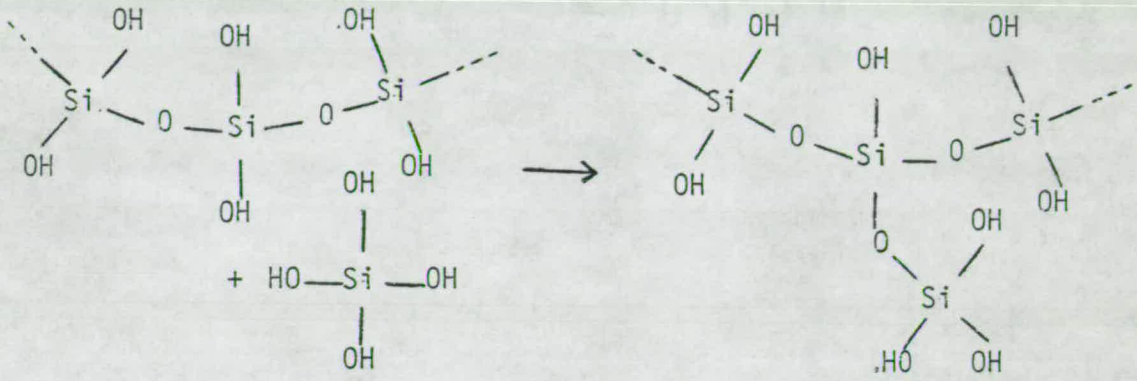


Fig. 3 Ionic species in equilibrium with amorphous silica at 25°C (after Stumm & Morgan, 1970)

□□□ pH & SiO_2 concentration in L. Neagh & L. Leven

2. More monosilicic acid is added at the surface of the colloidal polysilicic acid particles by condensation. The polysilicic acid particles are spheroid, amorphous and isotropic, (i.e. their physical properties do not vary with direction).



3. The particles then aggregate by crosslinking and physical bonding, the colloidal sol finally becoming a gel, (Greenberg & Sinclair, 1955).

The solubility of amorphous silica in water is also related to the specific surface area (i.e. the number of free SiOH groups) and is influenced by the presence of metallic contamination. Any loss of surface area results in the silica being more insoluble, (Eitel, 1975).

6. SILICON IN RELATION TO BIOLOGICAL SYSTEMS

Silicon has been shown to play a role in the growth of animals, plants and microorganisms.

a. PLANTS

Silicon is essential for the growth of diatoms (Bacilliarophycae) (See detailed description p. 22) and silicified flagellates (Chrysophycae) as well as for a few higher plants e.g. *Equisetum arvense*, *Oryza sativa* and *Nicotiana tabacum*. The silicon has a skeletal

role, being used to form spines, needles or shells. The silicon of these structures is in the form of hydrated amorphous silica (SiO_2nH_2O), similar to the structure of silica gel (Lewin and Reimann, 1969).

Silica is also present in some members of the green algae (Dinophycae) and cyanobacteria (Cyanophycae) isolated from marine plankton. There is no evidence that silica is essential for the development of these algae, and they do not form silicaeous shells. It has been suggested that silica uptake in this case may be a mechanism by which these algae limit the growth of diatoms (Furman et al., 1978). That other algae could go to such lengths to compete is perhaps some measure of the evolutionary success of diatoms.

b. BACTERIA AND FUNGI

Although there is as yet no evidence for an absolute requirement for silica by any bacterium, it has been shown that certain soil bacteria will take up silica from their growth medium. When grown in the absence of phosphorus they will exchange phosphorus for silica, suggesting some connection with the uptake mechanism. Certain carbohydrates and amino acids stimulate silica uptake, glucose being one of the most effective, (Heinen, 1962; 1963). Examination of cells and cell free extracts show that silica is initially found in the cell walls and, as growth continues, in the cell fluid, (Heinen, 1965). Of the silica taken up by a capsulate Loch Leven water bacterium grown on medium containing glucose, 27% was found to be present in the capsule and 60% in the acapsulate cells, (L. Foster, pers. comm.).

Polysaccharides of animal and plant origin have been found to contain silicon bound by ester and ether linkages, which is only released by hydrolysis with strong acid or alkali. It has been suggested that silicon may function as a cross-linking agent, forming $R_1 - O - Si - O-R_2$ or $R_1 - O - Si - O - Si - R_2$ bridges. The initial uptake of silica by polysaccharides may be an important stage in the metabolism of silicon in animals, plants and bacteria, (Schwarz, 1973; Varma et al., 1974; 1975).

Soil Bacteria and fungi have been found which can solubilise inorganic silicates by the production of either the chelating agent 2-keto-gluconic acid, or organic acids (e.g. oxalic and citric.) (Duff et al., 1963; Henderson & Duff, 1963; and Webley & Duff, 1965). An acid producing fungus and bacterium and an alkali producing bacterium were all reported as releasing soluble silica from minerals by Kutzova, (1969). Silicate solubilising bacteria were also isolated from the surfaces of minerals from tropical areas and their possible importance in the weathering of rocks in arid tropical regions suggested, (Sovostin, 1972). A *Proteus mirabilis* strain isolated from soil which actively removed silica from solution has also been shown to depolymerise and use silica from an 85% polymerised solution, (Lauwers & Heinen, 1974). Such solubilisation mechanisms could be of importance to diatoms growing in soil, and if they also occur in the sediments, to the cycling of silica in freshwater. Even where solubilisation is followed by bacterial uptake, the silica will be in a relatively more soluble form e.g. incorporated in a polysaccharide.

c. ANIMALS

Silica is used in the formation of skeletal structures of radiolarians (Protozoa) and sponges (Porifera) (Lewin, 1969). The

element has also been shown to be an essential dietary requirement for chicks and rats where silicon deficiency results in skull deformities and slower growth, (Schwarz & Milne, 1972).

Despite the variety of organisms which can use silica, diatoms are by far the major users as a consequence of their occurrence in a wide range of environments and their abundance within these environments. It has been estimated that 80 to 160 x 10^9 tons of silica is bound into diatom frustules each year in the oceans, and only a small proportion of this (0.3 x 10^9 tons) accumulates in the sediments, (Lisitzin, 1971). Therefore the mechanism by which frustule silica dissolves is of great importance.

7. SILICON REQUIREMENT AND UTILISATION BY DIATOMS

Silicon is an essential nutrient for diatoms, and may not only determine the size of the diatom crop, but also the content, as changes in the silica concentration have been found to correspond with changes in the diatom species present, (Kilham, 1971). Similar changes in algal flora have been associated with eutrophication, (see p. 9). Such changes are thought to relate to the different diatom species having different uptake kinetics for silicic acid (Werner, 1977).

a. THE FORMATION OF DIATOM CELL WALLS

i) The silica frustule

Diatoms are characterised by the nature of their cell wall which is composed of a silica shell or frustule and organic skin or coat, (Reimann et al., 1966). The structure of the frustule is complex with regularly arranged patterns of pores. This pattern is so regularly

repeated from one generation to another that it is used as the basis for diatom taxonomy, (Duke & Reimann, 1977).

The frustule is composed of two halves or thecae, which overlap, each theca having two elements, a valve and a girdle (See Fig. 4). Mitosis and the production of the frustules of daughter cells occurs within the parent cell; therefore with each new generation the cells become progressively smaller. Only after sexual reproduction and auxospore formation is there any increase in cell size.

The sequence of events which occur during silica uptake and culminate in the formation of the frustule can be summarised as follows:

1. Silica is generally assumed to be taken up in the form of monomeric silicic acid, however certain species are capable of using silica in a polymerised form, (Golterman, 1967). The uptake of silica is an energy requiring process, although after translocation it is considered that no further energy is required for the polycondensation of the silica within the cell, (Werner, 1977).

   Washing of *Navicula pelliculosa* cells with distilled water suppressed the uptake of silicic acid. However, this could be reversed by the addition of reduced sulphur compounds. This suggests that sulphydryl compounds, or enzymes containing such groups are involved in the uptake mechanism, (Lewin, 1967).
2. Mitosis of the daughter cells occurs within the parent frustule.
3. Silica deposition occurs within a membrane system, which is known as the silicalemma, derived from the Golgi apparatus. Paired vesicles containing the polycondensed silica appear along the plasmalemma of the two daughter cells, (Coombs & Volcani, 1968).

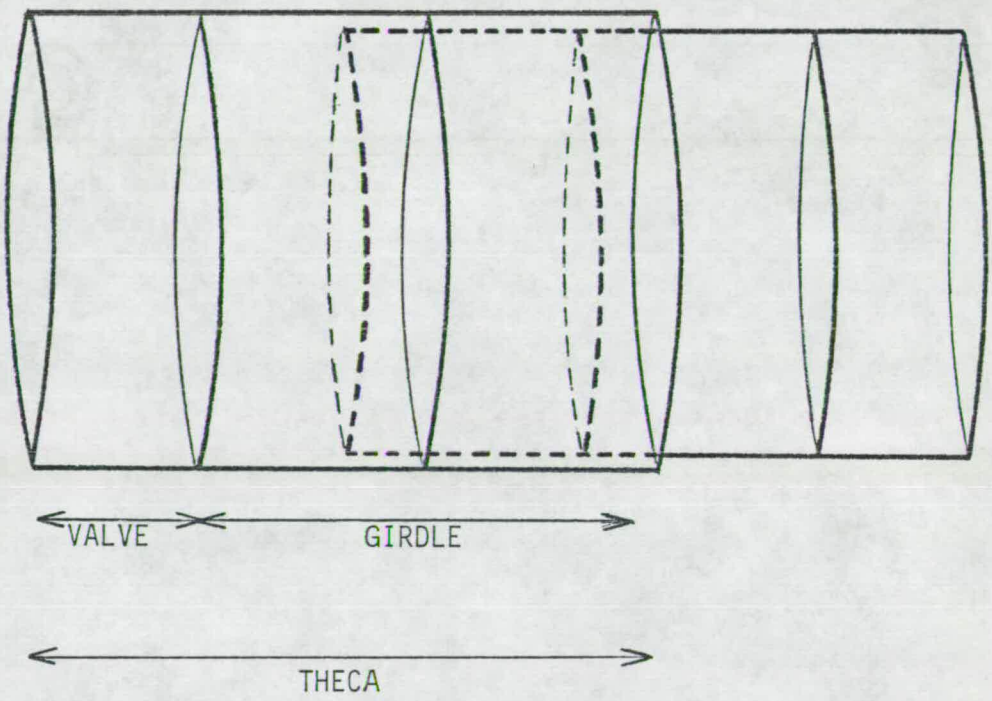


Fig. 4 Schematic diagram of a diatom frustule

4. The inner side of the silicalemma comes away from the silica shell and forms the new plasmalemma of the cell, and the outer side an outer organic coat. Subsequently more organic material is added to the inner surface of the frustule forming an inner, as well as an outer, organic coat (Lauritis et al., 1968). After deposition within the frustule the silica remains there until after the death of the diatom.
5. Protein is the major organic component being added to the wall during silica deposition, however, carbohydrate is continually added to mature cell walls, (Coombs & Volcani, 1968).

ii) The organic component

The silica of the cell wall is bound both outwardly and inwardly by organic material, forming a shell envelope, (Duke & Reimann, 1977). This organic material is closely associated with the silica component, as treatment with hydrofluoric acid vapour results in the removal of the silica, leaving empty 'skins' which retain the shape of the silica frustule, (Reimann et al., 1966).

The organic material stains specifically with Ruthenium red, suggesting the presence of polyglucuronic acids or their derivatives. This is of practical use to forensic scientists when determining if death was the result of drowning. If pink-staining diatoms are found in the alveolar spaces of the lungs it is likely that the person drowned. However, if the diatoms do not stain, they are probably derived from the inhalation of diatomaceous earth i.e. fossil diatoms (Duke & Reimann, 1977).

Examination of the carbohydrate content of the walls of *Phaedodactylum tricornutum* showed the presence of glucuronic acid (27%), and ester sulphate (0.5%) in addition to mannose. The polysaccharide was characterised as having a 1-3 linked mannopyranose backbone, occasional residues containing sulphate groups and short and long side chains containing glucopyranosyluronic acid and mannopyranose, (Ford & Percival, 1965a & b). It has been suggested that this type of polysaccharide may prove to be characteristic for this class of organism (Percival & McDowell, 1967).

The incorporation of ^{14}C labelled CO_2 into *Navicula pelliculosa* showed that 53% of the label was incorporated into amino acids, 17% into mannose, and the rest in glucuronic acid, xylose, fucose, glucose, galactose and rhamnose, during the period of silica uptake, prior to frustule formation (Coombs & Volcani, 1968). However these workers do not give any indication of the possible position of the polysaccharide or protein components in the wall.

A comparison of the amino acid and sugar content of the cell wall of the diatoms *Navicula pelliculosa, Melosira granulata, Cyclotella stelligera, Cyclotella cryptica* and *Nitzschia brevirostris* showed that the cell was enriched in serine, threonine and glycine and depleted in sulphur - containing, aromatic and acidic amino acids. The sugar content was found to be more variable, but had a tendency to be enriched in xylose and mannose in some species. From this work a model of the outer side of the wall has been suggested (see Fig. 5), the amino acids mediating the silica deposition and perhaps influencing the final shape of the frustule, (Hecky et al., 1973).

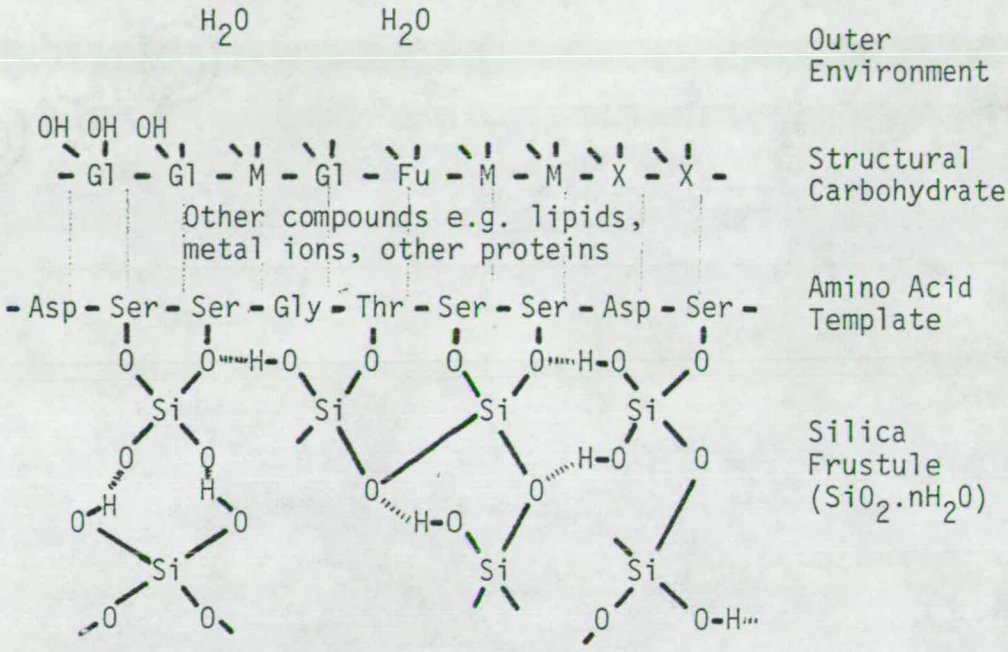


Fig. 5 Hypothetical arrangement of the organic layers in a diatom cell wall (Hecky et al., 1973).

Key:

Gl = glucose, M = mannose, Fu = fucose, X = Xylose, each sugar has two outwardly directed hydroxyl groups.

Asp = aspartic acid, Ser = serine, Thr = threonine, Gly = glycine.

Hatched lines = hydrogen bonds.

Hydroxy-proline, which is a characteristic component of higher plant cell walls was reported as being present in diatoms at low levels, (Gotelli & Clelland, 1968).

The presence of a number of distinctive polysaccharide and protein components in diatom cell walls has been reported by Nakajima & Volcani, including the non-protein amino acids ε-N-trimethyl - L- δ hydroxylysine phosphate and the non-phosphorylated form and 3,4 - dihydroxyproline. It was suggested that these amino acids serve as a matrix for silicification, in a similar manner to collagen in the process of calcification, (Nakajima & Volcani, 1969; 1970).

Lipid is a relatively minor component of the cell wall, accounting for as little as 0.8 to 1.3% of the organic matter in the wall, (Kates & Volcani, 1968).

The final picture of the organic component of the diatom cell wall is one of an outer polysaccharide layer, possibly rich in mannose, and also containing glucuronic acid, to which material is continually being added once the wall is mature, and possibly has a protective role. The second major component is protein which may act as a template for the silica component of the wall.

It remains to be determined if the structure of the organic part of the wall is a) similar for all diatoms, and b) identical on the outer and inner side of the silica frustule.

b. OTHER METABOLIC USES OF SILICA

The use of silica starvation synchrony of cell populations and ^{68}Ge and ^{31}Si as radioactive tracers by a number of investigators has

shown that silicon biochemistry can influence and control metabolism within the cell, other than in wall formation.

This has been seen to occur under laboratory conditions in the following four instances, (Werner, 1977)

1. In the citric acid cycle between acetyl - Co-A and 2-oxoglutarate
2. In the synthesis of proteins localised in cell organelles
   e.g. chromosomes, chloroplasts
3. The regulation of respiration and chrysolaminarin (a major storage carbohydrate) utilisation
4. The regulation of chlorophyll synthesis.

c. THE ADVANTAGE OF THE SILICA WALL TO THE DIATOM CELL

The process of polycondensation and polymerisation of the silica occurs as a physical process, brought about by concentration of the silica. Therefore energy is required only for uptake and translocation. It is possible that this relatively low energy requirement would put diatoms at a selective advantage when competing with organisms producing cell wall materials which have a higher demand on the cells energy resources, for example cellulose, pectin, chitin and mannans. Diatoms may also be at an advantage when competing for nutrients. The frustule has a large surface area as a result of its complex pore structure. This was calculated for the diatom *Coscinodiscus asteromphalus* as being one hundred times greater than a smooth cylinder of similar dimensions. Secondly the adsorptive properties of the silica gel-like frustule will allow them to adsorb essential nutrients from low concentrations in aquatic environments, (Werner, 1977).

8. THE SOLUBILITY OF DIATOM FRUSTULES

The release of soluble silica from diatom cultures, diatom dominated phytoplankton and fossil diatoms under a variety of different conditions has been examined to determine the importance of the re-cycling of frustule silica.

The findings of individual investigators are summarised below:

a. DIATOM CULTURES AND DIATOM DOMINATED PHYTOPLANKTON

The release of silica from cultures of methyl alcohol killed *Nitzschia linearis* and *Thalassiosira nana* was compared by Jørgensen (1955). He found that in both cases the solubility was dependent on the pH, with the optimum being in carbonate buffer at pH 10. At this pH the solubility of the *T. nana* reached a maximum after 40 days and had levelled out at the end of 60 days, with only 20% of the silica being dissolved. The *N. linearis* however was completely dissolved over the same period. The relative insolubility of the *T. nana*, even at this high pH is of interest suggesting that it cannot be assumed that a high pH alone will ensure the solubility of the frustules.

Silica dissolution from initially alive xenic cultures of *Skeletonema costatum*, *Chaetoceros gracilis* and *Thalassiosira decipiens* in sterilised natural sea water at 30°C showed that less than 10% of the silica was released from the *T. decipiens* over 10 days, whereas 50% was released from the other species over a similar period. However, the total amount of silica which had been released by the end of the experiment, expressed as a percentage of the initial silica content of the diatoms, was approximately the same, i.e. although the initial rates of release were different, the final amounts were similar. These

differences could again be attributable to differences in the species. However any possible differences in the bacteria seen to present within the "gelatinous aggregate" of decomposing cells were not taken into account, (Kamatani, 1969).

Measurement of the silica dissolution from acid-cleaned frustules (i.e. frustules with any organic material removed) of diatom cultures in sea water showed an increase with temperature. Differences were also found between the diatom *Thalassiosira decipiens* and *Rhizosolenia hebetata* during the period of silica release. Silica appeared to be released in two stages, the first being at a faster rate than the second. The *T. decipiens* dissolved at a rate seven times greater than the *R. hebetata*, but if the amount released is expressed as a percentage of the initial silica present only 45% of the *T. decipiens* was released during the first stage, compared to 80% from the *R. hebetata*. Microscopic examination of the *T. decipiens* showed that the relatively more fragile girdle region of the frustule disappeared before the more robust valve, (Kamatani & Riley, 1979).

Although it is difficult to directly compare the results of these investigators as dissolution was measured under different conditions in each instance, it is interesting to note that in all instances the silica of the *Thalassiosira* species dissolved to a lesser extent than the other diatom species examined.

Lewin (1961) examined dissolution of silica from an axenic culture of *Navicula pelliculosa* at pH 9 to 9.3 in tris buffer at 19^{o}C. She reported that any treatment which killed the cells, e.g. treatment with pepsin, trypsin, urea and bile salts (all protein-denaturing or solubilising agents) and heat-killing enhanced silica release. However

the amounts of silica released were low when compared with the release from acid-cleaned cells (i.e. 80 to 90% less).

The addition of solutions containing metal ions (0.0037M), including aluminium, iron, and titanium, to acid-cleaned cells retarded release. Metal contamination therefore lowers the solubility of silica, probably by reducing the number of free Si-OH groups (see p. 19). A similar effect was obtained when aluminium was added to heat-killed cells. Extraction of heat-killed cells with chelating agents prior to dissolution experiments enhanced silica release. Combined treatment with oxalate and EDTA resulted in silica release similar to that obtained from acid-cleaned cells.

When sea-water phytoplankton consisting predominantly of the diatoms *Skeletonema*, *Chaetoceros* and *Rhizosolenia* was heat-killed and the cells extracted with a mixture of EDTA and oxalate the silica release paralleled that obtained from acid-cleaned cells. Heat-killing alone resulted in about 70% more silica being released. It is interesting to note that this is much higher than the amount released from the axenic *N. pelliculosa*. The heat-killing of the plankton (5 minutes at 100°C) may not have been sufficient to sterilise it and the effect could be due to the growth of contaminating bacteria.

Dissolution of silica from concentrated diatom dominated plankton from Loch Leven, Kinross, suspended in filtered loch water, resulted in an increase in dissolved silica of approximately 30% of initial frustule silica, after 38 days incubation at 20°C, and 60 to 100% after 14 to 15 months. Silica release at 4 and 10°C was slower in comparison, being less than 5% after 50 days. Suspension of the plankton in de-ionised water resulted in a reduction of the percentage silica release to less than 5% at 20°C, (Bailey-Watts, 1976b).

Unfortunately differences in the pH and microbial activity were not considered. It is possible that removal of growth factors present in the loch water reduced microbial growth on the decaying diatoms and therefore also the silica release. However these experiments do show that silica can be released from diatoms present in the plankton in Loch Leven, and after a period of 14 to 15 months complete dissolution may take place.

Sterile autolysis of an axenic culture of *Stephanodiscus hantzschii* resulted in only 20 to 30% of the initial silica being released after 5 weeks. The cells were killed initially with chloroform, suspended in distilled water (pH7), and incubated at room temperature. However after the growth and death of an unspecified algal bloom in an experimental tank (1-2m^3) filled with bog water (pH8) 60% of the silica taken up by the algae was liberated over a period of 46 days, during the months of July and August, (Golterman, 1960). This difference could be attributable to microbial action, or differences in the diatom species present.

b. FOSSIL DIATOMS

The accumulation of large deposits of diatomaceous silica at various sites in the sediments of the oceans and freshwaters indicates that under certain conditions the amorphous silica of diatoms remains stable. In the oceans, regions of maximum diatom growth are mirrored by the accumulation of frustules in the bottom sediments. However the reflection is distorted in that fewer species are represented and there is a decrease in the total numbers, only 1/10 - 1/100 of the initial numbers of frustules reach the bottom, most of the dissolution occurring in the top 100m. It is likely that a similar re-cycling within the

water column occurs in the deeper lakes, the thinner frustules or parts of them dissolving before reaching the sediments, (Calvert, 1966).

A comparison of the rate of dissolution of fossil with 'recent' diatoms showed that the silica release from fossils was minimal. This could be related to the specific surface area of the frustule, it being much lower in the fossils (Lewin, 1961). Similar results were obtained by Kamatani, (1971), in which he found that recent diatom frustules dissolved in a similar manner to silica gel, whereas fossil diatomite behaved similarly to opaline silica. It therefore appears that fossil diatomite has undergone physical and possibly chemical changes which render it resistant to dissolution.

The major conclusions which can be drawn from these studies on silica dissolution are:

a. soluble silica is released from 'recent' diatom frustules, and that both the amounts and the rates of release may vary with the diatom species, pH, temperature, the manner in which the diatoms are killed, the presence or absence of the organic component of the wall and the composition of the suspending medium.
b. Different parts of the silica frustule may dissolve at different rates.
c. Fossil diatoms are relatively insoluble when compared with 'recent' frustules, probably due to changes in the nature of the silica.

However, in none of these studies was the possible effect of microbial activity examined.

9. ALGAL-BACTERIAL ASSOCIATIONS IN FRESHWATERS

Heterotrophic bacteria are usually found in association with the phytoplankton in freshwater, as they are dependent on fixed carbon compounds for growth and metabolism. In a review of the Planktonic Microflora of Freshwaters (Caldwell, 1977), an estimate of 97% epiphytic bacteria, to only 3% planktonic bacteria is quoted. These bacteria were present in the polysaccharide material associated with the algae, or directly attached, possibly with the aid of specialised structures (e.g. the holdfast and stalk of caulobacter-type bacteria). Gram negative bacteria dominate in freshwaters and the genera *Vibrio*, *Aeromonas*, *Flavobacterium*, *Achromobacter* and *Pseudomonas* are amongst those which have been reported as dominating.

The possible ecological interactions which are open to bacteria associated with a phytoplankton bloom include the following:

a. BENEFICIAL ASSOCIATIONS

These are associations which may benefit one or other of the participants, but are not detrimental to either.

Relationships can be divided into those of a general nature and those which are specific.

Phytoplankton have been shown to excrete a number of carbon containing compounds, including polysaccharides, organic acids, and amino acids. Glycollate is one of the most commonly secreted compounds and is therefore possibly the major bacterial substrate, (Caldwell, 1977). The diatom *Stephanodiscus hantzschii* has been shown to release amino acids and polysaccharide as well as glycollate, (Watt, 1969).

Utilisation of these compounds in microbial respiration could have the general effect of accelerating photosynthesis, as oxygen will be removed and carbon dioxide supplied to the algae. It has been found that the addition of sucrose to a xenic culture of *Microcystis* enhanced the growth of the algae. Sucrose also stimulated the growth of a xenic culture of *Oscillatoria rubescens*, but had no effect on an axenic culture. The addition of chelating agents to deteriorating xenic cultures which lacked sucrose partially restored viability. It therefore appears that the added carbon source prevented the bacterial degradation of metal chelates, which maintain the availability of trace elements essential for the algae. Other sugars and carbon compounds are reported as having a similar effect. Therefore algal excretion of carbon compounds which can be used by bacteria may help to prevent bacterial decomposition of other compounds necessary for algal growth, (Lange, 1967).

A more specific relationship has been reported by Paerl, (1976), in which bacteria have been shown to attach specifically to the polar regions of heterocysts of the cyanobacterium *Anabaena circinalis* and *Aphanizonemenon flos-aquae*. He suggested that respiration by the bacteria may maintain the anaerobic conditions necessary for nitrogen-fixation to occur, even while photosynthesis is taking place.

Pure cultures of *Pseudomonas* and *Flavobacterium* sp. were reported as stimulating the growth of a *Chlamydomonas* sp., also in pure culture (Delucca & McCracken, 1977). The production of vitamin B12 by bacteria has been suggested as a possible growth stimulant for phytoplankton in freshwater, (Stewart et al., 1977). It has been shown that the vitamin B12 requirement of several marine diatoms can be supplied by heterotrophic bacteria isolated from the same waters, under laboratory conditions, (Haines & Guillard, 1974).

b. INHIBITION OF ALGAE BY BACTERIA

A myxobacterium isolated from freshwater, with the ability to lyse and kill green algae, cyanobacteria and bacteria was reported by Stewart and Brown, (1969). Non-fruiting myxobacteria have also been found in association with phytoplankton in three Scottish Lochs. Fruiting myxobacteria, *Actinomyces* and *Cellovibrio* have been shown to lyse cyanobacteria under laboratory conditions. Such organisms will be less specific than lytic phage in their host range, and are generally capable of producing a wide range of degradative enzymes. However it is unlikely that the presence of such organisms can be assumed as being an indication that algal crops will be severely limited. These organisms probably co-exist with only slight variations in the populations. This has been shown to occur in continuous culture of *Anacystis nidulans* and a myxobacterium. Only when an imbalance occurs will large scale lysis of the algae ensue, (Stewart et al., 1977).

c. BACTERIAL DECOMPOSITION OF ALGAE

Examination of phytoplankton taken from Lake Windermere and Estwaithe Water (English Lake District) revealed that numbers of attached bacteria varied with algal type. Diatoms and dinoflagellates tended to have fewer attached bacteria than green algae or cyanobacteria, (Jones, 1972).

Observation of the diatom *Asterionella* in Blelham Tarn (English Lake District) during a bloom showed that heavy bacterial colonisation only occurred towards the end of its active growth phase, when 80 to 100% of algal cells examined had more than five bacteria attached. From this and observations on other algae (*Tabellaria* sp., a desmid and a

dinoflagellate) it would appear that attachment of bacteria only occurs during the decline and death of phytoplankton. This suggests the positive chemo-taxis of bacteria towards compounds, possibly leaking from a dead or dying cell, or alternatively, the breakdown of some algal 'defense mechanism' which prevents bacterial attachment, (Jones, 1976). Positive attraction of bacteria to the amino acids glycine, serine and threonine and to glucose at concentrations as low as 10^{-5}Molar have been reported by Paerl (Unpubl.).

Although the biochemical composition of diatoms varies from species to species, and also to some extent with the cultural conditions, they can offer a potentially wide range of substrates for bacterial growth. In general diatoms contain protein, carbohydrate and lipid in order of decreasing amount when grown under nutrient sufficient conditions. However when *Navicula pelliculosa* and *Cyclotella cryptica* were grown under silica limitation, the percentage carbohydrate content was reduced and the lipid increased, i.e.

	Protein		Carbohydrate		Lipid	
N. pelliculosa	42	39*	21	15*	25	34*
C. cryptica	31	25*	26	15*	12	30*

*growth silica limited

The amino acids alanine, aspartic acid, glutamic acid, glycine, leucine, and serine, are the predominant amino acids, each of these usually accounting for 7 to 10% of the total amino acids present. Glucose accounts for approximately 50% of the carbohydrate content, with galactose, mannose and rhamnose each accounting for approximately 10 to 15%. The majority of the fatty acids are C_{16} saturated and unsaturated chains, and C_{20} polyunsaturated chains. Diatoms are

therefore suitable food for bacteria with a C:N ratio varying from 3 to 7 under nutrient sufficient conditions, and reaching as high as 43 when nutrient limited, (Darley, 1977). Bacteria may attack the compounds present in the diatoms with hydrolytic enzymes or alternatively rely to some extent on the autolysis of the cells.

CONCLUSIONS

The interactions open to bacteria and algae appear to be diverse and difficult to categorise. For example, a bacterium although capable of inhibiting algal growth may be ecologically more important in processes of decomposition. The problems of determining how important interactions found in the laboratory may be in a lake are many, as laboratory work necessarily examines only a small part of the ecosystem. It should also be noted that interactions and changes can occur within micro-environments (for example the surface of a diatom frustule) and may remain undetected.

10. SUMMARY OF THE BASIS AND OBJECTIVES OF THIS STUDY

Limited published data have shown that variation occurs in the amount of silica solubilised from diatom frustules. However, the only direct evidence for bacterial involvement in this process was demonstrated by Krumbein (1978). Using scanning electron microscopy and EDX spot analysis he correlated a reduction in the silica content of the diatom frustules with the points at which bacteria were attached.

Frustule dissolution is clearly an important process in relation to the silica availability in Loch Leven, Lough Neagh and any other lake where the input of silica from external sources is not significant. As silica is essential for the growth of the important primary producers, diatoms, this is fundamental to the ecosystem of the lakes.

Four areas were considered in an attempt to obtain some insight into the bacterial role in frustule dissolution:

a. The bacterial populations attached to algae which may be involved in frustule dissolution and diatom decomposition. This study included aspects of nutrition and identification.

b. The release of soluble silica from diatom cultures under a range of laboratory conditions. This included a comparison of the silica released under sterile conditions and in the presence of bacterial populations.

c. Some possible mechanisms of bacterial action were investigated. The effect of the availability of nutrients, of static or moving incubation conditions and of disruption of diatom cells, on soluble silica release was determined.

d. Laboratory data were interpreted in relation to the physical, chemical and biological conditions in the two lakes.

II. MATERIALS AND METHODS

II MATERIALS AND METHODS

1. DIATOMS

a. CULTURES AND INCUBATION CONDITIONS FOR MAINTAINING CULTURES

The Cultures used in this study are listed in Table 2.1.

Table 2.1 Diatom Cultures and Sources

CULTURES	SOURCE
Tabellaria flocculosa var. *asterionelloides*	Culture Centre of Algae and Protozoa, Storey's Way, Cambridge
Asterionella formosa	
Navicula pelliculosa	
Cyclotella meneghiniana	River Cam, isolated by Dr. H. Belcher, CCAP
Melosira sp.	Loch Leven, isolated by Miss E. Rutowski, ITE, Edinburgh
Asterionella formosa	
Oscillatoria redekei	Lough Neagh, provided by Dr. C. Gibson, FBIU, NI.

These cultures were selected for the following reasons: *Asterionella formosa* makes an important contribution to the total phytoplankton biomass in Loch Leven. *Melosira* sp. and *Cyclotella meneghiniana* are also present, and another species of *Cyclotella*, *C. pseudostelligera*, is among the dominant phytoplankton (Bailey-Watts, 1974). *Asterionella formosa* and species of *Cyclotella*, *Tabellaria* and *Melosira*, have also been recorded in the phytoplankton of Lough Neagh, (Gibson et al., 1971). *Navicula pelliculosa* is not recorded among the phytoplankton of Loch Leven in 1968-1971; however this culture could be cultivated on solid medium. It was therefore hoped that it would be easily rendered bacteria-

free or axenic. The cyanobacterium, *Oscillatoria redekei*, is a common bloom-former in Lough Neagh and was available as an axenic culture.

All strains were sub-cultured once a month and maintained in a north or north-east facing window-sill, with a muslin screen as protection from direct sun-light. Duplicate cultures were kept in lighted incubators maintained at 12° or 15°C. The *Cyclotella meneghiniana* culture did not survive at this temperature, and was kept at 20°C. During the winter months cultures were also maintained in a 20°C green-house with supplementary illumination on an 18 hour light-dark cycle.

Incubation was static in cotton-wool stoppered 250ml. flasks containing approximately 150ml. of either Chu 10 medium or Woods Hole MBL (see pp. 44 & 45 for details). The *N. pelliculosa* was maintained on agar slopes in cotton-wool stoppered test-tubes.

Diatoms were grown in bulk in 2l. flasks containing 1l. of medium, or in the case of *N. pelliculosa* on solid medium in Roux bottles. All glass-ware was rinsed with distilled water before use.

b. ROUTINE MEDIA

Cultures were initially grown in Chu 10 medium, (Chu, 1942), with the modifications suggested in the CCAP List of Strains (George, 1976), and supplemented with soil extract as a growth factor and vitamin source (see below).

In an attempt to lower the bacterial contamination, as an aid to the production of axenic cultures, strains were subsequently maintained on the more defined medium Woods Hole MBL (Nichols, 1973). The

Oscillatoria redekei was grown on ASMI medium, as recommended by C. Gibson (pers. comm.).

The recipes for these media are detailed below.

