THE EFFECT OF PHOTOSYNTHETIC MICROORGANISMS ON THE PERMEABILITY OF MARINE SEDIMENTS

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SUMMARY

(1) The main aim of the present work was to study the effects of microorganisms on permeability of marine sands. A secondary aim was to review the principle and theory of primary production in aquatic habitats with particular reference to the ^{14}C assimilation technique for measuring primary production. The review is presented in the appendix.

(2) Darcy's equation was mathematically derived to measure permeability by the falling head permeameter.

(3) Three types of experiment were done. The first tested 3 core diameters and 4 bed heights in order to develop a suitable permeameter. The second tested the effects of fine material on permeability. The third tested the effects of different microorganisms in enrichment culture on permeability.

(4) The results of the first experiment showed that no combination of sediment bed height and core diameter was better than any other.

(5) The sediment treatments in the second experiment were natural sediment, natural sediment with fines removed, Rockware (quartz) sand, natural sediment with fines removed and then added, and Rockware sand with fines added. The results showed that the fine material reduced the permeability of sediment. Permeability also decreased with successive runs. This is probably due to sediment compaction.

(6) In the third experiment, sediment cores were enriched with photosynthetic (M) medium and heterotrophic bacterial (B) medium, and then incubated either in the light (L) or in the dark (D) to give 4 treatments (ML, MD; BL, BD). Control (C) cores were incubated in formalin. The experiment was run for 25 days.

(7) The permeability of the BL and BD cores fell dramatically during the experiment. The permeability of the ML and MD cores also fell but to a lesser extent. The permeability of the control (C) cores only fell slightly. This slight fall is attributed to compaction.

(8) Chlorophylls, bacteriochlorophylls, ^{14}C primary production and heterotrophic counts were measured at the end of the experiment. The ML cores had high chlorophylls and primary production. The BL cores had high bacteriochlorophylls and heterotrophic counts while the values for the control cores were very low or negligible.

INTRODUCTION

The activity of benthic micro- and macro-organisms in sediments has great ecological importance. As a result of this activity, dramatic changes occur in many of the physical and chemical properties of sediments (Fager, 1964; Rhoads, 1967; Frankel & Mead, 1973; Cadee, 1976; Aller, 1978 a, b; Aller, 1980a; Nowell et al., 1981; Aller, 1982; Larson & Rhoads, 1983; Thayer, 1983).

These biological effects can occur vertically and horizontally and can be on a microscale (micro-spatial variation), (Anderson et al., 1981; Meadows & Tait, 1985) or on a large scale over hundreds of metres (macro-spatial variation) (Cadee, 1976; Meadows & Tufail, 1986). There can also be marked temporal variations (Cadee, 1979; Frostick & McCave, 1979; Rhoads et al., 1978).

A considerable body of literature documents bioturbation in the marine environment and its ecological effect on the structure of fossil and present-day sediments (Webb, 1958, 1969; Schafer, 1972; Clifton & Hunter, 1973; Ruello, 1973; Frey, 1975; Golubic et al., 1975; Hollister et al., 1975; Reineck, 1977; Aller, 1978b; Reineck & Singh, 1980; Carney, 1981; Aller, 1982; Larson & Rhoads, 1983; Thayer, 1983; Weaver & Schultheiss, 1983; Miller, 1984; Curran, 1985; Pollard, 1988). Quantitative measurements of bioturbation in the Clyde Sea Area have been obtained from resin casting of large crustacean and fish burrows (Atkinson et al., 1982; Nash et al. 1984) and of smaller organisms (Tait in Meadows & Tufail, 1986). A recent report has shown that the burrowing hemichordate Saccoglossus kowaleskii, which ventilates its burrows, inhibits aerobic microbial activity in its burrow linings by secreting 2,4-dibromophenol (King, 1986; Meadows, 1986).

The activity of meiofauna and microorganisms in sediments is not

so obvious because of their size, although the metabolic effects of these organisms are often noticeable. For example aerobic chemoheterotrophs use up oxygen in the sediment and make it anaerobic so that it then becomes an ideal environment for Desulfovibrios and methanogenic bacteria (Redford, 1958; Stanier et al., 1977; Meadows & Campbell, 1988).

Field and laboratory studies have shown that many physical and chemical properties of sediments are dictated by biological and microbiological activity (Aller, 1978a; Aller & Yingst, 1978; McLachlan, 1978; Wormald & Stirling, 1979; Aller, 1980 a; Nowell et al., 1981; Grant, Bathman & Mills, 1986; Grant, Mills & Hopper, 1986). Chemical properties such as Eh, pH, sulphide, and O_2 and nutrients in pore water, may be altered by burrowing activity (Anderson & Meadows, 1978; Aller, 1978b; Wormald & Stirling, 1979; Aller, 1980b; Aller, 1982; Atkinson et al., 1982; Aller, 1983; Hennig et al., 1983; Meadows & Tufail, 1986). Physical properties such as sediment stability (shear strength and critical erosion velocity), particle sedimentation and permeability are all affected by bioturbation or microbial activity (Moore, 1931; Gingsburg & Lowenstam, 1958; Rhoads, 1963; Fager, 1964; Bathurst, 1967; Neumann et al., 1970; Scoffin, 1970; Aspiras et al., 1971; Frankel & Mead, 1973; Holland et al., 1974; Cadee, 1976, 1979; Frostick & McCave, 1979; Carney, 1981; Atkinson et al., 1982; Grant et al., 1982; Weaver & Schultheiss, 1983; Girling, 1984; Meadows & Tufail, 1986; Meadows & Campbell, 1988).

The objectives of my research were to investigate the effects of detrital material and of photosynthetic and heterotrophic microorganisms on the permeability of marine sediments. This was achieved by 3 series of experiments and by a brief review. The materials, methods and results of the first two series of experiments are presented together. These are followed by the materials, methods

and results of the third series of experiments. The review is presented in appendix 1.

The three series of experiments were concerned with a laboratory investigation of the effects of microbial growth on sediment permeability. In the first series I assessed the experimental design of the falling or variable-head permeameter. The second series tested the effects of fine detrital material on natural and artificial sediment. The third series, which was the major part of my experimental work, tested the effects of photosynthetic and heterotrophic microorganisms on the permeability of marine sediments using enrichment culture methods. This series is also referred to as the enrichment experiment.

In the third series, I used intertidal sand with its indigenous microorganisms, which I enriched with photosynthetic media and heterotrophic media and incubated under a diel light/ dark and dark regime. The aim of this methodology was to stimulate the growth of a range of autotrophic and heterotrophic microrganisms (see appendix 6 p 256). In particular it was hoped that blue-green algae and diatoms would become abundant in the cores containing photosynthetic medium and incubated in the light, and that aerobic gram-negative bacteria would become abundant in the cores containing bacterial medium and incubated in the dark. This proved to be so, and enabled me to assess the effects of these and other microorganisms on the permeability of sediment cores.

The fourth part of my thesis is a short review of primary production, photosynthesis and plant respiration in aquatic habitats, and of the ^{14}C technique for measuring primary production. This review was needed because details of the principles of primary production and of its measurement are not presented simply in the literature.

MATERIALS AND METHODS - First and second series of experiments

Two series of permeability experiments were carried out. The first was designed to test the best core diameter and sediment bed height, and the second to test the effects of interstitial detrital material on permeability. Throughout this thesis the term core will mean a glass column containing a core of sediment.

Natural surface sediment was collected at Ardmore low tide level (Nat. grid NS 320 792). It was kept overnight at 10°C and then sieved through a 1 mm sieve to remove macrofauna.

a) First series of experiments

Thirty six glass cores were prepared, 12 having a diameter of 11 mm, 12 of 18 mm, and 12 of 29 mm. Each core was 500 mm long. The lower end of each core was covered with a stainless steel mesh (80 squares/inch) below which was stretched a fine nylon mesh. The steel and nylon meshes were retained by a plastic clip around the base of the core. This arrangement allowed free flow of water but retained sediment and detrital material. Four sediment bed heights were tested: 25 mm, 50 mm, 100 mm and 200 mm. Three replicate cores were set up for each combination of sediment bed height and core diameter, giving 36 cores in all. 100 % saturated sediment was added to the cores to the appropriate height, sea water was added to the top of the core, and the core inverted 4 to 5 times. Each core was placed in a cylindrical glass basin filled with sea water. The water level in the core was adjusted to 200 mm above the sediment surface and the cores were clamped in the glass basins so that the water level inside and outside the core was the same. The cores were left for 24 hours at 20°C to allow the sediment to settle. During this time their tops were sealed with rubber bungs.

The next day the water levels in the cores were adjusted to 200 mm above the sediment surface. Runs were initiated by removing a core from the glass basin, clamping the core vertically and then removing the rubber bung. The water level immediately began to fall, and the time taken for it to fall 25 mm was recorded. Three readings were taken on each core, topping up the water level each time to the 200 mm mark with sea water. Permeability coefficients (K $mm.sec^{-1}$) were then calculated using the variable-head permeameter equation 8 (p 13).

b) Second series of experiments

A bed height of 50 mm and a core diameter of 29 mm was used throughout this series. The method of setting up the cores and measuring permeability was as described in the first series of experiments.

Five sediment treatments were tested. These were :

A Natural sediment

B Natural sediment with fines removed

C Rockware sand (a relict quartz sediment)

D Natural sediment from which fines had been removed and then added again.

E Rockware sand with fines added.

Fines = fine interstitial detrital material.

Fines were removed from the sediment as follows. 1000 ml of natural sediment was mixed with 300 ml of filtered sea water. This was left to settle for 30 sec. after which the supernatant was carefully decanted. The procedure was repeated 18 times until the supernatant was clear. The sediment from which the fines had been removed was used in treatments B and D. The solution containing the fines (18 x 300 ml = 5.4 l) was used in preparing sediments D and E. This solution was left

to stand for 24 hrs. at 20°C to allow the fines to settle. The supernatant was then decanted and the remaining volume containing the fines was made up to 1000 ml with filtered sea water.

Fifteen cores were prepared as in the first series of experiments, three for each of the five treatments. The general procedure for preparing the sediment treatments is shown in the flow diagram (figure 1). Filtered sea water was added to A, B, and C and the solution containing the fines to D and E, until the water level was 200 mm above the sediment surface. The cores were then inverted 4 to 5 times and left immersed in filtered sea water for 24 hrs. at 20°C. The next day 4 readings were taken on each of the cores in exactly the same way as in the first series of experiments, and permeability coefficients K ($mm.sec^{-1}$) calculated.

Note :

(1) The tables and figures in the main body of the text and the tables in the appendices are each numbered separately. Tables in the body of the text are referred to differently from those in the appendices. For example a text table is referred to as table 3 and an appendix table as appendix 5.4 table 2.

(2) Statistical analyses of the data are given in the results. The following code is used for probabilities * $0.05>P>0.01$, ** $0.01>P>0.001$, *** $P<0.001$

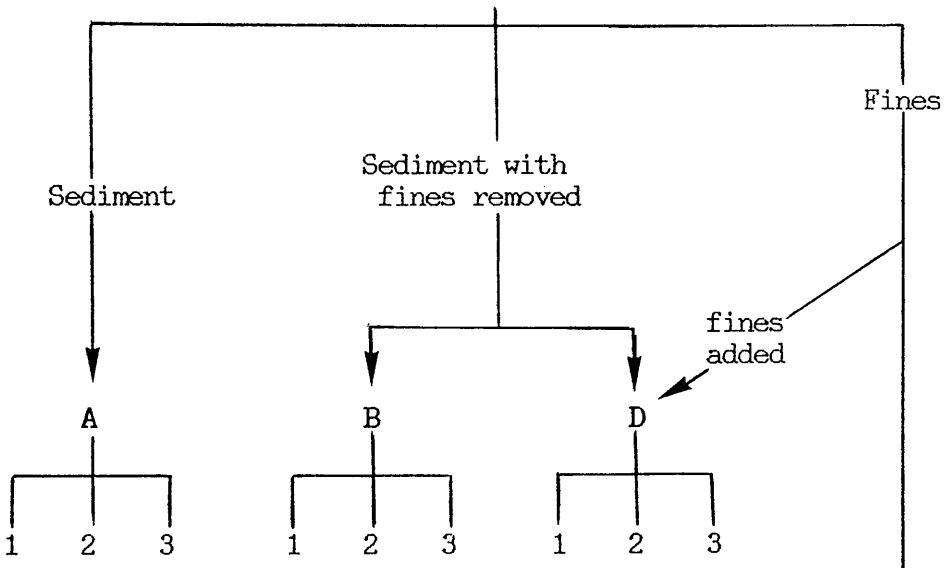
Figure 1

Second series of experiments.

Flow diagram showing the preparation of the five sediment treatments A, B, C, D, and E.

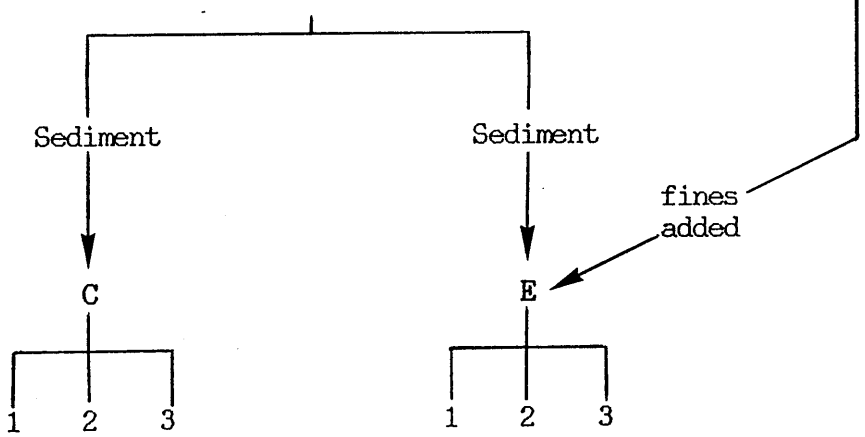
Natural sediment

(sieved through 1 mm sieve)



Replicates

Rockware sediment



Replicates

RESULTS - First and second series of experiments

Development of a permeability equation

The quantity of water flowing through a sediment per unit time can be regarded as the sediment's permeability. Clearly, it must be directly proportional to the height of the water above the sediment surface, since the greater the depth of the water the greater the pressure. It is also directly proportional to the cross-sectional area through which the water flows - the bigger the area the greater the water flow. It is inversely proportional to the length of the flow through the sediment - the longer the flow path the greater the frictional resistance of the sediment. These statements can be formalised in the following equation:

$$(Q/t) \propto (A.H)/l \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

$$(Q/t) = (k.A.H)/l \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

where k = a constant of proportionality called the permeability coefficient.

Q = quantity of water flowing.

t = time taken by water to flow.

A = cross-sectional area through which water flows.

H = hydraulic head across soil.

l = length of flow path through soil.

This is known as Darcy's equation after Darcy (1856) who first developed it in the 19th century (Smith, 1981, p. 41).

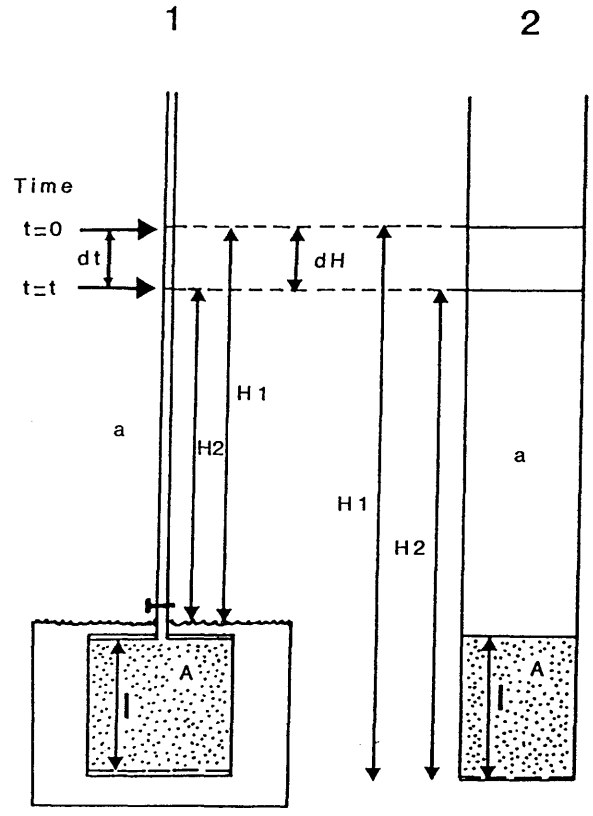
In practice there are two ways in which this equation can be used. The first is its use for calculating k for a coarse-grained sediment using a constant-head permeameter. The second is its use for calculating k for a fine-grained sediment using a variable-head permeameter. This latter method was the method used in the present

study and involves the derivation of a new equation for k from equation (2).

In the variable-head permeameter water is allowed to flow through the sediment for a small interval of time, dt, measuring at the same time the small fall in head height of water above the sediment, -dH. Hence the quantity of water (Q) flowing through the sediment in time dt is $Q = -a.dH$,

where a = surface area of water (since volume "Q" = surface area (a) x height (dH)). The negative sign is to allow for the fall in the water height.

The following diagram shows the commonly used variable-head permeameter (Smith, 1981) (1), and the modified variable-head permeameter I used in my experiments (2).



The derivation of the new equation for k is as follows.

Substituting $Q = -a.dH$ and $t = dt$ into equation (2) gives

$$-(a.dH)/dt = (k.A.H)/l \quad \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

This is a first order differential equation which can be solved for k by the separation of variables method as follows:

$$-adH = (k.A.H.dt)/l$$

or $$dt = -(a.l)/(A.k).(dH/H) \quad \ldots\ldots\ldots\ldots\ldots\ldots (4)$$

Equation 4 can be integrated for dt between limits 0 and t, and for dH between limits H_2 and H_1, where t is the time taken for the water level to fall from H_1 to H_2 (see above figure)

$$\int_0^t dt = \int_{H_1}^{H_2} -((a.l)/(A.k)).(dH/H) \quad \ldots\ldots\ldots\ldots\ldots\ldots (5)$$

Since $-(a.l)/(A.k)$ is a constant in this integration equation (5) can be rewritten as

$$\int_0^t l\,dt = -((a.l)/(A.k)).\int_{H_1}^{H_2} (1/H).dH \quad \ldots\ldots\ldots\ldots (6)$$

Solving the left-hand and right-hand integrals gives:

$$\Big| t \Big|_0^t = -(a.l)/(A.k) \Big| \ln H \Big|_{H_1}^{H_2}$$

$$t - 0 = -(a.l)/(A.k).(\ln H_2 - \ln H_1)$$

$$t = (a.l)/(A.k).(\ln H_1 - \ln H_2)$$

$$k = (a.l)/(A.t).\ln(H_1/H_2) \quad \ldots\ldots\ldots\ldots (7)$$

but since a = A in my permeameter

$$k = (l/t).\ln(H_1/H_2) \quad \ldots\ldots\ldots\ldots\ldots\ldots (8)$$

Equation (8) is the form I used for calculating permeability values.

The units of k in equation (8) can be worked out by dimensional analysis as $k = (L/T).\ln(L/L) = LT^{-1}$ where L = length and T = time. Since length has been measured in mm and time in seconds, the units of k are $mm.sec^{-1}$.

First series of experiments

The results of the first series of experiments are shown in tables 1 and 2 and figure 2. The statistical analysis of this data is given in tables 3, 4 and 5. The data in these tables will now be described.

Table 1 shows the time (sec) for the water column to drop 25 mm for the different core diameters and bed heights. Table 2 shows these times converted into the equivalent permeability coefficients (k in $mm.sec^{-1}$).

Figure 2 summarises the data from tables 1 and 2. It shows that permeability is lower at a bed height of 50 mm than at 25 mm, that it increases again at 100 mm, but then becomes variable at 200 mm. There is an indication in some instances that the permeability coefficient decreases with increasing core diameter. This latter effect is predictable from equation 7 where K ($mm.sec^{-1}$) is inversely proportional to A (the cross-sectional area of the sediment).

The significance of these results were tested by a two-way analysis of variance followed by a series of one-way breakdown analyses of variance. The two-way analysis of variance tested differences between core diameter(Factor A) and between bed heights (Factor B) (table 3).

The results of this analysis showed that Factor A and Factor B were significant. However nothing can be said about these main factor effects because the interaction was also significant. In order to obtain further information about core diameters and bed heights a series of one-way breakdown analyses of variance were done. Table 4 gives the results of the analyses testing differences in permeability between pairs of bed heights at each of the 3 core diameters (11, 18 and 29 mm) . The results show that 7 out of 18 comparisons were significant. However, none of the 18

Table 1. First series of experiments.
Original data of Time (sec) for water column to drop 25 mm for natural sediment using 11, 18 and 29 mm diameter cores.

Core diam. (mm)	Sed.bed ht.(mm)	Run 1	Run 2	Run 3
11	Core 1: 24	24	23	23
	no. 2: 25	22	23	20
	3: 25	20	15	15
18	Core 1: 24	34	39	44
	no. 2: 21	11	13	12
	3: 29	27	30	30
29	Core 1: 25	39	41	44
	no. 2: 25	25	26	29
	3: 28	38	42	48
11	Core 1: 53	38	44	46
	no. 2: 58	56	58	60
	3: 55	46	47	48
18	Core 1: 54	38	40	42
	no. 2: 43	57	61	58
	3: 49	57	53	57
29	Core 1: 50	57	64	69
	no. 2: 45	58	69	70
	3: 50	61	68	76
11	Core 1: 101	55	53	53
	no. 2: 95	59	66	77
	3: 108	64	71	74
18	Core 1: 95	73	85	103
	no. 2: 99	52	54	52
	3: 97	58	61	59
29	Core 1: 97	49	55	58
	no. 2: 120	92	103	112
	3: 99	50	54	58
11	Core 1: 220	120	125	130
	no. 2: 196	104	98	106
	3: 216	127	126	139
18	Core 1: 200	108	114	121
	no. 2: 202	117	117	124
	3: 192	115	102	111
29	Core 1: 215	133	149	145
	no. 2: 204	256	341	399
	3: 201	155	188	207

Table 2. First series of experiments.
Permeability coefficient k ($mm.sec^{-1}$) for natural sediment using 3 core diameters (11, 18, 29mm) and 4 sediment bed heights (25, 50, 100,200mm).

Theoritical bed ht. (mm)	Core diam. (mm)	Measured bed ht. (mm)	Run 1	Run 2	Run 3
	11	Core 1: 24	0.1183	0.1235	0.1235
		2: 25	0.1338	0.1280	0.1472
		3: 25	0.1472	0.1963	0.1963
25	18	Core 1: 24	0.08353	0.07283	0.06455
		2: 21	0.2292	0.1939	0.2101
		3: 29	0.1242	0.1117	0.1117
	29	Core 1: 25	0.07550	0.07182	0.06692
		2: 25	0.1178	0.1133	0.1015
		3: 28	0.08558	0.07743	0.06775
	11	Core 1: 53	0.1451	0.1253	0.1199
		2: 58	0.1056	0.1019	0.09852
		3: 55	0.1234	0.1207	0.1182
50	18	Core 1: 54	0.1472	0.1399	0.1332
		2: 43	0.08190	0.07653	0.08049
		3: 49	0.09096	0.09782	0.09096
	29	Core 1: 50	0.09242	0.08231	0.07635
		2: 45	0.08351	0.07019	0.06919
		3: 50	0.08636	0.07747	0.06932
	11	Core 1: 101	0.1592	0.1652	0.1652
		2: 95	0.1426	0.1275	0.1093
		3: 108	0.1429	0.1288	0.1235
100	18	Core 1: 95	0.1152	0.09897	0.08168
		2: 99	0.1662	0.1601	0.1662
		3: 97	0.1471	0.1398	0.1446
	29	Core 1: 97	0.1741	0.1551	0.1471
		2: 120	0.1061	0.09477	0.08716
		3: 99	0.1729	0.1601	0.1490
	11	Core 1: 220	0.1125	0.1080	0.1039
		2: 196	0.1229	0.1304	0.1206
		3: 216	0.1054	0.1062	0.09631
200	18	Core 1: 200	0.1195	0.1132	0.1067
		2: 202	0.1109	0.1109	0.1046
		3: 192	0.2132	0.2403	0.2208
	29	Core 1: 215	0.1004	0.08965	0.09213
		2: 204	0.05090	0.03822	0.03266
		3: 201	0.08348	0.06882	0.06251

Figure 2

First series of experiments

Mean permeability coefficients K(mm.sec^{-1}) at bed heights 25 mm, 50 mm, 100 mm, and 200 mm and core diameters 11 mm, 18 mm, and 29 mm. Vertical bars are standard deviations. n = 9 for each treatment.

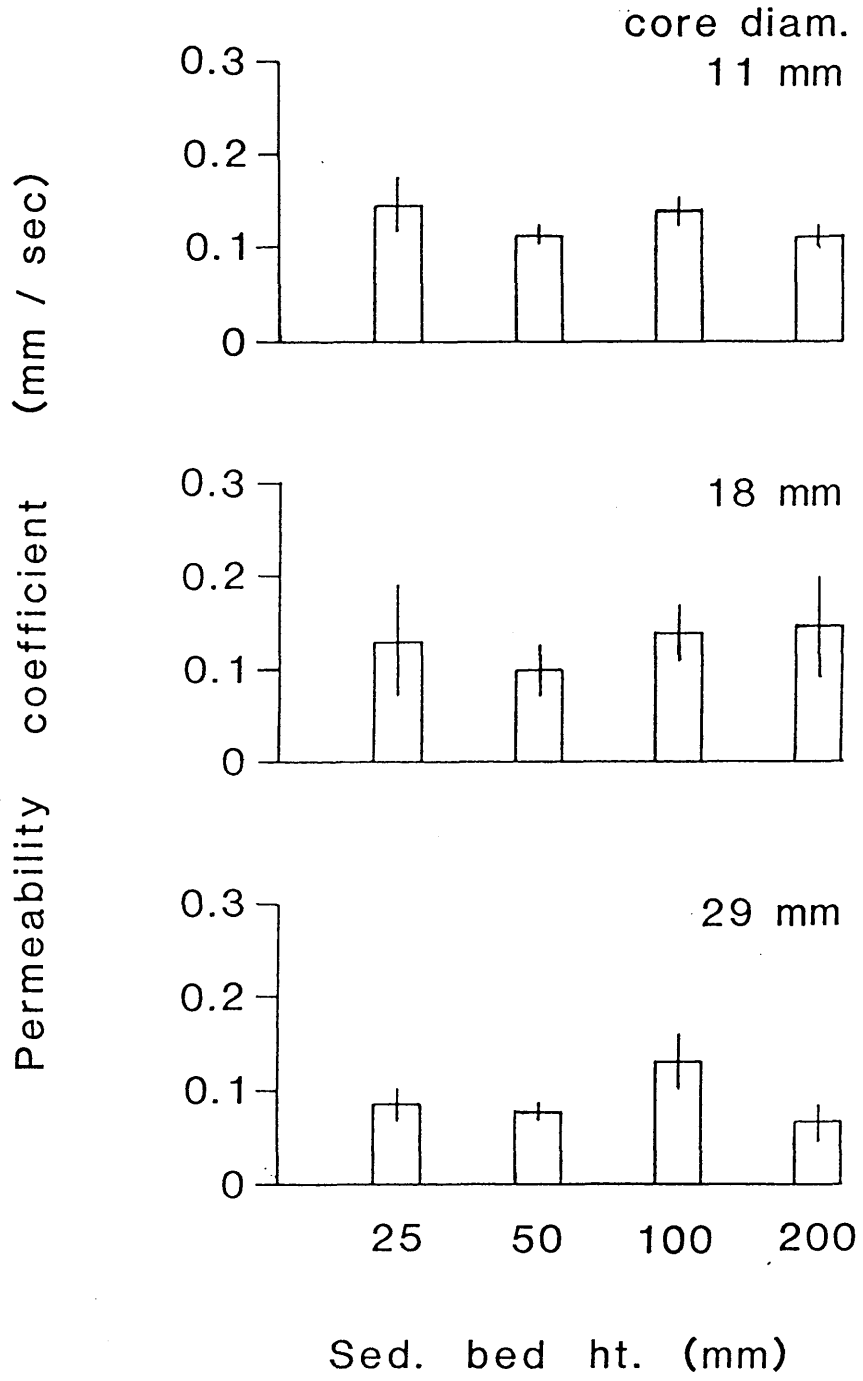

core diam.
11 mm
18 mm
29 mm
0.3
0.2
0.1
0
Permeability coefficient (mm / sec)
25
50
100
200
Sed. bed ht. (mm)

Table 3. First series of experiments.
Two-way analysis of variance of permeability ($mm.sec^{-1}$) testing differences between core diameter (Factor A; 11, 18, 29mm) and sediment bed height (Factor B; 25,50,100,200mm)

Source of variation	SS	MS=SS/df	d.f.	F-Ratio	P
Factor A Core diam.	0.03241	0.01621	2	15.41	
Factor B Bed height	0.02177	0.007257	3	6.899	
A x B interaction	0.02154	0.003590	6	3.413	$0.005 > P > 0.001$ **
Residual Error	0.1010	0.001052	96		
Total	0.1761		107		

Table 4. First series of experiments.
One-way analyses of variance testing differences in permeability ($mm.sec^{-1}$) between the 4 bed heights (25,50,100,200mm) at each of the 3 core diameters (11,18,29mm).

Core diam. (mm)	Bed height (mm)	F-Ratio	P
11	25 / 50	6.49	0.025>P>0.01 *
	25 / 100	0.210	0.75>P>0.50
	25 / 200	10.2	0.005>P>0.001 **
	50 / 100	7.88	0.025>P0.01 *
	50 / 200	0.96	0.50>P>0.25
	100 / 200	14.5	0.005>P>0.001 **
18	25 / 50	1.67	0.25>P>0.10
	25 / 100	0.01	P>0.75
	25 / 200	0.30	0.75>P>0.50
	50 / 100	5.18	0.05>P>0.025
	50 / 200	4.39	0.10>P>0.05
	100 / 200	0.38	0.75>P>0.50
29	25 / 50	1.22	0.50>P>0.25
	25 / 100	16.2	P<0.001 ***
	25 / 200	2.85	0.25>P>0.10
	50 / 100	27.2	P<0.001 ***
	50 / 200	1.30	0.50>P>0.25
	100 / 200	25.4	P<0.001 ***

Table 5. First series of experiments.
One-way analyses of variance testing differences in permeability ($mm.sec^{-1}$) between the 3 core diameters (11,18,29mm) at each of the 4 bed heights (25,50,100,200mm).

Bed height (mm)	Core diam. (mm)	F-Ratio	P
25	11 / 18	0.30	0.75>P>0.50
	11 / 29	24.6	P<0.001 ***
	18 / 29	4.74	0.05>P>0.025
50	11 / 18	1.63	0.25>P>0.10
	11 / 29	50.9	P<0.001 ***
	18 / 29	7.11	0.025>P>0.01 *
100	11 / 18	0.17	0.75>P>0.50
	11 / 29	0.02	P<0.75
	18 / 29	0.04	P<0.75
200	11 / 18	3.62	0.10>P>0.05
	11 / 29	23.3	P<0.001 ***
	18 / 29	14.8	0.005>P>0.001 **

mm core diameter comparisons were significant. In addition, the permeabilities of the 50 and 200 mm bed heights were never significantly different.

Table 5 gives the results of the one-way analyses of variance testing differences in permeability between pairs of core diameters at each of the 4 bed heights (25, 50, 100 & 200 mm). The results show that 5 out of the 12 comparisons were significant (table 5). However the results for the 100 mm bed height were never significant. In addition,the permeabilities of the 11 and 18 mm core diameters were never statistically different.

The overall results of this experiment did not give a clear cut indication as to which was the best core diameter and bed height to be used in the second permeability experiment. I therefore chose the 29 mm core diameter rather than 11 or 18 mm because there is likely to be less of a wall effect with this than with the smaller core diameters. I chose the 50 mm bed height for the following reasons. A bed height of 25 mm is rather small to set up, while a 200 mm bed height is too big. A 50 or a 100 mm bed height seemed more suitable. I eventually chose the 50 mm bed height because it had a lower standard deviation than the 100 mm bed height (figure 2) and therefore the results with it were likely to be less variable.

Second series of experiments

The results of the second series of experiments are shown in tables 6 and 7, and figures 3 and 4. The statistical analysis of this data is given in tables 8 and 9 and in appendix 2 table 1 (p 149).

Table 6 shows the time (sec) for the water column to drop 25 mm for the five different sediment treatments. Table 7 gives the permeability coefficients k ($mm.sec^{-1}$) calculated from the times in table 6. This data is summarised in figure 3. There were considerable differences between the permeability values for different sediment treatments. Natural sediment, natural sediment with fines removed and then added again, and Rockware sand with fines added, all had low permeabilities (treatments A, D & E). Natural sediment with fines removed and Rockware sand both had high permeabilities (treatments B & C). There were also differences in permeability between successive runs, the later runs having a lower permeability than the earlier runs.

The significance of these results were tested by a two-way analysis of variance followed by a series of one-way breakdown analyses of variance. The two-way analysis of variance tested differences between treatments (Factor A) and between runs 1 to 4 (Factor B) (table 8). The results of this analysis showed that both factors were highly significant. These main factor effects are meaningful because the interaction effect was not significant. The differences between the treatments was analysed in more detail by a series of one-way breakdown analyses of variance comparing differences in permeability between pairs of treatments (A, B, C, D, & E) for successive runs (1, 2, 3 & 4). The results of the 40 one-way analyses of variance are given in appendix 2 table 1 (p 149). A summary table of the F ratios from these one-way anovars is given in table 9. The F ratios in this table substantiate the general conclusions made above.

Table 6. Second series of experiments.
Original data of time (sec) for a drop of 25 mm in the water column level for the five sediment treatments using 29 mm diameter cores. The notations for sediment treatments are A = natural sediment, B = natural sediment - fines , C = Rockware sand, D = natural sediment - fines + fines, E = Rockware sand + fines. Four runs were taken on 3 replicate cores.

Run no.	A	B	C	D	E
I	38	18	15	60	71
II	53	26	19	75	97
III	78	27	20	79	112
IV	86	29	22	82	129
Sed.bed ht. (mm)	60	57	50	63	56
I	111	15	20	100	100
II	132	22	21	104	122
III	147	28	22	110	143
IV	139	31	24	119	163
Sed.bed ht. (mm)	58	50	50	60	60
I	70	28	16	94	44
II	102	39	16	109	62
III	127	41	16	109	70
IV	131	43	17	120	77
Sed.bed ht. (mm)	54	55	50	65	64

Table 7. Second series of experiments.
Permeability coefficient k ($mm.sec^{-1}$) of the five sediment treatments using 29 mm diameter cores. The notations for sediment treatments are A = natural sediment, B = natural sediment - fines, C = Rockware sand, D = natural sediment - fines + fines, E = Rockware sand + fines. Four runs (I,II,III,IV) were taken on 3 replicate cores (1,2,3).

Replicate cores	Run no.	A	B	C	D	E
1	I	0.1596	0.3241	0.3512	0.1049	0.08105
	II	0.1144	0.2244	0.2773	0.08390	0.05933
	III	0.07777	0.2160	0.2634	0.07966	0.05138
	IV	0.07053	0.2011	0.2395	0.07674	0.04461
	Sed.bed ht.(mm)	60	57	50	63	56
2	I	0.05326	0.3512	0.2634	0.06066	0.06066
	II	0.04478	0.2395	0.2509	0.05832	0.04972
	III	0.04021	0.1881	0.2395	0.05514	0.04242
	IV	0.04253	0.1699	0.2195	0.05097	0.03721
	Sed.bed ht.(mm)	58	50	50	60	60
3	I	0.07993	0.2027	0.3293	0.06852	0.1447
	II	0.05485	0.1455	0.3293	0.05909	0.1027
	III	0.04406	0.1384	0.3293	0.05909	0.09096
	IV	0.04271	0.1320	0.3099	0.05367	0.08269
	Sed.bed ht.(mm)	54	55	50	65	64

Figure 3

Second series of experiments.

Mean permeability coefficients (K $mm.sec^{-1}$) for the five treatments, A = natural sediment, B = natural sediment with fines removed, C = Rockware sediment, D = natural sediment with fines removed and then added, E = Rockware sand with fines added. Vertical bars are standard deviations. n = 12 for each treatment.

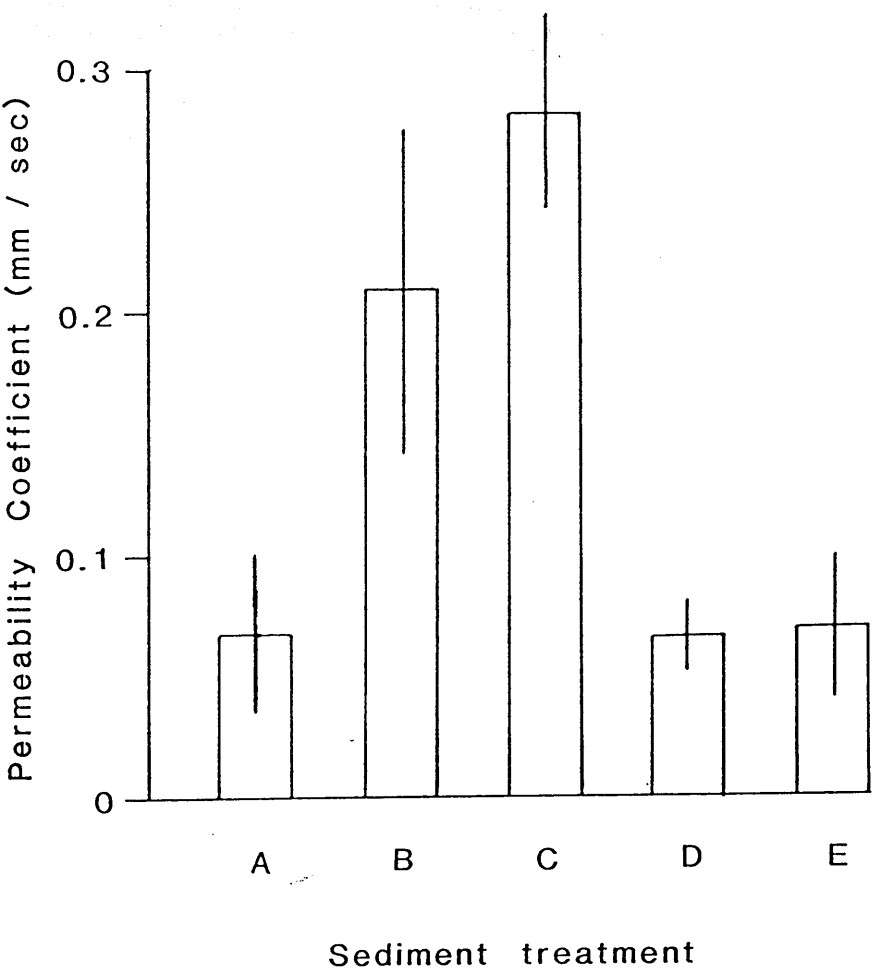

0.3
0.2
0.1
0
Permeability Coefficient (mm / sec)
A
B
C
D
E
Sediment treatment

Table 8. Second series of experiments.
Two-way analysis of variance of permeability ($mm.sec^{-1}$) testing differences between sediment treatments (Factor A; A,B,C,D,E see p 23) and runs 1 to 4 in the cores (Factor B).

Source of	SS	MS=SS/df	d.f.	F-Ratio	P
Factor A Treatment	0.4896	0.1224	4	81.59	P<0.001 ***
Factor B Runs	0.02847	0.00949	3	6.327	0.005>P>0.001 **
A x B interaction	0.01271	0.00106	12	0.7067	0.75>P>0.50
Residual Error	0.05986	0.00150	40		
Total	0.5906		59		

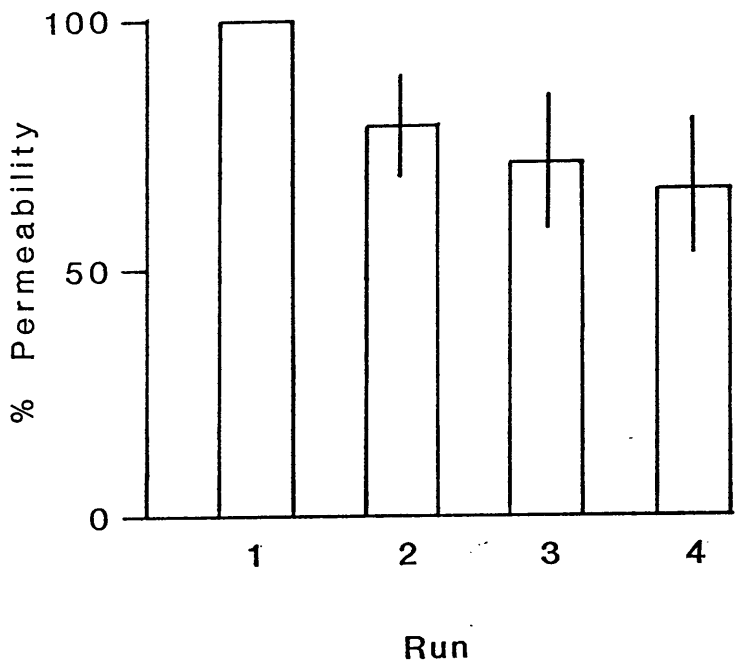
Table 9. Second series of experiments.
Comparisons of differences in permeability ($mm.sec^{-1}$) between pairs of treatments (A,B,C,D,E; see p 23) for each successive run (1,2,3,4). F-ratios from $1_{4} \times 2$ one-way anovars appendix 2 table 1 (p 149). Probalities are $0.05>P>0.01$*, $0.01>P>0.001$** , $P<0.001$***.

Treatment		B	C	D	E
	Run				
	1	12.25*	27.44**	0.32	0.000
A	2	13.14*	45.90**	0.03	0.000
	3	24.47**	57.80**	0.56	0.160
	4	27.58**	49.84**	0.47	0.030
	Run				
	1		0.17	20.29*	14.27*
B	2		4.96	20.11*	15.76*
	3		7.54	23.57**	19.28*
	4		6.83	24.66**	21.30**
	Run				
	1			63.48**	35.93**
C	2			79.61***	58.21**
	3			58.13**	49.37**
	4			48.86**	42.72**
	Run				
	1				0.37
D	2				0.04
	3				0.03
	4				0.12

Figure 4

Second series of experiments.

Percent permeability for runs 1 to 4. Permeability of run 1 for each replicate core was taken as 100 %. The permeabilities of runs 2, 3, and 4 were expressed as percentages of run 1. The means and standard deviations of these percentages are plotted. (n = 3 replicates x 5 treatments = 15 for runs 2, 3, and 4).


100
50
0
% Permeability
1
2
3
4
Run

There are no differences between the permeabilities of sediments containing fines (A, D & E) (F-ratios not significant) and no differences between the permeabilities of sediments not containing fines (B & C) (F-ratios not significant). However if the permeabilities of any of A, D and E are compared with the permeabilities of B and C, they are always significantly different (F-ratios significant).This means that once the fines have been removed from natural sediment (B) its permeability does not differ from that of the artificial Rockware sand (C); conversely if the fines are added to Rockware sand (E) then the permeability of the latter matches the permeability of the natural sediment (A) and the natural sediment from which fines have been removed and then returned (D). Overall therefore fines have a major effect in reducing permeability, presumably by progressively blocking the interstitial spaces of the sediment.

It is interesting to note that the permeability of successive runs decreased (see above). To illustrate this, the permeabilities of runs 2, 3 and 4 were expressed as percentages of run 1 for each core (figure 4). The data show clearly that permeability decreases with successive runs. This may be because successive runs cause a slight compaction of the sediment column.

MATERIALS AND METHODS - Third series of experiments (Enrichment experiment)

The principle behind the experiment was as follows. Sandy sediment was collected from Ardmore, and then put into ten glass columns in the laboratory. Four of these cores were filled with photosynthetic medium to stimulate photosynthetic growth, four with bacterial medium to stimulate bacterial growth and two with formalin to inhibit microbial growth. Two of the photosynthetic medium cores and two of the bacterial medium cores were kept in the light and the remaining two from each medium were kept in the dark. The control formalin cores were left in the light. The experiment was run for 25 days. Every two days the medium was changed and permeability measurements were taken.

The remainder of the materials and methods section contains a detailed description of the preparation and conduct of the experiment under the following headings:

1) Collection of sediment

2) Preparation of cores

3) Changing medium and taking permeability readings

4) Termination of experiment and parameters measured at that time.

Collection of sediment:

Sediment (0-2 cm) was collected from low tide at Ardmore, Clyde Estuary, Scotland (Nat. Grid. NS 320 792). It was sieved through a 500 µm sieve using 0.45 µm membrane filtered seawater and then gently mixed. Some of this sediment was kept aside at 10°C for certain measurements on the following day.

Preparation of sediment cores and permeability measurements at the beginning of the experiment

The experience obtained in preparing cores in the first two series of experiments led me to re-design the preparation procedure to obtain maximal packing uniformity between cores. This was necessary for detailed comparisons between treatments. I therefore describe my definitive method in detail. 10 cores were set up. Each core was prepared as follows.

(i) A 500 mm long glass column (I.D. 29 mm) was prepared by covering its lower end with nylon and stainless steel mesh and then sterilised. This column eventually contained sediment and formed the permeability core.

(ii) A wider glass column (I.D. 60 mm) was fitted with a no. 45 rubber bung at its lower end and clamped vertically in a plastic basin. The narrow glass column (I.D. 29 mm) was then lowered into the wider column and clamped.

(iii) The outer and the inner columns were filled to their tops with seawater that had been previously filtered through no. 1 Whatman paper.

(iv) Portions of the mixed sediment were then taken with a clean spatula. The spatula was held above the inner narrow glass column and gently tapped so that sediment progressively dropped into the inner column and settled through the water. This was repeated until the sediment core in the inner column had reached 50 mm height.

(v) The surface of the sediment was then levelled by gently moving a flat spatula backwards and forwards in the water just above the sediment surface.

(vi) The inner column, from here onwards called the core, was carefully removed from the outer column and clamped

vertically. The level of the seawater in the core was allowed to drop until it was 100 mm above the bottom of the core.

(vii) A 'U' tipped glass tube with a glass funnel attached at its upper end was then gently lowered into the core until the 'U' tip was immersed in the seawater. The appropriate medium was then poured slowly through the funnel. The level of the 'U' tube was gradually raised as the level of the liquid rose to the top of the core.

(viii) The time (sec) taken for the medium to fall successive vertical intervals of 25 mm from 500 to 100 mm above the bottom of the core was noted. This gave 17 times and hence 16 permeability readings using equation 8 (p 13). During this process the liquid in the interstitial spaces was replaced with fresh medium until the liquid level was 100 mm above the bottom of the core.

(ix) The core was then transferred gently to a 500 ml measuring cylinder containing 70 ml of medium. It was necessary to be extremely careful during the transfer into the measuring cylinder to avoid any disturbance of the sediment.

(x) The top of the core was then covered with a 5 cm sterile plastic petri dish and the annulus between the core and the measuring cylinder was covered with metal foil.

All the cores were set up in the same way and the appropriate medium was added to each.

Permeability measurements during the progress of the experiment

Every two days the media were changed and 16 permeability readings taken for each core as follows.

(i) The core was carefully removed from the measuring cylinder and clamped vertically (Plate 1).

Plate 1 : Enrichment experiment. Permeability readings being taken on a bacterial (BL) core on day 22 of the enrichment experiment. Vertical distance between marks on permeameter tube are 25 mm.

(ii) The appropriate media was added as per (vii) above.

(iii) Permeability readings were taken as per (viii) above.

(iv)The core was then transferred back to the measuring cylinder as per (ix) and (x) above.

During the latter part of the experiment the BL and BD cores developed a dense bacterial growth which slowed down the flow of the medium through the sediment. In these cases I was only able to take 3 to 7 times giving 2 to 6 permeabilities. (For example, a drop of 25 mm column height sometimes took over one hour).

The photosynthetic medium was a modification of the Medium M12 (Asher & Spalding, 1982) and contained 50 ml soil extract, 2 g $NaNo_3$ and 0.014 g of $Na_2HPO_4.2H_2O$ made up to 1 litre with artificial seawater. The bacterial medium contained 5 g bacteriological peptone (Oxoid L37) (Cruickshank et al., 1977) and 0.1 g $FePO_4$ (with water) made up to 1 litre with artificial seawater. The control medium contained 25 ml of 40 % formaldehyde completed to 70 ml with distilled water made up to 1 litre with 82 % ASW.

The final salinity of the seawater in all media was $26^{o}/_{oo}$. Media were autoclaved and filtered through sterile Whatman No. 1 filter paper before use.

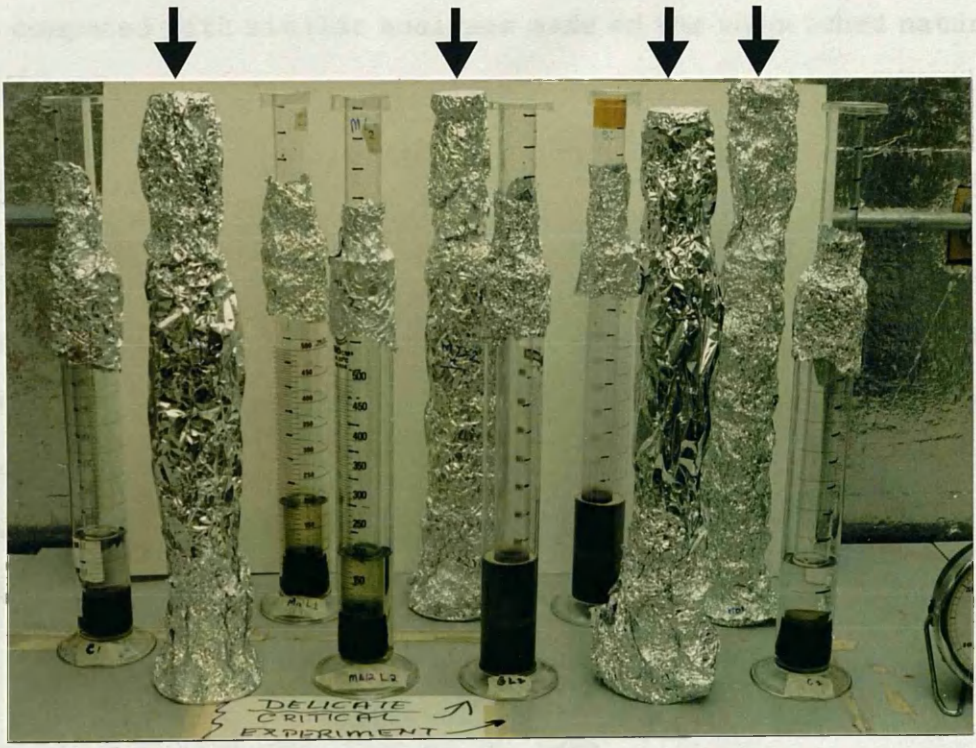
Two replicate cores of the photosynthetic and bacterial media were completely covered with metal foil to exclude light. These were called dark cores while the other two replicates were left uncovered and called light cores. The 2 light and 2 dark replicate cores of the photosynthetic (ML & MD) and bacterial (BL & BD) medium and 2 controls (C) were left at 18 ^{o}C under a 17h light/7h dark photoperiod (Plate 2).

