In situ Measurement of Microbial Biomass, Community Structure and Nutritional Status [and Discussion]
Author(s): David C. White, P. Meadows, G. Eglinton, M. L. Coleman
Source: *Philosophical Transactions: Physical Sciences and Engineering*, Vol. 344, No. 1670, Quantifying Sedimentary Geochemical Processes (Jul. 15, 1993), pp. 59-67
Published by: The Royal Society
Stable URL: http://www.jstor.org/stable/54099
Accessed: 12/02/2009 11:33

In situ measurement of microbial biomass, community structure and nutritional status

By David C. White

Center for Environmental Biotechnology, University of Tennessee/Oak Ridge National Laboratory, 10515 Research Drive, Suite 300, Knoxville, Tennessee 37932–2567, U.S.A.

In sediments and soils the extant microbiota that can be counted by direct microscopy have proved exceedingly difficult to isolate and culture. Classical tests are time consuming and provide little indication of the interactions within the community, the community nutritional status or metabolic activity. The *in situ* method is based on the extraction of 'signature' lipid biomarkers (SLB) from the cell membranes and walls of microorganisms. Lipids are cellular components that are recoverable by extraction with organic solvents. Lipids are an essential component of the membrane of all cells and play a role as storage materials. Extraction of the lipid components of the microbiota from soils and sediments provides both purification and concentration together with an *in situ* quantitative analysis of the microbial biomass, community structure, and nutritional status. The determination of the total phospholipid ester-linked fatty acids (PLFA) provides a quantitative measure of the viable biomass. Viable microbes have an intact membrane which contains phospholipids (and PLFA). With cell death enzymes hydrolyze the phosphate group within minutes to hours. The lipid core remains as diglyceride (DG). The resulting DG has the same signature fatty acids as the phospholipids (until it degrades) so a comparison of the ratio of PLFA to DG provides an indication of the viable and non-viable microbes. Analysis by SLB technique provides a quantitative definition of the microbial community structure as specific groups of microbes contain characteristic PLFA patterns. The analysis of other lipids such as the sterols (for the microeukaryotes – nematodes, algae, protozoa), glycolipids (for the phototrophs, gram-positive bacteria), or the hydroxy fatty acids in the lipopolysaccharide of the lipid A (gram-negative bacteria) can provide more detailed community structure analysis. The formation of poly β-hydroxyalkanoic acid (PHA) in bacteria or triglyceride (TG) in the microeukaryotes relative to the PLFA provides a measure of the nutritional status. Bacteria grown with adequate carbon and terminal electron acceptors form PHA when they cannot divide, because some essential component is missing. Rates of incorporation of ^{14}C-acetate into PHA relative to PLFA is a sensitive indicator of disturbance artifacts in estimates of metabolic activity in sediments with redox gradients. Exposure to toxic environments can lead to minicell formation and increases in specific PLFAs. Respiratory quinone structure indicates the proportions of aerobic/anaerobic activities in the community. The SLB technology provides quantitative *in situ* information that define the microbial ecology in sedimentary geochemical processes.