Modified Chu 10 (pH 7.3)

$Ca(NO_3)_2.4H_2O$	20.0mg.	
KH_2PO_4	6.2mg.	
$MgSO_4.7H_2O$	25.0mg.	
Na_2CO_3	20.0mg.	
$Na_2SiO_3.5H_2O$	43.4mg.	
HCl (1N)	0.25ml.	
$EDTA.Na_2$	2.0mg.	(Added as complex EDTA. Fe)
$FeCl_3$	1.0mg.	
H_3BO_3	2.48mg.	
$MnCl_2.2H_2O$	1.39mg.	
$(NH_4)_6Mo_7O_{24}.4H_2O$	1.00mg.	
Soil extract	50ml.	

Soil extract was prepared by adding 1000g. of garden soil to 1l of tap water and autoclaving for 30 minutes. Approximately 0.5g. of $CaCO_3$ was added to the soil suspension which was filtered through double filter paper (Whatman's No.1) until clear. It was then bottled and sterilised in 50ml. amounts (Allen, 1957).

Woods Hole MBL (pH 7.2)

Prepared as stock solutions, 1ml. of each added per litre.

a. Macronutrients - 1ml. each/l.

$CaCl_2.2H_2O$	36.76g./l.
$MgSO_4.7H_2O$	36.97g./l.
$NaHCO_3$	12.6 g./l.
K_2HPO_4	8.71g./l.
$NaNO_3$	85.01g./l.
$Na_2SiO_3.5H_2O$	0.0212g. added/l. of medium (not as stock solution)

b. Micronutrients - 1ml. each/l.

Na_2EDTA	4.36g./l.
$FeCl_3.6H_2O$	3.15 g./l.
$CuSO_4.5H_2O$	0.01 g./l
$ZnSO_4.7H_2O$	0.022 g./l.
$CoCl_2.6H_2O$	0.01 g./l.
$MnCl_2.4H_2O$	0.18 g./l.
$Na_2MoO_4.H_2O$	0.006 g./l.

c. vitamins

Thiamine.HCl	0.1mg./l.
Biotin	0.5µg./l.
Cyanocobalamin	0.5µg./l.

d. Tris - 2ml./l.

Tris (Hydroxymethyl)-aminomethane	50g./200ml.

Adjust pH to 7.2 with HCl

ASMI

Prepared as stock solutions, 1ml. of each added/l.

$NaNO_3$	170.0g./l.	$Na_2EDTA.2H_2O$	1.08 g./l.
$MgCl_2.7H_2O$	49.0 g./l.	$FeCl_3.6H_2O$	2.48 g./l.
$Ca\ Cl_2.2H_2O$	29.0 g./l.	H_3BO_3	2.48 g./l.
$MgSO_4.6H_2O$	41.0 g./l.	$MnCl_2.H_2O$	1.39 g./l.
K_2HPO_4	17.4 g./l.	$ZnCl_2$	0.436 g./l.
Na_2HPO_4	14.2 g./l.	$CoCl_2.6H_2O$	0.02 g./l.
		$CuCl_2.2H_2O$	0.00014 g./l.
		$Na_2MoO_4.2H_2O$	0.006 g./l.

The pH of the medium was set to 8.5 using dilute NaOH (approx. 0.05N)

c. AXENIC DIATOMS

A sterile culture was required so that the effect of microbial action on the solubility of the frustule could be determined. Axenic diatom cultures were not available from external sources. In some experiments mercuric chloride (1g./l.) was added to the controls. However, a sterile control which could remain untreated was preferable, as the release of silica from diatoms which were initially alive could then be investigated. Therefore the following methods were carried out in an attempt to obtain axenic diatom cultures.

i. Single Cell Isolation

Xenic diatom cultures were streaked onto water agar, (Difco Bacto-agar), and individual cells were picked off using a micro-needle and a micro-manipulator. These were then placed on fresh sterile agar blocks and transferred onto agar slopes or into a liquid medium.

Attempts were also made to isolate diatoms from Loch Leven phytoplankton. This method did not prove to be successful. Only in one instance did a *N. pelliculosa* clone grow, and this was not bacteria-free. It is possible that the diatoms which were cultured in liquid medium did not react favourably to attachment to the solid agar surface, (e.g. *A. formosa*, *T. flocculosa* and Loch Leven diatoms). However the *N. pelliculosa* was already in culture on solid medium. One explanation for the lack of growth may be that the cells were damaged by manoeuvering with the micro-needle. This was required to break the surface tension of the water film on the agar. When the needle was placed on a cell of for example, *A. formosa*, a sharp tap on the surface on which the microscope and micro-manipulator were sitting was sufficient to snap the frustule.

ii. Treatment with Antibiotics

The method outlined by Droop (1969) was employed, in which a concentrated algal culture was exposed for 24 hours to six, two-fold serial dilutions of the filter-sterilised antibiotic mixture:

	mg./ml.
Benzyl penicillin sulphate	8000
Streptomycin sulphate	1600
Chloramphenicol	200

These were then sub-cultured into both Woods Hole MBL and Chu 10 media. Duplicate cultures of *C. meneghiniana*, *N. pelliculosa*, *T. flocculosa* and *A. formosa* were treated.

In all instances the untreated control culture grew. In one case the *T. flocculosa* grew at the greatest dilution of antibiotic, but this culture was not axenic. Microscopic examination showed the presence of

bacteria, which suggests that at these concentrations the diatoms were sensitive to the antibiotics and the bacteria were not.

iii. Washing of Individual Cells

The isolation and washing of individual cells by successive transfer into fresh sterile medium as a means of obtaining axenic algal culture was described in detail by Pringsheim (1946).

Micro-pipettes were made by drawing out the tips of sterile pasteur pipettes over the pilot light of a Bunsen burner. Individual diatoms were then transferred through six drops of sterile medium on a large microscope slide, using a fresh pipette for each wash. The cells entered the pipettes by capillary action and were discharged by blowing out through a piece of rubber-tubing attached to the pipette. The final transfer was into a test-tube of sterile growth medium, which was incubated in the light for up to three months.

An attempt was made to clean the diatoms *A. formosa*, *T. flocculosa*, *C. meneghiniana* and *Melosira* sp. by this method. In a number of instances the diatoms grew, but only one axenic culture, (*C. meneghiniana*) was obtained. It therefore appears that this method does not damage the cells. The greater the number of transfers made, the greater the chance of freeing the cell of bacteria. However, the more manipulation, the less chance of recovering the cell. The lack of success with the other diatom types could be explained by these diatoms requiring the presence of bacteria for growth. Either bacteria which provide an excess of vitamins (e.g. B_{12}, Haines & Guillard, 1974) or bacteria which remove compounds excreted by the diatoms which may inhibit their growth.

Of the three methods the latter was the most time-consuming, however, this was the only method which was successful.

2. BACTERIA

a. ISOLATION AND ROUTINE MAINTENANCE

The isolation, routine maintenance and counting of bacteria were carried out on Casein Peptone Starch (CPS) medium (Jones, 1970) at 25°C.

The medium contained:

Soluble casein	0.5g.	(The starch and casein were autoclaved separately and added aseptically before pouring the agar)
Peptone	0.5g.	
Starch	0.5g.	
K_2HPO_4	0.2g.	
$MgSO_4.7H_2O$	0.05g.	
Fe EDTA	0.05g.	
Glycerol	1ml.	
Agar	15g.	
Distilled water to 1l.		

b. IDENTIFICATION AND BIOCHEMICAL TEST METHODS

1. *Flagella Staining*

Rhode's (1958) staining method was used. The smears were fixed with Kirkpatrick's fixative, ferric tannate mordant was added and the preparation finally washed with ammoniacal silver nitrate.

2. *Oxidase Test* (Kovac's)

This was carried out using filter paper to which 2 to 3 drops of 1% (w/v) tetramethyl-p-phenylenediamine dihydrochloride had been added. Cultures were then smeared onto this and the appearance of a dark purple colour within 10s. was scored positive (Sneath & Collins, 1974).

3. *Catalase Test*

This was carried out in test-tubes, by the addition of hydrogen peroxide to bacterial suspensions.

4. *Capsules*

The presence or absence of capsules was determined by relief staining with eosin (Method of Howie and Kirkpatrick, 1934 as described in Cruickshank, 1965).

5. *Oxidative/Fermentative*

The medium of Hugh & Leifson (1953) was used to differentiate between the oxidative and fermentative metabolism of glucose. Duplicate tubes of solid medium were stab inoculated, one of the duplicates was sealed with vaseline. The medium contained:

K_2HPO_4	0.3g.
Tryptone	1g.
Yeast extract	1g.
NaCl	5g.
Agar	3g.
Bromothymol blue	0.03g.
Distilled H_2O	1l.
Glucose	10g.

(Glucose was prepared separately as sterile 10% (w/v) solution and added aseptically to melted sterile base to give a final concentration of 1% (w/v)

6. *Gelatin liquefaction*

To test for proteolytic breakdown of gelatin, cultures were stab inoculated into nutrient gelatin. The test-tubes were cooled before examination for liquefaction. The composition of the medium was:

Oxoid Nutrient Broth	28g.
Gelatin	4g.
Distilled H_2O	1l.

Charcoal gelatin discs (Oxoid) were also added to broth cultures and agar slopes of cultures.

7. *Tributyrin and Casein breakdown*

Cultures were inoculated onto plates of tributyrin (for fat) and casein (for protein) agar and examined for clearance rings. These media contained the following components:

i. Casein Agar

(a)	Peptone	5g.
	Yeast Extract	3g.
	Agar	25g.
(b)	10% Skim Milk made with 100g. Skim Milk Powder in 1l. distilled H_2O	

a & b are prepared and autoclaved separately. Equal volumes of each are mixed prior to pouring plates.

ii. Tributyrin Agar

Peptone	5g.
Yeast extract powder	3g.
Tributyrin (glyceryl tributyrate)	10g.
Agar	15g.
Distilled H_2O	1l.

8. *Starch breakdown*

After inoculation and incubation starch agar plates were flooded with iodine and examined. The medium used contained:

Oxoid Nutrient Agar	28g.
Soluble Starch	10g.
Distilled H_2O	1l.

9. *Lipase assay*

This method was applied to determine if the bacteria were producing large amounts of lipase. The method used was M. Rowe's modification (pers. comm.) of the Sigma Diagnostic kit (Lipase No. 800 (Titrimetric)), which involves the breakdown of an olive oil substrate. 1ml. of bacterial culture supernatant was added to a mixture containing 3ml. of a standard olive oil suspension and 1ml. of Trizma Buffer solution. This was incubated for 6 hours at 37°C, and the reaction stopped by addition of 95% ethanol. The fatty acids liberated by the action of the lipase were then titrated to pH 9.3 with sodium hydroxide, using an automatic titrator, (Metrohm Herisau, Titrator E526 with a Dosimat E535 automatic burette). The volume of titrant required is a measure of the lipase activity.

10. *Hydrolase activity*

The presence or absence of a number of active enzymes in bacterial cultures was examined with the API ZYM System. This screens for the activity of the following 19 enzymes: acid and alkaline phosphatase; esterase (C_4); esterase/lipase (C_8); lipase (C_{14}); phosphoamidase; leucine, valine, and cysteine aryl amidase; trypsin; chymotrypsin; α and β galactosidase; β-glucuronidase; α-and β-glucosidase; N-acetyl-β glucosaminidase; α mannosidase; α fucosidase.

11. *The API 20 Enterobacteriaceae system*

This was used as an aid to the identification of the bacterial isolates. This involved the following 23 standard biochemical tests which are carried out in micro-tubes: the production of β-nitrophenyl, galactosidase (ONPG), arginine dihydrolase, lysine and ornithine decarboxylase, hydrogen sulphide, urease,

tryptophan deaminase and indole; the Vosges-Proskauer test and proteolysis of gelatin; The use of citrate as a carbon source; the utilisation of glucose, mannose, inositol, rhamnose, saccharose, melobiose, amylose, arabinose and sorbitol.

12. *Identification scheme*

The scheme of Gibson et al. (1977) was used for preliminary identification of bacteria (Table 2.2).

3. COUNTING METHODS FOR DIATOMS AND BACTERIA

1. Diatom cultures were counted under x400 magnification using a haemocytometer slide.

2. Direct counts of bacteria and algae were carried out during decomposition experiments using a modified Breed's smear technique. 0.1ml. of suspension was removed using a standard wire loop, and this was evenly spread over a marked circle of 1cm.^{2}. After rapid drying and heat-fixing the slides were stained with methylene blue (for *O. redekei*) and ruthenium red (for diatoms). Samples were taken in triplicate and the number of fields counted depended on the number of organisms per field.

3. Total viable counts of bacterial populations from water, and from diatoms at the end of decomposition experiments, were carried out according to the method of Cupples (1979). 10ml of sample was shaken up with 90ml. of $\frac{1}{4}$ strength Ringer's solution, containing glass beads. The latter were used to help to break up any clumps. Suitable ten-fold dilutions were then made and 0.1ml. spread plate on CPS agar in triplicate. These were incubated for 3 weeks at 25^{o}C.

Table 2.2 Identification of Aerobic, Heterotrophic, Gram Negative Rods
(after Gibson et al., 1977)

MOTILITY	FLAGELLA	OXIDASE	OXIDATION/ FERMENTATION OF GLUCOSE	PIGMENTS			
				DIFFUSABLE		NON-DIFFUSABLE	
				FLUORESCENT	NON-FLUORESCENT		
+	polar	+	oxidative or -	Green or -	blue-green, red, brown or -	- or yellow, red, brown, blue, pink	*Pseudomonas* and allied groups
+	polar	+	fermentative	-	- or brown	- or yellow, blue	Vibrionaceae
+	peritri-chous	+	- or oxidative	-	-	-	*Alcaligenes*
+	peritri-chous	+	- or oxidative	-	-	yellow	*Flavobacterium*
+	peritri-chous	-	fermentative	-	-	- or yellow, red	Enterobacteriaceae
-	none	-	fermentative	-	-	-	Enterobacteriaceae
-	none	+ or -	oxidative or -	-	-	-	*Moraxella*-like *Acinetobacter*
-	none	+ or -	- or oxidative or weak fermentative	-	-	yellow or orange	*Flavobacterium* *Cytophaga* *Flexibacter*

4. Viable counts of pure cultures of bacteria were carried out in a similar way, however they were not spread plated. Using a standard dropper, triplicate 0.02ml. drops were applied to the surface of agar plates and allowed to be absorbed before incubation.

4. SILICA ANALYSES

a. SOLUBLE

Monomeric ammonium molybdate reactive silica was determined colorimetrically by the method of Strickland & Parsons (1968).

Samples were mixed with an ammonium molybdate reagent, which resulted in the formation of silicomolybdate, phosphomolybdate and arsenomolybdate complexes. A reducing solution, containing metol and oxalic acid was then added to reduce the silicomolybdate complex to a blue reduction compound. The reducing solution simultaneously decomposed the phosphomolybdate and arsenomolybdate, thus eliminating interference from these compounds. The extinction of the resulting solution was then measured in 1cm. cells at a wavelength of 810nm, using a Pye Unicam SP6-400 U.V. spectrophotometer. Sodium silicofluoride (Na_2SiF_6) was used as a standard, as this solution was stable indefinitely.

To ensure that none of the soluble silica originated from external sources, all sampling, dilution and addition of molybdenum reagents were carried out using an automatic pipette with plastic tips. These were steeped in Decon 90 over night, thoroughly rinsed in tap-water and finally triple distilled water rinsed before use. A similar washing procedure was used for all the plastic containers which were used in the preparation of reagents and for the silica analyses.

Spectrophotometer readings above 1.0 on the absorbance scale (equivalent to approx. 2.5µgSi/ml.) are less accurate than in the range 0.1 to 1.0, as the scale is logarithmic (M. Saunders, pers. comm.). Therefore samples were diluted with distilled water to ensure that they came within this range.

b. TOTAL SILICA

To determine the percentage of soluble silica released from the diatom frustules, it was necessary to measure the initial total silica content of the diatom cells.

Samples of diatom cultures were washed three times by alternate centrifugation and re-suspension in sterile silica-free culture medium. Aliquots were then placed in platinum crucibles and dried overnight in an oven at 80°C. They were then ashed and fused with excess sodium carbonate (approx. 0.2g.). This was carried out initially over an oxidised Bunsen flame. However values for the total silica of diatom cultures appeared to be lower than the amount of soluble silica released as a result of decomposition. Therefore the methods described in Table 2.3 were compared using a *N. pelliculosa* culture.

Table 2.3 A Comparison of Ashing and Fusing Methods

	Method	Silica Content*
1.	Ashing by heating from cold to 1000°C in a furnace, followed by sodium carbonate fusion over a fully oxidised Bunsen flame	2.79 2.84
2.	Ashing, followed by fusion over fully oxidised Bunsen flame	2.83 2.49
3.	Addition of sodium carbonate before ashing over fully oxidised Bunsen flame, so that fusing and ashing occurred simultaneously.	2.72 2.64

*expressed as a percentage of the dry weight of the diatoms (results for duplicate samples)

The less consistent results from ashing over a Bunsen flame may possibly be caused by the loss of silicon along with carbon during rapid heating with the Bunsen flame, (M. Saunders, pers. comm.).

As a result of these findings, ashing was carried out in a furnace, and was followed by sodium carbonate fusion over a Bunsen flame. The melt produced was then dissolved in distilled water with gentle heating. The sides of the platinum crucible were thoroughly rubbed down with rubber coated glass rods, to ensure that all the silica dissolved. The resulting alkaline solution was neutralised with 2N H_2SO_4 and the solution made up to 25ml. in acid soaked (1 : 1 conc. H_2SO_4 : HNO_3) and distilled water rinsed volumetric flasks. It was then immediately diluted and analysed for soluble silica, to prevent any possible polycondensation of the concentrated silica solution. The range of dilutions tested were neat, 1 in 2, 1 in 5 and 1 in 10. A standard of dried, spectrophotometrically pure silicon dioxide gave 99% recovery of the silica.

5. EXPERIMENTS ON SILICA DISSOLUTION FROM DIATOMS

a. CULTURE PREPARATION AND TREATMENT

In general, diatoms were four weeks old when used for decomposition experiments, although culture conditions varied with the time of year (i.e. when growing on a window sill) and as, and when, a variety of lighted incubators were available for use. As a result of this the percentage of silica release in different experiments are not directly comparable, as silica release can vary with cultural conditions (Werner, 1977).

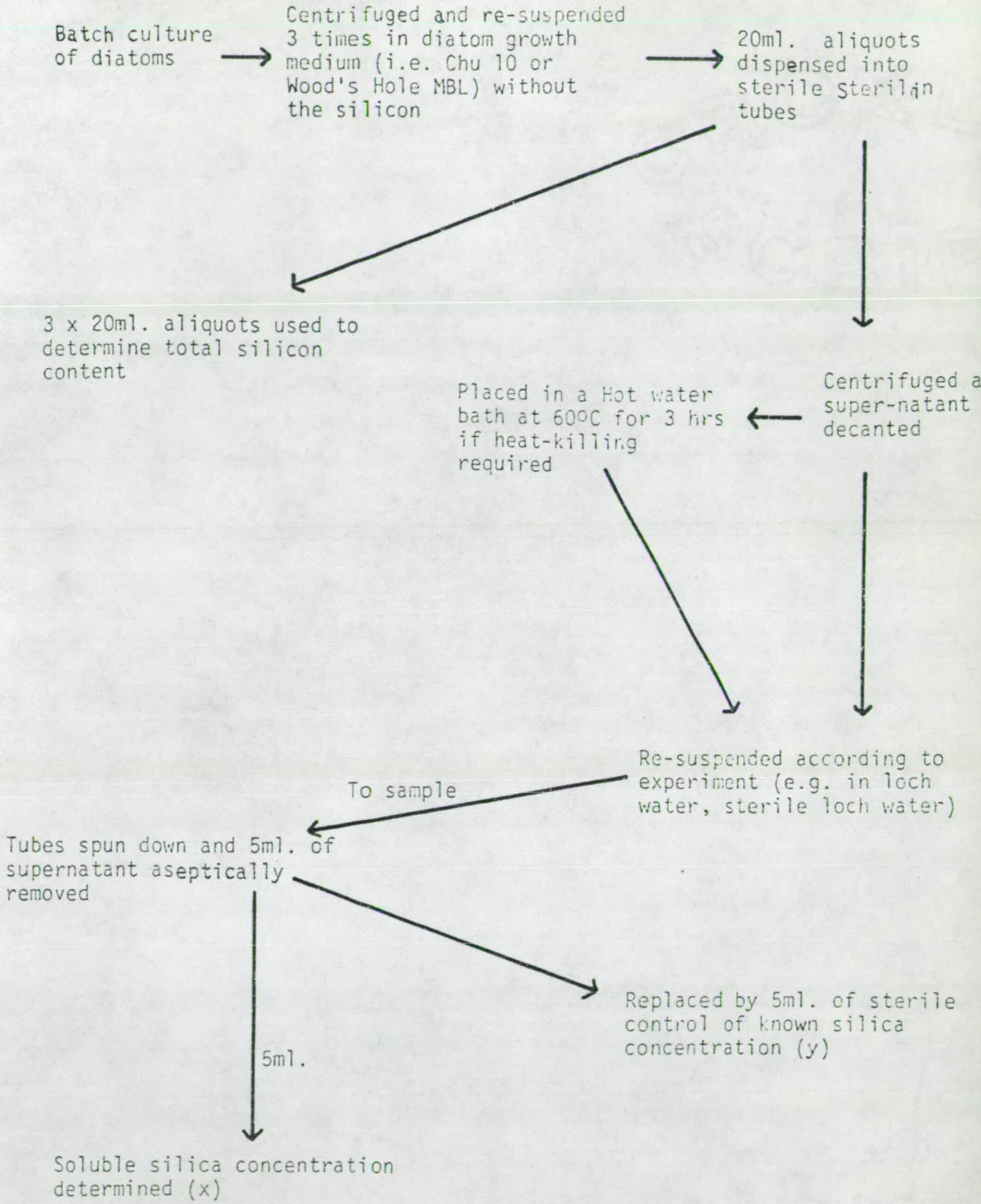
At the end of the incubation period the diatom cultures were examined microscopically to ensure that the cells were still intact. Cultures were washed three times by alternate centrifugation and resuspension in silica-free culture medium, and then 20ml. aliquots were pipetted into plastic sterilin tubes, spun down and the supernatant decanted.

Where heat killing of the diatoms was required they were placed in a water bath at 60^{o}C for three hours (Golterman, 1960). This would also reduce the bacterial contamination of xenic cultures.

The volume was then made up to 20ml. using appropriately treated lake water or growth medium, centrifuged, and a 5ml. sample removed to measure the initial soluble silica concentration. This was then replaced with 5ml. of the appropriate sterile control (e.g. heat sterilised lake water, filter sterilised lake water). This procedure of centrifugation, removal of a 5ml. sample, and replacement with 5ml. of a sterile control of known silica concentration, was repeated on each sampling day.

Lake water and sterile lake water controls, without the diatoms, were also set up and sampled. Any changes in the silica concentration in these tubes were taken into account when determining changes in the tubes with the diatoms, thus ensuring that the changes being measured were the result of the presence of the diatom cultures.

A diagrammatic summary of the sampling procedure and the calculations required to determine the changes in the concentration of silica in the vials is presented in Fig. 2.1.



To calculate final silica concentration in tube after sampling procedure:

$$x_i - \tfrac{1}{4}x_i + \tfrac{1}{4}y_i = z_i$$

Therefore any change in silica concentration on the next day of sampling, where x_{ii} is the new measured silica concentration, $= x_{ii} - z_i$.

Fig. 2.1 Outline of the Procedure used in Silica Dissolution Experiments

b. SAMPLING OF THE LAKES

Water samples were taken from the surface of the lakes, in general from the main body of water, using cleaned, sterile plastic containers. In the case of Loch Leven this was usually in the region of the Northern Deep area (see Fig. 1, p. 11). As both lakes are very well mixed, these samples were hoped to be representative of the whole water body.

c. ASSESSMENT OF ERRORS

In all experiments three or four replicates were set up for each treatment. Confidence limits (at $P = 0.05$) were calculated from the variation in soluble silica measurements between replicates and are represented on individual graphs.

d. PHOTOGRAPHY

Microscopic photography was carried out with a Leitz microscope using phase contrast and a camera attachment. Colour photographs were taken using Kodak Photomicrography Colour Slide film. The slides were then Cibachrome printed. Black and White photographs were taken using Kodak SO115 film.

III. A PRELIMINARY INVESTIGATION OF LOCH LEVEN WATER BACTERIA

III. A PRELIMINARY INVESTIGATION OF LOCH LEVEN WATER BACTERIA

1. THE ISOLATION OF THE DOMINANT BACTERIA PRESENT IN LOCH LEVEN WATER

INTRODUCTION

This investigation was carried out in an attempt to isolate the dominant bacteria present in Loch Leven water, and in particular those found in association with the indigenous diatoms. Although this was not a detailed taxonomic study, it was necessary to obtain some preliminary information on the types of bacteria present.

a. THE EXAMINATION OF COLONY TYPES ON CPS AGAR

METHODS

Loch Leven phytoplankton were concentrated by filtering 600ml of Loch Leven water through Whatman's No. 1 filter paper. The algae were scraped off the filter paper and the volume made up to 20ml. Plate counts as detailed in the Materials & Methods (p. 53) were carried out on this sample and unfiltered Loch Leven water. In this way, any differences between the dominant organisms in the water and associated with the bloom could be determined.

RESULTS & DISCUSSION

At the time of sampling a bloom of *Asterionella formosa*, unicellular centric diatoms, and *Melosira ambigua* was beginning to reduce and an *Anabaena flos-aquae* bloom was evident, (Bailey-Watts, 1977). It would therefore be expected that the bacteria isolated, would be those associated with the healthy cyanobacterial bloom, or alternatively those involved in the initial decomposition of the diatoms.

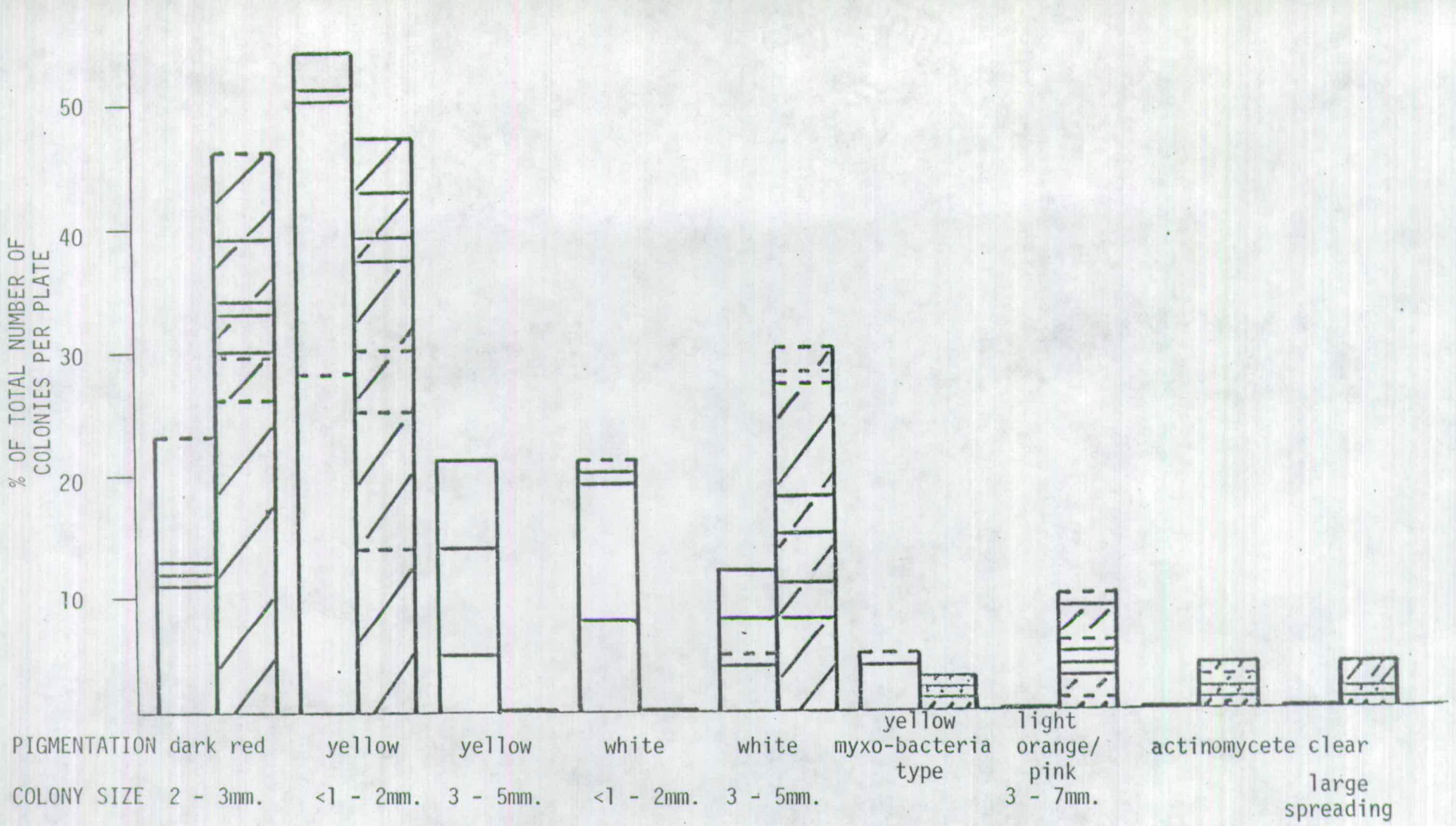


Fig. 3.1 A comparison of the colony types isolated from Loch Leven water and concentrated phytoplankton

KEY: = Loch Leven water sample; = Concentrated Loch Leven phytoplankton; ---- = Samples taken 16/11/79; —— = Samples taken 30/11/79 (each bar represents the mean of 2 or 3 replicate plates)

All the colonies were examined and differences in pigmentation and size were recorded. The total number of colonies varied from 20 to 70 (mean 42 ± 11; P = 0.05). This was therefore not an accurate measure of the total viable count on CPS as only plates with 40 to 60 colonies are considered to be statistically valid, (Jones, 1979). However they do give some measure of the relative numbers. Colony types are expressed as a percentage of the total number of colonies to give some indication of the dominant types, (Fig. 3.1).

The dominant organisms at the time of sampling appear to produce dark red, yellow and white colonies, other types accounting for less than 10%. Some of the colony types present in low percentages only appear in the concentrated sample. This suggests that they may be closely associated with the phytoplankton, and are not present in large enough quantities free in the water for them to be detected in the untreated water.

The small white and large yellow colonies are not found in the concentrated samples, which suggests that they are not closely associated with the algae, or at least not physically attached. The percentage of dark red colonies increases in the concentrated sample. These dark red organisms did not dominate in samples taken in October 1978 or July 1979 when *A. flos-aquae* was absent. However, they re-appeared in August 1979 along with the *A. flos-aquae* where they accounted for 50% of the colony types on CPS. They did not dominate in water samples taken from a plastic enclosure (5m. x 3m.) in which water had been isolated from the main body of water in the Loch and in which the *A. flos-aquae* bloom was not present. Bearing in mind the inaccuracy of the bacterial counts, it can be estimated that there were approximately 2-3000 dark red

pigmented organisms per ml, associated with only 4000 threads of *Anabaena flos-aquae* (Bailey-Watts, 1977). Therefore if these organisms are specifically associated with the cyanobacterium, the colonisation is less than one per thread. Jones, (1976) recorded more than five bacteria attached per cell of *Asterionella formosa*. However microscopic examination could not show if these bacteria were all the same type.

This preliminary investigation did not differentiate between the bacteria which were associated with diatom decomposition and those with a symbiotic or saprophytic association with healthy phytoplankton.

b. THE EXAMINATION OF PURE CULTURES OF LOCH LEVEN WATER BACTERIA

METHODS

Approximately 60 of the organisms which were numerically dominant on the CPS agar plates, and those which appeared only in the concentrated phytoplankton samples were purified and sub-cultured onto CPS slopes.

These were examined microscopically after 2 to 3 days to determine if the macroscopic and microscopic characteristics were related.

Several of these cultures were examined in greater detail so that a tentative identification could be made. Their ability to degrade the complex molecules tributyrin, starch and gelatin was also investigated, as this might give some indication of their activity in algal decomposition.

RESULTS & DISCUSSION

As anticipated, colony size could not be related to microscopic differences. For example, some of both the large and small yellow

colonies were stalked caulobacter- type organisms. The light orange and pink colonies which only appeared in the concentrated sample were also of this type. It is likely that these organisms are attached to the surfaces of the algae and use any organic material which is secreted. Caulobacter- type organisms can use soluble carbohydrates, amino acids and organic acids as carbon and energy sources (Stanier et al., 1971).

Gibson et al., (1977) presented a scheme for the preliminary division of Gram negative heterotrophic aquatic bacteria (see Table 2.2, p. 54). Although only limited tests were carried out on these organisms this scheme would place the dominant dark red pigmented organisms in the genus *Pseudomonas* or allied groups. Some of the yellow pigmented may belong to either *Pseudomonas* or *Alcaligenes*. They do not therefore appear to be as aggressive decomposers as the Cytophaga - Flexibacter type organisms, which produce a wide range of hydrolytic enzymes. However the latter group accounted for less than 10% of the total population, (Fig. 3.1).

Some of the bacteria which produced white colonies may be more active in decomposition, belonging to either the Vibrionaceae or Enterobacteriaceae, as one isolate was capable of hydrolysing all three media tested.

All of the isolates examined with one exception, are capable of breaking down tributyrin (see Table 3.1) which suggests that they may also degrade algal membranes and lipid storage products.

However, although the presence of clearance rings on these three media indicates the production of large amounts of extracellular enzymes, a negative result does not necessarily indicate complete inability to decompose these substrates or other complex molecules.

Table 3.1 Characteristics of Bacteria Isolated from Loch Leven Water in November 1977.

NO. OF ISOLATES EXAMINED	COLONY PIGMENT-ATION	MOTILITY	OXIDATION/ FERMENTATION OF GLUCOSE	GRAM REACTION	HYDROLYSIS OF			OTHER CHARACTER-ISTICS	POSSIBLE GROUPS
					STARCH	TRIBUTYRIN	GELATIN		
5	dark red	+	-	-	-	+	-	rods	*Pseudomonas* & allied groups
1	white	+	fermentative	-	+	+	+	rods	Vibrionaceae or* Enterobacteriaceae
1	white	+	fermentative	-	-	+	+	rods	
1	white	+	oxidative	-	-	-	-	rods	*Pseudomonas** and allied groups or *Alcaligenes*
4	yellow	+	-	-	-	+	-	rods	*Pseudomonas** & allied groups or *Flavobacterium*
1	yellow	+	-	±	-	+	-	pleomorphic	*Arthrobacter*
4	yellow	+ (Gliding)	oxidative	-	+	+	+	narrow rods	*Cytophaga** *Flexibacter*
1	pink	±	oxidative	-	-	+	±	motile & stalked bacteria, forming rosettes	*Caulobacter*
1	orange	±	-	-	+	+	-		
1	dark red	±	-	-	-	+	±		
1	light orange	±	oxidative	-	+	±	±		
1	pink	-	-	-	+	+	±	colonies tough-cells filamentous appearance	*Flavobacterium**

*Division according to Gibson et al., (1977) (see Table 2.2, p.54).

+ = positive

± = weakly positive

- = negative

The A.P.I. ZYM microsystem, produced by Analylab Products Inc. (USA), which screens for the production of 19 different enzymes, does not require large quantities of the enzyme to give a positive reaction. This method is used in a later part of this study (see Section VII).

2. AN INVESTIGATION OF THE BACTERIA ATTACHED TO INDIVIDUAL DIATOM CELLS

INTRODUCTION

To determine if the bacteria associated with the diatoms were aggressive decomposers, bacteria were isolated from individual diatom cells. The cultures were examined and compared with the dominant bacteria isolated from complete water samples.

METHODS

Individual diatom cells were transferred from one agar surface to another using a micro-needle and a micro-manipulator. The blocks of agar with the individual diatom cells attached were then placed onto CPS agar plates and incubated at 25^{o}C. Unfortunately under the magnification used to carry out the manipulation it was impossible to determine if the diatom cells picked off had bacteria attached or not.