Plate 2 : Enrichment experiment. Photograph of experiment in progress in the phytotron room (constant temperature (18°C), 17 hr natural light / 7 hr dark regime) showing the 10 cores.

Number of cores / treatment

Treatment	Code	Light	Dark
Photosynthetic medium	ML	2	0
	MD	0	2
Bacterial medium	BL	2	0
	BD	0	2
Control	C	2	0
Total number of cores		6	4 = 10

The 4 dark cores (2, MD; 2, BD) are arrowed.


DELICATE
CRITICAL
EXPERIMENT

Termination of the experiment and readings taken

At the end of the experiment the effects of the different media on the growth of photosynthetic and heterotrophic microorganisms were quantified by measuring chlorophyll and phaeopigment concentrations, heterotrophic bacterial counts and ^{14}C primary productivity rates. This was done by removing sediment from each replicate core into a sterile pot and mixing it with a sterile spatula. The mixed sediment was then divided into suitable portions for the analyses. The results were compared with similar analyses made on the unenriched natural sediment.

Measurement of algal chlorophylls a, b, & c, carotenes, phaeopigments and bacteriochlorophylls ab, c & d.

Chlorophylls a, b and c, carotenes, and phaeopigments were measured using the methods of Strickland & Parsons (1972) and bacteriochlorophylls ab, c & d using the methods of Takahashi & Ichimura (1968), with slight modifications for sediment. The details of the experimental procedure were as follows.

(i) 1 to 2 g of well-mixed sediment was weighed and transferred to a pre-weighed clean Mickle tube.

(ii) A pinch of magnesium carbonate was then added to the sediment. The magnesium carbonate should be light weight or "Levis" grade (Strickland & Parsons, 1972).

(iii) 10 ml of 90% acetone was added to the Mickle tube.

(iv) The Mickle tube was tightly sealed with a rubber bung and the sample was shaken in a Mickle disintegrator for 10 mins. at 4°C. The rubber bung was then removed, the top of

the Mickle tube was tightly sealed with parafilm, and the whole tube was covered with aluminium foil to keep the sample in total darkness.

(v) The tube was then left in the refrigerator at c. 1-2^{o}C for 20 hrs, to allow pigment extraction to take place.

(vi) The following day the Mickle tube was removed from the refrigerator, inverted x6 times, and left to stand for c. 2 min.

(vii) The supernatant was gently decanted into a pre-weighed graduated 15 ml centrifuge tube. If the volume of the extract was less than 10 ml it was made up to 10 ml with 90% acetone. A careful record of the volume of acetone was kept for the final calculations. The tube was sealed with parafilm to stop evaporation of the extract, and the entire tube covered with metal foil to prevent breakdown of chlorophylls.

(viii) The extract was centrifuged at 3000 rpm for 7 mins. at 4^{o}c.

(ix) The extract was then left at room temperature for c. 10 mins. before the absorbance readings were taken. This prevented any misting on the glass cuvette (Parsons et al. 1984). The extract was carefully poured into a 10 mm path-length glass cuvette and its absorbance measured against a reference cuvette containing 90% acetone. A SP6 550 spectrophotometer was used throughout. The absorbances were measured at the following wavelengths.

Pigment	Wavelength nm
Chlorophyll a	663 / 665
Chlorophyll b	645
Chlorophyll c	630
Carotenes	480
Carotenes	510
Phaeopigments	665 after acidification
Turbidity Blank	750 before & after acidification
Bacteriochlorophyll ab	772
Bacteriochlorophyll c	654
Bacteriochlorophyll d	662
Bacteriochlorophyll Blank	850

(x) Cell-to-cell blanks: cell-to-cell blanks were determined at all the above wavelengths as follows. The reference and sample cuvettes were filled with 90% acetone. The spectrophotometer was set at zero absorbance at a particular wavelength using the reference cuvette. The absorbance of the sample cuvette was then taken at the same wavelength. This absorbance gave the cell-to-cell blank for the wavelength. This procedure was repeated for all the wavelengths.

(xi) Turbidity blanks: a correction for turbidity is needed when colloidal material is likely to cause problems. For example, if glass fibre filters are used to filter phytoplankton from seawater, the filters disintegrate into pulp in acetone and

produce small amounts of colloidal material (Strickland & Parsons, 1972). Although I did not use filters, I thought it safe to take the turbidity blank measurements in case there was any colloidal material in the sediment sample. The turbidity absorbance was measured at 750 nm where there is no absorbance from chlorophylls, carotenes and phaeopigments. The turbidity absorbance was then corrected by subtracting the cell-to-cell blank at 750 nm to give the turbidity blank (Parsons et al., 1984).

(xii) Bacteriochlorophyll blank: the bacteriochlorophyll blank was taken as the absorbance at 850 nm minus the cell-to-cell blank at 850 nm (Jones, 1979).

(xiii) Calculation of corrected absorbances: the cell-to-cell blank at a given wavelength was subtracted from the absorbancy of the sample at that wavelength to give the corrected absorbance. The cell-to-cell blank was applied to all wavelengths. The turbidity blank was subtracted from the absorbances of chlorophyll a at 663 / 665 nm, chlorophyll b at 645 nm, chlorophyll c at 630 nm, carotenes at 480 nm and 510 nm and phaeopigments at 665 nm. The turbidity blank was the same for all wavelengths except at 510 nm (x2) and 480 nm (x3). The bacteriochlorophyll blank was subtracted from the absorbances of bacteriochlorophyll ab at 772 nm, bacteriochlorophyll c at 654 nm, and bacteriochloropyhll d at 662 nm.

(xiv) Phaeopigments: Two drops of 0.5N HCl were added to the reference and sample cuvettes. The solution was mixed by holding a piece of aluminium foil over the mouth of the

cuvette and inverting 3-4 times. The solution was left to stand for 6 mins. The absorbance was taken at 665 and 750 nm against the acidified blank. The cuvettes were thoroughly rinsed with 90% acetone before each sample was taken.

(xv) The weight of sediment used in the extraction was obtained by decanting extract from the Mickle and centrifuge tubes and letting the tubes - which contained the sediment - dry at 60°C for 24 hrs. The dry weight of sediment was obtained by subtraction, since the tubes had been preweighed.

(xvi) The concentrations of the different pigments were calculated as µg pigment / g dry weight of sediment by using equations 1 to 17. I wrote a computer program to conduct these calculations. The computer program is given in appendix 3 (pp 160-170) (Flow chart, listing, run).

Chlorophyll a:

1. $\mu g.g^{-1} = (15.6E_{665} - 2.0E_{645} - 0.8E_{630}) \times V / (LxS)$
2. $\mu g.g^{-1} = (11.6E_{665} - 1.31E_{645} - 0.14E_{630}) \times V / (LxS)$
3. $\mu g.g^{-1} = (11.64E_{663} - 2.16E_{645} + 0.10E_{630}) \times V / (LxS)$
4. $\mu g.g^{-1} = 26.7 \times (665_o - 665_a) \times V / (LxS)$

Chlorophyll b:

5. $\mu g.g^{-1} = (25.4E_{645} - 4.4E_{665} - 10.3E_{630}) \times V / (LxS)$
6. $\mu g.g^{-1} = (20.7E_{645} - 4.34E_{665} - 4.42E_{630}) \times V / (LxS)$
7. $\mu g.g^{-1} = (20.97E_{645} - 3.94E_{663} - 3.66E_{630}) \times V / (LxS)$

Chlorophyll c:

8. $\mu g.g^{-1} = (109E_{630} - 12.5E_{665} - 28.7E_{645}) \times V / (LxS)$
9. $\mu g.g^{-1} = (55E_{630} - 4.64E_{665} - 28.7E_{645}) \times V / (LxS)$
10. $\mu g.g^{-1} = (54.22E_{630} - 14.81E_{645} - 5.53E_{663}) \times V / (LxS)$

Carotenes:

11. $\mu g.g^{-1} = 7.6 \times (E_{480} - 1.49E_{510}) \times V / (LxS)$
12. $\mu g.g^{-1} = (4.0E_{480}) \times V / (LxS)$
    if predominantly Chlorophyta or Cyanophyta
13. $\mu g.g^{-1} = (10.0E_{480}) \times V / (LxS)$
    if predominantly Chrysophyta or Pyrrophyta

Phaeopigment:

14. $\mu g.g^{-1} = 26.7 \times (1.7x(665_a) - 665_o) \times V / (LxS)$

where 665_o is the absorbance at 665 nm before acidification and 665_a is the absorbance at 665 nm after acidification

Bacteriochlorophylls:

15. μg BChl.ab.$g^{-1} = 25.2 \times (E_{772}) \times V / (LxS)$
16. μg BChl.c.$g^{-1} = 10.2 \times (E_{654}) \times V / (LxS)$
17. μg BChl.d.$g^{-1} = 10.8 \times (E_{662}) \times V / (LxS)$

In these equations, E is the corrected absorbance and the subscripts indicate the wavelength at which the absorbance was measured. For example E_{662} = the corrected absorbance at 662 nm, and $15.6E_{665}$ = 15.6 x the corrected absorbance at 665 nm. In addition, V = the total volume (ml) of 90% acetone used for extraction, L = the cell path-length (cm), and S = the total dry weight (g) of sediment. Equations 1, 5, 8 and 11 are from Richards and Thompson (1952), 2, 6, 9, 12, and 13 are from Parsons & Strickland (1963), 3, 7, and 10 are from SCOR/UNESCO (UNESCO,1966), 4 and 14 are from Lorenzen (1967), and 15, 16 and 17 are from Takahashi & Ichimura (1968) with slight modifications for sediment.

Heterotrophic viable counts (Colony-forming units per gram dry

Heterotrophic viable counts (Colony-forming units per gram dry

weight sediment)

Heterotrophic viable counts were taken on the untreated natural sediment at the beginning of the experiment and on the enriched sediment samples at the end of the experiment. Standard plating techniques were used (Cruickshank et al. 1975) using Marine agar 2216 ("DIFCO" certified).

Before starting the serial dilution and plating, the bench was scrupulously cleaned with 95% alcohol and a bunsen flame was lit to keep conditions as sterile as possible. All the serial dilution and plating work was carried out near the flame. The detailed procedure is given below.

(i) 2 g wet sediment was weighed on a sterile piece of foil.

(ii) The sediment was transferred to a sterile 50 ml conical flask and 18 ml of sterile artificial seawater (ASW) was added. The seawater had been previously sterilised by membrane filtration.

(iii) The mouth of the conical flask was then covered with a double layer of aluminium foil and the contents thoroughly mixed 3-4 mins. by swirling.

(iv) 6 sterile universal bottles and a 10 ml sterile syringe were used for each sediment sample. 9 ml of sterile ASW was injected into each of the 5 universal bottles. The bottles were capped immediately after injection.

(v) 1 ml of sediment and seawater mixture from the conical flask was injected into one of the above bottles. The bottle was

immediately capped and the contents mixed. This gave the 10^{-1} dilution. The procedure was repeated with the 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} bottles to give the 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} dilutions.

(vii) A 0.1 ml aliquot was taken from the 10^{-6} dilution bottle and injected onto an agar plate.

(viii) A glass spreader was dipped in 95% alcohol and then sterilized in a flame and held in the air for a few seconds. This was then used to spread the 0.1 ml aliquot evenly over the agar surface.

(ix) Steps (vii) and (viii) were repeated for the 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2} and 10^{-1} dilutions. Two replicate spread plates were prepared for each of these dilutions.

(x) The spread plates from the light incubated samples were put in clear polyethylene bags and the plates from the dark samples were put in dark polyethylene bags. All the plates were incubated at 18°C for 6 days.

(xi) At the end of the incubation period the number of bacterial colonies on each plate were counted. The number of colonies (=colony forming units) were converted to c.f.u. g^{-1} dry weight of sediment. The following equation was used for this conversion:

$$\text{c.f.u. } g^{-1} \text{ dry wt.sed.} = (\text{No. of colonies per plate} \times \text{dil.factor}) / \text{dry wt.sed.}$$

Primary Production measurements (^{14}C uptake method)

Primary production was measured by the ^{14}C technique (Steeman Nielsen, 1952a; Unesco, 1966b; Strickland & Parsons, 1972; Unesco, 1973; Parsons et al., 1984). Before beginning the experiments the 0.5 ml of 1 mCi sodium [^{14}C] carbonate supplied from Amersham was diluted to 2.5 $\mu Ci.ml^{-1}$ ^{14}C. The details of this procedure are given in appendix 4.1 (p 171). The subsequent experimental procedure is described below.

(i) 15 g portions of wet sediment (Unesco, 1973, p. 34) were placed in dark and light bottles. This weight was obtained by an indirect volume method which was found to be more convenient. The method was tested before the experiment and was accurate.

The sediment was removed from the core and mixed gently with a spatula. The base of a 5 cm plastic petri dish was marked on the outside at 0.3 cm from the bottom. The base was then evenly filled with sediment to the mark. The wet weight of this sediment was 15 g. Two 15 g portions of sediment were taken from each core. One was transferred to a 150 ml clear glass bottle (the light bottle) and the other to a 150 ml dark glass bottle (the dark bottle). The dark bottle acts as a blank which determines any dark fixation of carbon due to heterotrophic activity.

(ii) 140 ml of sterile ASW was added to each bottle.

(iii) 2 ml of 5 μCi $Na_2{}^{14}CO_3$ was then injected into each bottle. The bottle was tightly screwed and then shaken.

(iv) 20 bottles were set up. There were 5 treatments each with 2 replicate cores, and 1 light and 1 dark bottle were set up

for each of the 2 cores in the 5 treatments (5 x 2 x 2 =20).

(v) The 20 bottles were incubated under a fluorescent light source at 18°C for 3 hours. These bottles were then put in a dark box. The flourescent light had an intensity and spectral composition equivalent to that of natural sunlight.

(vi) Three sediment samples were taken from each bottle for counting as follows. Each sediment sample was obtained by pipetting 3 aliquots of sediment onto a 25 mm GF/F glass fibre filter in a Millipore filtering apparatus. This was done using a Pasteur pipette. The sample was then vacuum filtered. The vacuum filtration removed the interstitial water. The sediment was then transferred to a plastic scintillation vial.

(vii) 20 ml of Unisolve I scintillation liquid (Kalbhen, 1980) was added to each vial. Unisolve I contains meta xylene, PPO and triton X-100 and was supplied by Koch-Light Ltd. 2 vials were also prepared for the background counts and 2 for original activity. All 4 contained 20 ml of scintillation liquid. In addition the 2 original activity vials contained 2 ml of 5 μCi $Na_2{}^{14}CO_3$.

All scintillation vials were left in the dark for 48 hours for any chemiluminescence before being counted (Grower & Bransome, 1970).

(viii) The samples were counted on a PW 4700 Philips liquid scintillation counter. Each sample was counted twice for 10 min. The printout from the liquid scintillation counter gave NDPM (net disintegrations per minute) for each sample. The NDPM value of the background was subtracted from the NDPM of the light bottle, the dark bottle and the original activity

to give the corrected NDPM of each sample. A computer program was written which calculated the mean NDPM and finally NDPM per gram dry weight of sediment. The flow chart, listing, and run of the computer program is given in appendix 4.2 (pp 174-178). These NDPM values were used with other variables to calculate mg of Carbon fixed per dry weight of sediment per hour. A flow chart showing this general procedure is given in appendix 4.3 (p 179).

(ix)After counting, the dry weight of sediment in the vials was obtained as follows.

1. The scintillation liquid was decanted leaving the sediment behind.
2. 4 ml of 95% alcohol was added to the vial. The contents of the vial were shaken and left to stand for 1 min.
3. After the sediment had settled the alcohol rinse was decanted. The sediment was rinsed 4 times in this way.
4. The sediment was then left to dry in the vials at $60^{o}C$ for 24 hrs. The dry weight of sediment was obtained by weighing the vials first with the sediment and then without the sediment.
5. The dry weight of the sediment in the bottle was obtained in a similar manner. The seawater and radio-isotope solution was decanted. The sediment was dried at $60^{o}C$ for 24 hrs. The bottle was first weighed with sediment and then after removing the sediment.
6. The dry weight of the sediment in the vials and the bottle was used in the computer program to calculate the DPM per dry weight (g) of sediment (appendix 4.2 p 176).

The Total Carbon Dioxide content of sea water

In order to calculate primary productivity using the ^{14}C technique one has to first determine the total carbonate content of the sea water in which the primary productivity is taking place. This requires a detailed understanding of (i) the carbonate system in and the total carbonate content of sea water, (ii) the concepts of total alkalinity and carbonate alkalinity and (iii) the experimental determination of total alkalinity and carbonate alkalinity. These parameters are all affected by temperature, salinity and pH.

(i) The carbonate system; the total carbonate content of sea water

The carbonate system in sea water consists of undissociated molecules of CO_2 (carbon dioxide in sol.), H_2CO_3 (carbonic acid), HCO_3^- (bicarbonate) and CO_3^{2-} (carbonate) ions (Harvey, 1957; Skirrow, 1965; Vollenweider, 1969; Gieskes, 1974). Dissolved carbon dioxide in sea water reacts with water to form the weak acid carbonic acid. The carbonic acid then dissociates into bicarbonate and carbonate ions. The carbonate ions react with alkaline earth ions, in particular calcium, forming solid carbonates (Gieskes, 1974). All of these are in equilibrium with each other and with hydrogen ions as follows.

$$CO_2\ (aq.) \longleftrightarrow H_2CO_3 \overset{K_1'}{\longleftrightarrow} H^+ + HCO_3^- \overset{K_2'}{\longleftrightarrow} CO_3^{2-} + H^+$$

(Edmond & Gieskes, 1970)

The summed concentration of these ions (except the H^+) is

called the total carbonate content or the total carbon dioxide content of sea water. K_1' and K_2' are the apparent dissociation constants (Mehrbach et al., 1973).

(ii) Concepts of Total alkalinity (TA) and Carbonate alkalinity (CA)

In sea waters with salinity greater than $10^o/_{oo}$, the total alkalinity (TA) is made up of the summed concentrations in milliequivalents of CO_3^{2-}, HCO_3^-, $H_2BO_3^-$, OH^- and, in a negative sense, H^+. This can be written as

$$TA = [HCO_3^-] + 2[CO_3^{2-}] + [H_2BO_3^-] + [OH^-] - [H^+]$$

in this equation $[HCO_3^-] + 2[CO_3^{2-}] = CA$

This can be written as $TA = CA + A$

where $A = [H_2BO_3^-] + [OH^-] - [H^+]$ and the units are milliequivalents.l^{-1}. Hence to find CA you have to calculate TA and A.

Technically, Total Alkalinity is defined as the number of milliequivalents of hydrogen ion present in an acid that are neutralized by 1 kg of seawater, when a large excess of the acid is added. Similarly, Carbonate Alkalinity is the number of milliequivalents of hydrogen ion that are neutralized in converting the carbonate and bicarbonate ions to carbonic acid and carbon dioxide in 1 kg of sea water, when a large excess of acid is added (Strickland & Parsons, 1972).

(iii) (a) Experimental determination of Total alkalinity

The principle behind this determination is the addition of more than enough acid to convert (i) all the CO_3^{2-} and HCO_3^- ions to H_2CO3 and CO_2 and (ii) all the borate ions to boric acid thus

(i) $HCO_3^- + CO_3^{2-} = H_2CO_3 + CO_2$

(ii) $H_2BO_3^- + H^+ = H_3BO_3$

The excess acid leaves free H^+ ions in solution which produce the lower pH. The practical determination is as follows:

1. 100 ml of the seawater sample is pipetted into a 200 ml polyethylene bottle. 25 ml of 0.01N HCl is added and the contents are thoroughly mixed.
2. The pH meter is standardized using a phthalate buffer (pH = 4.00 at 20-25°C). The preparation of the buffer used for the standardization is given in appendix 4.4 (p 181). The pH of the sea water sample is then measured. If the pH is equal to or less than 4.0 then the total alkalinity is calculated as,

$$\text{Total alkalinity} = 2.500 - (1250a_H / f)$$

In this equation a_H is the hydrogen ion activity of the pH that has just been measured and is obtained from Strickland & Parsons (1972, p. 296, table V). f is an empirical factor that ranges from 0.75 to 0.89 depending on temperature and salinity. It is obtained from Strickland & Parsons (1972, p. 297, table VI).

If the pH is greater than 4.0, an extra 5 ml of 0.01N HCl is added, the contents of bottle mixed and the pH of the solution taken again. The total alkalinity in this case is calculated as;

$$\text{Total alkalinity} = 3.000 - (1300a_H / f)$$

(b) Experimental determination of Carbonate alkalinity

The carbonate alkalinity is now determined from the relationship $CA = TA - A$

TA has already been calculated. A, the borate alkalinity and the hydroxyl and hydrogen part of the equation in milliequivalents.l^{-1}, is determined from the in situ pH which in turn is determined from the laboratory pH and the field and laboratory temperature. The procedure is to measure the laboratory temperature and pH (using standard phosphate buffer pH = 6.87 at 20-25^{0}C , see appendix 4.4 p 181) and to find the field pH from the following equation:

$$pH_S = pH_m - \alpha (t - t_m)$$

The value of α is found from Strickland & Parsons (1972, p. 294, table III). The value of A is then found from Strickland & Parsons (1972, p 298, table VIII) using the value of the in situ temperature, and salinity.

(c) Determination of total carbon dioxide (TC) in sea water

The total carbon dioxide content of the sea water is now calculated as $TC = 12000 \times CA \times F_T$ mgC.m^{-3}

The factor F_T converts CA to TC by the relationship

$TC = CA \times F_T$

(Mehrbach et al., 1973, p.898, equation 3). It is obtained from Strickland & Parsons (1972, p 299, table IX) knowing the in situ pH, temperature and salinity. The factor 12000 changes the units from milliequivalents.l^{-1} to mgC.m^{-3}.

F_T is calculated from the apparent dissociation constants of carbonic acid K_1' and bicarbonate K_2', (Mehrbach et al., 1973, p. 898) but the mathematics are complicated and not

pertinent to the present context.

(d) Determination of total carbon fixed g^{-1} sed. h^{-1}

Finally the amount of carbon (mg) fixed per gram dry weight of sediment per hour is calculated as:

mg C fixed g^{-1} dry wt. h^{-1} = $((DPM_L - DPM_D) \times W \times 1.05) / (DPM_O \times N)$

where DPM_L = (DPM of light bottle - DPM of background), DPM_D = (DPM of dark bottle - DPM of background), W = weight of total carbon dioxide mg C. m^{-3} in seawater as calculated above and 1.05 is the isotopic correction factor (since ^{14}C behaves differently from ^{12}C isotope it is thought that ^{14}C may be taken up more slowly than ^{12}C). DPM_O = DPM of entire activity added to the bottle and N = number of hours the sample has been incubated in the light.

Note: The methodology of calculating the factor A (meq/litre), the total alkalinity, and W - the weight of carbonate carbon present in seawater is given in three flow charts in appendices 4.5, 4.6, 4.7 (pp 183-188). W is used in the calculations of mg C fixed g^{-1} sed. h^{-1} as shown in the flow chart in appendix 4.3 (p 179) (see above).

RESULTS - Third series of experiments (Enrichment experiment)

The permeability experiment data falls into two parts;
(i) the original time data, that is the time taken for the water column to fall 25 mm in the cores; and (ii) the permeability coefficient data (K in mm sec^{-1}) which has been calculated from the time data. I shall deal with these in turn.

(i) The original time data is shown in appendix 5.1 (pp 189-194). The numeral 1 was added to all the data, because the first value of time was zero, and some transformations of zero have no meaning (e.g. log_{10}, log_e, and square root). The notations used in the original data are as follows;

column C1 serial number
column C2 water column height (mm)
columns C3-C4 time (sec) for replicates 1 and 2 of photosynthetic light medium ML1 and ML2
columns C5-C6 " " " " " " " " photosynthetic dark medium MD1 and MD2
columns C7-C8 " " " " " " " bacterial light medium BL1 and BL2
columns C9-C10 " " " " " " " bacterial dark medium BD1 and BD2
columns C11-C12 " " " " " " " control C1 and C2

This data was stored, transformed and statistically analysed by regression analysis on the University ICL Mainframe computer using the statistical package Minitab. C1 to C12 is the Minitab notation for column 1 to column 12. Details of the storage and treatment of the data on the Mainframe Minitab program are given in appendix 5.2 (pp 195-203).

The results of the time data are plotted in figure 5 as square root time (sec) (y-axis) against water column height (mm) (x-axis). The reason for the square root transformation is given in appendix 5.3 (p 204).

The graphs show 2 important points. Firstly, the time taken for the water column to drop 25 mm increases as the overall water column height decreases (x axis: read from right to left). In other words there is an inverse relationship between time and water column height. Regression analyses of the square root transformed data showed that all of these relationships were highly significant (appendices 5.4 and 5.5 tables 2 and 3 (pp 206-252).

Secondly, as the experiment progressed, the slopes of the experimental cores became steeper (figure 5). This effect is clearly seen from day 16 onwards in the BL and BD cores. The ML and MD cores were less affected, and the control cores showed no increase in slopes The increases in the slopes reflect the decreasing permeability of the experimental cores. The steeper the slope the more slowly the water runs through the sediment column.

(ii) Time (sec) and successive water column heights (mm) (appendix 5.1 pp 189-194) were used in equation 8 (p 13) to calculate 16 permeability coefficients (K in $mm.sec^{-1}$) for each replicate core. The means and standard deviations of these coefficients for each core, at each of the 9 days on which readings were taken, are given in table 10 and figure 6. The means of the two replicate core means for each medium are also plotted as a graph in figure 7. The table and figure show that the permeability coefficients fell in all the cores during the experiment. This effect was most marked in the BL cores, followed by the BD, MD, and ML cores, with the control cores showing the smallest fall. Differences between the treatments were established

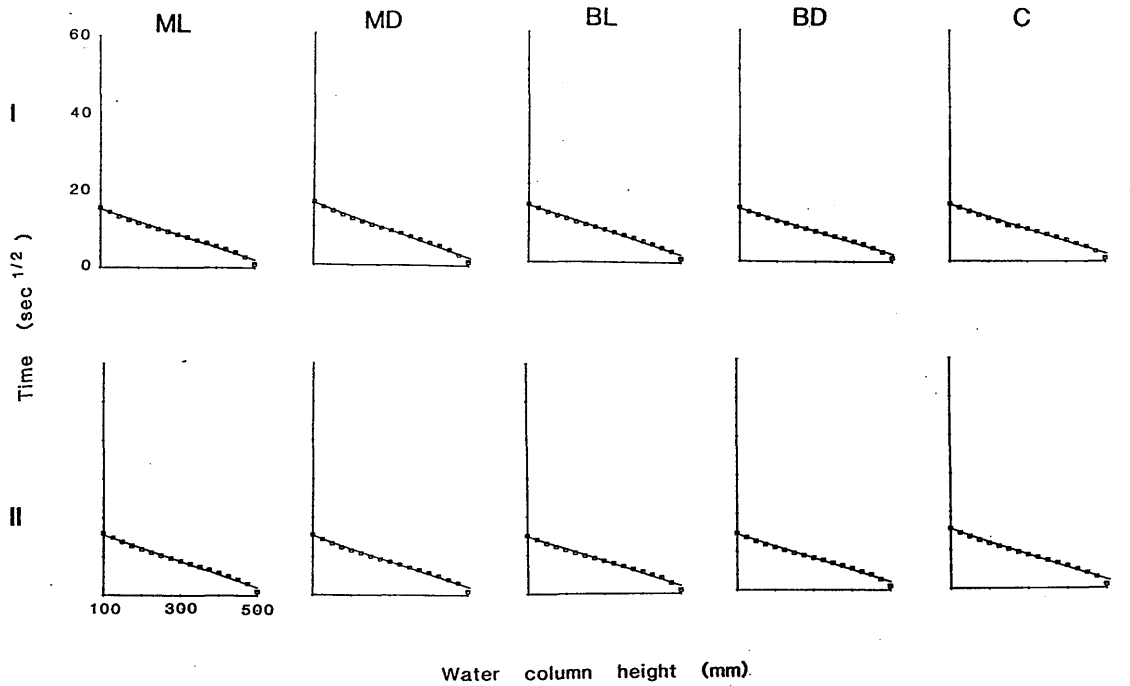
Figure 5

Third series of experiments (Enrichment experiment).

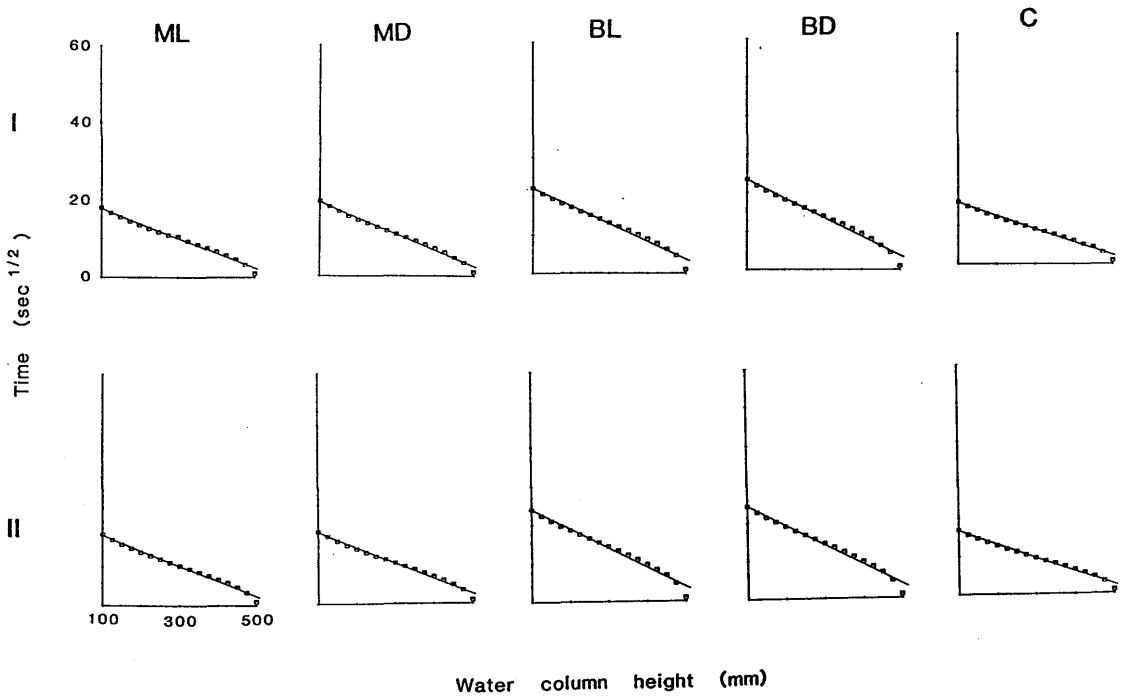
Time (sec) as square root (y-axis) against water column height (mm) (x-axis) for ML, MD, BL, BD, and C cores on days 1 to 25.

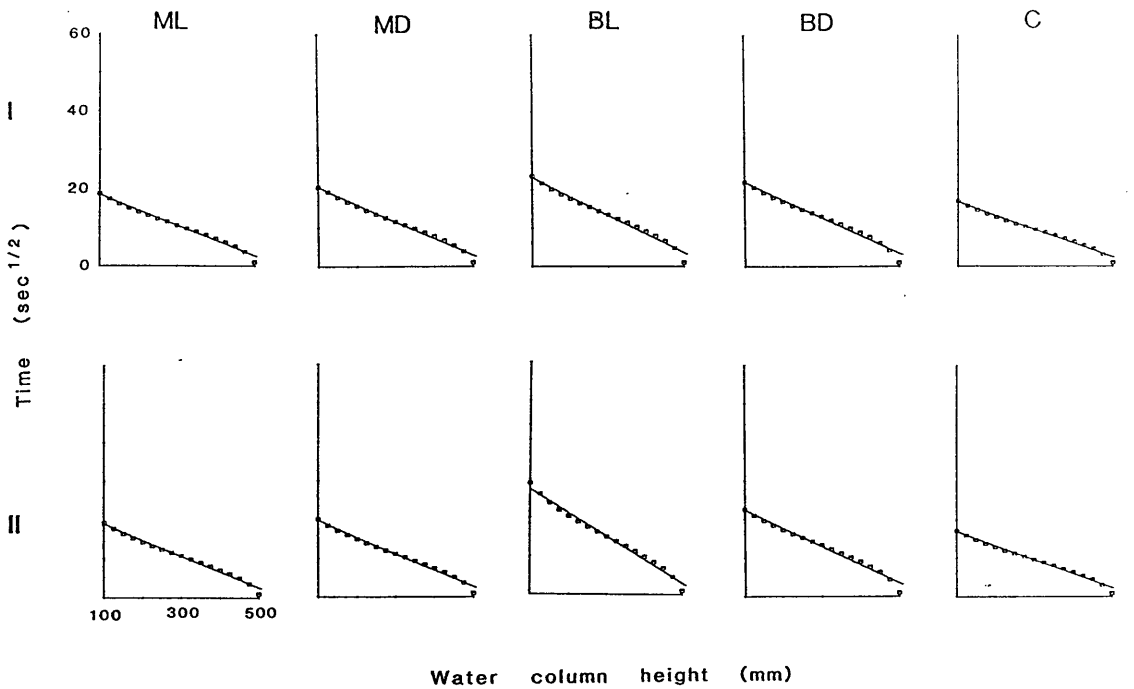
Note : x-axis read from right to left.

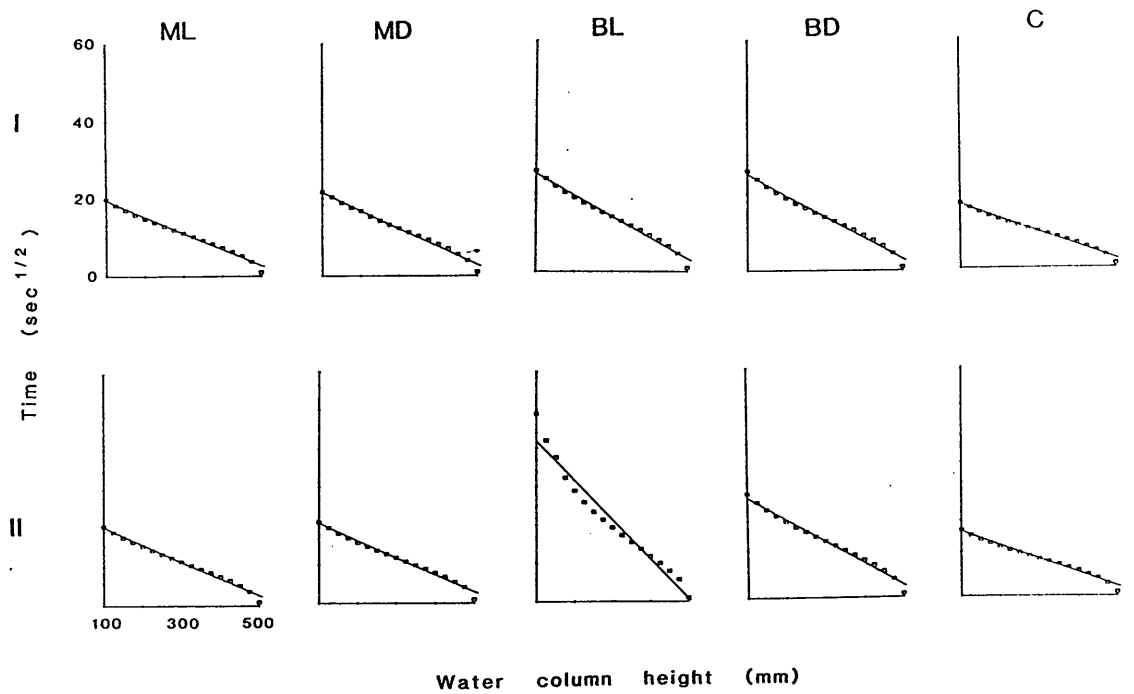
DAY 1

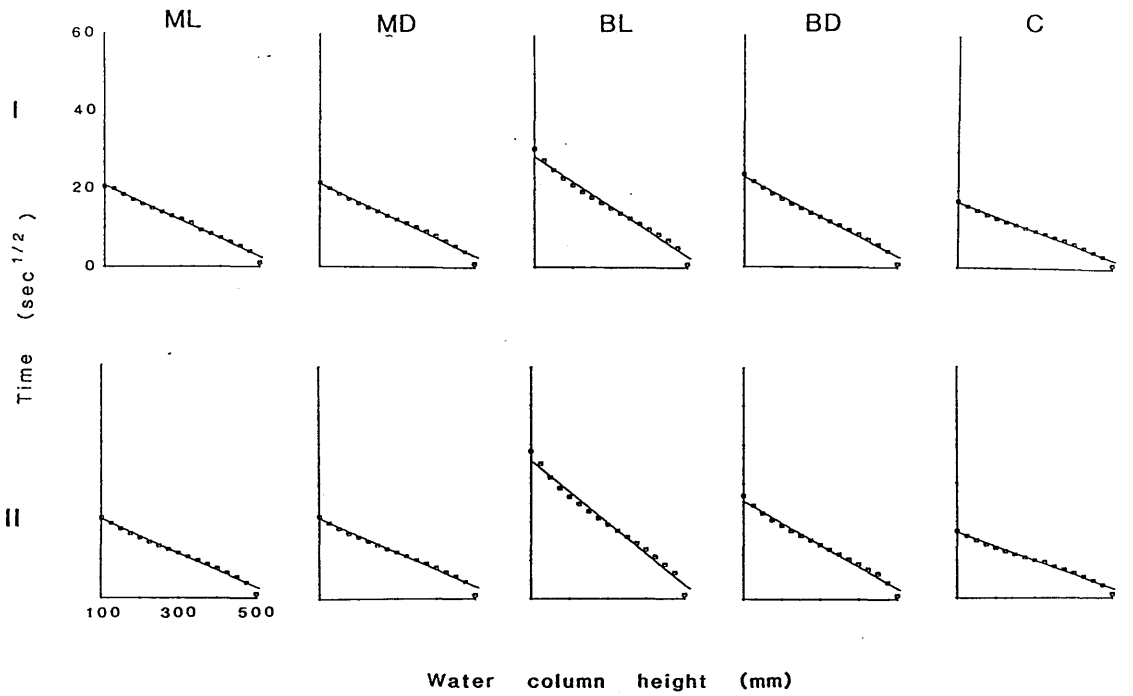

ML
MD
BL
BD
C
I
II
60
40
20
0
Time (sec 1/2)
100
300
500
Water column height (mm)

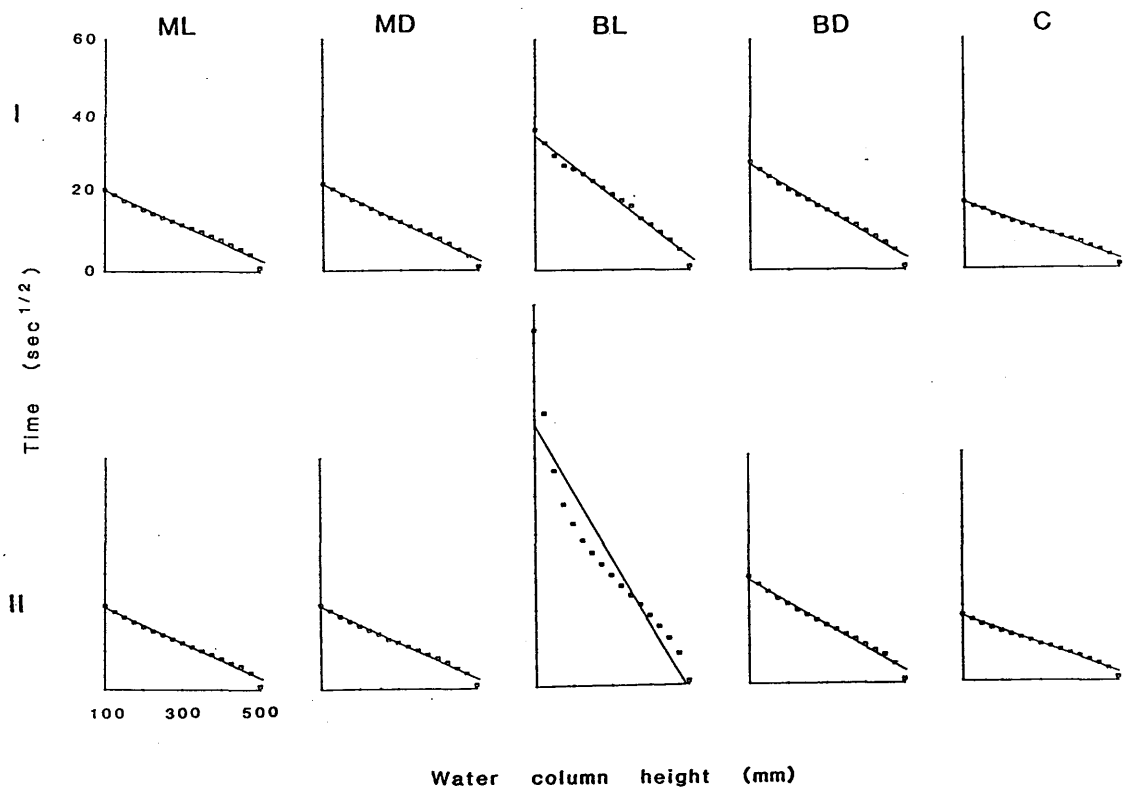
DAY 4

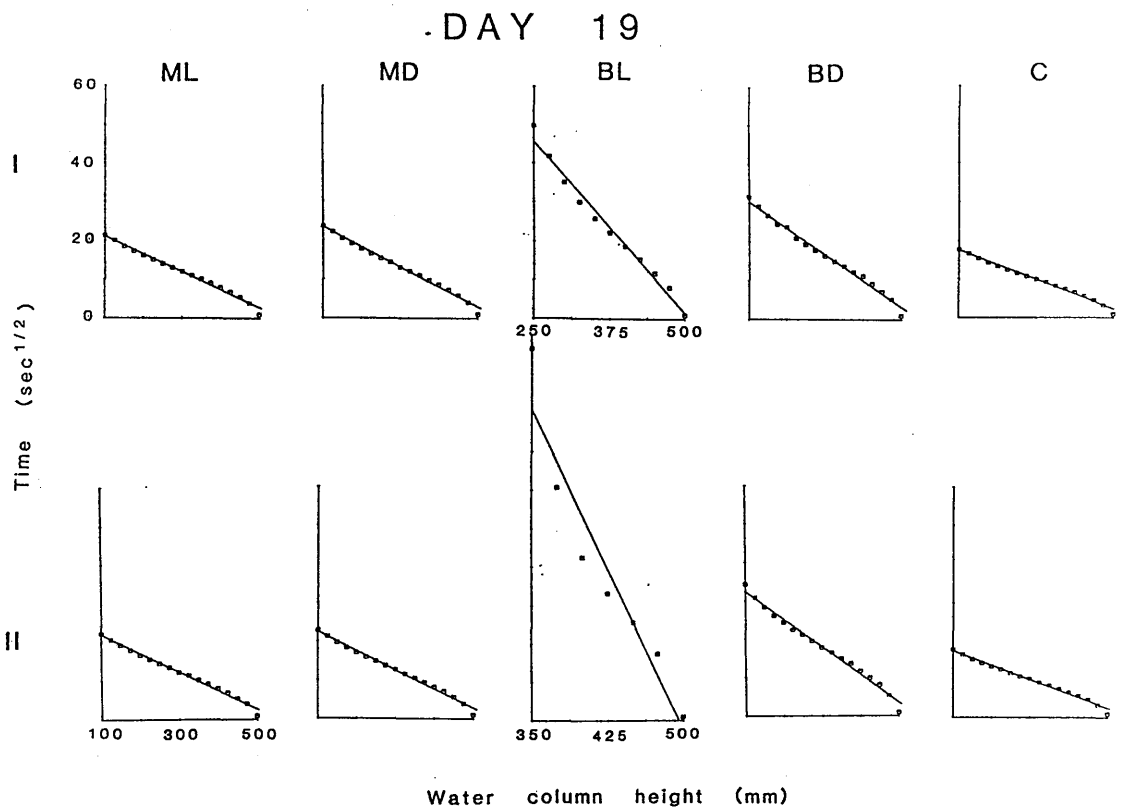

ML
MD
BL
BD
C
I
II
60
40
20
0
Time (sec 1/2)
100
300
500
Water column height (mm)

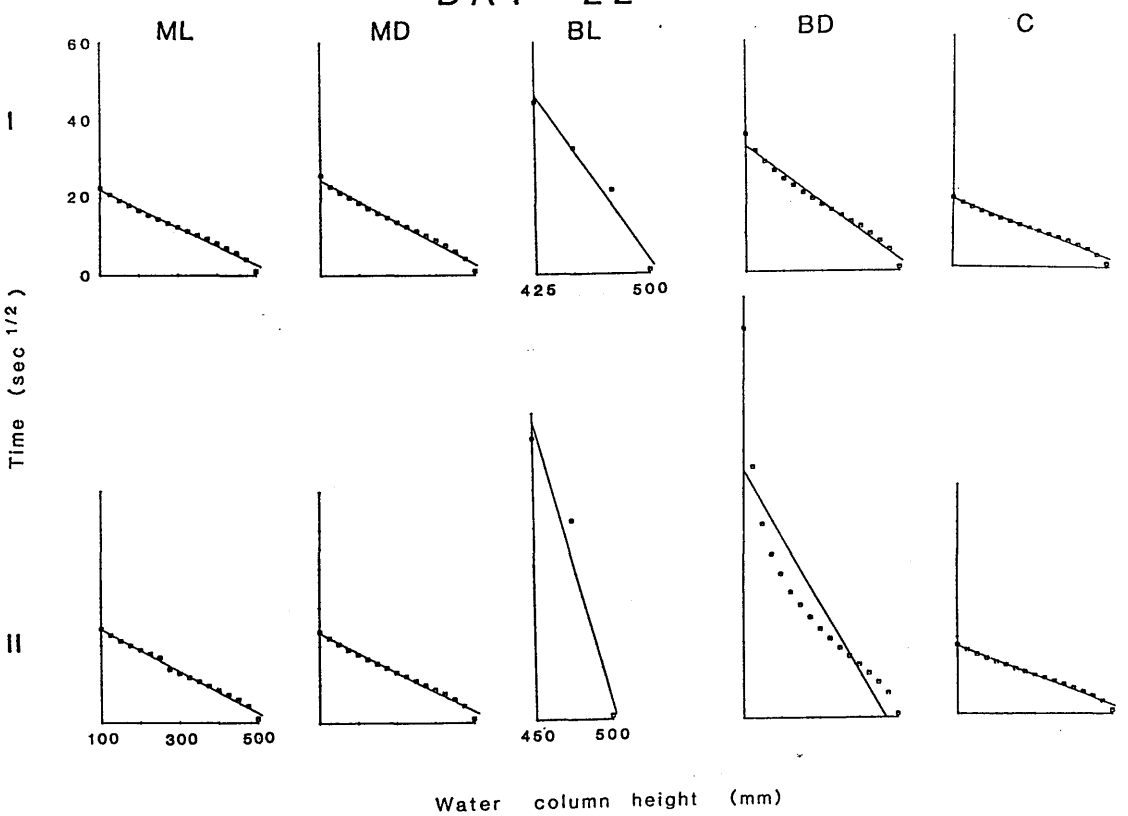

DAY 7
ML
MD
BL
BD
C
I
II
60
40
20
0
Time (sec 1/2)
100
300
500
Water column height (mm)


DAY 10
ML
MD
BL
BD
C
I
II
60
40
20
0
Time (sec 1/2)
100
300
500
Water column height (mm)


DAY 13
ML
MD
BL
BD
C
I
II
60
40
20
0
Time (sec 1/2)
100
300
500
Water column height (mm)


DAY 16
ML
MD
BL
BD
C
I
II
60
40
20
0
Time (sec 1/2)
100
300
500
Water column height (mm)


DAY 19
ML
MD
BL
BD
C
I
II
60
40
20
0
250
375
500
100
300
500
350
425
500
Time (sec 1/2)
Water column height (mm)


DAY 22
ML
MD
BL
BD
C
I
II
60
40
20
0
425
500
100
300
500
450
500
Time (sec 1/2)
Water column height (mm)

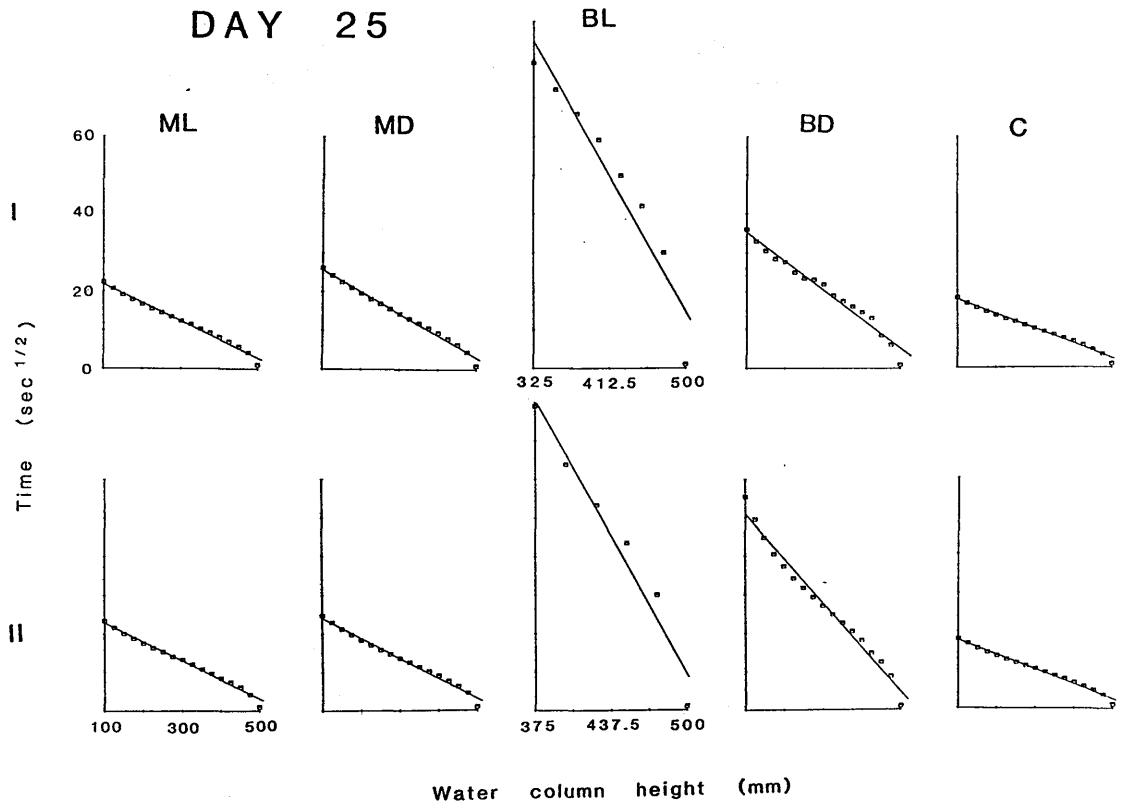

DAY 25
ML
MD
BL
BD
C
I
II
60
40
20
0
Time (sec 1/2)
325 412.5 500
100 300 500
375 437.5 500
Water column height (mm)

Table 10. Enrichment experiment. Mean permeability coefficients with standard deviations in brackets (K mm.sec^{-1} x 10^{-2}.)