Phil. Trans. R. Soc. Lond. A (1993) **344**, 59–67
Printed in Great Britain

1. Introduction

Sediments and soils contain about 10^9 cells per gram whether recovered from under the ice in Antarctica or tropical estuaries (Smith *et al.* 1986). The sedimentary microbial community is incredibly diverse metabolically with enzymatic activities and complex interactions. To begin to understand the ecology of this complex community methods must be developed that do not introduce artefacts of measurement and that preserve the interactions between metabolically diverse groups that could be essential in the functioning of the intact system. The classical microbiologic approach that was so successful in public health of isolating single pathogenic species is clearly not satisfactory when faced with a microbial consortium where interactions govern the multiple geochemical processes in the sediment. In the first place most of the organisms seen in the microscope will not grow in culture. It has been repeatedly documented in the literature that viable counts or direct counts of bacteria attached to sediment grains are inadequate and may represent 0.1 to 10% of the extant community (Tunlid & White 1991; White 1983, 1986, 1988). Classical tests are time consuming and provide little indication of the nutritional status or evidence of toxicity. Instead of microbial tests depending on growth or morphology, the microbial biomass can be determined in terms of universally distributed biomarkers that are characteristic of microbes. Our laboratory has concentrated upon lipid biomarkers as they are readily concentrated from sediments, and the concentration step results in significant purification. The lipid chemistry of the microbes is sufficiently diverse that it can provide quantitative estimates of the community structure and in some cases the nutritional status. The signature lipid biomarker (SLB) technique provides a quantitative means to measure the viable microbial biomass, the total community structure, and community nutritional status without the need for quantitatively recovering the microbes or isolating them for growth. SLB technique utilizes the lipid profiles of microbes so there is no need for culture or isolation. Determination of signature biomarkers with the lipids allows analysis over scales ranging from milligrams to kilograms of subsurface sediments. This has been a major benefit of the SLB analysis with the heterogeneity problems associated with *in situ* processes such as bioremediation. Quantitative estimates of heterogeneity in determining the most effective quadrat size for accurate estimation of the microbiota in estuarine mud-flats by the SLBT has been demonstrated (Federle *et al.* 1983). Classical methods involve the recovery and culture of organisms from soils or sediments for microbial assessment. Using SLB technique it is not necessary to quantitatively release the cells from the soil or sedimentary matrix nor to make the isolated cells grow. In soils and many sediments less than one percent of the organisms can be successfully cultured. This 'present but not culturable' problem has been increasingly recognized throughout the research community. For example the organism causing recent outbreaks of cholera in many cases can not be cultured.

The SLB method is based on the extraction of 'signature' lipid biomarkers from the cell membranes and walls of microorganisms (Guckert *et al.* 1985). Lipids are cellular components that are recoverable by extraction in organic solvents. Lipids are an essential component of the membrane of all cells and play a role as storage materials.

2. Signature lipid biomarker techniques

The SLB analysis provides quantitative insight into important attributes of microbial communities.

(*a*) *Viable biomass*

The determination of the total phospholipid ester-linked fatty acids (PLFA) provides a quantitative measure of the viable or potentially viable biomass. Viable microbes have an intact membrane which contains phospholipids (and PLFA). With cell death the cellular enzymes hydrolyze and thus release the phosphate group within minutes to hours of cell death (White *et al.* 1979). The lipid remaining is diglyceride (DG) (figure 1). The resulting diglyceride contains the same signature fatty acids as the phospholipids so a comparison of the ratio of PLFA to diglyceride fatty acids provides an indication of the viable and non-viable microbes. A careful study of subsurface sediment showed the viable biomass determined by PLFA was equivalent (but with a much smaller standard deviation) to that estimated by intercellular ATP, cell wall muramic acid and very carefully done acridine orange direct counts (Balkwill *et al.* 1988). PLFA provides an accurate measure of viable or potentially viable biomass as the phospholipids have a high natural turnover rate, are rapidly lost from viable cells, are found in all cellular membranes, and are found in reasonably constant amounts in bacterial cells as they occur in nature.

(*b*) *Community structure*

The analysis of SLB by capillary gas chromatography/mass spectrometry (GC/MS) provides sufficient information for detection of specific subsets of the microbial community that allows quantitative definition of the microbial community structure. Specific groups of microbes often contain characteristic lipids (Tunlid & White 1991; White 1986, 1988). For example, specific PLFA are prominent in the hydrogenase containing *Desulfovibrio* sulphate-reducing bacteria whereas the *Desulfobacter* type of sulphate-reducing bacteria contain distinctly different PLFA (Edlund *et al.* 1985; Dowling *et al.* 1986). Patterns of the prominent PLFA from isolated microbes after growth on standardized media are used to differentiate over 8000 species of organisms with the Microbial Identification System (MIDI, Newark, DE) (Welch 1991). Cluster analyses of PLFA patterns of whole microbial communities quantitatively define relatedness of different microbial communities. Cluster analysis of PLFA patterns of methane-oxidizing bacteria showed closeness that paralleled the phylogenetic sequence similarities in the 16S RNA (Guckert *et al.* 1991). Cluster analysis of the total viable microbial community showed that deep subsurface sediment microbiota from sandy horizons with hydrologic permeability were clearly different from the clay-rich aqualudes and both were different from the microbiota at the surface or in drilling fluids (White *et al.* 1991).