RESULTS & DISCUSSION

The water sample used in this investigation was taken from Loch Leven harbour (see Fig. 1, p.11) in January 1978, as the loch was frozen over. Bacteria grew from only 11 out of the 30 diatoms, which were picked at random. This suggests that the diatoms were not heavily colonised. Similarly from 8 *Scenedesmus* sp. cells which were transferred, only four bacteria were isolated.

Isolates were subjected to the same tests as the bacterial cultures examined in the previous section, to determine any major differences.

The bacteria isolated from the individual diatom cells seem to be similar to some of the types isolated from earlier water samples (see Table 3.2). The yellow pigmented organisms (possibly *Flavobacterium* or *Pseudomonas* type) only break down the tributyrin. The bacteria forming white colonies are again nutritionally more versatile.

Bacillus sp. were the only types which had not been previously isolated and it is likely that these organisms would be very active in decomposition.

It is interesting that all of the isolates from individual algal cells, except for one, either fermented or oxidised glucose. On the other hand, this occured with only half of the loch water bacteria previously examined (Table 3.1). Therefore it appears that there is some variation between the bacteria present in the water and those associated with algae, although some of them may be similar.

To determine if specific bacterial types are associated with a. healthy algae, b. dying algae or c. capable of growth in association with both, would require an intensive study during the rise and fall of the algal population. A major difficulty would be assessing the importance of bacteria (e.g. filamentous) which have been observed in association with decomposing diatoms (see p.151) but could not be isolated. These aspects were not investigated in this study.

Table 3.2 Characteristics of Bacteria Isolated from Individual Loch Leven Algal Cells in January, 1978.

SOURCE	COLONY PIGMENTATION	MOTILITY	OXIDATION/ FERMENTATION OF GLUCOSE	GRAM REACTION	HYDROLYSIS OF			OTHER CHARACTERISTICS	POSSIBLE GROUPS
					STARCH	TRIBUTYRIN	GELATIN		
Centric Diatom Pennate Diatom	clear	+	oxidative	-	-	+	-	rods	
Centric Diatom	white	+	oxidative	-	+	±	+	rods	*Pseudomonas** and allied groups or *Alcaligenes*
Pennate Diatom	white	+	oxidative	-	-	+	+		
2 *Scendesmus* sp.	white	+	oxidative	-	+	+	+		
Pennate Diatom	pink	+	oxidative	-	+	±	+	rods	*Pseudomonas**
2 *Asterionella* sp.	yellow	+	-	-	-	+	-	rods	*Pseudomonas** and allied groups or *Flavobacterium*
Melosira sp.	yellow	+	oxidative	-	-	N.G.	-		
Centric Diatom	yellow	+	oxidative	-	-	+	-		
Melosira sp.	white	+	fermentative	-	+	±	+	rods	Vibrionaceae* or Enterobacteriaceae
Centric Diatom 3 *Asterionella* sp.	white	-	fermentative	+	+	+	+	spore forming	*Bacillus*

*Division according to Gibson et al., (1977) (see Table 2.2, p.54)

+ = positive

± = weakly positive

- = negative

N.G. = no growth.

IV. THE RELEASE OF SOLUBLE SILICA FROM DIATOM CULTURES

IV. THE RELEASE OF SOLUBLE SILICA FROM DIATOM CULTURES

INTRODUCTION

The limited data available on the solubilisation of diatom silica have been discussed in the Introduction (p.30). However, the microbial decomposition of diatoms and the effect this process might have on the release of soluble silica has not been investigated. Experiments were therefore carried out to compare the release of soluble silica from diatoms under sterile conditions and in the presence of lake water, containing natural bacterial populations. The activity of pure cultures is reported in a later section (see p.112).

1. THE RELEASE OF SILICA FROM AN AXENIC CULTURE OF *CYCLOTELLA MENEGHINIANA*

METHODS

Batch cultures of axenic *C. meneghiniana* were prepared as detailed in the Materials & Methods (p. 59).

The following treatments were set up in quadruplicate.

1. Active culture, suspended in lake water,
2. Active culture, suspended in heat sterilised lake water,
3. Heat-killed culture, suspended in lake water,
4. Heat-killed culture, suspended in heat sterilised lake water,
5. Active culture, suspended in Woods Hole (MBL) salt solution without silica and
6. Heat-killed culture, suspended in Woods Hole (MBL) salt solution without silica.

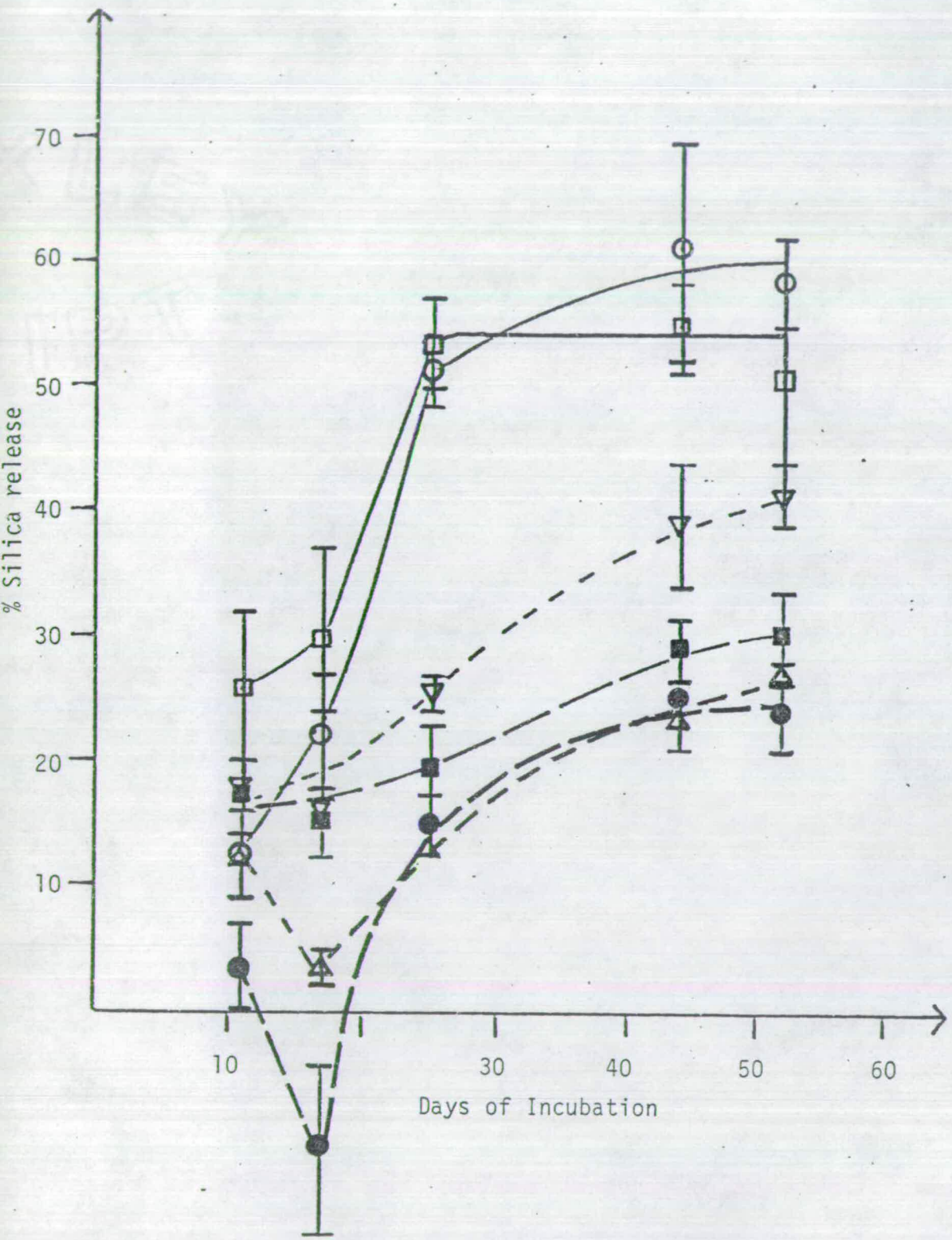


Fig. 4.1 The release of soluble silica from axenic *C. meneghiniana* at 25°C in the presence of Loch Leven water (——), heat sterilised Loch Leven water (— —) and Woods Hole MBL salt solution without silica (- - -)

KEY:

	L. Leven water	Sterile L. Leven water	Woods Hole MBL
Active culture	○	●	△
Heat-killed culture	□	■	▽

I confidence limits (P = 0.05)

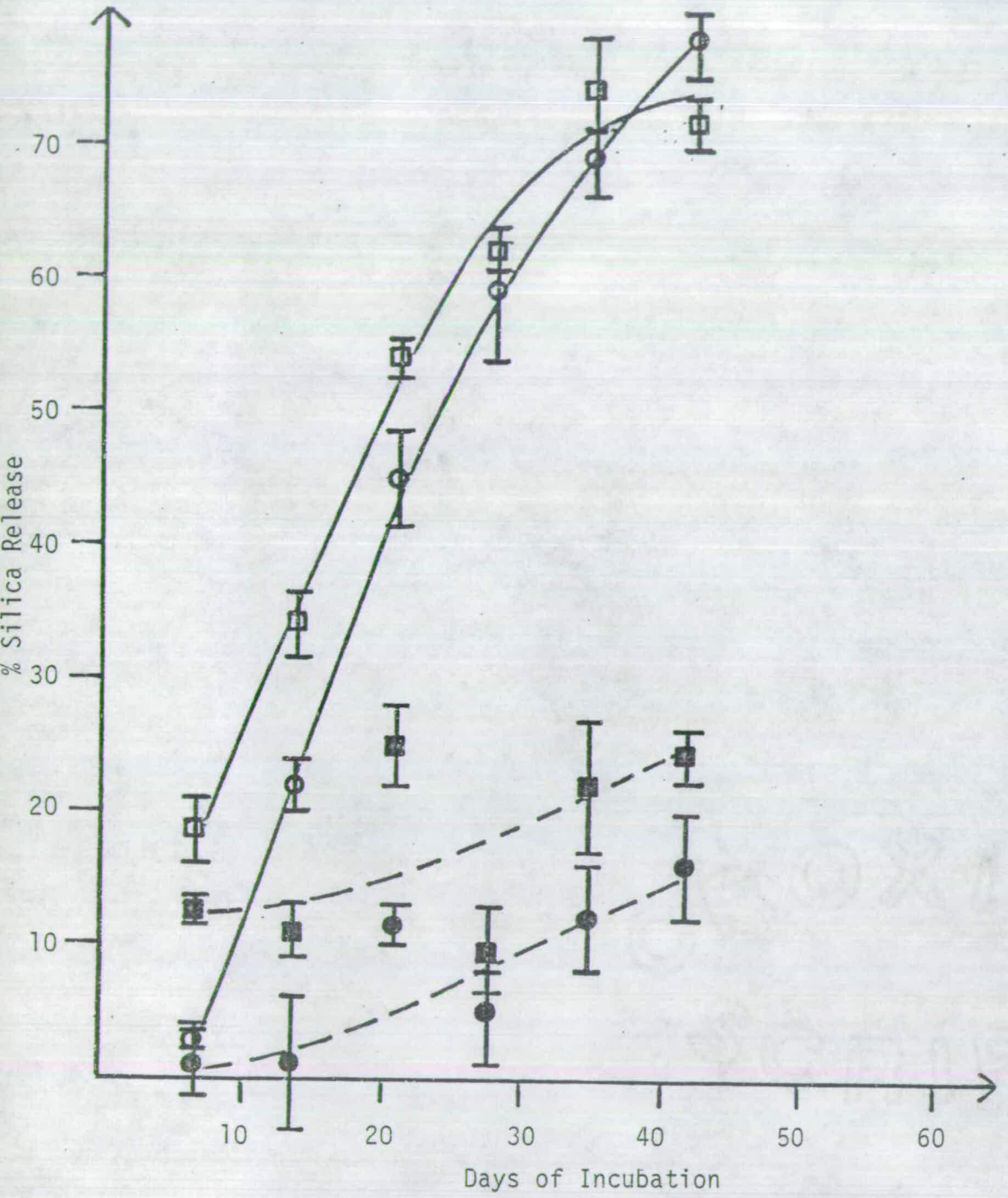


Fig 4.2 The release of soluble silica from axenic *C. meneghiniana* at 25°C in the presence of Lough Neagh water (——) and heat sterilised Lough Neagh water (— —)

KEY:	L. Neagh water	Sterile L. Neagh water
Active culture	○	●
Heat-killed culture	□	■

The effect of both Loch Leven and Lough Neagh water was examined, and the initial total silica content of diatom cultures, and the number of diatom cells added per ml. were as follows:

Loch Leven; 1.2µgSi/ml., 1.6×10^9 cells/ml.

Lough Neagh; 2.7µgSi/ml., 5×10^4 cells/ml.

Changes in the silica concentration in control tubes containing sterile and non-sterile lake water without added diatoms were subtracted before the percentage of silica released from the cultures was calculated. Tubes were incubated in the dark at 25°C.

RESULTS & DISCUSSION

The percentage of soluble silica released from axenic *C. meneghiniana* in the presence of sterile and non-sterile Loch Leven and Lough Neagh water can be seen in Fig. 4.1 (Loch Leven) and 4.2 (Lough Neagh). The results for the two experiments are not directly comparable because:

a. the diatoms were cultured under different conditions of light, daylength and temperature. The diatoms had also been sub-cultured a number of times during the period between the two experiments. These factors are assumed to be the reasons for the different amounts of silica obtained per cell;

b. the initial total amount of frustule silica was different; and

c. the water was taken from two lakes, at different times of year, and therefore differed both chemically and biologically.

However it can be clearly seen that in both cases the lake bacterial populations enhance soluble silica release when compared with sterile autolysis.

In the presence of the bacteria 55 to 60% of the silica was released within the first 25 to 30 days, with the rate of release increasing and then gradually decreasing, until there was no further release. In neither experiment was all the diatom silica solubilised, which suggests that either parts of the frustule are 'recalcitrant" in that they remain insoluble, or alternatively the mechanism of solubilisation requires bacterial growth, and this is being limited by the availability of nutrients. In the latter case the graphs of silica release would follow a similar pattern to that of bacterial growth.

The differences in the percentage silica released cannot be attributed to changes in the gross pH because the changes occurred in all the treatments, and the control tubes. In the experiment with Loch Leven water the pH increased from 7.3 to 8.0, and with Lough Neagh water it dropped from pH 8.3 to 8.0. Woods Hole (MBL) remained at pH 7.3.

The heat-killing of the diatoms resulted initially in a greater release of silica, approximately 15% more than from initially living axenic diatoms. This is in agreement with the findings of Lewin (1961) who reported that treatments which killed axenic *Navicula pelliculosa*, including heat-killing, resulted in enhanced silica release (see Introduction, p. 31). This masks any reduction in silica solubilisation which might occur as a result of denaturation of autolytic enzymes. It is possible that heat-killing disrupts the membrane systems of the cells and the relatively more soluble cytoplasmic silica is released quickly. The percentage of cytoplasmic silica will vary with the stage of the cell division cycle. The cells which have not been heat-killed may be able to take up silica from the lake water, although there is always

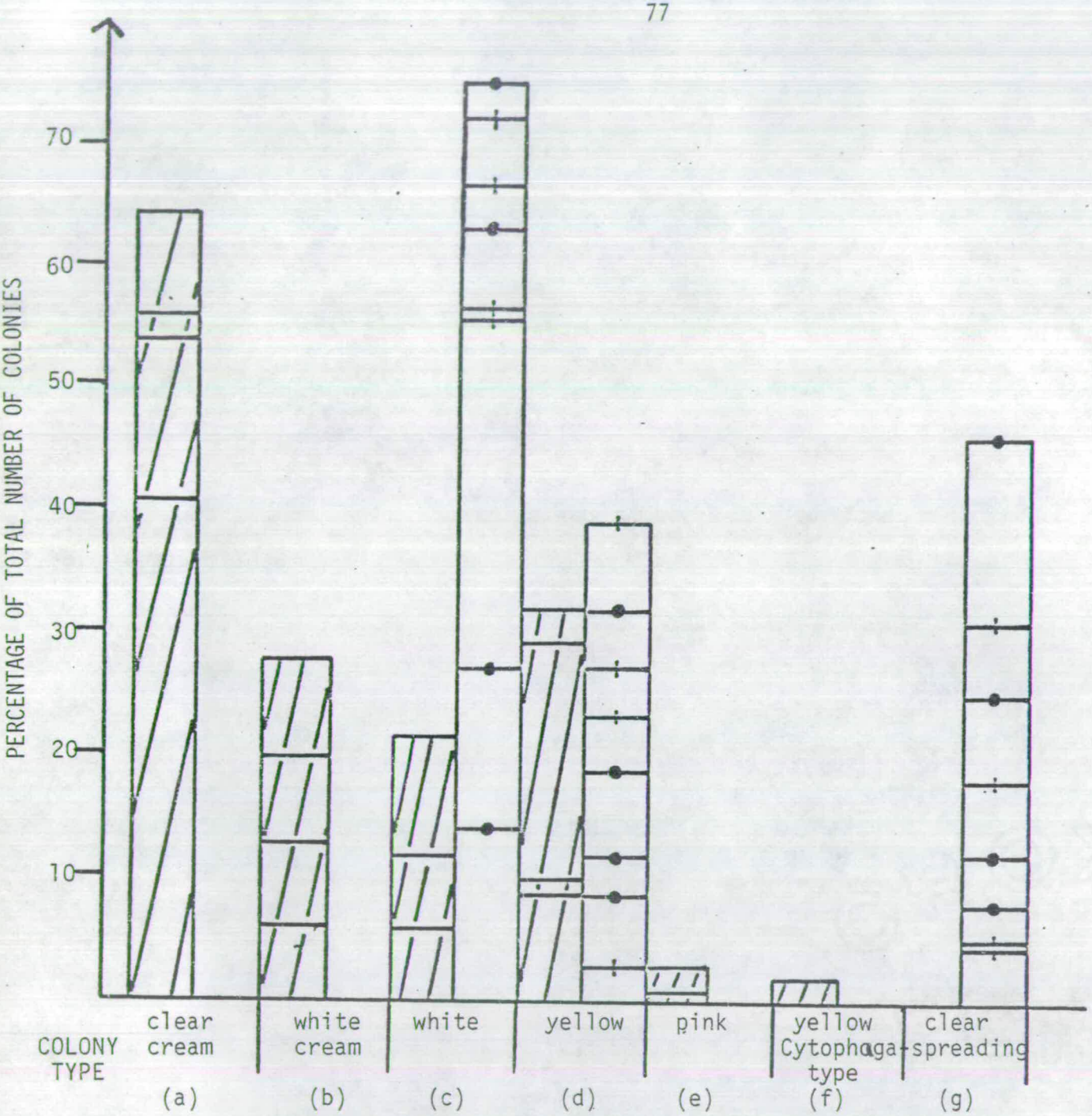


Fig 4.3 A comparison of the colony types obtained on spread plates of CPS agar, before and after incubation of Loch Leven water with axenic *C. meneghiniana*. Loch Leven water population at the beginning of the experiment (▨), each bar represents the mean of a water sample plated in triplicate; Loch Leven water after incubation with the diatoms for 50 days (▭), each bar represents the mean of one of the four replicate tubes, plated out in triplicate. Active culture (-●-), heat-killed culture (-+-).

Total Viable Counts Before and After Experiment

Loch Leven water (initial count)	6.2×10^4/ml.	(62 ± 16 colonies/plate)
After Incubation with:		
active *C. meneghiniana* culture	6.0×10^4/ml.	(60 ± 9 colonies/plate)
heat-killed *C. meneghiniana* culture	5.6×10^4/ml.	(56 ± 7 colonies/plate)

more silica being released than taken up. This occurred when living diatoms were incubated under sterile conditions, either suspended in sterile lake water or the diatom growth medium (Woods Hole MBL) without the silica added (Fig. 4.1).

The sequence of events could therefore be as follows: Suspensions of living diatoms which are placed in the dark subsequently die and a small amount of dissolved silica is released. Diatoms which are still metabolising remove this silica from solution, resulting in a reduction in the dissolved silica. Finally these diatoms also die and the dissolved silica concentration once more increases. Whether the measured silica concentration decreases, increases or remains constant will depend on the relative amounts of uptake and dissolution.

A comparison of the total viable counts on CPS medium (Fig. 4.3) for Loch Leven water at the beginning of the experiment and after 50 days incubation with the *C. meneghiniana* at 25^{o}C was made. The results show that although the total numbers of bacteria present are similar, there have been changes in the dominant colony types present. There are fewer colony types present than are normally found in total viable counts of fresh Loch Leven water (see SectionIII,p.64). Also clear spreading colonies, (g), appeared which were not amongst the dominant colony types in the loch water. It therefore appears that certain members of the loch bacterial population are better adapted to growth on decomposing diatoms than others. Pure cultures of the yellow pigmented, (d), white, (c), and clear spreading, (g), colonies were isolated and used in studies on the effect of pure cultures on silica release (see p.112).

These isolates represent the dominant members of the bacterial populations which are capable of growth on CPS medium and which occur at the end of 50 days incubation in the presence of *C. meneghiniana*. This does not take into account any possible succession of micro-organisms on the diatoms e.g. possible initial colonisation by bacteria utilising 'simple' compounds which may leach from a dying cell, followed by those which decompose more complex molecules.

During the course of these experiments, microscopic examination showed that the diatoms were forming clumps with the bacteria present in the lake water. However this clumping did not occur under sterile conditions. It was therefore not possible to relate changes in the numbers of diatoms present with changes in the silica concentration, found under non-sterile conditions. Filamentous, rod-shaped and caulobacter-type bacteria were amongst those observed in association with the clumps. The problems of correlating the bacteria isolated on CPS with those observed under the microscope and the importance of clump formation are further discussed in Section VIII.

It can be concluded that bacterial populations present in lake water can accelerate the solubilisation of silica from this diatom over the rate at which it would occur as a result of sterile autolysis.

2. THE RELEASE OF SILICA FROM A NUMBER OF XENIC DIATOM CULTURES

METHODS

The production of axenic diatom cultures proved to be very difficult (see p.46) and cultures which were obtained from culture collections were contaminated. Therefore to investigate the dissolution of a number of different diatom types it was necessary to use bacterially contaminated or xenic cultures. A sterile control was obtained by adding mercuric chloride (1g/l). The cultures were all heat-killed prior to the experiment in an attempt to reduce the bacterial population contaminating the cultures. Batch cultures of diatoms were prepared as detailed in the Materials and Methods (p.59) and treated in the following ways;

1. Heat-killed and suspended in lake water,
2. Heat-killed and suspended in heat sterilised lake water and
3. Suspended in lake water containing mercuric chloride (1g/l).

The initial total silica contents of the diatom cultures used in the experiments were as follows:

	Total Silica Content of Diatom Cultures (µg Si/ml) used in experiments on		
	a. The effect of Lake bacterial populations		b. The effect of bacteria contaminating the cultures
	L. Neagh	L. Leven	
1. *Asterionella formosa* (from CCAP)		2.8	2.8
Asterionella formosa (Loch Leven isolate)	2.0		
2. *Tabellaria flocculosa*		5.6*	5.6* 7.0
3. *Navicula pelliculosa*		14.9	8.9
4. *Cyclotella meneghiniana*		1.2	1.2

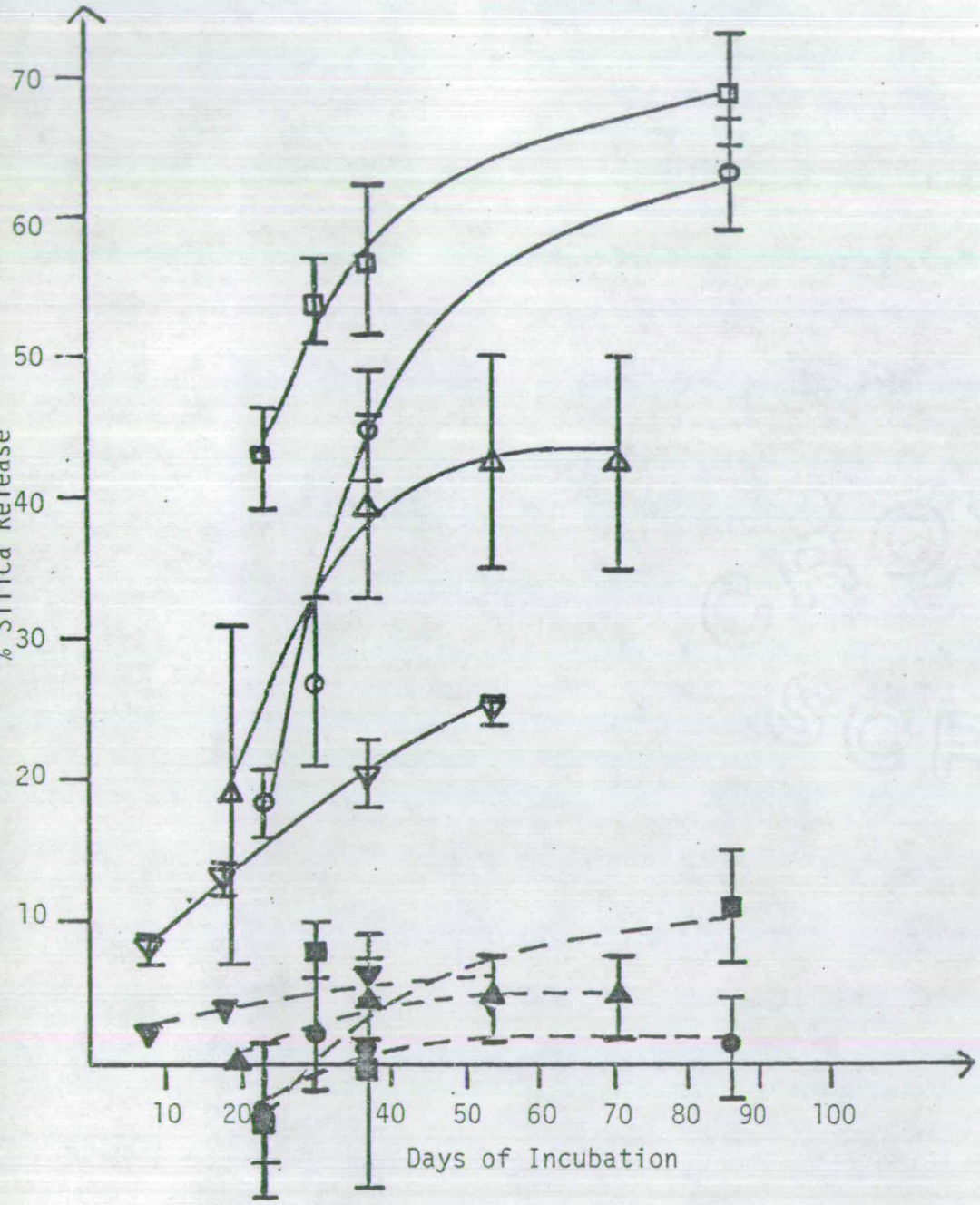


Fig. 4.4 The release of soluble silica from a number of diatoms in the presence of Loch Leven water (——) and Loch Leven water with mercuric chloride (1g./l.) added (— —). Incubation was at 25°C

KEY:

- ○ *T. flocculosa*
- □ *A. formosa*
- △ *C. meneghiniana*
- ▽ *N. pelliculosa*

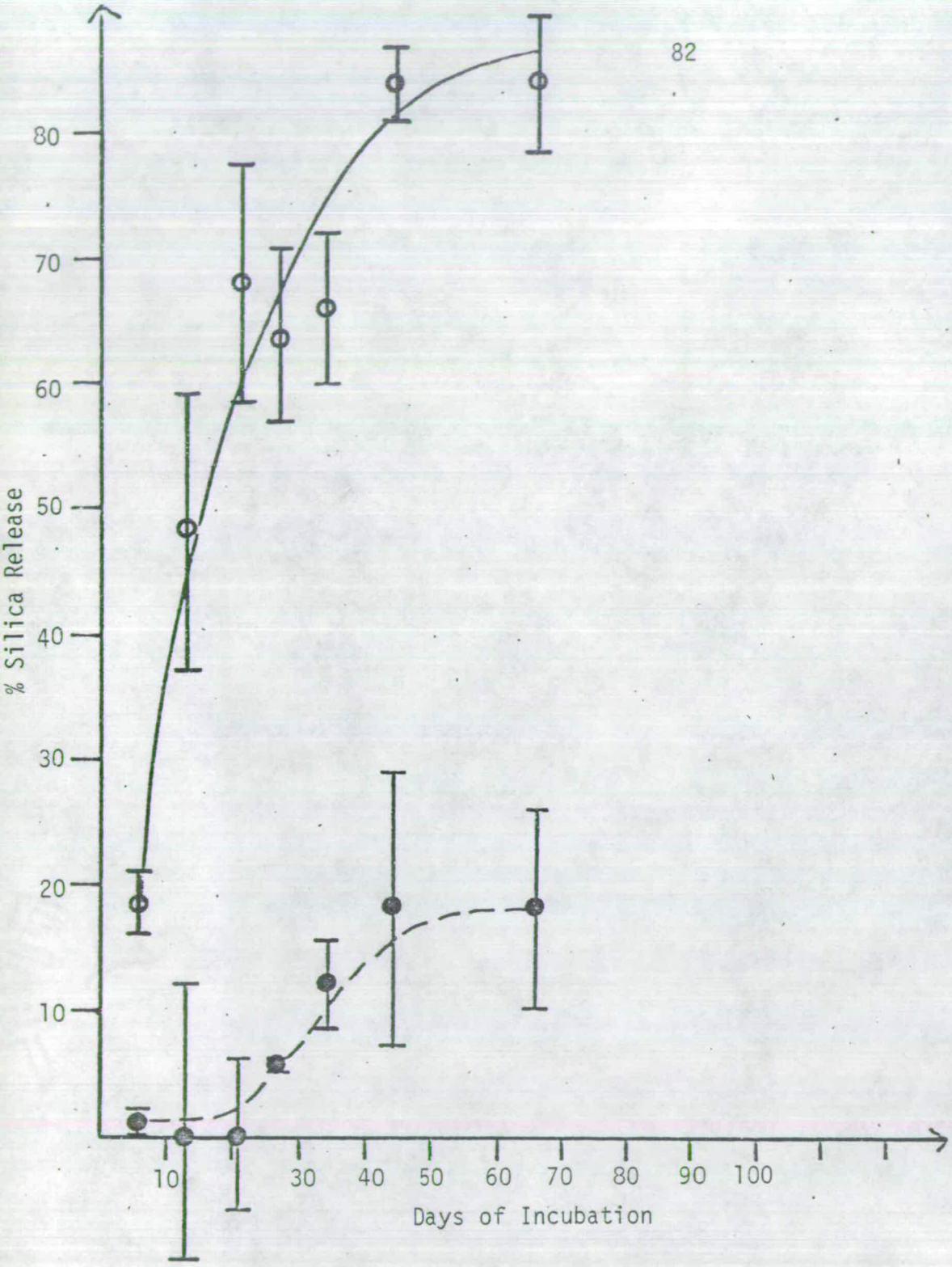


Fig. 4.5 The release of soluble silica from heat-killed *A. formosa* at 25°C in the presence of Lough Neagh water (O) and Lough Neagh water with mercuric chloride (1g./l.) added (●)

All experiments were incubated at 25°C. *A. formosa* and *T. flocculosa* were also incubated at 4°C (see *). Changes in the soluble silica concentration in controls of only lake water, heat sterilised lake water and lake water containing mercuric chloride (1g/l) were subtracted from changes in the tubes containing the diatoms, before the percentage silica released from the suspended diatoms was calculated.

RESULTS & DISCUSSION

a. THE EFFECT OF LAKE BACTERIAL POPULATIONS

The release of silica from diatoms in the presence of lake bacterial populations and from controls sterilised with mercuric chloride was compared. The results in Fig. 4.4 and 4.5 show that both Loch Leven and Lough Neagh bacterial populations enhance the release of soluble silica from these diatoms of which *A. formosa* is a common bloom former in Loch Leven (Bailey-Watts, 1974). The presence of the mercuric chloride did not appear to effect the silica concentration as addition of this to the suspensions of *A. formosa* and *T. flocculosa* in Loch Leven water at the end of the experiment did not cause any change in the silica concentration.

Release occurs to a different extent with different diatom types. A number of physical factors can effect the solubility of the frustule, (see Introduction, p. 30) and differences for various diatom types have been reported. For example, frustule thickness and the hydration of the frustule silica may influence the solubilisation. Strict comparison of the silica released from these diatoms may not be valid, as different cultural conditions can result in solubility differences with only one diatom type. However, it is interesting that *N. pelliculosa* which consistently resulted in a low level of release is one of the smaller diatoms.

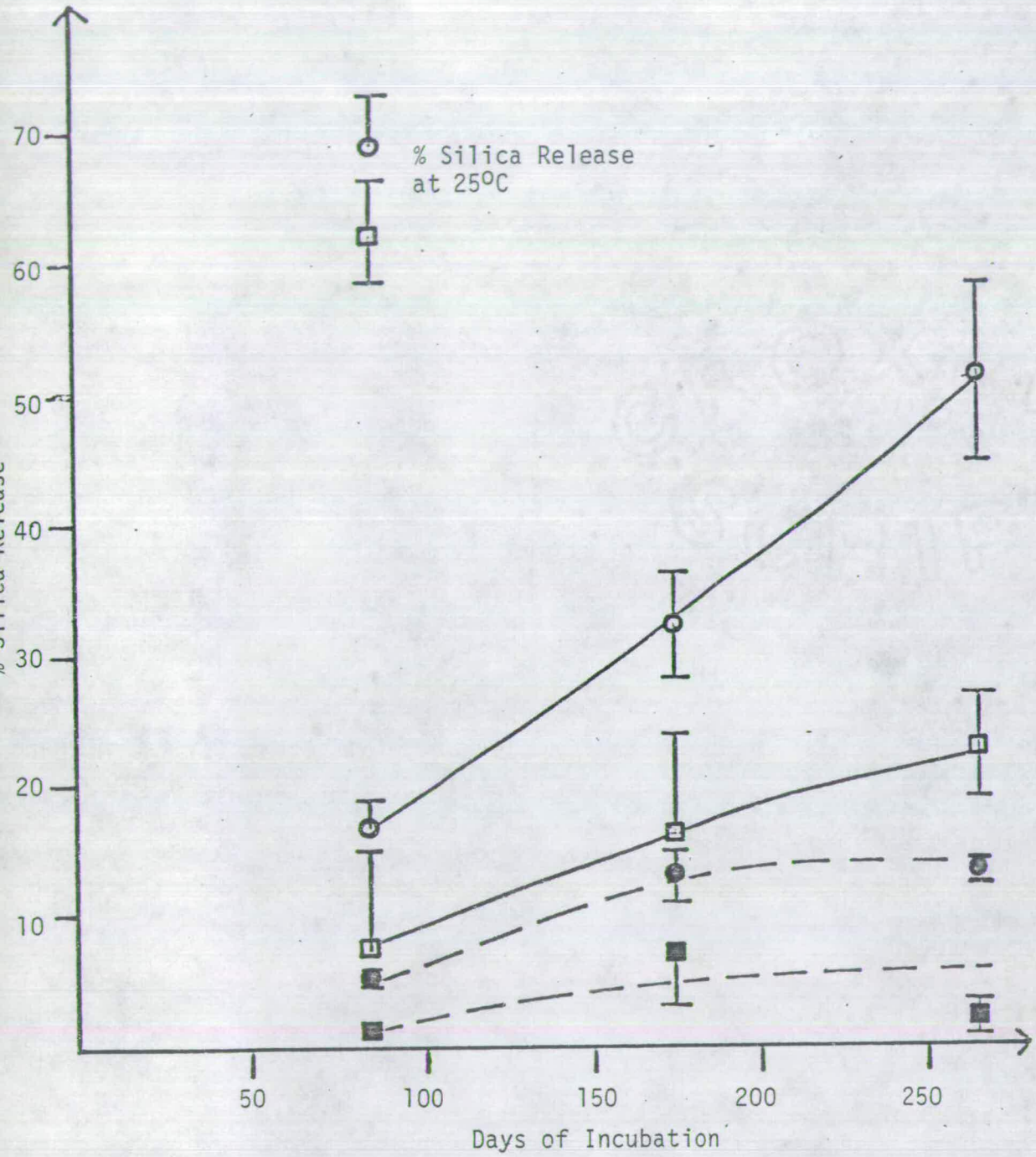


Fig. 4.6 The release of soluble silica from heat-killed *A. formosa* (O) and *T. flocculosa* (□) at 4°C suspended in Loch Leven water (——) and Loch Leven water with mercuric chloride (1g./l.) added (— —)

It would be interesting to determine if there was any relationship between the ratio of cell volume to frustule thickness and the amount of silica released.

