Measurement of biological oxygen demand in sandy beaches

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Measurements of biological oxygen demand in a sandy beach using conventional *in situ* techniques are compared with laboratory measurements of interstitial oxygen changes in intact cores. Oxygen uptake as measured in the laboratory was approximately three times that measured in the field despite the fact that the cores were undisturbed. In addition, the effect of disturbance on sediment cores was investigated and it was found that oxygen demand in disturbed cores increased by up to 60%, due mainly to an increase in bacterial activity. This effect was still evident after 24 hours of incubation. The applicability of conventional respirometer techniques to well-drained systems such as open sandy beaches is questioned and an alternative approach suggested.

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Bepalings van biologiese suurstof aanvraag deur gebruikmaking van *in situ* tegnieke word vergelyk met laboratorium bepalings van interstitiële suurstof verandering in intakte sediment kerne. Suurstof opname, soos bepaal in die laboratorium, was ongeveer drie maal hoër as wat in die veld bepaal is, ongeag van die feit dat die kerne ongesteurd gelaat is. Boonop was die effek van versteuring op die sediment kerne ondersoek en die resultate dui daarop dat suurstof opname in die versteurde kerne tot soveel as 60% verhoog, hoofsaaklik te wyte aan verhoogte bakteriese aktiwiteit. Hierdie effek was reeds waarneembaar na 24 uur inkubasie. Die toepasbaarheid van die konvensionele respirometer tegnieke op goed gedreineerde sisteme soos oop sandstrande word bevraagteken en 'n alternatiewe benadering word voorgestel.

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The measurement of benthic metabolism has been the subject of much study in recent years and the methods used are mainly variations of the "dark and light bottle" technique either *in situ* (Pamatmat 1968; Hargrave 1969; Biggs & Flemmer 1972; Smith *et al.* 1972; Dye *et al.* in press), or on intact cores in the laboratory (Hayes & MacAulay 1959; Knowles *et al.* 1962; Carey 1967; Pamatmat 1971; Riznyk & Phinney 1972). Other approaches include the use of radio-active tracers (Meyer-Reil 1978) and experimental sand columns (Johnson 1970; McIntyre *et al.* 1970).

Those methods relying on the measurement of oxygen changes in water overlying cores or areas of substrate restrict the supply of oxygen to diffusion only. In areas where large movements of the water table take place in the substrate oxygen is supplied primarily by water movement and to a much lesser extent by diffusion. It is thus clear that an alternative method is required for BOD measurements in such systems.

The purpose of this paper is to present such an alternative and to compare it with results obtained from a conventional surface respirometer. The method takes into account the three-dimensional nature of the system and the results are expressed in terms of volume of sediment or in terms of area to a specified depth.

Methods

Benthic oxygen consumption was measured at a point midway between mid-water and high water of springs (HWST) on a fine sandy beach at Port Elizabeth (34° S/25° 30′ E). This well sorted beach has a median particle size of 207 um and a porosity of 25% (McLachlan 1977). The benthos in the area studied consists mainly of micro-organisms and meiofauna and very low numbers of small sand mussels (*Donax serra* Röding).

Collection of samples

Twenty seven cores, randomly selected from a one square metre area, were taken in opaque PVC tubes 10,5 cm in length and 16,6 cm^2 in area. The tubes, filled with sand and sealed with rubber bungs, were transported to the laboratory for incubation. The state of the tide was such

that the sand was saturated with water and the cores remained in this condition throughout the experiment. In addition triplicate samples of sand were taken from the study area for enumeration of bacteria and protozoa. These were taken by means of a hand-held copper corer 10cm² in area and 20 cm in length. The upper 10 cm of each was retained and the three were pooled. The analyses were performed on 75 cm³ sub-samples from this fraction.

Oxygen consumption measurements

In situ oxygen consumption was measured using two respirometers, each consisting of a 500 ml opaque bottle attached to a 20 cm aluminium tube 7,3 cm in diameter. The respirometers were pushed into the sand to a depth of 20 cm, filled with sea-water from the surrounding area and sealed. Samples of the sea-water were taken for initial oxygen measurements. At the end of the experiment, which ran from 06h00 to 12h00 on a sunny day in December 1977, duplicate samples of 4 ml of water were withdrawn from each respirometer by syringe for final oxygen measurements. These were done using a radiometer unit connected to a Clark E 5046 oxygen electrode.

