A STUDY OF THE RESPIRATION AND MICROFLORA OF THE MUD OF THE HEATHCOTE RIVER, CHRISTCHURCH

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SUMMARY : Plate counts and CO_2-respiration rates for various added substrates were measured for samples of mud from the lower reaches of the Heathcote River, Christchurch. All samples tested contained large populations of aerobic and anaerobic micro-organisms able to metabolize glucose, succinate and benzoate. Our results suggest that the availability of utilizable carbon was the major limiting factor for cell growth.

INTRODUCTION

The object of this study was to attempt to gain some measure of the amount of microbiological and biochemical activity in the sediments of the Heathcote River, Christchurch, in a zone between Ensors Road and Tunnel Road Bridge (refer Fig. l) which might be affected by flood control measures. This portion of the Heathcote River has, in the past, received potentially toxic trade wastes and could receive accidental contamination in the future. Furthermore, the lower part of this zone receives tidal waters, so its metabolic activities might be affected by the presence of sea water concentration of NaCl. It was of interest to see if this previous history was reflected in the microbiological and metabolic activity of the sediments.

The assay of metabolic activity of mud and soil is usually accomplished by measuring the rate and quantity of CO_2 evolved using Warburg or other techniques. Heterotrophic micro-organisms grow on organic materials in soil or mud; part of the carbon assimilated is used to form biomass whilst a larger and relatively constant proportion is oxidized during cell respiration and ultimately released as CO_2. Aerobic organisms will also consume O_2 during these processes but this is less easily measured. Any factor that affects microbial growth will affect the yield of CO_2. Thus the rate and amount of CO_2 released will reflect the overall metabolic activities of the diverse range of micro-organisms present in a given sample of soil or mud.

In the present study we investigated the ability of mud samples taken from selected sites along the Heathcote River (Fig. l) to metabolize added nutrients. Plate counts of the aerobic and anaerobic bacteria present were also made. Additionally, the effect of supplements of different organic nutrients and O_2 supply on the rate of respiration was investigated for one site.

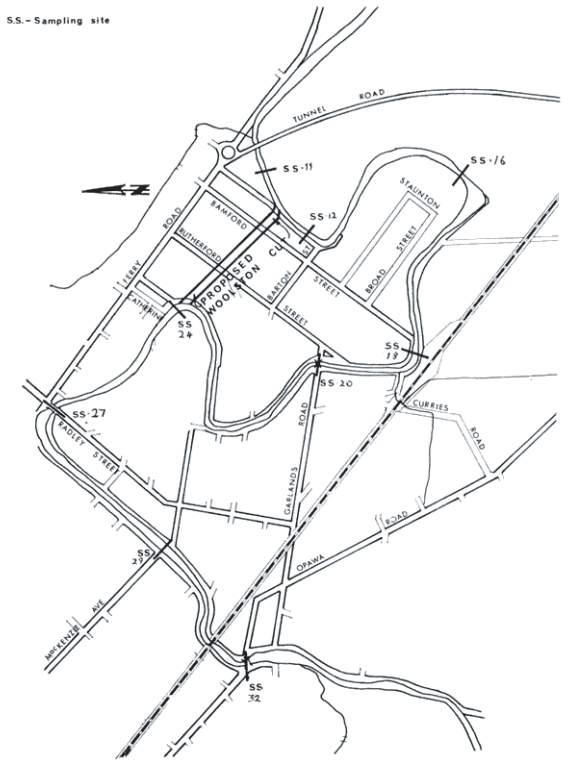

FIGURE 1. *Map of lower Heathcote River showing sampling sites and proposed Woolston Cut.*

MATERIALS AND METHODS

Mud samples

Samples of mud from the Heathcote River were supplied by Dr J. Robb and were from the same sites as those chosen for the concurrent chemical programme being conducted by the Christchurch Drainage Board's laboratory. Comparative control samples were taken from the River Avon at Dallington and at Ilam (University campus).

Measurement of CO_2 evolution

For preliminary experiments a standard Gilson Respirometer (U.S.A.) was used to attempt to measure the rates of O_2-uptake and CO_2-evolution from mud. Unfortunately, the metabolic rates of our samples proved to be too low for measurement by this technique.

Because of this problem recourse was made to a simple CO_2-absorption procedure for measuring CO_2-evolution from soil or mud: 100 g aliquots of mud were weighed out into 8 absorption vessels (500 ml "AGEE" jars) whilst an additional sample was used for the estimation of dry weight (after drying at 105° C).

The following additions were made to duplicate vessels:

a. 1 ml water (endogenous control)
b. 1 ml 5 % glucose
c. 1 ml 5% Na succinate
d. 1 ml 5 % $(NH_4)_2HPO_4$
e. 1 ml 5 % glucose + 1 ml 5 % $(NH_4)_2HPO_4$
f. 1 ml 5 % succinate + 1 ml $(NH_4)_2HPO_4$

An empty flask served as atmospheric control.