The amount of nutrient material available for bacterial growth will determine microbial activity. The sources of nutrients available to the bacteria include

a) by-products of diatom metabolism;

b) the contents of the diatom cells; and

c) other nutrients present in the water column.

This important question is considered in Section V.

Incubation at 4°C also resulted in enhanced release from *A. formosa* and *T. flocculosa* (Fig. 4.6) although to a lesser extent than at 25°C. This experiment again reflects the differences in the amount of silica released from the two diatoms. Although it is quite uncommon for Loch Leven to be frozen over for long periods, the temperature of the water will be approximately 4°C for 3 to 4 months in a normal winter. From these results it would seem that even at this temperature lake bacteria can have an effect on the solubilisation of silica, although this is less than at 25°C, i.e. after approximately 9 months incubation less silica was released from *T. flocculosa* than at 25°C after only 2½ months. However continued incubation at the lower temperature might result in similar final amounts of silica being solubilised.

b. THE EFFECT OF THE BACTERIA CONTAMINATING THE DIATOM CULTURES

The release of silica from diatoms in the presence of the bacteria contaminating the cultures, and in the presence of Loch Leven bacteria, were compared to determine if the contaminating bacterial populations had any effect. If they had not, diatoms without added mercuric chloride could be used as controls.

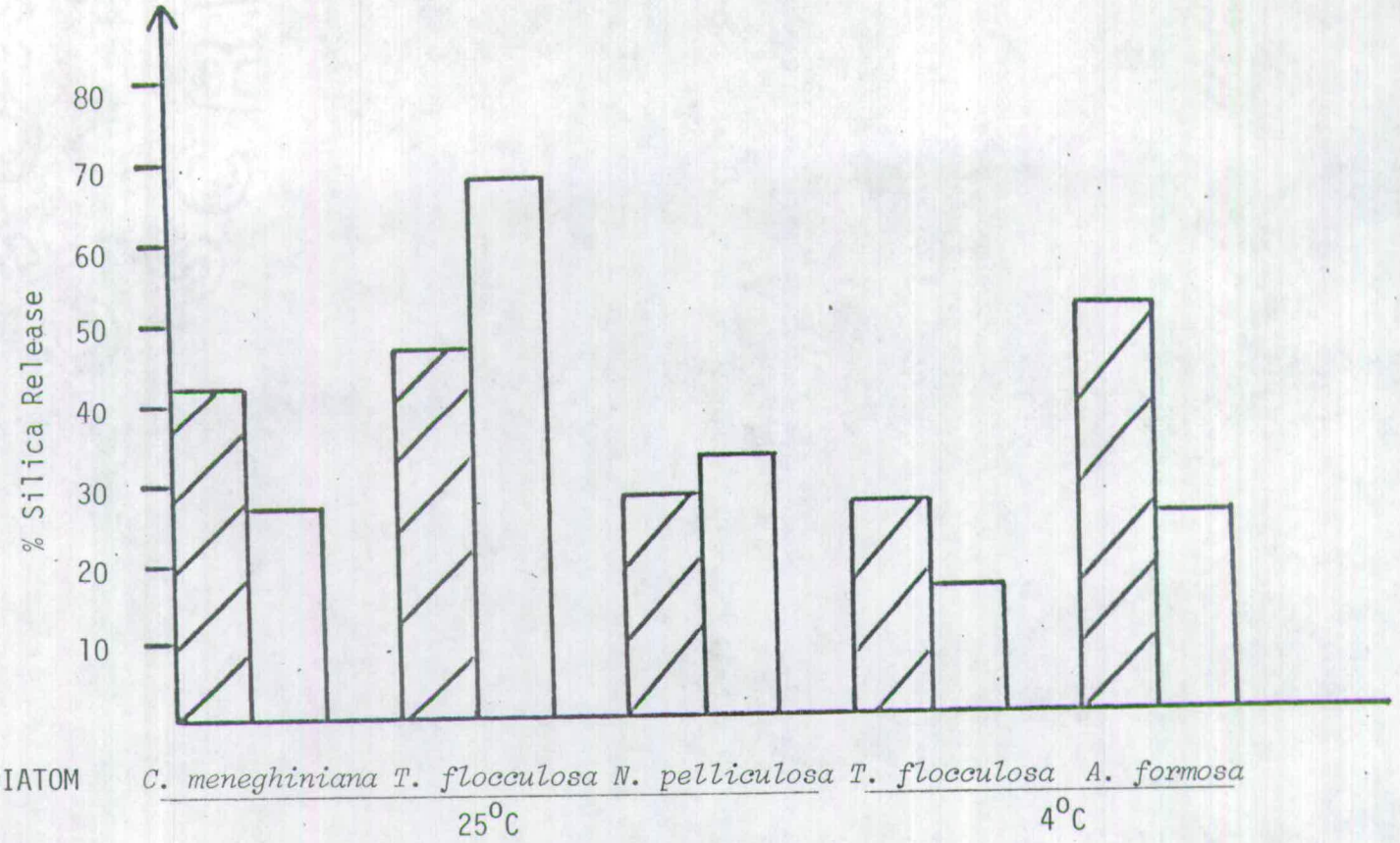


Fig. 4.7 The effect of Loch Leven bacteria and bacteria contaminating diatom cultures on the percentage silica release

Suspended in Loch Leven water

Suspended in heat sterilised L. Leven water

From Fig. 4.7 it can be seen that contaminating bacteria in the various diatom cultures are also capable of causing the release of soluble silica with a varying capacity relative to the loch populations, even after the diatoms have been heat-killed.

These differences could reflect the contaminating bacterial populations effectiveness' in decomposing diatoms. For example in the instances where less silica is being released from the diatoms (e.g. *C. meneghiniana* at 25°C) the bacteria could be adapted to growth on the nutrients released during diatom growth, rather than decomposition of the diatoms. On the other hand the bacteria contaminating the *T. flocculosa* appeared to be more successful at causing the release of soluble silica at 25°C, and less at 4°C.

SUMMARY OF RESULTS

1. Loch Leven and Lough Neagh water bacterial populations enhance silica release over sterile autolysis of an axenic *C. meneghiniana* culture.

2. There were fewer bacterial types present after 50 days incubation with the axenic *C. meneghiniana* than in the original Loch Leven water.

3. Loch Leven bacteria enhance silica release over mercuric chloride sterilised controls of *T. flocculosa, A. formosa, N. pelliculosa C. meneghiniana.* Similar results were obtained with Lough Neagh water and *A. formosa.*

4. The bacteria contaminating the diatom cultures and Loch Leven water populations have different effects on the amount of silica released. In some instances the contaminating populations have a greater effect and in others a lesser effect than the Loch Leven populations.

5. The rate of silica release from the diatoms *A. formosa* and *T. flocculosa* in the presence of Loch Leven bacteria, incubated at 4°C, is greater than from a mercuric chloride sterilised control although this is much slower than at 25°C.

V. AN INVESTIGATION OF THE POSSIBLE MODES OF BACTERIAL ACTION

V. AN INVESTIGATION OF THE POSSIBLE MODES OF BACTERIAL ACTION

In an attempt to gain some insight into the nutrient conditions associated with the bacterial dissolution of the diatom silica, the effect of nutritional amendments was investigated. The physical effects of agitation and sonication were also examined.

1. THE EFFECT OF THE AVAILABILITY OF BACTERIAL NUTRIENTS ON SOLUBLE SILICA RELEASE

INTRODUCTION

a. LOCH LEVEN WATER SUPPLEMENTED WITH CASAMINO ACIDS AT TWO DIFFERENT CONCENTRATIONS

A low concentration, approximately equivalent to the total organic nitrogen content of Loch Leven water (Holden & Caines, 1974) and a high concentration, based on the content of media used for nutritional studies of soil and herbage bacteria by Keddie et al. (1966) were used. Casamino acids were chosen because Hecky et al.'s (1973) model of the organic component of the wall includes an amino acid template. The use of the added amino acids might effectively 'spare' the frustule amino acids and reduce decomposition. Alternatively de-amination could result in a pH increase. An increase above 9 in the micro-environment might enhance silica release (see Introduction, p.17).

b. LOCH LEVEN WATER SUPPLEMENTED WITH GLUCOSE AT TWO DIFFERENT CONCENTRATIONS

Glucose is another potential nutrient found in diatoms. It is present in the organic coat and the major storage carbohydrate of

diatoms, chrysolaminarin, is a glucan. Secondly, Lewin (1961) found that the addition of chelating agents enhanced silica release. Therefore silica could be solubilised in a similar way to the bacterial solubilisation of insoluble inorganic phosphates and silicates in soils, i.e. by the production of chelating agents such as 2-keto-gluconic acid, which is synthesised from glucose, (Duff et al., 1963).

METHODS

Batch cultures of *N. pelliculosa*, *A. formosa*, *C. meneghiniana* and *T. flocculosa* were prepared as detailed in the Materials & Methods, p. 59.

The following treatments were then set up:

a. *N. pelliculosa*, heat-killed and suspended in;
   1. Loch Leven water,
   2. Heat sterilised Loch Leven water,
   3. Loch Leven water + glucose (.002g./l.),
   4. Loch Leven water + glucose (2g./l.),
   5. Loch Leven water + Casamino acids (0.002g./l.) and
   6. Loch Leven water + Casamino acids (0.5g./l.).

Unfortunately when this experiment was carried out the method used to determine the total insoluble silica was inaccurate (see p.56). Therefore there is no value for the initial total silica content of the *N. pelliculosa*. The results are expressed as the final soluble silica concentration in µgSi/ml., minus the soluble silica concentration in control tubes to which diatom cultures had not been added. Tubes were incubated at 25°C in the dark.

Treatments 1, 2 & 4 were repeated and the initial total silica concentration of the *N. pelliculosa* culture was 19.9µgSi/ml. Results

for this experiment are expressed as the percentage of the silica released and detected by the soluble silica analysis.

b. *C. meneghiniana*, *A. formosa* and *T. flocculosa*, heat-killed and suspended in:

1. Loch Leven water,
2. Heat sterilised Loch Leven water,
3. Loch Leven water + mercuric chloride (1g./l.) and
4. Loch Leven water + glucose (2g./l.).

The *T. flocculosa* and *A. formosa* were incubated at 4°C in the dark and the results expressed as a percentage of the initial silica content of the diatoms. These were 2.8µgSi/ml. (*A. formosa*) and 5.6µgSi/ml. (*T. flocculosa*). The *C. meneghiniana* was incubated at 25°C and the results are expressed as the final concentration of soluble silica (µgSi/ml.) minus the concentration in the control tubes.

RESULTS & DISCUSSION

The addition of these nutrients, with the exception of the high glucose concentration, did not influence the release of silica from the *N. pelliculosa* (see Table 5.1). Therefore the mechanism of bacterial dissolution may not be as simple as some of the hypotheses discussed on p. 90 . The use of simply a carbon or nitrogen source does not appear to increase the dissolution of the silica, which suggests that the degradation of specific compounds present in the diatoms may be important in the process of solubilisation. Release in the presence of the high concentration of glucose resulted in a lower final concentration of dissolved silica. This reduction did not occur in the control tubes which did not contain diatoms. Similar results were obtained when

Table 5.1 The Effect of the Addition of Glucose and Casamino Acids on the Release of Soluble Silica from Heat-killed *Navicula pelliculosa* Suspended in Loch Leven Water

	DIATOMS SUSPENDED IN:					
	LOCH LEVEN WATER	HEAT STERILISED L. LEVEN WATER	L. LEVEN WATER + GLUCOSE (.0002g./l.)	L. LEVEN WATER + GLUCOSE (2g./l.)	L. LEVEN WATER + CASAMINO ACIDS (.0002g./l.)	L. LEVEN WATER + CASAMINO ACIDS (0.5g./l.)
EXPERIMENT 1 (AFTER 63 DAYS AT 25°C)						
INCREASE IN SOLUBLE SILICA CONCENTRATION	3.94 ± 0.16	3.78 ± 0.22	4.00 ± 0.05	0.78 ± 0.026	3.95 ± 0.16	3.32 ± 0.47
TOTAL VIABLE COUNT ON CPS AGAR	10^6	10^8	10^6	10^8	10^6	10^6
NO. OF BACTERIAL TYPES*	10	2	12	4	8	5
EXPERIMENT 2 (AFTER 22 DAYS AT 25°C)						
PERCENTAGE SOLUBLE SILICA RELEASE	31	36	N.D.	7	N.D.	N.D.

*Based on colony morphology, microscopic observation and Gram stain.

N.D. = Not Done.

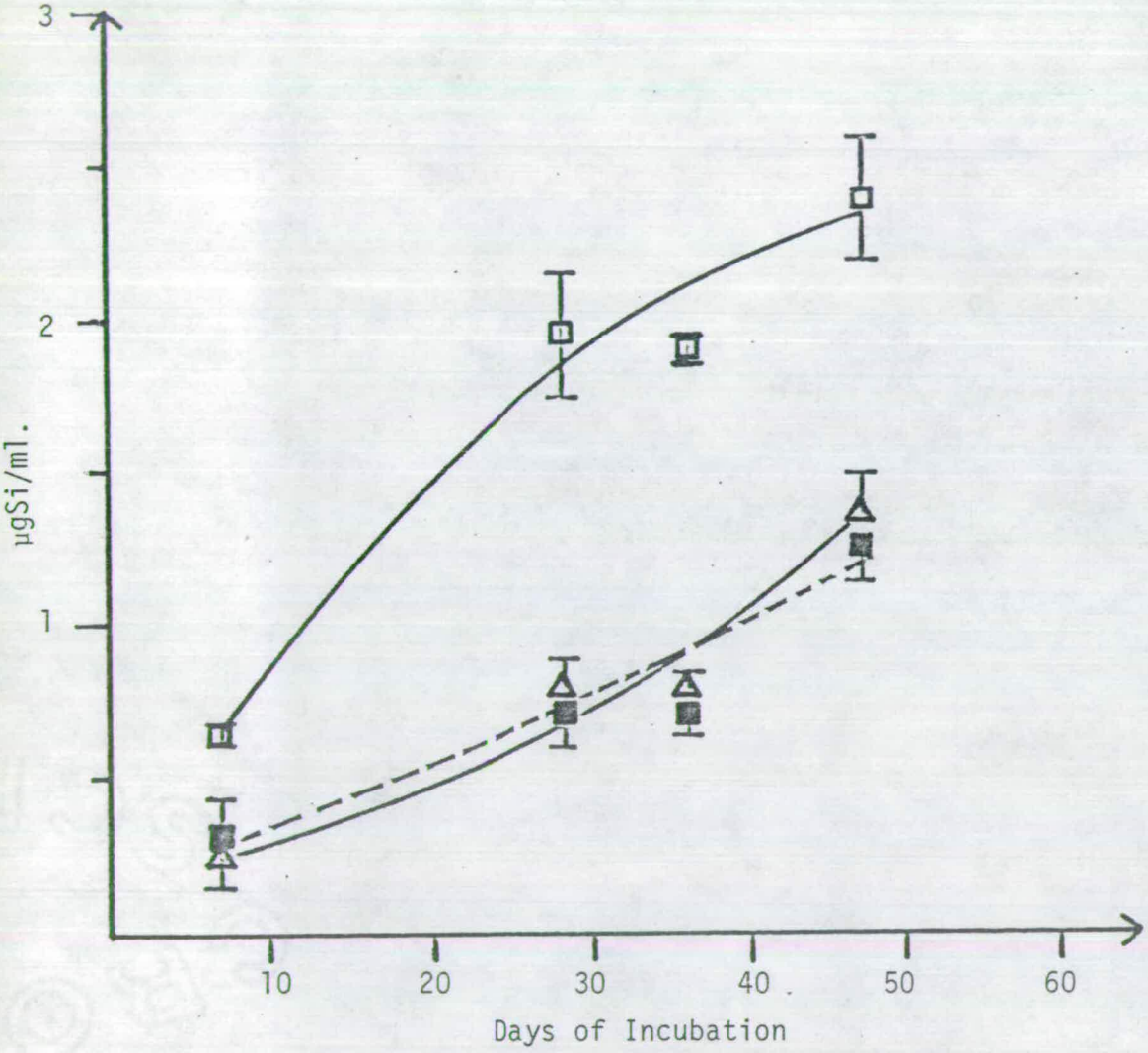


Fig. 5.1 The release of soluble silica from *C. meneghiniana* at 25°C in the presence of L. Leven water (□), L. Leven water with glucose (2g./l.) (△), and L. Leven water with mercuric chloride (1g./l.) (--■-)

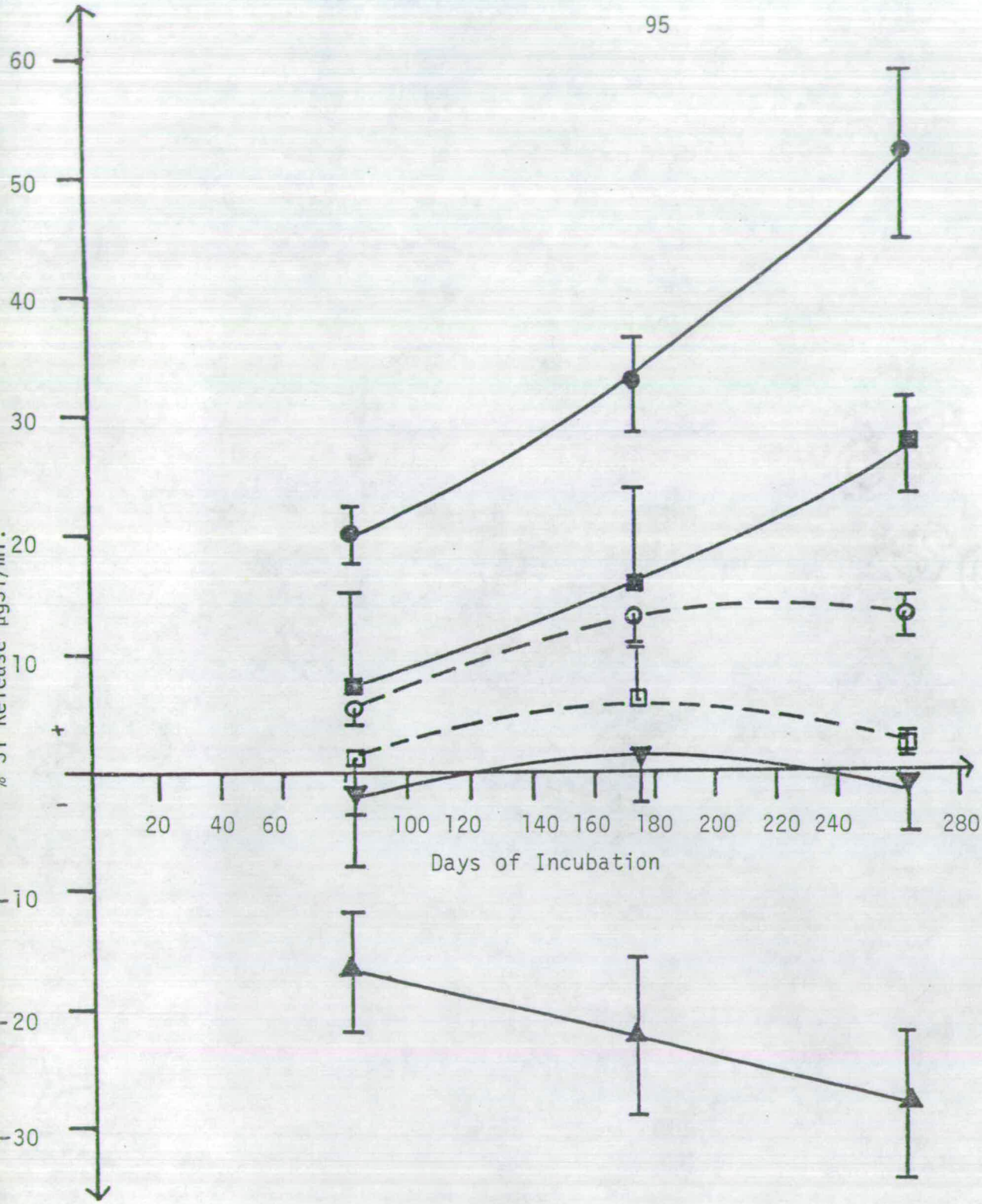


Fig. 5.2 The percentage of soluble silica released from suspensions of xenic *A. formosa* and *T. flocculosa* suspended in Loch Leven water and incubated at 4°C.
KEY: *A. formosa* suspended in loch water (●); *A. formosa* suspended in heat sterilised loch water (-○); *A. formosa* suspended in heat sterilised loch water + glucose (1g./l.) (▲); *T. flocculosa* suspended in loch water (■); *T. flocculosa* suspended in heat sterilised loch water (-□); *T. flocculosa* suspended in loch water + glucose (1g./l.) (▼)

C. meneghiniana was incubated in the presence of glucose (2g./l.)(see Fig. 5.1). In the experiments incubated at 4°C, with the diatoms *A. formosa* and *T. flocculosa*, there was a reduction in the amount of silica present, when compared to the mercuric chloride sterilised control (Fig. 5.2).

Possible explanations of this phenomenon include the following:

a. The soluble silica is undergoing polycondensation in the presence of the glucose. This is unlikely at these concentrations of silica and pH. The pH at the end of the incubation period at 4°C in the presence of glucose (2g./l.) dropped from 6.8 to 4.4 in the case of *T. flocculosa* and to 3.9 for the *A. formosa*. This change suggests that the micro-organisms are fermenting the glucose, however this should not effect the silica concentration.

b. The silica is being solubilised and then taken up by the bacteria. Heinen (1963) reported the uptake of dissolved silica by a *Proteus mirabilis* strain isolated from soil. This effect was enhanced in the presence of various nutrients including glucose. The uptake of silica in the presence of glucose by pure cultures isolated from the *N. pelliculosa* in experiment 1, (see Table 5.1), was demonstrated by L. Foster (unpubl.). She found that two isolates took up silica from a sodium metasilicate solution after incubation for 3 days in a medium containing Casamino acids (0.05%), yeast extract (0.05%) and glucose (0.2%). Silica was not taken up in the absence of glucose. One of these isolates was tentatively identified as a member of the Enterobacteriaceae. Two other isolates, thought to be *Pseudomonas* sp., did not take up silica from the medium. Examination of the silica content of one of the isolates showed that 70% of the silica lost from

solution was present in the bacterial cells and capsules. The reduction in the silica concentration was in the order of 0.36, 0.35 and 0.3µgSi/ml. from solutions with silica concentration of 3.2, 1.25 and 0.4µgSi/ml., at cell concentrations of 10^8 to 10^9/ml. There were approximately 10^8 bacteria/ml. after incubation of the *N. pelliculosa* at the high concentration of glucose (Table 5.1, Experiment 1). Assuming that these bacteria were taking up silica to a similar extent, this would not account for the reduction in the silica concentration (i.e. from 4.0 to 0.78µgSi/ml.).

c. It is possible that with the nutrient supplement the bacteria do not decompose parts of the diatom which, in so doing, would influence the solubilisation of the silica (e.g. the carbohydrate component of the wall). This 'sparing' effect could be the result of the use of the glucose as an alternative nutrient, or as a result of the selection of a slightly different microbial population. There was a reduction in the types of bacteria present at the high concentration of glucose when compared to the loch water alone. Therefore the bacteria which decompose the components of the diatoms, which in turn influence the solubility of the frustule, may not have been selected.

If this is the case, it may be that the carbohydrate component of the wall is important in protecting the frustule from dissolution. On the other hand, a similar effect was not obtained in the presence of the Cas-amino acids. It is possible that either, the Cas-amino acids do not supply the particular amino acids required by the bacteria, or that the protein component of the wall does not have as important a protective role as the carbohydrate.

2. THE IMPORTANCE OF NUTRIENTS PRESENT IN LOCH LEVEN WATER

INTRODUCTION

An attempt was made to determine the relative importance of the different nutrient sources available to the bacteria active in decomposition in the water column. Therefore the release of soluble silica from diatoms was examined in a number of different suspending media.

a. PARTICULATE MATTER PRESENT IN LOCH LEVEN WATER

Loch Leven water was filtered through Whatman's No. 1 filter paper to remove the larger particles present. Unfortunately this procedure would also have altered the populations of bacteria, in that many of those associated with the particulate matter remained in the filter paper (see Section III).

b. SOLUBLE NUTRIENTS PRESENT IN LOCH LEVEN WATER

Suspension of the xenic diatoms in filter sterilised Loch Leven water would also give some indication of the importance of particulate matter. Only the soluble nutrients present in the water would be available for the growth of the contaminating bacteria. Heat sterilised Loch water would contain all the particulate matter present in the water, however the process of sterilisation (15 min. at 121^{o}C) may destroy some of the growth factors present in the water.

c. NUTRIENTS PRESENT IN THE DIATOM CELLS

Suspension of the xenic diatoms in the salt solution of one of the diatom growth media (Chu 10) without any silica or growth factors added would result in the only nutrient source being the diatom cells.

METHODS

Batch cultures of *N. pelliculosa*, *T. flocculosa* and *C. meneghiniana* were prepared as in the Materials & Methods (p.59). The following treatments were then set up:

a. *N. pelliculosa* suspended as follows;

1. Initially active culture, in Loch Leven water,
2. " " " in filtered Loch Leven water,
3. Heat-killed culture in Loch Leven water,
4. " " in filtered Loch Leven water,
5. " " in heat sterilised Loch Leven water and
6. " " in filter sterilised Loch Leven water.

The initial total silica content of the diatoms was 9.0µgSi/ml. Filtered Loch Leven water was prepared by filtering, under pressure, through Whatman's No. 1. filter paper. This removed the particulate matter present in the water, including the algae. Filter sterilised water was prepared by filtering under pressure through Millipore membrane filters (pore size 0.22µm.).

b. *N. pelliculosa*, *T. flocculosa* and *C. meneghiniana* were heat-killed and suspended in some of the following media;

1. Loch Leven water,
2. Heat sterilised Loch Leven water,
3. Filter sterilised Loch Leven water,
4. Chu 10 salt solution (without silica or growth factors) and
5. Loch Leven water with mercuric chloride (1g./l.).

Tubes were incubated at 25°C in the dark. The

results are expressed as the percentage of initial total silica of the diatom cultures released in a soluble form. Changes in the silica concentration in control tubes with no added diatom culture were taken into account.

RESULTS & DISCUSSION

Filtering of the Loch Leven water through Whatman's No. 1 filter paper resulted in a reduction in the amount of silica released when compared with unfiltered water (see Fig. 5.3). This may be attributable to a reduction of the numbers of Loch bacteria when the water is filtered. Bacteria associated with particulate matter will remain in the filter. However the diatoms were not axenic and the bacteria contaminating the *N. pelliculosa* culture enhanced silica release to the same extent as the Loch Leven populations in previous experiments (see Fig. 4.7, p. 86). It is therefore possible that the reduction in the amount of silica released is a result of the removal of nutrients used for growth by the bacteria. Further evidence for this can be found in the smaller amount of silica released when the diatoms are suspended in filter sterile Loch Leven water, as opposed to heat sterilised Loch water. Suspension in the salt solution of Chu 10 also reduced the amount of silica released (see Table 5.2). It would appear that the reduction in available nutrients is limiting the growth of the contaminating bacteria, which subsequently effects the release of soluble silica.

However when the *N. pelliculosa* was re-suspended in 'fresh' heat sterilised Loch Leven water at the end of the period of silica release, no further silica was solubilised. This suggests that the bacteria are not relying only on the nutrients present in the Loch water for growth. They possibly utilise growth factors present in the water, but require

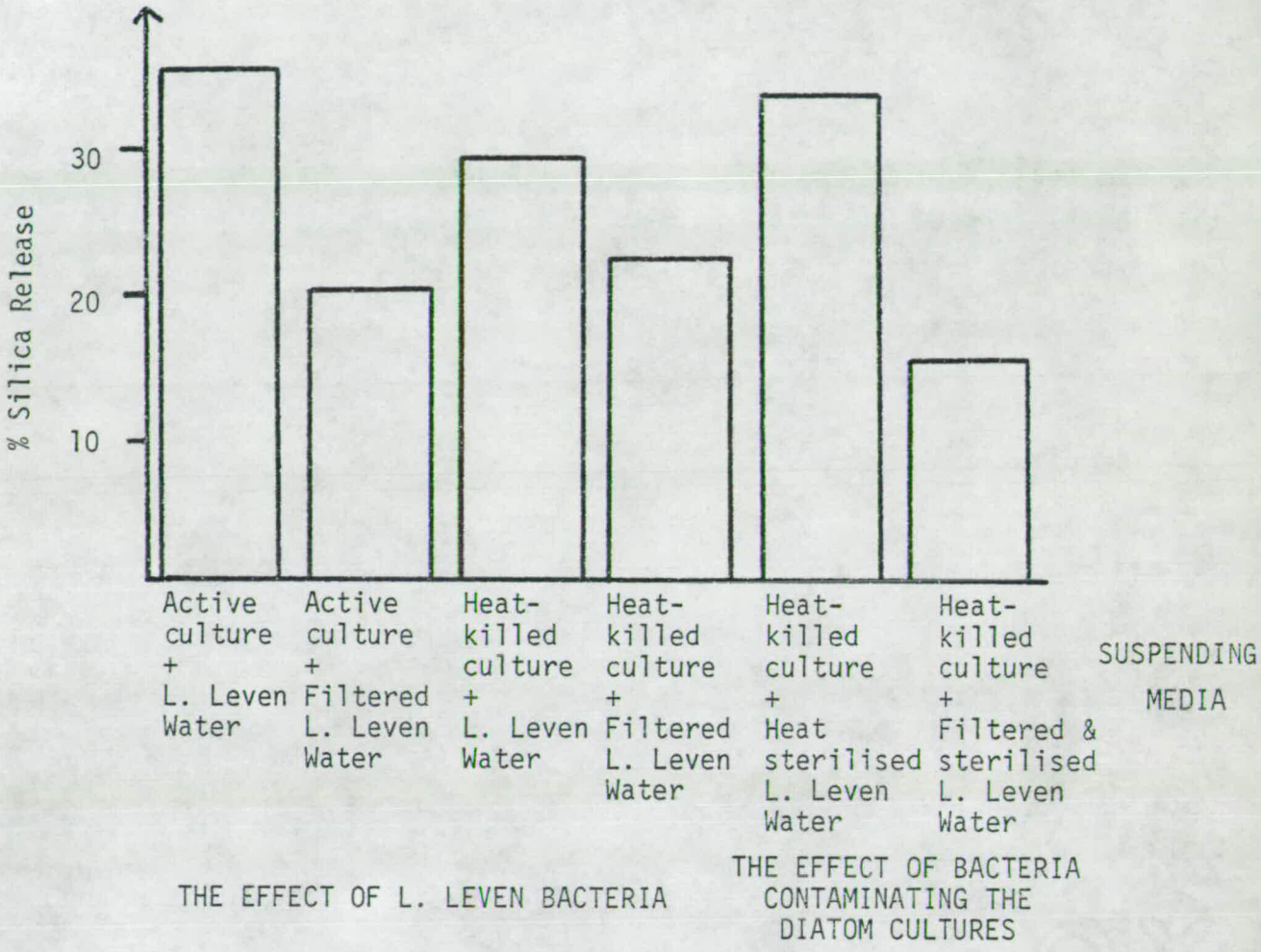


Fig. 5.3 The release of soluble silica from *N. pelliculosa* after 57 days incubation at 25°C.

Table 5.2 The Percentage of Soluble Silica Released from Heat-killed Diatoms after Suspension in a Variety of Media and Incubation at 25°C for 55-60 Days

DIATOM	SUSPENDING MEDIUM: LOCH LEVEN WATER	HEAT STERILISED L. LEVEN WATER	FILTER STERILISED L. LEVEN WATER	CHU SALT SOLUTION	L. LEVEN WATER + $HgCl_2$ (1g./T.)
N. pelliculosa	29	33	15	N.D.	N.D.
T. flocculosa	39	73	21	6	N.D.
C. meneghiniana	42	27	N.D.	18	4.5

N.D. = Not Done.

the diatom cells for the supply of carbon and nitrogen. Alternatively the solubility of only part of the frustule is influenced by bacterial growth. It would be interesting to determine if the addition of the cytoplasmic contents of diatom cells at the end of one of these experiments resulted in any further solubilisation of the silica, or if parts of the frustule were truly 'recalcitrant'.

Bailey-Watts (1976b) reported that less than 5% of the silica was released when Loch Leven phytoplankton was suspended in de-ionised water. It is possible that this was again the result of the reduction in growth factors, or alternatively loss of viability of the bacteria when suspended in de-ionised water.

In conclusion, it would appear that the growth of bacteria which appear to influence the solubility of the diatom frustules is dependent on nutrients present in the Loch water, as well as those present in the diatom cells.

3. THE EFFECT OF PHYSICAL AGITATION AND SONICATION OF THE DIATOM CELLS

INTRODUCTION

Suspensions were agitated during incubation, in an attempt to imitate the effects of turbulence in the lakes. The effect of sonication of the diatoms prior to incubation was also investigated. Breaking of the cells could result in greater dissolution as a larger surface area would be exposed. Grinding of quartz increases its solubility as the surface layer is disturbed (Henderson et al., 1970). It has also been suggested that the breaking of diatom cells by the grazing of zooplankton would enhance the solubility of the frustule (Cooper, 1952).