The analysis of other lipids such as the sterols (for the microeukaryotes – nematodes, algae, protozoa) (White *et al.* 1980), glycolipids (phototrophs, gram-positive bacteria), or the hydroxy fatty acids in the lipopolysaccharide of the lipid A (gram-negative bacteria) (LPS OHFA) (Parker *et al.* 1982) can provide more detailed community structure analysis (figure 1).

Analysis of the gram-negative bacteria by LPS OHFA can be particularly valuable if the patterns are unique. Two of the most interesting anaerobes which are related to sulphate-reducing bacteria have sufficiently unusual LPS OHFA that they can be

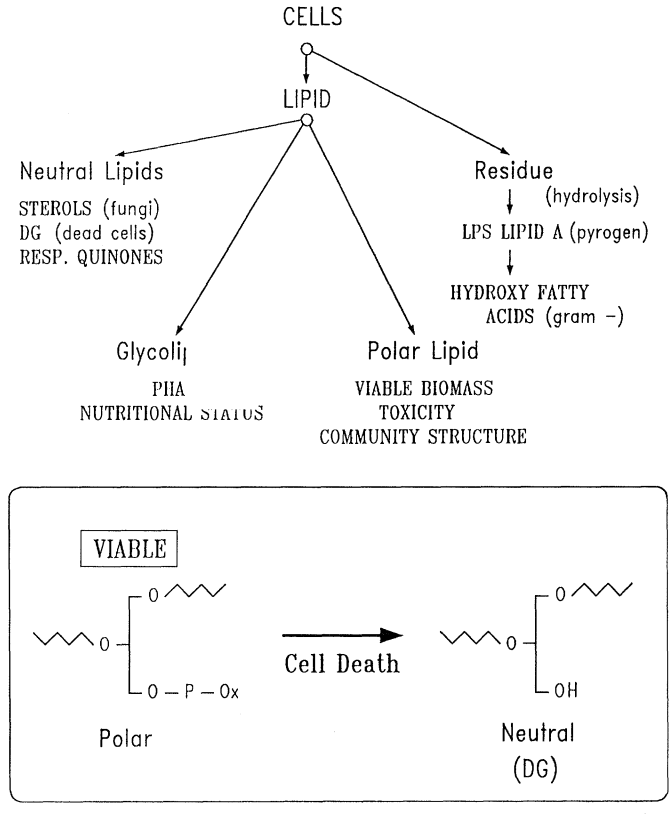

Figure 1. Scheme for the signature lipid biomarker (SBL) technique in which sediments are extracted, the lipids fractionated, and analysed by gas chromatography/mass spectrometry after derivatization (upper panel). Enzymatic hydrolysis after cell death of the membrane phospholipids resulting in diglycerides (DG) with the same ester-linked fatty acids (lower panel).

readily identified in sediments. *Desulfomonile tiedjei* is the only isolated anaerobic, aromatic-dehalogenating microbe. *Geobacter metallireducens* utilizes a narrow range of organic electron donors with iron, manganese, or uranium as the terminal electron acceptor (Lovely *et al.* 1992). The search for homologous anaerobes with different substrate utilization will be facilitated by the detection of their distinctive LPS OHFA patterns.

(*c*) *Nutritional status*

The proportion of poly β-hydroxyalkanoic acid (PHA in bacteria) (Nickels *et al.* 1979; Findlay & White 1983) or triglyceride (in the microeukaryotes) (Gehron & White 1982) relative to the PLFA provides a measure of the nutritional status. Bacteria exposed to adequate carbon and terminal electron acceptors form PHA when they cannot divide, because some essential component is missing (phosphate, nitrate, trace metal, etc.). TG is formed in a variety of conditions by microeukaryots in which plentiful carbon abounds.

In bacteria specific patterns of PLFA can indicate physiological stress (Guckert *et al.* 1986). Exposure to toxic environments can lead to minicell formation and a relative increase in specific *trans* momoenoic PLFAS. Formation of increasing proportions of *trans* fatty acids with exposure to increasing concentrations of phenol toxicants has been shown for *Pseudomonas* (Heipieper *et al.* 1992).