The laboratory oxygen consumption measurements were designed that the effect of disturbance could be assessed. The 27 cores were randomly divided into three sets of nine. The first set (A) was left undisturbed except for withdrawing 1 ml of water at intervals of eight hours for oxygen analysis. This was done by means of an 18 guage needle blocked at the end and provided instead with two rings of four 250 um holes. The needle was inserted to approximately half-way down the core and oxygen was measured as described. The second set (B) was disturbed by mixing each separately and repacking the cores thus destroying any stratification. Each core in the third set (C) was hand-shaken for three periods of 60 seconds in filtered seawater (0,45 um) removing most of the meiofauna and 93% of the protozoa (Dye 1979). Bacteria require rather severe shaking for efficient extraction (Dale 1974) or alternatively sonication (see below), and no decrease in bacterial numbers could be expected by the above procedure. The cores were resaturated and after allowing 30' minutes for stabilization the initial oxygen was measured. A 1 mm layer of water was left over each core to ensure saturation but only interstitial water was used for oxygen determinations.

After eight hours of incubation at 20° C, the oxygen content of the interstitial water was measured. Following this the first three cores in each set were pooled and the bacterial and protozoan populations determined on a subsample. The remaining six cores in each set were re-oxygenated to their initial levels by injecting aerated and filtered seawater by means of the needle described. In this way the interstices were flushed with fresh sea-water (approximately 5 mgl⁻¹ O_2) with the minimum of disturbance to the cores. At the end of 16 hours the above procedure was repeated with the next three cores in each set and at the end of 24 hours the last three were similarly analysed. analysed.

Chemical oxygen demand was determined on cores saturated with 5% formalin and allowed to stabilize for 24 hours before the rate of oxygen consumption was measured over the following six hours (Pamatmat 1971). This was subtracted from the total measurements to obtain the biological oxygen demand.

Bacterial and protozoa extraction

The bacteria were extracted from one gram of sand by a process similar to that of Dale (1974) except that low amplitude (23 KHz, 8 um) sonication was used instead of vortex shaking. Each sample was subjected to five periods of 60 seconds sonication using 50 ml of freshly filtered sea-water (0,45 um) each time. The efficiency of the procedure was calculated from the curve produced when one gram of sand was repeatedly sonicated until no further bacteria were extracted. It was found that after five sonications 85,5% were extracted. Subsequent microscopic examination of the sand failed to reveal appreciable numbers of bacteria.

Counting was done on 25 ml subsamples by staining them with an equal volume of a 10 mgl⁻¹ solution of Acridine Orange for three minutes and filtering through Gelman 0,45 um black membrane filters. Pieces of these filters (approximately 0,5 cm²) were mounted in emmersion oil on glass slides and counting was done under an epi-fluorescence microscope. The field area was $1{,}8 \times 10^{-3}$ mm² and the mean of 20 such fields constituted a count. The counts were converted to cells/g by the following formula:

$$\text{cells/g} = \frac{672269 \times C \times 250 \times 1{,}16}{25 \times 1}$$

where: $\frac{1}{672269}$ is the fraction of the total functional filter area counted. C is the mean count (n = 20). 25 is the volume of sample filtered. 1 is the mass of the sand used in grammes. 250 is the dilution factor. 1,16 is the efficiency correction factor.

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The effect of sonication on the cells was determined by counting the cells present in a sample of aged seawater and comparing this with samples from the same source which had been subjected to two periods of 60 seconds sonication with one gram of pre-ashed sand. Most of the bacteria are extracted after two such sonications.

The protozoa were extracted by hand-shaking 50 g of sand for three periods of 60 seconds in 100 ml of filtered seawater and filtering the suspension through 5 um membrane filters for counting as described. This process was found to be 93% efficient (Dye 1979).

Calculation of oxygen consumption rates

Since the radiometer gives readings in partial pressure of oxygen (mm Hg), these have to be converted to volumes of oxygen and the formulae used depend on the method and the way in which samples were taken. In the case of the *in situ* surface respirometers the following formula was used:

$$V_{O_2} = 6{,}28 \times 10^{-3} \times C \times P_{O_2} \times V_w \text{ in ml.}$$

where: $6{,}28 \times 10^{-3} \times C$ is the solubility coefficient of oxygen in water at 20° C and 35‰ salinity (Truesdale *et al.* 1955).