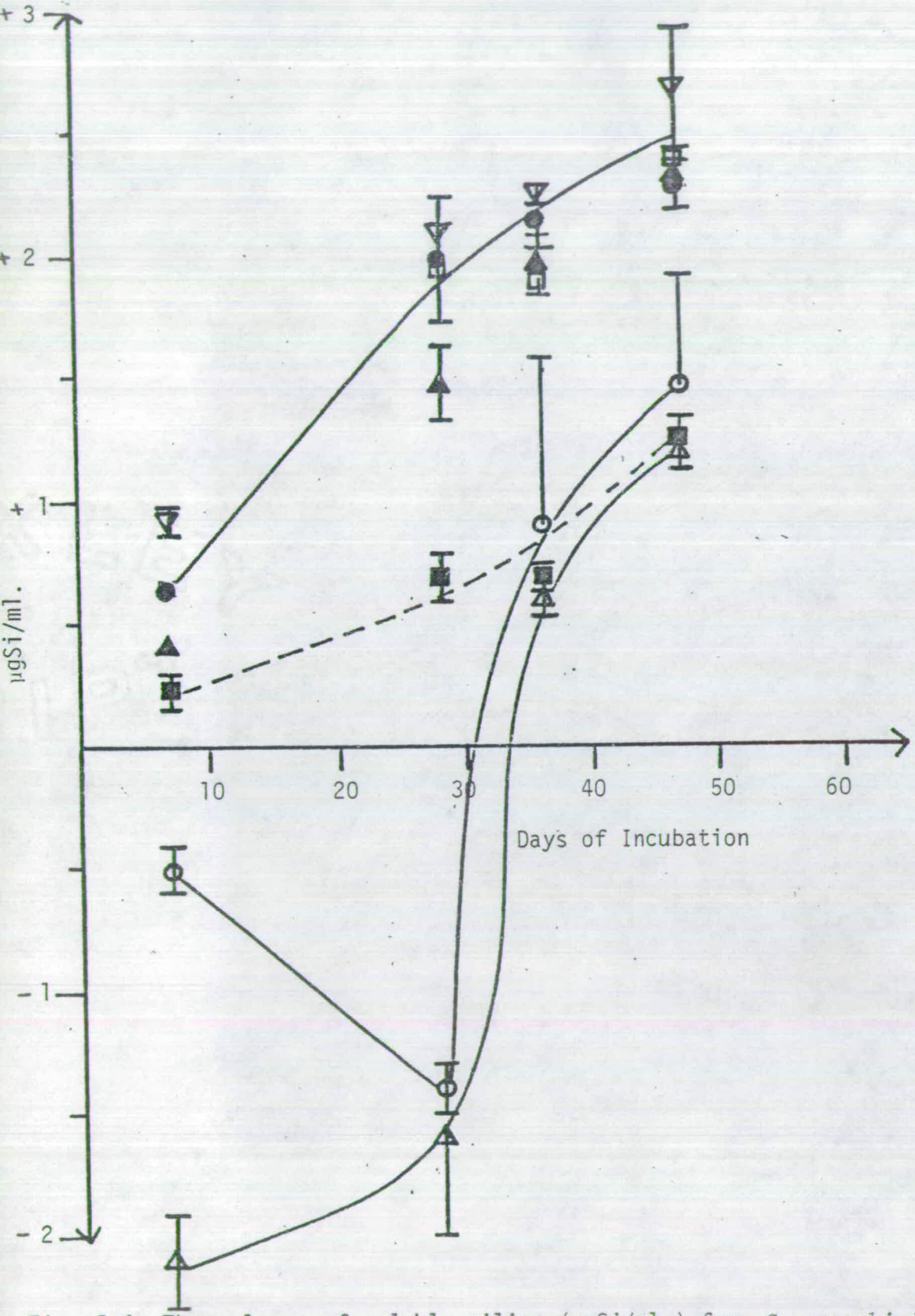


Fig. 5.4 The release of soluble silica (µgSi/ml.) from *C. meneghiniana* incubated under the following conditions at 25°C

a) Initially active culture,
   1. agitated in the dark ●
   2. statically in the dark ▲
   3. agitated in the light ○
   4. statically in the light △

b) Heat-killed culture,
   1. agitated in the dark ▽
   2. statically in the dark □
   3. agitated in the dark with mercuric chloride (1g./l.) -■-

a. THE EFFECT OF AGITATION ON SILICA RELEASE IN THE LIGHT AND DARK

METHODS

A batch culture of xenic *C. meneghiniana* was prepared as detailed in the Materials and Methods section, p. 59. The following treatments were set up:

a. Initially active cultures were suspended in Loch Leven water and incubated as follows;
   1. agitated in the dark,
   2. statically in the dark,
   3. agitated in the light and
   4. statically in the light.

b. Heat killed cultures were suspended in Loch Leven water and incubated as in treatments 1 and 2 and also in Loch Leven water with mercuric chloride (1g./l.) added.

All the tubes were incubated at 25°C. Moving incubation was mediated by placing the tubes on a Spiromix. The results are expressed as concentration of silica (µgSi/ml.) after subtraction of the control tubes to which diatoms had not been added.

RESULTS & DISCUSSION

Incubation in the light under static and agitating conditions resulted in an initial uptake of silica by the diatoms (see Fig. 5.4). This suggests that they remain viable in the light for the first 20 to 25 days of incubation. It therefore appears that the bacteria present are not inhibitory to diatom growth, i.e. they do not kill active diatom cells, but colonise the cells once they become inactive.

Agitation of the diatom suspensions might be expected to enhance the silica release as a result of either a. increased aeration affecting microbial growth; b. the movement causing physical damage to the cells; or c. even distribution of the dissolved silica. However the silica released from both alive and heat killed cells in the dark was similar, whether incubation was static or on the Spiromix (Fig. 5.4).

b. THE EFFECT OF ULTRA-SONICATION ON THE RELEASE OF SOLUBLE SILICA

METHODS

Batch cultures of the diatoms *N. pelliculosa* and *C. meneghiniana* were prepared as in the Materials & Methods, p. 59. The following treatments were set up;

1. Heat-killed culture suspended in Loch Leven water,
2. Heat-killed culture, ultra-sonicated and suspended in Loch Leven water,
3. Heat-killed culture, ultra-sonicated, centrifuged and the supernatant decanted to remove the diatom cell contents released after sonication. Re-suspension in Loch Leven water,
4. Heat-killed culture suspended in Loch Leven water with mercuric chloride (1g./l.) added,
5. Heat-killed culture ultra-sonicated and suspended in Loch Leven water with mercuric chloride (1g./l.) and
6. Treated as in (3), but with the final re-suspension in Chu 10 salt solution, without silica.

These treatments would produce the following suspensions;

1. Whole diatom cells and Loch Leven water,
2. Fragmented diatom cells and Loch Leven water,

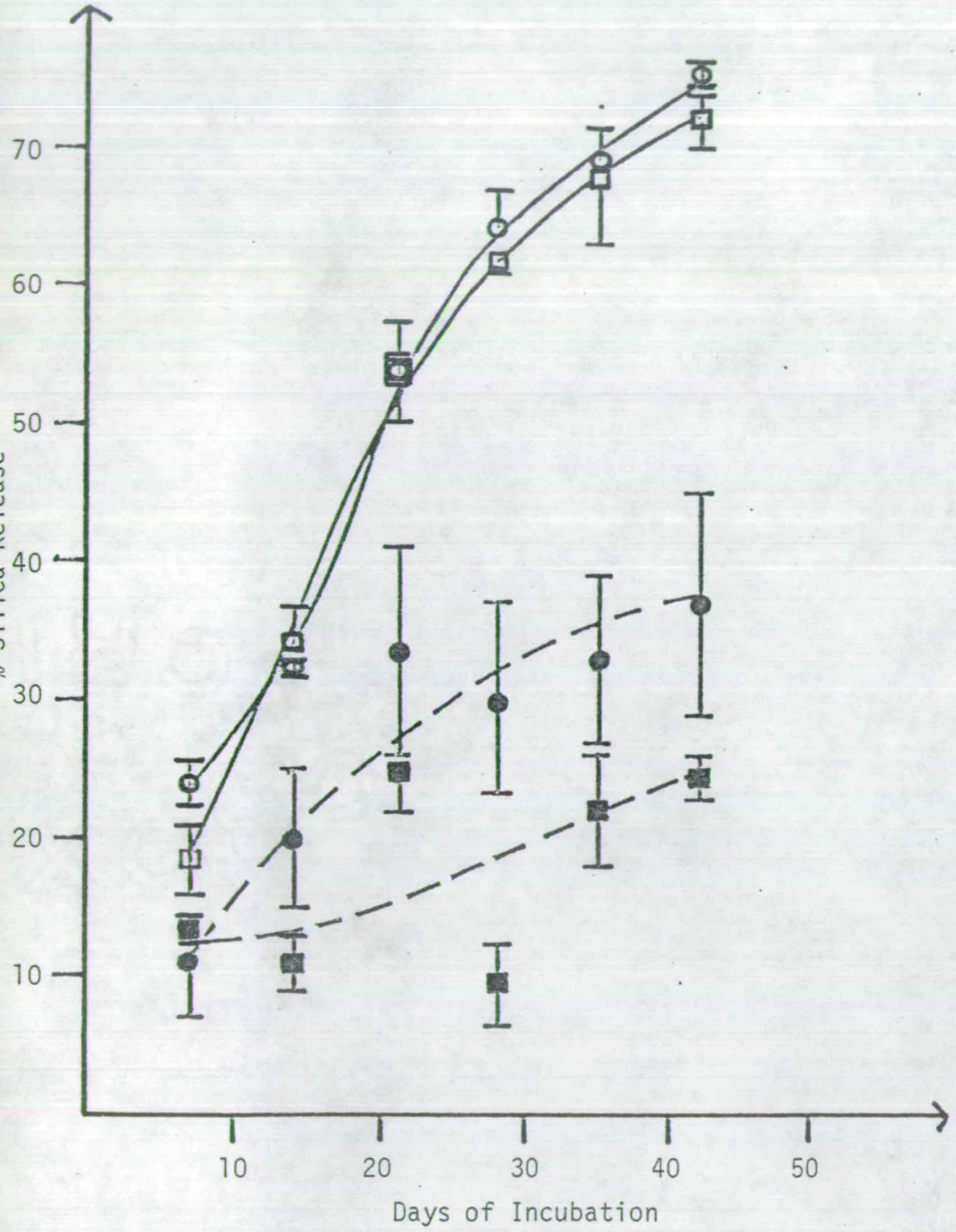


Fig. 5.5 The release of soluble silica from heat-killed (□) and ultra-sonicated (○) axenic *C. meneghiniana*, at 25°C, suspended in Lough Neagh water (——) and heat sterilised Lough Neagh water (– – –).

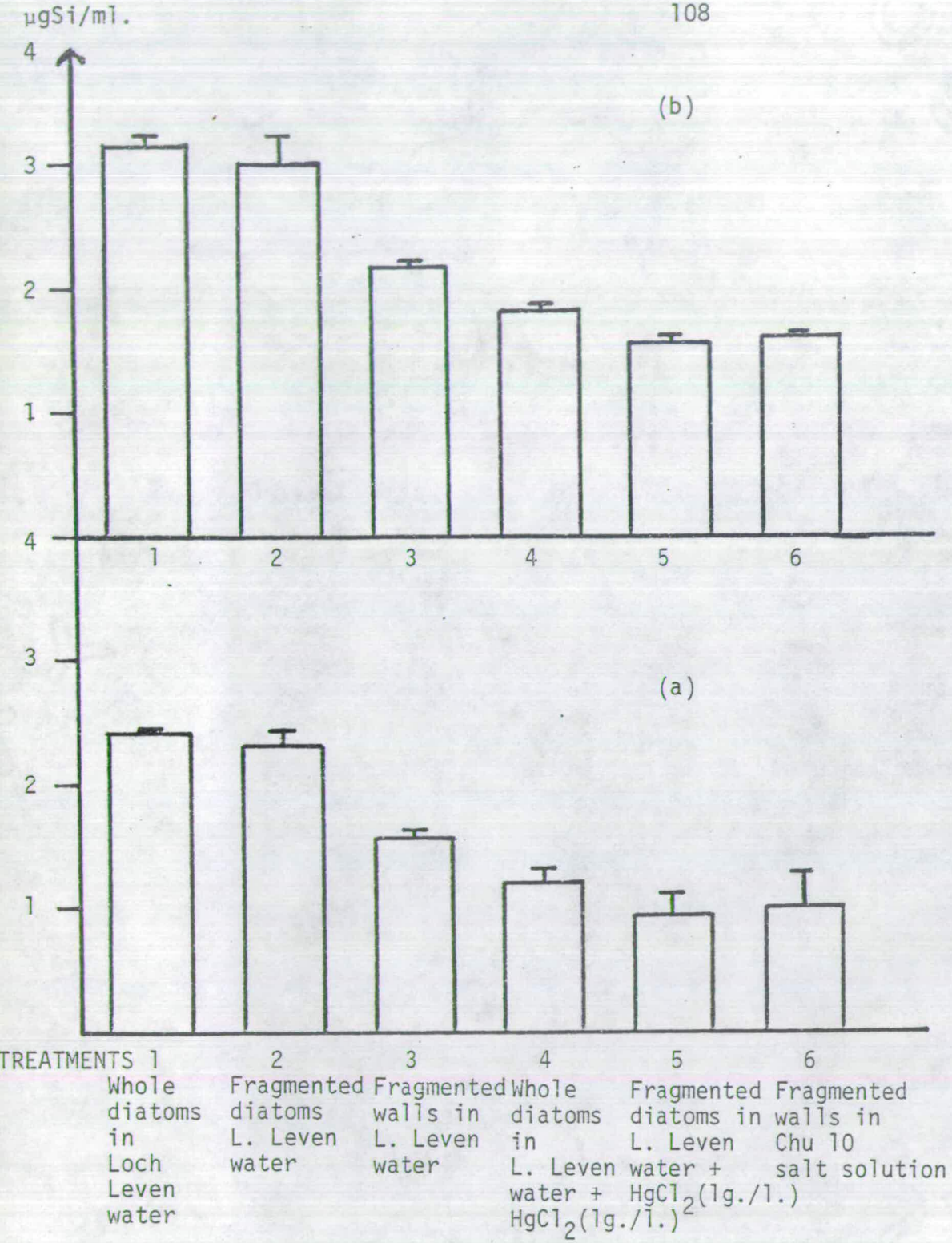


Fig. 5.6 The effect of ultra-sonication on the amount of silica released from the diatoms *N. pelliculosa* (a) and *C. meneghiniana* (b)

3. Fragmented walls (i.e. without most of the cytoplasmic contents) and Loch Leven water,
4. & 5. Suspensions as in 1 & 2, under sterile conditions and
6. Fragmented walls suspended under sterile conditions.

A batch culture of axenic *C. meneghiniana* was treated in the following way;

1. Heat-killed and suspended in Lough Neagh water,
2. Heat-killed and suspended in heat-sterilised Lough Neagh water,
3. Ultra-sonicated and suspended in Lough Neagh water and
4. " " " " in heat sterilised Lough Neagh water.

Ultra-sonication was carried out until at least 90% of the diatom cells no longer appeared whole under the light microscope. The *C. meneghiniana* required 10 x 30 second periods, and the *N. pelliculosa* 20 x 30 second periods. Under the microscope approximately 60% of the fragments were less than half the size of a complete frustule, although they were still recognisable as parts of a frustule.

The experiments were incubated at 25°C in the dark. Results are expressed as either the increase in silica concentration in µgSi/ml., or as the percentage of the initial total silica content of the axenic *C. meneghiniana* culture (2.7µgSi/ml.).

RESULTS & DISCUSSION

It is apparent from Figs. 5.5 and 5.6 that the amount of cell breakage brought about by ultra-sonication did not enhance the solubilisation of the silica. The amounts released were similar to those of whole cells incubated under similar conditions. In the experiment using the axenic *C. meneghiniana* culture there is a slight

increase in the silica released from sterile sonicated cells when compared to whole cells (Fig. 5.5). However a similar effect was not found when comparing the mercuric chloride sterilised controls (Fig. 5.6). The reduction in silica released from washed *C. meneghiniana* and *N. pelliculosa* (Treatments 3 & 6, Fig. 5.6), i.e. with most of the cytoplasmic contents removed, may be attributable to the loss of the cytoplasmic silica. Any silica associated with cytoplasmic contents will not be present and therefore not solubilised. Also the nutrients normally available for bacterial growth will be absent, and this factor may limit the extent to which the frustule silica is solubilised. There was no detectable soluble silica in the supernatant after the cells had been sonicated and spun down, which suggests that the silica present in the cytoplasm is not in a soluble form. However, it may be relatively more soluble than the frustule silica, as the initial amount of silica released is similar to that from the heat-killed cells (Fig. 5.5) (see Section IV, p. 76). This would also explain the reduction in the amount of silica released from washed frustules in the Chu salt solution (Fig. 5.6).

SUMMARY OF RESULTS & CONCLUSIONS

From these results one of the major factors effecting the release of soluble silica from diatoms would appear to be the growth of bacterial populations present. Silica was released to the greatest extent when all nutrients present in lake water and in the diatom cells were available for bacterial growth. Nutrient supplements did not enhance the silica release. This suggests that the relationship between the diatoms, the bacteria and the solubility of the frustule is specific to the decomposition of parts of the diatoms by the bacteria. The addition of glucose (2g./l.) resulted in less silica being released. Therefore

the decomposition of the carbohydrate component of the wall may be important in the solubilisation of the frustule. Silica uptake by bacteria in the presence of glucose is unlikely to be of importance in the water column. However, in the sediments where nutrients will be more concentrated it is possible that this may take place. This ability does not seem to be common to all bacterial types. It is therefore difficult to speculate how important bacterial uptake of silica is in relation to the silica cycle, when compared to the amounts utilised by diatoms.

VI. THE EFFECT OF THE INOCULATION OF PURE CULTURES OF BACTERIA ON SILICA RELEASE FROM DIATOMS

VI. THE EFFECT OF THE INOCULATION OF PURE CULTURES OF BACTERIA ON SILICA RELEASE FROM DIATOMS

INTRODUCTION

As it has been shown that complete bacterial populations from lake water enhance silica release from diatoms (Sections IV & V) the effect of pure cultures of individual members of Loch Leven populations was investigated. Pure cultures of bacteria were inoculated onto diatom cultures to determine their effect on silica release. The possible relationship between clump formation and silica release was also investigated. It was hoped that this study might show if differences in the physiological characteristics of the isolates could be related to their ability to release silica. The taxonomy of these organisms and aspects of their nutrition were also investigated (see Section VII).

METHODS

Four of the five isolates examined in this study were obtained from CPS spread plates of samples taken at the end of dissolution experiments. In all cases the experiments had been carried out using Loch Leven water. Organisms with yellow, white and thin spreading 'clear' colony types were frequently dominant at the end of these experiments (see Section IV, Fig. 4.3). The fifth isolate, organism Y2, was isolated from a surface water sample of Loch Leven water. It was thought that it may be important in clump formation because of the tough, sticky nature of its colonies. Organism W2 had also caused clump formation, (see Section VIII, Table 8.3).

Bacterial culture	Isolated from:
White 1 (W1)	*C. meneghiniana* suspension
Yellow 1 (Y1)	"
Clear (C)	"
White 2 (W2)	*N. pelliculosa* suspension
Yellow 2 (Y2)	Loch Leven water, surface sample.

Batch cultures of axenic *C. meneghiniana* and xenic *A. formosa* (Loch Leven isolate) were prepared as detailed in the Materials & Methods (p. 59). The *A. formosa* culture was suspended at two different concentrations; 2 and 4 x 10^7 cells/ml. The initial total silica concentrations were 1 and 2 µgSi/ml. respectively. Suspensions of the axenic *C. meneghiniana* contained 4 x 10^4 cells/ml. with an initial total silica content of 2.7µgSi/ml.

The following treatments were set up;

The *A. formosa* culture was heat-killed and suspended in

1. Lough Neagh water,
2. Lough Neagh water + mercuric chloride (1g./l.),
3. Heat sterilised Lough Neagh water and
4. Heat sterilised Lough Neagh water, inoculated with pure cultures of the five bacteria. Organism Y1 was only inoculated at the high concentration of diatoms.

Spread plating on CPS of samples taken from the treatments at the end of the experiment resulted in recovery of only the inoculated organisms. The suspensions in heat sterilised Lough Neagh water proved to be sterile. This suggests that the bacteria contaminating this culture did not survive the heat-killing of the diatoms. Therefore any

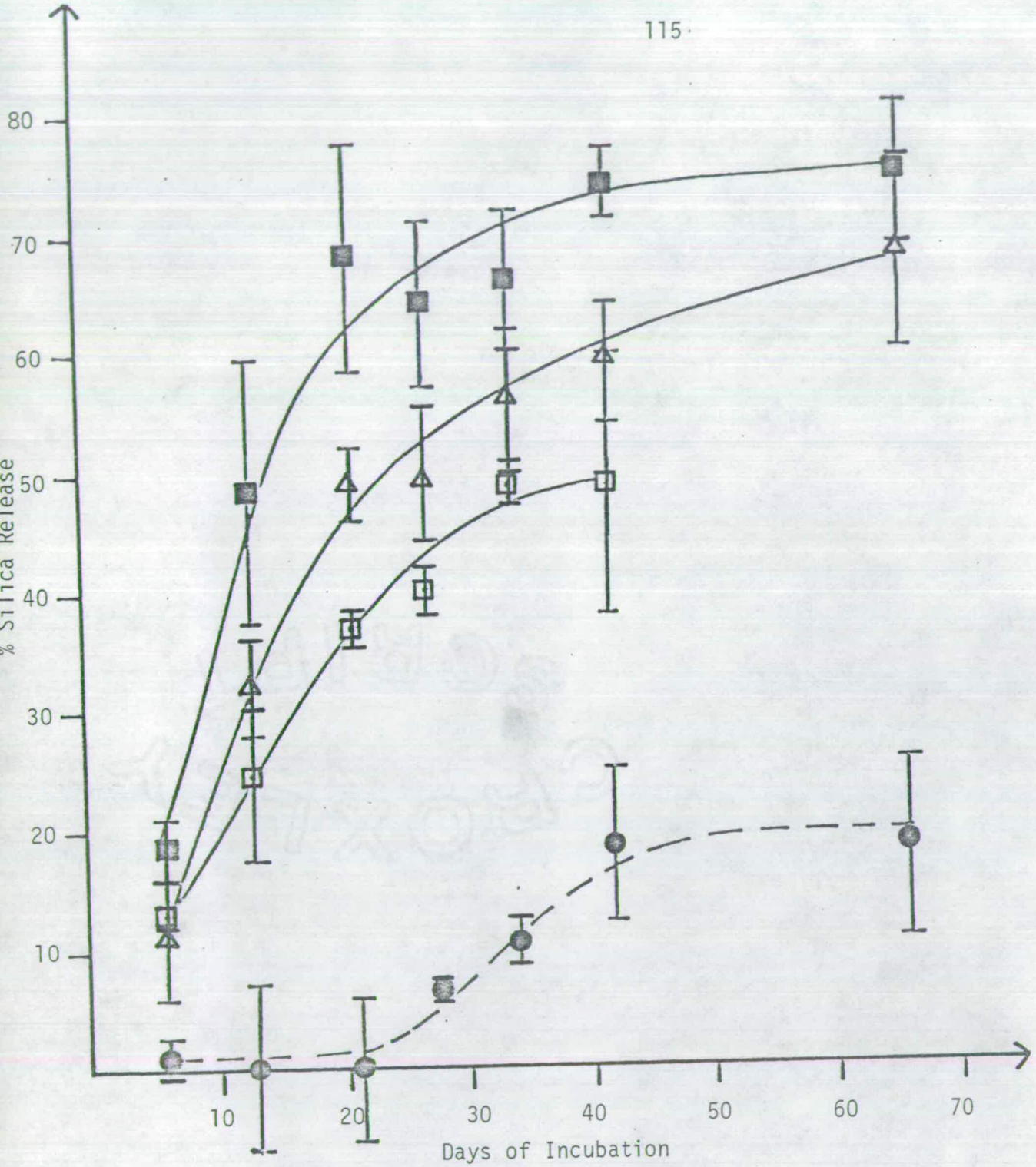


Fig. 6.1 The release of soluble silica from heat-killed *A. formosa* at 25°C in the presence of (a) Lough Neagh water (■), (b) Heat sterilised L. Neagh water inoculated with organism Y1 (△), (c) Heat sterilised L. Neagh water inoculated with organism W2 (□), (d) Heat sterilised L. Neagh water with mercuric chloride (1g./l.) added (-●-)

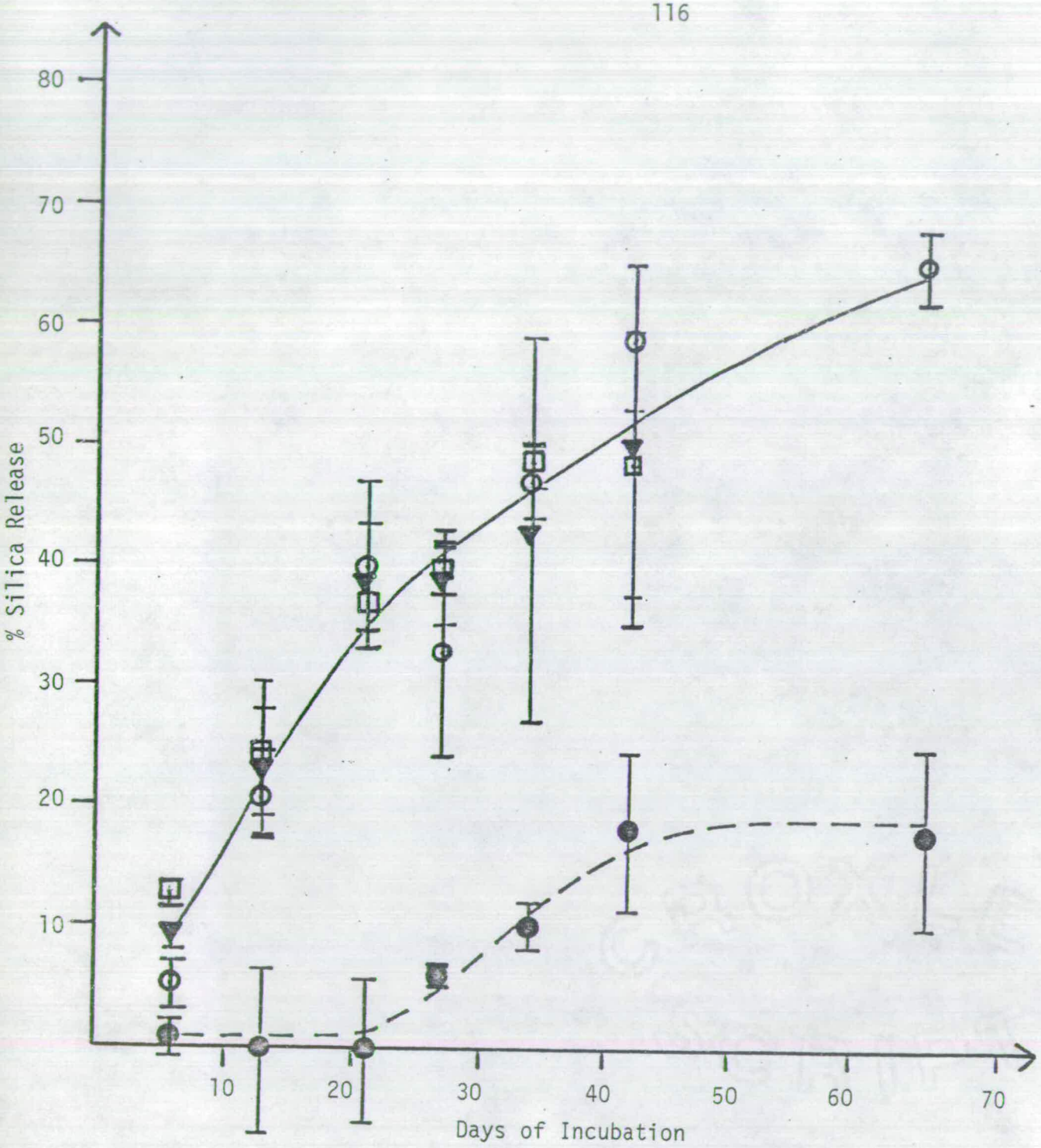


Fig. 6.2 The release of soluble silica from heat-killed *A. formosa* (4×10^7 cells/ml.), at 25°C, in the presence of heat-sterilised Lough Neagh water (●), and heat-sterilised L. Neagh water inoculated with (a) organism W1 (▼), (b) organism W2 (□) and (c) organism Y2 (○)

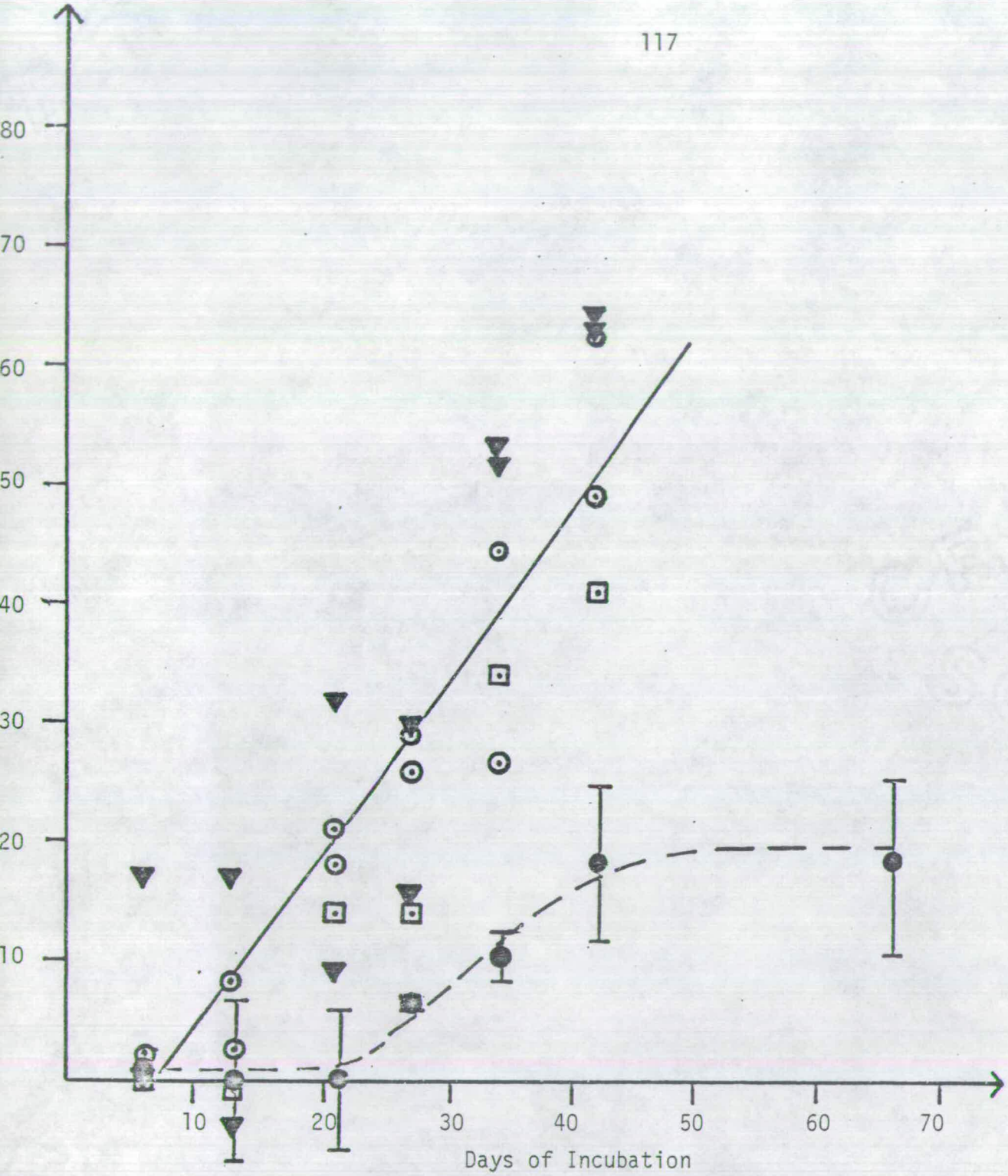


Fig. 6.3 The release of soluble silica from heat-killed *A. formosa* (2×10^7 cells/ml.) at 25°C, in the presence of heat-sterilised Lough Neagh water (●) and heat-sterilised L. Neagh water inoculated with (a) organism W1 (▼), (b) organism W2 (□) and (c) organism Y2 (○) ($r = 0.890$)

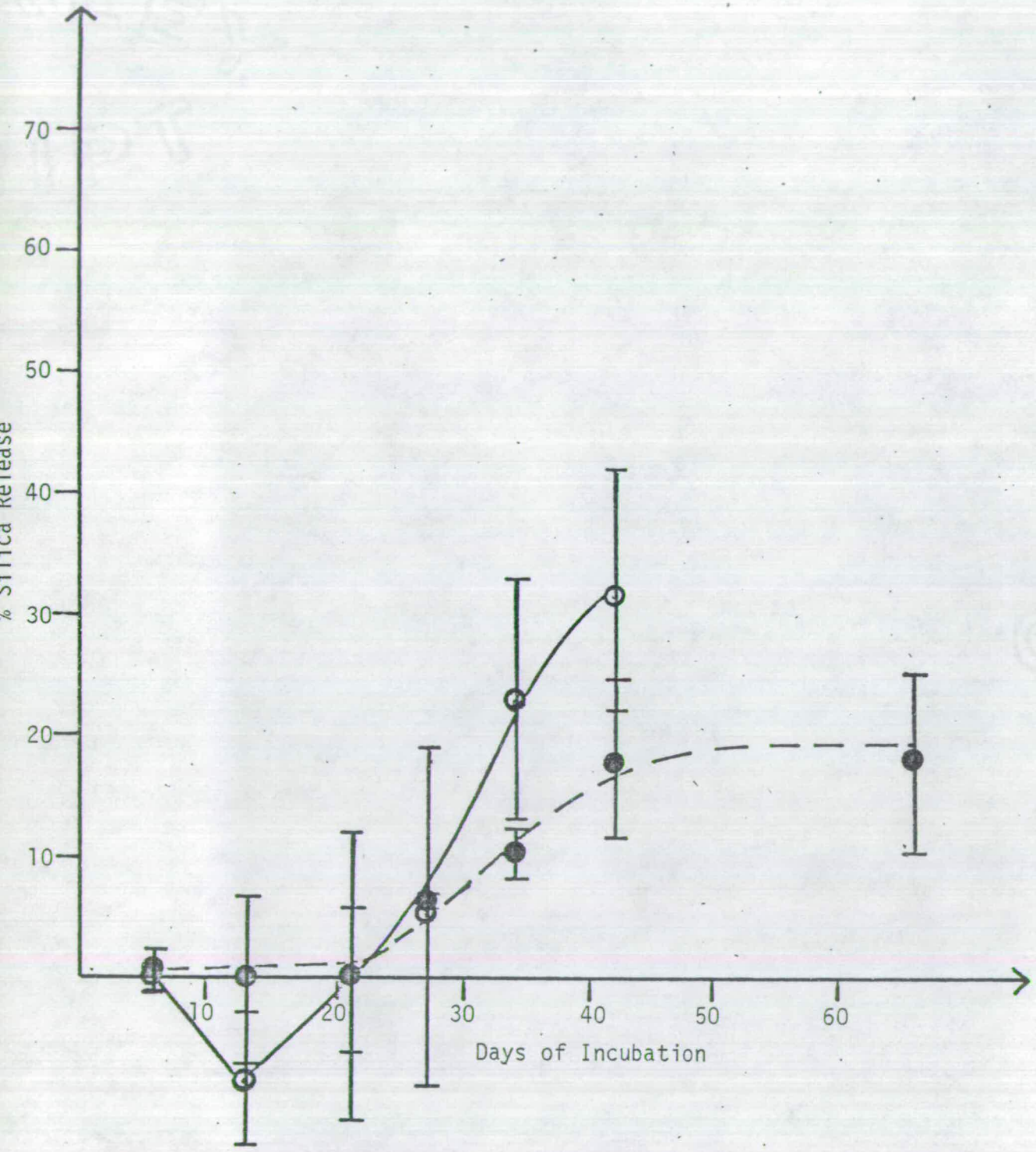


Fig. 6.4 The release of soluble silica from heat-killed *A. formosa*, at 25°C, in the presence of heat sterilised Lough Neagh water (●) and heat sterilised L. Neagh water inoculated with organism C (○)

differences in the silica release can be attributed to the growth of only the inoculated organisms.

The axenic *C. meneghiniana* was suspended as follows;

1. Active culture in Lough Neagh water,
2. Active culture in heat sterilised Lough Neagh water, inoculated with pure cultures of organisms Y1 and W2 and
3. Active culture in heat sterilised Lough Neagh water.

In both experiments the diatoms were inoculated by the addition of 2ml. of a slightly turbid suspension of bacterial culture which had been grown on CPS slopes for two days. There were therefore approximately 10^5 bacteria/ml. at the start of the experiments. Cultures Y2 and W2 grow in clumps and do not form turbid suspensions even after prolonged 'whirly-mixing'. Therefore it was impossible to determine the numbers in these inocula.

RESULTS & DISCUSSION

The amounts of silica released at the high and low concentrations of *A. formosa* were similar in the presence of lough water, under sterile conditions and in the presence of organism C. Therefore the graphs of these readings are expressed as the mean of four replicates (Figs. 6.1 & 6.4). The release of silica differed at the two different diatom concentrations in the presence of organisms Y2, W1 and W2. Therefore the mean of the two replicates at the high concentrations are represented in Fig. 6.2

In Fig. 6.3 each individual value for the duplicate tubes at the low concentration is plotted. There was no significant difference between organisms Y2, W1 and W2. The line of the graph represents the best straight line, calculated using regressional analysis ($r = 0.890$).