Day	Replic. core	Treatment ML	MD	BL	BD	C
1	1	34.07 [2.485]	31.34 [2.754]	37.41 [3.188]	41.12 [2.971]	36.12 [2.048]
	2	31.64 [1.580]	33.70 [3.641]	35.64 [2.589]	36.48 [2.051]	33.16 [1.506]
4	1	25.06 [0.7552]	21.56 [1.843]	16.09 [1.726]	13.27 [1.502]	30.69 [3.422]
	2	23.97 [1.427]	22.65 [1.023]	13.60 [1.477]	14.16 [1.529]	28.09 [1.400]
7	1	23.12 [1.170]	18.97 [1.015]	14.67 [1.235]	16.52 [1.412]	28.30 [1.639]
	2	21.61 [0.7730]	20.25 [0.8323]	10.51 [1.424]	15.55 [1.507]	27.80 [1.869]
10	1	20.65 [1.221]	17.38 [2.167]	12.15 [0.8607]	12.64 [1.229]	27.58 [1.405]
	2	19.37 [1.090]	18.41 [1.353]	5.334 [2.240]	11.38 [0.7223]	26.81 [1.890]
13	1	18.44 [2.974]	17.13 [1.167]	9.668 [1.765]	14.47 [1.037]	27.34 [3.090]
	2	18.95 [1.058]	17.84 [1.967]	6.173 [1.116]	12.09 [1.886]	27.35 [5.856]
16	1	18.12 [1.831]	16.21 [1.936]	7.053 [2.285]	10.45 [1.021]	27.10 [5.550]
	2	17.35 [2.629]	17.25 [1.853]	2.297 [1.061]	10.68 [1.628]	26.71 [2.438]

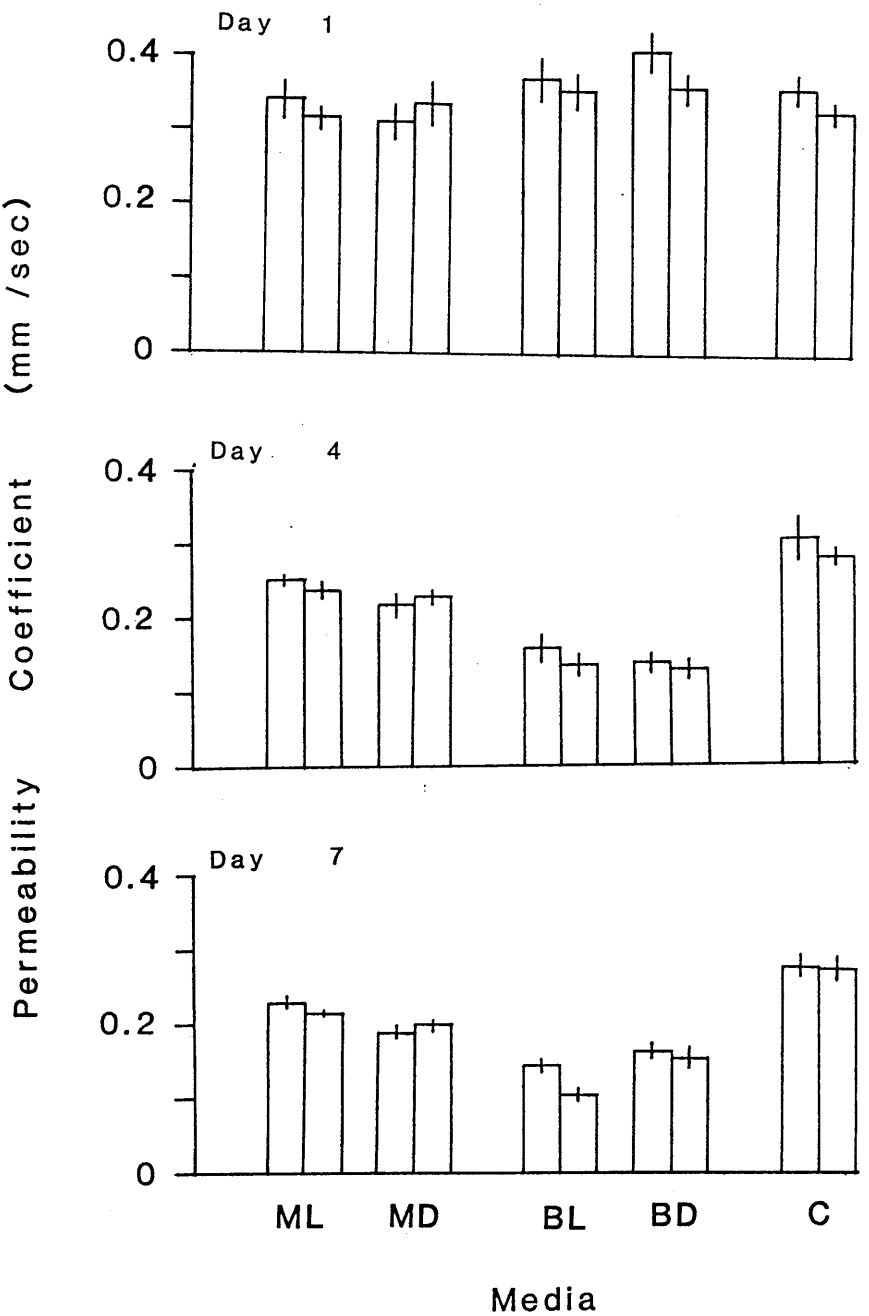
Table 10 contd.

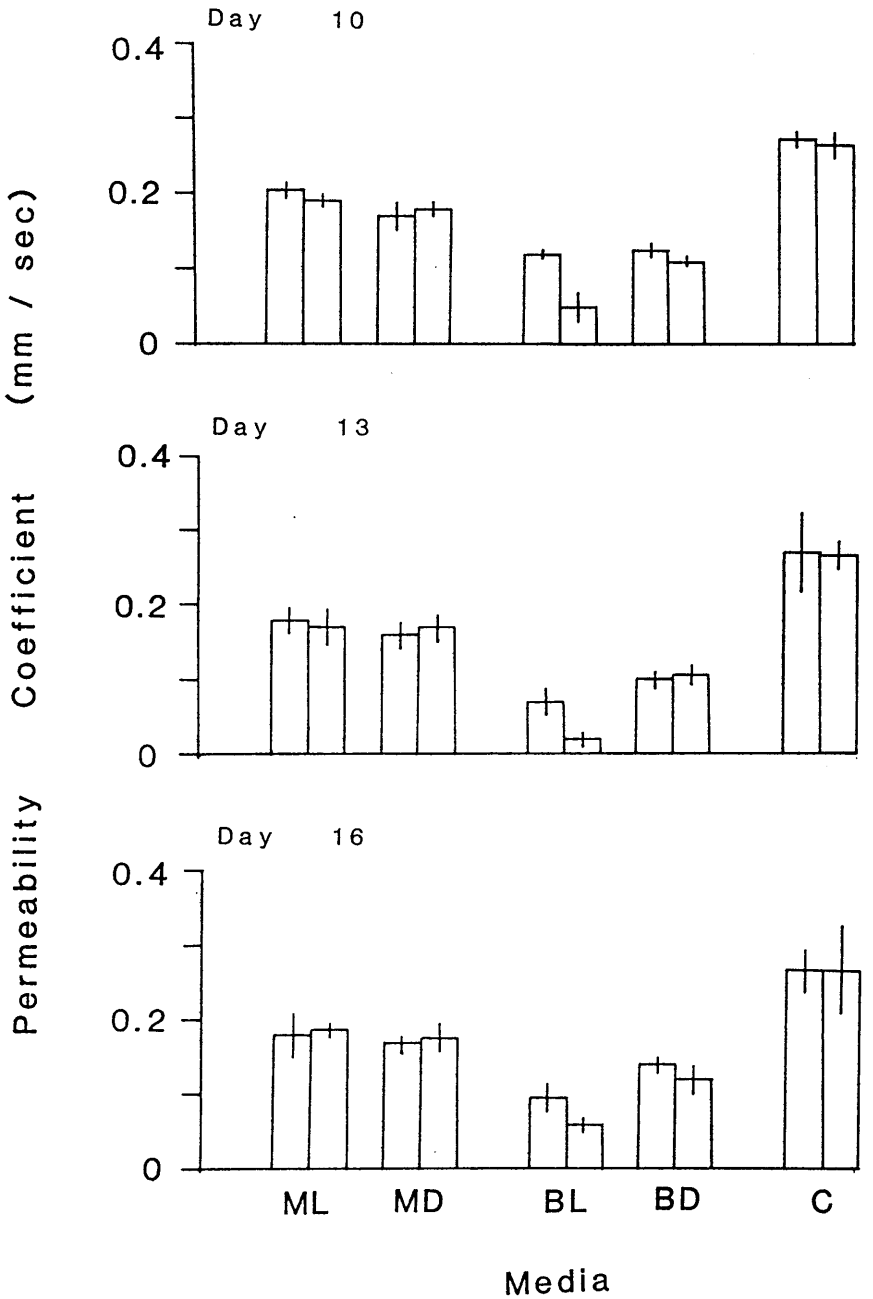
Day	Replic. core	Treatment ML	MD	BL	BD	C
19	1	17.73 [0.9366]	14.65 [1.336]	2.176 [1.137]	9.397 [3.702]	25.99 [2.327]
	2	16.39 [1.758]	15.75 [1.116]	0.4862 [0.3215]	7.436 [1.207]	26.01 [2.956]
22	1	16.12 [0.7789]	13.35 [0.8513]	0.4367 [0.1155]	7.452 [1.540]	24.74 [0.9217]
	2	14.80 [2.676]	14.54 [0.8090]	0.009794 [0.003572]	3.338 [1.982]	24.98 [1.866]
25	1	16.28 [1.053]	11.98 [0.7705]	0.007022 [0.001396]	8.806 [8.657]	24.89 [1.817]
	2	14.98 [1.707]	13.49 [0.8852]	0.002777 [0.0008307]	3.099 [0.6981]	24.81 [1.687]

Figure 6

Third series of experiments (Enrichment experiment).

Means and standard deviations of permeability coefficients (K $mm.sec^{-1}$) for the two replicate cores for treatments ML, MD, BL, BD, and C on days 1 to 25. n = 16 for each replicate.


Day 1
Day 4
Day 7
0.4
0.2
0
Permeability Coefficient (mm /sec)
ML
MD
BL
BD
C
Media


Day 10
0.4
0.2
0
Day 13
0.4
0.2
0
Day 16
0.4
0.2
0
ML
MD
BL
BD
C
Media
Permeability Coefficient (mm / sec)

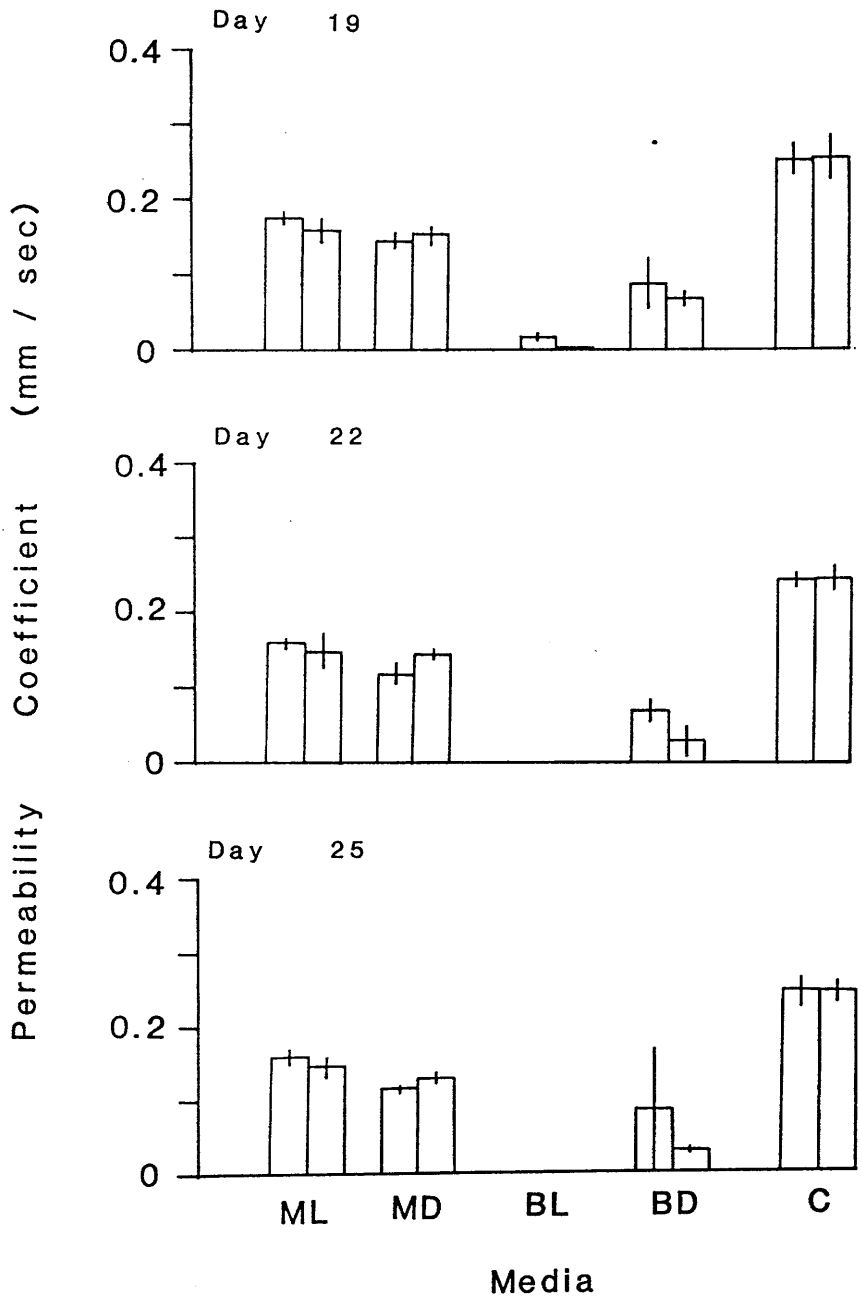
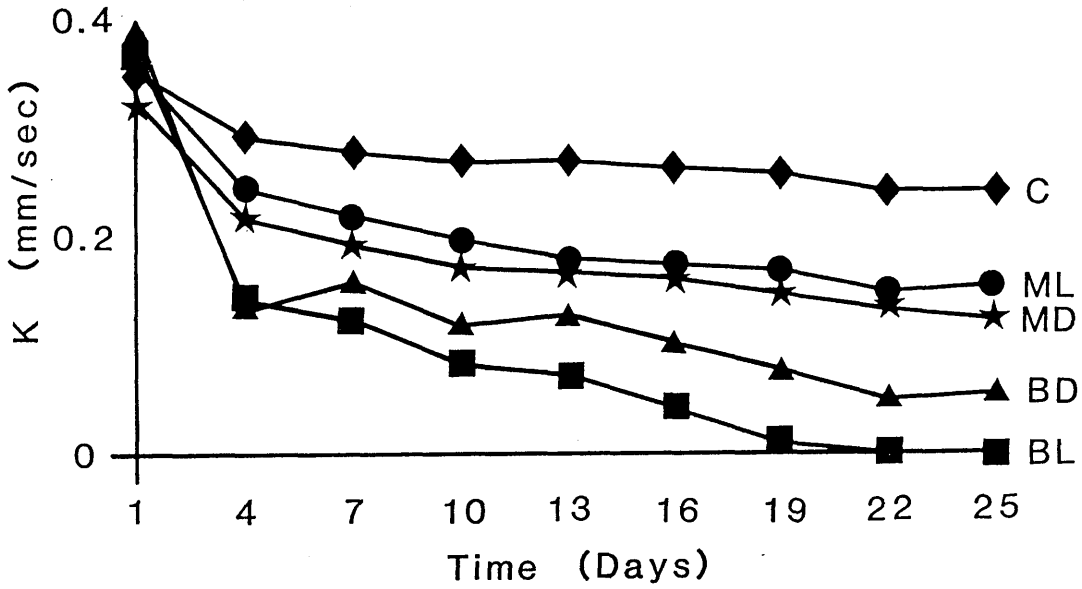

Day 19
0.4
0.2
0
Day 22
0.4
0.2
0
Day 25
0.4
0.2
0
Permeability Coefficient (mm / sec)
ML
MD
BL
BD
C
Media

Figure 7

Third series of experiments (Enrichment experiment).

Mean permeability coefficients (K mm.sec^{-1}) of the 2 replicate cores for ML, MD, BL, BD, and C for days 1 to 25. n = 2 for each treatment.


0.4
0.2
0
K (mm/sec)
1
4
7
10
13
16
19
22
25
Time (Days)
C
ML
MD
BD
BL

within a week. Microorganisms growing in the heterotrophic bacterial medium in the light (BL) and the dark (BD) had a larger effect in reducing permeability than did microorganisms growing in the photosynthetic medium in the light (ML) and in dark (MD). The former (BL, BD) would have consisted mainly of heterotrophic microorganisms, and the latter (ML, MD) of photosynthetic and chemoautotrophic microorganisms.

The results were analysed statistically by two series of analyses of variance. In the first series, two-way analyses of variance were applied to each medium in turn, comparing differences between replicates (Factor A) and differences between days (Factor B) (table 11). In all but one of these analyses, the factor A and factor B interaction was not significant, and so meaningful statements could be made about the two main factors. In the 4 comparisons that could be made, there were always statistically significant differences in the permeabilities between the two replicate cores (Factor A) and in the permeabilities on the successive days at which the readings were taken (Factor B). This means that there was always significantly more variability between replicate cores than within the cores, and that the declines in permeability in the different treatments during the experiment were all significant.

In the second series, one-way analyses of variance with two levels were applied to the readings obtained at the end of the experiment. These compared the permeability of pairs of media in turn (table 12). All paired comparisons were highly significant, and so by the end of the experiment the permeability of the cores containing the different media were all different from each other.

An interesting effect was noted in the variability of the permeabilities between replicates of each medium. There was some

Table 11. Enrichment experiment. Five two-way analyses of variance, one for each medium, comparing differences between replicates (Factor A) and between days (Factor B).

Medium : ML

Source of variation	SS	MS=SS/df	d.f.	F-Ratio	P
Factor A Replicates	0.1013	0.01013	1	34.67	P<0.001
Factor B Days	0.7869	0.09836	8	336.9	P<0.001
A x B interaction	0.004084	0.00051	8	1.747	0.01 >P> 0.05
Residual Error	0.07895	0.000292	270		
Total	0.8801		287		

Medium : MD

Source of variation	SS	MS=SS/df	d.f.	F-Ratio	P
Factor A Replicates	0.01143	0.01143	1	39.69	P<0.001
Factor B Days	0.8955	0.1119	8	388.7	P<0.001
A x B interaction	0.001390	0.0001740	8	0.6042	P>0.75
Residual Error	0.07766	0.0002880	270		
Total	0.9859		287		

Table 11 contd.

Medium : BL

Source of variation	SS	MS=SS/df	d.f.	F-Ratio	P
Factor A Replicates	0.007722	0.007722	1	19.65	P<0.001
Factor B Days	0.4056	0.05070	8	129.0	P<0.001
A x B interaction	0.004751	0.0005940	8	1.511	0.25 >P> 0.10
Residual Error	0.007067	0.000393	18		
Total	0.4252		35		

Medium : BD

Source of variation	SS	MS=SS/df	d.f.	F-Ratio	P
Factor A Replicates	0.02064	0.02064	1	8.821	0.005 >P> 0.001
Factor B Days	2.495	0.3119	8	133.3	P<0.001
A x B interaction	0.05951	0.007440	8	3.179	0.005 >P> 0.001 **
Residual Error	0.6329	0.002340	270		
Total	3.208		287		

Table 11 contd.

Medium : C

Source of variation	SS	MS=SS/df	d.f.	F-Ratio	P
Factor A Replicates	0.004446	0.004446	1	5.835	0.025 >P> 0.01
Factor B Days	0.2279	0.02848	8	37.38	P<0.001
A x B interaction	0.008875	0.001109	8	1.455	0.25 >P> 0.10
Residual Error	0.2056	0.0007620	270		
Total	0.4468		287		

Table 12. Enrichment experiment. Ten one-way analyses of variance comparing 2 media at a time on day 25 of permeability experiment. Main factor = media compared. 2 media are compared in each analysis (= 2 levels).

Media compared	Source of variation	SS	MS=SS/df	d.f.	F-ratio	P
ML / MD	Main Factor	0.01338	0.01338	1	73.42	P<0.001
	Residual Error	0.01130	0.0001820	62		
	Total	0.02468		63		

Media compared	Source of variation	SS	MS=SS/df	d.f.	F-Ratio	P
ML / BL	Main Factor	0.1859	0.1859	1	1006	P<0.001
	Residual Error	0.007393	0.0001850	40		
	Total	0.1933		41		

Media compared	Source of variation	SS	MS=SS/df	d.f.	F-Ratio	P
ML / BD	Main Factor	0.1498	0.1498	1	63.34	P<0.001
	Residual Error	0.1466	0.002360	62		
	Total	0.2963		63		

Table 12 contd.

Media compared	Source of variation	SS	MS=SS/df	d.f.	F-ratio	P
ML / C	Main Factor	0.1361	0.1361	1	507.9	P<0.001
	Residual Error	0.01662	0.0002680	62		
	Total	0.1528		63		

Media compared	Source of variation	SS	MS=SS/df	d.f.	F-Ratio	P
MD / BL	Main Factor	0.1235	0.1235	1	1264	P<0.001
	Residual Error	0.003907	0.00009770	40		
	Total	0.1274		41		

Media	Source of variation	SS	MS=SS/df	d.f.	F-Ratio	P
MD / BD	Main Factor	0.07360	0.07360	1	31.85	P<0.001
	Residual Error	0.1431	0.002310	62		
	Total	0.2167		63		

Table 12 contd.

Media compared	Source of variation	SS	MS=SS/df	d.f.	F-Ratio	P
MD / C	Main Factor	0.2349	0.2349	1	1109	P<0.001
	Residual Error	0.01313	0.0002120	62		
	Total	0.2480		63		

Media compared	Source of variation	SS	MS=SS/df	d.f.	F-Ratio	P
BL / BD	Main Factor	0.02695	0.02695	1	7.750	0.01>P>0.005
	Residual Error	0.1392	0.003480	40		
	Total	0.1661		41		

Media compared	Source of variation	SS	MS=SS/df	d.f.	F-Ratio	P
BL / C	Main Factor	0.4703	0.4703	1	2040	P<0.001
	Residual Error	0.009224	0.0002310	40		
	Total	0.4796		41		

Table 12 contd.

Media compared	Source of variation	SS	MS=SS/df	d.f.	F-Ratio	P
BD / C	Main Factor	0.5714	0.5714	1	238.7	P<0.001
	Residual Error	0.1484	0.002390	62		
	Total	0.7199		63		

indication that inter-replicate variability increased as the experiment progressed. This increase can be interpreted as progressively more unpredictable and uneven growth of microorganisms in the interstices of the sediment.

The concentrations ($\mu g.\ g^{-1}$ dry wt. sed.) of chlorophylls a, b, c, phaeopigments, carotenes, bacteriochlorophylls ab, c, and d were measured on the sediment before enrichment and on the enriched sediment at the end of the incubation. The concentrations of the chlorophylls and carotenes were calculated using a number of equations This was done to gain experience. The means and standard deviations calculated from these equations are given in appendix 5.6 table 4 (pp 253-254). Table 13 shows the means and standard deviations for chlorophylls a, b, c, and carotenes computed from one of these - Parsons and Strickland's equation (PS) (Strickland & Parsons, 1972), together with the means and standard deviations of phaeopigment and bacteriochlorophylls ab, c, and d. The data in table 13 are plotted in figures 8, 9 and 10 to facilitate comparisons of the data. Chlorophylls a and c, phaeopigment and carotenes were highest in the ML cores, and chlorophyll b was highest in the MD cores. Bacteriochlorophyll ab was highest in the BL cores and bacteriochlorophyll c and d were highest in the ML cores.

The heterotrophic bacterial colony counts per plate and their converted values of c.f.u. g^{-1} dry wt. sed. are given in table 14. The original data of the colony counts per plate at 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} dilutions are given in appendix 5.7 table 5 (p 255). The data show that heterotrophic counts were highest in the BL and BD cores.

Primary production was measured on the enriched cores at the end of the experiment. The means and standard deviations of these are given in table 15. The ML cores showed the highest primary production

Table 13. Enrichment experiment.
Chlorophyll a, b, c, phaeopigment, carotenes, and bacteriochlorophyll ab, c and d concentrations ($\mu g\ g^{-1}$ dry wt. sed.) in the unenriched sediment (NS) and enriched ML, MD, BL, BD and control C cores. The equations are as follows: PS = Parsons and Strickland, L = Lorenzen (see Strickland & Parsons, 1960). Further data using other equations are given in appendix 5.6 table 4 (p 253).

Pigment		Treatment NS	ML	MD	BL	BD	C
Chlorophyll a							
(PS)	mean	5.961	7.388	5.253	3.003	4.462	2.699
	s.d.	0.1934	2.659	0.2297	1.082	0.3306	0.1427
Chlorophyll b							
(PS)	mean	0.0013	0.3170	0.3865	0.0000	0.1303	0.1734
	s.d.	0.0562	0.0820	0.0662	0.0000	0.2372	0.1156
Chlorophyll c							
(PS)	mean	2.218	2.702	1.683	0.0000	1.703	0.9121
	s.d.	0.1554	0.7514	0.2895	0.0000	0.0018	0.0209
Phaeopigment							
(L)	mean	1.176	2.160	1.795	0.0000	1.419	1.903
	s.d.	0.2776	0.2347	0.7185	0.0000	0.4780	1.058
Carotenes							
(PS)	mean	2.575	3.098	2.168	0.9108	2.124	0.7653
	s.d.	0.0547	1.167	0.0351	0.8797	0.0653	0.1900
Bacteriochlorophyll							
Bchl. ab	mean	0.0000	0.4624	0.1191	6.211	0.0000	0.2259
	s.d.	0.1986	0.1477	0.1684	4.079	0.0000	0.0112
Bchl. c	mean	2.864	3.762	2.599	2.683	2.415	1.507
	s.d.	0.0352	1.428	0.1403	0.1097	0.1131	0.0101
Bchl. d	mean	5.488	7.106	5.054	4.589	4.469	2.804
	s.d.	0.0020	2.457	0.2137	0.0128	0.2687	0.0022

Figure 8

Third series of experiments (Enrichment experiment).

Mean concentrations ($\mu g\ g^{-1}$ dry wt. sed.) of chlorophylls a, b, and c in natural sediment (NS) and ML, MD, BL, BD, and C. Vertical bars are standard deviations. n = 2 for each treatment.

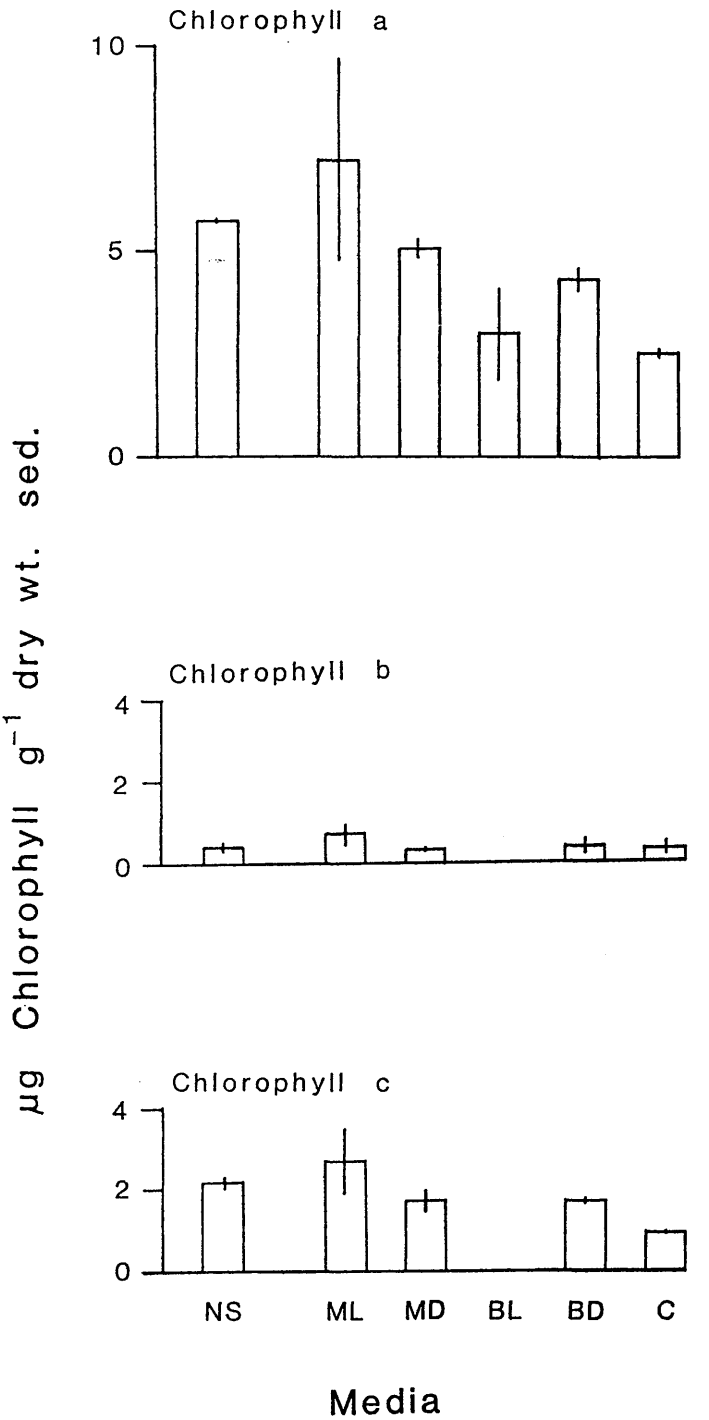

Chlorophyll a
10
5
0
Chlorophyll b
4
2
0
Chlorophyll c
4
2
0
NS
ML
MD
BL
BD
C
Media
µg Chlorophyll g⁻¹ dry wt. sed.

Figure 9

Third series of experiments (Enrichment experiment).

Mean concentrations ($\mu g\ g^{-1}$ dry wt. sed.) of Carotenes and phaeopigments for natural sediment (NS) and ML, MD, BL, BD, and C. Vertical bars are standard deviations. n = 2 for each treatment.

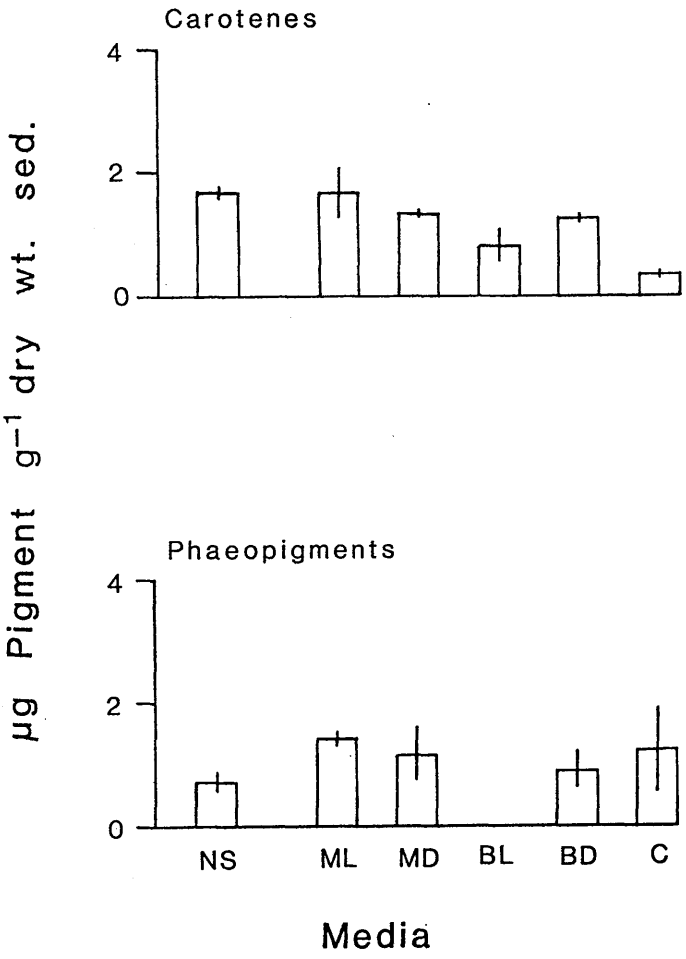

Carotenes
4
2
0
Phaeopigments
4
2
0
μg Pigment g⁻¹ dry wt. sed.
NS
ML
MD
BL
BD
C
Media

Figure 10

Third series of experiments (Enrichment experiment).

Mean concentrations ($\mu g\ g^{-1}$ dry wt. sed.) of bacteriochlorophylls ab, c and d for natural sediment (NS), and ML, MD, BL, BD, and C. Vertical bars are standard deviations. n = 2 for each treatment.

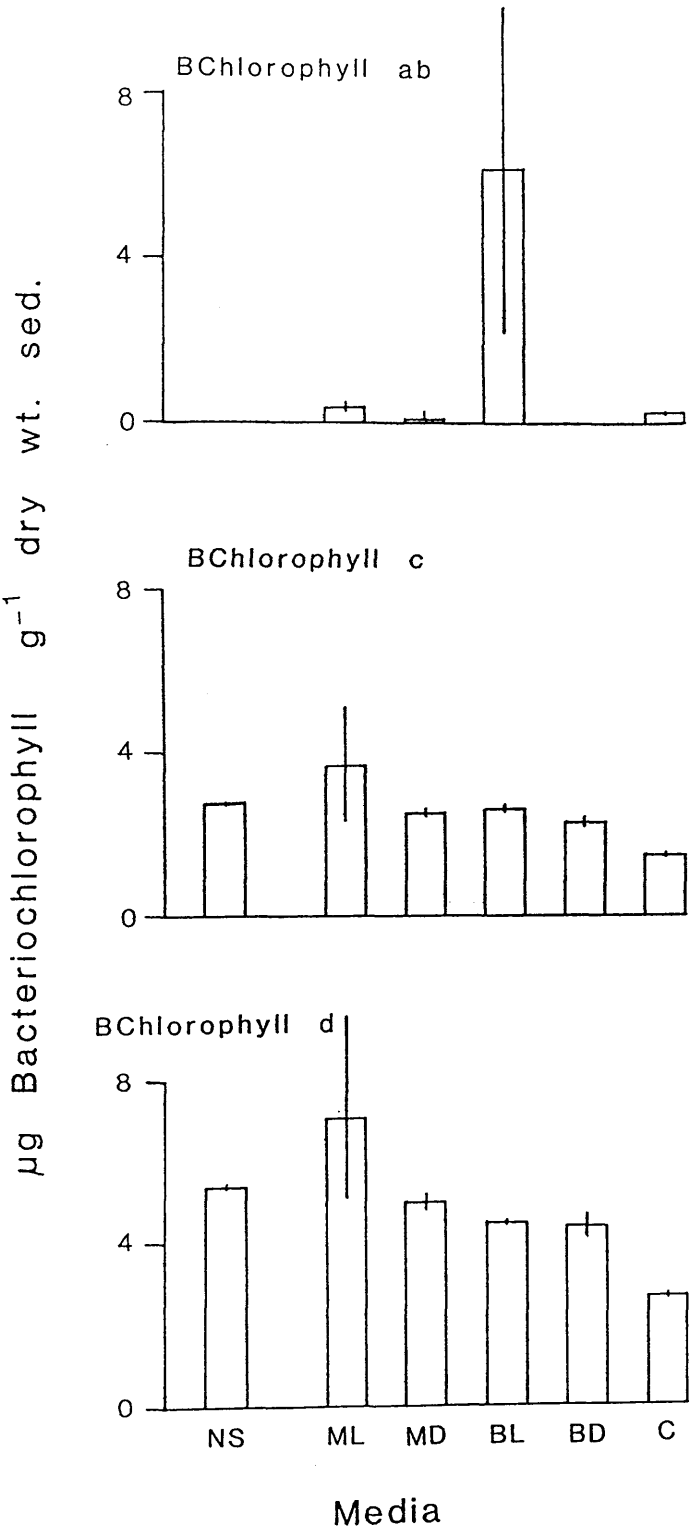

BChlorophyll ab
8
4
0
BChlorophyll c
8
4
0
BChlorophyll d
8
4
0
NS
ML
MD
BL
BD
C
Media
µg Bacteriochlorophyll g⁻¹ dry wt. sed.

Table 14. Enrichment experiment.
Heterotrophic bacterial counts in unenriched natural sediment (NS) and the enriched ML, MD, BL, and BD cores and the control C cores (there were 2 replicate plates per core). Plates were set up at 10^{0}, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}. As far as possible; plates were selected for counting that had 30 to 300 colonies. The table gives the dilutions of these plates, colonies/plate, and colony forming units/plate (c.f.u./plate). More detailed data are given in appendix 5.7 table 5 (p 255).

Medium	Repl.	Dilution	Colonies per plate	c.f.u. g^{-1} dry wt. sed.
NS	i	10^{-2}	74	7.40×10^5
	ii	"	67	6.70×10^5
ML1	i	10^{-3}	300	3.00×10^7
	ii	"	78	7.80×10^6
ML2	i	"	25	2.50×10^6
	ii	"	53	5.30×10^6
MD1	i	10^{-3}	240	2.40×10^7
	ii	"	118	1.18×10^7
MD2	i	"	28	2.80×10^6
	ii	"	34	3.40×10^6
BL1	i	10^{-5}	109	1.09×10^9
	ii	"	110	1.10×10^9
BL2	i	"	32	3.20×10^8
	ii	"	103	1.03×10^9
BD1	i	10^{-5}	38	3.80×10^8
	ii	"	115	1.15×10^9
BD2	i	"	93	9.30×10^8
	ii	"	40	4.00×10^8
C1	i	10^{-2}	0	$< 0.10 \times 10^5$
	ii	"	28	2.80×10^5
C2	i	"	11	1.10×10^5
	ii	"	0	$< 0.10 \times 10^5$

Table 15. Enrichment experiment.
Primary production (mg C. g^{-1} dry wt. sed. h^{-1}) for the enriched ML, MD, BL, BD, and C control cores. Means, standard deviations, and means as a percentage of the ML mean. n = 2 for each medium.

	Treatment				
	ML	MD	BL	BD	C
mean	1.851	0.5769	0.0823	0.0382	0.0000
s.d.	1.136	0.0841	0.0978	0.0540	0.0000
% of ML	100	31	5	2	0

followed by the MD cores (30% of ML). The BL (5%) and BD (2%) showed very low primary production and the control cores showed none.

In summary, the chlorophyll and bacteriochlorophyll concentrations, heterotrophic counts and primary production values reflect the treatments of the different cores. Cores containing photosynthetic medium incubated in the light, had high chlorophyll a and c, bacteriochlorophyll c and d and primary production, and low heterotrophic counts. Cores containing photosynthetic medium incubated in the dark, had high chlorophyll b concentrations, 30% of ML primary production and low heterotrophic counts. Cores containing bacterial medium incubated in the light, had high bacteriochlorophyll ab concentrations and heterotrophic counts, but low chlorophyll concentrations and primary production. Cores containing bacterial medium incubated in the dark, had high heterotrophic counts and low chlorophyll and bacteriochlorophyll concentrations and primary production. The values for control cores were extremely low or nonexistent as was to be expected.

DISCUSSION

The measure of the ease with which a fluid flows through soil or sediment (gravel, sand, silt, clay etc.) is known as permeability. The common term used to define permeability is the permeability coefficient K, sometimes also called hydraulic conductivity or percolation rate. The units of K are $cm.sec^{-1}$ or $mm.sec^{-1}$ (Fair & Hatch, 1933; Fraser, 1935; Granton & Fraser, 1935; Childs & Collis-George, 1950; Reeve et al., 1957; Kinori, 1970; Kessler & Oosterbaan, 1974; Lambe & Whitman, 1979; Dunn et al., 1980; Smith, 1981; Lee et al., 1983; Sleath, 1984).

Another equation is sometimes used for measuring permeability - this is called the specific or absolute permeability K" (Lambe & Whitman, 1979), and is given by $K'' = (K.\mu) / \gamma$, in which K = permeability coefficient, μ = the viscosity of the liquid and γ = the unit weight of the liquid.

The permeability coefficient K which I have used in this thesis can be converted to specific permeability as follows,

K'' in cm^2 = K in $cm.sec^{-1} \times 1.02 \times 10^{-5}$

K'' in darcys = K in $cm.sec^{-1} \times 1.035 \times 10^{3}$

Permeability is commercially important in solving soil seepage, settlement and stability problems such as leakage under dams, the rate at which buildings settle, and the rate at which the strength of a deposit increases after it has been subjected to consolidating pressure (Christiansen, 1947; Jones, 1955; Lambe, 1955; Luthin, 1966).

Permeability also plays an important part in the field of oil recovery. This is evident from the in situ growth of bacteria in cracks of sandstone which reduce the rock permeability and obstructs

the recovery of oil. Permeability is measured regularly during Enhanced Oil Recovery (EOR) work. In this technique sandstone is shaped into a core and injected with one or more strains of bacteria, - sometimes also called "plugging" (Hart et al., 1960; Crawford, 1961; Kalish et al., 1964; Raleigh & Flock, 1965; Finnerty & Singer, 1983; Jenneman et al., 1984).

There are a large number of methods available for the measurement of permeability. Field measurements can be made in saturated or non-saturated soils by Auger holes, piezometers, pumped boreholes, double tubes, or infiltrometers (Donnan, 1957; Luthin, 1957; Kinori, 1970; Kessler & Oosterbaan, 1974; Dunn et al., 1980; Smith, 1981; Lee et al., 1983). Laboratory measurements are made on sediment in a core, or on a field sample taken by coring (Goode & Christiansen, 1945; Wit, 1967), and use a falling-head or a constant-head permeameter, or an oedometer (Fraser, 1935; Childs & Collis-George, 1950; Jones, 1955; Reeve, 1957; Reeve et al., 1957; Dettmann & Emerson, 1959; Webb, 1969; Kessler & Oosterbaan, 1974; Lambe & Whitman, 1979; Dunn et al. 1980; Smith, 1981; Lee et al., 1983). I used a falling-head permeameter (see results p 12).

Permeability is affected by a number of factors including the physical characteristics of the sediment itself (table 16), and the characteristics of the liquid flowing through the sediment (table 17). The main purpose of my research, however, was to investigate microbiological effects on permeability.

There is a scattered literature on the effects of biological and microbiological activity on permeability, and most of this is on terrestrial soils. For example, the permeability of soils can be affected by cracks and holes made by roots and worms (Reeve et al., 1957). The effects of microorganisms on soil permeability have been

Table 16. Physical characteristics of sediments and soil affecting permeability. Author code; 1 : Fraser (1935), 2 : Granton & Fraser (1935), 3 : Christiansen et al. (1946), 4 : Wallace (1948), 5 : Lambe (1955), 6 : Reeve et al. (1957), 7 : Lambe & Whitman (1979), 8 : Webb (1969), 9 : Beard & Weyl (1973), 10 : Dunn et al. (1980), 11 : Deans et al. (1982).

Physical Factor	Author
1) Particle size	1, 6, 7, 9, 10
2) Particle shape	1, 7, 9, 10
3) Porosity	1, 6, 9
4) Void ratio	2, 5, 7, 10
5) Compaction	5, 7, 8
6) Soil fabric and flocculation	5, 7
7) Degree of saturation	5, 7
8) Packing, consolidation, and cementation	1, 2, 8
9) Ion exchange capacity	7, 10
10) Entrapment of air	3, 6
11) Dispersion and aggregation	5
12) Fines	4, 7, 11

Table 17. Characteristics of liquids flowing through sediments and soils that affect permeability. Author code; 1 : Fraser (1935), 2 : Graton & Fraser (1935), 3 : Cristiansen (1947), 4 : Wallace (1948), 5 : Lambe (1955), 6 : Quirk & Schofield (1955), 7 : Reeve et al. (1957), 8 : Dettmann & Emerson (1959), 9 : Lambe & Whitman (1979), 10 : Webb (1969), 11 : Lee et al., (1983).

Characteristic	Author
1) Temperature	1, 2, 4, 10
2) Capillary action	2, 4
3) Hydraulic gradient	1, 11
4) Viscosity	2, 5, 9, 11
5) Density	2, 5, 11
6) Unit weight	5, 11
7) Ionic concentration	7, 8
8) Polarity	5
9) Acidity	8
10) Salt content	3, 6, 7
11) Cohesion, adhesion, absorption, fluidity, surface tension, pressure, inertia, momentum, velocity, acceleration, impact, friction, direction and distance	2, 10

extensively examined. For example bacteria and fungi or their metabolic products (e.g. polysaccharides) are known to clog soil pores and hence reduce permeability (Bodman, 1937; Fireman & Bodman, 1939; Alderfer & Merkle, 1941; Fireman, 1944; Pillsbury & Appleman, 1945; McCalla, 1946, 1950; Allison, 1947).

There has been less work done on the effect of biological and microbiological activity on the permeability of freshwater and marine sediments. Webb (1958, 1969) conducted an interesting field study in which he investigated the relationship between permeability of sediments and the abundance and the distribution of Branchiostoma lanceolatum and the interstitial harpacticoid copepod Evansula incerta. He showed that high numbers of B. lanceolatum were found in sands having high specific permeabilities. He concluded that both species react to differences in specific permeability but the way in which they are affected needed further investigation. (Webb defines specific permeability as the ratio of the rate of change in the drainage factor to porosity. The drainage factor is the rate of flow of water). Aller (1983) investigated the differential permeability of animal burrow linings to various solutes under experimental conditions. Amongst other interesting results he showed that the diffusion coefficients of small inorganic solutes across burrow linings were 10 to 40% of those in free solution. Weaver and Schultheiss (1983) measured permeability of burrows in deep-sea cores. They showed that the presence of open burrows increased the permeability of clay to that characteristic of coarse sand. Gupta and Swartzendruber (1962) showed in laboratory experiments that the permeability of quartz sand decreased dramatically when the numbers of bacteria were greater than 400,000 g^{-1} and that this occurred mainly in the superficial layers of the core. Mitchell and Nevo (1964) studied the reduction in permeability of coarse beach sand caused by

bacterial activity and showed that polysaccharide-producing bacteria - most of which were Flavobacterium species - had the greatest effect. Shaw et al. (1985) conducted permeability experiments with pure and mixed natural populations of stream water bacteria in glass bead cores. Their results showed that exopolysaccharide produced by bacteria drastically reduced permeability.

I used enrichment media to stimulate the growth of microorganisms in naturally occurring sediments, which then allowed me to investigate the effect of these microorganisms on sediment permeability.This approach does not appear to have been used previously.

Enrichment culture is a microbiological technique that promotes selective growth of microorganisms from natural habitats such as soil, sediment or water. The method uses an enriched or selective medium, which is suitable for the growth of a specific organism or group of organisms. The chemical composition of enrichment media and variation in factors such as temperature, pH, ionic strength, light and aeration, determine the types of microorganisms that will grow. Different combinations of variables result in different microbial populations (Boney, 1975; Fogg, 1975; Stanier et al., 1977). An enrichment medium that is not initially highly selective may acquire increased selectivity for a particular type of microorganism. For example, if fermentative bacteria and yeasts are grown with other microorganisms in a carbohydrate-rich medium, the bacteria and yeasts produce organic end-products. Other microorganisms present are less tolerant of these end-products than are the fermentative bacteria and yeasts, and hence their growth is suppressed (Stanier et al., 1977).

The conditions and enrichment media used in this study were designed to enhance the growth of microorganisms such as blue-green algae and diatoms (ML), nitrifying and sulphur bacteria (MD), purple

and green bacteria (BL), and aerobic gram-negative and nitrifying bacteria, and fungi (BD) in the respective cores (table 18). This will only happen if these microorganisms were originally present in the unenriched sediment.