P_{O_2} is the change in partial pressure (mm Hg).

V is the volume of water in the respirometer (500 ml).

Oxygen consumption was calculated from:

$$O_2 \text{ uptake (ml } O_2 \text{ m}^{-2} \text{ h}^{-1}) = \frac{V_{O_2} \times 10^4}{2At}$$

where: V_{O_2} is as above.
A is the area of the respirometer (41,83 cm²).
t is the duration of the experiment (6 h).

To express the results in terms of a core length of 10 cm a factor of 0,5 is introduced. The factor 10^4 extrapolates the results to one square metre.

Oxygen consumption in the cores was calculated as follows:

$$O_2 \text{ uptake (ml } O_2 \text{ m}^{-2} \text{ h}^{-1}) = \frac{6{,}28 \times 10^{-3} \times C \times P_{O_2} \times V_s \times P_s \times 10^4}{A \times t}$$

where:
$6{,}28 \times 10^{-3} \times C$ and P_{O_2} are as above.
V is the volume of sand (166 cm³).
P is the porosity of the sand (25%).
t is the duration of the experiment (8 h).
A is the area of the core (16,6 cm²).

In addition to the above a series of cores were taken horizontally every 20 cm from the surface to the water table during low tide at three tidal levels, *viz.*, HWST, mid-water (MW) and LWST. Oxygen consumption was measured as described and the results plotted against depth. The oxygen consumption of the sand column was calculated by integrating these depth profiles. The above formula has to be modified slightly to express the results in terms of cm³ of sand thus:

$$O_2 \text{ uptake (ml } O_2 \text{ m}^{-2} \text{ h}^{-1}) = \frac{6{,}28 \times 10^{-3} \times C \times P_{O_2} \times P_S}{t}$$

with the symbols the same as above. It will be seen from the last equation that the actual volume of sand used is not important as long as the porosity is known.

Results

Table 1 gives the results of the field and laboratory measurements of benthic oxygen consumption. For the sake of clarity only the results are adjusted for a COD of 2,5%. It can be seen that there is a considerable difference between the field and laboratory results, with the latter almost three times higher than the former. The mean uptake as measured in the field was 4,36±0,30 ml O_2 $m^{-2}h^{-1}$ as opposed to 11,58±0,18 ml O_2 $m^{-2}h^{-1}$ in the laboratory incubated cores.

Table 1 Results of field and laboratory measurements of BOD in a sandy beach

Duration of measurement	Field 6 h	Laboratory 8 h[a]	8 h[b]	8 h[c]
Initial O_2 ml l^{-1}	4,99	3,50	3,25	3,40
Final O_2 ml l^{-1}	4,80	1,84	1,55	1,30
O_2 uptake ml $O_2 m^{-2}$ h^{-1}	4,36 ±0,30 n = 4	10,46 ±0,81 n = 9	10,72 ±0,28 n = 6	13,34 ±0,21 n = 3
Mean	4,36	11,58 ± 0,18		n = 18

[a] 0 - 8 h [b] 8 - 16 h [c] 16 - 24 h

Figure 1 shows the variation in BOD as measured on a series of horizontal cores taken from the surface to the water table. Benthic oxygen consumption varies with depth in a rather unpredictable fashion. At high water, for instance, a considerable increase occurred below 60 cm. The total oxygen uptake, calculated by integration, was 24,26 ml O_2 m^{-2} h^{-1} (HWST); 8,6 ml O_2 m^{-2} h^{-1} (MW) and 0,5 ml O_2 m^{-2} h^{-1} (LWST), all to the depths indicated.

Figure 2 shows the results of incubation experiments on cores subjected to varying degrees of disturbance. The oxygen uptake in the undisturbed cores (A) remained fairly constant for 16 hours and increased only slightly after this time. The hand mixed cores (B) showed an immediate increase in oxygen uptake and by eight hours their activity was 48% higher than the undisturbed level. This activity peaked at 71% above the undisturbed level after 16 hours. A decrease occurred after this time but the activity was still 37% higher than the cores in (A).