Table 1. *Disturbance artefacts in sandy sediments*

(Artefacts detected as increases in rate of incorporation of ^{14}C-acetate into membrane lipid (PLFA) relative to bacterial storage lipid (PHA) determined after a 10 min incubation given as mean (standard deviation, $n = 5$). Enteropneust (benthic sediment processing animal) feces show disturbance of the survivors of digestion followed by rapid regrowth on the sediment of the fecal pellet.)

ambient sand	0.31 (0.03)
raked sand	0.50 (0.05)
sieved sand	0.60 (0.02)
sting ray pits (2–3 h)	0.70 (0.10)
sand with tide and waves	1.00 (0.20)
slurried sand	1.40 (0.30)
enteropneust fecal pellet	0.40 (0.20)
sediment (food)	
2 h	1.00 (0.90)
4 h	0.30 (0.10)
6 h	0.09 (0.06)

The respiratory quinone structure indicates the degree of aerobic activity (Hedrick & White 1986). Environments with high potential terminal electron acceptors (oxygen, nitrate) induce formation of benzoquinones in bacteria in contrast to microbes respiring on organic substrates which form naphthoquinones. Fermentative organisms may form no respiratory quinones. The SLB methods can determine proportions of aerobic, anaerobic respiratory, or anaerobic fermentative growth in the communities under study. With SLB technology it was possible to show that anaerobic respiratory metabolism was responsible for siderite formation in estuarine concretions (Coleman *et al.* 1993). Knowing whether aerobic or exclusively anaerobic metabolic processes are available can be of great importance in bioremediation prediction.

(*d*) *Metabolic activity and the disturbance artefact*

Since the SLB technique involves the separation and assay of distinctive lipid biomarkers, rates of formation from precursors or turnover during growth may be determined (White & Tucker 1969). Radioactive or mass labelled precursors are added to the sediment and the incorporation or metabolic activity determined at timed intervals after isolation of the product. Adding the labelled precursor to sediments in slurries, or by injection with various degrees of disturbance resulted in progressively greater apparent metabolic rates (Moriarty *et al.* 1985; Findlay *et al.* 1985, 1990).

Natural disturbances by benthic sediment processing invertebrates, sting ray feeding, or wave action on tidal flats can be detected both as increased rates of incorporation of labelled precursors and/or shifts in PLFA/PHA ratios if the gentlest methods of labelled precursor application are utilized (Findlay *et al.* 1990). With these methods the sedimentary microbiota has been shown to be remarkably responsive to disturbances that allow metabolic activity (table 1). Microbes in sediments exist like coiled springs awaiting metabolic opportunities thereby creating opportunities for determinations of activity that are much greater than the actual basal rates. Measurements at the surface with subsurface sediment slurries gave metabolic rates five orders of magnitude higher than would allow for the measured presence of oxygen in a deep subsurface aquifer with very minimal recharge from the

surface (Phelps *et al.* 1993). Since estimates of carbon dioxide production and methane by soil and benthic microbes are important in calculations of greenhouse effects, these disturbance artefacts possibly have introduced serious errors.

3. Utility of the signature lipid biomarker technique

Despite the complexities of the sedimentary microbiota, the SLB technique allows *in situ* determinations that provide insights into sedimentary processes. By sampling just ahead and just behind a echinoderm sand dollar *Mellita quinquiesperforata* as they slowly move through the sediments processing the sand, it was possible to show with the SLB that the feeding was selective for protozoans and bacteria to some extent (Findlay & White 1983). Diatoms passed through the sand dollar alimentary tract intact. Excluding the top predators (fish and crabs) from an estuary by caging induced changes in the sedimentary microbiota (Federle *et al.* 1983). There was an immediate overgrowth of the opportunistic polychaete *Mediomastus ambiseta* with markedly decreased polyenoic PLFA characteristic of nematodes and algae. There was a concomitant increase in bacteria especially anaerobic sulphate-reducing bacteria as detected in specific PLFA patterns. Shortly after the increase in bacteria the proportions of linoleic acid characteristic of bacteriovorus protozoa increased. The overgrowth of the polychaetes grazed the nematodes and algae which allowed the bacterial overgrowth. The change in bioturbation decreased the aeration of the sediment with an increase in the proportions of anaerobes.