My results show that the microorganisms expected to grow under such conditions, grew in large numbers. For example the high chlorophyll a and c in the ML cores indicated high numbers of photosynthetic microorganisms such as diatoms and blue-green algae and high bacteriochlorophyll c and d indicated the presence of green bacteria. This was further substantiated by the high primary production in these cores as a result of diatoms and blue-green algae.

The BL cores show high bacteriochlorophyll ab indicating large numbers of purple bacteria. The high heterotrophic counts in these cores was additional evidence of a large number of bacteria and is in agreement with the high bacteriochlorophyll.

The most effective conditions and microorganisms which reduced the permeability of sand in my cores were the bacteria in the BL and the BD cores. Hence bacteria may be the most important sedimentary microorganisms blocking pores and reducing permeability in the field. This may be due to their very small size, their ability to form a plug in the interstices, or their secretion of extracellular metabolic products such as polysaccharides. The microorganisms that grew in the ML cores such as diatoms and blue-green algae, also clog the pores of sediment but to a lesser extent.

My results broadly agree with the work of Gupta and Swartzendruber (1962), Mitchell and Nevo (1964), and Shaw et al. (1985) (see above) who showed that bacterial growth clogs the pores of sediment and reduces its permeability. The latter two papers show that the polysaccharide produced by the bacteria rather than the bacterial cells themselves block the pores. I cannot confirm this because I did

Table 18. Types of microorganisms expected to grow under different incubation conditions in enrichment media cores. M = photosynthetic medium, B = bacterial medium; L = incubated in the light, D = incubated in the dark. Modified from Stanier et al. (1977).

		Photoauto-trophs	Chemoauto-trophs	Photohetero-trophs	Chemohetero-trophs
Principal energy source	(i) Light	+	-	+	-
	(ii) Chemical	-	+	-	+
Principal carbon source	(i) Inorganic	+	+	- + or - +	-
	(ii) Organic	-	-		+
Media used to enrich sediment cores		ML	MD	BL	BD
Microorganisms likely to develop in enrichment cores		blue-green algae and diatoms	nitrifying bacteria, sulphur-oxidizing bacteria, hydrogen bacteria, methanogenic bacteria	purple bacteria, green bacteria	aerobic gram-negative bacteria, denitri-fying bacteria, fungi and protozoa

not measure polysaccharide. On the other hand I have studied the effects of blue-green algae and diatoms and have shown that these organisms have a significant effect in reducing permeability, although to a lesser extent.

Two other factors in my experiments need comment. One is the effect of fine material in the sediment, and the other is the method of packing the cores. Fine material is known to reduce permeability. This can be shown by removing the fines (Lambe & Whitman, 1979; Deans et al., 1982). Deans et al., (1982) tested permeability of sediment which had been pretreated in 4 different ways. They showed that both autoclaving and mixing dramatically reduced permeability while removing the fine material increased it. The results of the second series of experiments (p 32) show that the presence of fines has a major effect in reducing sediment permeability. Bodman (1937) and Fireman and Bodman (1939) describe the fine material problem in detail and suggest that as the liquid flows downward it causes the dispersion and rearrangement of fine clay particles which then block the pores and reduce permeability. This effect may also account for the slight decrease in permeability of the control cores during the progress of the enrichment experiment (figure 7, p 72).

I packed my cores by allowing wet sediment to settle through seawater, in contrast to most other workers who have used dry sediment (Granton & Fraser, 1935; Fireman, 1944; Gupta & Swartzendruber, 1962; Mitchell & Nevo, 1964; Webb, 1969). This ensured that the indigenous microorganisms in the sediment remained viable and capable of growing in the enrichment media.

APPENDICES

Note: "text p 5, 50" means see text on pages 5 and 50. This notation is used throughout the appendices where appropriate.

PHOTOSYNTHESIS **Appendix 1.1**

Introduction

When light energy is absorbed by chlorophyll molecules in the cells of photosynthetic organisms a complex sequence of events is set in motion which eventually leads to the fixation of CO_2 as carbohydrate. This process is called photosynthesis. The light energy is transferred to electrons in the chlorophyll molecule and as a result, the electrons are raised from their normal stable energy to a higher unstable one. The electrons in the higher energy level then have a tendency to return to their normal level; this is a multi-stage process in which the electrons move through a number of steps from molecule to molecule loosing energy as they do so. In this chain of reactions each step is catalysed by an enzyme. The overall effect is that energised electrons release their absorbed energy which is utilised by other cellular components to reduce carbon dioxide to carbohydrates.

The process is rather complex. Photosynthesis can be divided into 3 main parts. The first two are light-dependent processes and are called light reactions. They utilise the energy in sunlight to add inorganic phosphate to ADP thus forming ATP.

$$ADP + P_i + \text{energy} \xrightarrow{\text{enzyme}} ATP + H_2O$$

This process which is called photophosphorylation, can either be cyclic or noncyclic. The third part, consists of carbohydrate synthesis in which CO_2 is reduced to carbohydrate via the Calvin cycle. It occurs in the light or dark and is called a dark reaction.

The three parts are most easily understood by considering the microanatomic and molecular, and then the biochemical aspects of photosynthesis, in that order. The biochemical aspects cover cyclic

photophosphorylation, noncyclic photophosphorylation and carbohydrate synthesis. The following account on photosynthesis is taken from Lehninger (1975), Gregory (1977), Hatch and Boardman (1981), Styrer (1981), Govindjee (1982), and Keeton and Gould, (1986).

Microanatomic and molecular aspects

Chloroplasts are the centres of photosynthesis in the plant cell and contain all the biochemical machinery and chlorophylls necessary for photosynthesis. They consist of an outer membrane, an inner membrane which is smooth and flat and follows the contours of the outer membrane, and grana. The grana consist of stacks of interconnected compartments called thylakoids. The thylakoid membrane contains the chlorophyll pigments, reaction centers, antenna pigments and the electron transport systems which are necessary for photosynthesis. The thylakoid membrane separates the interior of the thylakoid, which has a low pH of about 4, from the rest of the chloroplast called the stroma, which has a pH of about 8. When light energy impinges on the chloroplast, the photosynthetic machinery located in the thylakoid membrane sets up an electrochemical gradient which supplies energy for the synthesis of ATP. As a result H^+ ions , accumulate in the thylakoid interior thus producing the low pH there, and OH^- ions accumulate in the outer compartment (the stroma) and thus produce a high pH there.

Chlorophyll and related pigments in the chloroplasts are organised into photosynthetic units. Each unit contains about 300 pigment molecules including chloropyll a, chlorophyll b, and carotenoids. One pigment molecule in each unit is a specialised form of chlorophyll a and acts as a reaction center for the photosynthetic process. The other pigment molecules in the photosynthetic unit act rather like

antennas responsive to light energy.

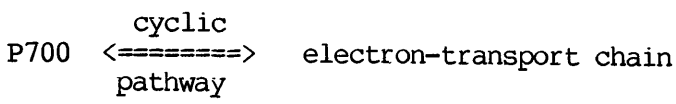
There are two types of photosynthetic unit. The first has a chlorophyll molecule called P700 as its reaction center. The second has a chlorophyll molecule called P680 as its reaction center. P700 and P680 refer to the inability of the chlorophyll a molecule at the reactor center to absorb light of wavelengths greater than 700nm and 680nm respectively.

Cyclic photophosphorylation uses the P700 system and the noncyclic photophosphorylation uses the P700 and P680 system.

Biochemical aspects

(i) Cyclic photophosphorylation

Cyclic photophosphorylation utilises the photosynthetic unit containing the P700 chlorophyll molecule as its reaction center. When a photon of light hits an antenna pigment, it raises an electron in the pigment to a higher energy level. This energized electron passes from one pigment molecule to another until it reaches P700. The energized electron is transferred from P700 to an acceptor molecule, the enzyme FeS, which contains iron and sulphur. During this transfer the acceptor molecule is reduced and P700 is oxidized. The electron passes along an electron-transport chain catalysed by a series of enzymes: from FeS to Fd (ferredoxin), to cytochrome b_6, to plastoquinone (PQ), to cytochrome f and finally to plastocyanin (PC). When the electron reaches PC it waits for an opening in P700. As soon as another electron is energized and transferred to FeS, a gap is made in P700 and the waiting electron fills it. This step completes the cyclic path. During each transfer step in this process energy is released, and the electrons move from and then return to the P700 chlorophyll.



Cyclic photophosphorylation is believed to be the first form of photosynthesis to have evolved. It is the only form of photosynthesis found in most photosynthetic bacteria. Cyclic photophosphorylation is not an efficient system. 25 kcal.mole^{-1} is gained by the excitation of P700, but only part of this, 3.4 kcal.mole^{-1} (13.6%) released during passage of electrons from plastoquinone (PQ) to cytochrome f, is utilised by the cell to synthesise ATP. The remainig energy released during the other steps of the cycle is wasted.

(ii) Noncyclic photophosphorylation

Noncyclic photophosphorylation contains two photosystems. Photosystem I contains the P700 reaction-center with its associated antenna molecules and also an electron transport chain from FeS to Fd, to the flavoprotein FAD. Photosystem II contains the P680 reaction-center with its antenna molecules and also a special set of electron transporting molecules (Q, PQ, cytochrome f, PC). An associated part involves the splitting of water during the last stage of photosystem II.

Photosystems I and II are initiated when photons strike P700 and P680. Photosystem I starts at the reaction center P700 and follows the electron transport chain which is the same as the cyclic reaction up to the electron acceptor Fd. In photosystem I the excited electron is transferred from Fd to FAD flavoprotein. It is at this stage that energy is indirectly used to synthesise ATP. The electron then passes to NADP which is reduced. Unlike cyclic photophosphorylation the electron is not returned to P700 but retained by $NADP_{re}$ and later used

to reduce CO_2. This leaves an electron hole in P700 of photosystem I.

Photosystem II also involves a light event and is associated with splitting of water. This system starts at the reaction center P680. The excited electron is transferred along an electron-transport chain first to substance Q, then to PQ. From here it follows the same path as the cyclic pathway to the P700 molecule. In P700 it fills the electron hole produced in photosystem I. This now leaves the P680 of the photosystem II with a vacancy for an electron.

The electron hole left in photosystem II is filled by an electron from water. This occurs when the P680 attracts the electrons from water by the help of enzyme Z. As a result, free protons (H^+) and oxygen are released. The electrons from water go to P680 of photosystem II and are passed to substance Q and along the electron transport chain of photosystem II and photosystem I. The electrons which ultimately reduce CO_2 to carbohydrate therefore come from water.

During the entire process ATP and $NADP_{re}$ is formed and O_2 is released. Since the electrons do not follow a circular path this system is called noncyclic photophosphorylation.

The overall transfer of electrons is as follows:

H_2O ----> P680 ----> Photosystem II transport chain ----> P700

Photosystem I transport chain ----> $NADP_{re}$ ----> (Calvin cycle) carbohydrate

Cyclic and noncyclic photophosphorylation as described above occurs in green plants and blue-green bacteria (Stanier et al., 1977). Purple and green bacteria contain a different type of chlorophyll, bacteriochlorophyll ab, c, d or e) and use light energy in the synthesis of ATP and $NADP_{re}$ in a similar way. But they do not use water as the source of electrons; they use hydrogen sulphide (H_2S) and hence produce sulphur rather than oxygen.

(iii) Carbohydrate synthesis

The third part of photosynthesis is the dark reaction of carbon fixation. This reaction takes place in the stroma of the chloroplast. The CO_2 is reduced to form glucose by a sequence of steps, each one being catalysed by an enzyme. This is called the Calvin cycle and in it the CO_2 goes up an energy gradient passing through a series of intermediate compounds. These compounds can be unstable but the final end-product, carbohydrate, is stable.

The major steps that occur in the reduction of carbon dioxide to carbohydrate via the Calvin cycle are briefly as follows. During the stepwise carbohydrate synthesis energy is derived from light via ATP and $NADP_{re}$. The CO_2 combines with a five-carbon compound called ribulose bisphosphate (RuBP); the hypothetical six-carbon compound so formed is unstable and is broken into two three-carbon molecules called phosphoglyceric acid (PGA). Each of the three-carbon molecules of PGA is phosphorylated by ATP. The phosphorylated three-carbon compound thus formed is reduced by the addition of hydrogen from $NADP_{re}$. This results in the formation of an energy-rich three-carbon compound, phosphoglyceraldehyde (PGAL). PGAL is the stable sugar and end product of photosynthesis. Five out of the six molecules of PGAL are used in the formation of new ribulose (RuBP) using energy supplied by ATP. Hence more CO_2 can be used with new RuBP. The sixth PGAL molecule is used in the synthesis of glucose, which is considered as the ultimate end product of photosynthesis. In higher plants glucose is said to be utilised as soon as it is synthesised, in building-blocks for starch, cellulose and sugars. Part of the PGAL is used in lipid, amino acid and nucleotide production.

Respiration and Photorespiration

The rate of respiration of plants in the light is usually much greater than in the dark. This is because two respiratory mechanisms are involved, one occurring only in the light (photorespiration) and the other occurring in the light and dark (dark respiration). (Lehninger, 1975; Zelitch, 1971; Goldsworthy, 1976; Gregory, 1977; Halliwell, 1981; Govindjee, 1982).

Dark respiration (mitochondrial respiration) takes place in mitochondria by the usual respiratory routes of glycolysis, the Kreb's cycle, and oxidative phosphorylation to provide ATP, at the same time releasing CO_2. Carbon dioxide in dark respiration can also be produced by the oxidative pentose phosphate pathway. Dark respiration occurs in the dark and in the light, but the rate in the light may be reduced by up to 75% (Raven, 1972; Halliwell, 1981).

Photorespiration takes place only in the light (Decker, 1955, 1959; Jackson & Volk, 1970; Bidwell, 1977; Burris, 1977, 1980; Peterson, 1980; Halliwell, 1981). It is not sensitive to mitochondrial respiratory inhibitors and thus is not based on the same biochemical pathways as mitochondrial respiration. C_3 plants show photorespiration and mitochondrial respiration, but C_4 plants only show mitochondrial respiration (see below for definition of C_3 and C_4 plants).

In photorespiration, oxygen attaches to the active site of the enzyme ribulose 1,5-bisphosphate carboxylase. The enzyme then catalyses the oxygenation of ribulose 1,5-bisphosphate (5 carbon atoms = 5C) to yield 3-phosphoglycerate (3C) and phosphoglycolate (2C). The phosphoglycolate is hydrolysed to glycolate by a phosphate. From here onwards the metabolism of glycolate occurs in peroxisomes (also termed microbodies) - cell organelles bounded by a single membrane and loosely associated with chloroplasts. The glycolate is then oxidised

by molecular oxygen, catalysed by the enzyme glycolate oxidase to produce glyoxylate (2C). Finally, the glyoxylate is metabolised to other products such as glycine, oxalate, formate or CO_2 in different species.

Photosynthesis and photorespiration can be regarded as competitive processes because CO_2 and O_2 compete for the same binding sites on the enzyme ribulose 1,5-bisphosphate carboxylase. If CO_2 binds to the enzyme, photosynthesis occurs, while if O_2 binds to the enzyme, photorespiration occurs. This competition between CO_2 and O_2 has important implications. If the CO_2 concentration in the environment is high and the O_2 concentration low, photosynthesis is most likely to occur. If the reverse is true, photorespiration is most likely to occur. Photorespiration also occurs more readily as the temperature increases.

It is interesting to note that the rate of photorespiration in the light is about 5 times the rate of mitochondrial respiration in the dark. Furthermore photorespiration is often regarded as a wasteful process because it does not result in the production of ATP while mitochondrial respiration does.

P/R ratios

Photosynthetic rates (P) and respiration rates (R) are often expressed relative to each other as P/R (or P:R) ratios. P/R ratios vary with species and depend on the stage of growth of the algae and on nutrient availability (Syrett, 1953; Coombs et al., 1967; Stewart & Alexander, 1971; Pickett, 1975). These studies show that algal cultures have different P/R ratios under different environmental conditions. For example, Stewart and Alexander (1971) found that in phosphate-deficient cultures of blue-green algae, the addition of phosphate increased the rate of respiration by 82 %. Ryther (1954)

has shown that P/R ratios vary considerably in Chlamydomonas grown in a nutrient-limited culture. During the exponential stage the P/R ratio was 10/1, during the stationary phase it dropped to 2/1 and after cell division had stopped it fell to 1/1.

There are problems associated with measuring P/R ratios of natural populations in the field, because the respiration rate of photosynthetic organisms cannot be separated from that of the nonphotosynthetic organisms (Ryther, 1956a). Ryther (1956a) using an indirect argument, suggests that P/R ratios may be as high as 20:1 in summer, but may fall to 5:1 or less in winter. He concludes that under winter conditions photosynthesis by the entire population is only able to compensate for its respiration on days of highest incident radiation.

Dark respiration in marine algae

Dark respiration is known to occur in a number of marine algae and as in terrestrial plants occurs in the dark and to a reduced extent in the light (Hoch et al., 1963; Forrester et al., 1966; Brown & Tregunna, 1967; Sargent & Taylor, 1972; Ried et al., 1973; Laing et al., 1974; Lloyd, 1974; Mangat et al., 1974; Peterson, 1980). Dark respiration can either be expressed as absolute rates of respiration, or in relative terms as percent net or gross photosynthesis, or as the ratio of photosynthesis to respiration (P/R ratio).

A number of authors have considered the potential importance of dark respiration in determining overall primary productivity. Steemann Nielsen and Jensen (1957) reported that 40 % of the gross photosynthesis in the ocean was lost in dark respiration. Eppley and Sharp (1975) found that in phytoplankton from oligotrophic waters, the ratio of incorporation of $^{14}CO_2$ to apparent dark loss of $^{14}CO_2$ over a

24 hour period was 1.3. Most of this dark loss was attributed to dark respiration. This low ratio indicates the importance of dark respiration in controlling primary productivity in nutrient deficient waters (oligotrophic waters) where phytoplankton have low growth rates. Finally, Ryther (1956a) and Eppley and Sloan (1965) have conducted experiments on a number of species under laboratory conditions. Ryther's experiments showed that in Dunaliella euchlora, 20 % of the carbon fixed was lost over 24 hrs in the dark, and that in Chlamydomonas, Nannochloris, and Nitzschia 10 to 40 % ^{14}C fixed in a 2 hour light period was lost in a succeeding 4 to 6 hrs in dark period. Eppley and Sloan (1965) studied 9 species of algae, measured net photosynthesis and dark respiration, and calculated P / R ratios that were between 6/1 to 10/1. These are fairly high ratios. In other words carbon fixed during photosynthesis was 6 to 10 times that lost during dark respiration. In spite of this, one species Dunaliella tertielecta showed dark CO_2 losses in 5 hrs which were from 36 to 46 % of the carbon fixed during the proceeding 5 hrs of light.

All of this work shows that dark respiration can be highly significant under field conditions, that P/R ratios in marine phytoplankton can range from 12/1 to 1/1, and that between 10 and 46 % of gross photosynthesis in the sea may be lost as dark respiration.

There are very few methods for measuring dark respiration. The current method for determining dark respiration in marine algae involves the measurement of O_2 consumption by an O_2 electrode (Burris, 1977). Although an earlier technique of Steemann Nielsen and Hansen (1959) can be used. This involves measuring the rates of photosynthesis by ^{14}C at different light intensities, plotting the two variables and extrapolating the rate of respiration. These authors used this technique to calculate respiration and subtracted it from gross production to obtain net production in natural phytoplankton.

Photorespiration in marine algae

Photorespiration has been described in a wide range of marine algae (Turner et al., 1956; Tolbert, 1974; Black et al., 1976; Burris et al., 1976; Burris, 1977), although there is some debate whether it occurs in all species (Fock et al., 1971; Beardall & Morris, 1975; Lloyd et al., 1977; Bidwell, 1977, Burris, 1980)

There are obvious difficulties in measuring photorespiration. This is because photosynthesis, dark respiration and photorespiration (light respiration) occur at the same time, and between them the three processes take up or release of O_2 and CO_2 (Goldsworthy, 1976; Zelitch, 1968; Jackson & Volk, 1970; Zelitch, 1971; Burris, 1980; Halliwell, 1981). Indirect methods are therefore used both for terrestrial plants and for freshwater and marine algae. Furthermore Halliwell (1981) recommends always using two methods when assessing the effects of environmental parameters on photorespiration. There are a number of indirect methods of which I shall describe three (Burris, 1980; Halliwell, 1981). The first relies on measuring the inhibition of photosynthesis produced by high O_2 concentrations in the environment, sometimes called O_2 inhibition of photosynthesis; the second uses postillumination "bursts" of CO_2 production or of O_2 consumption, and the third measures decreases in the photosynthetic quotient.

Method 1. O_2 Inhibition of photosynthesis

This method depends on the progressive inhibition of photosynthesis as the O_2 concentrations in the environment increases. Photosynthetic rates are measured at different concentrations of O_2 in the external medium. The progressive inhibition of photosynthesis at increasing concentrations of O_2, so called Warburg effect (Warburg,

1920) is assumed to be an increase of photorespiration. This effect is reviewed in Jackson and Volk (1970), Ludlow and Jarvis (1971), Burris et al., (1976), and Burris (1980) and has been used to measure photorespiration in marine algae by Turner et al. (1956), Black et al. (1976), Burris et al. (1976), and Burris (1977). Turner et al. (1956) measured rates of photosynthesis at different O_2 concentrations and demonstrated that high concentrations of O_2 inhibit photosynthesis. Burriss et al. (1976) measured incorporation of $^{14}C_2$ into glycine and serine, the intermediates of the glycollate pathway, in 6 marine plants under different O_2 concentrations as estimates of photorespiration. Burris (1977) showed that the rates of photosynthesis were lowest at high O^2 intensities in 5 marine algae. However some caution is needed because Bidwell (1977) and Lloyd et al (1977) have been unable to demonstrate the effect with some marine species.

Method 2. Post-illumination "bursts" (PIB) of CO_2 production or of O_2 consumption

The principle behind this method is that when a plant is transferred from the light to the dark there is a rapid increase in CO_2 release which lasts for 1-2 mins gradually falling to the rate characteristic of dark respiration. The size of this CO_2 "burst" is increased by increasing the light intensity or temperature or by decreasing the O_2 concentration before darkening the plant (Decker, 1955, 1959; Burris, 1980; Halliwell, 1981). This leads to the assumption that the burst is a short continuation of photorespiration in the dark which arises by the breakdown of residual glycolate and compounds derived from glycollate such as glycine and serine to CO_2. Since these substrate pools are exhausted very quickly the "bursts" only last for a short time. The burst is followed by O_2 consumption and CO_2 production at a steady rate; this is dark respiration.

Photorespiration is the difference between the steady state rate of dark respiration and the rate of the "burst" (Zelitch, 1971). For example Downton et al. (1976) showed an increased burst of O_2 consumption during the postillumination period in marine algae. The "burst" was seen to increase at higher concentrations of O_2. Burris (1977) demonstrated postillumination bursts of O_2 consumption and CO_2 production in 6 marine algae.

There is however an important objection to the use of the method. The residual pool of the chemicals in the leaf need not be related to the rate at which carbon is passing through them (Halliwell, 1981, p. 149). However, although the pool size of glycollate is very small, that of glycine and serine can be significant. In spite of this objection results from the PIB method correlate fairly well with photorespiration rates obtained by other methods, and it is easy to perform. The PIB method and criticism of it are described clearly by Halliwell (1981).

Method 3. Photosynthetic quotient (PQ)

In this method the photosynthetic quotient (O_2 produced / CO_2 consumed) is measured at increasing O_2 concentrations or decreasing CO_2 concentrations. Although the method has not been widely used, it is in principle a useful approach, because during photorespiration O_2 is consumed and CO_2 produced. This means that the photosynthetic quotient decreases as photorespiration increases. This is because the O_2 produced during photosynthesis will be reduced by increased O_2 consumption caused by photorespiration, and similarly the CO_2 consumed during photosynthesis will be decreased by CO_2 production during photorespiration. Hence the numerator of PQ will decrease and the denominator will increase, as photorespiration increases, so the PQ

decreases as photorespiration increases. Since photorespiration increases at increased O_2 concentrations and at decreased CO_2 concentrations, the PQ, will decrease and be an estimate of increased photorespiration.

Several marine algae show a trend in which the photosynthetic quotient drops under increased O_2 concentrations and lowered CO_2 concentrations (Fock et al., 1968, 1969, 1971; Burris, 1980).

In addition two other methods which are less frequently used have been reported. For example Birmingham et al. (in Burris, 1980) measured photorespiration as the differnce between true and apparent photosynthesis. Coughlan and Tattersfield (1977) used a modification of Zelitch's (1968) method in which the $^{14}CO_2$ used in fixation is allowed to release as $^{14}CO_2$ into $^{14}CO_2$-free air. The rate of $^{14}CO_2$ release in the light versus the rate of release in the dark is an indicator of the amount of photorespiration

C_3 and C_4 photosynthesis

All that I have described so far occurs in what are called C_3 plants. There is, however, another group of plants termed C_4 that are found in the tropics. C_3 plants get their name from the three-carbon compound, phosphoglyceric acid (PGA) which is produced in the Calvin cycle during photosynthesis. The C_4 plants obtain their name from the four-carbon compound, oxalacetate which is formed when CO_2 and phosphoenolpyruvate (PEP) combine in the mesophyll cell during the Hatch-Slack pathway (which is an alternate route of CO_2 fixation, see below). C_4 plants do not undergo photorespiration in the light and show a slightly different photosynthetic pathway. The result of this is that primary production by C_4 plants can occur at much lower CO_2 concentrations and is not inhibited by increasing O_2 concentrations. In addition, unlike C_3 plants CO_2 uptake leading to photosynthesis by

C_4 plants is not affected by temperature.

The biochemistry of the C_4 photosynthesis is interesting. It is separated into 2 linked cycles, one in the mesophyll cells and the other in the bundle-sheath cells. CO_2 enters the cycle by combining with the three-carbon compound (C_3) phosphoenolpyruvate (PEP) to form a four-carbon compound (C_4). The enzyme that catalyses this carboxylation, unlike ribulose 1,5-bisphosphate carboxylase, is not inhibited by high O_2 concentrations. In other words O_2 does not compete with CO_2 for the active site on the enzyme. The C_4 compound then enters the bundle-sheath cell and is oxidised by $NADP_{ox}$ ----> $NADP_{re}$, thus forming CO_2 and C_3 compound PEP. The CO_2 enters the Calvin cycle in the normal way and is reduced to C_6 glucose; this occurs in the bundle-sheath cells. The C_3 compound PEP reenters the mesophyll cell and then recombines with atmospheric CO_2 to form more C_4. This is sometimes called the Hatch-Slack pathway of C_4 photosynthesis.

It is important to note that in both C_3 and C_4 plants the final step in the assimilation of CO_2 into carbohydrate is by the Calvin cycle. In C_3 plants the Calvin cycle is the only pathway of CO_2 fixation. In C^4 plants there is a preliminary fixation step which is not sensitive to O_2 concentration. C_4 plants are therefore more suited to conditions of high temperature and intense light than are C_3 plants. C_4 photosynthesis has evolved independently in a number of unrelated tropical plant groups and is an excellent example of natural selection and adaptation to environment.

The following two equations give the overall reaction for the synthesis of hexose in the C_3 and C_4 plants (Lehninger, 1975).

C_3 plants;

$$6CO_2 + 18ATP + 12NADPH + 12H^+ + 12H_2O \longrightarrow$$
$$\text{hexose} + 18ADP + 18P_i + 12NADP^+$$

C_4 plants;

$$6CO_2 + 30ATP + 12NADPH + 12H^+ + 24H_2O \longrightarrow$$
$$\text{hexose} + 30ADP + 30P_i + 12NADP^+$$

where ADP = adenosine diphosphate; ATP = adenosine triphosphate;
NADPH = nicotinamide adenine dinucleotide hydrogen phosphate;
NADP = nicotinamide adenine dinucleotide phosphate; H^+ = proton;

Note: In some books these abbreviations apply,

NADPH = $NADP_{re}$ = $NADP_{reduced}$ (reduction is defined as, either addition of H^+ or electrons)

NADP = $NADP_{ox}$ = $NADP_{oxidised}$ (oxidation is defined as, either removal of H^+ or electrons)

P_i = inorganic phosphate; hexose = a six-carbon sugar;

Having said all this it is not clear whether C_4 photosynthesis occurs in marine plants.

Photoinhibition

The phenomenon of photoinhibition has been known for many years (Rabinowitch, 1951; Steemann Nielsen, 1952b). As light intensity increases from low levels, the rate of photosynthesis increases to a maximum (P_{max}). The light intensity at P_{max} is termed the saturation light intensity (I_{sat} or I_{max}) (Ryther,1956b, p. 62; Strickland, 1960, p. 115). At light intensities greater than I_{sat} the photosynthetic rate falls again. This fall is termed photoinhibition. Photoinhibition has been demonstrated in a large number of freshwater and marine phytoplanktonic species (Ryther, 1956b; Ryther & Menzel, 1959; Steemann Nielsen & Hansen, 1959; Armitage & House, 1962; Goldman et al., 1963; Ganf, 1975; Belay & Fogg, 1978; Belay, 1981).

The photosynthetic rate P_I at a given light intensity I is sometimes measured as a proportion of the maximum rate of photosynthesis, P_{max}. Obviously, P_I is always less than or equal to P_{max}, ($P_I \leq P_{max}$). The ratio P_I / P_{max} is termed relative photosynthesis by Ryther (1956b) and f by Strickland (1960, p.115). f = 1 when $P_I = P_{max}$, that is at I_{sat}; f is less than 1 at light intensities which are lower than or higher than I_{sat}.

Smith (1936) introduced an empirical equation that related the photosynthetic rates (P_I) and (P_{max}), to light intensity (I),

$$K.I = P_I / (P^2_{max} - P_I^2)^{\wedge}1/2$$

where K is a constant. This equation can be used to describe the complex curve that relates I to P_I (Winokur, 1948; Talling, 1957).

In general, photoinhibition occurs in surface waters when the incident visible radiation exceeds about 0.1 $cal.cm^{-1}.min^{-1}$ $\simeq$ 17000 lux (17 klux) of photosynthetically active light (Belay & Fogg, 1978).

Cellular and biochemical mechanisms of photoinhibition

There appear to be 3 sequential responses to increasing light intensities above I_{sat} (Goldman et al., 1963). The first is a drop in photosynthetic rate and involves the inactivation of either light or dark reactions or both. It is not affected by temperature and is reversible (Kok, 1956; Zurzycki, 1957; Steemann Nielsen, 1962). The second occurs just before pigment breakdown, and may involve chloroplast proteins. This is also a reversible reaction and is oxygen-dependent (Aach, 1954; Sironval & Kandler, 1958). Finally, the third response is a photolytic breakdown of the photosynthetic pigments chlorophyll and carotene (Sironval & Kandler, 1958).

Satoh (1970 a,b,c) has conducted a detailed investigation of photoinactivation at moderate light intensities which approximate to those in situ, and has demonstrated 2 types of photoinhibition operating at different points in the photosynthetic machinery of the chloroplast. The first disturbs an early step of photosystem I, is oxygen-dependent and is partially prevented by the presence of photosynthetic inhibitors such as 3-(4'-chlorophenyl)-1,1-dimethylurea (CMU), 3-(3',4'-dichlorophenyl)-1,1-dimethylurea (DCMU) and o-phenanthroline. The second inactivates photosystem II, is oxygen-independent, and is not inhibited by photosynthetic inhibitors.

Photoinhibition and light wavelength

A number of authors have tested the effects of different wavelengths of light on the degree of photoinhibition. Ultraviolet light causes photoinhibition in planktonic algae that are adapted to low light intensities (Steemann Nielsen, 1964; Belay, 1981). Near infrared energy can also cause photoinhibition when it is absorbed by photosynthetic pigments (Govindjee et al., 1961). Visible light which remains after absorption by chloroplasts is also destructive (Goldman et al., 1963).

Ecological effects of photoinhibition

The light intensity at the surface of natural waters is often high enough to cause photoinhibition. For example Armitage and House (1962) showed that photosynthetic rates were higher below the surface of antarctic Lakes and attributed this to photoinhibition of cells in the surface layers. In a similar investigation, Stadelmann et al. (1974) found that photoinhibition occurring in the surface layers of Lake Ontario was associated with high light intensities. These authors also noted that in surface layers, lower photosynthetic rates occurred in the afternoon than in the morning at the same light intensity.

Phytoplankton living at high light intensities such as those at the surface of the sea can show adaptation to the intensities there (see footnote). At all light intensities, they photosynthesise at higher rates and show a higher P_{max} and I_{sat} than do deeper living forms (Hellebust, 1970; Belay, 1981). For example, Ryther and Menzel (1959) demonstrated adaptation of marine phytoplankton at 3 depths in a stratified water column during summer. The surface plankton showed a higher P_{max} and I_{sat} than the subsurface phytoplankton when exposed to a range of light intensities (i.e. had a lower P_{max} and I_{sat}). This indicates that in a stratified and stable water column the phytoplankton living at different depths become adapted to the light intensities found there. Steemann Nielsen and Hansen (1959) reported

Footnote: There are in principle 2 ways of detecting adaptation. The first is by collecting phytoplankton from different depths of the water column which will therefore have different light intensities. Both populations are then exposed to a range of light intensities. The sample which comes from the higher light intensity/shallower depth will show a higher I_{sat} and also a higher P_{max} than the samples taken from lower light intensity. The second is to take a laboratory culture of a phytoplankton species, divide it into 2 samples, and expose 1 sample to a high light intensity and the other to a low light intensity, both for the same length of time. Each of the sample is then exposed to a range of light intensities. As in the field example, the population that had been previously exposed to the high light intensity will show a higher I_{sat} and a higher P_{max} than the sample previously exposed to the low light intensity.

similar observations in vertically stable water columns which showed that marine phytoplankton were adapted to the light intensity at the depth at which they lived. Ryther and Menzel (1959) also studied an unstratified water column in winter and showed that the phytoplankton from all the 3 depths became fully light saturated at the same light intensity (5000 foot candles). Ryther and Menzel (1959) interpreted this as showing that in a well mixed water column the phytoplankton are circulated continuously throughout the water and do not have enough time to adapt to the light intensity at any particular depth.

Phytoplankton can recover from photoinhibition (Hellebust, 1970). In other words if the stress of high light intensity is removed, the cells recover and eventually photosynthesise at their original rate. This recovery can take place at low light intensities or in the dark (Belay & Fogg, 1978). The recovery time depends on the time of exposure and on the previous light conditions (Belay & Fogg, 1978). For example Goldman et al. (1963) showed that antarctic freshwater phytoplankton exposed to half hour light recovered in about 3 h, while those exposed for 1 h recovered in 6 h. Similarly, Belay (1981) demonstrated recovery times of 2 h and 20 h for freshwater phytoplankton which were exposed to bright sunlight for 2 h and 6 h respectively. This means that phytoplankton carried to the surface during upwelling will take longer to recover under conditions of bright sun light than when the sky is overcast.

Finally, there are sometimes major differences between the effects of photoinhibition on different taxonomic groups of phytoplankton. Ryther (1956b) plotted relative photosynthesis against light intensity for three groups of marine phytoplankton: green algae, diatoms and dinoflagellates. The organisms within each group behaved very similarly, but there were striking differences between the groups

(loc.cit. p 64). The green algae showed a saturation light intensity of 500-750 foot candles, the diatoms 1000-2000 foot candles, and the dinoflagellates 2500-3000 foot candles. Ecologically this means that the dinoflagellates will be most resistant to high light intensities, the diatoms intermediate, and the green algae least resistant. Other studies have shown that diatoms taken from different localities at different times of the year showed different rates of photoinhibition (Harris, 1973; Harris & Piccinin, 1977).

PRIMARY PRODUCTION AND ITS MEASUREMENTS — Appendix 1.2

Primary production (primary productivity) is the rate of production of new organic material by photosynthetic organisms (phytoplankton, macroalgae) and is expressed symbolically in equation (1) as carbohydrate $(H_2CO)_n$. It is sometimes also called carbon assimilation or carbon fixation. The units of primary production are weight of carbon fixed per unit volume or unit area of seawater or sediment, per unit time (for example $mgC.m^{-3}h^{-1}$, $mgC.m^{-3}day^{-1}$, $mgC.m^{-2}day^{-1}$, and $gC.m^{-2}yr^{-1}$) (Goldman & Wetzl, 1963; Raymont, 1963; Goldman, 1966; Vollenweider, 1969; Russell-Hunter, 1970; Unesco, 1973; Lieth & Whittaker, 1975; Morris, 1980; Harris, 1986; Meadows & Campbell, 1988).

It is important to distinguish between gross and net primary production. Gross primary production is the total production of organic material by photosynthetic organisms. Net primary production is that part of gross primary production that remains after some of the organic material has been used in respiration by the photosynthetic organisms (Whittaker et al., 1975). In other words it is the gross production minus the organic material used in respiration (Hall & Moll, 1975). For example in coastal waters, if the gross primary production is 120 $gC.m^{-2}yr^{-1}$ and respiration uses 20 $gC.m^{-2}yr^{-1}$, then the net primary production is 100 $gC.m^{-2}yr^{-1}$ (Ryther, 1969).

Net primary production is usually measured by one of two methods: the rate of O_2 production during photosynthesis or the rate of ^{14}C incorporation into organic matter during photosynthesis. In both methods parallel samples are incubated in the light and dark; this is often called the light and dark bottle method because clear and dark bottles are used. Photosynthesis and respiration occurs in the light

bottle and respiration by photosynthetic and nonphotosynthetic organisms as well as chemoautotrophic fixation occurs in the dark bottle (Strickland, 1960; Thomas, 1961; Vollenweider, 1969; Sestak et al., 1971; Strickland & Parsons, 1972; Unesco, 1973; Hall & Moll, 1975; Peterson, 1980).

Photosynthesis:

$$nCO_2 + nH_2O \longrightarrow (H_2CO)_n + nO_2 \qquad (1)$$

Respiration:

$$nO_2 + (H_2CO)_n \longrightarrow nCO_2 + nH_2O \qquad (2)$$

O_2 method

The method is the same in principle for photosynthetic organisms in seawater (phytoplankton) or in sediment (phytobenthos). Three bottles are set up, two of which are clear and one of which is dark. Each contains seawater with phytoplankton or membrane filtered seawater to which sediment is added. The O_2 concentration in one of the clear bottles is measured immediately. The remaining two are termed the light and dark bottles and are incubated in the light (see below). At the end of the incubation time the O_2 concentration of both bottles is measured. The O_2 concentration in the dark bottle has decreased due to respiration. The O_2 concentration in the light bottle has increased due to photosynthesis in spite of the respiration that also takes place. The O_2 produced and consumed are then calculated as follows (data modified from Czaplewski & Parker, 1973; Hall & Moll, 1975).

Net oxygen production due to photosynthesis = LB - IB

$$= 8.40 - 8.05 = 0.35 \text{ ppm } O_2 . \text{ hr}^{-1}$$

Respiration = IB - DB

$$= 8.05 - 8.00 = -0.05 \text{ ppm } O_2 . \text{ hr}^{-1}$$

Gross oxygen production due to photosynthesis = LB - DB

$$= 8.40 - 8.00 = 0.40 \text{ ppm } O_2 . \text{hr}^{-1}$$

where IB = O_2 concentration (ppm) in the unincubated clear bottle

LB = " " " " " incubated clear bottle

DB = " " " " " incubated dark bottle

In order to find the gross and net primary production and respiration, two relationships are needed. Firstly, the oxygen produced during photosynthesis is related to the CO_2 - carbon fixed or assimilated into organic material during photosynthesis. Secondly, the oxygen consumed during respiration is related to the carbon released as CO_2 during respiration. These two relationships are given by the photosynthetic quotient (PQ) and the respiratory quotient (RQ) both of which are dimensionless (Strickland & Parsons, 1972).

$$PQ = \frac{\text{Molecules of } O_2 \text{ liberated during photosynthesis}}{\text{Molecules of } CO_2 \text{ assimilated during photosynthesis}}$$

$$RQ = \frac{\text{Molecules of } CO_2 \text{ liberated during respiration}}{\text{Molecules of } O_2 \text{ consumed during respiration}}$$

The PQ is then used with the O_2 light and dark bottle measurements to calculate gross primary production and with the O_2 light and initial bottle measurements to calculate the net primary production.

The RQ is used with the O_2 initial bottle and the dark bottle measurements to calculate respiration. (Details are given in Strickland & Parsons, 1972 pp. 261-266).

^{14}C method

As in the O_2 method, the ^{14}C method is the same in principle for photosynthetic organisms in seawater or in sediment. Two bottles are set up one of which is clear and one of which is dark. Each contains seawater or membrane filtered seawater to which is added sediment. ^{14}C as carbonate or bicarbonate is added to both. The bottles are then incubated in one of 3 ways.

(i) The in situ method. The bottles are suspended in the sea at the sampling point.

(ii) The simulated in situ method. The bottles are incubated under natural light on board ship.

(iii) The incubator method. The bottles are incubated under artificial light in the laboratory. I used this method.

Incubation times usually range from 2 to 6 hours, during which time ^{14}C fixation occurs. The ^{14}C fixation in the two bottles is measured at the end of the incubation period. If primary production in seawater is being measured, the seawater containing the organisms is filtered onto a membrane filter, and the membrane filter and organisms are counted in a liquid scintillation counter. If primary production in sediment is being measured, the sediment is filtered off and counted in a liquid scintillation counter.

^{14}C fixation in the light bottle measures light fixation (fixation by photosynthetic organisms). The ^{14}C fixation in the dark bottle measures dark fixation (fixation by chemoautotrophic organisms) and also light-initiated fixation by photosynthetic organisms.

The carbon assimilated by the photosynthetic organisms is

calculated as follows. The total amount of CO_2 in the seawater used for incubation is obtained by a series of calculations after having measured the *in situ* temperature and salinity, the laboratory temperature, and the pH before and after acidification. The amount of ^{14}C added as $^{14}CO_2$ at the beginning of the incubation period is known. The $^{14}CO_2$ incorporated by photosynthetic organisms during photosynthesis is obtained by subtracting the dark bottle ^{14}C value from the light bottle value. The resultant figure is then multiplied by the ratio of the total CO_2 in the seawater sample to the ^{14}C added as $^{14}CO_2$, which gives the carbon assimilated during photosynthesis (Thomas, 1961; Schlieper, 1972; Strickland & Parsons, 1972; Unesco 1973; Bougis, 1976).

There is some debate about whether the ^{14}C method measures net or gross primary production, or some intermediate value. Ryther (1956a) showed that *Dunaliella* lost no ^{14}C in 24 hours in the light but respired 20% in the 24 hours of darkness, thus indicating that respired $^{14}CO_2$ was reassimilated by photosynthesis with 100% efficiency. He thus concluded that ^{14}C measures net photosynthesis. On the other hand Steemann Nielsen and Al Kholy (1956) compared the ^{14}C and the oxygen techniques using phosphorus and nitrogen deficient algae. Their results showed that after correcting for respiration, ^{14}C compared exactly with gross photosynthesis. Later Antia *et al.* (1963) proved by their experiments that during diatom blooms in coastal waters the ^{14}C method measured the net production of particulate matter whereas the O_2 method measured the gross production of organic material. Hobson *et al.* (1976) have conducted a field study which showed that the ^{14}C technique only measures net production when cells are exposed for 24 hours to optimal irradiation and nutrient concentrations. These authors suggest that in temperate latitudes

during summer the incubation should be carried out for about 48 hours.

It is therefore open to question as to whether the ^{14}C method measures gross or net primary production. However most authors favour the view that the method gives production estimates that are closer to net than to gross primary production. This complicated issue is well reviewed by Peterson (1980).

Sources of error in the ^{14}C method

There are several potential sources of error in the ^{14}C method. Five of these are described below. Only the first two are normally corrected for.

(a) Dark fixation

Some of the $^{14}CO_2$ incorporated into organic matter occurs by non-photosynthetic routes in the cells of chemosynthetic microorganisms and is called dark fixation. It occurs in the light and in dark. Taguchi and Platt (1977) have used a blank for the measurement of dark fixation by introducing a second dark bottle to which 0.2 mg of mercuric chloride is added. The dark fixation of $^{14}CO_2$ is the difference in activity between the dark bottle without mercuric chloride and the bottle with mercuric chloride. Some of the dark fixation however could be due to light-initiated dark reactions in photosynthetic organisms. To test this possibility the samples should be kept in the dark for various time periods before dark incubation with ^{14}C (Taguchi & Platt, 1977).

The incorporation of $^{14}CO_2$ into organic matter by microorganisms in the dark means that these organisms utilize chemical energy from reduced inorganic compounds and are therefore chemoautotrophs (Stanier et al., 1977). The activity of these microorganisms is usually less than 5% of the light fixation but may reach 50% in

oligotrophic waters (Bougis, 1976). Dark fixation increases significantly with temperature (Brouardel, 1973) and the ratio between dark and light fixation increases with decreasing cell density (Morris et al., 1971).

The rate of chemoautotrophic bacterial incorporation of $^{14}CO_2$ may be significantly greater in the dark than in the light. For example light in the visible range is lethal to nitrifying microorganisms - which are chemoautotrophic (Muller-Neugluck & Engel, 1961; Schon & Engel, 1962). Tuttle and Jannasch (1977) demonstrated an increased dark $^{14}CO_2$ fixation in the presence of sodium thiosulphate. This acts as a chemical energy source for chemoautotrophic activity.

Parsons et al. (1984) have erroneously referred to dark fixation as heterotrophic activity. This is incorrect because the principal carbon source in heterotrophic activity is organic (Stanier et al., 1977, p. 34) and the carbon source used in dark fixation is inorganic $^{14}CO_2$.

The dark fixation is corrected for by subtracting the dark bottle fixation from the light bottle fixation.

(b) Differences in uptake rates of ^{14}C and ^{12}C

Radioactive $^{14}CO_2$ is slightly heavier than $^{12}CO_2$ and so is taken up slightly more slowly; the difference is 5 to 6 % (Steeman Nielsen, 1955). It has been called an isotope discrimination factor (Peterson, 1980) and means that $^{14}CO_2$ is fixed at about 95 % of the fixation rate of $^{12}CO_2$. A correction factor of 1.05 or 1.06 (100/95) is therefore included in the calculation of primary production.

(c) Respiratory loss and reassimilation of $^{14}CO_2$

There is some debate about the loss of $^{14}CO_2$ by respiration. The $^{14}CO_2$ which is fixed during photosynthesis can be respired as $^{14}CO_2$ and reassimilated either within the cell (endogenous reassimilation) or from the external medium (exogenous reassimilation) (Weigl et al.,

1951; Jackson & Volk, 1970; Raven, 1972; Harris, 1978). However it is not clear what proportions are reassimilated by two routes (Thomas, 1961; Raven, 1970, 1972, p 1009,1010).

If all the $^{14}CO_2$ is reassimilated then the in situ $^{14}CO_2$ technique measures net photosynthesis. If only part of the $^{14}CO_2$ is reassimilated then the technique measures a rate somewhere between the net and gross rate of photosynthesis. If none of the $^{14}CO_2$ is reassimilated the technique measures the gross rate of photosynthesis (Ryther & Vaccaro, 1954; Ryther, 1956a; Strickland, 1960; Thomas, 1961). Macroalgae reassimilate more at low O_2 levels (Meidner, 1962; Brown & Tregunna, 1967; Downton & Tregunna, 1968; Bidwell et al., 1969). In $^{14}CO_2$ experiments, unicellular algae reassimilate only part of the $^{14}CO_2$ that is released (Brown & Weis, 1959; Weis & Brown, 1959; Bunt, 1965; Raven, 1972). Harris and Piccinin (1977) found that $^{14}CO_2$ uptake measures gross photosynthesis over 10 minute incubations at light intensities below I_K.

Thomas (1961) has introduced sets of respiratory correction factors to obtain net or gross photosynthesis at 3 light intensities. This correction becomes very important at low light intensities, when the P/R ratio decreases. The raw ^{14}C data is multiplied by 1.15, 1.25 and 2.0 to give a rough estimate of gross photosynthesis, or by 1.05, 1.05 and 1.0 to give a rough estimate of net photosynthesis.

(d) Release of dissolved organic carbon by phytoplankton

Losses of ^{14}C by the release of dissolved organic carbon from phytoplankton may lead to underestimates of primary production (Gieskes & van Bennehom, 1973). Marine and freshwater phytoplankton are known to excrete dissolved organic carbon - for example as mucus (Fogg, 1958; Hellebust, 1965; Thomas, 1971). Anderson and Zentschel (1970) measured ^{14}C incorporated into dissolved organic material in

the filtrate during light and dark bottle experiments. These authors found that it was 10 to 20% of the amount incorporated into particulate material (i.e. living cells) retained on the filter, and so the effect may be highly significant. Furthermore Hellebust (1965) has shown that algae exposed to direct sunlight may have very high excretion rates - possibly because of cell damage by photo-oxidation. Dissolved organic carbon may also be released by breakage of cells during incubation in the light and dark bottles and during subsequent filtration although this latter effect can be reduced if the vacuum does not exceed 1/4 to 1/3 atm. (Strickland & Parsons, 1972).

The organic carbon lost in this way can be estimated by measuring the dissolved organic carbon in the filtrate. First the unused ^{14}C in the filtrate is removed by the method of "stripping". 5 ml of the filtrate is acidified with 0.2 ml of 3% H_2PO_4, the solution is then bubbled with N_2 gas which removes the active $^{14}CO_2$. The dissolved organic ^{14}C activity which is retained in the solution is measured using a scintillation counter (Schlinder et al., 1972; Unesco, 1973; Parsons et al., 1984).

(e) Retention of dissolved ^{14}C by membrane filters

There are a number of ways in which dissolved inorganic ^{14}C could be retained by membrane filters. This unfixed ^{14}C could adsorb to or absorb into the filter (McMohan, 1973). It could bind to other substances which would be retained on the filter (Nalewajko & Lean, 1972) or could precipitate inorganically in the presence of iron (Goldman & Mason, 1962).