The activity of the shaken cores (C) showed a 62% increase over the undisturbed level after 8 hours. This was followed by a decrease to 59% after 16 hours but the activity was still 26% above (A) after 24 hours.

Table 2 Comparison of bacteria counts from sonicated and unsonicated cell suspensions. Data expressed as counts per microscopic field (n = 10)

Unsonicated	Sonicated 60 seconds	120 seconds
10,2 ± 1,21	7,6 ± 0,73	10,1 ± 1,21
8,9 ± 1,54	8,7 ± 0,86	8,7 ± 1,09
7,8 ± 1,20	9,3 ± 1,23	8,1 ± 0,90
8,97 ± 0,74	8,53 ± 0,55	8,97 ± 0,62

Table 2 gives the results of a comparison of bacteria numbers in sonicated and unsonicated samples of aged seawater. Statistical analysis of this data revealed that no significant decrease in numbers resulted from this extraction process (variance analysis: p=0,005).

The changes in bacterial and protozoan numbers during the incubations are shown in Fig. 3. Bacteria in the undisturbed cores remained constant at 30 to 32 x 10^6 cells/g for the first eight hours after which the population slowly increased to 64 x 10^6 after 24 hours. In contrast, the mixed and shaken cores exhibited a rapid increase in bacteria so that by 8 hours the populations were 47 and 56 x 10^6 cells/g respectively. Both reached 71 x 10^6 cells/g after 16 hours. After this the mixed set (B) remained constant while the shaken set (C) decreased slightly to 66 x 10^6 cells/g.

A totally different picture was presented by the protozoa. The undisturbed and mixed cores had an initial population of 7 x 10^3 cells/g which decreased to 3,9 x 10^3 and 3 x 10^3 cells/g after eight hours respectively. This trend continued so that by the end of the experiment both sets had a protozoan density of 1,2 x 10^3 cells/g. Since 93% of the protozoa in set (C) had been extracted the initial level in this case was 0,5 x 10^3 cells/g. This slowly increased to 0,9 x 10^3 cells/g over the 24 hour incubation.