Organism Y1 was only inoculated at the high concentration of diatoms, therefore the results are expressed as the mean of four replicates (Fig. 6.1).

From Fig. 6.2 it would appear that silica release in the presence of organisms Y2, W2 and W1 is very similar, and greater than that obtained under sterile conditions. Inoculation with Y1 resulted in a slightly greater release of silica (Fig. 6.1). However this did not equal the release in the presence of the complete Lough Neagh population. A slower rate of silica release was obtained with organisms Y2, W1 and W2 at the lower concentrations of diatoms (Fig. 6.3). This suggests that the ratio of bacteria to diatom cells, or the inoculum potential, is important. The complete Lough Neagh population would appear to have the greatest inoculum potential as similar high rates of silica release were obtained at the high and low concentrations of diatoms. The differences in silica release at the two different diatom concentrations could relate to the different concentrations of nutrients available for the growth of the organisms. Alternatively, it may relate to the decreased chances of contact of the bacteria with the diatom cells.

In all instances the release was greater than that obtained in the sterile controls. However release in the presence of organism C resulted in low initial release of silica, at both concentrations of diatom cells (Fig. 6.4). The initial reduction in silica concentration suggests that there may be some uptake of dissolved silica. However the final effect is less efficient solubilisation of the diatom silica. Total viable counts on CPS carried out at the end of the dissolution experiment are presented in Table 6.1. The low release of silica with organism C is therefore not attributable to loss of viability of the

Table 6.1 Final Total Viable Counts of the Five Isolates on CPS Agar after Inoculation onto Heat-killed *A. formosa*

ORGANISM	TOTAL VIABLE COUNT ($\times 10^6$) AFTER INOCULATION ONTO:	
	4×10^7 DIATOMS/ML.	2×10^7 DIATOMS/ML.
Y1	3.0	N.D.
Y2	4.1	0.035
	0.17	
W1	0.6	0.45
	0.3	
W2	4.3	5.7
	5.1	4.7
C	2.2	3.3
	1.3	

Counts represent the mean of 3 replicate plates, sampled from one tube.

N.D. = Not Done.

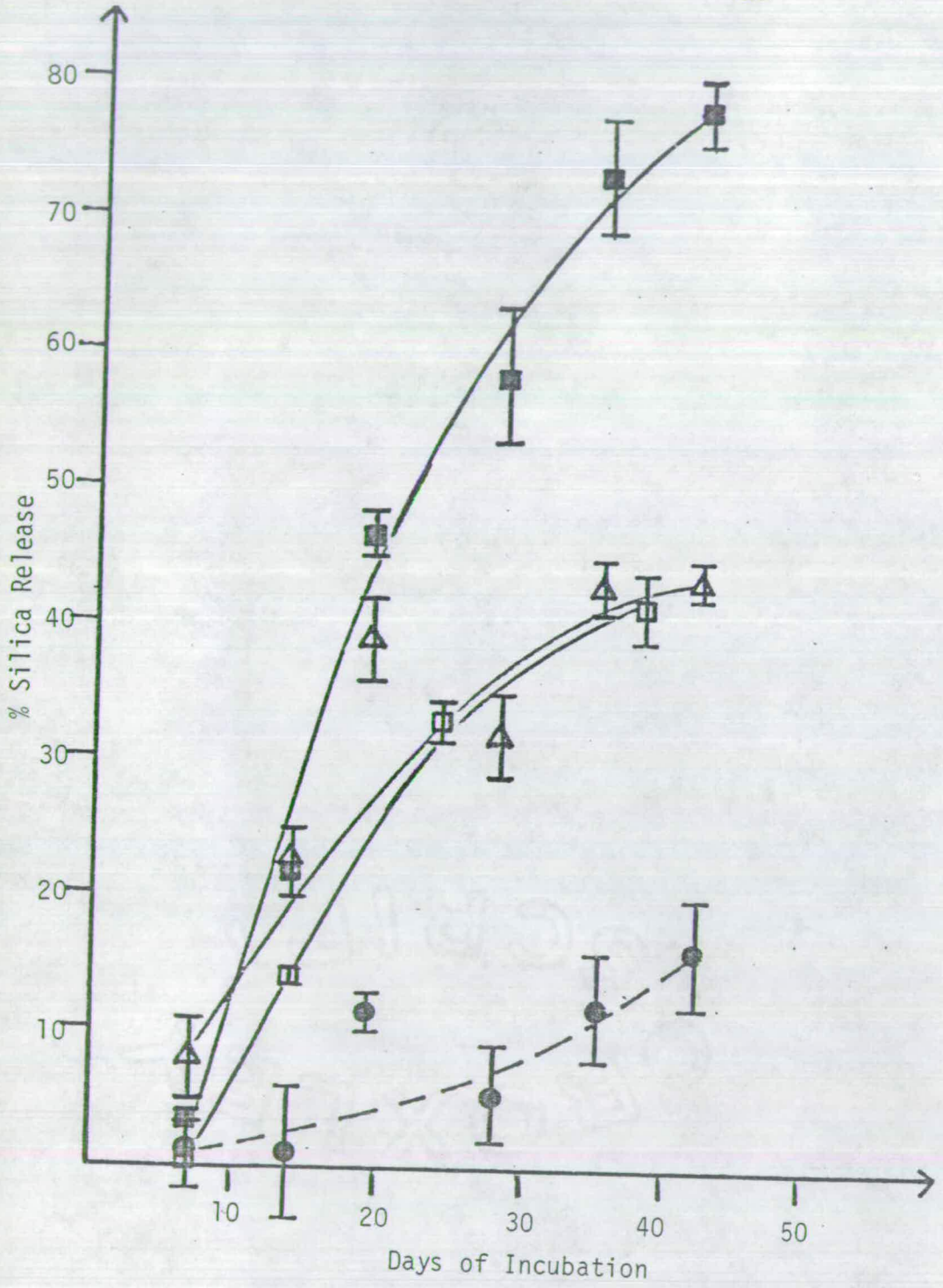


Fig. 6.5 The release of soluble silica from active cultures of *C. meneghiniana* in the presence of
a) Lough Neagh water (■)
b) Heat sterilised L. Neagh water inoculated with organism Y1 (△)
c) Heat sterilised L. Neagh water inoculated with organism W2 (□)
d) Heat sterilised L. Neagh water (– –●)
Incubation was at 25°C.

organism. The slower release of silica at the lower concentration did not relate to a final lower count on CPS. The irregularity of the final counts obtained for Y2 suggests that shaking with glass beads was not sufficient to break up the bacteria into individual cells. More consistent results could perhaps have been obtained if the suspensions had been mildly sonicated.

Organisms W2 and Y2 form clumps. However, under these experimental conditions this does not appear to influence the solubilisation of the silica.

Inoculation of organisms Y1 and W2 onto axenic *C. meneghiniana* again increased the silica released over that of the sterile control Fig. 6.5. However this was again not as great as the effect of the complete Lough Neagh population. In this instance the silica released by these organisms was similar. Again the ability to form clumps did not appear to influence the amount of silica released.

The higher rate of silica release in the presence of the complete Lough Neagh population may be due to the synergistic action of members of the population, or members of the population which have not been isolated being more efficient at diatom decomposition.

VII. AN EXAMINATION OF THE TAXONOMY AND NUTRITIONAL CHARACTERISTICS OF THE FIVE BACTERIAL ISOLATES USED IN THE INOCULATION STUDIES OF SECTION VI

VII. AN EXAMINATION OF THE TAXONOMY AND NUTRITIONAL CHARACTERISTICS OF THE FIVE BACTERIAL ISOLATES USED IN THE INOCULATION STUDIES OF SECTION VI

INTRODUCTION

As the five isolates (see p.114) appear to differ in their ability to enhance silica release and form clumps with the diatoms (see Section VIII), their taxonomy and the effect of their growth on a number of different substrates were examined. Any enzymic or nutritional characteristics which could relate to the decomposition of the organic coat of the frustule could be of particular interest. The rate of degradation of the organic coat has been suggested as the rate controlling step in frustule dissolution (Hecky, 1973).

1. TAXONOMY

Identification to the possible genus or generic group was carried out using the scheme for the preliminary identification of Gram negative rods devised by Gibson et al. (1977) (see Table 2.2 p. 54). The tests used in this study are detailed in the Materials and Methods Section p. 49 .

The major characteristics of the isolates are illustrated in Table 7.1.

a. ORGANISM Y1

This organism probably belongs to the family Enterobacteriaceae. The optimum temperature for growth was 24°C and the maximum 31°C (A. Kelly, pers. comm.). It therefore seems that this organism is an *Erwinia* sp. as only this group of the Enterobacteriaceae have optimum

Table 7.1 Some Characteristics of the Five Bacterial Isolates

CULTURE	MICROSCOPIC MORPHOLOGY	MOTILITY	FLAGELLA	OXIDASE REACTION	OXIDATION/ FERMENTATION OF GLUCOSE	PIGMENT	CAPSULE	COLONIAL APPEARANCE	VOSGES-PROSKAUER TEST	NO_3 REDUCTION	ONPG PRODUCTION
Y1	rods, sometimes paired 1.25 - 2.15 x 1.0μm.	+	peritrichous	-	fermentative	yellow	+	glossy, convex, opaque	+	-	+
W1	rods, rounded, sometimes paired 1.5 - 3 x 0.8μm.	+	peritrichous	-	weak fermentative	none	+	glossy, convex, opaque	+	-	-
W2	rods, frequently clumped 1.3 x 1.0μm.	+	peritrichous	-	weak fermentative	none	+	glossy, convex, opaque	-	+	-
Y2	rods, in clumps, older cultures in chains or filaments 1.25 - 3 x 1.0μm. Filaments up toμ13 m. long	-	-	-	-	yellow	+	glossy, hard, convex, opaque	-	-	-
C	rods, sometimes curved 0.7 - 1.5 x 0.6μm.	+	polar	+	-	none	-	flat, transluscent (spread over agar surface)	-	-	-

All negative for arginine dihydrolase; lysine & ornithine de-carboxylase; utilisation of citrate; H_2S production; urease, tryptophan de-aminase & indole production. All isolates were catalase positive and Gram negative.

growth temperatures of less than 30°to 37°C (Buchanan & Gibbons, 1974). It may be a member of the 'herbicola group' of *Erwinia* as it is yellow pigmented. This identification was confirmed using the API 20E system which involves 20 miniaturised biochemical tests. This system was developed for use at 35° to 37°C. However, the incubation was carried out at 25°C and 30°C for this organism. Only very weak acid reactions were obtained for the sugar fermentation. However, if these are considered positive the pattern of results nearest to those obtained for this organism agrees with the identification of an *Erwinia* sp. Yellow pigmented organisms, similar to *Erwinia*, have been isolated from water samples taken from rivers, springs, wells and distribution water (Leclerc, 1962). In this study more than 8000 samples were examined. These organisms were most frequently isolated in spring, in particular during March. It was therefore concluded that these organisms are members of the saprophytic populations found in natural waters and are not associated with faecal contamination. *Xanthomonas* are another group of yellow pigmented organisms which are associated with plants and plant material. However, these organisms are strict aerobes and polarly flagellated (Buchanan & Gibbons, 1974).

b. ORGANISM Y2

This organism can be placed in the heterogeneous group *Flavobacterium* as it is yellow pigmented and respiratory, and gliding, twitching or flexing have not been observed (Gibson et al., 1977). However, it is unusual in its formation of hard colonies and clumps in broth culture. In this respect it resembles organisms which have been isolated from sewage (e.g. *Zoogloea ramigera*) which are considered important in floc formation. Although 'clump' formation in these

organisms is thought to be associated with the production of extra-cellular fibrils, isolate Y2 produces capsules. Some *Pseudomonas* sp. have also been reported as forming 'flocs'. Out of twelve floc-forming sewage isolates eight were considered to be *Zoogloea* and four *Pseudomonas* sp. (Crabtree & McCoy, 1967).

c. ORGANISMS W1 & W2

These isolates were only weakly fermentative in closed Hugh & Leifson's stab cultures and did not produce acid from glucose in the open tube, in which an alkaline reaction occurred. The significance of this is difficult to determine as alkaline reactions in this medium were found to be erratic and not reproducible when similar tests were carried out by different laboratories (Sneath & Collins, 1974). These organisms were also screened using the API 20E system and gave negative results for all the sugar utilisation tests. A total of fifteen different patterns of fermentation end-products from *Erwinia* sp. have been reported, half of these including the production of 2,3-butandiol as one of the end-products. Therefore in these fermentation reactions less acid was produced. *Erwinia amylovora* was reported as being a very slow fermenter, therefore only minor quantities of end-products were present. It was thought that this might explain why multiple end-product patterns were obtained for one species (White & Starr, 1971). It is possible that similar weak fermentation reactions were occurring with these organisms.

Members of the genus *Alcaligenes* are reported as being isolated from fresh-water and important in decomposition and mineralisation processes. This group obtains their energy from the tricarboxylic acid cycle and compounds chemically similar to those in the cycle. They

therefore tend to be unreactive in standard bacteriological tests, especially in their utilisation of carbon sources (Gibson et al., 1977). However these organisms are strictly aerobic and oxidase positive (Buchanan & Gibbons, 1974).

d. ORGANISM C

This organism was least effective in releasing dissolved silica from the diatoms. It would appear to be a *Pseudomonas* sp. (Hendrie & Shewan, 1979). As it does not produce acid in Hugh & Leifson's medium or a fluorescent pigment on Kings 'B' medium it can be considered a member of either the Group III or Group IV according to Shewan's Classification system (Shewan et al., 1960). Later studies showed that all the Group I & II organisms came within the description of *Pseudomonas* sp. in Bergey's Manual of Determinative Bacteriology (Buchanan & Gibbons, 1974). However the organisms in Groups III and IV were heterogeneous (Gibson et al., 1977). Isolates from these groups could be placed in two genera, *Pseudomonas* or *Alteromonas*, the latter having a lower %GC (Lee et al., 1977). It is interesting that enzymes of the Entner-Doudoroff and pentose phosphate pathways were found to be present in strains which gave negative reactions with glucose in Hugh & Leifson's medium. It would therefore appear that these organisms can metabolise glucose but do not always use it as a principal energy yielding pathway (Gibson et al., 1977).

Therefore this organism cannot be more precisely identified without a more detailed investigation.

2. THE PRODUCTION OF EXTRA-CELLULAR HYDROLASES

The ability of these organisms to breakdown tributyrin, olive oil, gelatin, starch and casein was investigated and the presence of enzymes detectable by the API ZYM system examined. Details of media and methods used can be found in the Materials and Methods section p. 49.

From Table 7.2 it can be seen that the production of clearance rings in tributyrin agar is the only characteristic which appears to relate to enhanced silica release. Tributyrin breakdown may be attributable to the action of either esterase or lipase enzymes. Therefore the bacterial cultures were examined for their ability to breakdown longer chain fatty acids. Supernatant liquid taken after the growth of these organisms was assayed for lipase; olive oil being the substrate.

There was no detectable lipase present after *Asterionella formosa* (Loch Leven isolate) had been incubated with Lough Neagh water at 25°C, or when the same diatom was inoculated with organism Y1.

Alford & Elliot (1959) reported that growth on 1% Bacto-peptone resulted in maximum lipase production by *Pseudomonas fluorescens*. Therefore all five organisms were grown in this medium, both statically and shaken, at 25°C. Lipase was again undetectable. Incubation in tributyrin broth which was shaken twice every 24 hours resulted in no detectable lipase after four days. However, after two weeks, minimal activity (less than 10 units/ml.) was detectable in organisms Y1 and W1. The low level of activity may be attributable to low numbers of bacteria, there being approximately 10^4 organisms/ml. (see Table 7.2). It therefore appears that tributyrin breakdown may be mediated to a great extent by an esterase enzyme.

Table 7.2 Extracellular Hydrolase Production by the Five Bacterial Isolates

CULTURE	BREAKDOWN OF: TRIBUTYRIN	GELATIN		STARCH	CASEIN	OLIVE OIL (LIPASE ASSAY AFTER GROWTH IN TRIBUTYRIN BROTH)		% SILICA RELEASE FROM *A. FORMOSA* AFTER 25 DAYS INCUBATION AT 25°C
		STAB INOCULATION	CHARCOAL DISC			ACTIVITY (units/ml.)	FINAL TOTAL VIABLE COUNT ON CPS AGAR (organisms/ml.)	
W1	+	-	-	-	+	6	3.5×10^4	40
W2	+	-	-	-	+	0	1×10^5	40
Y1	++	-	-	-	+	7	1.5×10^4	50
Y2	+	-	-	-	-	0	$< 10^1$	40
C	-	-	-	-	-	0	3×10^3	10

It may be important that all these techniques rely on the production of reasonably large amounts of extra-cellular enzymes. The ecological disadvantages of an organism producing large quantities of, for example, a constitutive lipase enzyme, in an aquatic environment where there is little fat present, when compared to milk, for example, could be quite considerable. It has been suggested that enzyme activity in aquatic systems would be most efficient if the enzyme is either cell surface bound or closely associated with capsular or slime material. The proteinase of a *Cytophaga* sp. has been reported to be complexed with the acidic polysaccharide of the slime layer of this organism. It has been suggested that this may aid the stability of the enzyme. Therefore the polysaccharide may not only allow the organism to remain in contact with the substrate by adhering, but also ensure that the enzymes come into contact with the substrate. Fifty percent of the proteolytic activity of a periphytic *Pseudomonas* sp. isolated from the marine environment was lost by three sea water washes, which suggests that it is loosely bound to the cell envelope (Corpe & Winters, 1972). They also noted that the greatest amount of proteinase, esterase, phosphatase and β-glucosidase enzymes was located within the cells and released only as a result of autolysis or mechanical breakage of the cells. These authors suggested that the mineralisation by enzymes released from dead bacteria may also be important in the turn-over of organic material in the sea. This may also aid the digestion of grazing and detritus feeding invertebrates.

The enzymic activity of these organisms was also examined with the API ZYM system which involves inoculation of a range of substrates with cell suspensions. The recommended incubation is at 37^{o}C for three hours.

Therefore, in the case of these isolates only enzyme activity was measured and not growth. It would have been interesting to have examined this system at a lower temperature. However, Alford & Elliot (1960) found that lipase production per cell of *P. fluorescens* was greater at 20°C than 30°C, although the optimum temperature of the lipase enzyme was near 40°C. This was not influenced by the temperature at which the cells had been grown.

From the results detailed in Table 7.3, it can be seen that all these isolates produced both acid and alkaline phosphatase. It has been suggested that regions of high alkaline phosphatase activity correspond with regions of maximum mineralisation of algal remains in fresh waters (Reichardt et al., 1967) and that this activity could be important in the supply of inorganic phosphate for algal growth (Berman, 1969). The presence of alkaline phosphatase activity was also correlated with algal and bacterial activity by Jones (1972) although the origins of this remain to be established. A neutral phosphatase, only slightly inhibited by phosphate, has been shown to be produced by two different bacterial types, (*Chromobacter* and *Pseudomonas*) isolated from a stream. As the maximum activity of this enzyme is at pH 7 it is possible that they may also play a significant role in the hydrolysis of organic phosphate in the natural environment (Matavauj & Flint, 1980). However the relationship of phosphatase production to silica solubilisation is not clear.

The API ZYM system indicates that all five isolates are capable of hydrolysing fatty acids, with a range of different chain lengths, from short chain water soluble to long chain non-water soluble. The negative result and low lipase activity measured using the olive oil lipase assay must therefore be due to the low levels of production of these enzymes.

Table 7.3 Enzyme Production by the Five Bacterial Isolates, Tested Using the API ZYM System.

CULTURE	PHOSPHATASE		ESTERASE (C_4)	ESTERASE/ LIPASE (C_8)	LIPASE (C_{14})	PHOSPHO-AMIDASE	ARYLAMIDASE			TRYPSIN	CHYMO-TRYPSIN	GALACTO-SIDASE		β-GLUCURON-IDASE	GLUCO-SIDASE	
	ALKALINE	ACID					Leu	Val	Cys			α	β		α	β
W1	++	++++	+	+++	+++	+	+++++	+++	++	-	-	-	-	-	-	+
W2	+++	++	+++	++	+	++	++	++	+	+	-	-	+	+	-	+
Y1	+++++	+++++	++	+	+	++++	+++++	++	+	+	+++	-	++	++++	+++++	++
Y2	+++	+++	+	+	+	+	++++	++	-	-	+	+	-	-	-	-
C	+++++	+	+	++	+	-	-	-	-	-	-	-	-	-	-	-

All -ve for N-acetyl-β-glusosaminidase, α mannosidase, α fucosidase.

Reaction scored from + to +++++, according to API ZYM system.

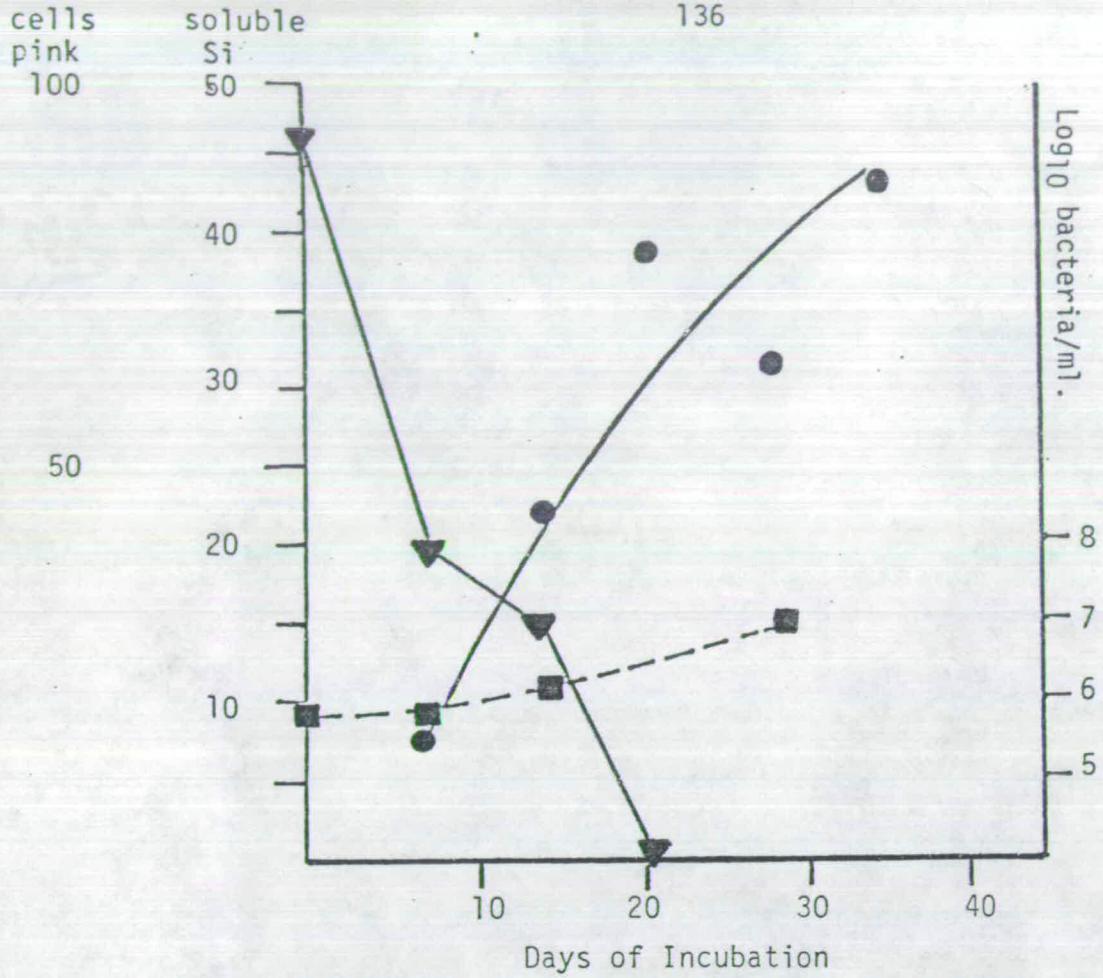
Noticable differences do occur in the range of amidases, sugar hydrolases and proteolytic enzymes produced by the different isolates. Organism C which was least efficient in the solubilisation of silica did not produce any of these enzymes. On the other hand organism Y1 which enhanced silica release to the greatest extent produced the widest range of enzymes. These experiments give some indication of differences in the ability of these organisms to produce hydrolases. However the enzymes tested for may not necessarily be active against components of the diatom cells.

In Hecky's proposed structure of the outer organic component of diatom walls (Fig. 5, p. 27), amino acids and polysaccharides are the major components. Lipids only accounted for 0.8% of the wall organic matter in freshwater species examined by Kates & Volcani (1968). However the presence of lipid droplets within the cytoplasm has been reported in *Melosira varians* (Crawford, 1973) and *Gomphonema parvulum* (Dawson, 1973). This suggests that although perhaps not directly related to the solubilisation of the frustule, lipid may still be present as a substrate for growth.

It is therefore possible that the enhanced silica release obtained in the presence of these organisms is a direct result of their decomposition of the protein and polysaccharide component of the diatom wall.

3. RUTHENIUM RED STAINING OF THE DIATOM WALL

Ruthenium Red stains specifically for acidic polysaccharides in the organic coat (Werner, 1977). Therefore the percentage of *C. meneghiniana* cells which stained pink after inoculation with organism



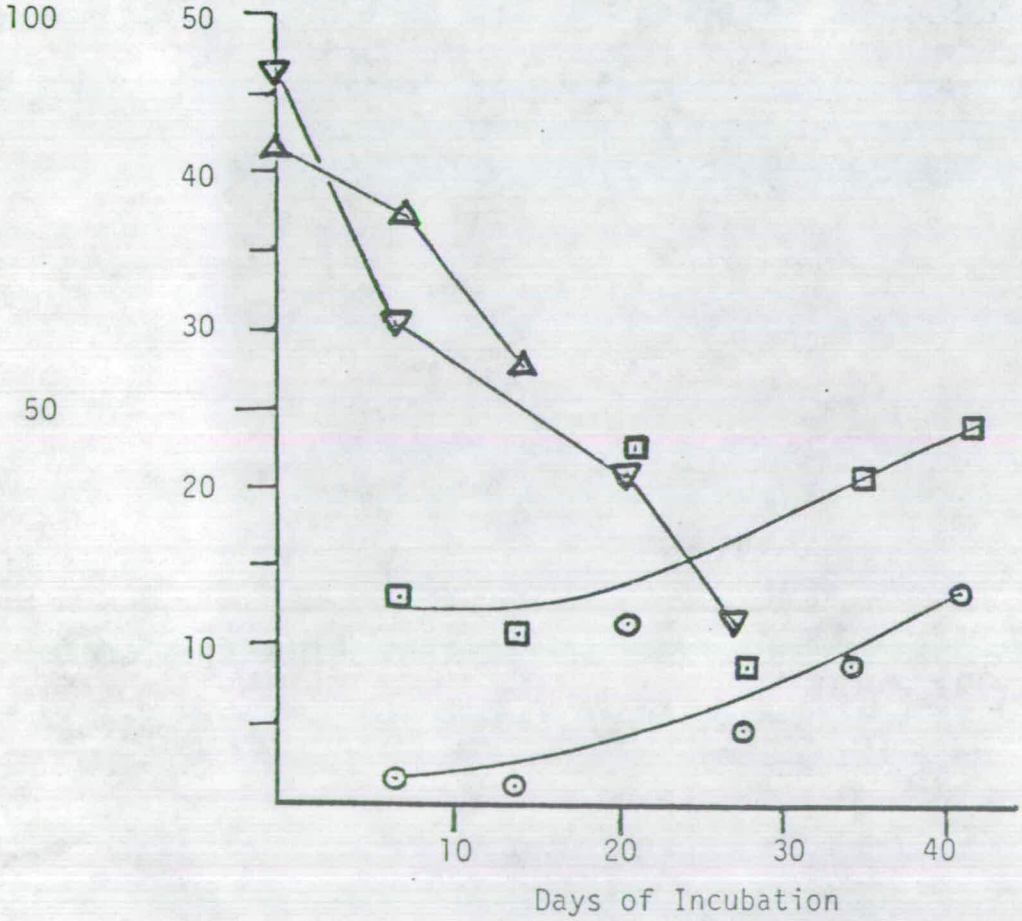


Fig. 7.1 The relationship of soluble silica release with Ruthenium Red-staining cells of axenic *C. meneghiniana*, suspended in heat-sterilised L. Neagh water.

(a) Active culture, inoculated with organism Y1. % soluble silica released (●); % diatoms which stain pink (▼); $\log_{10}$ of the number of bacteria/ml. (direct count (■).

(b) Active culture (▽ & ○) and heat-killed cultures (△ & □), both uninoculated. % soluble silica released (○ & □); % diatom cells which stain pink (▽ & △).

Y1 was measured. This was carried out during the experiment described in Section VI, p.119. Breed's smears were made with samples taken from the tubes, stained and examined under the light microscope (details in Materials and Methods, p. 53). The following treatments of the axenic *C. meneghiniana* were examined:

1. Active culture suspended in heat sterilised Lough Neagh water and inoculated with organism Y1,
2. Active culture suspended in heat sterilised Lough Neagh water and
3. Heat-killed culture suspended in heat sterilised Lough Neagh water.

The release of silica can be seen to form an inverse relationship with the number of diatoms staining pink (Fig. 7.1). This can also be related to a ten-fold increase in the number of bacteria present. A similar relationship of pink-staining cells and silica release can be seen to occur in the sterile controls, which suggests that once active growth of the diatom ceases the wall decomposes. However this degradation appears to be accelerated by organism Y1. The reduction in the number of pink-staining heat-killed diatoms is initially not as great as from the initially active autolysing culture. This could be the result of de-naturation of the autolytic enzymes of the diatoms as a result of heat-killing. The initial higher concentration of soluble silica which occurred after heat-killing did not appear to be caused by the removal of the Ruthenium Red staining organic component. However, it may have effected the integrity of this, and the membrane systems of the cell, allowing leakage of intra-cellular silica.

Table 7.4 illustrates the association of the bacteria with the diatom cells. The larger the numbers of bacteria associated with the diatoms the less accurate the determination. Total viable counts measured at

Table 7.4 Data Obtained from Microscopic Observation of Axenic *C. meneghiniana* after Inoculation with Organism Y1 and Incubation at 25°C

DAY	% OF DIATOM CELLS WITH BACTERIA ASSOCIATED	NO OF BACTERIA/ DIATOM (MEAN & RANGE OF VALUES)	NO OF DIATOMS/ML. X10^4	% OF DIATOM CELLS WITH PARTS MISSING	
				INOCULATED WITH Y1	STERILE DIATOMS
0	1	0	4.2	4.5	4.5
7	3	6 (2 - 10)	4.2	11.5	15.5
14	90	6 (2 - 20)	4.2	2.5	N.D.
21	85	7 (3 - 23)	3.8	68	13.5
28	90	8 (6 - 48)	4.2	48	15.0

N.D. = Not Done.

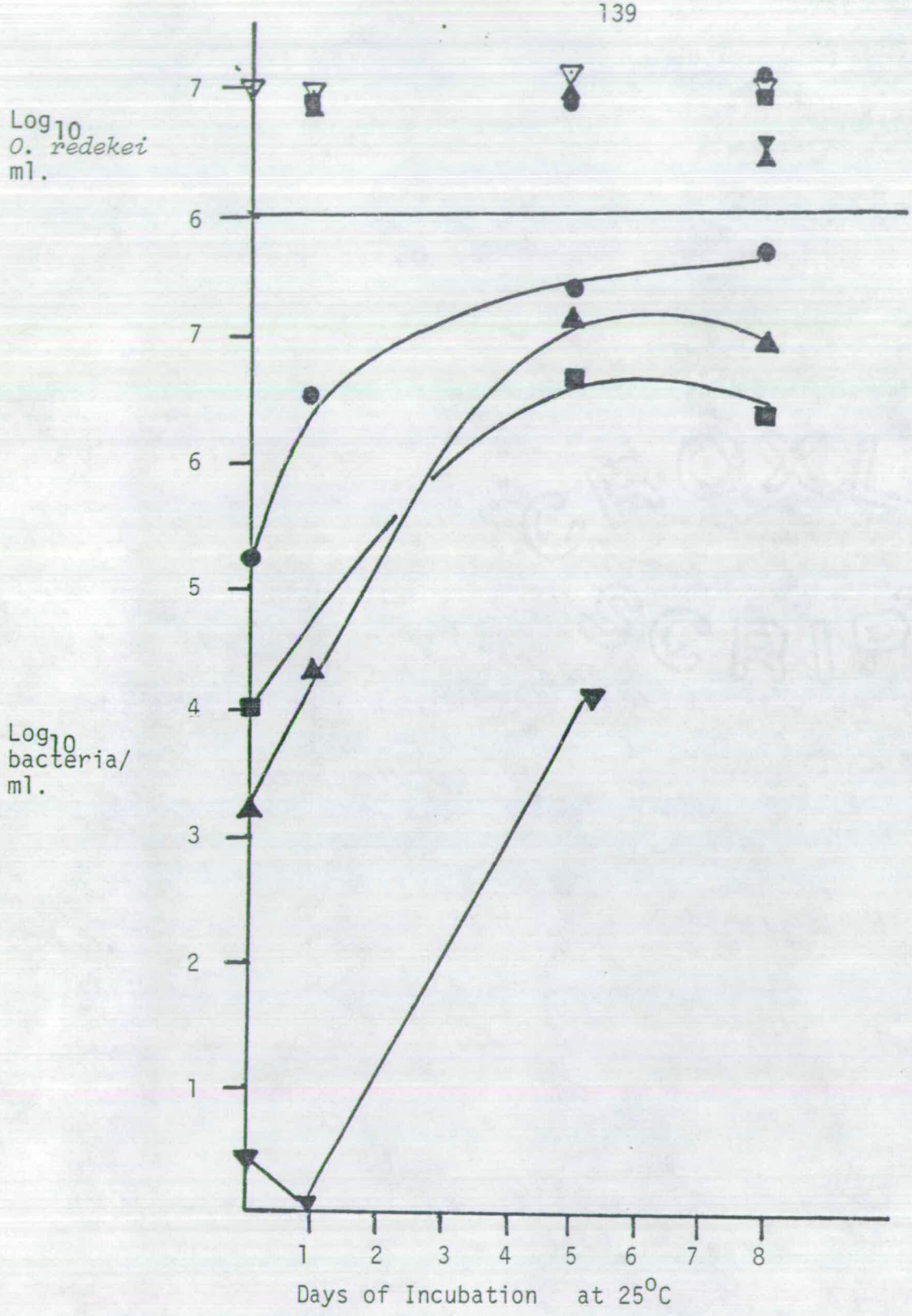


Fig. 7.2 The growth of four of the bacterial isolates on the cyanobacterium *Oscillatoria redekei*. Y1 (●); Y2 (▼); W2 (▲); C (■), uninoculated control of *O. redekei* (▽)

the end of the incubation period indicated that there were more than 100 bacteria present per diatom cell. This suggests that this bacterium does not become irreversibly attached to the diatom cells and that the majority of the bacteria are not attached. It is also interesting that the percentage of diatom cells which appeared to be damaged was greater in the presence of organism Y1 than in the sterile control. There was therefore visible evidence of the dissolution of the diatom frustules.

Although it is difficult to draw firm conclusions from one experiment in which only one diatom to bacteria ratio was examined, it does appear that the active growth of the organism Y1 results in faster decomposition of the organic component of the wall, and therefore enhanced silica release.

4. AN INVESTIGATION OF THE GROWTH OF THE FIVE ISOLATES ON THE CYANOBACTERIUM *OSCILLATORIA REDEKEI*

To determine if these bacteria were capable of growth on other algae which might be present in the lakes, they were inoculated into suspensions of an active axenic culture of *Oscillatoria redekei*. The results of total viable counts of bacteria on CPS agar and direct microscopic counts of *O. redekei* (as detailed in Materials and Methods, p. 53) are presented in Fig. 7.2. These results indicate that although the bacteria appear to be growing on the *O. redekei*, they do not cause any drastic reduction in the number of cells. This suggests that they are not as aggressive as the organisms which have been reported to lyse cyanobacterial blooms in freshwaters. However the *O. redekei* does provide suitable substrates for growth. Where there is a slight reduction in the numbers of *O. redekei* (organisms Y2 and W1) it is likely that this relates to the formation of clumps with these bacteria.