Into each vessel was placed a vial containing 10 ml 0.1 M-NaOH to absorb any CO_2 released and the lid sealed with silicone grease. The samples were then incubated at 25 °C and the NaOH titrated at intervals of two or three days against standard 0.1 M-HCl and the decrease in NaOH titre converted to mg CO_2 released.

Metabolic studies

In order to compare the ability of mud samples to metabolize different organic substrates under aerobic and anaerobic conditions a sequence of bubbler tubes was set up as shown below:

AIR or N_2 → 10% KOH → Mud+ Substrate → Standard 0.1 M-NaOH

The inflowing air or N_2 was passed through 10% KOH to remove any traces of CO_2, then led into a bubbler tube containing 20 g mud plus the organic substrate. Any CO_2 evolved was trapped in a second bubbler tube containing 20 ml of 0.1 M-NaOH.

Microbiological tests

Estimates of the populations of aerobic and anaerobic micro-organisms present in each sample were made by conventional microbiological plate count techniques: 1 ml aliquots from serial dilution being plated out on plate count agar (Difco, U.S.A.). Plates were incubated at 25°C and counted after 3 days.

RESULTS AND DISCUSSION

A preliminary series of CO_2-evolution determinations was made to check that the rate of CO_2. evolution gave a valid measure of the mud's metabolic activity. Factors such as the effect of substrate, added nitrogen and phosphate and the time course of CO_2-production were studied; these results are shown in Figure 2.

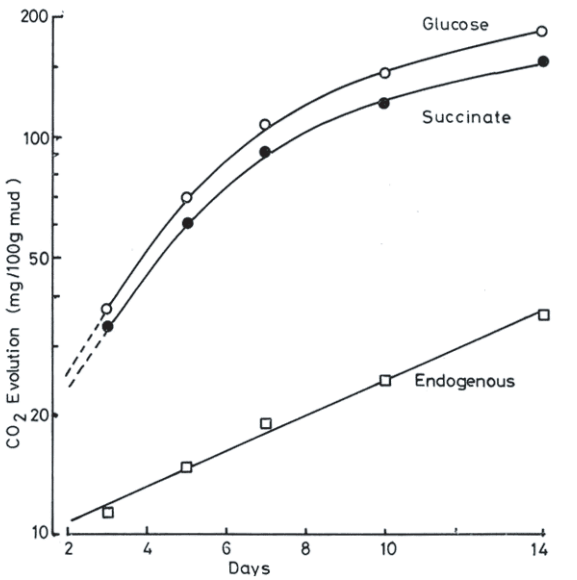

FIGURE 2. *CO_2 -evolution from mud Ensors Road sample) in presence of added substrate.*

From Figure 2 it may be seen that the rate of CO_2-release was exponential between days 3-7 and this is typical of CO_2-release by actively growing cells. This being the case, a comparison of the yields of respiratory CO_2, and hence microbial growth rates, may be made by using the equation:

$$\text{Growth Rate (as rate of increase of } CO_2 \text{ production)} = \frac{k.\text{Log}_{10}\,\Delta CO_2}{t \text{ (days)}}$$

where 'ΔCO_2' equals the amount of CO_2 produced during 't' days. The endogenous rate of respiration

gave a measure of the basal population utilizing endogenous carbon sources for growth. When extra carbon substrates such as glucose or succinate were added, the rate of cell growth, and hence CO_2-release, was markedly increased, whereas supplements of extra N or P did not significantly affect CO_2-evolution. Thus, it may be concluded that in these samples microbial growth was limited chiefly by the availability of utilizable carbon sources.

Metabolic experiments

On the basis of the above data, samples of mud from selected sites along the Heathcote River were assayed for CO_2-evolution over 6 days, both with and without added substrate. The same mud samples were also used for plate counts; the results of these tests are summarized in Table 1.

In the course of these experiments it was noticed that the colour of the mud became lighter at the surface and this was most marked when supplemented with glucose. A possible explanation could be the change in oxidation state of heavy metals and/ or sulphur compounds. In general, the samples supplemented with glucose were the most odoriferous.

The metabolic activity of the Ensors Road sample (Site 33) was investigated in more detail using a greater range of substrates under aerobic and anaerobic conditions. The metabolic interrelationships between these substrates are shown below, whilst the results are compared in Figure 3.