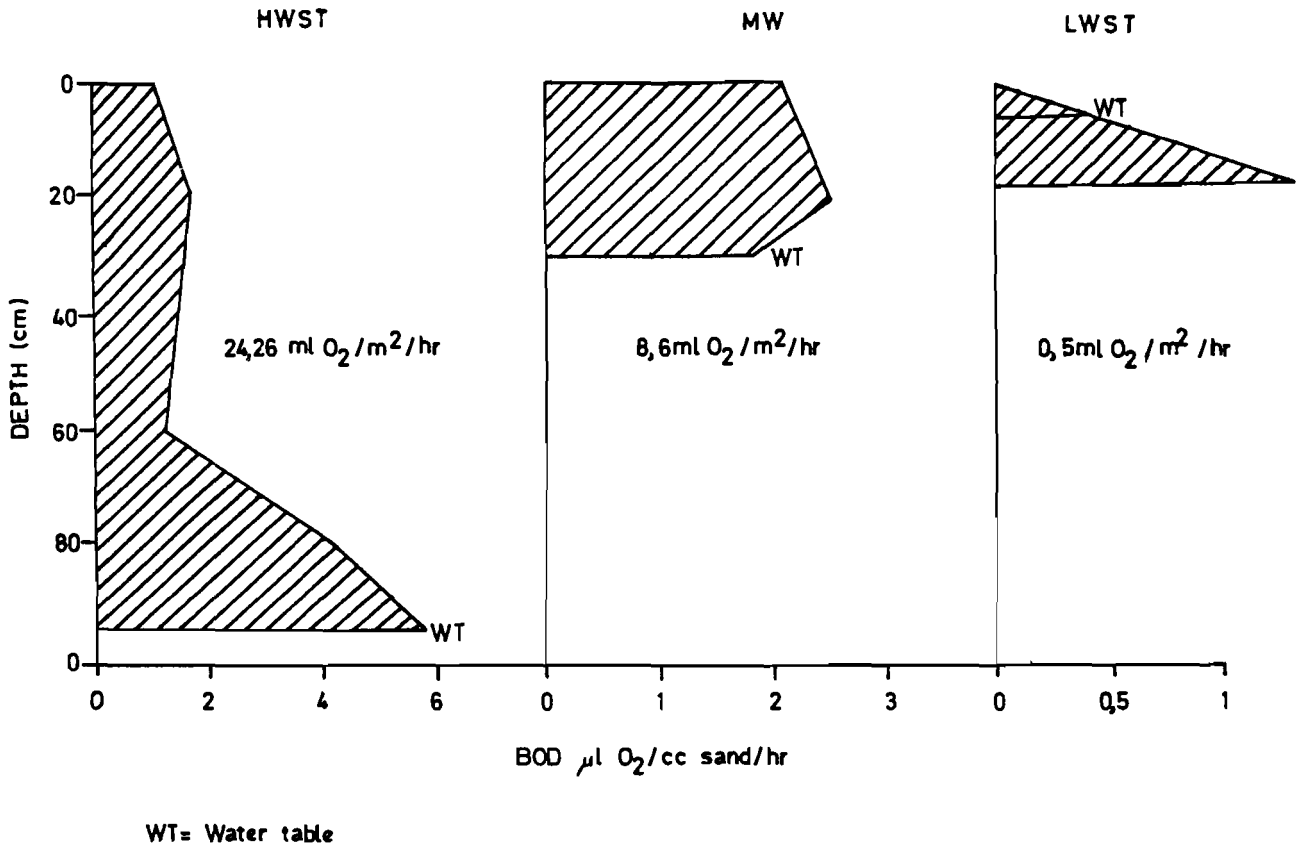

Fig. 1 Vertical BOD profiles at three tidal levels on a sandy beach.

