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23

METHODS FOR MICROBIAL BIOMASS, COMMUNITY STRUCTURE AND METABOLIC ACTIVITIES ON SURFACES

D.C. WHITE.

Center for Biomedical and Toxicological Research, 310 Nuclear Research Building, TALLAHASSEE, Florida 32306 (USA)
Current address : Institute for Applied Microbiology, P.O.B. X, BLDg 1503 OAKRIDGE, Tennessee 37831 (USA)

ABSTRACT - Microbes in nature are often concentrated on surfaces where they exist in microcolonies of mixed composition. The assay of these mixed microbial consortia by utilizing biochemical components eliminates the problems associated with cultural selectivity of viable counts or the requirement for quantitative release from surfaces required in some microscopic techniques. The assay of cellular components restricted to subsets of the microbial community gives insights into the community structure of the biofilm whereas assay of cellular components common to all cells can be used for biomass estimations. Formation of endogenous storage materials can be utilized as measures of nutritional status and rates of formation or turnover of specific "signatures" from labeled precursors can give insight into metabolic activities. Extracellular polymers involved in adhesion are also important components of biofilms. These analyses are destructive. They can now be correlated with non-destructive analyses by Fourier transforming infrared spectrometry which may provide real-time analyses of biofilm dynamics.

Key words : biochemical analysis, biomass, biofilms, community structure, FT/IR, metabolic activity, nutritional status, signature components.

RÉSUMÉ - Les bactéries en milieu naturel sont souvent concentrées sur des surfaces où elles existent en microcolonies de composition variée. L'étude de ces populations microbiennes mixtes par l'utilisation de composants biochimiques élimine les problèmes liés à la sélectivité des milieux de culture pour la numération des bactéries viables ou encore les problèmes liés à l'obligation de décrocher quantitativement les microorganismes fixés aux surfaces pour certaines techniques de microscopie. L'étude des composants cellulaires caractéristiques donne un aperçu sur la structure de la communauté du biofilm alors que l'étude des composants cellulaires communs à toutes les cellules peut servir, elle, à des estimations de biomasse. La formation de réserves endogènes peut être utilisée pour mesurer l'état nutritionnel. Le taux de formation ou turn-over de signatures spécifiques à partir de précurseurs marqués peut apporter des informations sur leurs activités métaboliques. Les polymères extracellulaires ayant un rôle dans l'adhésion sont également d'importants composants du biofilm. Mais leur analyse les détruit. Cependant elle peut maintenant être comparée aux analyses non destructives en spectrophotométrie infrarouge à transformée de Fourier, qui apporte des renseignements en temps réel sur la dynamique du biofilm.

Mots clés : analyses biochimiques, biomasse, biofilm, structure de la communauté, FT/IR, activité métabolique, état nutritionnel, marqueurs.

The problem

Microbes adherent to surfaces in biofilms present a complex problem for assay. The classical methods of microbiology involving the isolation and subsequent culturing of organisms lead to great underestimations of the total microbes present. Microscopic methods that require quantitative release of the bacteria from the biofilm can be shown

not to remove all cellular components from the surface. *In situ,* microscopic methods often fail because morphology in microbial biofilms may have little relationship to the metabolic function of the cells. The combination of autoradiography and microscopy however can provide a powerful tool in the study of biofilms.

Biochemical analysis

Our laboratory has been involved in the development of assays for biofilms of microbial consortia in which the bias of cultural selection of the classical plate count is eliminated. The total community is examined in these procedures and by using labeled precursors it is also possible to determine the metabolic activities of specific components in the microbial communities. The concept of "signatures" for subsets of the community based on the limited distribution of specific components has been validated by using antibiotics and cultural conditions to manipulate the community structure. The resulting changes agreed both morphologically and biochemically with the expected results (White *et al.*, 1980). Other validation experiments that involved isolation and analysis of specific organisms and finding them in appropriate mixtures, utilization of specific inhibitors and noting the response and changes in the local environment such as the light intensity are summarized in the review (White, 1983).