Dissolved ^{14}C on membrane filters may also come from cell rupture during filtration (Lasker & Holmes, 1957; Guillard & Wangerky, 1958; McAllister, 1961; Kuenzler & Ketchum, 1962; Arthur & Rigler, 1967; Nalewajko & Lean, 1972; Strickland & Parsons, 1972; McMohan, 1973). However McMohan (1973) considers that much of this can be attributed

to absorption of unfixed ^{14}C to unknown substances in water. In carefully controlled experiments McMohan (1973) showed that this category of ^{14}C can be eluted by washing the filter with at least 100 ml of water and advises this as a routine step. Hall and Moll (1975) suggest fuming the filter pads over HCl fumes in a dessicator for 2 hours. The HCl fumes convert all $^{14}CO_3^{2-}$ or $H^{14}CO_3^{1-}$ to $^{14}CO_2$ which diffuses off the filter pads.

Productivity Index

The standing crop or the short-term net primary production of a phytoplankton population may give little indication of its production capacity. Sometimes a large crop may photosynthesise at a low rate while a small crop may photosynthesise at a high rate (Verduin, 1956). This problem is particularly important when comparing temporally or geographically separated populations of phytoplankton, or comparing the relative fertility of water masses and their endemic populations. The problem is partly resolved by using an index of productivity.

The Productivity Index (PI) relates net primary production at a standard light intensity to primary standing crop, and is expressed as primary production per unit standing crop. The accepted standard light intensity is one langley.min^{-1} (= 1 cal.cm^{-2}. min^{-1}).

$$PI = \frac{\text{primary production (at unit light intensity)}}{\text{standing crop}}$$

$$= \frac{dp}{dt} \times \frac{1}{p} \text{ at unit light intensity}$$

where dp/dt = primary production (mg C. m^{-3}. hr^{-1}), p = standing crop (mg C.m^{-3}). The units of the productivity index are therefore h^{-1} x (langley.min^{-1})$^{-1}$. If h is converted to min, the units become min^{-1} x (langley.min^{-1})$^{-1}$, which equals cm^{2}.cal^{-1} (Strickland, 1960).

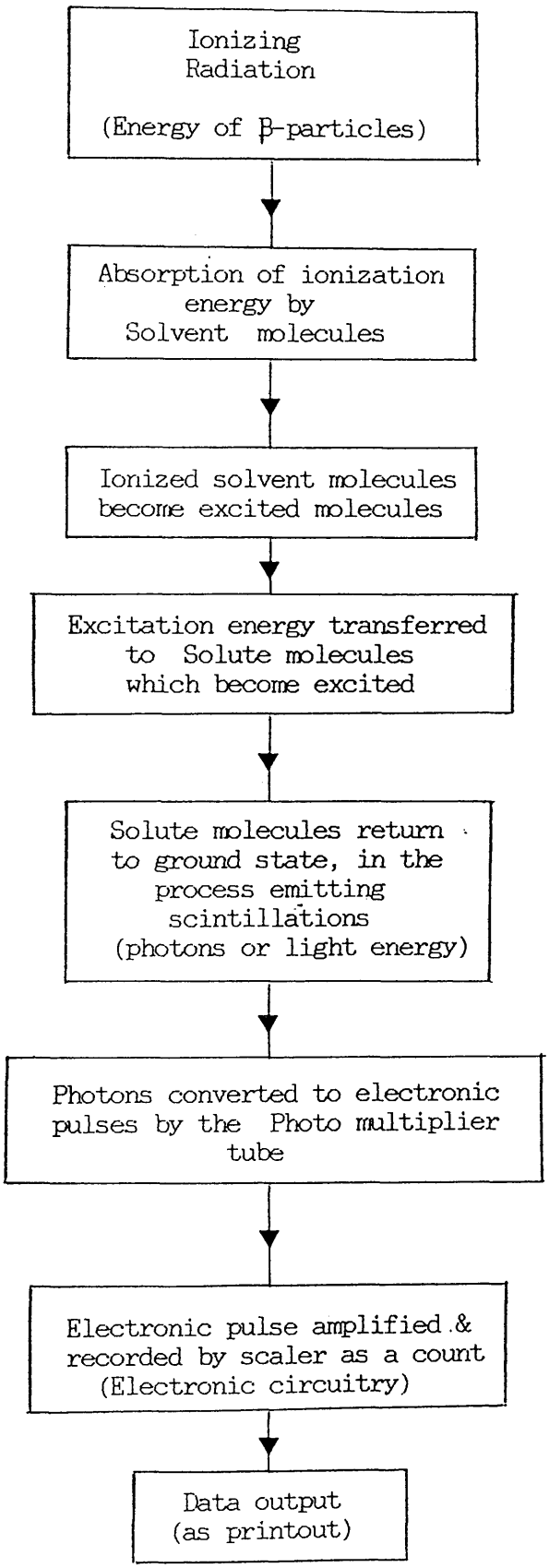
Productivity indices are often fairly constant with depth in the euphotic zone, but are greater in oceanic areas than in coastal zones (Currie, 1957). The productivity index for most waters lies in the range of 0.1 to 5 h^{-1} (ly.min^{-1})$^{-1}$. If in a given area the productivity index is constant with depth and location, primary production can be calculated from the standing crop, since primary production = standing crop x PI (Strickland, 1960). This is useful, because measuring the standing crop of a field population is quicker and easier than measuring its primary production.

PRINCIPLE OF LIQUID SCINTILLATION COUNTING — Appendix 1.3

The principle of Liquid Scintillation is that some organic substances can absorb the energy of bombarding beta particles from a radioactive source and transform the energy into light photons. These photons or scintillations in turn are detected and converted to electrical pulses by a photomultiplier tube.

The sequence of events is as follows. The energy of the beta particle emitted from a radioactive source is first absorbed by molecules of the solvent which in turn become excited. This excitation energy is then transferred to the scintillator (solute or phosphor) - causing the scintillator molecules to become excited. When the scintillator molecules return to their ground state they emit photons (light). The photons enter the photomultiplier tube giving rise to an electronic pulse, which can be amplified and recorded by a scaler (part of the instrument which records the total number of pulses). The height of the pulse coming from the photomultiplier tube will depend on the number of photons emitted from the scintillator molecules, which in turn will depend on the efficiency with which the solvent excitation energy is transferred from one molecule to another. In principle the pulse height is a function of the energy of the beta particle. The term "liquid scintillation counting" means that the scintillators are usually dissolved in a suitable solvent containing the radioactive material that is being counted. The counting process can be affected by interfering factors such as quenching , chemiluminescence, and by spurious counts from the solute and the walls of the container (Lambie, 1964; Horrocks, 1970; Stubbs, 1973; Dyer, 1974; Kobayashi & Maudsley, 1974; Chapman & Ayrey, 1981).

The following flow chart shows the sequence of events that take place during Liquid Scintillation Counting.



Quenching

Quenching is a phenomenon that reduces the light output (photon production) in the system. This loss in photon production causes a reduction of pulse height.

There are 4 different kinds of quenching.

a) Self quenching

Self quenching occurs when the concentration of the solute exceeds a certain level and causes a reduction in counting efficiency (Dyer, 1974).

b) Impurity quenching

Many radioactive samples are insoluble in solvents like toluene or xylene, and so a secondary solvent or a solubilizer is used to blend the sample with or suspend the sample in the scintillant. The addition of any substance to the 'ideal' scintillant reduces the scintillation efficiency (number of photons emitted per particle) by competing with the primary solute for energy transfer. This phenomenon is called impurity quenching. Substances such as methanol or ethanol have a very mild effect while chloroform and carbon tetrachloride can reduce counting efficiencies drastically (Chapman & Ayrey, 1981).

c) Colour quenching

Colour quenching occurs when a sample contains coloured materials. Coloured materials absorb the light emitted by the solute and prevent it from reaching the photomultipier tube (Chapman & Ayrey, 1981).

d) Photon quenching

If a cocktail contains refractory substances then a heterogeneous counting mixture is obtained. This prevents maximum interaction between the beta energy from ^{14}C decay and the solvent-solute mixture, and is called photon quenching (Dyer, 1974).

Quench correction methods:

There are 3 common methods of correcting for quenching.

1) Internal standardization

In this method the sample is counted alone , then a specific amount of a nonquenching radioactive standard is added and the sample is recounted. A comparison of the count before and after the radioactive standard is added, allows the evaluation of the correction factor for quenching. The counting efficiency of the quenched sample is determined as follows:

Efficiency = (netCPM(int.std.+sample) - netCPM sample) / DPM int.std.

The disadvantages of this method are that double counts are needed, the standard needs dispensing very accurately, and the standard may contaminate the sample. In spite of the disadvantages, the internal standard method is accepted for heterogeneous samples - in particular for highly quenched samples.

2) Sample Channels Ratio method (SCR) (pulse-height shift method)

This method is based on the principle that the energy of the beta pulse spectrum decreases with increasing quenching and so the entire beta spectrum shifts towards a lower energy level (Wang & Willis, 1965; Chapman & Ayrey, 1981). The outline of the method is as follows.

The counter is set to give 2 pulse windows. The first channel, channel 1, covers all beta particles having energies from 0 to 156 Kev and the second channel, channel 2 is set to cover all beta particles having energies from 50 to 156 Kev. This method needs a calibration curve using $\frac{dn}{\lambda}$ internal standard.

A series of vials is then prepared. Each one contains the same activity of radioactive standard and the same amount of scintillator but different concentrations of quenching agent. The samples are

counted and any shift in the spectrum is detected by a change in the ratio of the counts in the two channels. A calibration curve is obtained by plotting the sample channels ratio (R), where R is

R = (net counts in channel 2) / (net counts in channel 1),

against efficiency (E):

E = (net count rate in channel 1) / DPM of standard.

The channels ratio of an unknown sample is computed from the sample counts, and the efficiency is extrapolated from this calibration curve. This method has the advantage that only one count is needed, it is independent of the volume of sample, its accuracy is good, and the sample is not contaminated by the addition of a radioactive internal standard. The drawbacks are that it is not suitable for low activity samples, and the level of quenching has to be low - otherwise a separate curve is needed for colour quenched samples.

3) External standard channels ratio (ESR)

The ESR method is used when a low activity sample with count rates near to background is being analysed. The technique for calibration is basically the same as the SCR method where a series of vials are prepared using different amounts of quenching agent. In the ESR method, the samples in the vials are firstly counted. Then an external standard is positioned mechanically below each sample vial in turn and another count taken.

The way the external standard works is as follows. The external standard consists of a small pellet containing a gamma emitting nuclide such as ^{133}Ba. The gamma rays irradiate the vial and its contents. This interaction produces high energy Compton electrons which interact with the scintillator-solvent mixture. The energy

spectrum of the Compton electrons resembles the shape of the beta spectrum of soft beta emitters such as ^{14}C. Because of this the Compton electrons are quenched to approximately the same extent as the photons produced by the beta particles from the decay of ^{14}C. This is usually referred to as proportionate quenching of the Compton electrons (Kobayashi & Maudsley, 1974). The ESR is calculated by the following equation:

$$ESR = \frac{CPM(\text{sample + Ext.std.})\ Ch.2 - CPM(\text{sample})\ Ch.2}{CPM(\text{sample + Ext.std.})\ Ch.1 - CPM(\text{sample})\ Ch.1}$$

or

$$= \frac{CPM(\text{Ext.std.})\ Ch.2}{CPM(\text{Ext.std.})\ Ch.1}$$

The efficiency is calculated as in the SCR method. A calibration curve is plotted using the ESR and efficiency for the above series of quenched standards. Unknown samples are then counted and their ESR calculated. The counting efficiency is extrapolated from the calibration curve. The disintegrations per minute (DPM) is found using the following equation:

DPM = CPM (sample) Ch.2 / Efficiency

Some liquid scintillation counters have computers which can be programmed to calculate the ESR, efficiency and DPM. The liquid scintillation counter I used had this facility.

The disadvantages of the ESR method are that it is slightly less reproducible than the SCR method, and that the external standard has to be counted separately thus increasing counting time. The main advantages of the method are that it can be used for heavily quenched or low activity samples and for the simultaneous counting of 2 isotopes (ISOCAP / 300 Liquid Scintillation System operating manual).

Common terms used in Liquid Scintillation Counting

Activity

Activity is defined as the rate of disintegration of a radioactive nuclide. The units of activity are the becquerel (1 Bq = 1 disintegration sec^{-1}) and the curie (Chapman & Ayrey, 1981).

Beta particles

The emission of a β^- particle results in the gain of a positive charge by the nucleus. This emission is associated with the conversion of a neutron (n) to a proton (p), that is,

$$n \longrightarrow p + \beta^-$$

For example, the radioactive carbon isotope ^{14}C whose nucleus contains six protons and eight neutrons decays to the stable nitrogen isotope ^{14}N whose nucleus contains seven protons and seven neutrons.

$$^{14}C\ (6p + 8n) \longrightarrow {}^{14}N\ (7p + 7n) + \beta^-$$

The other type of emission is of a β^+ particle, which results in the loss of a positive charge by the nucleus. In this case a proton is converted to a neutron,

$$p \longrightarrow n + \beta^+$$

For example, the radioactive carbon isotope ^{11}C whose nucleus contains six protons and five neutrons decays to the stable boron isotope ^{11}B whose nucleus contains five protons six neutrons.

$$^{11}C\ (6p + 5n) \longrightarrow {}^{11}B\ (5p + 6n) + \beta^+$$

(Neame & Homewood, 1974).

Chemiluminescence

Chemiluminescence is caused by inorganic species in some cocktails. In chemiluminescence, spurious light pulses or photons are generated as a result of chemical interaction between various sample components (Kobayashi & Maudley, 1974; Chapman & Ayrey, 1981). The

decay of chemiluminescence is faster at low temperatures, and because of this Bransome and Grower (1970) suggest leaving samples in the dark for 2-3 days before counting.

Cocktail

A cocktail is a mixture of solutes and solvents (Dyer, 1974).

Compton effect

If a gamma or X-ray photon hits a planetary electron, both the photon and the electron are deflected. The photon loses energy to the electron and forms a gamma ray, and the electron gains energy from the photon and is then called a "Compton electron". The amount of energy transferred depends on the angle of deflection. Electrons with a wide range of energies are produced up to a maximum energy given by a gamma ray rebounding at an angle of 180^{o} (Chapman & Ayrey, 1981).

Curie

A curie (Ci) is a unit of activity. It is the amount of radioactive isotope in which 37,000,000,000 atoms decay or disintegrate every second. A millicurie (mCi) is 10^{-3} curie and a microcurie (μm) is 10^{-6} curie (Overman & Clark, 1960; Neame & Homewood, 1974; Chapman & Ayrey, 1981). 1 Ci = 3.7×10^{10} Bq.

Excitation

This occurs when a nucleus, atom or molecule absorbs energy and changes from its ground state to an excited state (Chapman & Ayrey, 1981).

Half-life

The half-life of an isotope is the time taken for its radioactivity to decay from a given value to exactly half that value. The half-life of ^{14}C is 5,730 years (Chapman & Ayrey, 1981).

Isotopes

A series of nuclides with the same atomic number (i.e. same number of protons) but differing numbers of neutrons (Chapman & Ayrey, 1981).

Million electron volts (MeV)

The particles and electromagnetic radiation emitted by a decaying nucleus carry a certain amount of energy. This energy is measured in units of million electron volts (MeV) or thousand electron volts (keV). One electron volt is equivalent to the energy acquired by an electron moving through a potential of one volt. Beta particles carry kinetic energy, and this energy is related to the velocity of the particle (Neame & Homewood, 1974).

Nucleon

The collective term for the two major particles (proton and neutron) which are found in the nucleus (Chapman & Ayrey, 1981).

Nuclide

An atomic species with a specified nucleon content (Chapman & Ayrey, 1981).

Photon

A photon is a quantum of light or electromagnetic energy which has properties similar to a particle (Chapman & Ayrey, 1981).

Radioisotope

A nuclide whose nucleus is unstable and eventually undergoes radioactive decay (Chapman & Ayrey, 1981).

Scintillator or Solute

A scintillator is a material which emits flashes of light when excited by ionizing radiation (Chapman & Ayrey, 1981). A scintillator can be a primary scintillator which facilitates sample preparation or

a secondary scintillator which gives increased counting efficiencies.

A primary scintillator should have the following properties.

(a) It should have a high efficiency of light production when activated by radiation.

(b) It must emit light at a wavelength that is within the region of maximum sensitivity of the photomultiplier tube.

(c) It should be soluble and stable under the sample conditions and the working temperature of the counter.

(d) There should be no concentration quenching.

(e) It should be economical and readily available.

The most commonly used primary scintillator is:

2,5 - Diphenyloxazole which is abbreviated as PPO (Kobayashi & Maudsley, 1974).

A secondary scintillator absorbs the emitted light of the primary scintillator and re-emits it at a longer wavelength. It also increases the light output from the sample. The highest counting efficiency is obtained at a maximum concentration of the secondary scintillator whether the sample is quenched or unquenched. Bush & Hansen (1965) suggest using a secondary scintillator only when conditions similar to those listed below prevail.

(a) The sample contains a compound that brings about direct quenching of the primary scintillator.

(b) The primary scintillator has a high enough concentration to produce strong self-quenching.

(c) The counter in use has a better response at longer wavelengths.

(d) The sample being counted has a significant absorption in the near ultraviolet.

An example of a commonly used secondary scintillator is 1,4-Bis-2-(5-phenyloxazolyl) benzene which is abbreviated as POPOP (Kobayashi & Maudsley, 1974).

Solvents

Solvents are of two types, primary and secondary. The function of a primary solvent is to dissolve a solute. A primary solvent has to be an aromatic solvent (non-polar solvent) - usually toluene or a mixture of xylenes. A secondary solvent helps in the miscibility between a radioactive sample and a primary solvent-solute system (Dyer, 1974). For samples which exist as aqueous solutions a more polar solvent such as ethanol is needed; this is an example of a secondary solvent (Chapman & Ayrey, 1981).

The solubility of the scintillator in the solvent and the efficiency in transferring energy from the source of beta-emission have to be considered when choosing a solvent (Kobayashi & Maudsley, 1974).

Specific activity

The specific activity is the ratio of the number of radioactive atoms to the number of atoms of the same element in a given compound. This is sometimes more loosely defined as activity per unit weight (Chapman & Ayrey, 1981).

Relationship between the biological sample, primary solvent, secondary solvent and solute

Many biological samples occur as aqueous solutions and are polar. Such samples have a very low solubility in non-polar aromatic solvents such as toluene or xylene. Since the primary solvent is usually toluene or xylene, a secondary solvent which is more polar is used to aid miscibilty between the radioactive sample and the primary solvent. The primary solute, like the primary solvent, is non-polar and aromatic; this makes the primary solvent and solute compatible (Neame & Homewood, 1974; Chapman & Ayrey, 1981).

Appendix 2

Table 1. Second series of experiments. 1 x 2 one-way analyses of variance comparing differences in permeability ($mm.sec^{-1}$) between pairs of treatments (A, B, C, D, E) for successive runs (1,2,3,4) (text p 23).
Pairs of treatments compared on the following 10 pages.

A x B	B x C	C x D	D x E
A x C	B x D	C x E	
A x D	B x E		
A x E			

Thus:

A x B	4 comparisons: runs 1,2,3, and 4.
A x C	4 comparisons: runs 1,2,3, and 4.
A x D	4 comparisons: runs 1,2,3, and 4.
A x E	4 comparisons: runs 1,2,3, and 4.
B x C	4 comparisons: runs 1,2,3, and 4.
B x D	4 comparisons: runs 1,2,3, and 4.
B x E	4 comparisons: runs 1,2,3, and 4.
C x D	4 comparisons: runs 1,2,3, and 4.
C x E	4 comparisons: runs 1,2,3, and 4.
D x E	4 comparisons: runs 1,2,3, and 4.

A x B Comparison

Run	Source of variation	SS	MS=SS/df	d.f.	F-ratio	P
1	Factor	0.0571	0.0571	1	12.3	0.025>P>0.01 *
	Residual	0.0186	0.00466	4		
	Total	0.0757		5		
2	Factor	0.0261	0.0261	1	13.1	0.025>P>0.01 *
	Residual	0.00793	0.00198	4		
	Total	0.0340		5		
3	Factor	0.0241	0.0241	1	24.5	0.01>P>0.005 **
	Residual	0.00394	0.000986	4		
	Total	0.0281		5		
4	Factor	0.0201	0.0201	1	27.6	0.01>P>0.005 **
	Residual	0.00291	0.000729	4		
	Total	0.0230		5		

A x C Comparison

Run	Source of variation	SS	MS=SS/df	d.f.	F-ratio	P
1	Factor	0.0707	0.0707	1	27.4	0.01>P>0.005 **
	Residual	0.00103	0.00258	4		
	Total	0.0810		5		
2	Factor	0.0690	0.0690	1	45.9	0.005>P>0.001**
	Residual	0.0601	0.00150	4		
	Total	0.0750		5		
3	Factor	0.0749	0.0749	1	57.8	0.005>P>0.001 **
	Residual	0.00518	0.00129	4		
	Total	0.0800		5		
4	Factor	0.0627	0.0627	1	49.8	0.005>P>0.001 **
	Residual	0.00503	0.00126	4		
	Total	0.0677		5		

A x D Comparison

Run	Source of variation	SS	MS=SS/df	d.f.	F-ratio	P
1	Factor	0.00057	0.00057	1	0.32	P<0.75
	Residual	0.00724	0.00181	4		
	Total	0.00781		5		
2	Factor	0.000027	0.000027	1	0.03	P<0.75
	Residual	0.00325	0.000814	4		
	Total	0.00328		5		
3	Factor	0.000169	0.000169	1	0.56	0.50>P>0.25
	Residual	0.00120	0.000300	4		
	Total	0.00137		5		
4	Factor	0.000109	0.000109	1	0.47	0.75>P>0.50
	Residual	0.000921	0.000230	4		
	Total	0.00103		5		

A x E Comparison

Run	Source of variation	SS	MS=SS/df	d.f.	F-ratio	P
1	Factor	0.00001	0.00001	1	0.00	P>0.75
	Residual	0.00997	0.00249	4		
	Total	0.00997		5		
2	Factor	0.0000	0.0000	1	0.00	P>0.75
	Residual	0.00442	0.00111	4		
	Total	0.00443		5		
3	Factor	0.000086	0.000086	1	0.16	0.75>P>0.50
	Residual	0.00219	0.000547	4		
	Total	0.00228		5		
4	Factor	0.000013	0.000013	1	0.03	P>0.75
	Residual	0.00171	0.000428	4		
	Total	0.00172		5		

B x C Comparison

Run	Source of variation	SS	MS=SS/df	d.f.	F-ratio	P
1	Factor	0.00072	0.00072	1	0.17	0.75>P>0.50
	Residual	0.0167	0.00417	4		
	Total	0.0174		5		
2	Factor	0.0103	0.0103	1	4.96	0.10>P>0.05
	Residual	0.00828	0.00207	4		
	Total	0.0185		5		
3	Factor	0.0140	0.0140	1	7.54	0.10>P>0.05
	Residual	0.00742	0.00185	4		
	Total	0.0214		5		
4	Factor	0.0118	0.0118	1	6.83	0.10>P>0.05
	Residual	0.00690	0.00173	4		
	Total	0.0187		5		

B x D Comparison

Run	Source of variation	SS	MS=SS/df	d.f.	F-ratio	P
1	Factor	0.0691	0.0691	1	20.3	0.025>P>0.01 *
	Residual	0.0136	0.00341	4		
	Total	0.0827		5		
2	Factor	0.0278	0.0278	1	20.1	0.025>P>0.01 *
	Residual	0.00552	0.00138	4		
	Total	0.0333		5		
3	Factor	0.0203	0.0203	1	23.6	0.01>P>0.005 **
	Residual	0.00344	0.00859	4		
	Total	0.0237		5		
4	Factor	0.0172	0.0172	1	24.7	0.01>P>0.005 **
	Residual	0.00280	0.000699	4		
	Total	0.0200		5		

B x E Comparison

Run	Source of variation	SS	MS=SS/df	d.f.	F-ratio	P	
1	Factor	0.0583	0.0583	1	14.3	0.025>P>0.01	*
	Residual	0.0164	0.00409	4			
	Total	0.0747		5			
2	Factor	0.0264	0.0264	1	15.8	0.025>P>0.01	*
	Residual	0.00669	0.00167	4			
	Total	0.0330		5			
3	Factor	0.0213	0.0213	1	19.3	0.025>P>0.01	*
	Residual	0.00442	0.00111	4			
	Total	0.0258		5			
4	Factor	0.0191	0.0191	1	21.3	0.01>P>0.005	**
	Residual	0.00359	0.000897	4			
	Total	0.0227		5			

C x D Comparison

Run	Source of variation	SS	MS=SS/df	d.f.	F-ratio	P
1	Factor	0.0840	0.0840	1	63.5	0.005>P>0.001 **
	Residual	0.00529	0.00132	4		
	Total	0.0893		5		
2	Factor	0.0718	0.0718	1	79.6	P<0.001 ***
	Residual	0.00361	0.000902	4		
	Total	0.0754		5		
3	Factor	0.0679	0.0679	1	58.1	0.005>P>0.001 **
	Residual	0.00467	0.00117	4		
	Total	0.0726		5		
4	Factor	0.0575	0.0575	1	46.9	0.005>P>0.001 **
	Residual	0.00491	0.00123	4		
	Total	0.0624		5		

C x E Comparison

Run	Source of variation	SS	MS=SS/df	d.f.	F-ratio	P
1	Factor	0.0721	0.0721	1	35.9	0.005>P>0.001 **
	Residual	0.00802	0.00201	4		
	Total	0.0801		5		
2	Factor	0.0695	0.0695	1	58.2	0.005>P>0.001 **
	Residual	0.00478	0.00119	4		
	Total	0.0743		5		
3	Factor	0.0699	0.0699	1	49.4	0.005>P>0.001 **
	Residual	0.00566	0.00142	4		
	Total	0.0755		5		
4	Factor	0.0609	0.0609	1	42.7	0.005>P>0.001 **
	Residual	0.00570	000143	4		
	Total	0.0666		5		

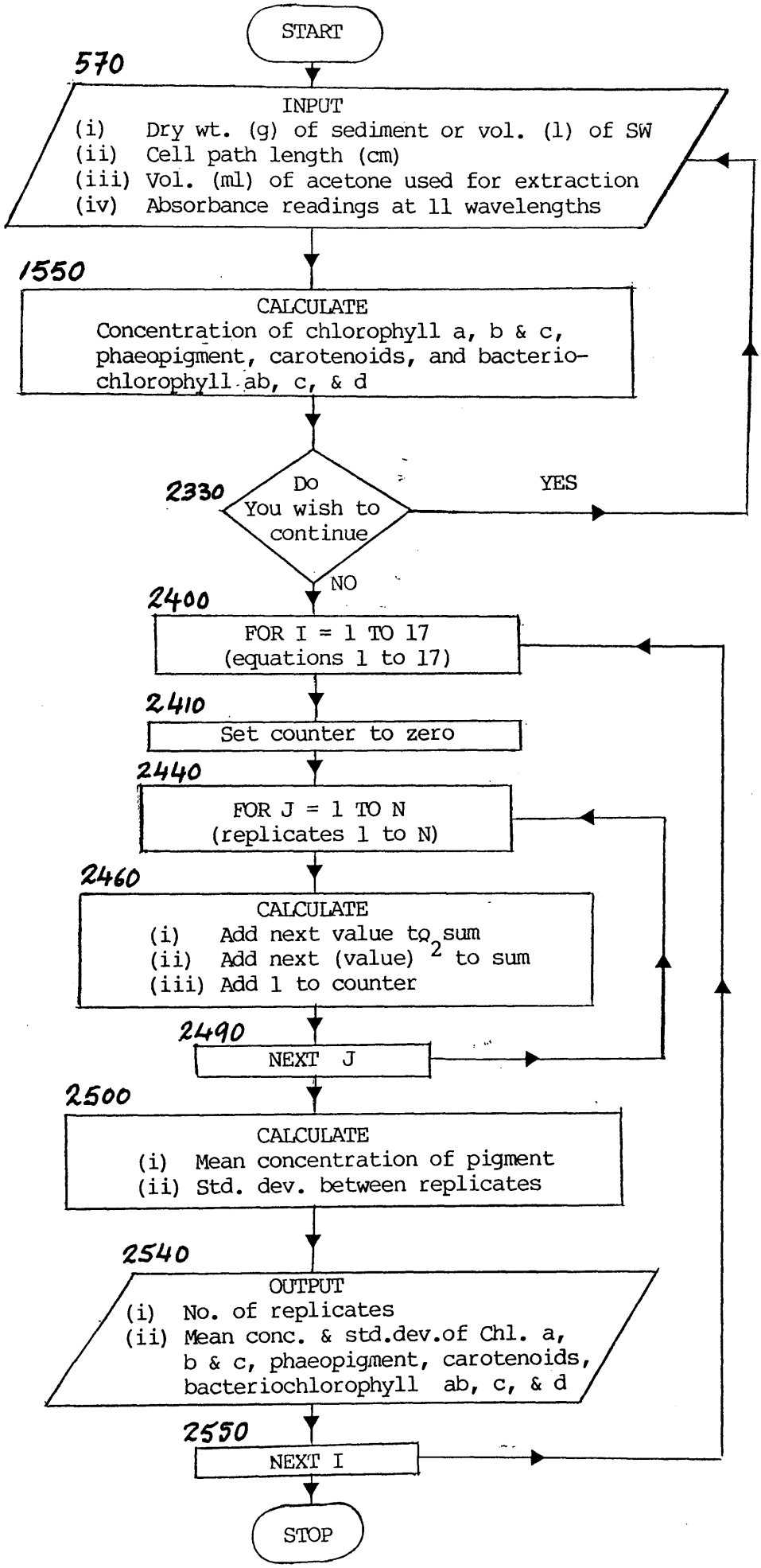
D x E Comparison

Run	Source of variation	SS	MS=SS/df	d.f.	F-ratio	P
1	Factor	0.00046	0.00046	1	0.37	0.75>P>0.50
	Residual	0.00496	0.00124	4		
	Total	0.00541		5		
2	Factor	0.000018	0.000018	1	0.04	P>0.75
	Residual	0.00202	0.000504	4		
	Total	0.00204		5		
3	Factor	0.000014	0.000014	1	0.03	0.75>P>0.50
	Residual	0.00168	0.000420	4		
	Total	0.00170		5		
4	Factor	0.000047	0.000047	1	0.12	0.75>P>0.50
	Residual	0.00159	0.000398	4		
	Total	0.00164		5		

Appendix 3

Enrichment experiment.

Computer program for calculating chlorophyll a, b, c, phaeopigment, carotenoids and bacteriochlorophyll ab, c and d, in sea water or sediment (text p 45).


START
570
INPUT
(i) Dry wt. (g) of sediment or vol. (l) of SW
(ii) Cell path length (cm)
(iii) Vol. (ml) of acetone used for extraction
(iv) Absorbance readings at 11 wavelengths
1550
CALCULATE
Concentration of chlorophyll a, b & c, phaeopigment, carotenoids, and bacterio-chlorophyll ab, c, & d
2330
Do You wish to continue
YES
NO
2400
FOR I = 1 TO 17
(equations 1 to 17)
2410
Set counter to zero
2440
FOR J = 1 TO N
(replicates 1 to N)
2460
CALCULATE
(i) Add next value to sum
(ii) Add next (value)^2 to sum
(iii) Add 1 to counter
2490
NEXT J
2500
CALCULATE
(i) Mean concentration of pigment
(ii) Std. dev. between replicates
2540
OUTPUT
(i) No. of replicates
(ii) Mean conc. & std.dev.of Chl. a, b & c, phaeopigment, carotenoids, bacteriochlorophyll ab, c, & d
2550
NEXT I
STOP

LISTING:

```
10 DIM A(17,20),X(20)
20 REM***PSM/RM/AT***8/3/83
30 POKE  59468!,14
40 A$="CHLOROPHYLL,CAROTENOIDS,PHAEOPIGMENT AND BACTERIOCHLOROPHYLL ANALYSIS  "
50 B$="IN MARINE                                    SEDIMENTS/SEAWATER"
60 C$="THIS PROGRAMME HAS BEEN DEVELOPED BY AZRA TUFAIL 1983/4 DEPARTMENT OF ZOO
LOGY.  "
70 CD$="GLASGOW UNIVERSITY;FROM TWO SOURCES."
80 D$="(A)FOR CHLOROPHYLLS A,B,C,PHAEOPIGMENTS AND PLANT CAROTENOIDS:"
90 DE$="   STRICKLAND AND PARSONS 1972.A PRACTICAL HANDBOOK OF SEAWATER "
100 E$="ANALYSIS,FISH.      RES.BD.CANAD.BULL.167 2ND ED.PP.188-192;     "
110 LPRINT,""
120 EA$="(B)FOR BACTERIOCHLOROPHYLLS:           "
130 EB$="  JONES 1979.A GUIDE TO METHODS FOR ESTIMATING MICROBIAL NUMBERS AND "
140 EC$="BIOMASS       IN FRESH WATERS;FBA;80"
150 F$="THE PROGRAMME CALCULATES CHLOROPHYLLS A,B,C PHAEOPIGMENTS,PLANT CAROTENO
IDS   "
160 G$="AND BACTERIOCHLOROPHYLS IN UG/G OF SEDIMENT OR MG/CUBIC METRE OF SEAWATE
R."
170 H$="IF ABSORBANCE READINGS HAVE ONLY BEEN TAKEN FOR SOME "
180 I$="EQUATIONS,ENTER 9E9 FOR MISSING ABSORBANCE READINGS"
190 J$="APPLY CELL-TO-CELL BLANK CORRECTIONS TO ABSORBANCE READINGS "
200 K$="BEFORE ENTERING THEM (STRICKLAND AND PARSONS P191)"
210 L$="DO NOT MULTIPLY THE ABSORBANCE READING BY ANY FACTOR "
220 M$="TO ALLOW FOR ACETONE VOLUME OR PATH LENGTH"
230 PRINT  A$;B$
240 PRINT C$;CD$
250 PRINT D$;DE$;E$;EA$;EB$;EC$
260 PRINT  F$;G$
270 PRINT  H$;I$
280 PRINT  J$;K$
290 PRINT "DO NOT SUBTRACT 750NM OR 850NM READING FROM ANY OTHER ABSORBANCE READ
INGS"
300 PRINT L$;M$
310 PRINT "SAMPLE DETAILS"
320 LPRINT A$;B$
330 LPRINT
340 LPRINT C$;CD$
350 LPRINT
360 LPRINT D$
370 LPRINT DE$;E$
380 LPRINT
390 LPRINT,"WHERE (PS)=PARSON AND STRICKLAND EQUATIONS"
400 LPRINT,"       (R)=RICHARDS AND THOMPSON EQUATIONS"
410 LPRINT,"       (SU)=SCOR-UNESCO EQUATIONS"
420 LPRINT,"       (L)=LORENZEN EQUATIONS"
430 LPRINT," "
440 LPRINT," "
450 LPRINT EA$
460 LPRINT EB$;EC$
470 LPRINT," "
480 LPRINT F$;G$
490 LPRINT, " "
500 LPRINT," "
510 LPRINT,
520 N=0
530 LPRINT," "
540 INPUT "DATE OF COLLECTION";X$
550 INPUT "NATURE OF MATERIAL COLLECTED";Y$
```

```
560 INPUT "SAMPLE NUMBER";Z
570 N$="DRY WEIGHT OF SEDIMENT (GRAMS) OR VOLUME OF SEAWATER                "
580 O$="                (LITRES) USED:(S)"
590 PRINT  N$;O$:INPUT  S
600 INPUT "CELL PATH LENGTH IN CM";L
610 INPUT "VOLUME OF ACETONE (MILLILITRES) USED TO EXTRACT PIGMENT";V
620 N=N+1
630 LPRINT,"DATE OF COLLECTION: ";X$
640 LPRINT," "
650 LPRINT,"NATURE OF MATERIAL COLLECTED: ";Y$
660 LPRINT,"SAMPLE NUMBER: ";Z
670 LPRINT,N$;O$;S
680 LPRINT,"CELL PATH LENGTH (CM) :";L
690 LPRINT,"VOLUME OF ACETONE USED (ML) TO EXTRACT PIGMENT:";V
700 LPRINT," "
710 INPUT "ABSORBANCE AT 750NM";W1
720 INPUT "ABSORBANCE AT 750NM AFTER ACIDIFICATION";W
730 INPUT "ABSORBANCE AT 665NM";W2
740 INPUT "ABSORBANCE AT 665NM AFTER ACIDIFICATION";W3
750 INPUT "ABSORBANCE AT 663NM";W4
760 INPUT "ABSORBANCE AT 645NM";W5
770 INPUT "ABSORBANCE AT 630NM";W6
780 INPUT "ABSORBANCE AT 510NM";W7
790 INPUT "ABSORBANCE AT 480NM";W8
800 INPUT "ABSORBANCE AT 850NM ";W9
810 INPUT "ABSORBANCE AT 772NM ";W10
820 INPUT "ABSORBANCE AT 654NM ";W11
830 INPUT "ABSORBANCE AT 662NM ";W12
840 LPRINT,"ABSORBANCE AT 750NM:";W1
850 LPRINT,"ABSORBANCE AT 750NM AFTER ACIDIFICATION:";W
860 LPRINT,"ABSORBANCE AT 665NM:";W2
870 LPRINT,"ABSORBANCE AT 665NM AFTER ACIDIFICATION:";W3
880 LPRINT,"ABSORBANCE AT 663NM:";W4
890 LPRINT,"ABSORBANCE AT 645NM:";W5
900 LPRINT,"ABSORBANCE AT 630NM:";W6
910 LPRINT,"ABSORBANCE AT 510NM:";W7
920 LPRINT,"ABSORBANCE AT 480NM:";W8
930 LPRINT,"ABSORBANCE AT 850NM:";W9
940 LPRINT,"ABSORBANCE AT 772NM:";W10
950 LPRINT,"ABSORBANCE AT 654NM:";W11
960 LPRINT,"ABSORBANCE AT 662NM:";W12
970 LPRINT
980 IF  W2=9E+09  THEN  1000
990  GOTO  1080
1000 A(1,N)=9E+08
1010 A(2,N)=9E+08
1020 A(4,N)=9E+08
1030 A(5,N)=9E+08
1040 A(6,N)=9E+08
1050 A(8,N)=9E+08
1060 A(9,N)=9E+08
1070 A(11,N)=9E+08
1080 IF  W3=9E+09  THEN  1100
1090  GOTO  1120
1100 A(4,N)=9E+08
1110 A(11,N)=9E+08
1120 IF  W4=9E+09  THEN  1140
1130  GOTO  1170
1140 A(3,N)=9E+08
1150 A(7,N)=9E+08
```

```
1160 A(10,N)=9E+08
1170 IF  W5=9E+09  THEN  1190
1180  GOTO  1280
1190 A(1,N)=9E+08
1200 A(2,N)=9E+08
1210 A(4,N)=9E+08
1220 A(5,N)=9E+08
1230 A(6,N)=9E+08
1240 A(7,N)=9E+08
1250 A(8,N)=9E+08
1260 A(9,N)=9E+08
1270 A(10,N)=9E+08
1280 IF  W6=9E+09  THEN  1300
1290  GOTO  1390
1300 A(1,N)=9E+08
1310 A(2,N)=9E+08
1320 A(3,N)=9E+08
1330 A(5,N)=9E+08
1340 A(6,N)=9E+08
1350 A(7,N)=9E+08
1360 A(8,N)=9E+08
1370 A(9,N)=9E+08
1380 A(10,N)=9E+08
1390 IF  W7=9E+09  THEN  1410
1400  GOTO  1420
1410 A(14,N)=9E+08
1420 IF  W8=9E+09  THEN  1440
1430 GOTO 1460
1440 A(12,N)=9E+08
1450 A(13,N)=9E+08
1460 IF W10=9E+09 THEN 1480
1470 GOTO 1490
1480 A(15,N)=9E+08
1490 IF W11=9E+09 THEN 1510
1500 GOTO 1520
1510 A(16,N)=9E+08
1520 IF W12=9E+09 THEN 1540
1530 GOTO 1550
1540 A(17,N)=9E+08
1550 A(1,N)= (11.6*(W2-W1)-1.31*(W5-W1)-.14*(W6-W1))*V/L/S
1560 A(2,N)=(15.6*(W2-W1)-2*(W5-W1)-.8*(W6-W1))*V/L/S
1570 A(3,N)=(11.64*(W4-W1)-2.16*(W5-W1)+.1*(W6-W1))*V/L/S
1580 A(4,N)=26.7*((W2-W1)-(W3-W))*V/L/S
1590 A(5,N)=(20.7*(W5-W1)-4.34*(W2-W1)-4.42*(W6-W1))*V/L/S
1600 A(6,N)=(25.4*(W5-W1)-4.4*(W2-W1)-10.3*(W6-W1))*V/L/S
1610 A(7,N)=(20.97*(W5-W1)-3.94*(W4-W1)-3.66*(W6-W1))*V/L/S
1620 A(8,N)=(55*(W6-W1)-4.64*(W2-W1)-16.3*(W5-W1))*V/L/S
1630 A(9,N)=(109*(W6-W1)-12.5*(W2-W1)-28.7*(W5-W1))*V/L/S
1640 A(10,N)=(54.22*(W6-W1)-14.81*(W5-W1)-5.53*(W4-W1))*V/L/S
1650 A(11,N) =26.7*(1.7*(W3-W)-(W2-W1))*V/L/S
1660 A(12,N)=4*(W8-3*W1)*V/L/S
1670 A(13,N)=10*(W8-3*W1)*V/L/S
1680 A(14,N)=7.6*((W8-3*W1)-1.49*(W7-2*W1))*V/L/S
1690 A(15,N)=25.2*(W10-W9)*V/L/S
1700 A(16,N)=10.2*(W11-W9)*V/L/S
1710 A(17,N)=10.8*(W12-W9)*V/L/S
```

```
1720 PRINT "1 CONC OF CHLOROPHYLL A(PS):";A(1,N)
1730 PRINT "2 CONC OF CHLOROPHYLL A(R):";A(2,N)
1740 PRINT "3 CONC OF CHLOROPHYLL A(SU):";A(3,N)
1750 PRINT "4 CONC OF CHLOROPHYLL A(L):";A(4,N)
1760 PRINT "5 CONC OF CHLOROPHYLL B(PS):";A(5,N)
1770 PRINT "6 CONC OF CHLOROPHYLL B(R):";A(6,N)
1780 PRINT "7 CONC OF CHLOROPHYLL B(SU):";A(7,N)
1790 PRINT "8 CONC OF CHLOROPHYLL C(PS):";A(8,N)
1800 PRINT "9 CONC OF CHLOROPHYLL C(R):";A(9,N)
1810 PRINT "10 CONC OF CHLOROPHYLL C(SU):";A(10,N)
1820 PRINT "11 CONC OF PHAEOPIGMENT (L):";A(11,N)
1830 PRINT "12 CONC OF PLANT CAROTENOIDS EC(PS):";A(12,N)
1840 PRINT "13 CONC OF PLANT CAROTENOIDS EP(PS):";A(13,N)
1850 PRINT "14 CONC OF PLANT CAROTENOIDS E2(R):";A(14,N)
1860 PRINT "15 CONC.OF BACT.CHL.AB:";A(15,N)
1870 PRINT "16 CONC.OF BACT.CHL.C:";A(16,N)
1880 PRINT "17 CONC.OF BACT.CHL.D:";A(17,N)
1890 P$="THE CONC OF CHLOROPHYLL A,B,C,PHAEOPIGMENT AND PLANT CAROTENOIDS "
1900 Q$="                AND BACTERIOCHLOROPHYLLS AB,C&D(IN MICROGRAMS PER GRAM "
1910 QR$="                DRY WEIGHT OF SEDIMENT OR MILLIGRAM PER CUBIC METRE OF S
EAWATER) "
1920 Q$="                 ARE CODED FROM 1-17 AS FOLLOWS:"
1930 LPRINT,P$;Q$;QR$;QS$
1940 LPRINT," "
1950 LPRINT,"1 CONC OF CHLOROPHYLL A(PS):";A(1,N)
1960 LPRINT,"2 CONC OF CHLOROPHYLL A(R):";A(2,N)
1970 LPRINT,"3 CONC OF CHLOROPHYLL A(SU):";A(3,N)
1980 LPRINT,"4 CONC OF CHLOROPHYLL A(L):";A(4,N)
1990 LPRINT," "
2000 LPRINT,"5 CONC OF CHLOROPHYLL B(PS):";A(5,N)
2010 LPRINT,"6 CONC OF CHLOROPHYLL B(R):";A(6,N)
2020 LPRINT,"7 CONC OF CHLOROPHYLL B(SU):";A(7,N)
2030 LPRINT," "
2040 LPRINT,"8 CONC OF CHLOROPHYLL C(PS):";A(8,N)
2050 LPRINT,"9 CONC OF CHLOROPHYLL C(R):";A(9,N)
2060 LPRINT,"10 CONC OF CHLOROPHYLL C(SU):";A(10,N)
2070 LPRINT," "
2080 LPRINT,"11 CONC OF PHAEOPIGMENT (L):";A(11,N)
2090 LPRINT," "
2100 LPRINT,"12 CONC OF PLANT CAROTENOIDS EC(PS):";A(12,N)
2110 LPRINT,"13 CONC OF PLANT CAROTENOIDS EC(PS):";A(13,N)
2120 LPRINT,"14 CONC OF PLANT CAROTENOIDS E2(R):";A(14,N)
2130 LPRINT," "
2140 LPRINT," '
2150 LPRINT,"15 CONC OF BACT.CHL.AB:";A(15,N)
2160 LPRINT,"16 CONC OF BACT.CHL.C:";A(16,N)
2170 LPRINT,"17 CONC OF BACT.CHL.D:";A(17,N)
2180 LPRINT," "
2190 LPRINT, " "
2200 LPRINT, " "
2210 LPRINT,"_____________________________________________________________________"
2220 LPRINT, " " " " " "
2230 LPRINT,"_____________________________________________________________________"
2240 LPRINT," "
2250 LPRINT," "
2260 LPRINT," "
2270 LPRINT," "
2280 LPRINT," "
2290 PRINT,"---------------------------------------------------------------------"
```

```
2300 INPUT "PRINT YES TO CONTINUE AND NO TO TERMINATE PROGRAMME";T$
2310 IF T$="YES" THEN 540
2320 AB$="REPLICATES"
2330 AC$="MEAN"
2340 AD$="STD DEV"
2350 PRINT TAB(7);AB$;TAB(31);AC$;TAB(48);AD$
2360 LPRINT,"_____"
2370 LPRINT,TAB(12);AB$;TAB(30);AC$;TAB(50);AD$
2380 LPRINT,"_____"
2390 LPRINT," "
2400 FOR I=1 TO 17
2410 NX=0
2420 T=0
2430 R=0
2440 FOR  J=1 TO N
2450 IF  A(I,J)=9E+08  THEN  2490
2460 T=T+A(I,J)
2470 R=R+A(I,J)^2
2480 NX=NX+1
2490 NEXT  J
2500 M=T/NX
2510 U=SQR((R-T^2/NX)/(NX-1))
2520 PRINT TAB(2);(I);TAB(12);NX;TAB(28);M;TAB(48);U
2530 LPRINT
2540 LPRINT TAB(2);(I);TAB(12);NX;TAB(28);M;TAB(48);U
2550 NEXT  I
2560 LPRINT,CHR$(12)
```

RUN :

CHLOROPHYLL,CAROTENOIDS,PHAEOPIGMENT AND BACTERIOCHLOROPHYLL ANALYSIS IN MARINE
SEDIMENTS/SEAWATER

THIS PROGRAMME HAS BEEN DEVELOPED BY AZRA TUFAIL 1983 DEPARTMENT OF ZOOLOGY, GLASGOW UNIVE
FROM TWO SOURCES.

(A)FOR CHLOROPHYLLS A,B,C,PHAEOPIGMENTS AND PLANT CAROTENOIDS:
STRICKLAND AND PARSONS 1972.A PRACTICAL HANDBOOK OF SEAWATER ANALYSIS,FISH. RES.BD.CANAD.
BULL.167 2ND ED.PP.188-192;

WHERE (PS)=PARSON AND STRICKLAND EQUATIONS
(R)=RICHARDS AND THOMPSON EQUATIONS
(SU)=SCOR-UNESCO EQUATIONS
(L)=LORENZEN EQUATIONS

(B)FOR BACTERIOCHLOROPHYLLS:
JONES 1979.A GUIDE TO METHODS FOR ESTIMATING MICROBIAL NUMBERS AND BIOMASS IN FRESH WATERS;

THE PROGRAMME CALCULATES CHLOROPHYLLS A,B,C PHAEOPIGMENTS,PLANT CAROTENOIDS
AND BACTERIOCHLOROPHYLS IN µG/G OF SEDIMENT OR MG/CUBIC METRE OF SEAWATER.