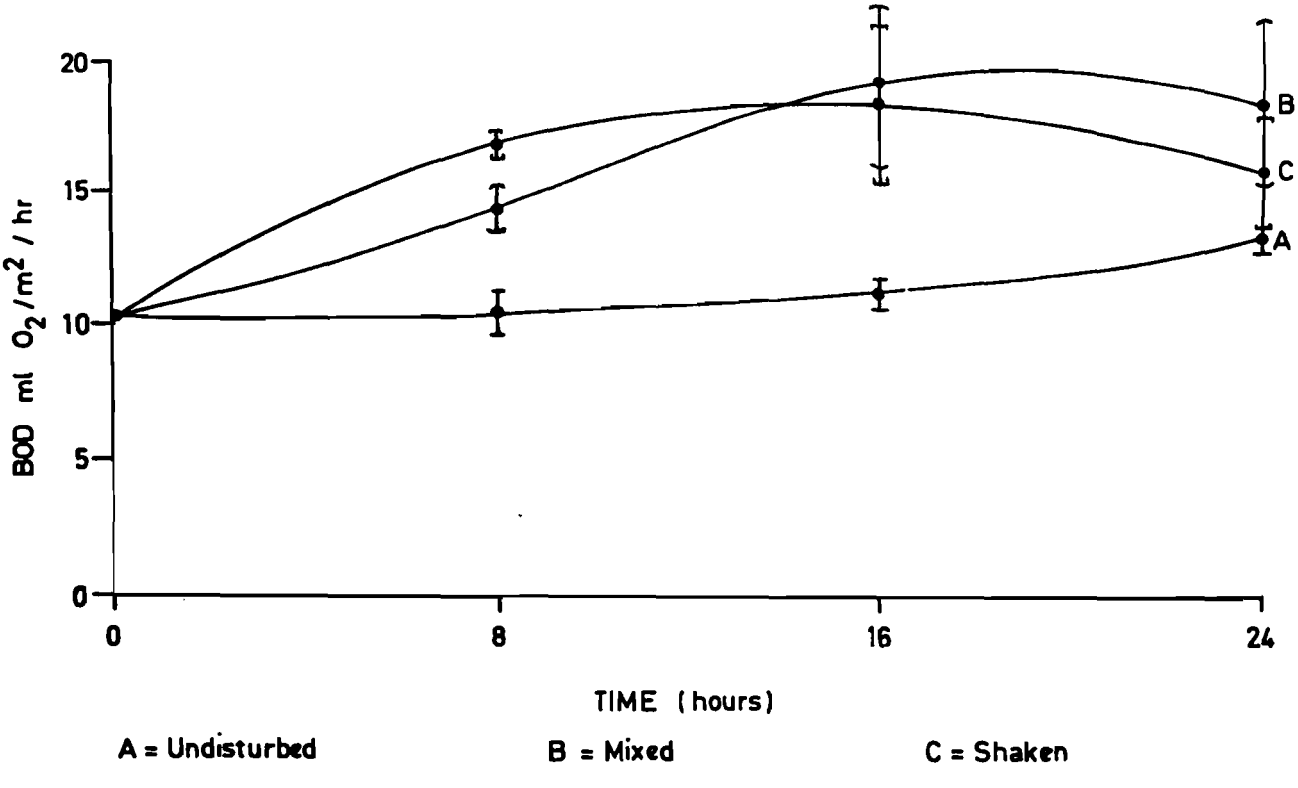

Fig. 2 The effect of disturbance on BOD as measured in incubated cores.

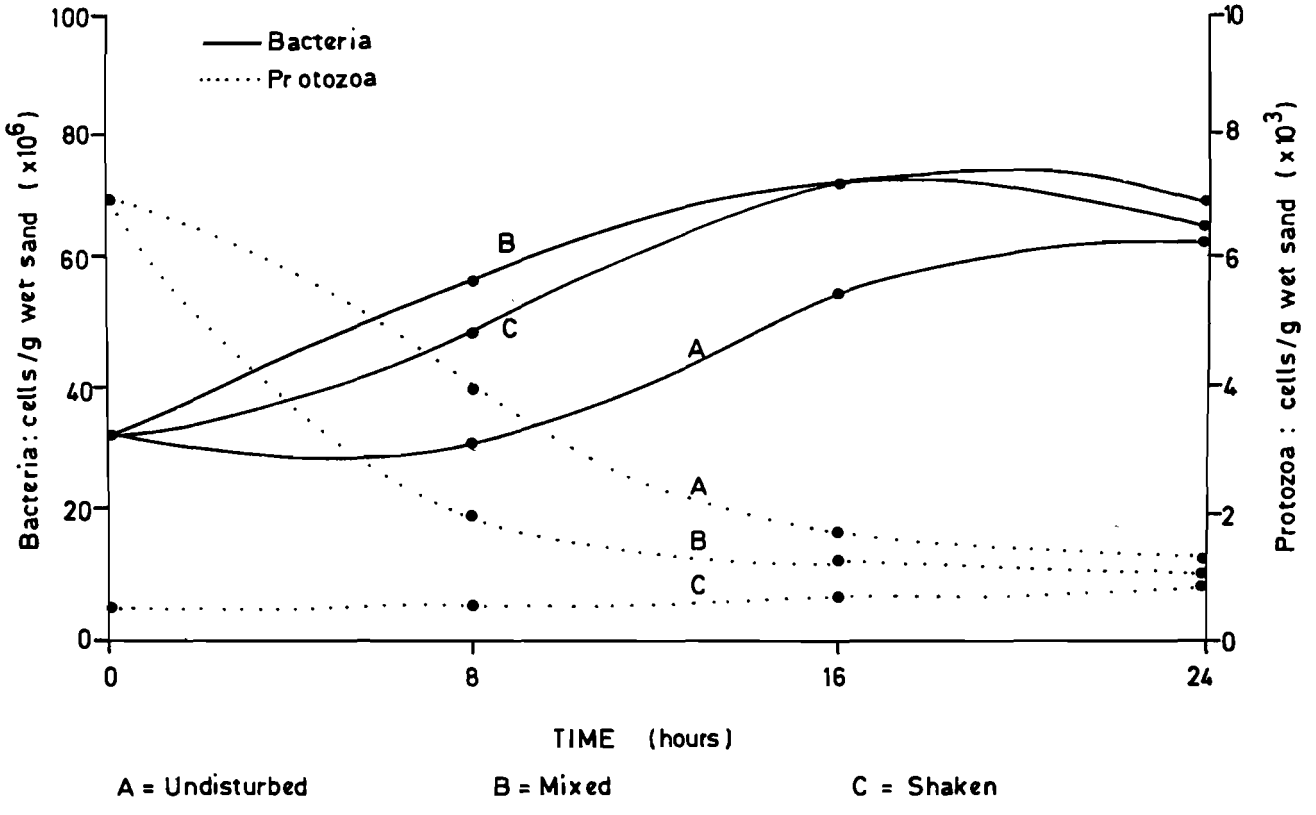

Fig. 3 Changes in bacteria and protozoa populations in cores subjected to varying degrees of disturbance.