With these techniques, we have been able to show succession in marine biofouling films (Morrison *et al.*, 1977 ; Nickels *et al.*, 1981a), the effects of substratun biodegradability (Bobbie *et al.*, 1978), the effects of substratum microtopology (Nickels *et al.*, 1981b), the effects of mechanical disturbance (Nickels *et al.*, 1981c), the effects of amphipod grazing and resource partitioning (Morrison and White, 1980 ; Smith *et al.*, 1982a), the effects of sand dollar bioturbation and predation (Findlay and White, 1983a), the effects of essential elemental chelation (Nickels *et al.*, 1979), the effects of oil and gas well drilling fluids (Smith *et al.*, 1982b), the effects of light (Bobbie *et al.*, 1981) and the effects of epibenthic predation (Federle *et al.*, 1983) on microbial films.

In Table 1, the sensitivity of some of the methods by which biomass and community structure can be determined is listed. These methods utilize fluorimetric detection after high pressure liquid chromatography (HPLC) (phytanyl glycerol ether phospholipid assay) or detection by flame ionization after gas-liquid chromatography (GLC). With these techniques it is possible to detect 10^3 to 10^5 organisms the size of *E. coli.* The use of more sensitive assays based on derivatives that give strong responses in electron capture detection capillary GLC should increase the sensitivity of these assays.

The phospholipids are found in the membranes of all cells. The membrane phospholipids have a relatively rapid turnover so the assay of these lipids gives a measure of the "viable" cellular biomass (White *et al.*, 1979a). The phosphate of the phospholipids or the glycerol-phosphate and acid labile glycerol from phosphatidyl glycerol has been assayed (Gehron and White, 1983). The ester-linked phospholipid fatty acids are both the most sensitive and the most useful measures thus far developed (Bobbie and White, 1980). The usefulness of this assay has been greatly increased by the determination of the configuration and position of double bonds in monoenoic fatty acids (Nichols *et al.*, 1985). Samples from marine sediments often yield 300 fatty acids which give deep insight into the community structure as well as an estimate of the biomass. "Signatures" (components restricted to subsets of the microbial community with similar physiological functions) for some of the microbial groups involved in anaerobic fermentations have been developed.

The rate limiting step in fermentations is the degradation of polymers that is carried out by the anaerobic fermenters. A portion of these organisms contain plasmalogen phospho-

Component	Concentration nmoles/g dry wt.	Sensitivity moles	Equivalence (10^{12} *E. coli*/g dry wt.)
Extractable Phospholipid			
Phosphate	50	10^{-9}	2×10^{7}
Glycerol Phosphate	50	10^{-11}	2×10^{5}
Ester-linked Extractable Fatty Acids	100	3×10^{-13}	3×10^{3}
Muramic acid/glucosamine	14.5	3×10^{-13}	2×10^{4}
LPS-Lipid a hydroxy Fatty Acids	13.5	2×10^{-13}	1×10^{4}
Teichoic Acid Glycerol/Ribitol	341	10^{-11}	3×10^{4}
Diphytanyl Glycerol Ether	1.7	4×10^{-14}	4×10^{4}

Table 1 : Sensitivity of biomass measures in microbial biofilms.

lipids that are limited to this physiological class of anaerobes (Goldfine and Hagen, 1972). Plasmalogens can be essayed by their resistance to alkaline methanolysis and extreme sensitivity to mild acid (White *et al.*, 1979b). Other groups of anaerobic fermenters contain phosphosphingolipids with unusual sphingosine bases. These were described in *Bacteriodes* (Rizza *et al.*, 1970). Sphingosines are readily assayed in acid hydrolysates of the polar lipids by their amino groups or by GLC of the long chain bases (White *et al.*, 1969).

Phytanyl glycerol ethers found in the Archaebacteria can be assayed by HPLC after appropriate derivatization (Martz *et al.*, 1983). C. Mancuso in this laboratory has improved and resolution of the analysis of the diphytanylglycerol ether lipids of the methanogenic bacteria by HPLC. She has also been able to show the presence of isoprenologues of the aliphatic side chains of the ether lipids using highly sensitive GC/MS techniques with the aim of identifying the specific types of methanogenic bacteria.

The sulfate-reducing bacteria contain lipids which can be utilized to identify at least a portion of this class. They contain a unique profile of branched saturated and monounsaturated as well as hydroxy fatty acids in ester linkage to the phospholipids (Fredrickson, H., Ph. D. thesis, FSU). These organisms are active even in fermentations in which there is no added sulfate as they can recycle organic sulfur in the feed-stock (Smith and Klug, 1981).