Microcosms isolated from the environment they are designed to mimic are created for assessing the toxicity of xenobiotics on benthic biota. The quantitative definition of the sedimentary microbial community structure in these microcosms can be compared directly with that in the field by SLB technique. Experiments showed microbial biomass and community structure were detectably different but the degree of difference was not large and did not increase with time when compared with the field with microcosms from a shallow, turbid, highly disturbed bay enriched with riverain runoff that is characterized by low macroscopic species diversity and high biomass (Federle *et al.* 1986). Microcosms prepared from a more stable, higher salinity system with much greater diversity that is controlled by epibenthic predators showed greater differences between replicate microcosms themselves as well as to the field. Moreover the divergences increased with time.

4. Conclusions

The SLB can provide quantitative definition of the viable biomass, community structure, and nutritional status of the sedimentary microbiota giving answers to questions of how much, who, and the state of nutrition of what is there. The specificity of who is there can be greatly increased by using nucleic acid probes to specific bacterial 16S RNA or enzymes. The proportions of specific genes in the community can also be determined with these probes. Unfortunately these measures give a picture of the potential metabolic activity of the microbial community rather than the actual activity at the time of sampling. This second key parameter of the metabolic activities is much more difficult to address. Additions of labelled substrates or precursors can involve serious disturbance artifacts. The future may lie in implanting ultramicro biosensors or genetically engineering bacteria that faithfully 'report' specific metabolic activities. Reporters for the synthesis of extracellular

polysaccharide polymers in *Pseudomonas* have been genetically engineered utilizing a gene cassette for bioluminescence. These bacteria bioluminese when the gene is active and exopolymer is formed (Wallace *et al.* 1992).

I acknowledge receiving the following grants: N00014-91-J-1598, from the Office of Naval Research, RP-8011-2, from the Electric Power Research Institute, DE-FGO5-91ER61193 and DE-FG05-90ER60988 from the Department of Energy for partial support of this research.

References

Balkwill, D. L., Leach, F. R., Wilson, J. T., McNabb, J. F. & White, D. C. 1988 Equivalence of microbial biomass measures based on membrane lipid and cell wall components, adenosine triphosphate, and direct counts in subsurface sediments. *Microb. Ecol.* **16**, 73–84.

Coleman, M. E., Hedrick, D. B., Lovely, D. R., White, D. C. & Pye, K. 1993 Reduction of Fe (III) in sediments by sulphate reducing bacteria. *Nature, Lond.* **361**, 436–438.

Dowling, N. J. E., Widdel, F. & White, D. C. 1986 Phospholipid ester-linked fatty acid biomarkers of acetate-oxidizing sulfate reducers and other sulfide forming bacteria. *J. gen. Microbiol.* **132**, 1815–1825.

Edlund, A., Nichols, P. D., Roffey, R. & White, D. C. 1985 Extractable and lipopolysaccharide fatty acid and hydroxy acid profiles from *Desulfovibrio* species. *J. Lipid Res.* **26**, 982–988.

Federle, T. W., Hullar, M. A., Livingston, R. J., Meeter, D. A. & White, D. C. 1983 Spatial distribution of biochemical parameters indicating biomass and community composition of microbial assemblies in estuarine mud flat sediments. *Appl. envir. Microbiol.* **45**, 58–63.

Federle, T. W., Livingston, R. J., Meeter, D. A. & White, D. C. 1983 Modification of estuarine sedimentary microbiota by exclusion of epibenthic predators. *J. exp. mar. Biol. Ecol.* **73**, 81–94.

Federle, T. W., Livingston, R. J., Wolfe, L. E. & White, D. C. 1986 A quantitative comparison of microbial community structure of estuarine sediments from microcosms and the field. *Canad. J. Microbiol.* **32**, 319–325.

Findlay, R. H., Pollard, P. C., Moriarty, D. J. W. & White, D. C. 1985 Quantitative determination of microbial activity and community nutritional status in estuarine sediments: evidence for a disturbance artifact. *Canad. J. Microbiol.* **31**, 493–498.