5. CONCLUSIONS

The organisms which enhance the solubility of diatom frustules produce a wider range of hydrolases than the less efficient bacteria. The period during which silica release occurs can be related to the growth period of one of the isolates examined and this can also be related to the removal of the Ruthenium Red staining component of the wall. All five isolates examined are also capable of growing on the cyanobacterium *O. redekei*. These isolates appear to be members of the Loch Leven bacterial flora which can be active decomposers.

VIII. AGGREGATION OR CLUMPING OF DIATOMS

VIII. AGGREGATION OR CLUMPING OF DIATOMS

INTRODUCTION

During the course of the silica solubilisation experiments it was noted that under certain conditions the diatoms and bacteria were forming aggregates or clumps. As the term aggregate has been used to describe non-living particulate matter, formed mainly by the adsorption of dissolved organic matter onto bubbles and other interfaces in the sea (Riley, 1963), these detrital particles containing diatoms and bacteria shall be referred to as 'clumps'.

The colonisation of a submerged surface by bacteria occurs in three stages, (Floodgate, 1972), the first stage being a reversible attachment influenced by physical criteria (e.g. surface charge). This stage is followed by irreversible attachment, often associated with the production of specialised structures by the organisms. For example, fimbriae, flagella and polysaccharide material in the form of sheets, pads, trails or coalesced capsules have all been associated with the adhesion of bacteria in aquatic environments (Hirsch & Pankratz, 1970; Paerl, 1975; Jones, 1980). The final stage is one of multiplication of the organisms and the production of a micro-ecosystem.

The presence of diatom frustules within detrital clumps has been noted by Bothby, Wiebe & Pomeroy, (1972) and Paerl, (1973, 1974, 1975). Polary flagellated Gram negative bacteria, fungal mycelia and filamentous bacteria have been observed in diatom clumps in the oligotrophic Lake Tahoe (USA). Attachment of microorganisms was only seen to occur when diatoms showed signs of partial decomposition. When plankton were suspended in dialysis bags under sterile conditions clumps did not form to the same extent as in the presence of a living lake population. The small amount of clumping which did occur under sterile conditions could

be attributed to physical effects (Paerl, 1973). It was therefore concluded that microorganisms are important in clump formation. A comparison of detrital clumps in Lake Tahoe with samples from the Pacific Ocean showed that filamentous bacteria were numerically dominant in Lake Tahoe where diatoms dominated the phytoplankton. The bacteria observed in these particles appear to be metabolically active as incubation in the presence of ^{3}H - labelled glucose and acetate for four hours resulted in accumulation of the label in bacterial cells and filaments (Paerl, 1974).

The formation of these clumps would therefore appear to be a phenomenon common to aquatic ecosystems. Paerl (1980) provides a comprehensive review of the 'Attachment of Microorganisms to Living and Detrital Surfaces in Freshwater Systems'. In deeper lakes and in the oceans it is likely that much of the decomposition and re-cycling of minerals occurs within these clumps, long before they reach the sediments. For example, in Lake Tahoe, which is approximately 440m. deep, it is likely that it occurs in the first 150m. (Paerl, 1973). However in shallower lakes such clump formation could increase the rate of sedimentation of the algae, leading to sediment microbial activity.

Consequently microscopic examination of the diatoms in the dissolution experiments was carried out to determine how the findings of these authors related to the formation of clumps in Loch Leven and Lough Neagh.

METHODS

Examination of wet-mounts of the diatom suspensions in the dissolution experiments was carried out by phase contrast microscopy.

It was also hoped to gain information on a. the extent of bacterial colonisation of the diatoms, b. the types of bacteria present, and c. the relationship of the increase in dissolved silica with the number of diatom cells present. To determine if clumping was mediated by micro-organisms under loch conditions a batch culture of *Asterionella formosa* (Loch Leven isolate) was suspended under the following conditions;

1. Active culture in Loch Leven water,
2. Heat-killed culture in Loch Leven water and
3. Active culture in Loch Leven water with mercuric chloride (1g./l.) added.

300ml. of these suspensions were placed in dialysis tubing (2.5inch diameter). These were knotted at either end, and the knots subsequently dipped in paraffin wax (Paerl, 1973). Bags were set up in triplicate and tied together with string. These were placed inside a bottomless drum which also had a small hole in the top, thus allowing free passage of the Loch water, but little light penetration. This was attached to a buoy at the Northern Deep area of Loch Leven (see Fig. 1) and left at this point for the month of August 1979.

RESULTS & DISCUSSION

1. FACTORS EFFECTING CLUMP FORMATION

Clumps were formed in the presence of Loch Leven and Lough Neagh water which had not been sterilised, but not in the presence of bacteria contaminating xenic cultures (see Table 8.1). These clumps were generally visible to the naked eye after three to five days incubation at 25^{o}C. When suspended in heat sterilised Loch water they did not clump, suggesting that the populations contaminating the diatom cultures were incapable of causing clumping, or that some heat labile component of Loch water was

Table 8.1 The Effect of Different Bacterial Populations and Incubation Conditions on Clump Formation

DIATOM TYPE	LOCH LEVEN POPULATION	BACTERIA CONTAMINATING DIATOM CULTURES	STERILE CONTROL
1. *A. formosa*	+	-	-
2. *T. flocculosa*	+	-	-
3. *N. pelliculosa*	+	-	-
4. *C. meneghiniana*	+	-	-
5. *Melosira* sp.	+	-	-
6. *Asterionella formosa* (Loch Leven isolate)	+	-	-

Incubation at 25^{o}C; + = clumps formed; - = clumps not formed.

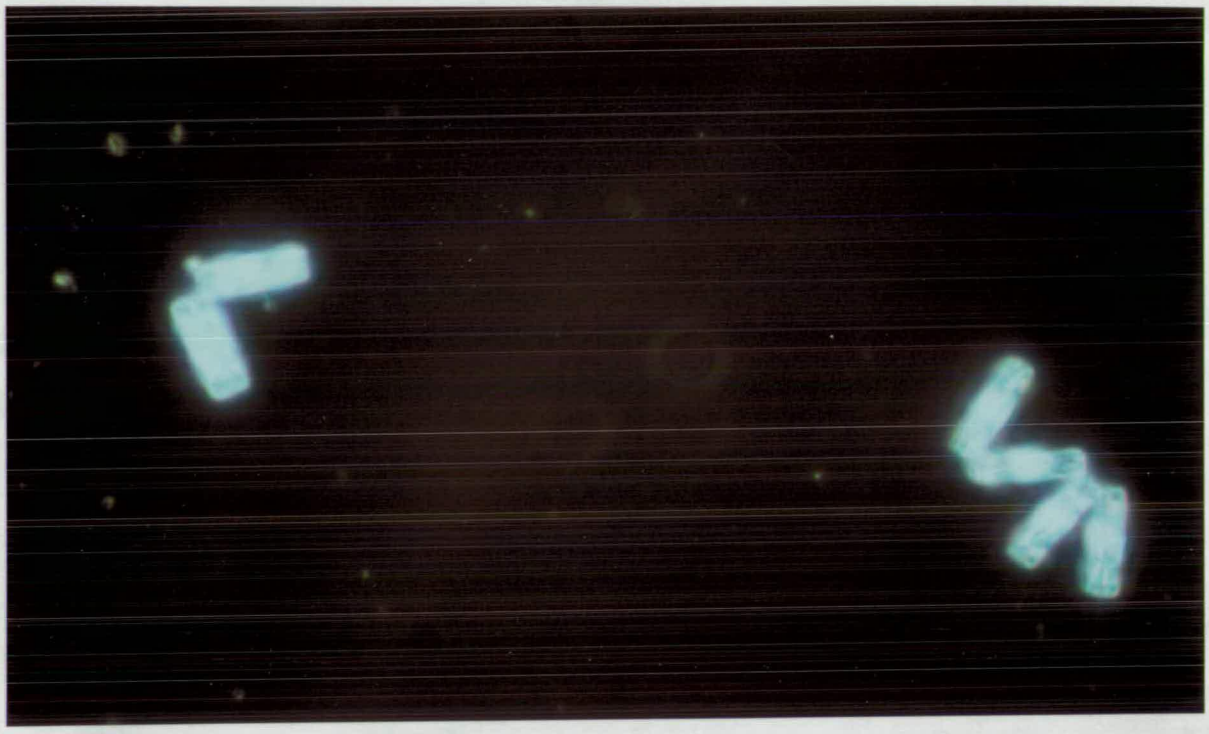
Similar results were obtained when diatoms 1, 2 and 3 were incubated at 4^{o}C, whether incubation was static or agitated, and for diatoms 4 and 6 in the presence of Lough Neagh water bacteria.

responsible (Photographs 1 & 2). However, filter sterilised Loch water did not cause them to clump, which implies that this is a microbial effect.

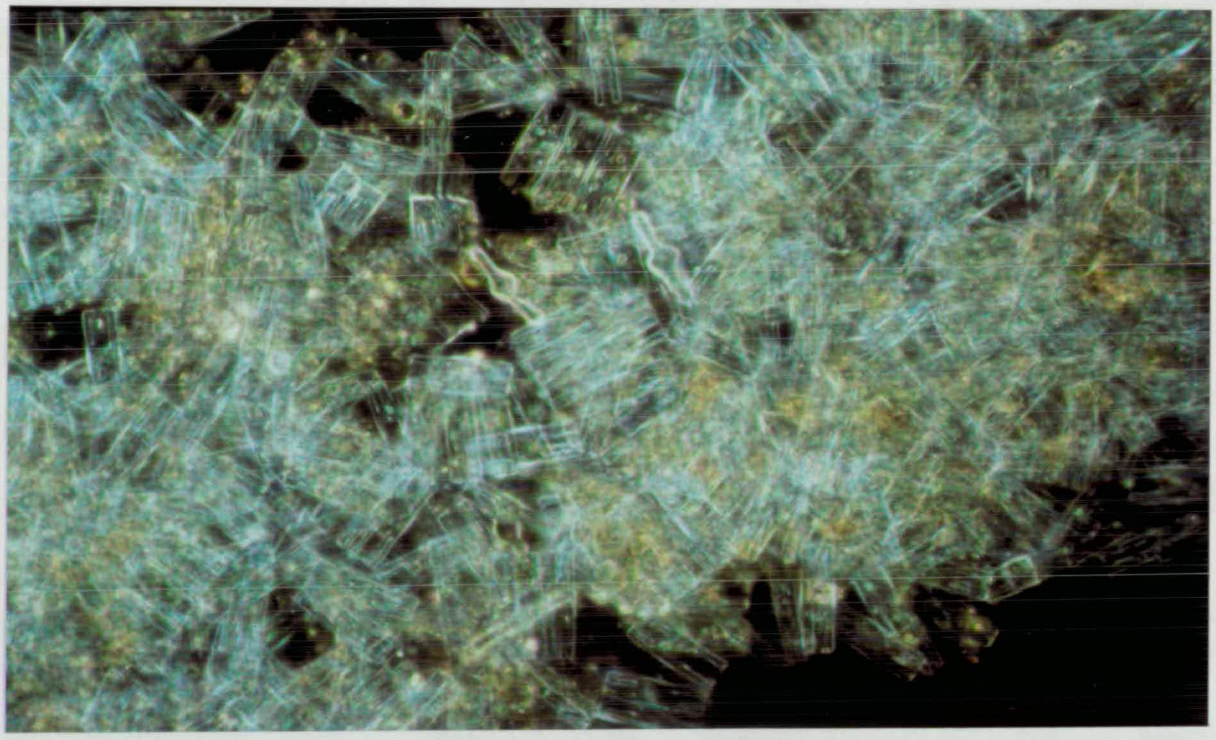
The suspension of xenic *N. pelliculosa* in heat-sterilised Loch Leven water resulted in the release of soluble silica, and this was attributed to the action of the contaminating bacteria (Section IV, p. 85). Under these conditions clumps were not formed, and the diatoms were counted using a haemocytometer slide. Over the period of silica release there was no significant reduction in the total number of diatom cells present. Examination of the larger diatom *C. meneghiniana*, when incubated with the 'non-clumping' organism Y1, revealed that parts of the frustule were missing. However it does seem that although silica is lost from the frustules, they remain recognisable under the light microscope. It was therefore impossible to try to relate silica release with a reduction in the numbers of diatoms present, where clumps were being formed.

Clumping also occurred in the dialysis bags containing the *A. formosa* (culture) and Loch Leven water which had been incubated within the Loch. Clumping did not occur in similar bags which had been sterilised in mercuric chloride prior to placing in the Loch (Photograph 3).

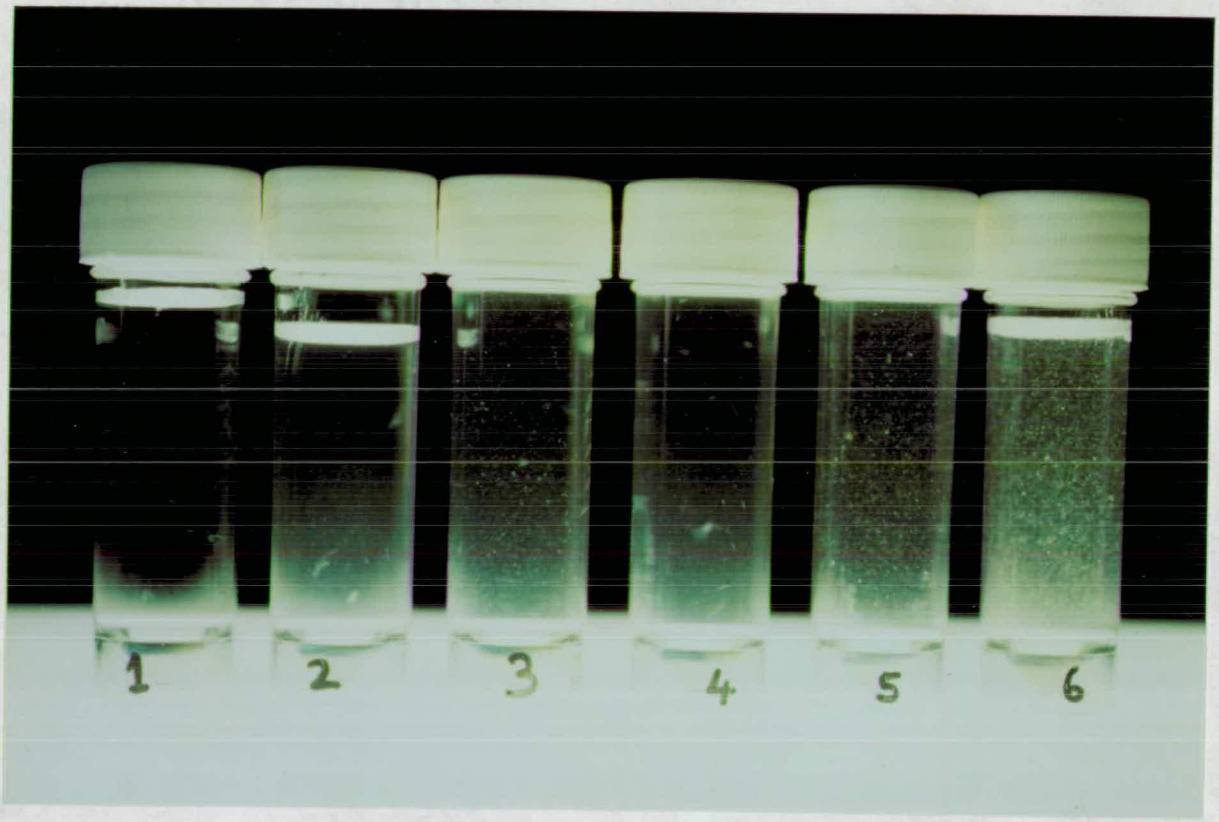
In general clumps varied in size from 0.1 to 0.8mm. and contained from 3 to more than 30 diatom cells. It was very difficult to determine microscopically any physiological changes which were occurring within the clumps. The clumping suggested that the insoluble cytoplasmic contents were leaking from the cells, but remaining within the clump, which was formed from frustules, cytoplasm, bacteria and occasionally other algae which had been present in the lake water. Bacterial polysaccharide



Photograph 1. *T. flocculosa* (xenic, heat-killed culture) after incubation at 25°C in the presence of heat sterilised Loch Leven water (X100 magnification).



Photograph 2. *T. flocculosa* (xenic, heat-killed culture) after incubation at 25°C in the presence of Loch Leven water (X100 magnification).



Photograph 3. *A. formosa* (xenic culture) after incubation within dialysis bags in Loch Leven in August 1979.

KEY
1. Distilled water
2. L. Leven water, $HgCl_2$ sterilised
3. L. Leven water
4. *A. formosa* + L. Leven water, $HgCl_2$ sterilised
5. *A. formosa* + L. Leven water
6. *A. formosa* + L. Leven water.

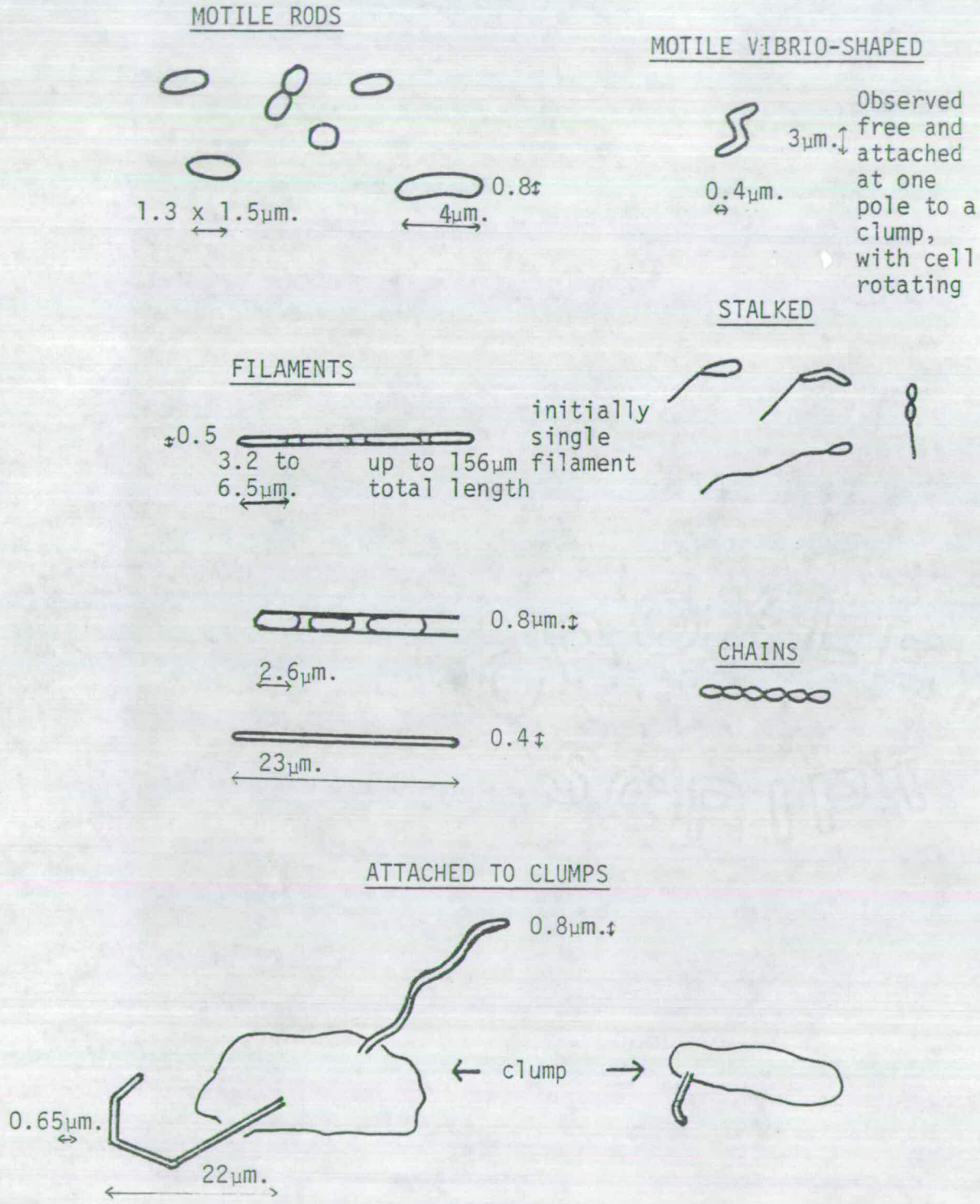


Fig. 8.1 The microscopic appearance of some of the bacteria observed in association with the diatom clumps

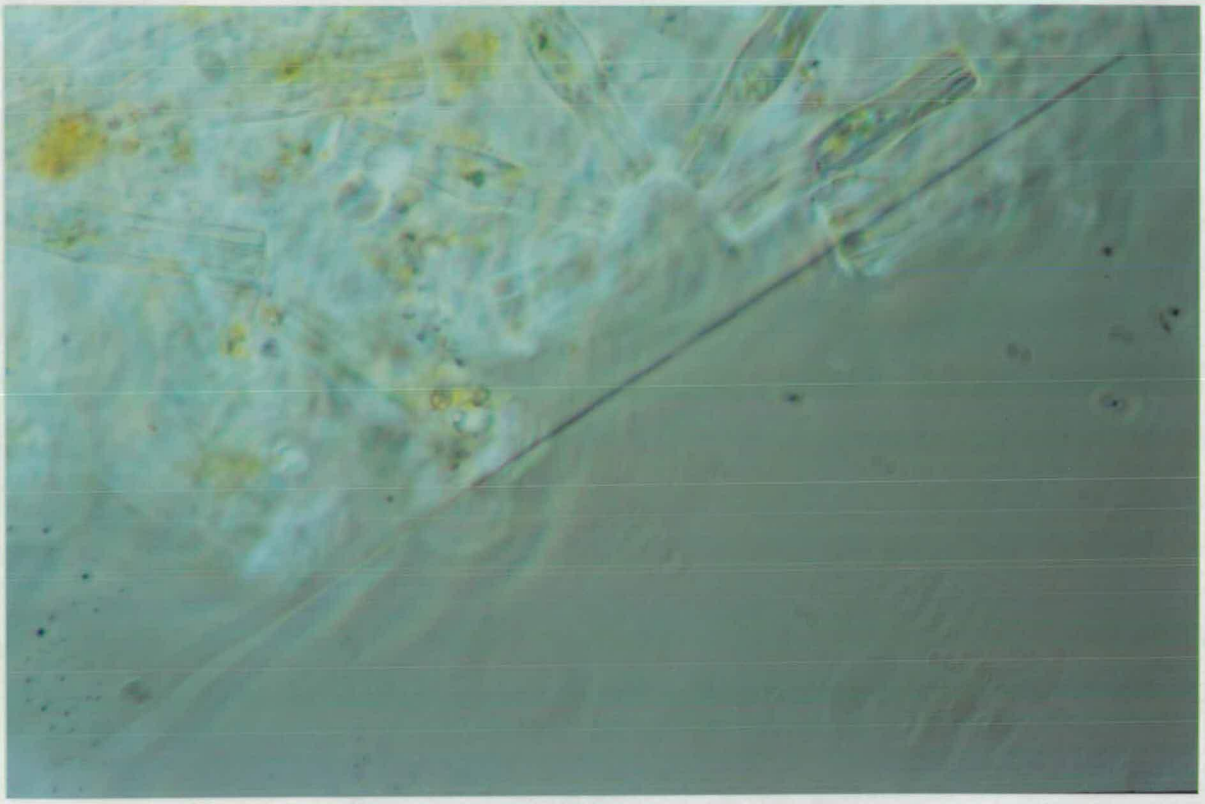
material would also be likely to be present. Clumps remained intact for at least 60 days, by which time the frustules appeared empty, but bacteria were still present.

Examples of some of the bacteria which were observed in association with these clumps are illustrated in Fig. 8.1. Filamentous forms were common, and appeared in some cases to ramify throughout the clumps. Photographs 4 & 5 demonstrate some of the filamentous organisms. Initial observation of these filamentous organisms suggested that they were unsheathed. However, in some instances if a microscope preparation was examined after 24 hours, the filaments split into individual cells and the organisms then appeared to be sheathed. Photograph 5 is an example of one of these organisms, which initially appeared as a complete filament. Photographs 6 & 7 are also examples of clumps under higher magnification, illustrating the many rod-shaped bacteria also seen in the clumps. Photograph 8 is an example of a clump formed in the presence of the Lough Neagh water population.

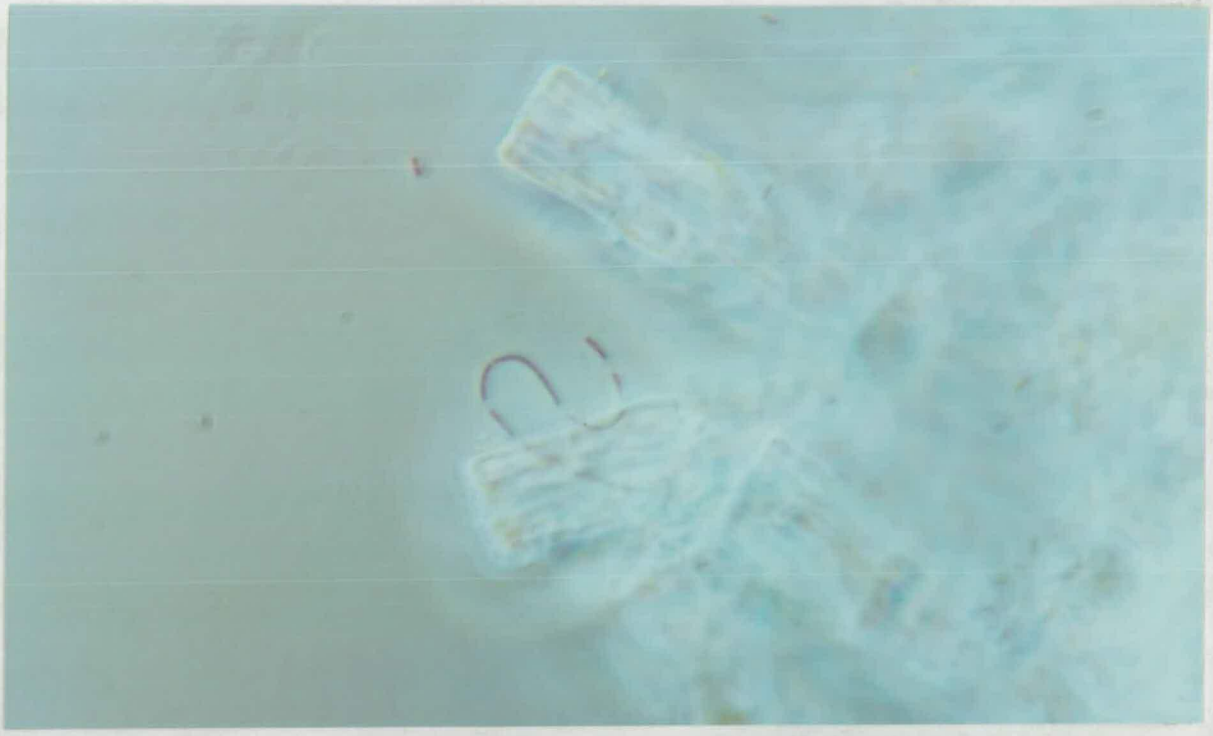
Filamentous bacteria, which have been noted in freshwaters include *Vitreoscillae* (Pringsheim, 1951), *Leptothrix* (Jones, 1978), *Sphaerotilus* (Stokes, 1954) and some members of the Cytophagaceae (Buchanan & Gibbons, 1974).

2. THE ISOLATION OF BACTERIA FROM CLUMPING ALGAE

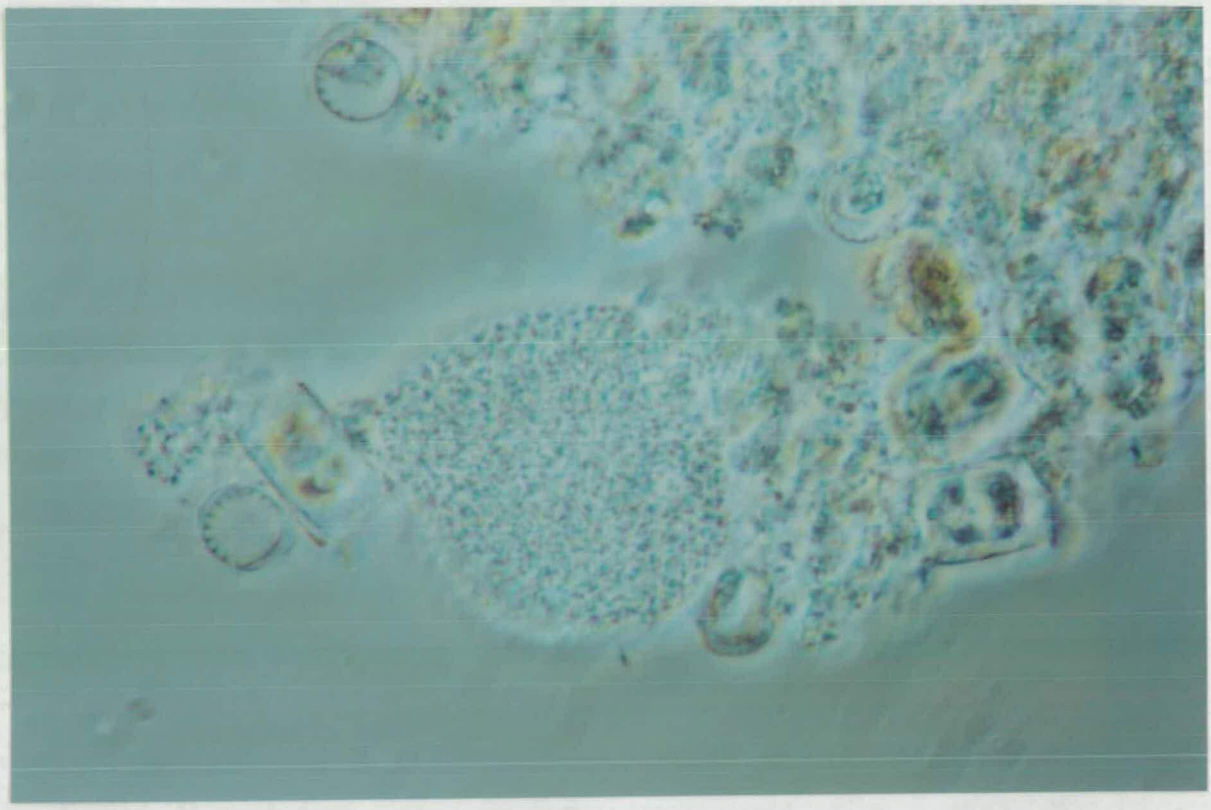
An attempt was made to isolate these filamentous forms by inoculation of the clumps onto CPS and dilute media containing 0.1% Bacto-peptone with and without 0.1% yeast extract, as recommended by Pringsheim (1951). A medium containing 0.1% Bacto-peptone made up with Loch Leven water and surface spread with sterile *N. pelliculosa* was also tested. The diatom may be providing a source of growth factors.



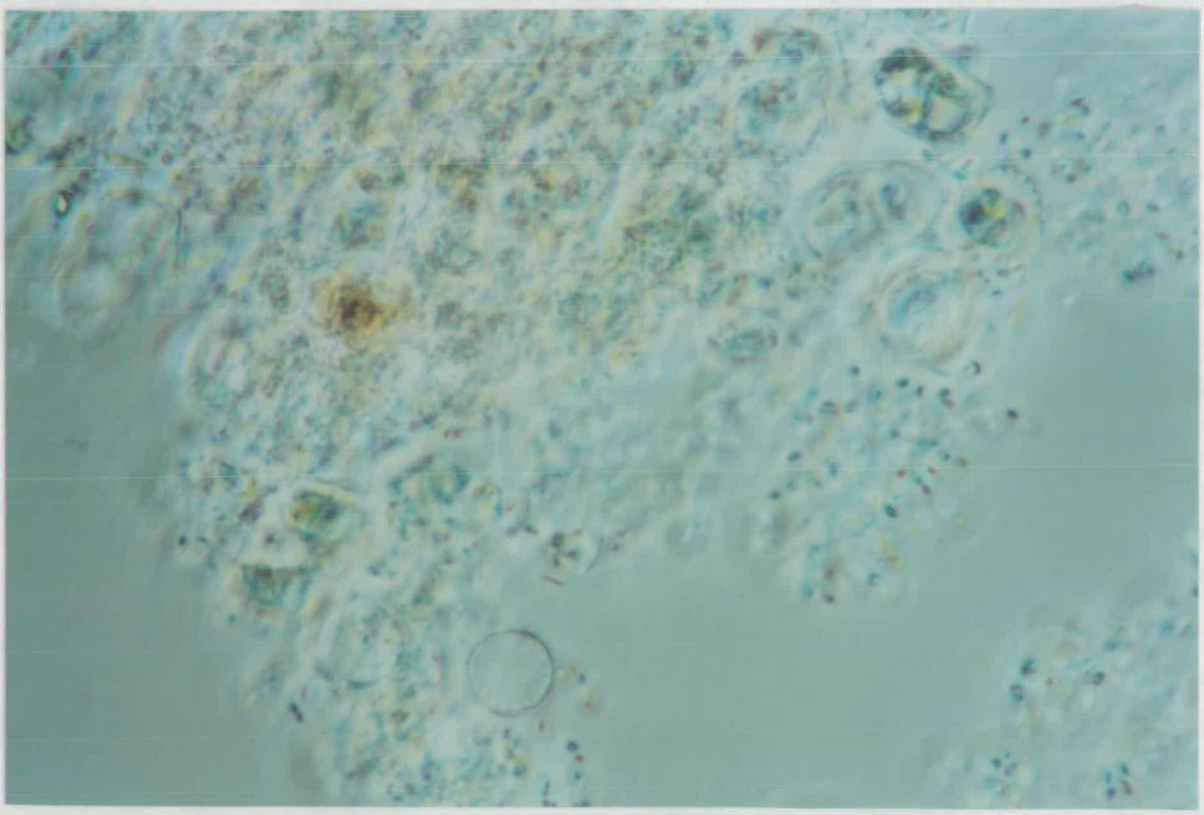
Photograph 4. *T. flocculosa* (xenic, heat-killed culture) after incubation at 25°C with Loch Leven water. Note filamentous bacterium (X900 magnification).