DATE OF COLLECTION: 24.7.83

NATURE OF MATERIAL COLLECTED: Ardmore L.T. sand
SAMPLE NUMBER: 1
DRY WEIGHT OF SEDIMENT (GRAMS) OR VOLUME OF SEAWATER (LITRES) USED:(S) 1.056

CELL PATH LENGTH (CM) : 1
VOLUME OF ACETONE USED (ML) TO EXTRACT PIGMENT: 10

ABSORBANCE AT 750NM: .001
ABSORBANCE AT 750NM AFTER ACIDIFICATION: .004
ABSORBANCE AT 665NM: .053
ABSORBANCE AT 665NM AFTER ACIDIFICATION: .04
ABSORBANCE AT 663NM: .053
ABSORBANCE AT 645NM: .016
ABSORBANCE AT 630NM: .014
ABSORBANCE AT 510NM: .023
ABSORBANCE AT 480NM: .063
ABSORBANCE AT 850NM: .0005
ABSORBANCE AT 772NM: .002
ABSORBANCE AT 654NM: .029
ABSORBANCE AT 662NM: .053

THE CONC OF CHLOROPHYLL A,B,C,PHAEOPIGMENT AND PLANT CAROTENOIDS
ARE CODED FROM 1-17 AS FOLLOWS:
DRY WEIGHT OF SEDIMENT OR MILLIGRAM PER CUBIC METRE OF SEAWATER)

1 CONC OF CHLOROPHYLL A(PS): 5.50829
2 CONC OF CHLOROPHYLL A(R): 7.29855
3 CONC OF CHLOROPHYLL A(SU): 5.4368
4 CONC OF CHLOROPHYLL A(L): 4.04507

5 CONC OF CHLOROPHYLL B(PS): .259066
6 CONC OF CHLOROPHYLL B(R): .173278
7 CONC OF CHLOROPHYLL B(SU): .587917

8 CONC OF CHLOROPHYLL C(PS): 2.17044
9 CONC OF CHLOROPHYLL C(R): 3.18625
10 CONC OF CHLOROPHYLL C(SU): 1.84784

11 CONC OF PHAEOPIGMENT (L): 2.32592

12 CONC OF PLANT CAROTENOIDS EC(PS): 2.27251
13 CONC OF PLANT CAROTENOIDS EC(PS): 5.68128
14 CONC OF PLANT CAROTENOIDS E2(R): 2.06605

15 CONC OF BACT.CHL.AB: .357921
16 CONC OF BACT.CHL.C: 2.75258
17 CONC OF BACT.CHL.D: 5.36881

DATE OF COLLECTION: 24.7.83

NATURE OF MATERIAL COLLECTED: Ardmore L.T. sand
SAMPLE NUMBER: 2
DRY WEIGHT OF SEDIMENT (GRAMS) OR VOLUME OF SEAWATER (LITRES) USED:(S)

CELL PATH LENGTH (CM) : 1
VOLUME OF ACETONE USED (ML) TO EXTRACT PIGMENT: 10

ABSORBANCE AT 750NM: .002
ABSORBANCE AT 750NM AFTER ACIDIFICATION: .005
ABSORBANCE AT 665NM: .094
ABSORBANCE AT 665NM AFTER ACIDIFICATION: .064
ABSORBANCE AT 663NM: .0925
ABSORBANCE AT 645NM: .028
ABSORBANCE AT 630NM: .024
ABSORBANCE AT 510NM: .048
ABSORBANCE AT 480NM: .115
ABSORBANCE AT 850NM: .001
ABSORBANCE AT 772NM: .0035
ABSORBANCE AT 654NM: .053
ABSORBANCE AT 662NM: .092

THE CONC OF CHLOROPHYLL A,B,C,PHAEOPIGMENT AND PLANT CAROTENOIDS
ARE CODED FROM 1-17 AS FOLLOWS:
DRY WEIGHT OF SEDIMENT OR MILLIGRAM PER CUBIC METRE OF SEAWATER)

1 CONC OF CHLOROPHYLL A(PS): 9.26813
2 CONC OF CHLOROPHYLL A(R): 12.2872
3 CONC OF CHLOROPHYLL A(SU): 8.9928
4 CONC OF CHLOROPHYLL A(L): 7.92784

5 CONC OF CHLOROPHYLL B(PS): .375023
6 CONC OF CHLOROPHYLL B(R): .260932
7 CONC OF CHLOROPHYLL B(SU): .972917

8 CONC OF CHLOROPHYLL C(PS): 3.23304
9 CONC OF CHLOROPHYLL C(R): 4.51503
10 CONC OF CHLOROPHYLL C(SU): 2.76512

11 CONC OF PHAEOPIGMENT (L): 1.99397

12 CONC OF PLANT CAROTENOIDS EC(PS): 3.92298
13 CONC OF PLANT CAROTENOIDS EC(PS): 9.80745
14 CONC OF PLANT CAROTENOIDS E2(R): 2.97052

15 CONC OF BACT.CHL.AB: .566853
16 CONC OF BACT.CHL.C: 4.77236
17 CONC OF BACT.CHL.D: 8.8429

	REPLICATES	MEAN	STD DEV
1	2	7.38821	2.65862
2	2	9.79288	3.5275
3	2	7.2148	2.51448
4	2	5.98646	2.74553
5	2	.317044	.0819936
6	2	.217105	.0619808
7	2	.780417	.272236
8	2	2.70174	.751372
9	2	3.85064	.939584
10	2	2.30648	.648616
11	2	2.15994	.234719
12	2	3.09775	1.16706
13	2	7.74437	2.91764
14	2	2.51829	.639555
15	2	.462387	.147737
16	2	3.76247	1.42819
17	2	7.10586	2.45654

Appendix 4.1

Enrichment experiment.

Sequence of dilution of 0.5 ml of 1 mCi sodium [^{14}C] carbonate to obtain an activity of 2.5 μCi.ml^{-1} (text p 50).

Sequence of dilution of 0.5 ml of 1 mCi sodium [^{14}C] carbonate to obtain an activity of 2.5 μCi.ml^{-1} (text p 50).

The procedure for diluting 0.5 ml 1mCi of sodium [^{14}C] carbonate ($Na_2{}^{14}CO_3$) to 2.5 μCi ml^{-1} is explained below.

The sodium [^{14}C] carbonate was supplied as an aqueous solution of 0.5 ml of 1mCi $Na_2{}^{14}CO_3$ in a glass ampoule by Amersham. The specific activity of this solution was 55.6 mCi mmol^{-1}. This radioactive solution had to be diluted to an activity of 2.5 μCi ml^{-1}.

The solution (diluent) used for diluting the 1 mCi of sodium [^{14}C] carbonate was prepared as follows. 50 g of analytical reagent quality sodium chloride (NaCl) were dissolved in distilled water and the volume was made up to the 1 litre mark. This gave a 5 % w/v sodium chloride solution. 0.3 g of anhydrous sodium carbonate (Na_2CO_3) and 1 pellet (c.0.2g) of sodium hydroxide (NaOH) were added to this solution (Strickland & Parsons, 1972).

Method of dilution

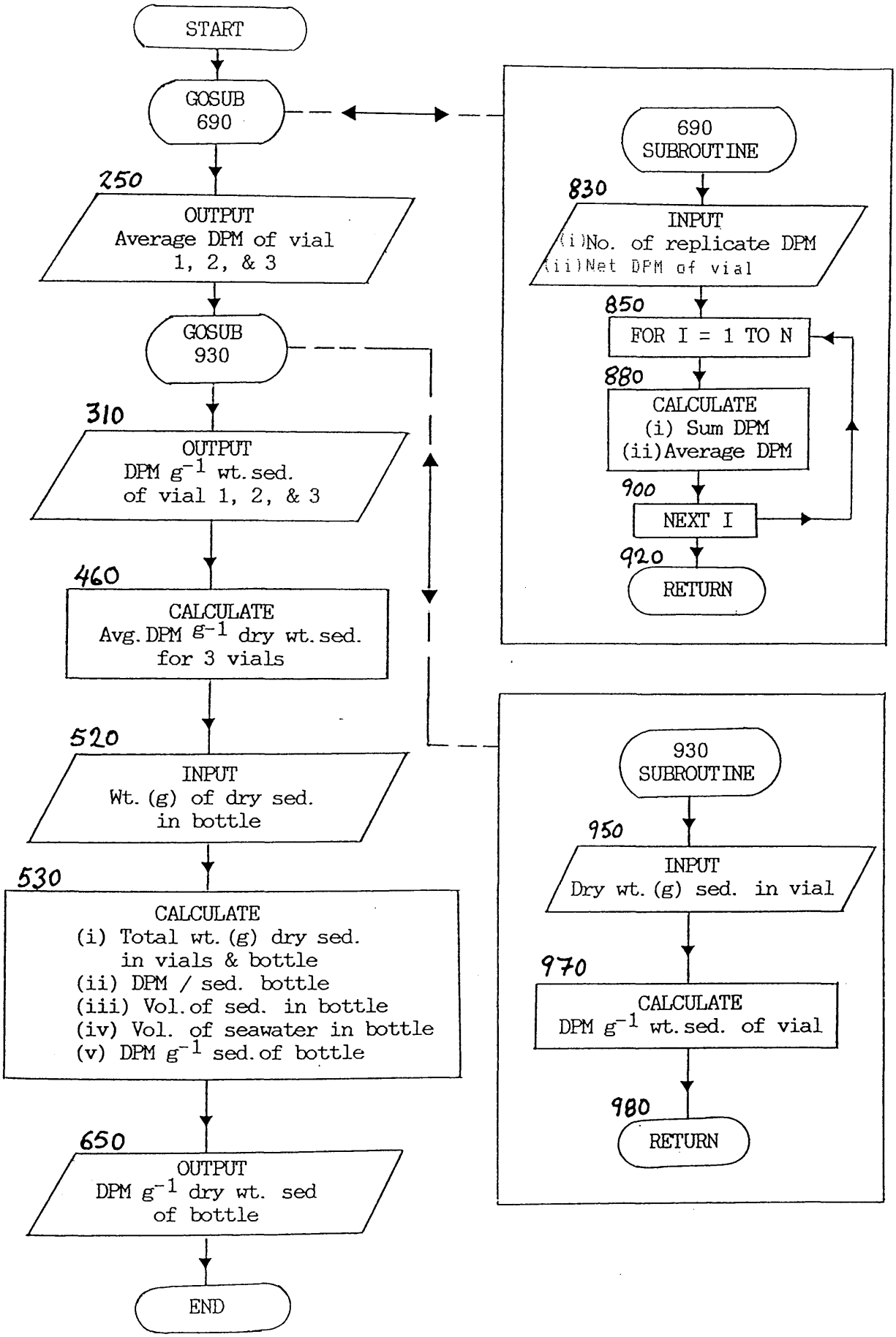
1. 150 ml of diluent was transferred to a 200 ml volumetric flask.
2. The 0.5 ml of 1 mCi sodium [^{14}C] carbonate was then injected into the volumetric flask containing the 150 ml diluent.
3. The ampoule which originally contained the 0.5 ml $Na_2{}^{14}CO_3$ solution was rinsed with small volumes of diluent, and the rinses were transferred to the volumetric flask.
4. The volume in the flask was then made up to the 200 ml mark with more diluent. The flask was inverted three times for complete mixing.
5. The dilute solution in 4 above was transferred to a sterile 500 ml volumetric flask which had been calibrated and marked at the 400 ml mark.

6. 200 ml of fresh diluent was measured in a sterile 200 ml volumetric flask. Portions of this diluent were used to rinse the 200 ml flask used in 4 and the rinses were transferred to the 500 ml volumetric flask containing the dilute ^{14}C solution. This step was repeated until the entire 200 ml of diluent had been used for rinsing. The contents of the 500 ml flask were then thoroughly mixed.
7. Following these dilutions, the radioactivity of the solution was assumed to be 2.5 μCi ^{14}C per millilitre.

Appendix 4.2

Enrichment experiment.

Computer program for calculating disintegrations per minute (DPM) per gram dry weight of sediment (text p 52).


START
GOSUB 690
250
OUTPUT Average DPM of vial 1, 2, & 3
GOSUB 930
310
OUTPUT DPM g-1 wt.sed. of vial 1, 2, & 3
460
CALCULATE Avg. DPM g-1 dry wt.sed. for 3 vials
520
INPUT Wt. (g) of dry sed. in bottle
530
CALCULATE
(i) Total wt. (g) dry sed. in vials & bottle
(ii) DPM / sed. bottle
(iii) Vol.of sed. in bottle
(iv) Vol. of seawater in bottle
(v) DPM g-1 sed.of bottle
650
OUTPUT DPM g-1 dry wt. sed of bottle
END
690 SUBROUTINE
830
INPUT
(i)No. of replicate DPM
(ii)Net DPM of vial
850
FOR I = 1 TO N
880
CALCULATE
(i) Sum DPM
(ii)Average DPM
900
NEXT I
920
RETURN
930 SUBROUTINE
950
INPUT Dry wt. (g) sed. in vial
970
CALCULATE DPM g-1 wt.sed. of vial
980
RETURN

LISTING:

```
10 REM**THIS PROGRAM CALCULATES MEAN OF NET DPM AND DPM PER DRY WT.SED (A.TUFAIL
20 REM**USE WHEN DPM CALC. BY COPMUTER (PERMIABILITY EXP.) FEB.1986,JULY 1986**
30 INPUT "SAMPLE NUMBER ";E
40 INPUT "SAMPLE DATE (OR DETAILS) ";E$
50 LPRINT "-----------------------------------------------------------------------
----------"
60 LPRINT : LPRINT "SAMPLE NUMBER = ";E
70 LPRINT "SAMPLE DATE (OR DETAILS) ";E$
80 LPRINT
90 Q=1
100 GOSUB 690
110 PRINT "MEAN DPM OF VIAL a ";L
120 PRINT
130 LPRINT "MEAN DPM OF VIAL a ";L
140 LPRINT
150 GOSUB 930
160 VA=U
170 PRINT : PRINT "DPM PER GRAM SED.VIAL a ";G
180 PRINT "-----------------------------------------------------------------------
190 LPRINT "DPM PER GRAM DRY WT.SED.VIAL a ";G
200 LPRINT : LPRINT "-----------------------------------------------------------------------
----------"
210 WA=G
220 GOSUB 690
230 PRINT "MEAN DPM OF VIAL b ";L
240 PRINT
250 LPRINT "MEAN DPM OF VIAL b ";L
260 LPRINT
270 GOSUB 930
280 VB=U
290 PRINT : PRINT "DPM PER GRAM SED.VIAL b ";G
300 PRINT "-----------------------------------------------------------------------
310 LPRINT "DPM PER GRAM SED.VIAL b ";G
320 LPRINT : LPRINT "-----------------------------------------------------------------------
----------"
330 WB=G
340 GOSUB 690
350 PRINT "MEAN DPM OF VIAL c ";L
360 PRINT
370 LPRINT "MEAN DPM OF VIAL c ";L
380 LPRINT
390 GOSUB 930
400 VC=U
410 PRINT : PRINT "DPM PER GRAM SED.VIAL c ";G
420 LPRINT "DPM PER GRAM DRY SED. VIAL c ";G
430 PRINT "-----------------------------------------------------------------------
440 LPRINT : LPRINT "-----------------------------------------------------------------------
--------"
450 WC=G
460 V=(WA+WB+WC)/3
470 LPRINT
480 PRINT
```

```
490 PRINT "MEAN DPM PER GRAM SED. FOR VIALS a,b & c = ";V
500 PRINT
510 LPRINT "MEAN DPM PER GRAM SED. FOR VIALS a,b & c = ";V
520 INPUT "WEIGHT OF DRY SED. IN BOTTLE ";X
530 TWS=(VA+VB+VC+X)
540 PRINT "TOTAL WEIGHT OF SED. (IN BOTTLE + IN 3 VIALS) g= ";TWS
550 LPRINT "TOTAL WEIGHT OF SED. (IN BOTTLE + IN 3 VIALS) g= ";TWS
560 Z=V*TWS
570 PRINT "DPM PER SED.BOTTLE ";Z
580 LPRINT "DPM PER SED.BOTTLE = ";Z
590 LPRINT
600 VS=(TWS/2.65)/10^6
610 VSW=.00015-(VS-(VS*.444))
620 DPMG=(Z*VSW)/TWS
630 LPRINT "VOLUME OF SEAWATER IN BOTTLE= ";VSW
640 PRINT "DPM PER g SEDIMENT BOTTLE= "; DPMG
650 LPRINT "DPM PER g SEDIMENT BOTTLE= ";DPMG
660 LPRINT "==========================================================================
==========="
670 LPRINT
680 END
690 PRINT
700 REM**SUBROUTINE TO CALCULATE MEAN DPM FROM NET DPM**
710 A$="FIRST NET DPM OF VIAL"
720 B$="SECOND NET DPM OF VIAL "
730 C$="THIRD NET DPM OF VIAL "
740 IF Q=1 THEN PRINT A$
750 IF Q=1 THEN LPRINT A$
760 IF Q=2 THEN PRINT B$
770 IF Q=2 THEN LPRINT B$
780 IF Q=3 THEN PRINT C$
790 IF Q=3 THEN LPRINT C$
800 PRINT :PRINT "--------------------------------------------------------------------
----------"
810 LPRINT : LPRINT
820 N=0 : M=0
830 INPUT "NUMBER OF REPLICATE NET DPM COUNTS PER VIAL ";N
840 PRINT
850 FOR I=1 TO N
860 INPUT "NET DPM OF VIAL ";A
870 LPRINT "NET DPM OF VIAL ";A
880 M=M+A
890 L=M/N
900 NEXT I
910 Q=Q+1
920 RETURN
930 PRINT
940 REM**SUBROUTINE TO CALCULATE DPM PER GRAM SED.**
950 INPUT "DRY WEIGHT OF SED. IN VIAL ";U
960 LPRINT : LPRINT "DRY WT. OF SED. IN VIAL ";U
970 G=L/U
980 RETURN
```

RUN:

SAMPLE NUMBER = 7
SAMPLE DATE (OR DETAILS) 24.9.84 MD2 LIGHT

FIRST NET DPM OF VIAL

NET DPM OF VIAL 2167.9
NET DPM OF VIAL 2165.4
MEAN DPM OF VIAL a 2166.65

DRY WT. OF SED. IN VIAL .8416
DPM PER GRAM DRY WT.SED.VIAL a 2574.44

SECOND NET DPM OF VIAL

NET DPM OF VIAL 1784.3
NET DPM OF VIAL 1788.8
MEAN DPM OF VIAL b 1786.55

DRY WT. OF SED. IN VIAL .7596
DPM PER GRAM SED.VIAL b 2351.96

THIRD NET DPM OF VIAL

NET DPM OF VIAL 1904.6
NET DPM OF VIAL 1888.3
MEAN DPM OF VIAL c 1896.45

DRY WT. OF SED. IN VIAL 1.0375
DPM PER GRAM DRY SED. VIAL c 1827.9

MEAN DPM PER GRAM SED. FOR VIALS a,b & c = 2251.44
TOTAL WEIGHT OF SED. (IN BOTTLE + IN 3 VIALS) g= 11.0689
DPM PER SED.BOTTLE = 24920.9

VOLUME OF SEAWATER IN BOTTLE= 1.47678E-04
DPM PER g SEDIMENT BOTTLE= .332487

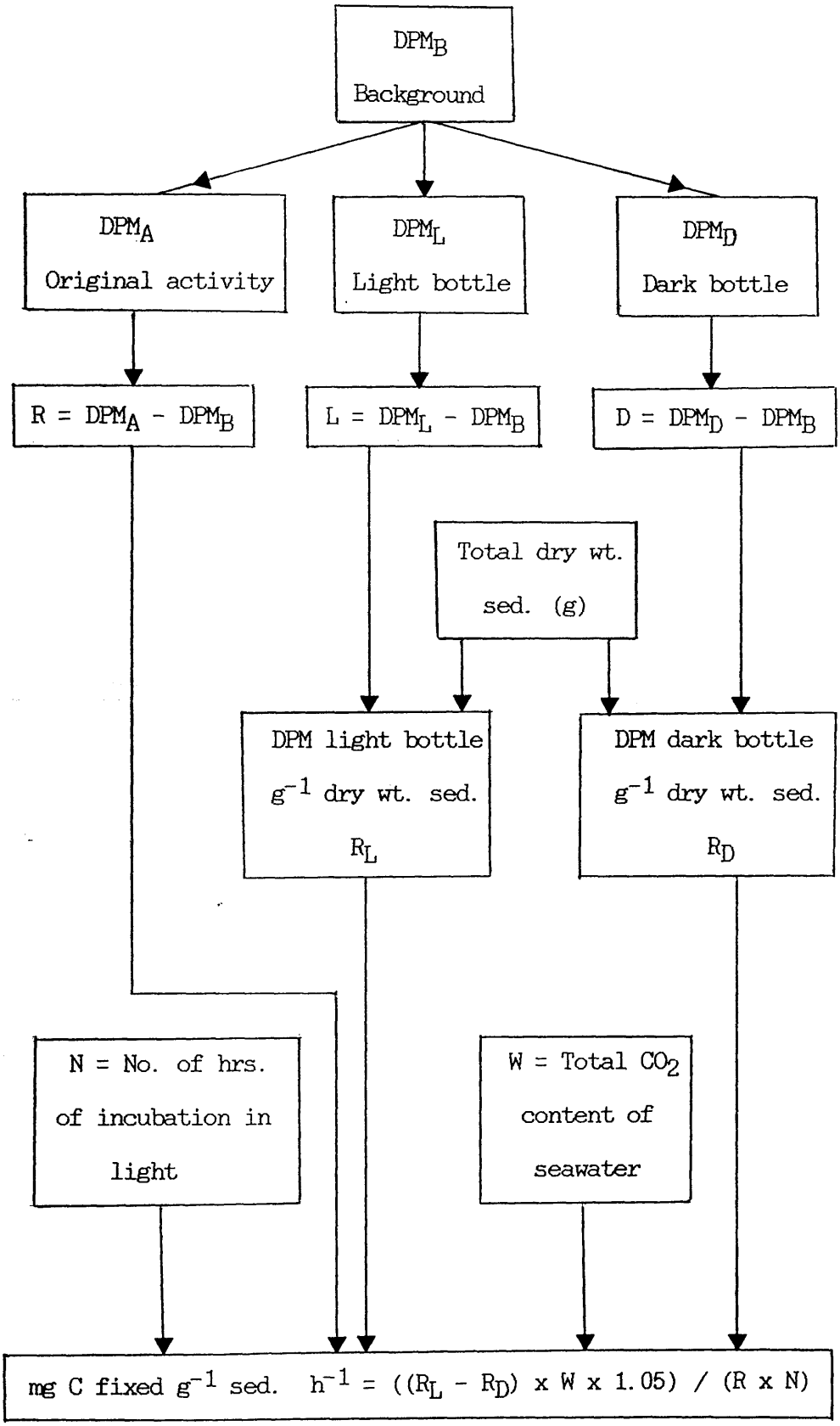
==========

Appendix 4.3

Enrichment experiment.

Flow chart showing general procedure for calculating mg.C fixed per dry weight of sediment per hour. DPM = disintegrations per minute, B = background, A = original activity, L = light bottle, D = dark bottle, R = corrected original activity, R_L = DPM per gram weight sediment of light bottle, R_D = DPM per gram weight sediment of dark bottle (text p 52, 57).

Note : W is obtained from the flow charts in appendices 4.5, 4.6, and 4.7, pp. 183-188.


DPM$_B$
Background
DPM$_A$
Original activity
DPM$_L$
Light bottle
DPM$_D$
Dark bottle
R = DPM$_A$ - DPM$_B$
L = DPM$_L$ - DPM$_B$
D = DPM$_D$ - DPM$_B$
Total dry wt.
sed. (g)
DPM light bottle
g^{-1} dry wt. sed.
R$_L$
DPM dark bottle
g^{-1} dry wt. sed.
R$_D$
N = No. of hrs.
of incubation in
light
W = Total CO$_2$
content of
seawater
mg C fixed g^{-1} sed. h^{-1} = ((R$_L$ - R$_D$) x W x 1.05) / (R x N)

Appendix 4.4

Enrichment experiment.

Preparation of standard phthalate buffer for total alkalinity determination (text p 55).

Preparation of standard phosphate buffer for pH determination (text p 56).

Preparation of standard phthalate buffer for total alkalinity determination (pH 4.00 at 20-25°C) (Strickland & Parsons, 1972).

0.05M potassium hydrogen phthalate (text p 55).

10.21 g of analytical reagent quality (primary buffer-standard specification) potassium hydrogen phthalate ($KHC_8H_4O_4$) were dissolved in distilled water and the volume was made up to 1 litre. This solution was stored in a glass bottle.

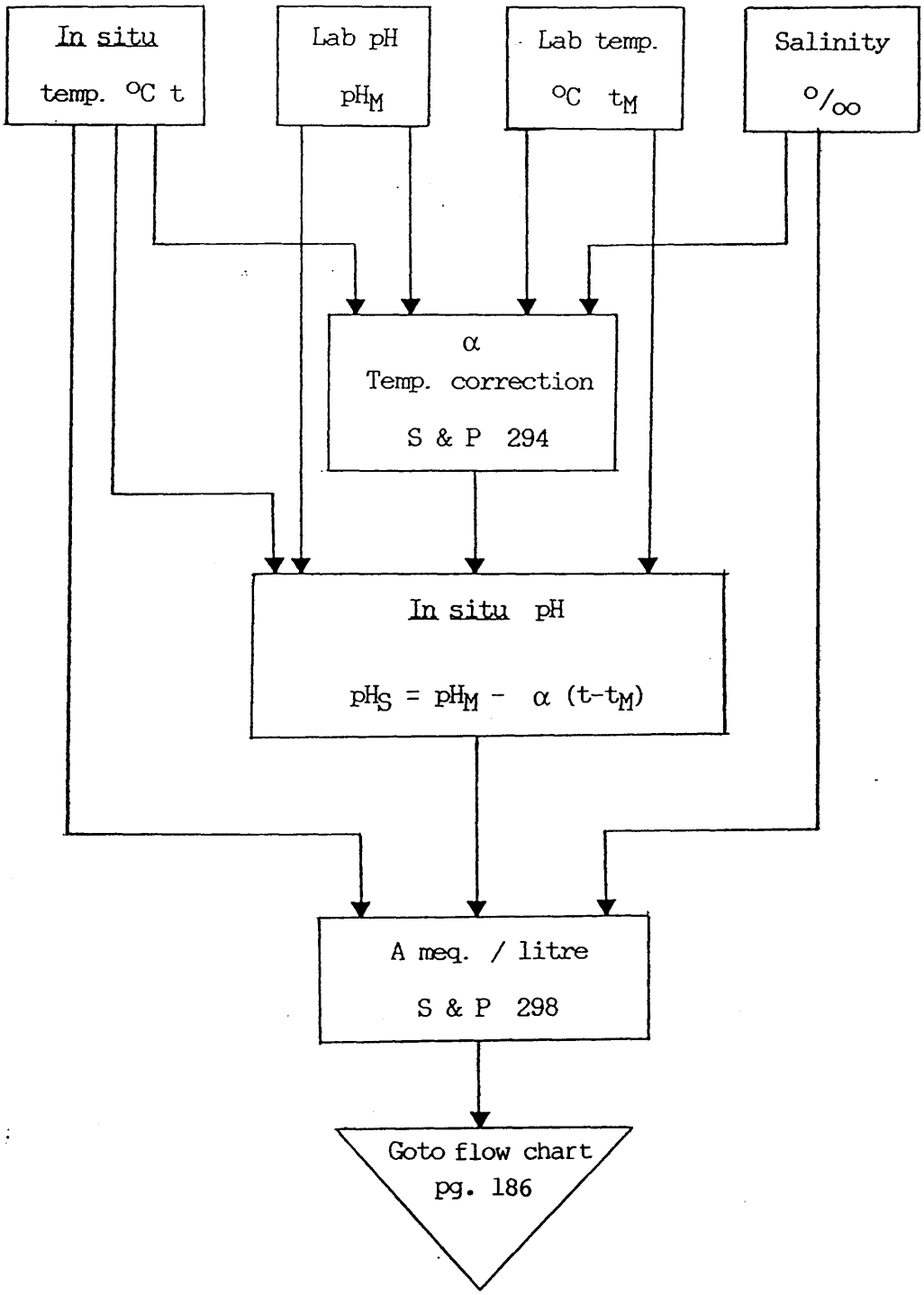
Preparation of standard phosphate buffer for pH determination (pH 6.87 at 20-25°C) (Strickland & Parsons, 1972) (text p 56).

34.0 g of analytical reagent quality potassium dihydrogen phosphate (monobasic) (KH_2PO_4) and 35.5 g of analytical reagent quality anhydrous disodium hydrogen orthophosphate (dibasic) (Na_2HPO_4) were dissolved in distilled water and the volume was made up to 1 litre. 100 ml of this solution was diluted to 1 litre with distilled water and the solution was stored in a polyethylene bottle.

Appendix 4.5

Enrichment experiment.

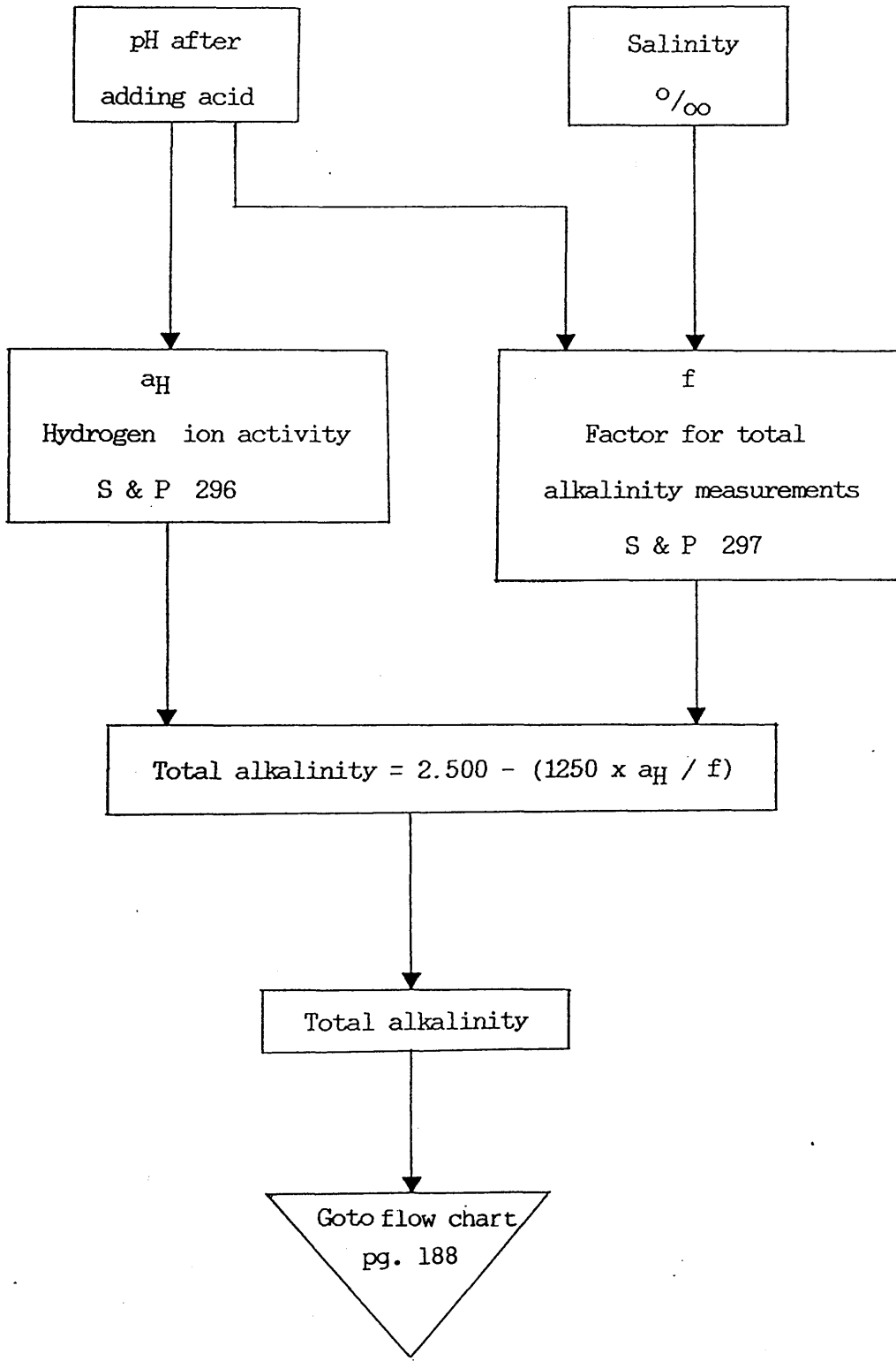
Flow chart showing methodology for calculating factor A (meq / litre) (text p 57). S & P = Strickland and Parsons, 1972.


In situ
temp. °C t
Lab pH
pH_M
Lab temp.
°C t_M
Salinity
°/oo
α
Temp. correction
S & P 294
In situ pH
pH_S = pH_M - α (t-t_M)
A meq. / litre
S & P 298
Goto flow chart
pg. 186

Appendix 4.6

Enrichment experiment.

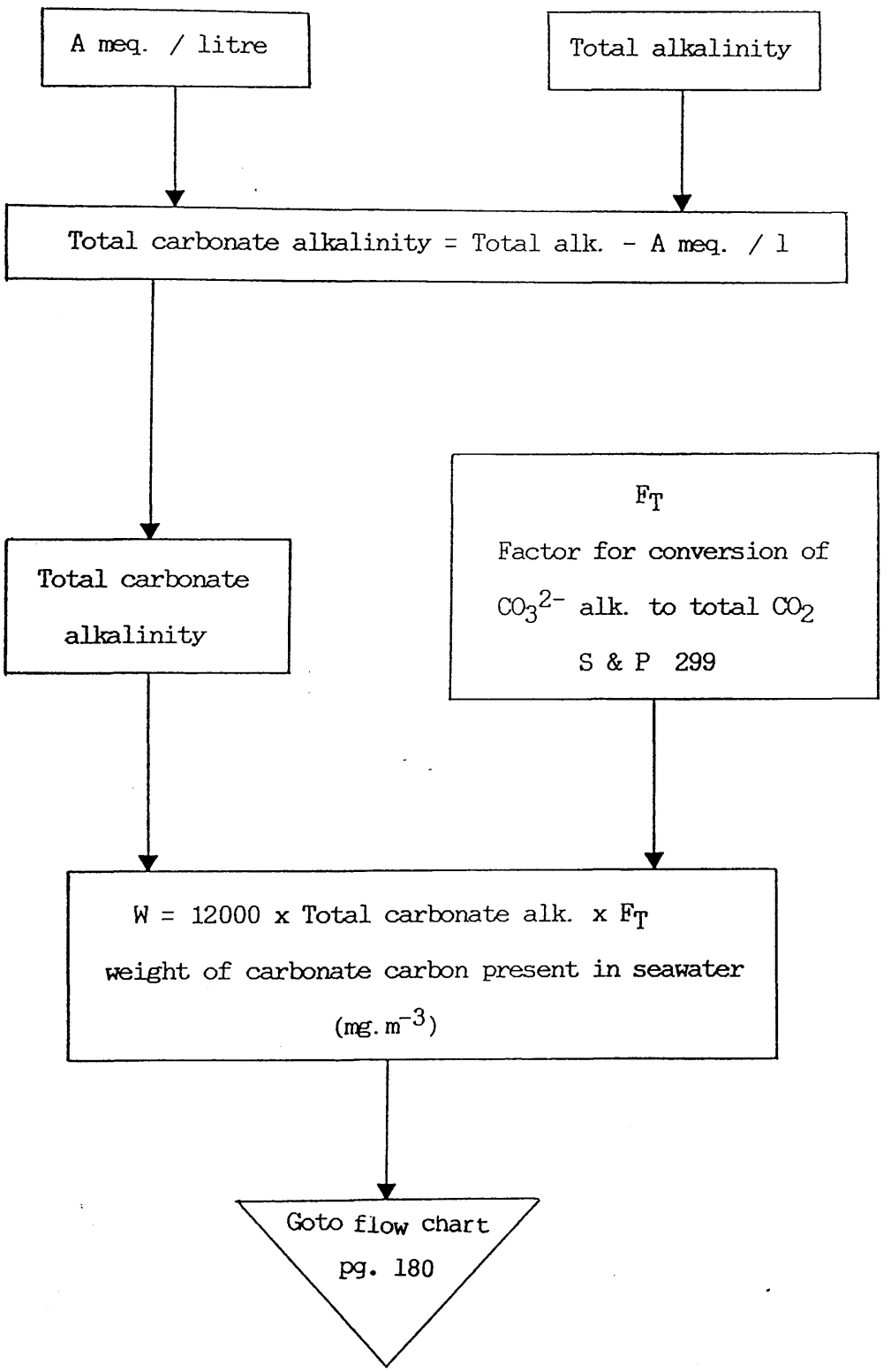
Flow chart showing methodology for calculating total alkalinity (text p 57). S & P = Strickland and Parsons, 1972.


pH after adding acid
Salinity ‰
a_H Hydrogen ion activity S & P 296
f Factor for total alkalinity measurements S & P 297
Total alkalinity = 2.500 - (1250 x a_H / f)
Total alkalinity
Goto flow chart pg. 188

Appendix 4.7

Enrichment experiment.

Flow chart showing methodology for calculating W - weight of carbonate carbon present in seawater ($mg.m^{-3}$). Factor A is in meq/litre (text p 57). S & P = Strickland and Parsons, 1972.


A meq. / litre
Total alkalinity
Total carbonate alkalinity = Total alk. - A meq. / l
Total carbonate alkalinity
F_T
Factor for conversion of
CO_3^{2-} alk. to total CO_2
S & P 299
W = 12000 x Total carbonate alk. x F_T
weight of carbonate carbon present in seawater
(mg. m^{-3})
Goto flow chart
pg. 180

Appendix 5.1

Enrichment experiment.

Original data of the enrichment experiment.

C1 = serial number, C2 = water column height (mm), C3-C4 = time (sec) for replicates 1 and 2 of photosynthetic light medium ML1 and ML2, C5-C6 = time (sec) for replicates 1 and 2 of photosynthetic dark medium MD1 and MD2, C7-C8 = time (sec) for replicates 1 and 2 of bacterial light medium BL1 and BL2, C9-C10 = time (sec) for replicates 1 and 2 of bacterial dark medium BD1 and BD2, C11-C12 = time (sec) for replicates 1 and 2 of control medium (text p 58).

DAY 1

COLUMN	C1	C2	C3	C4	C5	C6
COUNT	17	17	17	17	17	17
ROW						
1	1.	500.	1.	1.	1.	1.
2	2.	475.	8.	9.	8.	10.
3	3.	450.	16.	17.	17.	16.
4	4.	425.	24.	25.	27.	25.
5	5.	400.	32.	35.	35.	34.
6	6.	375.	41.	45.	46.	43.
7	7.	350.	51.	56.	57.	53.
8	8.	325.	63.	67.	70.	64.
9	9.	300.	74.	80.	82.	77.
10	10.	275.	88.	94.	95.	90.
11	11.	250.	101.	109.	111.	104.
12	12.	225.	117.	126.	128.	121.
13	13.	200.	136.	145.	147.	138.
14	14.	175.	155.	167.	169.	157.
15	15.	150.	178.	193.	196.	182.
16	16.	125.	209.	223.	225.	211.
17	17.	100.	242.	259.	262.	243.

COLUMN	C7	C8	C9	C10	C11	C12
COUNT	17	17	17	17	17	17
ROW						
1	1.	1.	1.	1.	1.	1.
2	8.	8.	7.	8.	8.	9.
3	14.	17.	13.	16.	16.	17.
4	22.	24.	21.	23.	23.	25.
5	29.	32.	28.	32.	32.	35.
6	38.	40.	36.	41.	41.	44.
7	48.	50.	44.	50.	50.	54.
8	58.	61.	54.	61.	61.	65.
9	69.	72.	63.	71.	72.	77.
10	81.	84.	74.	83.	84.	90.
11	94.	98.	85.	96.	97.	105.
12	107.	113.	99.	111.	111.	121.
13	125.	130.	114.	127.	128.	140.
14	142.	149.	129.	145.	147.	160.
15	164.	171.	149.	166.	170.	184.
16	189.	198.	170.	192.	195.	211.
17	219.	229.	198.	222.	224.	245.

DAY 4

COLUMN	C1	C2	C3	C4	C5	C6
COUNT	17	17	17	17	17	17
ROW						
1	1.	500.	1.	1.	1.	1.
2	2.	475.	11.	11.	12.	13.
3	3.	450.	22.	23.	23.	25.
4	4.	425.	33.	35.	38.	38.
5	5.	400.	45.	46.	52.	51.
6	6.	375.	57.	60.	68.	65.
7	7.	350.	71.	74.	84.	81.
8	8.	325.	86.	91.	100.	97.
9	9.	300.	111.	107.	120.	115.
10	10.	275.	119.	126.	140.	135.
11	11.	250.	138.	147.	163.	154.
12	12.	225.	159.	168.	188.	178.
13	13.	200.	183.	192.	214.	204.
14	14.	175.	210.	221.	241.	232.
15	15.	150.	242.	253.	284.	268.
16	16.	125.	278.	292.	327.	308.
17	17.	100.	325.	339.	379.	355.

COLUMN	C7	C8	C9	C10	C11	C12
COUNT	17	17	17	17	17	17
ROW						
1	1.	1.	1.	1.	1.	1.
2	22.	23.	21.	22.	10.	11.
3	40.	48.	41.	47.	20.	21.
4	61.	70.	66.	70.	27.	31.
5	81.	95.	91.	95.	38.	42.
6	102.	121.	116.	123.	49.	53.
7	126.	148.	144.	151.	61.	65.
8	147.	176.	168.	181.	73.	77.
9	172.	207.	197.	210.	85.	92.
10	199.	238.	228.	244.	100.	108.
11	227.	271.	261.	279.	115.	125.
12	258.	308.	296.	316.	132.	143.
13	294.	349.	333.	362.	152.	165.
14	331.	391.	378.	408.	175.	190.
15	376.	444.	426.	456.	202.	216.
16	425.	503.	483.	517.	228.	247.
17	488.	574.	553.	588.	264.	287.

DAY 7

COLUMN	C1	C2	C3	C4	C5	C6
COUNT	17	17	17	17	17	17
ROW						
1	1.	500.	1.	1.	1.	1.
2	2.	475.	13.	13.	15.	14.
3	3.	450.	25.	26.	29.	27.
4	4.	425.	37.	39.	45.	41.
5	5.	400.	49.	52.	61.	56.
6	6.	375.	63.	67.	77.	71.
7	7.	350.	78.	83.	96.	87.
8	8.	325.	93.	100.	115.	107.
9	9.	300.	110.	119.	135.	126.
10	10.	275.	131.	139.	159.	147.
11	11.	250.	151.	160.	184.	171.
12	12.	225.	173.	183.	212.	197.
13	13.	200.	198.	211.	245.	227.
14	14.	175.	227.	243.	279.	260.
15	15.	150.	261.	280.	321.	297.
16	16.	125.	303.	323.	372.	344.
17	17.	100.	351.	376.	424.	402.

COLUMN	C7	C8	C9	C10	C11	C12
COUNT	17	17	17	17	17	17
ROW						
1	1.	1.	1.	1.	1.	1.
2	23.	21.	17.	19.	10.	10.
3	44.	47.	36.	40.	21.	21.
4	63.	71.	57.	60.	30.	30.
5	83.	98.	76.	81.	41.	41.
6	106.	127.	96.	103.	52.	53.
7	130.	160.	119.	125.	65.	66.
8	153.	191.	141.	150.	78.	80.
9	180.	227.	165.	179.	92.	93.
10	207.	268.	189.	202.	107.	110.
11	239.	310.	216.	232.	123.	127.
12	272.	360.	247.	263.	141.	145.
13	312.	416.	283.	298.	163.	166.
14	353.	485.	318.	340.	186.	190.
15	406.	571.	364.	386.	213.	218.
16	469.	682.	418.	442.	245.	253.
17	551.	836.	480.	510.	287.	291.

DAY 10

COLUMN	C1	C2	C3	C4	C5	C6
COUNT	17	17	17	17	17	17
ROW						
1	1.	500.	1.	1.	1.	1.
2	2.	475.	14.	14.	16.	18.
3	3.	450.	28.	28.	31.	31.
4	4.	425.	40.	44.	49.	47.
5	5.	400.	55.	58.	67.	62.
6	6.	375.	70.	75.	86.	80.
7	7.	350.	87.	92.	106.	98.
8	8.	325.	105.	111.	127.	118.
9	9.	300.	125.	132.	151.	141.
10	10.	275.	145.	155.	173.	164.
11	11.	250.	167.	179.	202.	190.
12	12.	225.	194.	206.	235.	217.
13	13.	200.	221.	239.	280.	249.
14	14.	175.	254.	271.	309.	287.
15	15.	150.	292.	312.	354.	328.
16	16.	125.	336.	360.	410.	382.
17	17.	100.	395.	420.	466.	440.

COLUMN	C7	C8	C9	C10	C11	C12
COUNT	17	17	17	17	17	17
ROW						
1	1.	1.00	1.	1.	1.	1.
2	22.	32.00	21.	23.	11.	11.
3	44.	63.00	42.	46.	20.	22.
4	67.	99.00	63.	70.	30.	32.
5	90.	140.00	85.	95.	41.	45.
6	117.	188.00	113.	122.	53.	56.
7	142.	240.00	136.	151.	66.	69.
8	172.	299.00	164.	183.	80.	82.
9	203.	371.00	193.	217.	93.	96.
10	239.	455.00	227.	254.	109.	112.
11	276.	547.00	265.	296.	127.	130.
12	319.	673.00	304.	340.	145.	149.
13	370.	835.00	355.	395.	167.	172.
14	425.	1045.00	409.	454.	192.	200.
15	495.	1410.00	474.	528.	220.	226.
16	583.	1761.00	561.	617.	253.	260.
17	685.	2394.00	667.	732.	294.	302.

DAY 13

COLUMN	C1	C2	C3	C4	C5	C6
COUNT	17	17	17	17	17	17
ROW						
1	1.	500.	1.	1.	1.	1.
2	2.	475.	15.	15.	17.	20.
3	3.	450.	28.	29.	31.	35.
4	4.	425.	40.	42.	48.	50.
5	5.	400.	55.	59.	68.	67.
6	6.	375.	71.	75.	86.	85.
7	7.	350.	88.	94.	106.	103.
8	8.	325.	126.	113.	128.	125.
9	9.	300.	148.	135.	150.	147.
10	10.	275.	170.	158.	176.	170.
11	11.	250.	196.	183.	206.	196.
12	12.	225.	226.	210.	236.	226.
13	13.	200.	258.	242.	271.	260.
14	14.	175.	296.	278.	311.	289.
15	15.	150.	344.	319.	357.	338.
16	16.	125.	399.	371.	412.	393.
17	17.	100.	426.	430.	469.	457.

COLUMN	C7	C8	C9	C10	C11	C12
COUNT	17	17	17	17	17	17
ROW						
1	1.	1.00	1.	1.	1.	1.
2	28.	46.00	18.	19.	11.	11.
3	52.	79.00	35.	45.	19.	20.
4	76.	120.00	53.	62.	30.	31.
5	100.	163.00	74.	86.	42.	43.
6	130.	208.00	96.	111.	56.	56.
7	162.	257.00	119.	139.	66.	68.
8	196.	313.00	142.	168.	81.	89.
9	234.	373.00	171.	201.	95.	98.
10	279.	439.00	200.	238.	111.	113.
11	330.	516.00	233.	274.	129.	132.
12	385.	605.00	268.	318.	147.	150.
13	455.	703.00	312.	368.	170.	173.
14	533.	824.00	362.	424.	194.	198.
15	635.	993.00	420.	499.	225.	228.
16	768.	1230.00	489.	595.	259.	265.
17	941.	1466.00	575.	721.	299.	307.

DAY 16

COLUMN	C1	C2	C3	C4	C5	C6
COUNT	17	17	17	17	17	17
ROW						
1	1.	500.	1.	1.	1.	1.
2	2.	475.	20.	20.	14.	16.
3	3.	450.	34.	38.	29.	29.
4	4.	425.	50.	49.	49.	48.
5	5.	400.	67.	65.	67.	64.
6	6.	375.	83.	84.	87.	80.
7	7.	350.	105.	103.	109.	102.
8	8.	325.	125.	125.	131.	123.
9	9.	300.	144.	149.	159.	147.
10	10.	275.	168.	174.	184.	167.
11	11.	250.	193.	204.	215.	203.
12	12.	225.	223.	233.	256.	234.
13	13.	200.	255.	269.	297.	270.
14	14.	175.	292.	308.	337.	310.
15	15.	150.	335.	354.	386.	357.
16	16.	125.	390.	409.	446.	413.
17	17.	100.	445.	474.	502.	477.

COLUMN	C7	C8	C9	C10	C11	C12
COUNT	17	17	17	17	17	17
ROW						
1	1.00	1.00	1.	1.	1.	1.
2	29.00	69.00	28.	26.	12.	11.
3	62.00	154.00	51.	56.	23.	21.
4	99.00	242.00	76.	74.	32.	31.
5	139.00	338.00	102.	103.	48.	43.
6	180.00	449.00	138.	133.	56.	53.
7	280.00	559.00	168.	162.	68.	67.
8	331.00	682.00	204.	196.	82.	82.
9	393.00	840.00	239.	232.	97.	95.
10	462.00	1011.00	280.	274.	116.	114.
11	540.00	1211.00	331.	321.	133.	132.
12	627.00	1454.00	377.	369.	152.	152.
13	689.00	1814.00	433.	435.	176.	173.
14	741.00	2274.00	499.	496.	201.	199.
15	889.00	3199.00	589.	582.	241.	229.
16	1098.00	5130.00	681.	678.	266.	266.
17	1332.00	8693.00	791.	784.	307.	305.

DAY 19

COLUMN	C1	C2	C3	C4	C5	C6
COUNT	17	17	17	17	17	17
ROW						
1	1.	500.	1.	1.	1.	1.
2	2.	475.	15.	17.	16.	15.
3	3.	450.	30.	31.	34.	31.
4	4.	425.	46.	51.	53.	50.
5	5.	400.	62.	66.	74.	68.
6	6.	375.	81.	86.	96.	89.
7	7.	350.	101.	107.	121.	111.
8	8.	325.	122.	131.	146.	133.
9	9.	300.	144.	152.	171.	161.
10	10.	275.	170.	184.	206.	188.
11	11.	250.	197.	213.	238.	221.
12	12.	225.	227.	245.	275.	254.
13	13.	200.	261.	282.	317.	291.
14	14.	175.	299.	326.	369.	336.
15	15.	150.	344.	376.	421.	387.
16	16.	125.	400.	433.	491.	451.
17	17.	100.	455.	501.	556.	520.

COLUMN	C7	C8	C9	C10	C11	C12
COUNT	11	7	17	17	17	17
ROW						
1	1.00	1.00	1.00	1.00	1.	1.
2	65.00	302.00	27.00	29.00	11.	11.
3	139.00	660.00	52.00	66.00	20.	22.
4	238.00	1099.00	85.00	99.00	31.	32.
5	351.00	1809.00	126.00	138.00	44.	43.
6	498.00	3696.00	149.00	187.00	55.	56.
7	670.00	9341.00	187.00	226.00	68.	71.
8	899.00		224.00	274.00	84.	84.
9	1231.00		270.00	319.00	99.	102.
10	1740.00		321.00	385.00	116.	116.
11	2468.00		376.00	452.00	134.	135.
12			440.00	511.00	156.	160.
13			573.00	602.00	179.	179.
14			605.00	693.00	204.	204.
15			722.00	819.00	238.	232.
16			856.00	962.00	276.	272.
17			1001.00	1181.00	313.	315.