Discussion

Most of the published data on benthic metabolism illustrates a great spatial and temporal variation. Oxygen uptake rates vary from 19 ml O_2 $m^{-2}h^{-1}$(Smith *et al.* 1972: Bermuda) to 92 ml O_2 $m^{-2}h^{-1}$ (Smith 1973: Georgia coast). These areas supported macrofauna equivalent to 4 and 20 g m^{-2} dry mass respectively. Intertidal data are also very variable. Wieser & Kanwisher (1961) measured oxygen uptake rates of 33 and 26 ml O_2 $m^{-2}h^{-1}$ in a salt marsh and Pamatmat (1968), working on a sand flat, obtained values between 0 and 160 ml O_2 $m^{-2}h^{-1}$ at 5°C and 15° C respectively. Dye *et al.* (1978) measured benthic oxygen demand in clean sand and estuarine muddy sand and obtained rates of 3,6 to 22 ml O_2 $m^{-2}h^{-1}$ and 14 to 189 ml O_2 $m^{-2}h^{-1}$ respectively, both at 18° C. There are no published data for beaches but the present data compare well with sand column studies. Johnson (1970) obtained an oxygen consumption equivalent to 10 ml O_2 $m^{-2}h^{-1}$ which is almost identical to that obtained from vertical cores in the present study (Table 1).

Similarly the results from the surface respirometer compare with published data using similar methods, although at the lower end of the range. However, these results are approximately one third of the mean value obtained from the vertical cores. This difference arises from the fact that any system which relies on oxygen changes in a column of water overlying the substrate restricts the supply of oxygen to diffusion only. In well-drained systems dissolved oxygen is carried by the water and penetrates the substrate much more rapidly than can be accounted for by diffusion alone. The result of this must surely be an underestimate of benthic oxygen consumption. In addition surface respirometers, by their very position in the system, can at best only measure the oxygen consumption in the top few centimeters of the substrate. Since the respirometer is an "open" system it is not possible to define the volume of substrate being measured. The activity in the deeper layers cannot be measured by this method.

These criticisms are, however, not limited to well-drained beaches. In areas where large numbers of burrowing macrofaunal organisms occur, particularly those with permanent burrows extending from the surface, oxygen is also transported rapidly to the deeper layers by the organism (Dye & Furstenberg 1978). In such cases the applicability of surface respirometers is doubtful. Water movements have also been noted in subtidal sediments (Branch *pers. comm.*) and here again oxygen is transported more by mechanical means than by diffusion.

A possible solution to this problem has been presented in this paper. Instead of measuring oxygen changes in water above the substrate, oxygen changes in the interstitial water were measured. The distance over which oxygen must travel to reach the consumer organism is measured in um rather than cm and changes in the interstitial oxygen content more rapidly reflect the oxygen consumption of the system.

However, since the most active metabolism of the beach system takes place in the zone of active water movement, i.e. between the surface and the water table, measurements of oxygen consumption will still under-estimate the total (even if measured on cores) if such measurements are restricted to the top few centimeters only. This can be overcome by taking cores at intervals from the surface to the water table and integrating over the whole sand column. In this way the activity, even at depths of one

metre can be measured and taken into account. Measurements made in this way will, of course, exceed those made at the surface but will more accurately reflect the activity of the sand column. The problem of defining the volume of the substrate being studied is eliminated and true cognizance is taken of the three-dimensional nature of the system.

Bacterial populations are usually in a steady state in undisturbed sediments over the short term. This is due to the fact that gradients of oxygen, nutrients, waste materials and mutual inhibitors are established. If such a sediment is disturbed these gradients are broken down and, for a short period at least, the bacterial activity increases until a new steady state is reached. It is thus important that cores taken for the purpose of oxygen consumption measurements be undisturbed to avoid a possible over estimation of benthic respiration.

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