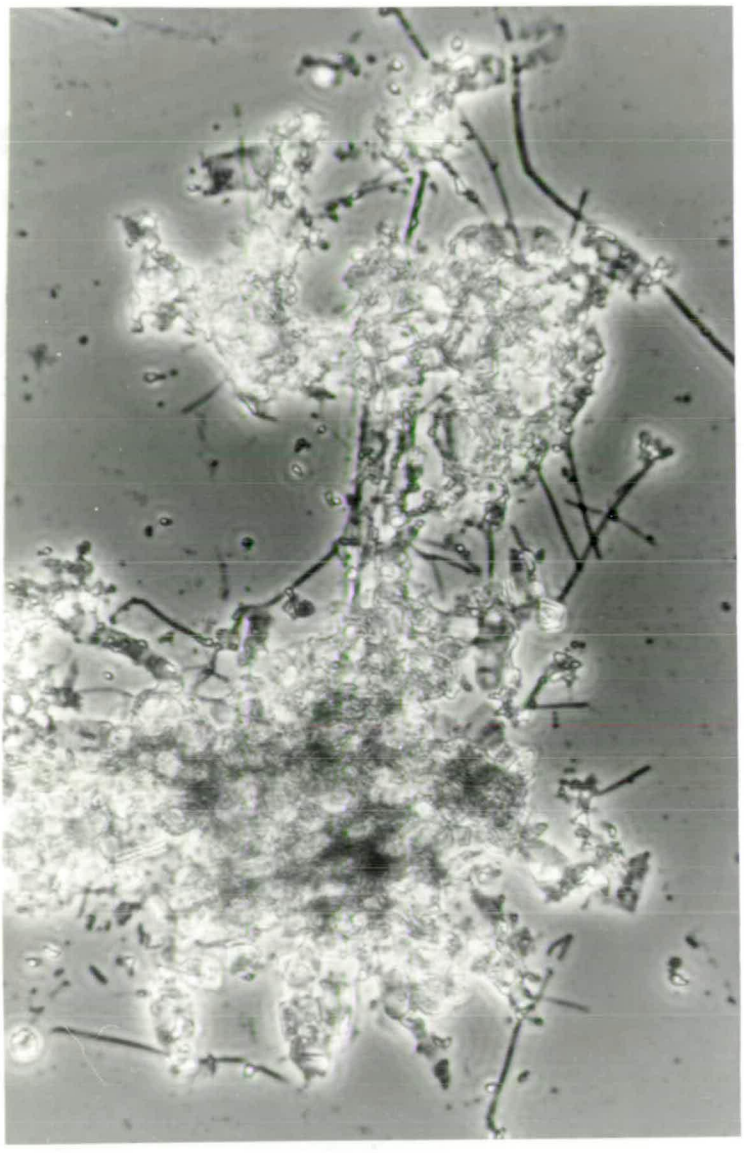


Photograph 5. *T. flocculosa* (xenic, heat-killed culture) after incubation at 25°C with Loch Leven water. Note sheathed filamentous bacterium (X900 magnification).

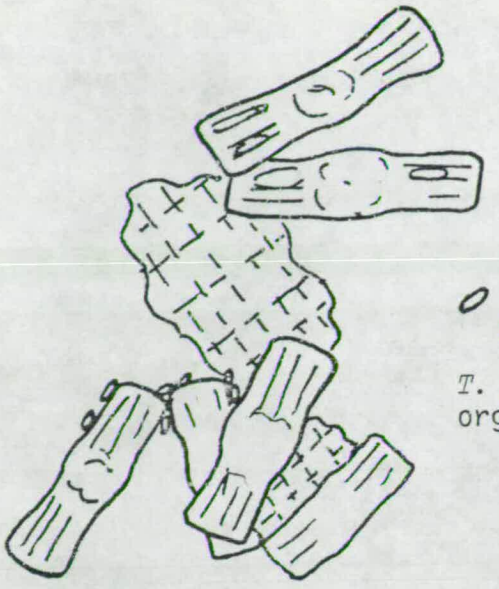


Photographs 6 & 7. *C. meneghiniana* (axenic, heat-killed) after incubation at 25°C with Loch Leven water (X900 magnification).



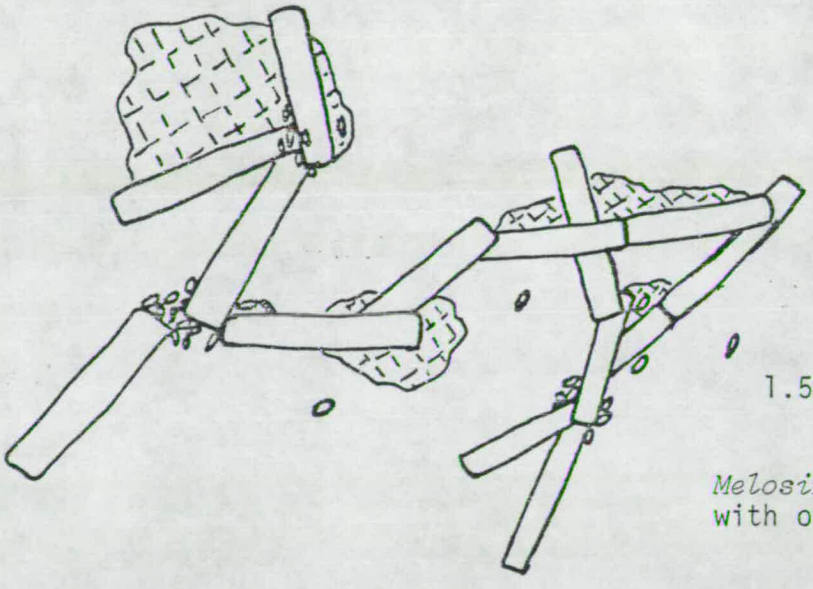


Photograph 8. *C. meneghiniana* (axenic, heat-killed) after incubation at 25°C with Lough Neagh water (X1000 magnification).



1.3µm. X
2µm.

T. flocculosa, inoculated with organism W2



1.56µm. X 0.8µm.

Melosira sp., inoculated with organism W2

Fig. 8.2 Drawings of clumps observed after the inoculation of *T. flocculosa* and *Melosira* sp. with organism W2

represents detrital matter which could not be drawn accurately (see photographs).

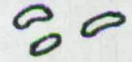
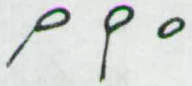
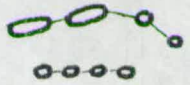
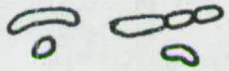
Examples of the organisms isolated are described in Table 8.2 . However none of these isolates were filamentous. Other workers have experienced difficulty in the isolation of filamentous organisms from freshwaters (J. G. Jones, pers. comm.).

To determine if any of those isolates could cause the diatom cells to form clumps, and also form filaments on re-inoculation, they were added to heat-killed *T. flocculosa* and *Melosira* sp. suspended in Chu 10 salt solution. There was no evidence of filament formation under these conditions, however two of the isolates did produce clumping of the diatoms (Table 8.3). Unfortunately the cytophaga-type organisms did not grow on re-inoculation. This may be attributed to the lack of growth requirements which would normally be supplied by the Loch water. The clumps formed are illustrated in Fig. 8.2. It should be noted that although these diatoms were not axenic, clumping did not occur in the presence of only the contaminating bacteria.

From this experiment it can be concluded that clumping is specific to the type of bacteria present, but not necessarily to the type of diatoms. The role of filamentous organisms observed in association with the diatom clumps, in either clump formation or diatom decomposition, remains to be determined.

Two of the isolates which formed clumps were examined in greater detail, (Y2 & W2 see Section VII, p.125) and tentatively identified as members of the *Enterobacteriaceae* and *Flavobacterium* respectively. The detailed examination of these cultures, which concerned their taxonomy and nutritional characteristics, did not reveal any factors related to their ability to promote clumping. They are both capsulate organisms

Table 8.2 Characteristics of Some of the Bacteria Associated with the Clumps

POSSIBLE TYPE	COLONY TYPE	MOTILITY	GRAM REACTION	MICROSCOPIC APPEARANCE UNDER PHASE CONTRAST	SIZE (μm)	SOURCE**
Cytophaga sp.	yellow* spreading	+ (gliding)	-		0.3 x 3 - 6	1,2,3,4
Vibrio shaped	clear* spreading	+	-		0.4 x 1.3 - 1.8	1,2,3,4
Caulobacter type	yellow orange white pink	±	-		0.5 x 2.0 0.4 x 1.3	1,2,3,4
Prosethecate	yellow white*	+ - -	- -		0.5 x up to 15.0 0.6 x 1.3 - 2.0	3
Coryneform	cream white mucoid*	+ +	variable		0.6 - 1.2 x 2 - 3 1 - 1.3 x 3 - 5	1,2,3 1,2
Rod shaped	white* yellow white clear	+ + - -	- - - -		0.7 x 1.5 - 3 0.4 x 1 - 2	3 1,3 1 2

** 1 = *T. flocculosa*; 2 = *A. formosa*; 3 = *N. pelliculosa*; 4 = *C. meneghiniana*.

*These isolates were re-inoculated onto diatom cultures, see Table 8.3.

Table 8.3 The Effect of the Inoculation of Diatoms with Pure Cultures of Bacteria on Clump Formation

ISOLATE	SOURCE	CLUMP FORMATION	RE-ISOLATION
Yellow spreading	*T. flocculosa*	-	-
	C. meneghiniana	-	-
Coryneform	*T. flocculosa*	+	+
White (W2)	*N. pelliculosa*	+	+
Vibrio shaped	*T. flocculosa*	-	+
Prosthecate	*N. pelliculosa*	-	-
Yellow (Y2)	Loch Leven water	+	+

and it may be that their stickiness can be attributable to this factor. However organisms which did not cause diatoms to clump were also capsulate. Examination with the electron microscope would perhaps shed more light on this aspect.

IX. GENERAL DISCUSSION

IX. GENERAL DISCUSSION

The primary objective of this study was to determine the effect of bacteria on the solubilisation of diatom frustules under different conditions in the laboratory. Evidence has been presented to show that lake bacterial populations and pure cultures of lake isolates enhance the release of silica when compared to sterile autolysis. A full discussion of the results in relation to *in vitro* microbial interactions has been provided in the relevant sections.

The main approach in the General Discussion is to attempt to relate the laboratory data to processes which may occur in the natural environment and in particular in Loch Leven and Lough Neagh.

1. DIATOMS AS A NICHE FOR BACTERIAL COLONISATION

a. THE FRUSTULE AS A SURFACE

The importance of surfaces to bacteria in all environments is well recognised. Examples of the wide-spread nature of bacterial associations with surfaces include attachment to plant and animal surfaces in relation to both pathogenicity and commensalism, and attachment to particles in soils, (Reading Symposium, 1980). Surfaces are of particular importance in aquatic environments because nutrients, including glycoproteins, proteins and other organic molecules, accumulate at the solid/liquid interface. They are therefore important sites for microbial growth in nutrient deficient and fast flowing aquatic environments, (Marshall, 1980 a & b). Attachment of bacteria to such surfaces results in a more stable environment in which the bacteria have the time and opportunity to utilise the substrates present, (Fletcher, 1980).

The surface characteristics of the diatom frustule include a large available surface area resulting from the complex pore structure and sculpturing. The silica gel-like nature of the amorphous silica has also been suggested as an advantage to living diatoms, by enabling them to concentrate nutrients, (Werner, 1977). This attribute may also be of advantage to the bacteria colonising the diatoms. Thus, the frustule may help to retain substrates in localised concentration. This effect would not occur with the breakdown of a cyanobacterium or green alga.

The adsorption of nutrients could limit their availability to microorganisms. Stotzky (1980) reported that the formation of clay protein complexes reduced the availability of the proteins for microbial use. If nutrients were strongly bound to the frustule, dissolution of the silica by bacteria could make them available. Uptake of the dissolved silica by the bacteria is probably not of major importance, although Heinen (1962), reported that silica can be used in place of phosphorus under conditions of phosphorus limitation. Silica may also form part of the structures of exopolysaccharides where it acts as a cross-linking agent. This may aid the rigidity of the structure (Dudman, 1977).

Bacterial solubilisation of the silica may be a secondary effect of growth on substrates associated with the frustule. These substrates would in the first instance be derived from the diatom cell. Thereafter other nutrients may accumulate at the frustule surface and be subjected to microbial decomposition. This 'secondary' association of nutrients with the large quantities of frustules present in the sediments of the lakes (Battarbee, 1978a; Haworth, 1972) could be of major importance in the solubilisation of the silica. It could be speculated that the influx of a non-diatom algal bloom into the sediments may

provide nutrients which also mediate the solubilisation of the frustule. The association of these nutrients with the diatom frustules and their subsequent microbial utilisation, if concurrent with silica solubilisation, may be as important to the silica cycle as the initial colonisation of the diatoms. The latter resulted in the release of only 60 to 80% of the total silica present under laboratory conditions (See Section IV).

Another aspect of bacterial attachment to the frustule is the formation of clumps of diatoms (see Section VIII, p.142). It is possible that the secretion of compounds by the bacteria mediates the "stickiness" of the cells. The major advantage to the bacteria of having two or more cells in close proximity, rather than one, is that far more algal nutrients would be available in that particular microhabitat.

b. NUTRIENTS FOR BACTERIA

The substrates which may be associated with the diatom frustule fall into two categories, a. nutrients derived from the diatom cell and b. nutrients present in the lakes which may accumulate at the frustule surface after death of the diatom. The possible importance of the latter group has been speculated on (see above), and will include the extra-cellular excretions and the contents of other algae in the lakes. This phenomenon was not investigated in this project and will be discussed (p.179).

A diatom cell contains a range of possible substrates suitable for utilisation by bacteria, including carbohydrate, lipid and protein (see Introduction). It therefore presents a niche suitable for bacterial colonisation. Bacteria may be initially attracted to diatoms

by the release of dissolved nutrients when the cells are no longer active (Jones, 1976). The complex molecules present may be subsequently degraded. In studies on the sterile autolysis of chloroform killed cells of *Scenedesmus* sp. and the diatom *Stephanodiscus hantzschii* 70 to 80% of the phosphorus content of the cells was liberated within the first few days. However only 20 to 30% of the nitrogen containing compounds was broken down by autolysis, the rest remained as undissolved protein-nitrogen (Golterman, 1960). A *Pseudomonas* sp. which was isolated from lake water decomposed the remains of the *Scenedesmus* cells after sterile autolysis (Golterman, 1972). The release and breakdown of carbohydrate and lipid material was not examined in these studies, however they do show the possible importance of microbial action in the breakdown of algal cells.

The results of this project indicate that bacterial decomposition of the diatoms enhances silica solubility. The only direct evidence for bacterial solubilisation of diatom frustules was obtained by Krumbein (1978). This work was carried out on Solar Lake, Sinai, and does not directly relate to Loch Leven or Lough Neagh. The diatoms examined were within cyanobacterial mats which were at the anoxic bottom of the lake, and the maximum temperature was in the region of 55 to 60^{o}C. The diatoms *Navicula, Nitzschia* and *Amphora* sp. which were present in the upper layers of the mats disappeared in the deeper layers. Scanning electron micrographs showed depressions in the frustules where bacteria were attached, and could be correlated with lower amounts of silica using EDX spot analysis. The author attributed the solubilisation to 'microbial mediated reactions', suggesting that exchange of phosphorus for silica in organic compounds may be important. However, although the pH never exceeds 7, this does not

exclude the possibility that the pH in the area surrounding the bacteria was higher, and therefore enhanced the solubility. The substrates used for microbial growth and the decomposition process resulting in silica solubilisation were not further investigated in this study.

The examination of the action of pure bacterial cultures (Section VII, p.124) indicates that the organisms which produce the widest range of hydrolases enhanced silica release to the greatest extent. These organisms were capable of breaking down lipid, proteins and carbohydrates. Whether the growth of bacteria utilising diatom substrates relates to the dissolution of the silica remains unclear. The data presented in Section VII, Fig.7.1 indicate that bacterial decomposition of the organic component of the wall is important. Other authors have shown that chemical removal of this component enhanced frustule solubility (e.g. Lewin, 1961). The reduced silica release in the presence of glucose (1g./l.) suggests that the availability of an alternative sugar 'spares' the decomposition of carbohydrate which may protect the frustule to some extent from rapid dissolution. Bacteria may exist which are capable of producing enzymes which specifically degrade the cell wall mannose enriched polysaccharide. Bacteria have been found which produce hydrolytic enzymes specific for the polysaccharides produced by higher algae, (Percival & McDowell, 1967), and those of other bacteria (Mitchell & Nevo, 1965). However it is not certain that these organisms can utilise the products as a carbon source (Sutherland, 1977). The ecological advantage of bacterial production of such specific enzymes is unclear, unless in the case of a diatom wall, it allowed quicker access to substrates which are suitable. For example, 1. the inner components of the wall, 2. enhanced diffusion of dissolved compounds present in

the cell and 3. dissociation of the two halves of the frustule and entry of bacteria.

The limitation of the silica release in the presence of bacteria does not relate to the lack of carbon or nitrogen as the addition of these nutrients did not increase the amount of silica released (Section V, p.92). This strongly suggests that the silica release is related to the utilisation of particular substrates which are closely associated with the frustules. The limiting factor may well be the degree of contact of bacteria with the frustule and associated substrate.

2. LAKE ENVIRONMENTAL FACTORS AND SILICA RELEASE

a. THE RATE OF SILICA SOLUBILISATION

The rate of silica solubilisation ($\mu gSiO_2 l^{-1} d^{-1}$) under the conditions of the experiments and the rates of increased silica concentration measured in the water column of Lough Neagh and Loch Leven are presented in Table 9.1

The rates of silica release in the lakes are calculated from graphs of silica flux, (see Table 9.1), during the periods of rapid rise in the silica concentration and low diatom productivity. During these months the lake temperatures would be in the region of 7^{o} to $20^{o}C$. It is interesting that the rates of release are of the same order as those obtained from diatom cultures under laboratory conditions at $25^{o}C$. However, they are greater than those obtained under sterile laboratory conditions. It therefore appears that autolysis of the diatoms alone, independent of other biological activity, cannot account for all the re-cycling of the silica within the lakes, and that bacteria and other

Table 9.1 A Comparison of the Rates of Silica Release in the Two Lakes and Under Laboratory Conditions

	DIATOM	LAKE	RATE OF SILICA RELEASE ($\mu gSiO_2 l^{-1} d^{-1}$)	TEMPERATURE (^oC)	MONTH & YEAR	REFERENCE
(a)	LABORATORY					
	C. meneghiniana	Leven	71	25^{o}C		Fig. 4.1
	(active culture)	Neagh	154	"		Fig. 4.2
	C. meneghiniana	Leven	59	"		Fig. 4.1
	(heat-killed)	Neagh	148	"		Fig. 4.2
	C. meneghiniana	Leven	13	"		Fig. 4.1
	(sterile)	Neagh	21	"		Fig. 4.2
	A. formosa	Leven	144	"		Fig. 4.4
	(heat-killed)	Leven	6	4		Fig. 4.6
		Neagh	175	25		Fig. 4.5
	A. formosa (sterile)	Neagh	26	"		Fig. 4.5
(b)	NATURAL ENVIRONMENT					
		Leven	126	10	October, 1972	Bailey-Watts (1976)
		Leven	190	7	April, 1978	Harriman (1978)
		Neagh	70	17	August, 1977	Gibson (1978)
		Neagh	45	15	June-October, 1970	Battarbee (1978a)

biological factors play an important role. The importance of bacterial solubilisation of silicon containing compounds, other than frustules, remains to be determined.

b. THE INTERACTION OF PHYSICAL, CHEMICAL AND BIOLOGICAL FACTORS

i. pH

The solubility of amorphous silica increases rapidly above pH 9 (see Fig. 3, p.18). The pH ranges of Loch Leven and Lough Neagh have maxima of 9.3 and 9.4 respectively, therefore there are occasions when a sharp increase in the silica solubilisation would be expected. The pH of Lough Neagh rose from 8 to just over 9 and returned to 8.4 in June 1970, with no effect on the dissolved silica concentration. The dissolved silica was increasing most rapidly when the pH was between 7.8 and 8.4. Therefore there did not appear to be a causal relationship between gross pH and silica release (Dickson, 1975). An investigation of the rates of silica released from Lough Neagh sediment core samples was carried out (Rippey, 1977). Seven cores were examined across the pH range 7.8 to 9.05, and the rate of silica release increased 21% over this range. It therefore appears that the pH can influence the solubility of the silica, but that the gross pH of the lakes is not a major factor influencing silica release.

In the experiments carried out in this study the pH did not exceed 9, therefore the solubilisation of the silica was not brought about by pH differences. Differences observed in the amounts of silica released under sterile and non-sterile conditions did not relate to differences in the pH (see Section IV, p. 76). Any pH change which occurred during

the course of these experiments occurred to the same extent under both sterile and non-sterile conditions.

It appears that considerable caution should be exercised over the interpretation of gross changes in the pH in the natural environment, as the localised pH in microhabitats may be substantially higher or lower. For example the de-amination of amino acids by a bacterium at the frustule surface might increase the pH sufficiently to enhance solubility.

ii. Oxygen Availability

The exposed position and morphometry of both Loch Leven and Lough Neagh ensures that the deeper waters rarely become anoxic (Bailey-Watts, 1976a; Bailey-Watts & Lund, 1973; Battarbee, 1978a). In Loch Leven sediments at least the top few millimetres remain more or less continuously oxidised (Bailey-Watts, 1976b). In Lough Neagh it has been reported that phosphorus release occurred from the sediments when the overlying waters were oxygen depleted, but not anoxic, the oxygen saturation of the water only rarely dropping below 50% (Rippey, 1977). In 1970 the lowest oxygen concentration was 65% and this only lasted for a few days during August (Wood & Gibson, 1973). It therefore seems that the release of dissolved silica under anaerobic conditions is not of importance in these lakes. Therefore anaerobic conditions were not examined in the laboratory. The effect of increased aeration by agitating the tubes (see Section V, p.105) did not increase the amount of silica released. This suggests that oxygen was not limiting the growth of the bacteria involved in the diatom decomposition.

Tessenow (1966) found little differences in the amounts of silica released from cores kept in the dark under aerobic and anaerobic conditions. This could be explained if facultative and obligate anaerobes were as important in silica dissolution as aerobes, and the process of solubilisation was as efficient anaerobically. At least one of the bacterial isolates which enhanced silica release was a facultative anaerobe (see Section VII, p.125).

iii. Temperature

Temperature will have a direct effect on the physical solubility of the silica (e.g. Kamatani & Riley, 1979; Tessenow, 1966) as well as on the growth of bacterial populations. The temperature ranges for the two lakes are presented in Table 9.2 (Gibson & Smith, 1974). The dissolved silica released from Lough Neagh core samples incubated over the temperature range from 4^{o} to $20^{o}C$ was carried out by Rippey (1977). The rate of silica release increased slowly from 4^{o} to 10^{o} (an increase of $40mgSim^{-2}d^{-1}$) after which it rose dramatically (an increase of $160mgSim^{-2}d^{-1}$). This study suggested that temperature was the most important physical factor involved in the release of soluble silica. The release from core samples under different conditions of pH, agitation of the sediment surface, and aqueous concentration of dissolved silica having also been compared. However, the rates of silica release from cores did not fit any standard physical pattern and the release could only be explained in physical terms if some of the soluble silica was being re-absorbed by clays. The possible influence of microbial action was not considered. The results presented in Fig. 4.6 indicate that bacteria enhance the release of silica at temperatures as low as $4^{o}C$, although not to the same extent as at $25^{o}C$. The differentiation between the physical effect of temperature on the solubility of the silica and the influence of microbial growth remains a major unsolved problem.

Table 9.2 The Annual Temperature Cycle in Loch Leven and Lough Neagh

MONTH		J	F	M	A	M	J	J	A	S	O	N	D
APPROXIMATE TEMPERATURE (°C)	L. LEVEN* (average for 1968-1971)	2	2	3	7	12	16	16	16	15	11	6	3
	L. NEAGH** (1969)	2	2	2	6	12	15	16	16	15	14	7	3

*after Smith (1974); **after Gibson & Stewart (1974).

iv. The Content of the Diatom Crop

The quality and content of algal blooms will to a large extent govern the nutrients available for microbial growth. The composition of diatoms varies with the conditions of growth and the growth limiting factors. This relates to the silica content of the frustule as well as to other cell components (Werner, 1977 and Section IV, p.71). These factors may be important in determining which diatoms remain within the sediments and eventually fossilise.

v. Invertebrate Activity

Daphnia hyalina var. *lacustris* has been a dominant member of the zooplankton in Loch Leven since 1970 (Johnson & Walker, 1974). This zooplankton grazes on the diatoms, as fractured *Asterionella formosa* cells have been observed in the guts of the *Daphnia*. The grazing may explain some of the anomalies which occurred in the usual inverse relationship between silica concentration and planktonic diatom populations (Bailey-Watts, 1976a).

Chironamid larvae, which are also filter-feeders, are abundant in the benthos of Loch Leven (Maitland & Hudspith, 1974) and also appear in Lough Neagh (Wood & Gibson, 1973). The feeding mechanism of these invertebrates will result in the exposure of more frustule surfaces which might physically enhance the solubility of the silica (Bailey-Watts, 1976a). Tessenow (1966) obtained enhanced silica release from *Asterionella* suspensions when incubated in the presence of *Daphnia magna*, over that obtained from *Asterionella* suspensions alone. Microscopic observation showed that the frustules had been fragmented by the feeding action of the *Daphnia*. Similar enhanced release was observed in the

presence of Chironamid larvae taken from the benthos. However, these experiments did not distinguish between the effects of invertebrate activity and the possible effects of bacterial action. The breaking of the frustules by sonication (see Section V, p.103) did not increase the release of silica to a greater extent than unbroken cells, in the presence of bacterial populations. It is possible that the digestion of organic matter within the guts of these animals may produce what is effectively an acid-cleaned frustule, which will be more readily soluble. However, this would decrease the bacterial colonisation of the diatoms and prevent any microbial induced solubilisation.

A variety of zooplankton have been observed in Lough Neagh (Graham, 1970). The dominant members of the population are the copepods, *Cyclops* and *Diaptomus*, and several species of rotifers. *Cyclops* sp. and rotifers also graze on the diatoms of Loch Leven, although their numbers are considered to be too low to have an appreciable effect on the diatom populations. Recognisable parts of diatom frustules have been observed in the faecal pellets of *Cyclops* sp. (E. Rutowski, pers. comm.). Organic material excreted with the pellet will present a different range of substrates available for bacterial growth. The bacterial decomposition of copepod faecal pellets containing diatom frustules and surrounded by a polysaccharide membrane was reported to occur within six to fourteen days in Lake Michigan (Ferrante & Parker, 1977). However, these authors did not relate this effect to any possible solubilisation of the frustules.

Rotifers feed on *Asterionella formosa* by breaking off the end of the frustule and sucking out the contents (L. May, pers. comm.) and on *Melosira* sp. by piercing the cells and again sucking out the contents,

(May, 1981). This suggests that the outer organic component of the frustule may be left intact, but that there will be a substantial reduction in the nutrients available for bacterial growth.

vi. The Relationship of These Factors with the Case History of a Spring in Lough Neagh

A comprehensive study of the sediments of Lough Neagh was carried out by D. Jewson and co-workers (Pers. comm.) after the settling out of a spring bloom of *Melosira* in 1979. The bloom crashed during a calm period, and the *Melosira* cells reached the sediment in what was thought to be a resting state, similar to that described by Lund (1954). *Melosira* is unusual in this characteristic as other common bloom formers do not appear to produce a resting state. This is one example of how the content of the diatom crop may influence re-solution of the silica.

The *Melosira* remained on the sediment surface until the temperature began to rise near the beginning of the summer. This coincided with greater activity of benethic invertebrates and increased aeration of the sediments. It was only at this point that dissolved silica began to appear in the water column. It can be postulated that the increase in temperature and oxidation of the sediments enhanced microbial activity.

The major factors influencing release under these conditions appear to be:

1. Temperature, which will influence microbial and invertebrate activity.
2. Aeration and disturbance of the sediments by invertebrates.
3. Invertebrate activity physically breaking up the *Melosira* resting stages.

The relative importance of these remains to be determined.

3. SOLUBILITY VS. INSOLUBILITY

Complete dissolution of frustules from concentrated diatom dominated phytoplankton from Loch Leven occurred after incubation in the light at 20°C for 15 months (Bailey-Watts, 1976b). The results of this project (Section IV) indicated that a maximum of 60 to 80% of the initial frustule silica became soluble after up to 4 months incubation, and complete dissolution did not occur. On the other hand, diatom frustules do accumulate in the sediments of Loch Leven (Haworth, 1972), and Lough Neagh (Battarbee, 1978a), where the taxa present in the top sediments correlate with the observed phytoplankton. A dramatic increase in the volume of the influx into the sediments in Lough Neagh can be correlated with the increased nutrient input of the last 100 years, relative to the input for the previous 500. This reflects the changes brought about by cultural eutrophication. This anomaly could be explained if the sediments qualitatively, but not quantitatively, reflect the phytoplankton populations. Oceanic deposits of diatoms do not reflect the phytoplankton directly, either quantitatively or qualitatively because more of the thinner shelled diatoms are absent and thought to dissolve in the water column (Lisitzin, 1971).

Electron micrographs of the diatoms from the sediments of Lough Neagh revealed the presence of pitted and coroded surfaces, the enlargement of pores and the absence of girdle bands. This suggests that partial solubilisation of the frustules can occur without them becoming completely unrecognisable (Battarbee, 1978a). Observations of the dissolution of silica from acid-cleaned frustules of *N. pelliculosa*

in alkaline solution commenced with the enlargement of the structural pores of the frustule (C. M. Sullivan, pers. comm.). This again suggests that a certain amount of the silica can become soluble, although the frustule may be left recognisable. It has been reported that the structure of silica deposits in rye straw remains clearly visible microscopically when 82% of the silica has been removed by alkali extraction (Golterman, 1960).

4. THE FATE OF A FRUSTULE - AN HYPOTHESIS

This hypothesis is based on the information produced during this study.

The diatoms die, probably as a result of silica limitation, and are colonised by a heterotrophic population of bacteria, in the water column. These may be attracted chemotactically to the diatoms by released dissolved organic compounds. The colonisation results in the formation of clumps and increases the sedimentation rate, (the living resting stages of *Melosira* sp. excepted, under calm conditions). The clumps are composed of frustules, cell contents, detrital matter (e.g. other phyto- and zoo-plankton), and bacteria. The bacteria using the other components of the clumps as a food source. This action leads to some solubilisation of the silica. How much occurs within the water columns depends on the sedimentation rate which is an important but unknown factor. Grazing of the diatoms will occur in the water column, but may not have a large impact on the total bloom.

Once in the sediments the frustule will be influenced in other ways. Invertebrate activity and aeration possibly become more important and nutrient availability less so. The influence of the accumulation of

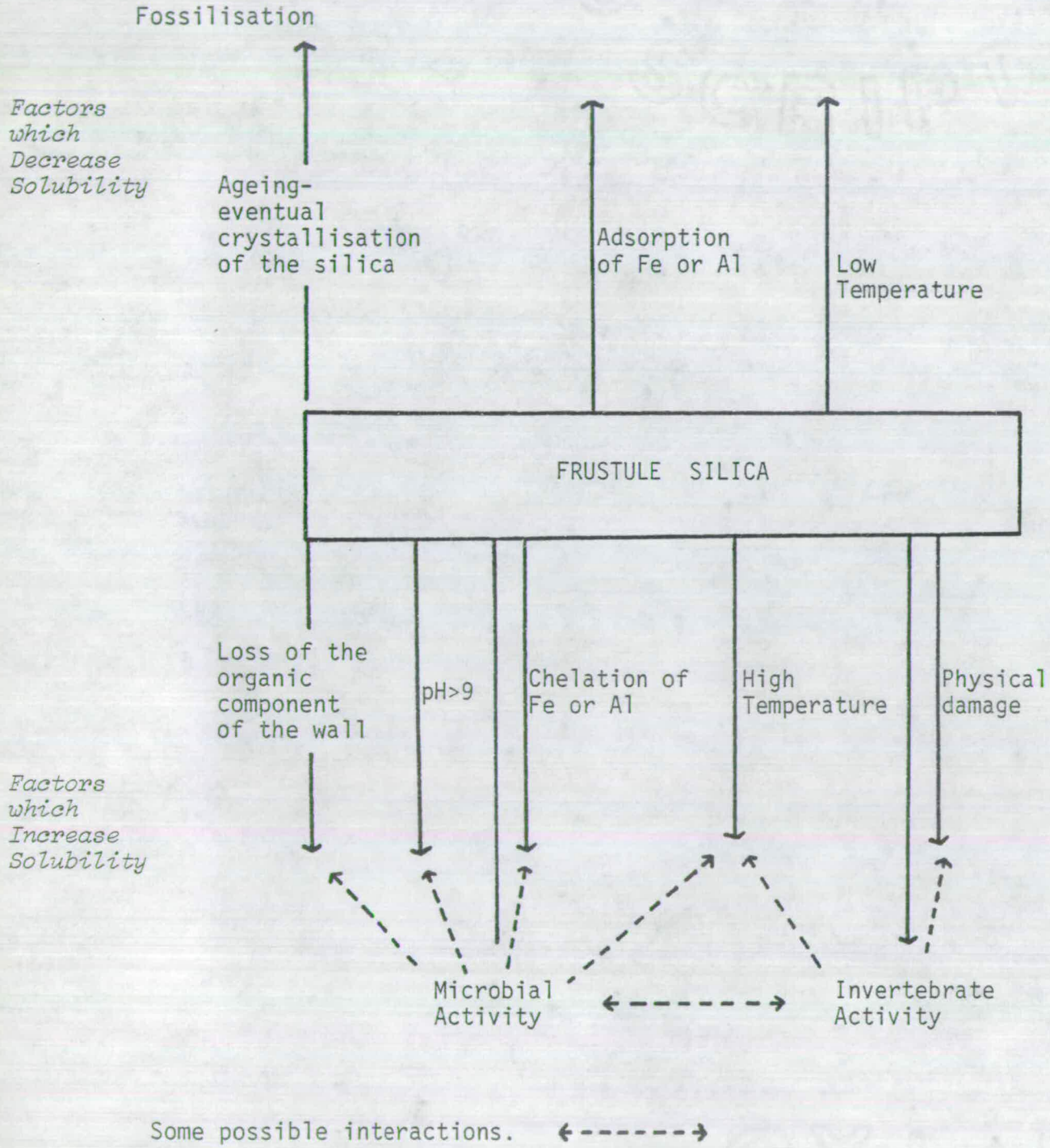


Fig. 9.1 Summary of the factors which may influence the solubility of a diatom frustule

other nutrients at the frustule surface and their use by bacteria may also be important. The adsorption of any iron or aluminium present would decrease the solubility by reducing the numbers of free -OH groups present.

Assuming that the mechanism of solubilisation is directly related to a particular aspect of microbial growth it might be possible to predict the extent to which a frustule could be solubilised. The potential for solubilisation could be related to the frustule silicon to total carbon ratio, which could be calculated for a particular environment. Where most of the nutrient carbon is supplied by the diatom cells, (e.g. the water column) this could be related to a particular diatom type, relating diatom volume and frustule thickness to the potential solubility.

The fate of a diatom frustule can be seen as an equilibrium which may be positively influenced by a number of factors (Fig. 9.1). From the results of this project and the findings of other authors microbial activity, invertebrate activity and temperature seem to be major factors controlling the release of soluble silica.

5. UNSOLVED PROBLEMS AND APPROACHES

This study was carried out almost entirely under laboratory conditions. It would therefore be interesting to determine if similar results could be obtained in the lakes.

Dialysis bags were used to partially restrict the environmental factors which would influence *in situ* experiments on clump formation (Section VII). Similar experiments could monitor any change in the total silica content of the diatom suspensions.

The importance of diatom dissolution in the sediments is an important area which was not investigated in this project. The separation of the effects of invertebrate and microbial activity, and the influence of temperature and oxygen availability on these is of major importance.

An investigation of the relationship between the adsorption, or accumulation, of nutrients at the frustule surface, and microbial growth could determine the relative importance of the initial colonisation of diatom cells by bacteria and subsequent silica release, compared with the secondary growth of microorganisms in the sediments. This study would involve an examination of the adsorptive properties of frustules in the sediments.

The assessment of the bacterial populations or types active in silica solubilisation remains a large problem. A technique for screening the bacterial population for organisms active in silica solubilisation could determine the ecology of these organisms, and their distribution in a space and time. However this leads to the general problem in microbial ecology of assessing which members of the population are active *in situ* and using, for example, growth on CPS agar, as an indication of activity. In this project filamentous bacteria were observed in close association with the decaying diatoms. However, these could not be isolated (Section VII). Re-inoculation with pure cultures of other bacterial isolates proved to be effective in enhancing silica release, which suggests that the filamentous types may be unimportant. However, the significance of their presence *in situ* remains to be determined. The use of such techniques as autoradiography could aid in an investigation of the metabolic activity of bacteria associated with the diatoms.

6. CONCLUSION

This General Discussion has considered the factors in the fresh-water aquatic ecosystem that are likely to influence the solubilisation and cycling of silica. It is clear that these factors are complex and dependent on the interaction of physical, chemical and biological parameters. The results reported here have attempted to demonstrate the possible role of bacteria associated with diatoms in the silica cycle.

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