DAY 22

COLUMN	C1	C2	C3	C4	C5	C6
COUNT	17	17	17	17	17	17
ROW						
1	1.	500.	1.	1.	1.	1.
2	2.	475.	17.	19.	18.	19.
3	3.	450.	33.	36.	37.	37.
4	4.	425.	50.	53.	60.	57.
5	5.	400.	68.	73.	83.	75.
6	6.	375.	88.	93.	107.	97.
7	7.	350.	108.	115.	132.	120.
8	8.	325.	130.	139.	160.	147.
9	9.	300.	155.	164.	190.	173.
10	10.	275.	182.	193.	222.	205.
11	11.	250.	212.	285.	258.	237.
12	12.	225.	243.	321.	299.	274.
13	13.	200.	282.	356.	350.	316.
14	14.	175.	326.	401.	404.	362.
15	15.	150.	374.	451.	457.	418.
16	16.	125.	435.	514.	525.	484.
17	17.	100.	510.	588.	669.	560.

COLUMN	C7	C8	C9	C10	C11	C12
COUNT	4	3	17	17	17	17
ROW						
1	1.00	1.00	1.00	1.0	1.	1.
2	476.00	2689.00	32.00	42.0	11.	11.
3	1066.00	5380.00	63.00	88.0	22.	22.
4	1982.00		96.00	138.0	34.	33.
5			136.00	196.0	47.	45.
6			168.00	262.0	60.	59.
7			211.00	335.0	73.	72.
8			255.00	429.0	88.	87.
9			301.00	540.0	104.	103.
10			358.00	691.0	122.	121.
11			420.00	868.0	142.	140.
12			496.00	1081.0	162.	166.
13			577.00	1416.0	186.	186.
14			685.00	1826.0	214.	212.
15			814.00	2574.0	246.	244.
16			988.00	4327.0	282.	280.
17			1278.00	10356.0	326.	325.

DAY 25

COLUMN	C1	C2	C3	C4	C5	C6
COUNT	17	17	17	17	17	17
ROW						
1	1.	500.	1.	1.	1.	1.
2	2.	475.	17.	18.	21.	21.
3	3.	450.	33.	38.	42.	40.
4	4.	425.	49.	56.	64.	59.
5	5.	400.	67.	73.	91.	82.
6	6.	375.	87.	94.	116.	104.
7	7.	350.	107.	117.	145.	128.
8	8.	325.	131.	146.	174.	157.
9	9.	300.	153.	177.	208.	185.
10	10.	275.	182.	200.	245.	216.
11	11.	250.	212.	237.	290.	250.
12	12.	225.	243.	269.	332.	292.
13	13.	200.	280.	308.	386.	336.
14	14.	175.	322.	354.	445.	388.
15	15.	150.	368.	406.	510.	450.
16	16.	125.	431.	470.	586.	525.
17	17.	100.	506.	548.	684.	610.

COLUMN	C7	C8	C9	C10	C11	C12
COUNT	8	6	17	17	17	17
ROW						
1	1.00	1.00	1.00	1.00	1.	1.
2	901.00	901.00	38.00	77.00	12.	12.
3	1771.00	1901.00	74.00	156.00	22.	23.
4	2501.00	2851.00	174.00	220.00	34.	33.
5	3511.00	4081.00	215.00	328.00	46.	46.
6	4351.00	6241.00	261.00	420.00	58.	59.
7	5201.00		308.00	511.00	72.	72.
8	6256.00		359.00	615.00	86.	88.
9			478.00	730.00	103.	105.
10			533.00	861.00	121.	121.
11			545.00	1005.00	142.	141.
12			619.00	1170.00	160.	162.
13			763.00	1392.00	185.	187.
14			808.00	1637.00	212.	214.
15			928.00	2000.00	244.	246.
16			1086.00	2453.00	279.	285.
17			1294.00	3061.00	328.	326.

Appendix 5.2

Enrichment experiment.

Details of storage and treatment of permeability data on the ICL 3980 Mainframe computer using the Minitab statistical package (text p 58).

Treatment of permeability data on the ICL 3980 Mainframe Computer

The original time (sec) taken for the water column height to fall intervals of 25 mm was recorded for the five media every alternate day, over a period of 25 days. This data was then entered into the Glasgow University ICL 3980 Mainframe Computer. The data was arranged into columns using the package MINITAB81 for statistical methods. The total number of columns was 12.

Column one had the integers 1 to 17 which represented the total number of times (sec) for the water column height to drop 25 mm. Column two had the values for the water column height in millimetres. The values ranged from 500 to 100 mm with intermediate values at a constant interval of 25 mm. Columns 3 to 12 had values of time (sec) of the two replicates for the five media types. One was added to each value of time before it was entered into the computer. This was done so that the data could be transformed to Log_{10}, Ln and squareroot. The notations for the different media and the corresponding data column numbers are:

C3	ML1
C4	ML2
C5	MD1
C6	MD2
C7	BL1
C8	BL2
C9	BD1
C10	BD2
C11	C1
C12	C2

where M = photosynthetic medium, B = bacterial medium, C = control, L = incubation in the light, D = incubation in the dark, 1 = replicate 1, 2 = replicate 2.

Instructions for entering data and application of some statistical methods using the **MINITAB81** statistics package on the ICL 3980 Mainframe Computer (Ryan et al. 1976; Sharp, 1986)

1) Switch on the mainframe terminal at the mains.

2) Wait for the cursor to appear on the top left hand corner of the screen (the screen will be referred to as VDU = visual display unit in the following text).

3)Press the RETURN key (<R> will be used for this operation in the following text).

4) VDU PAD>

Type CALL VME <R>

5) VDU ***Call connected

6) VDU Username : (or press <SEND>)

(Username is the users personal number e.g. GBZA99 which is issued by Glasgow University Computing Service)

Type :GBZA99 <R>

7) VDU Password:

If you are a new user, the default password is PASSWORD itself. To change the MAC Password,

Type CHANGE-PASSWORD ("Your new password") or CPW ("Your new password") <R>

8) VDU Please supply old password?

Type your old password <R>

(A password is a string of up to 12 characters including `space` and `underline`. It is important that you do not let others know your password. To stop your password being displayed on the VDU, press and hold the CONTROL key and press P then press E alone. Now enter your password. A safe practice is to change your password regularly).

9) VDU Which service : (Type ? if in doubt)

Type MAC <R>

10) VDU Session name :

A session name is a name given to the current session. It should not exceed 12 letters or digits and must start with a letter.

Type Session name (e.g. MAC2)

11) VDU Options : (Reply? for details)

Press <R>

12) VDU VME SESSION STARTS AT 10 : 31 : 35

VDU - (- is the Standard Command Prompt. At this stage the system expects a System Control Language command, e.g. DUD, INTRLB, DF, XLB, DLBD, see First Guide to VME, 1986).

13) - Type DUD <R>

This command stands for DISPLAY_USER_DETAILS and gives an alphabetical list of the user's libraries and files on the mainframe.

(A LIBRARY is a named collection of files. Libraries help to reduce the size of the catalogue, so create as many files as possible in a library. Files which do not belong to a library are called stand-alone files).

14) To create a permanent, empty library

Type INTRLB (Libraryname) <R>

(A libraryname can consist of up to 31 characters or digits starting with a letter. The name should preferably contain the characters LIB, to indicate that the name refers to a library e.g. MYLIB.

15) Type MINITAB81 <R>

This command starts a session in MINITAB.

There are two versions of the MINITAB commands: the full version and the short version. Either the full or the short version can be used. The full version is easier to understand but the short version is quicker to use. In the following text, the short versions of MINITAB commands are given in brackets, but the brackets are not used when entering the command. For example GENERATE 17 C1 is the full version for generating the integers 1 to 17 in column C1. The short version is GENE 17 C1.

16) VDU Minitab 81.1 is now being loaded;

17) VDU /- (/- is the alien-data prompt)

If you wish to get a printout of the current session then:

/- Type OUTUNIT 10 <R>

and if you want to stop a printout and only wish to have a screen dislpay then;

/- Type OUTUNIT 6 <R>

18) /- Type GENERATE 17 C1 <R>

The GENERATE (GENE) command generates 17 integers into column C1.

19) /- Type SET C2 <R>

The SET command is used to enter data in a specified column.

20) /- Type data values as such,

/- 1 4 6 7 9 10 12 14 15 17 18 20 23 25 28 29 32 <R>

Note when data values are entered a space is left between each value.

21) /- Type SET C3 <R>

Enter data values for column C3.

Repeat the same instructions for entering data for the remaining columns in turn i.e. C4-C12.

22) /- Type END <R>

This command comes at the end of data entry.

23) /- Type SAVE `Libraryname.Filename` <R>

e.g. SAVE `MYLIB.DAY1` <R>

This command saves the data file `DAY1` in the library `MYLIB`

Note MINITAB does not automatically create backup files or old generations of files as Wordstar and ECCE do. If the file has already been saved and you have retrieved it for editing, then the following procedure should be used. The newly edited file should be saved under a different file name than the previous version. In this way, the previous version serves as a backup file to the edited version in case the latter is lost.

24) /- Type PRINT C1-C12 <R>

This gives a listing of the data columns C1-C12 on the screen if you are in OUTUNIT 6, mode or a printout if you are in the OUTUNIT 10 mode.

25) /- Type LOGT C3 C13 <R>

This command transforms values in C3 to Log_{10} and puts them in column C13.

26) /- Type LOGE C3 C14 <R>

This command transforms values in C3 to natural log and puts them in column C14.

27) /- Type SQRT C3 C15 <R>

This command transforms values in C3 to squareroot and puts them in column C15.

28) /- Type DESCRIBE C2 <R>

This command (DESC) gives the mean, standard deviation and total number of values in column C2.

29) /- Type REGRESS C9 1 C2 <R>

This command (REGR) regresses the y-values in column C9 on 1 predictor in column C2 containing the x-values. It gives the

equation for the least squares line predicting y from x, an analysis of variance table, and other relevant information.

30) /- Type PLOT C3 C2 <R>

This command plots values of column C2 on the x-axis and of column C3 on y-axis.

31) /- Type ONEWAY C1,C2

This command (ONEW) performs a one-way analysis of variance. Here all the data is put into one column C1, and a second column C2 defines which level or group each value belongs to. The numbers used for levels or groups must be integers.

AOVONEWAY (AOVO) this is an alternative command to ONEWAY for the one-way analysis of variance. The data input varies from the command ONEWAY in that the 1st column C1 contains data from the first population (sometimes called group, or level), the 2nd column C2 contains data from the second population, the 3rd column C3 contains data from the third population, and so on. (The sample sizes need not be equal).

32) To end a session in MINITAB

/- Type STOP <R>

33) VDU - Type LGT <R>

LGT is a command to LOGOUT

34) VDU Session ends at 11 : 28 : 21

35) *** Cleared

36) Switch OFF the terminal at the mains.

Instructions to EDIT, RETRIEVE and SAVE a data file in MINITAB81

Edit data in one or more columns

/- CHOOSE ROWS WITH THE NUMBER K IN C1, CORRESPONDING ROWS OF C2, C3, PUT INTO C11, C12, C13

(CHOO)

This command chooses rows with the same value K in a column, and the corresponding rows of the other specified columns, and puts them into new columns.

/- CHOOSE 1 TO 4 IN C1, CORRESPONDING ROWS OF C2, C3, PUT INTO C11, C12, C13

The above example shows the second use of the command CHOOSE. This command chooses rows in a column with values ranging from 1 to 4 and the corresponding rows of other specified columns, and puts them into new columns.

/- ERASE C1 <R>

This command erases column C1 and you have to use the command SET C1 to enter new data in column C1.

(ERAS)

/- JOIN C2 TO THE BOTTOM OF C1, PUT INTO C3

This command joins C2 to the bottom of C1 and puts the joined columns into a new column C3.

/- JOIN C1 TO 2.4 TO 4.3 TO C2 TO C3, PUT INTO C4

This command joins columns and constants into a new column. In this example the new column C4 will have values (starting from the top of the column) in the following order: C3, C2, 4.3, 2.4 and C1.

/- PICK ROWS 1 TO 16 OF C1, PUT INTO C10

This command picks rows 1 to 16 of column C1 and puts them into a new column C10. The PICK command can be used to select any number of rows from a column and puts them into a new column.

Note The first row number must be less than or equal to the second row number.

/- SET C1 <R>

After you have SET a specified column (C1 in this example) you can type in data values for that column.

/- SUBSTITUTE 82 9 C5

This command substitutes the figure 82 into row 9 of column C5.

(SUBS)

Retrieve a file

/- RETRIEVE `Libraryname. Filename` <R>

This command retrieves a libraryfile for your access.

(RETR)

Save a file

/- SAVE `Libraryname. Filename` <R>

This command saves a libraryfile.

View data columns on the screen or obtain a printout

/- PRINT C1-C12

This lists the columns C1 to C12 on the screen if you are in OUTUNIT 6 mode or prints them on the printout if you are in the OUTUNIT 10 mode.

Appendix 5.3

Enrichment experiment.

Assessment of the best transformation of the time data in the enrichment experiment (original data in appendix 5.1) (text p 59).

Assessment of best transformation of time data in the enrichment experiment. Original data (Appendix 5.1 p 189)

The untransformed time data was subjected to 3 transformations: $\log_{10}$, $\log_e$ and square root. The untransformed and transformed data and the water column height were submitted to a series of regression analyses. Time (sec) (y) was regressed against water column height (mm) (x). This gave a total of 360 regression analyses. There were 2 replicate cores per medium, 5 media, 1+3 transformations, on each of the 9 days in the experiment on which readings were taken (2 x 5 x 4 x 9 = 360). Appendix 5.4 (Table 2 p 206) gives the results of the one-way analyses of variance of these regressions, and appendix 5.5 (Table 3 p 243) gives the regression equations and the F-ratios. The results in the two tables show that the square root transformation always gives the highest F-ratio except for the BL medium on day 22 and 25. The square root transformation was therefore chosen as the most appropriate one to use.

Appendix 5.4

Enrichment experiment.

Table 2. Regression analyses. One-way analyses of variance of the regressions of water column height (x axis) against time (y axis). Untransformed (none), and $\log_{10}$, $\log_e$, and square root transformed data.
9 days x 10 cores x 4 transformations = 360 one-way analyses of variance.
(text p 59).

Day, Medium, Replic.	Source of variation	df	SS	MS=SS/DF	F-ratio	P
1.ML.1 (None)	Regression	1	80500	80500	271.4	P<0.001
	Residual	15	4449	296.6		
	Total	16	84950			
1.ML.1 (Log)	Regression	1	4.731	4.731	65.17	P<0.001
	Residual	15	1.089	0.07260		
	Total	16	5.820			
1.ML.1 (Ln)	Regression	1	25.09	25.09	65.17	P<0.001
	Residual	15	5.774	0.3849		
	Total	16	30.86			
1.ML.1 (Sqrt)	Regression	1	277.0	277.0	2091	P<0.001
	Residual	15	1.988	0.1325		
	Total	16	279.0			
1.ML.2 (None)	Regression	1	92520	92520	279.0	P<0.001
	Residual	15	4974	331.6		
	Total	16	97500			
1.ML.2 (Log)	Regression	1	4.757	4.757	62.27	P<0.001
	Residual	15	1.146	0.07640		
	Total	16	5.903			
1.ML.2 (Ln)	Regression	1	25.22	25.22	62.26	P<0.001
	Residual	15	6.076	0.4051		
	Total	16	31.30			
1.ML.2 (Sqrt)	Regression	1	296.8	296.8	2020	P<0.001
	Residual	15	2.203	0.1469		
	Total	16	299.0			
1.MD.1 (None)	Regression	1	94760	94760	291.0	P<0.001
	Residual	15	4885	325.6		
	Total	16	99650			
1.MD.1 (Log)	Regression	1	4.828	4.828	61.32	P<0.001
	Residual	15	1.181	0.07873		
	Total	16	6.009			
1.MD.1 (Ln)	Regression	1	25.60	25.60	61.32	P<0.001
	Residual	15	6.262	0.4174		
	Total	16	31.86			
1.MD.1 (Sqrt)	Regression	1	301.5	301.5	1875	P<0.001
	Residual	15	2.412	0.1608		
	Total	16	303.9			

Table 2 contd.

Day, Medium, Replic.	Source of variation	df	SS	MS=SS/DF	F-ratio	P
1.MD.2 (None)	Regression	1	81800	81800	295.2	P<0.001
	Residual	15	4157	277.1		
	Total	16	85960			
1.MD.2 (Log)	Regression	1	4.573	4.573	61.05	P<0.001
	Residual	15	1.124	0.07491		
	Total	16	5.697			
1.MD.2 (Ln)	Regression	1	24.25	24.25	61.06	P<0.001
	Residual	15	5.957	0.3971		
	Total	16	30.20			
1.MD.2 (Sqrt)	Regression	1	275.3	275.3	1888	P<0.001
	Residual	15	2.188	0.1458		
	Total	16	277.5			
1.BL.1 (None)	Regression	1	66710	66710	298.9	P<0.001
	Residual	15	3348	223.2		
	Total	16	70060			
1.BL.1 (Log)	Regression	1	4.620	4.620	67.36	P<0.001
	Residual	15	1.029	0.6858		
	Total	16	5.648			
1.BL.1 (Ln)	Regression	1	24.49	24.49	67.36	P<0.001
	Residual	15	5.454	0.3636		
	Total	16	29.95			
1.BL.1 (Sqrt)	Regression	1	250.9	250.9	2286	P<0.001
	Residual	15	1.647	0.1098		
	Total	16	252.6			
1.BL.2 (None)	Regression	1	72350	72350	293.0	P<0.001
	Residual	15	3703	246.9		
	Total	16	76050			
1.BL.2 (Log)	Regression	1	4.558	4.558	62.33	P<0.001
	Residual	15	1.097	0.07313		
	Total	16	5.655			
1.BL.2 (Ln)	Regression	1	24.17	24.17	62.34	P<0.001
	Residual	15	5.816	0.3877		
	Total	16	29.98			
1.BL.2 (Sqrt)	Regression	1	258.8	258.8	1807	P<0.001
	Residual	15	2.148	0.1432		
	Total	16	260.9			

Table 2 contd.

Day, Medium, Replic.	Source of variation	df	SS	MS=SS/DF	F-ratio	P
1.BD.1 (None)	Regression	1	54330	54330	319.9	P<0.001
	Residual	15	2548	169.8		
	Total	16	56870			
1.BD.1 (Log)	Regression	1	4.497	4.497	67.51	P<0.001
	Residual	15	0.9991	0.06661		
	Total	16	5.496			
1.BD.1 (Ln)	Regression	1	23.84	23.84	67.50	P<0.001
	Residual	15	5.297	0.3532		
	Total	16	29.14			
1.BD.1 (Sqrt)	Regression	1	224.5	224.5	2015	P<0.001
	Residual	15	1.670	0.1114		
	Total	16	226.1			
1.BD.2 (None)	Regression	1	68070	68070	318.5	P<0.001
	Residual	15	3205	213.7		
	Total	16	71275			
1.BD.2 (Log)	Regression	1	4.515	4.515	61.72	P<0.001
	Residual	15	1.097	0.07315		
	Total	16	5.612			
1.BD.2 (Ln)	Regression	1	23.94	23.94	61.73	P<0.001
	Residual	15	5.818	0.3878		
	Total	16	29.75			
1.BD.2 (Sqrt)	Regression	1	250.2	250.2	1732	P<0.001
	Residual	15	2.168	0.1445		
	Total	16	252.4			
1.C.1 (None)	Regression	1	70100	70100	313.8	P<0.001
	Residual	15	3351	223.4		
	Total	16	73450			
1.C.1 (Log)	Regression	1	4.557	4.557	62.49	P<0.001
	Residual	15	1.094	0.07292		
	Total	16	5.650			
1.C.1 (Ln)	Regression	1	24.16	24.16	62.49	P<0.001
	Residual	15	5.799	0.3866		
	Total	16	29.96			
1.C.1 (Sqrt)	Regression	1	254.9	254.9	1838	P<0.001
	Residual	15	2.081	0.1387		
	Total	16	257.0			

Table 2 contd.

Day, Medium, Replic.	Source of variation	df	SS	MS=SS/DF	F-ratio	P
1.C.2 (None)	Regression	1	83050	83050	300.9	P<0.001
	Residual	15	4140	276.0		
	Total	16	87190			
1.C.2 (Log)	Regression	1	4.614	4.614	60.51	P<0.001
	Residual	15	1.144	0.07624		
	Total	16	5.758			
1.C.2 (Ln)	Regression	1	24.46	24.46	60.52	P<0.001
	Residual	15	6.064	0.4042		
	Total	16	30.52			
1.C.2 (Sqrt)	Regression	1	277.9	277.9	1825	P<0.001
	Residual	15	2.285	0.1523		
	Total	16	280.3			

Table 2 contd.

Day, Medium, Replic.	Source of variation	df	SS	MS=SS/DF	F-ratio	P
4.ML.1 (None)	Regression	1	145000	145000	306.2	P<0.001
	Residual	15	7102	473.5		
	Total	16	152100			
4.ML.1 (Log)	Regression	1	4.891	4.891	53.44	P<0.001
	Residual	15	1.373	0.09152		
	Total	16	6.264			
4.ML.1 (Ln)	Regression	1	25.93	25.93	53.44	P<0.001
	Residual	15	7.279	0.4853		
	Total	16	33.21			
4.ML.1 (Sqrt)	Regression	1	371.6	371.6	1547	P<0.001
	Residual	15	3.603	0.2402		
	Total	16	375.2			
4.ML.2 (None)	Regression	1	159600	159600	311.3	P<0.001
	Residual	15	7692	512.8		
	Total	16	167300			
4.ML.2 (Log)	Regression	1	4.968	4.968	53.11	P<0.001
	Residual	15	1.403	0.09354		
	Total	16	6.371			
4.ML.2 (Ln)	Regression	1	26.34	26.34	53.11	P<0.001
	Residual	15	7.439	0.4959		
	Total	16	33.78			
4.ML.2 (Sqrt)	Regression	1	391.7	391.7	1699	P<0.001
	Residual	15	3.459	0.2306		
	Total	16	395.2			
4.MD.1 (None)	Regression	1	199200	199200	305.2	P<0.001
	Residual	15	9790	652.7		
	Total	16	209000			
4.MD.1 (Log)	Regression	1	5.128	5.128	51.72	P<0.001
	Residual	15	1.487	0.09915		
	Total	16	6.616			
4.MD.1 (Ln)	Regression	1	27.19	27.19	51.72	P<0.001
	Residual	15	7.886	0.5257		
	Total	16	35.08			
4.MD.1 (Sqrt)	Regression	1	441.3	441.3	1639	P<0.001
	Residual	15	4.039	0.2692		
	Total	16	445.3			

Table 2 contd.

Day, Medium, Replic.	Source of variation	df	SS	MS=SS/DF	F-ratio	P
4.MD.2 (None)	Regression	1	175400	175400	328.7	P<0.001
	Residual	15	8005	533.7		
	Total	16	183400			
4.MD.2 (Log)	Regression	1	4.866	4.866	49.13	P<0.001
	Residual	15	1.486	0.09905		
	Total	16	6.352			
4.MD.2 (Ln)	Regression	1	25.80	25.80	49.12	P<0.001
	Residual	15	7.877	0.5252		
	Total	16	33.68			
4.MD.2 (Sqrt)	Regression	1	405.8	405.8	1438	P<0.001
	Residual	15	4.233	0.2822		
	Total	16	410.0			
4.BL.1 (None)	Regression	1	333100	333100	476.9	P<0.001
	Residual	15	10480	698.4		
	Total	16	343500			
4.BL.1 (Log)	Regression	1	4.769	4.769	36.24	P<0.001
	Residual	15	1.974	0.1316		
	Total	16	6.743			
4.BL.1 (Ln)	Regression	1	25.29	25.29.	36.25	P<0.001
	Residual	15	10.46	0.6979		
	Total	16	35.75			
4.BL.1 (Sqrt)	Regression	1	542.9	542.9	817.2	P<0.001
	Residual	15	9.967	0.6644		
	Total	16	552.9			
4.BL.2 (None)	Regression	1	467400	467400	530.0	P<0.001
	Residual	15	13230	881.9		
	Total	16	480700			
4.BL.2 (Log)	Regression	1	4.989	4.989	34.79	P<0.001
	Residual	15	2.151	0.1434		
	Total	16	7.140			
4.BL.2 (Ln)	Regression	1	26.45	26.45	34.80	P<0.001
	Residual	15	11.40	0.7602		
	Total	16	37.85			
4.BL.2 (Sqrt)	Regression	1	650.4	650.4	791.9	P<0.001
	Residual	15	12.32	0.8212		
	Total	16	662.7			

Table 2 contd.

Day, Medium, Replic.	Source of variation	df	SS	MS=SS/DF	F-ratio	P
4.BD.1 (None)	Regression	1	435000	435000	530.0	P<0.001
	Residual	15	12310	820.7		
	Total	16	447300			
4.BD.1 (Log)	Regression	1	5.071	5.071	36.56	P<0.001
	Residual	15	2.082	0.1388		
	Total	16	7.153			
4.BD.1 (Ln)	Regression	1	26.89	26.89	36.54	P<0.001
	Residual	15	11.04	0.7358		
	Total	16	37.94			
4.BD.1 (Sqrt)	Regression	1	633.7	633.7	838.3	P<0.001
	Residual	15	11.34	0.7560		
	Total	16	645.1			
4.BD.2 (None)	Regression	1	499200	499200	552.8	P<0.001
	Residual	15	13540	902.9		
	Total	16	512700			
4.BD.2 (Log)	Regression	1	5.123	5.123	35.72	P<0.001
	Residual	15	2.151	0.1434		
	Total	16	7.274			
4.BD.2 (Ln)	Regression	1	27.16	27.16	35.72	P<0.001
	Residual	15	11.41	0.7604		
	Total	16	38.57			
4.BD.2 (Sqrt)	Regression	1	679.8	679.8	859.2	P<0.001
	Residual	15	11.87	0.7912		
	Total	16	691.7			
4.C.1 (None)	Regression	1	97340	97340	331.1	P<0.001
	Residual	15	4410	294.0		
	Total	16	101800			
4.C.1 (Log)	Regression	1	4.606	4.606	55.71	P<0.001
	Residual	15	1.240	0.08267		
	Total	16	5.846			
4.C.1 (Ln)	Regression	1	24.42	24.42	55.71	P<0.001
	Residual	15	6.575	0.4383		
	Total	16	30.99			
4.C.1 (Sqrt)	Regression	1	299.5	299.5	1594	P<0.001
	Residual	15	2.819	0.1879		
	Total	16	302.3			

Table 2 contd.

Day, Medium, Replic.	Source of variation	df	SS	MS=SS/DF	F-ratio	P
4.C.2 (None)	Regression	1	114000	114000	322.7	P<0.001
	Residual	15	5296	353.1		
	Total	16	119200			
4.C.2 (Log)	Regression	1	4.642	4.642	53.24	P<0.001
	Residual	15	1.308	0.08718		
	Total	16	5.949			
4.C.2 (Ln)	Regression	1	24.61	24.61	53.23	P<0.001
	Residual	15	6.934	0.4623		
	Total	16	31.54			
4.C.2 (Sqrt)	Regression	1	323.7	323.7	1505	P<0.001
	Residual	15	3.226	0.2151		
	Total	16	327.0			

Table 2 contd.

Day, Medium, Replic.	Source of variation	df	SS	MS=SS/DF	F-ratio	P
7.ML.1 (None)	Regression	1	169100	169100	297.7	P<0.001
	Residual	15	8518	567.9		
	Total	16	177600			
7.ML.1 (Log)	Regression	1	4.843	4.843	50.11	P<0.001
	Residual	15	1.450	0.09665		
	Total	16	6.293			
7.ML.1 (Ln)	Regression	1	25.68	25.68	50.11	P<0.001
	Residual	15	7.687	0.5124		
	Total	16	33.36			
7.ML.1 (Sqrt)	Regression	1	398.0	398.0	1469	P<0.001
	Residual	15	4.063	0.2709		
	Total	16	402.1			
7.ML.2 (None)	Regression	1	193800	193800	294.4	P<0.001
	Residual	15	9873	658.2		
	Total	16	203600			
7.ML.2 (Log)	Regression	1	4.961	4.961	49.61	P<0.001
	Residual	15	1.501	0.1000		
	Total	16	6.462			
7.ML.2 (Ln)	Regression	1	26.30	26.30	49.58	P<0.001
	Residual	15	7.957	0.5305		
	Total	16	34.26			
7.ML.2 (Sqrt)	Regression	1	428.9	428.9	1474	P<0.001
	Residual	15	4.367	0.2911		
	Total	16	433.4			
7.MD.1 (None)	Regression	1	253300	253300	319.1	P<0.001
	Residual	15	11910	793.7		
	Total	16	265200			
7.MD.1 (Log)	Regression	1	5.062	5.062	46.53	P<0.001
	Residual	15	1.632	0.1088		
	Total	16	6.694			
7.MD.1 (Ln)	Regression	1	26.84	26.84	46.53	P<0.001
	Residual	15	8.652	0.5768		
	Total	16	35.49			
7.MD.1 (Sqrt)	Regression	1	491.8	491.8	1442	P<0.001
	Residual	15	5.115	0.3410		
	Total	16	496.9			

Table 2 contd.

Day, Medium, Replic.	Source of variation	df	SS	MS=SS/DF	F-ratio	P
7.MD.2 (None)	Regression	1	221300	221300	295.4	P<0.001
	Residual	15	11240	749.2		
	Total	16	232600			
7.MD.2 (Log)	Regression	1	5.032	5.032	48.76	P<0.001
	Residual	15	1.548	0.1032		
	Total	16	6.581			
7.MD.2 (Ln)	Regression	1	26.68	26.68	48.75	P<0.001
	Residual	15	8.209	0.5473		
	Total	16	34.89			
7.MD.2 (Sqrt)	Regression	1	460.1	460.1	1518	P<0.001
	Residual	15	4.547	0.3032		
	Total	16	464.7			
7.BL.1 (None)	Regression	1	403500	403500	315.9	P<0.001
	Residual	15	19150	1277		
	Total	16	422600			
7.BL.1 (Log)	Regression	1	4.923	4.923	37.18	P<0.001
	Residual	15	1.986	0.1324		
	Total	16	6.909			
7.BL.1 (Ln)	Regression	1	26.10	26.10	37.17	P<0.001
	Residual	15	10.53	0.7021		
	Total	16	36.63			
7.BL.1 (Sqrt)	Regression	1	603.2	603.2	902.4	P<0.001
	Residual	15	10.03	0.6685		
	Total	16	613.3			
7.BL.2 (None)	Regression	1	872600	872600	175.5	P<0.001
	Residual	15	74570	4971		
	Total	16	947200			
7.BL.2 (Log)	Regression	1	5.940	5.940	43.33	P<0.001
	Residual	15	2.057	0.1371		
	Total	16	7.997			
7.BL.2 (Ln)	Regression	1	31.49	31.49	43.31	P<0.001
	Residual	15	10.91	0.7271		
	Total	16	42.40			
7.BL.2 (Sqrt)	Regression	1	947.7	947.7	1195	P<0.001
	Residual	15	11.90	0.7933		
	Total	16	959.6			

Table 2 contd.

Day, Medium, Replic.	Source of variation	df	SS	MS=SS/DF	F-ratio	P
7.BD.1 (None)	Regression	1	320500	320500	408.0	P<0.001
	Residual	15	11780	785.5		
	Total	16	332300			
7.BD.1 (Log)	Regression	1	4.987	4.987	39.61	P<0.001
	Residual	15	1.889	0.1259		
	Total	16	6.876			
7.BD.1 (Ln)	Regression	1	26.44	26.44	39.60	P<0.001
	Residual	15	10.02	0.6677		
	Total	16	36.46			
7.BD.1 (Sqrt)	Regression	1	543.8	543.8	929.3	P<0.001
	Residual	15	8.778	0.5852		
	Total	16	552.6			
7.BD.2 (None)	Regression	1	360200	360200	419.2	P<0.001
	Residual	15	12890	859.3		
	Total	16	373100			
7.BD.2 (Log)	Regression	1	4.975	4.975	38.06	P<0.001
	Residual	15	1.961	0.1307		
	Total	16	6.936			
7.BD.2 (Ln)	Regression	1	26.38	26.38	38.05	P<0.001
	Residual	15	10.40	0.6932		
	Total	16	36.77			
7.BD.2 (Sqrt)	Regression	1	574.3	574.3	895.2	P<0.001
	Residual	15	9.623	0.6415		
	Total	16	583.9			
7.C.1 (None)	Regression	1	112200	112200	311.3	P<0.001
	Residual	15	5404	360.3		
	Total	16	117600			
7.C.1 (Log)	Regression	1	4.697	4.697	54.17	P<0.001
	Residual	15	1.301	0.08671		
	Total	16	5.997			
7.C.1 (Ln)	Regression	1	24.90	24.90	54.17	P<0.001
	Residual	15	6.896	0.4597		
	Total	16	31.80			
7.C.1 (Sqrt)	Regression	1	322.7	322.7	1473	P<0.001
	Residual	15	3.285	0.2190		
	Total	16	325.9			

Table 2 contd.

Day, Medium, Replic.	Source of variation	df	SS	MS=SS/DF	F-ratio	P
7.C.2 (None)	Regression	1	117700	117700	322.1	P<0.001
	Residual	15	5482	365.5		
	Total	16	123200			
7.C.2 (Log)	Regression	1	4.764	4.764	54.67	P<0.001
	Residual	15	1.307	0.08713		
	Total	16	6.071			
7.C.2 (Ln)	Regression	1	25.26	25.26	54.67	P<0.001
	Residual	15	6.930	0.4620		
	Total	16	32.19			
7.C.2 (Sqrt)	Regression	1	332.5	332.5	1591	P<0.001
	Residual	15	3.135	0.2090		
	Total	16	335.7			

Table 2 contd.

Day, Medium, Replic.	Source of variation	df	SS	MS=SS/DF	F-ratio	P
10.ML.1 (None)	Regression	1	211800	211800	294.5	P<0.001
	Residual	15	10790	719.2		
	Total	16	222600			
10.ML.1 (Log)	Regression	1	4.965	4.965	48.20	P<0.001
	Residual	15	1.545	0.1030		
	Total	16	6.510			
10.ML.1 (Ln)	Regression	1	26.32	26.32	48.20	P<0.001
	Residual	15	8.193	0.5462		
	Total	16	34.52			
10.ML.1 (Sqrt)	Regression	1	447.8	447.8	1426	P<0.001
	Residual	15	4.710	0.3140		
	Total	16	452.5			
10.ML.2 (None)	Regression	1	242600	242600	300.8	P<0.001
	Residual	15	12100	806.4		
	Total	16	254700			
10.ML.2 (Log)	Regression	1	5.093	5.093	47.87	P<0.001
	Residual	15	1.596	0.1064		
	Total	16	6.689			
10.ML.2 (Ln)	Regression	1	27.00	27.00	47.87	P<0.001
	Residual	15	8.462	0.5641		
	Total	16	35.47			
10.ML.2 (Sqrt)	Regression	1	482.9	482.9	1482	P<0.001
	Residual	15	4.887	0.3258		
	Total	16	487.8			
10.MD.1 (None)	Regression	1	310400	310400	336.6	P<0.001
	Residual	15	13830	922.2		
	Total	16	324200			
10.MD.1 (Log)	Regression	1	5.196	5.196	45.30	P<0.001
	Residual	15	1.721	0.1147		
	Total	16	6.916			
10.MD.1 (Ln)	Regression	1	27.55	27.55	45.29	P<0.001
	Residual	15	9.123	0.6082		
	Total	16	36.67			
10.MD.1 (Sqrt)	Regression	1	548.1	548.1	1460	P<0.001
	Residual	15	5.632	0.3754		
	Total	16	553.7			

Table 2 contd.

Day, Medium, Replic.	Source of variation	df	SS	MS=SS/DF	F-ratio	P
10.MD.2 (None)	Regression	1	266700	266700	302.4	P<0.001
	Residual	15	13230	882.0		
	Total	16	279900			
10.MD.2 (Log)	Regression	1	4.951	4.951	44.48	P<0.001
	Residual	15	1.669	0.1113		
	Total	16	6.620			
10.MD.2 (Ln)	Regression	1	26.25	26.25	44.50	P<0.001
	Residual	15	8.848	0.5899		
	Total	16	35.10			
10.MD.2 (Sqrt)	Regression	1	499.2	499.2	1318	P<0.001
	Residual	15	5.679	0.3786		
	Total	16	504.9			
10.BL.1 (None)	Regression	1	623900	623900	234.1	P<0.001
	Residual	15	39980	2665		
	Total	16	663900			
10.BL.1 (Log)	Regression	1	5.518	5.518	41.30	P<0.001
	Residual	15	2.004	0.1336		
	Total	16	7.522			
10.BL.1 (Ln)	Regression	1	29.26	29.26	41.31	P<0.001
	Residual	15	10.62	0.7082		
	Total	16	39.88			
10.BL.1 (Sqrt)	Regression	1	783.1	783.1	1263	P<0.001
	Residual	15	9.296	0.6197		
	Total	16	792.4			
10.BL.2 (None)	Regression	1	5900000	5900000	60.12	P<0.001
	Residual	15	1472000	98140		
	Total	16	7372000			
10.BL.2 (Log)	Regression	1	8.272	8.272	56.97	P<0.001
	Residual	15	2.178	0.1452		
	Total	16	10.45			
10.BL.2 (Ln)	Regression	1	43.86	43.86	56.97	P<0.001
	Residual	15	11.55	0.7698		
	Total	16	55.41			
10.BL.2 (Sqrt)	Regression	1	2712	2712	360.4	P<0.001
	Residual	15	112.9	7.525		
	Total	16	2824			

Table 2 contd.

Day, Medium, Replic.	Source of variation	df	SS	MS=SS/DF	F-ratio	P
10.BD.1 (None)	Regression	1	581600	581600	219.2	P<0.001
	Residual	15	39800	2653		
	Total	16	621400			
10.BD.1 (Log)	Regression	1	5.519	5.519	42.55	P<0.001
	Residual	15	1.946	0.1297		
	Total	16	7.464			
10.BD.1 (Ln)	Regression	1	29.26	29.26	42.54	P<0.001
	Residual	15	10.32	0.6878		
	Total	16	39.58			
10.BD.1 (Sqrt)	Regression	1	757.4	757.4	1260	P<0.001
	Residual	15	9.015	0.6010		
	Total	16	766.4			
10.BD.2 (None)	Regression	1	711100	711100	237.0	P<0.001
	Residual	15	45000	3000		
	Total	16	756100			
10.BD.2 (Log)	Regression	1	5.617	5.617	41.00	P<0.001
	Residual	15	2.055	0.1370		
	Total	16	7.673			
10.BD.2 (Ln)	Regression	1	29.78	29.78	40.99	P<0.001
	Residual	15	10.90	0.7265		
	Total	16	40.68			
10.BD.2 (Sqrt)	Regression	1	840.4	840.4	1330	P<0.001
	Residual	15	9.478	0.6319		
	Total	16	849.9			
10.C.1 (None)	Regression	1	119379	119379	310.3	P<0.001
	Residual	15	5771	384.7		
	Total	16	125150			
10.C.1 (Log)	Regression	1	4.748	4.748	55.08	P<0.001
	Residual	15	1.293	0.08620		
	Total	16	6.041			
10.C.1 (Ln)	Regression	1	25.17	25.17	55.06	P<0.001
	Residual	15	6.856	0.4571		
	Total	16	32.03			
10.C.1 (Sqrt)	Regression	1	334.6	334.6	1669	P<0.001
	Residual	15	3.008	0.2005		
	Total	16	337.6			

Table 2 contd.

Day, Medium, Replic.	Source of variation	df	SS	MS=SS/DF	F-ratio	P
10.C.2 (None)	Regression	1	125400	125400	312.0	P<0.001
	Residual	15	6030	402.0		
	Total	16	131500			
10.C.2 (Log)	Regression	1	4.701	4.701	52.10	P<0.001
	Residual	15	1.353	0.09023		
	Total	16	6.054			
10.C.2 (Ln)	Regression	1	24.92	24.92	52.10	P<0.001
	Residual	15	7.176	0.4784		
	Total	16	32.10			
10.C.2 (Sqrt)	Regression	1	340.4	340.4	1398	P<0.001
	Residual	15	3.651	0.2434		
	Total	16	344.1			

Table 2 contd.

Day, Medium, Replic.	Source of variation	df	SS	MS=SS/DF	F-ratio	P
13.ML.1 (None)	Regression	1	285934	285934	370.7	P<0.001
	Residual	15	11569	771.3		
	Total	16	297503			
13.ML.1 (Log)	Regression	1	5.394	5.394	51.52	P<0.001
	Residual	15	1.570	0.1047		
	Total	16	6.964			
13.ML.1 (Ln)	Regression	1	28.60	28.60	51.54	P<0.001
	Residual	15	8.324	0.5549		
	Total	16	36.92			
13.ML.1 (Sqrt)	Regression	1	539.6	539.6	2163	P<0.001
	Residual	15	3.743	0.2495		
	Total	16	543.4			
13.ML.2 (None)	Regression	1	255200	255200	291.7	P<0.001
	Residual	15	13121	874.8		
	Total	16	268321			
13.ML.2 (Log)	Regression	1	5.117	5.117	48.14	P<0.001
	Residual	15	1.595	0.1063		
	Total	16	6.712			
13.ML.2 (Ln)	Regression	1	27.13	27.13	48.12	P<0.001
	Residual	15	8.457	0.5638		
	Total	16	35.59			
13.ML.2 (Sqrt)	Regression	1	496.4	496.4	1574	P<0.001
	Residual	15	4.731	0.3154		
	Total	16	501.1			
13.MD.1 (None)	Regression	1	312411	312411	329.6	P<0.001
	Residual	15	14217	947.8		
	Total	16	326628			
13.MD.1 (Log)	Regression	1	5.168	5.168	45.06	P<0.001
	Residual	15	1.720	0.1147		
	Total	16	6.888			
13.MD.1 (Ln)	Regression	1	27.40	27.40	45.06	P<0.001
	Residual	15	9.122	0.6081		
	Total	16	36.52			
13.MD.1 (Sqrt)	Regression	1	548.7	548.7	1469	P<0.001
	Residual	15	5.604	0.3736		
	Total	16	554.3			

Table 2 contd.

Day, Medium, Replic.	Source of variation	df	SS	MS=SS/DF	F-ratio	P
13.MD.2 (None)	Regression	1	280507	280507	297.5	P<0.001
	Residual	15	14145	943.0		
	Total	16	294652			
13.MD.2 (Log)	Regression	1	4.826	4.826	41.35	P<0.001
	Residual	15	1.750	0.1167		
	Total	16	6.576			
13.MD.2 (Ln)	Regression	1	25.59	25.59	41.36	P<0.001
	Residual	15	9.279	0.6186		
	Total	16	34.87			
13.MD.2 (Sqrt)	Regression	1	504.5	504.5	1071	P<0.001
	Residual	15	7.067	0.4711		
	Total	16	511.6			
13.BL.1 (None)	Regression	1	1089851	1089851	142.3	P<0.001
	Residual	15	114863	7658		
	Total	16	1204714			
13.BL.1 (Log)	Regression	1	5.987	5.987	43.45	P<0.001
	Residual	15	2.066	0.1378		
	Total	16	8.054			
13.BL.1 (Ln)	Regression	1	31.74	31.74	43.46	P<0.001
	Residual	15	10.96	0.7304		
	Total	16	42.70			
13.BL.1 (Sqrt)	Regression	1	1060	1060	1035	P<0.001
	Residual	15	15.36	1.024		
	Total	16	1075			
13.BL.2 (None)	Regression	1	2670248	2670248	140.9	P<0.001
	Residual	15	284200	18947		
	Total	16	2954445			
13.BL.2 (Log)	Regression	1	6.321	6.321	36.37	P<0.001
	Residual	15	2.607	0.1738		
	Total	16	8.927			
13.BL.2 (Ln)	Regression	1	33.51	33.51	36.38	P<0.001
	Residual	15	13.82	0.9213		
	Total	16	47.33			
13.BL.2 (Sqrt)	Regression	1	1660	1660	925.3	P<0.001
	Residual	15	26.90	1.794		
	Total	16	1686			

Table 2 contd.

Day, Medium, Replic.	Source of variation	df	SS	MS=SS/DF	F-ratio	P
13.BD.1 (None)	Regression	1	446558	446558	240.6	P<0.001
	Residual	15	27845	1856		
	Total	16	474402			
13.BD.1 (Log)	Regression	1	5.491	5.491	46.07	P<0.001
	Residual	15	1.788	0.1192		
	Total	16	7.279			
13.BD.1 (Ln)	Regression	1	29.11	29.11	46.07	P<0.001
	Residual	15	9.480	0.6320		
	Total	16	38.59			
13.BD.1 (Sqrt)	Regression	1	667.9	667.9	1568	P<0.001
	Residual	15	6.391	0.4261		
	Total	16	674.3			
13.BD.2 (None)	Regression	1	660420	660420	189.2	P<0.001
	Residual	15	52362	3491		
	Total	16	712782			
13.BD.2 (Log)	Regression	1	5.745	5.745	44.38	P<0.001
	Residual	15	1.942	0.1295		
	Total	16	7.687			
13.BD.2 (Ln)	Regression	1	30.46	30.46	44.38	P<0.001
	Residual	15	10.30	0.6863		
	Total	16	40.76			
13.BD.2 (Sqrt)	Regression	1	818.7	818.7	1265	P<0.001
	Residual	15	9.709	0.6472		
	Total	16	828.4			
13.C.1 (None)	Regression	1	124146	124146	308.1	P<0.001
	Residual	15	6044	402.9		
	Total	16	130190			
13.C.1 (Log)	Regression	1	4.812	4.812	55.42	P<0.001
	Residual	15	1.302	0.08682		
	Total	16	6.114			
13.C.1 (Ln)	Regression	1	25.51	25.51	55.42	P<0.001
	Residual	15	6.904	0.4603		
	Total	16	32.42			
13.C.1 (Sqrt)	Regression	1	342.9	342.9	1664	P<0.001
	Residual	15	3.091	0.2061		
	Total	16	345.9			

Table 2 contd.

Day, Medium, Replic.	Source of variation	df	SS	MS=SS/DF	F-ratio	P
13.C.2 (None)	Regression	1	129292	129292	307.9	P<0.001
	Residual	15	6297	419.9		
	Total	16	135589			
13.C.2 (Log)	Regression	1	4.814	4.814	54.01	P<0.001
	Residual	15	1.337	0.08914		
	Total	16	6.152			
13.C.2 (Ln)	Regression	1	25.53	25.53	54.01	P<0.001
	Residual	15	7.090	0.4726		
	Total	16	32.61			
13.C.2 (Sqrt)	Regression	1	349.4	349.4	1478	P<0.001
	Residual	15	3.546	0.2364		
	Total	16	352.9			

Table 2 contd.

Day, Medium, Replic.	Source of variation	df	SS	MS=SS/DF	F-ratio	P
16.ML.1 (None)	Regression	1	272747	272747	316.7	P<0.001
	Residual	15	12919	861.3		
	Total	16	285667			
16.ML.1 (Log)	Regression	1	4.805	4.805	41.28	P<0.001
	Residual	15	1.746	0.1164		
	Total	16	6.551			
16.ML.1 (Ln)	Regression	1	25.48	25.48	41.28	P<0.001
	Residual	15	9.257	0.6172		
	Total	16	34.73			
16.ML.1 (Sqrt)	Regression	1	496.9	496.9	1081	P<0.001
	Residual	15	6.898	0.4599		
	Total	16	503.9			
16.ML.2 (None)	Regression	1	308109	308109	287.1	P<0.001
	Residual	15	16091	1073		
	Total	16	324200			
16.ML.2 (Log)	Regression	1	4.946	4.946	42.75	P<0.001
	Residual	15	1.736	0.1157		
	Total	16	6.682			
16.ML.2 (Ln)	Regression	1	26.22	26.22	42.74	P<0.001
	Residual	15	9.202	0.6135		
	Total	16	35.43			
16.ML.2 (Sqrt)	Regression	1	534.6	534.6	1262	P<0.001
	Residual	15	6.352	0.4235		
	Total	16	540.9			
16.MD.1 (None)	Regression	1	371170	371170	32.08	P<0.001
	Residual	15	17351	1157		
	Total	16	388521			
16.MD.1 (Log)	Regression	1	5.598	5.598	49.28	P<0.001
	Residual	15	1.704	0.1136		
	Total	16	7.302			
16.MD.1 (Ln)	Regression	1	29.68	29.68	49.28	P<0.001
	Residual	15	9.034	0.6023		
	Total	16	38.71			
16.MD.1 (Sqrt)	Regression	1	617.7	617.7	209.2	P<0.001
	Residual	15	4.430	0.2953		
	Total	16	622.1			

Table 2 contd.