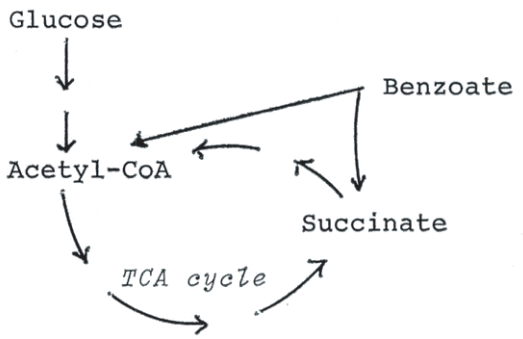

TABLE 1. *Respiration rates and bacterial populations; Heathcote River.*

Site No.	Location	Ammonia -N*	Available -P*	Rate of increase in CO_2 production. Mg/day/g dry wt Mud only (endogenous)	Mud + succinate	Mud + glucose	Plate counts (colonies x 10^6/ 1 g dry wt) Aerobic	Anaerobic
	Ensors Rd		-	0.19	0.22	0.25	2.44	0.84
32	Opawa Rd bridge, upstream	105	96	0.24	0.30	0.30	0.56	0.21
32A	downstream	18	46	0.27	0.30	0.31	0.25	0.35
29	McKenzie Ave.	101	56	0.28	0.27	0.29	1.36	0.76
27	Radley St	-	-	0.27	0.30	0.29	7.76	2.5
		-	-	0.30	0.31	0.31	9.36	1.32
24	Catherine St							
	edge	112	146	0.23	0.30	0.32	1.7	1.4
	midstream upper sediment	104	578	0.23	0.32	0.32	1.93	0.39
	midstream lower sediment	-	660	0.19	0.32	0.33	1.34	1.04
10A	Garlands Rd							
	edge	94	186	0.24	0.30	0.31	12.9	3.25
	midstream	150	328	0.21	0.31	0.31	0.26	0.15
18	Bamford St	326	473	0.27	0.31	0.32	4.0	0.64
16	Staunton St	476	336	0.25	0.31	0.31	1.73	0.35
14		-	-	0.21	0.29	0.31	1.24	0.91
12	Barton St	-	-	0.31	0.35	0.37	2.42	2.07
11	Tunnel Rd	46	242	0.27	0.36	0.37	1.07	0.50
	R. Avon: Dallington St	-	-	0.26	0.28	0.28	2.44	0.84
	R. Avon: Ilam campus	-	-	0.23	0.44	0.46	-	-

* Results from Board's laboratory

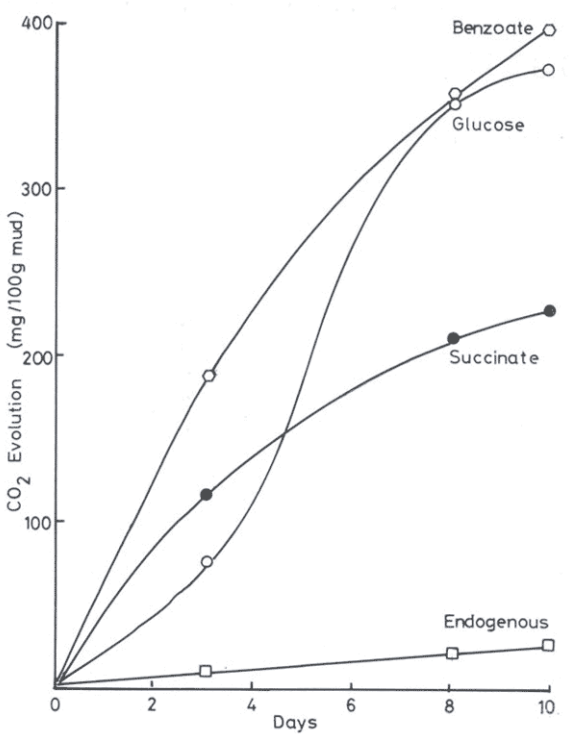

FIGURE 3. *Comparison of rate of metabolism of different substrates. Mud sample from Ensors Road site.*

In this experiment CO_2-free air was bubbled through the reaction mixture and it will be seen that benzoate, glucose and succinate were all readily metabolized. By contrast, under anaerobic conditions (N_2-bubbled) only glucose was fermented. These results are as expected since the metabolism of benzoate and succinate are essentially aerobic processes.

In view of the tidal conditions pertaining at the lower parts of the river, the effect of 2.68% NaCl (equivalent seawater) upon CO_2-release was investigated and found to be negligible.

Plate counts

The results of the plate counts are also included in Table 1 but unfortunately seem to show little obvious correlation with the values for CO^2 evolution. This can be attributed to the presence of many "spreading" organisms which make it difficult to obtain accurate counts. Nevertheless, the plate counts are valuable in as much as they demonstrate the presence of high levels of viable cells in all the samples.

There was little difference in final appearance between the aerobic and anaerobic plates but the latter were slower growing. Fungi appeared to be absent from the culture plates but fungal growth was observed on the surface of the mud in the respiration vessels for sites 18, 20A, 24 and 29.

CONCLUSIONS

The results presented in this report show that the samples of mud taken from the Heathcote River between Ensors Road and Tunnel Road all possessed similar levels of aerobic and anaerobic micro-organisms. These populations gave rise to low but significant basal rates of CO^2-release from the mud and the rates were enhanced when metabolizable carbon sources were present.

Supplements of phosphate and nitrogen (as NH_4+) did not significantly enhance respiration rates. Thus, it may be concluded that utilizable carbon is the limiting factor to microbial growth rather than N or P and this is in agreement with the results of the chemical analysis.

In other words, the river mud may be considered to be "healthy" and can be expected to metabolize most added nutrients provided it is not subjected to inorganic poisons that might harm the microflora and fauna.

ACKNOWLDEGMENTS

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