Findlay, R. H., Trexler, M. B., Guckert, J. B. & White, D. C. 1990 Laboratory study of disturbance in marine sediments: response of a microbial community. *Mar. ecol. Prog. Ser.* **61**, 121–133.

Findlay, R. H., Trexler, M. B. & White, D. C. 1990 Response of a benthic microbial community to biotic disturbance. *Mar. ecol. Prog. Ser.* **61**, 135–148.

Findlay, R. H. & White, D. C. 1983 Polymeric beta-hydroxyalkanoates from environmental samples and *Bacillus megaterium*. *Appl. envir. Microbiol.* **45**, 71–78.

Findlay, R. H. & White, D. C. 1983 The effects of feeding by the sand dollar *Mellita quinquiesperforata* on the benthic microbial community. *J. exp. mar. Biol. Ecol.* **72**, 25–41.

Gehron, M. J. & White, D. C. 1982 Quantitative determination of the nutritional status of detrital microbiota and the grazing fauna by triglyceride glycerol analysis. *J. exp. mar. Biol.* **64**, 145–158.

Guckert, J. B., Antworth, C. P., Nichols, P. D. & White, D. C. 1985 Phospholipid, ester-linked fatty acid profiles as reproducible assays for changes in prokaryotic community structure of estuarine sediments. *Microbiol. Ecology* **31**, 147–158.

Guckert, J. B., Hood, M. A. & White, D. C. 1986 Phospholipid, ester-linked fatty acid profile changes during nutrient deprivation of *Vibrio cholerae*: increases in the *trans/cis* ratio and proportions of cyclopropyl fatty acids. *Appl. envir. Microbiol.* **52**, 794–801.

Guckert, J. B., Ringelberg, D. B., White, D. C., Henson, R. S. & Bratina, B. J. 1991 Membrane fatty acids as phenotypic markers in the polyphasic taxonomy of methylotrophs within the proteobacteria. *J. Gen. Microbiol.* **137**, 2631–2641.

Hedrick, D. B. & White, D. C. 1986 Microbial respiratory quinones in the environment. I. A sensitive liquid chromatographic method. *J. microbiol. Meth.* **5**, 243–254.

Heipieper, H.-J., Diffenbach, R. & Keweloh, H. 1992 Conversion of *cis* unsaturated fatty acids to

trans, a possible mechanism for the protection of phenol degrading *Pseudomonas putida* P8 from substrate toxicity. *Appl. envir. Microbiol.* **58**, 1847–1852.

Lovely, D. R., Giovannoni, S. J., White, D. C., Champine, J. E., Phillips, E. J. P., Gorby, Y. A. & Goodwin, S. 1992 *Geobacter metallireducens* gen. nov. sp. nov., a microorganism capable of coupling the complete oxidation of organic compounds to the reduction of iron and other metals. *Appl. envir. Microbiol.* (In the press.)

Moriarty, D. J. W., White, D. C. & Wassenberg, T. J. 1985 A convenient method for measuring rates of phospholipid synthesis in seawater and sediments: its relevance to the determination of bacterial productivity and the disturbance artifacts introduced by measurements. *J. microbiol. Meth.* **3**, 321–330.

Nickels, J. S., King, J. D. & White, D. C. 1979 Poly-beta-hydroxybutyrate accumulation as a measure of unbalanced growth of the estuarine detrital microbiota. *Appl. envir. Microbiol.* **17**, 459–465.

Parker, J. H., Smith, G. A., Fredrickson, G. A., Fredrickson, H. L., Vestal, J. R. & White, D. C. 1982 Sensitive assay, based on hydroxy-fatty acids from lipopolysaccharide lipid A for gram negative bacteria in sediments. *Appl. envir. Microbiol.* **44**, 1170–1177.

Phelps, T. J., Murphy, E. M., Pfiffner, S. M. & White, D. C. 1993 Comparison of geochemical and biological estimates of subsurface microbial activity. *Geomicrobiology*. (Submitted.)