Day, Medium, Replic.	Source of variation	df	SS	MS=SS/DF	F-ratio	P
16.MD.2 (None)	Regression	1	318361	318361	279.2	P<0.001
	Residual	15	17098	1140		
	Total	16	335459			
16.MD.2 (Log)	Regression	1	5.307	5.307	48.16	P<0.001
	Residual	15	1.653	0.1102		
	Total	16	6.960			
16.MD.2 (Ln)	Regression	1	28.14	28.14	48.16	P<0.001
	Residual	15	8.765	0.5843		
	Total	16	36.90			
16.MD.2 (Sqrt)	Regression	1	560.8	560.8	1730	P<0.001
	Residual	15	4.862	0.3241		
	Total	16	565.7			
16.BL.1 (None)	Regression	1	2279273	2279273	216.8	P<0.001
	Residual	15	157721	10515		
	Total	16	2436993			
16.BL.1 (Log)	Regression	1	6.749	6.749	39.98	P<0.001
	Residual	15	2.532	0.1688		
	Total	16	9.282			
16.BL.1 (Ln)	Regression	1	35.78	35.78	39.98	P<0.001
	Residual	15	13.43	0.8950		
	Total	16	49.21			
16.BL.1 (Sqrt)	Regression	1	1589	1589	1305	P<0.001
	Residual	15	18.25	1.217		
	Total	16	1607			
16.BL.2 (None)	Regression	1	50784683	50784683	25.59	P<0.001
	Residual	15	29770698	1984712		
	Total	16	80555372			
16.BL.2 (Log)	Regression	1	9.628	9.628	45.37	P<0.001
	Residual	15	3.182	0.2122		
	Total	16	12.81			
16.BL.2 (Ln)	Regression	1	51.05	51.05	45.37	P<0.001
	Residual	15	16.87	1.125		
	Total	16	67.92			
16.BL.2 (Sqrt)	Regression	1	7785	7785	107.4	P<0.001
	Residual	15	1087	72.48		
	Total	16	8873			

Table 2 contd.

Day, Medium, Replic.	Source of variation	df	SS	MS=SS/DF	F-ratio	P
16.BD.1 (None)	Regression	1	854973	854973	256.5	P<0.001
	Residual	15	50000	3333		
	Total	16	904974			
16.BD.1 (Log)	Regression	1	5.623	5.623	38.89	P<0.001
	Residual	15	2.170	0.1446		
	Total	16	7.793			
16.BD.1 (Ln)	Regression	1	29.81	29.81	38.87	P<0.001
	Residual	15	11.50	0.7669		
	Total	16	41.32			
16.BD.1 (Sqrt)	Regression	1	918.8	918.8	1337	P<0.001
	Residual	15	10.31	0.6872		
	Total	16	929.1			
16.BD.2 (None)	Regression	1	841204	841204	240.9	P<0.001
	Residual	15	52383	3492		
	Total	16	893588			
16.BD.2 (Log)	Regression	1	5.617	5.617	39.14	P<0.001
	Residual	15	2.152	0.1435		
	Total	16	7.769			
16.BD.2 (Ln)	Regression	1	29.78	29.78	39.14	P<0.001
	Residual	15	11.41	0.7608		
	Total	16	41.19			
16.BD.2 (Sqrt)	Regression	1	910.7	910.7	1303	P<0.001
	Residual	15	10.48	0.6984		
	Total	16	921.2			
16.C.1 (None)	Regression	1	132155	132155	302.1	P<0.001
	Residual	15	6561	437.4		
	Total	16	138717			
16.C.1 (Log)	Regression	1	4.685	4.685	51.59	P<0.001
	Residual	15	1.362	0.09082		
	Total	16	6.047			
16.C.1 (Ln)	Regression	1	24.84	24.84	51.59	P<0.001
	Residual	15	7.223	0.4815		
	Total	16	32.06			
16.C.1 (Sqrt)	Regression	1	348.8	348.8	1393	P<0.001
	Residual	15	3.754	0.2503		
	Total	16	352.6			

Table 2 contd.

Day, Medium, Replic.	Source of variation	df	SS	MS=SS/DF	F-ratio	P
16.C.2 (None)	Regression	1	130040	130040	302.9	P<0.001
	Residual	15	6439	429.3		
	Total	16	136479			
16.C.2 (Log)	Regression	1	4.820	4.820	55.15	P<0.001
	Residual	15	1.311	0.08739		
	Total	16	6.131			
16.C.2 (Ln)	Regression	1	25.55	25.55	55.16	P<0.001
	Residual	15	6.950	0.4633		
	Total	16	32.50			
16.C.2 (Sqrt)	Regression	1	350.9	350.9	1760	P<0.001
	Residual	15	2.992	0.1994		
	Total	16	353.9			

Table 2 contd.

Day, Medium, Replic.	Source of variation	df	SS	MS=SS/DF	F-ratio	P
19.ML.1 (None)	Regression	1	293900	293900	313.5	p<0.001
	Residual	15	14060	937.5		
	Total	16	307900			
19.ML.1 (Log)	Regression	1	5.239	5.239	47.37	p<0.001
	Residual	15	1.660	0.1106		
	Total	16	6.898			
19.ML.1 (Ln)	Regression	1	27.77	27.77	47.35	p<0.001
	Residual	15	8.799	0.5866		
	Total	16	36.57			
19.ML.1 (Sqrt)	Regression	1	536.3	536.3	1637	P<0.001
	Residual	15	4.915	0.3277		
	Total	16	541.3			
19.ML.2 (None)	Regression	1	350800	350800	286.6	P<0.001
	Residual	15	18360	1224		
	Total	16	369100			
19.ML.2 (Log)	Regression	1	5.3240	5.3240	46.70	P<0.001
	Residual	15	1.7110	0.1140		
	Total	16	7.0340			
19.ML.2 (Ln)	Regression	1	28.22	28.22	46.68	P<0.001
	Residual	15	9.070	0.6047		
	Total	16	37.29			
19.ML.2 (Sqrt)	Regression	1	588.0	588.0	1677	P<0.001
	Residual	15	5.259	0.3306		
	Total	16	593.3			
19.MD.1 (None)	Regression	1	444700	444700	300.5	P<0.001
	Residual	15	22200	1480		
	Total	16	466900			
19.MD.1 (Log)	Regression	1	5.594	5.594	46.54	P<0.001
	Residual	15	1.804	0.1202		
	Total	16	7.398			
19.MD.1 (Ln)	Regression	1	29.66	29.66	46.53	P<0.001
	Residual	15	9.563	0.6375		
	Total	16	39.22			
19.MD.1 (Sqrt)	Regression	1	672.5	672.5	1833	P<0.001
	Residual	15	5.502	0.3668		
	Total	16	677.9			

Table 2 contd.

Day, Medium, Replic.	Source of variation	df	SS	MS=SS/DF	F-ratio	P
19.MD.2 (None)	Regression	1	378800	378800	285.7	P<0.001
	Residual	15	19890	1326		
	Total	16	398700			
19.MD.2 (Log)	Regression	1	5.514	5.514	47.90	P<0.001
	Residual	15	1.727	0.1151		
	Total	16	7.240			
19.MD.2 (Ln)	Regression	1	29.23	29.23	47.90	P<0.001
	Residual	15	9.155	0.6103		
	Total	16	38.39			
19.MD.2 (Sqrt)	Regression	1	618.9	618.9	1795	P<0.001
	Residual	15	5.172	0.3448		
	Total	16	624.1			
19.BL.1 (None)	Regression	1	5215000	5215000	54.30	P<0.001
	Residual	9	864300	96040		
	Total	10	6080000			
19.BL.1 (Log)	Regression	1	6.602	6.602	25.92	P<0.001
	Residual	9	2.293	0.2547		
	Total	10	8.895			
19.BL.1 (Ln)	Regression	1	35.01	35.01	25.91	P<0.001
	Residual	9	12.16	1.351		
	Total	10	47.16			
19.BL.1 (Sqrt)	Regression	1	2131	2131	492.2	P<0.001
	Residual	9	38.96	4.329		
	Total	10	2170			
19.BL.2 (None)	Regression	1	46180000	46180000	12.21	0.01 >P> 0.005
	Residual	5	18910000	3781000		
	Total	6	65080000			
19.BL.2 (Log)	Regression	1	7.534	7.534	14.47	0.01 >P> 0.005
	Residual	5	2.604	0.5208		
	Total	6	10.14			
19.BL.2 (Ln)	Regression	1	39.95	39.95	14.47	0.01 >P> 0.005
	Residual	5	13.81	2.761		
	Total	6	53.75			
19.BL.2 (Sqrt)	Regression	1	5449	5449	56.56	P<0.001
	Residual	5	481.8	96.35		
	Total	6	5931			

Table 2 contd.

Day, Medium, Replic.	Source of variation	df	SS	MS=SS/DF	F-ratio	P
19.BD.1 (None)	Regression	1	1360000	1360000	191.5	P<0.001
	Residual	15	106600	7104		
	Total	16	1467000			
19.BD.1 (Log)	Regression	1	6.234	6.234	42.09	P<0.001
	Residual	15	2.222	0.1481		
	Total	16	8.456			
19.BD.1 (Ln)	Regression	1	33.05	33.05	42.08	P<0.001
	Residual	15	11.78	0.7855		
	Total	16	44.83			
19.BD.1 (Sqrt)	Regression	1	1201	1201	1457	P<0.01
	Residual	15	12.35	0.8240		
	Total	16	1213			
19.BD.2 (None)	Regression	1	1767000	1767000	188.2	P<0.001
	Residual	15	140800	9387		
	Total	16	1908000			
19.BD.2 (Log)	Regression	1	6.283	6.283	38.50	P<0.001
	Residual	15	2.448	0.1632		
	Total	16	8.730			
19.BD.2 (Ln)	Regression	1	33.31	33.31	38.51	P<0.001
	Residual	15	12.98	0.8651		
	Total	16	46.29			
19.BD.2 (Sqrt)	Regression	1	1359	1359	1240	P<0.001
	Residual	15	16.44	1.096		
	Total	16	1376			
19.C.1 (None)	Regression	1	139000	139000	300.4	P<0.001
	Residual	15	6942	462.8		
	Total	16	146000			
19.C.1 (Log)	Regression	1	4.926	4.926	56.13	P<0.001
	Residual	15	1.316	0.08776		
	Total	16	6.242			
19.C.1 (Ln)	Regression	1	26.12	26.12	56.13	P<0.001
	Residual	15	6.980	0.4653		
	Total	16	33.10			
19.C.1 (Sqrt)	Regression	1	365.9	365.9	1896	P<0.001
	Residual	15	2.894	0.1930		
	Total	16	368.8			

Table 2 contd.

Day, Medium, Replic.	Source of variation	df	SS	MS=SS/DF	F-ratio	P
19.C.2 (None)	Regression	1	137000	137000	314.3	P<0.001
	Residual	15	6536	435.7		
	Total	16	143500			
19.C.2 (Log)	Regression	1	4.841	4.841	53.80	P<0.001
	Residual	15	1.350	0.08999		
	Total	16	6.191			
19.C.2 (Ln)	Regression	1	25.67	25.67	53.80	P<0.001
	Residual	15	7.157	0.4771		
	Total	16	32.82			
19.C.2 (Sqrt)	Regression	1	360.2	360.2	1640	P<0.001
	Residual	15	3.296	0.2197		
	Total	16	363.5			

Table 2 contd.

Day, Medium, Replic.	Source of variation	df	SS	MS=SS/DF	F-ratio	P
22.ML.1 (None)	Regression	1	353200	353200	263.8	P<0.001
	Residual	15	20080	1339		
	Total	16	373300			
22.ML.1 (Log)	Regression	1	5.286	5.286	45.97	P<0.001
	Residual	15	1.725	0.1150		
	Total	16	7.011			
22.ML.1 (Ln)	Regression	1	28.03	28.03	45.96	P<0.001
	Residual	15	9.147	0.6098		
	Total	16	37.17			
22.ML.1 (Sqrt)	Regression	1	587.3	587.3	1497	P<0.001
	Residual	15	5.884	0.3923		
	Total	16	593.2			
22.ML.2 (None)	Regression	1	522500	522500	285.5	P<0.001
	Residual	15	27450	1830		
	Total	16	550000			
22.ML.2 (Log)	Regression	1	5.756	5.756	49.07	P<0.001
	Residual	15	1.759	0.1173		
	Total	16	7.515			
22.ML.2 (Ln)	Regression	1	30.52	30.52	49.09	P<0.001
	Residual	15	9.326	0.6217		
	Total	16	39.84			
22.ML.2 (Sqrt)	Regression	1	741.0	741.0	1822	P<0.001
	Residual	15	6.103	0.4068		
	Total	16	747.1			
22.MD.1 (None)	Regression	1	558200	558200	211.8	P<0.001
	Residual	15	39530	2635		
	Total	16	597700			
22.MD.1 (Log)	Regression	1	5.674	5.674	44.92	P<0.001
	Residual	15	1.894	0.1263		
	Total	16	7.568			
22.MD.1 (Ln)	Regression	1	30.08	30.08	44.93	P<0.001
	Residual	15	10.04	0.6695		
	Total	16	40.12			
22.MD.1 (Sqrt)	Regression	1	751.6	751.6	1309	P<0.001
	Residual	15	8.615	0.5744		
	Total	16	760.3			

Table 2 contd.

Day, Medium, Replic.	Source of variation	df	SS	MS=SS/DF	F-ratio	P
22.MD.2 (None)	Regression	1	435200	435200	286.7	P<0.001
	Residual	15	22770	1518		
	Total	16	458000			
22.MD.2 (Log)	Regression	1	5.356	5.356	43.65	P<0.001
	Residual	15	1.840	0.1227		
	Total	16	7.196			
22.MD.2 (Ln)	Regression	1	28.40	28.40	43.67	P<0.001
	Residual	15	9.755	0.6503		
	Total	16	38.15			
22.MD.2 (Sqrt)	Regression	1	652.6	652.6	1503	P<0.001
	Residual	15	6.514	0.4343		
	Total	16	659.1			
22.BL.1 (None)	Regression	1	2134000	2134000	83.93	0.025
	Residual	2	50850	25430		>P>
	Total	3	2185000			0.01
22.BL.1 (Log)	Regression	1	5.244	5.244	6.162	0.25
	Residual	2	1.702	0.8511		>P>
	Total	3	6.947			0.01
22.BL.1 (Ln)	Regression	1	27.81	27.81	6.162	0.25
	Residual	2	9.025	4.513		>P>
	Total	3	36.83			0.10
22.BL.1 (Sqrt)	Regression	1	999.6	999.6	76.65	0.025
	Residual	2	26.09	13.04		>P>
	Total	3	1026			0.01
22.BL.2 (None)	Regression	1	14470000	14470000	7233000	P<0.001
	Residual	1	2.000	2.000		
	Total	2	14470000			
22.BL.2 (Log)	Regression	1	6.959	6.959	4.267	0.50
	Residual	1	1.631	1.631		>P>
	Total	2	8.590			0.25
22.BL.2 (Ln)	Regression	1	36.90	36.90	4.267	0.50
	Residual	1	8.648	8.648		>P>
	Total	2	45.55			0.25
22.BL.2 (Sqrt)	Regression	1	2617	2617	18.21	0.25
	Residual	1	143.7	143.7		>P>
	Total	2	2761			0.10

Table 2 contd.

Day, Medium, Replic.	Source of variation	df	SS	MS=SS/DF	F-ratio	P
22.BD.1 (None)	Regression	1	1871000	1871000	121.3	P<0.001
	Residual	15	231400	15430		
	Total	16	2102000			
22.BD.1 (Log)	Regression	1	6.345	6.345	40.81	P<0.001
	Residual	15	2.333	0.1555		
	Total	16	8.678			
22.BD.1 (Ln)	Regression	1	33.64	33.64	40.80	P<0.001
	Residual	15	12.37	0.8246		
	Total	16	46.01			
22.BD.1 (Sqrt)	Regression	1	1401	1401	827.2	P<0.001
	Residual	15	25.40	1.694		
	Total	16	1427			
22.BD.2 (None)	Regression	1	51430000	51430000	14.73	0.005 >P> 0.001
	Residual	15	52360000	3491000		
	Total	16	103800000			
22.BD.2 (Log)	Regression	1	10.98	10.98	69.98	P<0.001
	Residual	15	2.353	0.1569		
	Total	16	13.33			
22.BD.2 (Ln)	Regression	1	58.22	58.22	70.01	P<0.001
	Residual	15	12.47	0.8316		
	Total	16	70.69			
22.BD.2 (Sqrt)	Regression	1	7912	7912	57.67	P<0.001
	Residual	15	2058	137.2		
	Total	16	9971			
22.C.1 (None)	Regression	1	148200	148200	321.2	P<0.001
	Residual	15	6920	461.3		
	Total	16	155100			
22.C.1 (Log)	Regression	1	4.888	4.888	52.51	P<0.001
	Residual	15	1.396	0.09308		
	Total	16	6.284			
22.C.1 (Ln)	Regression	1	25.92	25.92	52.51	P<0.001
	Residual	15	7.403	0.4935		
	Total	16	33.32			
22.C.1 (Sqrt)	Regression	1	375.1	375.1	1565	P<0.001
	Residual	15	3.595	0.2397		
	Total	16	378.7			

Table 2 contd.

Day, Medium, Replic.	Source of variation	df	SS	MS=SS/DF	F-ratio	P
22.C.2 (None)	Regression	1	147500	147500	323.5	P<0.001
	Residual	15	6839	455.9		
	Total	16	154300			
22.C.2 (Log)	Regression	1	4.914	4.914	53.66	P<0.001
	Residual	15	1.374	0.09159		
	Total	16	6.288			
22.C.2 (Ln)	Regression	1	26.06	26.06	53.66	P<0.001
	Residual	15	7.284	0.4856		
	Total	16	33.34			
22.C.2 (Sqrt)	Regression	1	375.7	375.7	1728	P<0.001
	Residual	15	3.261	0.2174		
	Total	16	378.9			

Table 2 contd.

Day, Medium, Replic.	Source of variation	df	SS	MS=SS/DF	F-ratio	P
25.ML.1 (None)	Regression	1	346700	346700	269.2	P<0.001
	Residual	15	19330	1288		
	Total	16	366100			
25.ML.1 (Log)	Regression	1	5.275	5.275	46.03	P<0.001
	Residual	15	1.719	0.1146		
	Total	16	6.994			
25.ML.1 (Ln)	Regression	1	27.97	27.97	46.04	P<0.001
	Residual	15	9.112	0.6075		
	Total	16	37.08			
25.ML.1 (Sqrt)	Regression	1	581.8	581.8	1513	P<0.001
	Residual	15	5.767	0.3845		
	Total	16	587.6			
25.ML.2 (None)	Regression	1	414000	414000	301.5	P<0.001
	Residual	15	20590	1373		
	Total	16	434600			
25.ML.2 (Log)	Regression	1	5.328	5.328	43.53	P<0.001
	Residual	15	1.836	0.1224		
	Total	16	7.164			
25.ML.2 (Ln)	Regression	1	28.25	28.25	43.53	P<0.001
	Residual	15	9.735	0.6490		
	Total	16	37.98			
25.ML.2 (Sqrt)	Regression	1	635.6	635.6	1443	P<0.001
	Residual	15	6.607	0.4404		
	Total	16	642.3			
25.MD.1 (None)	Regression	1	652900	652900	284.1	P<0.001
	Residual	15	34470	2298		
	Total	16	687300			
25.MD.1 (Log)	Regression	1	5.682	5.682	42.75	P<0.001
	Residual	15	1.994	0.1329		
	Total	16	7.676			
25.MD.1 (Ln)	Regression	1	30.13	30.13	42.74	P<0.001
	Residual	15	10.57	0.7049		
	Total	16	40.70			
25.MD.1 (Sqrt)	Regression	1	812.9	812.9	1683	P<0.001
	Residual	15	7.244	0.4830		
	Total	16	820.2			

Table 2 contd.

Day, Medium, Replic.	Source of variation	df	SS	MS=SS/DF	F-ratio	P
25.MD.2 (None)	Regression	1	507400	507400	255.2	P<0.001
	Residual	15	29820	1988		
	Total	16	537200			
25.MD.2 (Log)	Regression	1	5.410	5.410	42.73	P<0.001
	Residual	15	1.899	0.1266		
	Total	16	7.308			
25.MD.2 (Ln)	Regression	1	28.68	28.68	42.74	P<0.001
	Residual	15	10.07	0.6710		
	Total	16	38.75			
25.MD.2 (Sqrt)	Regression	1	704.8	704.8	1409	P<0.001
	Residual	15	7.504	0.5003		
	Total	16	712.3			
25.BL.1 (None)	Regression	1	32630000	32630000	5586	P<0.001
	Residual	15	35050	5841		
	Total	16	32660000			
25.BL.1 (Log)	Regression	1	5.981	5.981	7.062	0.05 >P> 0.025
	Residual	15	5.082	0.8470		
	Total	16	11.06			
25.BL.1 (Ln)	Regression	1	31.71	31.71	7.061	0.05 >P> 0.025
	Residual	15	26.94	4.491		
	Total	16	58.66			
25.BL.1 (Sqrt)	Regression	1	4181	4181	69.96	P<0.001
	Residual	15	358.6	59.76		
	Total	16	4539			
25.BL.2 (None)	Regression	1	24830000	24830000	125.7	P<0.001
	Residual	15	790300	197600		
	Total	16	25620000			
25.BL.2 (Log)	Regression	1	6.373	6.373	6.738	0.10 >P> 0.05
	Residual	15	3.783	0.9458		
	Total	16	10.16			
25.BL.2 (Ln)	Regression	1	33.79	33.79	6.737	0.10 >P> 0.05
	Residual	15	20.06	5.015		
	Total	16	53.85			
25.BL.2 (Sqrt)	Regression	1	3591	3591	93.28	P<0.001
	Residual	15	154.0	38.50		
	Total	16	3745			

Table 2 contd.

Day, Medium, Replic.	Source of variation	df	SS	MS=SS/DF	F-ratio	P
25.BD.1 (None)	Regression	1	2189000	2189000	347.0	P<0.001
	Residual	15	94640	6309		
	Total	16	2284000			
25.BD.1 (Log)	Regression	1	5.886	5.886	28.95	P<0.001
	Residual	15	3.050	0.2033		
	Total	16	8.936			
25.BD.1 (Ln)	Regression	1	31.21	31.21	28.95	P<0.001
	Residual	15	16.17	1.078		
	Total	16	47.38			
25.BD.1 (Sqrt)	Regression	1	1447	1447	541.8	P<0.001
	Residual	15	40.06	2.671		
	Total	16	1487			
25.BD.2 (None)	Regression	1	11000000	11000000	120.0	P<0.001
	Residual	15	1375000	91670		
	Total	16	12380000			
25.BD.2 (Log)	Regression	1	7.146	7.146	31.05	P<0.001
	Residual	15	3.452	0.2301		
	Total	16	10.60			
25.BD.2 (Ln)	Regression	1	37.89	37.89	31.05	P<0.001
	Residual	15	18.30	1.220		
	Total	16	56.19			
25.BD.2 (Sqrt)	Regression	1	3425	3425	762.9	P<0.001
	Residual	15	67.33	4.489		
	Total	16	3492			
25.C.1 (None)	Regression	1	147000	147000	299.7	P<0.001
	Residual	15	7356	490.4		
	Total	16	154300			
25.C.1 (Log)	Regression	1	4.835	4.835	52.74	P<0.001
	Residual	15	1.375	0.09168		
	Total	16	6.210			
25.C.1 (Ln)	Regression	1	25.63	25.63	52.73	P<0.001
	Residual	15	7.291	0.4861		
	Total	16	32.93			
25.C.1 (Sqrt)	Regression	1	372.3	372.3	1599	P<0.001
	Residual	15	3.493	0.2328		
	Total	16	375.8			

Table 2 contd.

Day, Medium, Replic.	Source of variation	df	SS	MS=SS/DF	F-ratio	P
25.C.2 (None)	Regression	1	149000	149000	310.8	P<0.001
	Residual	15	7194	479.6		
	Total	16	156200			
25.C.2 (Log)	Regression	1	4.841	4.841	52.57	P<0.001
	Residual	15	1.381	0.09209		
	Total	16	6.223			
25.C.2 (Ln)	Regression	1	25.67	25.67	52.57	P<0.001
	Residual	15	7.324	0.4883		
	Total	16	32.99			
25.C.2 (Sqrt)	Regression	1	375.1	375.1	1645	P<0.001
	Residual	15	3.420	0.2280		
	Total	16	378.6			

Appendix 5.5

Enrichment experiment.

Table 3. Regression equations of water column height (x axis) against time (y axis). Untransformed (none) and $\log_{10}$, $\log_e$, and square root transformed data. F-ratios taken from appendix 5.4 table 2 p 206.
9 days x 10 cores x 4 transformations = 360 regression equations (text p 59).

Day, Medium, Replic.	Trans-forma-tion	Linear regression	F-ratio
1.ML.1	None	y = -0.5619x + 258.9	271.4
	Log	y = -0.004308x + 3.014	65.17
	Ln	y = -0.009919x + 6.941	65.17
	Sqrt	y = -0.03296x + 18.49	2091
1.ML.2	None	y = -0.6024x + 277.8	279.0
	Log	y = -0.004319x + 3.049	62.27
	Ln	y = -0.009945x + 7.020	62.26
	Sqrt	y = -0.03412x + 19.15	2020
1.MD.1	None	y = -0.6096x + 281.5	291.0
	Log	y = -0.04351x + 3.063	61.32
	Ln	y = -0.01002x + 7.052	61.32
	Sqrt	y = -0.03439x + 19.30	1875
1.MD.2	None	y = -0.5664x + 262.2	295.2
	Log	y = -0.004235x + 3.008	61.05
	Ln	y = -0.009751x + 6.927	61.06
	Sqrt	y = -0.03286x + 18.57	1888
1.BL.1	None	y = -0.5115x + 236.3	298.9
	Log	y = -0.004256x + 2.966	67.36
	Ln	y = -0.009801x + 6.829	67.36
	Sqrt	y = -0.03137x + 17.66	2286
1.BL.2	None	y = -0.5327x + 246.7	293.0
	Log	y = -0.004228x + 2.981	62.33
	Ln	y = -0.009735x + 6.863	62.34
	Sqrt	y = -0.03186x + 18.01	1807
1.BD.1	None	y = -0.4616x + 214.1	319.9
	Log	y = -0.004200x + 2.914	67.51
	Ln	y = -0.009670x + 6.710	67.50
	Sqrt	y = -0.02967x + 16.79	2015
1.BD.2	None	y = -0.5167x + 240.0	318.5
	Log	y = -0.004208x + 2.967	61.72
	Ln	y = -0.009689x + 6.832	61.73
	Sqrt	y = -0.03133x + 17.77	1732
1.C.1	None	y = -0.5243x + 243.2	313.8
	Log	y = -0.004227x + 2.976	62.49
	Ln	y = -0.009734x + 6.852	62.49
	Sqrt	y = -0.03162x + 17.89	1838
1.C.2	None	y = -0.5707x + 264.3	300.9
	Log	y = -0.004254x + 3.017	60.51
	Ln	y = -0.009794x + 6.946	60.52
	Sqrt	y = -0.03302x + 18.66	1825

Table 3 contd.

Day, Medium, Replic.	Trans-formation	Linear regression	F-ratio
4.ML.1	None	y = -0.7540x + 349.2	306.2
	Log	y = -0.004380x + 3.166	53.44
	Ln	y = -0.01009x + 7.290	53.44
	Sqrt	y = -0.03818x + 21.50	1547
4.ML.2	None	y = -0.7912x + 365.9	311.3
	Log	y = -0.004414x + 3.193	53.11
	Ln	y = -0.01016x + 7.351	53.11
	Sqrt	y = -0.03919x + 22.02	1699
4.MD.1	None	y = -0.8839x + 408.4	305.2
	Log	y = -0.004485x + 3.255	51.72
	Ln	y = -0.01033x + 7.495	51.72
	Sqrt	y = -0.04160x + 23.30	1639
4.MD.2	None	y = -0.8294x + 385.3	328.7
	Log	y = -0.004368x + 3.209	49.13
	Ln	y = -0.01006x + 7.390	49.12
	Sqrt	y = -0.03990x + 22.57	1438
4.BL.1	None	y = -1.143X + 539.9	476.9
	Log	y = -0.004325X + 3.362	36.25
	Ln	y = -0.009958X + 7.741	36.25
	Sqrt	y = -0.04614X + 26.67	817.2
4.BL.2	None	y = -1.354X + 639.5	530.0
	Log	y = -0.004423X + 3.458	34.79
	Ln	y = -0.01019X + 7.962	34.80
	Sqrt	y = -0.05050X + 29.09	792.0
4.BD.1	None	y = -1.306X + 615.5	530.0
	Log	y = -0.004460X + 3.447	36.54
	Ln	y = -0.01027X + 7.937	36.54
	Sqrt	y = -0.04985X + 28.59	838.3
4.BD.2	None	y = -1.399X + 659.2	552.8
	Log	y = -0.004482X + 3.482	35.72
	Ln	y = -0.01032X + 8.017	35.72
	Sqrt	y = -0.05163X + 29.59	859.2
4.C.1	None	y = -0.6179X + 287.2	331.1
	Log	y = -0.004250X + 3.056	55.71
	Ln	y = -0.009786X + 7.036	55.71
	Sqrt	y = -0.03427X + 19.45	1594
4.C.2	None	y = -0.6684X + 310.8	322.7
	Log	y = -0.004267X + 3.094	53.24
	Ln	y = -0.009824X + 7.123	53.23
	Sqrt	y = -0.03563X + 20.23	1505

Table 3 contd.

Day, Medium, Replic.	Trans-forma-tion	Linear regression	F-ratio
7.ML.1	None	y = -0.8143X + 377.5	297.7
	Log	y = -0.004358X +3.196	50.11
	Ln	y = -0.01004X + 7.358	50.11
	Sqrt	y = -0.03951X + 22.32	1469
7.ML.2	None	y = -0.8717X + 403.6	294.4
	Log	y = -0.004411X + 3.235	49.61
	Ln	y = -0.01016X + 7.450	49.58
	Sqrt	y = -0.04102X + 23.10	1474
7.MD.1	None	y = -0.9967X + 461.9	319.1
	Log	y = -0.004456X + 3.305	46.53
	Ln	y = -0.01026X + 7.611	46.53
	Sqrt	y = -0.04392X + 24.74	1442
7.MD.2	None	y = -0.9317x + 431.0	295.4
	Log	y = -0.004442x + 3.270	48.76
	Ln	y = -0.01023x + 7.530	48.75
	Sqrt	y = -0.04248x + 23.89	1518
7.BL.1	None	y = -1.258x + 588.7	315.9
	Log	y = -0.004394x + 3.406	37.18
	Ln	y = -0.01012x + 7.843	37.17
	Sqrt	y = -0.04864x + 27.83	902.4
7.BL.2	None	y = -1.850x + 841.5	175.5
	Log	y = -0.004826x + 3.629	43.33
	Ln	y = -0.01111x + 8.356	43.31
	Sqrt	y = -0.06096x + 33.46	1195
7.BD.1	None	y = -1.121x + 525.9	408.0
	Log	y = -0.004422x + 3.366	39.61
	Ln	y = -0.01018x + 7.751	39.60
	Sqrt	y = -0.04618x + 26.39	929.3
7.BD.2	None	y = -1.189x + 558.4	419.2
	Log	y = -0.004417x + 3.392	38.06
	Ln	y = -0.01017x + 7.811	38.05
	Sqrt	y = -0.04746x + 27.18	895.2
7.C.1	None	y = -0.6632x + 308.1	311.3
	Log	y = -0.004292x + 3.095	54.17
	Ln	y = -0.009882x + 7.126	54.19
	Sqrt	y = -0.03557x + 20.16	1474
7.C.2	None	y = -0.6795x + 315.3	322.1
	Log	y = -0.004322x + 3.111	54.67
	Ln	y = -0.009952x + 7.163	54.67
	Sqrt	y = -0.03611x + 20.41	1591

Table 3 contd.

Day, Medium, Replic.	Trans-forma-tion	Linear regression	F-ratio
10.ML.1	None	y = -0.9114x + 422.2	294.5
	Log	y = -0.004413x + 3.256	48.20
	Ln	y = -0.01016x + 7.497	48.20
	Sqrt	y = -0.04191x + 23.62	1426
10.ML.2	None	y = -0.9753x + 451.2	300.8
	Log	y = -0.004469x + 3.297	47.87
	Ln	y = -0.01029x + 7.590	47.87
	Sqrt	y = -0.04352x + 24.46	1482
10.MD.1	None	y = -1.103x + 511.2	336.6
	Log	y = -0.004514x + 3.362	45.30
	Ln	y = -0.01039x + 7.742	45.29
	Sqrt	y = -0.04636x + 26.06	1460
10.MD.2	None	y = -1.023x + 474.6	302.4
	Log	y = -0.004406x + 3.307	44.48
	Ln	y = -0.01015x + 7.615	44.50
	Sqrt	y = -0.04425x + 25.03	1319
10.BL.1	None	y = -1.564x + 719.2	234.1
	Log	y = -0.004652x + 3.533	41.30
	Ln	y = -0.01071x + 8.136	41.31
	Sqrt	y = -0.05542x + 30.88	1264
10.BL.2	None	y = -4.810x + 2064	60.12
	Log	y = -0.005696x + 4.125	56.97
	Ln	y = -0.01312x + 9.499	56.97
	Sqrt	y = -0.1031x + 52.26	360.4
10.BD.1	None	y = -1.510x + 693.1	219.2
	Log	y = -0.004652x + 3.515	42.55
	Ln	y = -0.01071x + 8.094	42.54
	Sqrt	y = -0.05450x + 30.31	1260
10.BD.2	None	y = -1.670x + 767.1	237.0
	Log	y = -0.004694x + 3.567	41.00
	Ln	y = -0.01081x + 8.220	40.99
	Sqrt	y = -0.05741x + 31.92	1330
10.C.1	None	y = -0.6842x + 317.2	310.3
	Log	y = -0.004315x + 3.110	55.08
	Ln	y = -0.009936x + 7.162	55.06
	Sqrt	y = -0.03623x + 20.46	1669
10.C.2	None	y = -0.7014x + 326.0	312.0
	Log	y = -0.004294x + 3.120	52.10
	Ln	y = -0.009886x + 7.185	52.10
	Sqrt	y = -0.03654x + 20.73	1399

Table 3 contd.

Day, Medium, Replic.	Trans-forma-tion	Linear regression	F-ratio
13.ML.1	None	y = -1.059x + 487.5	370.7
	Log	y = -0.004599x + 3.354	51.52
	Ln	y = -0.01059x + 7.722	51.54
	Sqrt	y = -0.04600x + 25.54	2163
13.ML.2	None	y = -1.000x + 462.1	291.7
	Log	y = -0.004480x + 3.308	48.14
	Ln	y = -0.01032x + 7.616	48.12
	Sqrt	y = -0.04412x + 24.75	1574
13.MD.1	None	y = -1.107x + 512.8	329.6
	Log	y = -0.004502x + 3.361	45.06
	Ln	y = -0.01037x + 7.739	45.06
	Sqrt	y = -0.04639x + 26.09	1469
13.MD.2	None	y = -1.049x + 488.9	297.5
	Log	y = -0.004350x + 3.312	41.35
	Ln	y = -0.01002x + 7.626	41.36
	Sqrt	y = -0.04448x + 25.35	1071
13.BL.1	None	y = -2.067x + 932.3	142.3
	Log	y = -0.004846x + 3.667	43.45
	Ln	y = -0.01116x + 8.443	43.46
	Sqrt	y = -0.06448x + 35.12	1035
13.BL.2	None	y = -3.236x +1461	140.9
	Log	y = -0.004979x + 3.894	36.37
	Ln	y = -0.01146x + 8.965	36.38
	Sqrt	y = -0.08068x + 43.98	925.3
13.BD.1	None	y = -1.323x + 606.9	240.6
	Log	y = -0.004641x + 3.454	46.07
	Ln	y = -0.01069x + 7.954	46.07
	Sqrt	y = -0.05118x + 28.40	1568
13.BD.2	None	y = -1.609x + 733.9	189.2
	Log	y = -0.004748x + 3.554	44.37
	Ln	y = -0.01093x + 8.184	44.38
	Sqrt	y = -0.05666x + 31.22	1265
13.C.1	None	y = -0.6978x + 323.2	308.1
	Log	y = -0.004344x + 3.124	55.42
	Ln	y = -0.01000x + 7.194	55.42
	Sqrt	y = -0.03667x + 20.67	1664
13.C.2	None	y = -0.7121x + 330.3	307.9
	Log	y = -0.004345x + 3.135	54.01
	Ln	y = -0.01001x + 7.220	54.01
	Sqrt	y = -0.03701x + 20.90	1478

Table 3 contd.

Day, Medium, Replic.	Trans-forma-tion	Linear regression	F-ratio
16.ML.1	None	y = -1.034 + 482.6	316.7
	Log	y = -0.004341x + 3.305	41.28
	Ln	y = -0.009995x + 7.610	41.28
	Sqrt	y = -0.04415x + 25.19	1081
16.ML.2	None	y = -1.099x + 509.7	287.1
	Log	y = -0.004404x + 3.337	42.75
	Ln	y = -0.01014x + 7.683	42.74
	Sqrt	y = -0.04579x + 25.91	1262
16.MD.1	None	y = -1.206x + 554.2	32.08
	Log	y = -0.004685x + 3.427	49.28
	Ln	y = -0.01079x + 7.891	49.28
	Sqrt	y = -0.04922x + 27.24	209.2
16.MD.2	None	y = -1.117x + 514.1	279.3
	Log	y = -0.004562x + 3.368	48.16
	Ln	y = -0.01051x + 7.756	48.16
	Sqrt	y = -0.04690x + 26.14	1730
16.BL.1	None	y = -2.990x + 1361	216.8
	Log	y = -0.005145x + 3.910	39.98
	Ln	y = -0.01185x + 9.002	39.98
	Sqrt	y = -0.07895x + 42.91	1306
16.BL.2	None	y = -14.11x + 5888	25.59
	Log	y = -0.006145x + 4.615	45.37
	Ln	y = -0.01415x + 10.63	45.37
	Sqrt	y = -0.1747x + 86.07	107.4
16.BD.1	None	y = -1.831x + 842.7	256.5
	Log	y = -0.004696x + 3.613	38.89
	Ln	y = -0.01081x + 8.320	38.87
	Sqrt	y = -0.06003x + 33.46	1337
16.BD.2	None	y = -1.816x + 834.4	240.9
	Log	y = -0.004693x + 3.606	39.14
	Ln	y = -0.01081x + 8.304	39.14
	Sqrt	y = -0.05976x + 33.27	1304
16.C.1	None	y = -0.7199x + 334.3	302.1
	Log	y = -0.004286x + 3.128	51.59
	Ln	y = -0.009870x + 7.203	51.59
	Sqrt	y = -0.03698x + 20.97	1394
16.C.2	None	y = -0.7141x + 330.4	302.9
	Log	y = -0.004348x + 3.133	55.15
	Ln	y = -0.01001x + 7.215	55.16
	Sqrt	y = -0.03710x + 20.89	1760

Table 3 contd.

Day, Medium, Replic.	Trans-forma-tion	Linear regression	F-ratio
19.ML.1	None	y = -1.074x + 495.9	313.5
	Log	y = -0.004533x + 3.350	47.37
	Ln	y = -0.01044x + 7.714	47.35
	Sqrt	y = -0.04586x + 25.67	1637
19.ML.2	None	y = -1.173x + 540.2	286.6
	Log	y = -0.004569x + 3.392	46.70
	Ln	y = -0.01052x + 7.811	46.68
	Sqrt	y = -0.04802x + 26.79	1677
19.MD.1	None	y = -1.321x + 607.1	300.5
	Log	y = -0.004684x + 3.467	46.54
	Ln	y = -0.01079x + 7.983	46.53
	Sqrt	y = -0.05135x + 28.48	1833
19.MD.2	None	y = -1.219x + 560.2	285.7
	Log	y = -0.004650x + 3.424	47.90
	Ln	y = -0.01071x + 7.884	47.90
	Sqrt	y = -0.04927x + 27.34	1795
19.BL.1	None	y = -8.710x + 4021	54.31
	Log	y = -0.009800x + 6.137	25.92
	Ln	y = -0.02257x + 14.13	25.91
	Sqrt	y = -0.1761x + 89.63	492.2
19.BL.2	None	y = -51.37x + 24250	12.21
	Log	y = -0.02075x + 11.55	14.47
	Ln	y = -0.04778x + 26.60	14.47
	Sqrt	y = -0.5580x + 276.8	56.56
19.BD.1	None	y = -2.310x + 1047	191.5
	Log	y = -0.004945x + 3.746	42.09
	Ln	y = -0.01139x + 8.625	42.08
	Sqrt	y = -0.06862x + 37.39	1457
19.BD.2	None	y = -2.632x + 1198	188.2
	Log	y = -0.004964x + 3.814	38.50
	Ln	y = -0.01143x + 8.782	38.51
	Sqrt	y = -0.07301x + 40.00	1240
19.C.1	None	y = -0.7383x + 340.9	300.4
	Log	y = -0.004395x + 3.157	56.13
	Ln	y = -0.01012x + 7.266	56.13
	Sqrt	y = -0.03788x + 21.25	1896
19.C.2	None	y = -0.7329x + 339.6	314.3
	Log	y = -0.004357x + 3.149	53.80
	Ln	y = -0.01003x + 7.251	53.80
	Sqrt	y = -0.03759x + 21.19	1640

Table 3 contd.

Day, Medium, Replic.	Trans-formation	Linear regression	F-ratio
22.ML.1	None	y = -1.177x + 542.2	263.8
	Log	y = -0.004553x + 3.390	45.97
	Ln	y = -0.01048x + 7.807	45.96
	Sqrt	y = -0.04799x + 26.81	1497
22.ML.2	None	y = -1.431x + 653.1	285.6
	Log	y = -0.004751x + 3.504	49.07
	Ln	y = -0.01094x + 8.069	49.09
	Sqrt	y = -0.05391x + 29.58	1822
22.MD.1	None	y = -1.480x + 677.5	211.8
	Log	y = -0.004717x + 3.518	44.92
	Ln	y = -0.01086x + 8.100	44.93
	Sqrt	y = -0.05429x + 30.03	1309
22.MD.2	None	y = -1.306x + 602.6	286.7
	Log	y = -0.004533x + 3.445	43.65
	Ln	y = -0.01055x + 7.932	43.67
	Sqrt	y = -0.05059x + 28.29	1503
22.BL.1	None	y = -26.13x + 12970	83.93
	Log	y = -0.04097x + 21.20	6.162
	Ln	y = -0.09433x + 48.81	61.61
	Sqrt	y = -0.5656x + 286.6	76.65
22.BL.2	None	y = -107.6x + 53790	7233000
	Log	y = -0.07462x + 37.83	4.267
	Ln	y = -0.1718x + 87.10	4.267
	Sqrt	y = -1.447x + 729.4	18.21
22.BD.1	None	y = -2.709x + 1217	121.3
	Log	y = -0.004988x + 3.810	40.81
	Ln	y = -0.01149x + 8.774	40.80
	Sqrt	y = -0.07413x + 40.15	827.2
22.BD.2	None	y = -14.20x + 5741	14.73
	Log	y = -0.006562x + 4.592	69.98
	Ln	y = -0.01511x + 10.57	70.01
	Sqrt	y = -0.1762x + 82.75	57.67
22.C.1	None	y = -0.7623x + 353.4	321.2
	Log	y = -0.004378x + 3.172	52.51
	Ln	y = -0.01008x + 7.304	52.51
	Sqrt	y = -0.03835x + 21.63	1565
22.C.2	None	y = -0.7605x + 352.1	323.5
	Log	y = -0.004390x + 3.172	53.66
	Ln	y = -0.01011x + 7.303	53.66
	Sqrt	y = -0.03838x + 21.60	1728

Table 3 contd.

Day, Medium, Replic.	Trans-forma-tion	Linear regression	F-ratio
25.ML.1	None	y = -1.166x + 537.4	269.2
	Log	y = -0.004548x + 3.386	46.03
	Ln	y = -0.01047x + 7.797	46.04
	Sqrt	y = -0.04777x + 26.70	1513
25.ML.2	None	y = -1.274x + 588.9	301.5
	Log	y = -0.004571x + 3.434	43.53
	Ln	y = -0.01053x + 7.907	43.53
	Sqrt	y = -0.04993x + 27.97	1443
25.MD.1	None	y = -1.600x + 735.3	284.1
	Log	y = -0.004721x + 3.558	42.75
	Ln	y = -0.01087x + 8.193	42.74
	Sqrt	y = -0.05646x + 31.33	1683
25.MD.2	None	y = -1.411x + 649.3	255.2
	Log	y = -0.004606x + 3.480	42.73
	Ln	y = -0.01061x + 8.013	42.74
	Sqrt	y = -0.05257x + 29.34	1409
25.BL.1	None	y = -35.26x + 17600	5586
	Log	y = -0.01510x + 9.264	7.062
	Ln	y = -0.03476x + 21.33	7.061
	Sqrt	y = -0.3991x + 214.6	69.96
25.BL.2	None	y = -47.65x + 23510	125.7
	Log	y = -0.02414x + 13.41	6.738
	Ln	y = -0.05558x + 30.88	6.737
	Sqrt	y = -0.5730x + 295.9	93.28
25.BD.1	None	y = -2.930x + 1378	347.0
	Log	y = -0.004805x + 3.872	28.95
	Ln	y = -0.01106x + 8.916	28.95
	Sqrt	y = -0.07533x + 42.89	541.8
25.BD.2	None	y = -6.569x + 2949	120.0
	Log	y = -0.005294x + 4.262	31.05
	Ln	y = -0.01219x + 9.814	31.05
	Sqrt	y = -0.1159x + 62.57	762.9
25.C.1	None	y = -0.7592x + 351.6	299.7
	Log	y = -0.004354x + 3.163	52.74
	Ln	y = -0.01003x + 7.283	52.73
	Sqrt	y = -0.03821x + 21.55	1599
25.C.2	None	y = -0.7645x + 354.1	310.8
	Log	y = -0.004357x + 3.167	52.57
	Ln	y = -0.01003x + 7.293	52.57
	Sqrt	y = -0.03836x + 21.63	1645

Appendix 5.6

Enrichment experiment.

Table 4. Chlorophyll concentrations (μg Chl. g^{-1} dry wt. sed.) in the unenriched sediment NS, and enriched ML, MD, BL, BD and control C cores. The equations used are as follows: PS = Parsons and Strickland; R = Richards; SU = SCOR/UNESCO; L = Lorenzen (Strickland & Parsons, 1972) (text p 82).

		Treatment					
Pigment		NS	ML	MD	BL	BD	C
Chlorophyll a							
(PS)	mean	5.961	7.388	5.253	3.003	4.462	2.699
	s.d	0.1934	2.659	0.2297	1.082	0.3306	0.1427
(R)	mean	7.906	9.793	6.970	4.064	5.915	3.577
	s.d.	0.0209	3.528	0.3112	1.363	0.4455	0.1876
(SU)	mean	5.794	7.215	5.085	3.023	4.407	2.611
	s.d.	0.0259	2.514	0.2264	1.016	0.3366	0.0651
(L)	mean	5.146	5.986	4.089	3.739	3.544	1.535
	s.d.	0.1866	2.746	0.6402	0.1779	0.0254	0.7692
Chlorophyll b							
(PS)	mean	0.0013	0.3170	0.3865	0.0000	0.1303	0.1734
	s.d.	0.0562	0.0820	0.0662	0.0000	0.2372	0.1156
(R)	mean	0.0000	0.2171	0.0000	0.0000	0.0539	0.1528
	s.d.	0.0504	0.0620	0.0000	0.0000	0.2687	0.1374
(SU)	mean	0.3810	0.7804	0.3764	0.0000	0.3935	0.3505
	s.d.	0.0581	0.2722	0.0599	0.0000	0.2270	0.1502

Table 4 contd.

Pigment		Treatment NS	ML	MD	BL	BD	C
Chlorophyll c							
(PS)	mean	2.218	2.702	1.683	0.0000	1.703	0.9121
	s.d.	0.1554	0.7514	0.2895	0.0000	0.0018	0.0209
(R)	mean	3.139	3.851	2.232	0.0000	2.457	1.267
	s.d.	0.3156	0.9396	0.6385	0.0000	0.1156	0.0526
(SU)	mean	1.901	2.306	1.418	0.0000	1.437	0.7823
	s.d.	0.1517	0.6486	0.3027	0.0000	0.0354	0.0120
Carotenes							
(PS) e_c	mean	2.575	3.098	2.168	0.9108	2.124	0.7653
	s.d.	0.0547	1.167	0.0351	0.8797	0.0653	0.1900
(PS) e_p	mean	6.438	7.744	5.419	2.277	5.310	1.913
	s.d.	0.1369	2.918	0.0878	2.200	0.1634	0.4751
(R)	mean	2.520	2.518	1.969	1.260	1.931	0.5475
	s.d.	0.1005	0.6396	0.0801	0.4278	0.1090	0.1192

Appendix 5.7

Enrichment experiment.

Table 5. Heterotrophic bacterial counts in unenriched natural sediment NS, and the enriched ML, MD, BL, and BD cores and the control C cores (there were 2 replicate plates per core). Plates were set up at 10^{0}, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}. As far as possible; plates were selected for counting that had 30 to 300 colonies. Numbers of colonies/plate. Two replicate plates per core. * : plate was overgrown (text p 82).

Medium	Rep. no.	Dilution 10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
NS	i	74	13	3	0	0
	ii	67	10	0	0	0
ML1	i	*	300	39	1	1
	ii	*	78	0	1	0
ML2	i	*	25	17	2	1
	ii	*	53	32	1	1
MD1	i	*	240	19	2	0
	ii	*	118	125	7	1
MD2	i	*	28	22	2	0
	ii	*	34	20	2	0
BL1	i	*	*	120	109	127
	ii	*	*	*	110	8
BL2	i	*	*	250	32	5
	ii	*	*	362	103	7
BD1	i	*	*	289	38	1
	ii	*	*	*	115	17
BD2	i	*	*	231	93	1
	ii	*	*	260	40	27
C1	i	0	0	1	3	0
	ii	28	0	0	0	0
C2	i	11	1	0	0	1
	ii	0	3	0	0	0

Appendix 6

Major groups of organisms using different energy and carbon sources. Modified from Stanier et al., (1977) (text p 5).

Principal carbon source	Energy source: Light	Energy source: Chemical
Inorganic carbon	Photoautotrophs: blue-green algae, diatoms, green plants on land	Chemoautotrophs: nitrifying bacteria, sulphur-oxidizing bacteria, hydrogen bacteria, methanogenic bacteria
Organic carbon	Photoheterotrophs: purple bacteria, green bacteria	Chemoheterotrophs: aerobic gram-negative bacteria, denitrifying bacteria, fungi, protozoa, all animals

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