Smith, G. A., Nichols, P. D. & White, D. C. 1986 Fatty acid composition and microbial activity of benthic marine sediments from McMurdo Sound, Antarctica. *FEMS Microbiol. Ecol.* **32**, 219–231.

Tunlid, A. & White, D. C. 1991 Biochemical analysis of biomass, community structure, nutritional status, and metabolic activity of the microbial communities in soil. In *Soil Biochemistry* (ed. J.-M. Bollag & G. Stotzky), vol. 7, pp. 229–262.

Wallace, W. H., Fleming, J. T., White, D. C. & Sayler, G. S. 1993 An *algD*-bioluminescent reporter plasmid to monitor alginate production in biofilms. *FEMS Microbiol. Ecol.* (Submitted.)

Welch, D. F. 1991 Applications of cellular fatty acid analysis. *Clin. Microbiol. Rev.* **4**, 422–438.

White, D. C. 1983 Analysis of microorganisms in terms of quantity and activity in natural environments. In *Microbes in their natural environments* (ed. J. H. Slater, R., Whittenbury & J. W. T. Wimpenny). *Society for General Microbiology Symposium*, **34**, pp. 37–66.

White, D. C. 1986 Environmental effects testing with quantitative microbial analysis: Chemical signatures correlated with *in situ* biofilm analysis by FT/IR. *Toxicity Assessment* **1**, 315–338.

White, D. C. 1988 Validation of quantitative analysis for microbial biomass, community structure, and metabolic activity. *Adv. Limnology* **31**, 1–18.

White, D. C., Bobbie, R. J., Nickels, J. S., Fazio, S. D. & Davis, W. M. 1980 Nonselective biochemical methods for the determination of fungal mass and community structure in estuarine detrital microflora. *Botanic Marina* **23**, 239–250.

White, D. C., Davis, W. M., Nickels, J. S., King, J. D. & Bobbie, R. J. 1979 Determination of the sedimentary microbial biomass by extractible lipid phosphate. *Oecologia* **40**, 51–62.

White, D. C., Ringelberg, D. B., Guckert, J. B. & Phelps, T. J. 1991 Biochemical markers for *in situ* microbial community structure. In *Proceedings of the First International Symposium on Microbiology of the Deep Subsurface* (ed. C. B. Fliermans & T. C. Hazen), pp. 4–45 to 4–56, 15–19 January 1990, Orlando, Fl. WSRC Information Services, Aiken, Sc.

White, D. C. & Tucker, A. T. 1969 Phospholipid metabolism during bacterial growth. *J. Lipid Res.* **10**, 220–233.

Discussion

P. Meadows. Dr White suggests minimum disturbance to samples for measuring microbial activity and biomass. But surely his method for isolating bacteria, growing a biofilm and then measuring the activity cannot be applied to sediments. Deep-sea studies by Jannasch and Wirsen have demonstrated *in situ* methods for measuring microbial activity. What is his opinion on this and how would he isolate and measure the bacterial activity in marine sediments without disturbing the sediment?

D. C. WHITE. The marine sedimentologist must look at the whole community. The biofilm approach more readily minimizes the disturbance artefact which gives an artificially high rate in sediments. The SLB method can be used to carefully measure the presence of organisms and estimate the rates of PHA biosynthesis relative to PLFA biomass which gives an estimate of disturbance. One of the least disturbing methods is to add the labelled precursor via a natural means such as adding to leaves of plants and subsequently examining the rhizosphere microbes for new synthesis by incorporating label. In some deep subsurface sediments, rates determined at the surface exceeded what was predicted based on oxygen utilisation by five orders of magnitude.

G. EGLINTON. What scope is there for devising analytical methodology that will provide estimates of not only the microbial biomass in a sediment but also the dead and decaying organisms, the necromass?

D. C. WHITE. We have just begun to examine the fatty acids patterns of the diglycerides which are formed when the polar phospholipids break down with cell death.

M. L. COLEMAN. Can the biochemical assay be used with inorganic products to calibrate the extent of disturbance?

D. C. WHITE. Yes; for example geochemical activity of microbes such as the conversions of $^{35}SO_4^{2-}$ into $^{35}HS^-$ could give an estimate of disturbance, particularly if several levels of disturbance were tested.