From the residue of the lipid extracted biofilm muramic acid, a unique component of the bacterial cell wall can be recovered (Findlay *et al.*, 1983). Muramic acid in the bacterial cell wall exists in a 1:1 molar ratio with glucosamine. Since the analysis gives both glucosamine and muramic acid and the chitin walls of many microeukaryotes yield glucosamine, the glucosamine to muramic acid ratio gives insight into the prokaryote to eukaryote ratio. This complements the information developed from the ester-linked phospholipid fatty acids. Gram negative bacteria contain distinctive patterns of amide or ester linked hydroxy fatty acids in the lipid A of their lipopolysaccharide wall polymers (Parker *et al.*, 1982). This has proved to be an extremely valuable assay. Teichoic acid polymers such as the substituted poly-glycerol or ribitol phosphate esters are found in some gram positive bacteria (Gehron *et al.*, 1984). We have also developed assays for extracellular polysaccharide polymer based on the specific content of uronic acids (Fazio

et al., 1982). This assay has been utilized to show that poor growth conditions stimulate the formation of uronic acid containing exopolymers (Uhlinger and White, 1983) and their role in sediment stability (Nowell *et al.*, 1985).

The nutritional status of biofilms can be estimated by monitoring the proportions of specific endogenous storage compounds relative to the cellular biomass. The nutritional status of microeukaryotes (algae, fungi, or protozoa) in biofilms can be monitored by measuring the ratio of triglyceride glycerol to the cellular biomass (Gehron and White, 1982). Certain bacteria form the endogenous lipid poly-beta-hydroxy alkanoate (PHA) under conditions of nutritional stress (Nickels *et al.*, 1979). A more sensitive assay based on GLC of the components of the polymer showed the presence of 3-OH acids longer than 4-carbons in these polymers (Findlay and White, 1983b). The ratio of the rate of formation of phospholipid fatty acids to PHA has been shown to be an extraordinarily sensitive measure of the nutrient environment in the bacterial niche (Findlay and White, 1984 ; Findlay *et al.*, 1985). This ratio of incorporation can be utilized to measure the "disturbance artifact" involved in the application of labeled precursors to highly stratified environments such as sediments (Findlay *et al.*, 1985).

The analyses described above all involve the isolation of signature components of microbial biofilms. Since each of the components are isolated, the incorporation of labeled isotopes from precursors can be utilized to provide rates of synthesis or turnover. This has proved useful in the quantitative description of the effects of predation on detrital microbial biofilms (King *et al.*, 1977 ; Morrison and White, 1980). Analysis of signatures by GC/MS makes possible the utilization of mass labeled precursors that are non-radioactive, have specific activities approaching 100 %, include isotopic marker for nitrogen, and can be efficiently detected using the selective ion mode. The high specific activity makes possible the assay of critical reactions using substrate concentrations in the biofilms that are just above the natural levels. This is not possible with radioactive precursors. Improvements in analytical techniques have increased the sensitivity of this analysis. Utilizing a chiral derivative and fused silica capillary GLC with chemical ionization and negative ion detection of selected ions, it proved possible to detect 8pg (90 femtomoles) of D-alanine from the bacterial cell wall (the equivalent of 10^3 bacteria) the size of *E. coli* (Tunlid *et al.*, 1985). In this analysis it proved possible to reproducibly detect a 1 % enrichment of ^{15} N-D-alanine in the ^{14} N-D-alanine.

Non-destructive infrared analysis

The analysis of biofilms based on the isolation of chemical signatures is a destructive analysis and cannot be readily automated or utilized to give real time monitoring of biofilms. The possibility of utilizing a non-destructive technique to monitor the chemistry of living biofilms is now available in the Fourier transforming infrared spectrometers (FT/IR).

The infrared portion of the spectrum is extraordinarily rich in information regarding the vibrational and rotational motions of atoms in molecules. Not only specific infrared absorption be assigned to particular types of covalent bonds but the modifications of these bonds by the local electronic environment can be detected in the details of the spectra (Bellamy, 1958 ; Parker, 1971). The infrared spectrum of a compound has long been accepted as one of the best mondestructive absolute proofs of identity in organic chemistry.

One of the problems restricting the application of infrared spectroscopy has been that the atomic interactions sensed in the infrared portion of the spectrum are at relatively low

energies and the detection is relatively inefficient. This has precluded the full usage of the power of the analysis using complex materials isolated from the environment.

The advent of fast computers has made possible a new type of infrared spectral analysis. This has made it possible to utilize the far infrared portions of the spectrum, to follow rapid reaction rates with changes in spectral intensity, and to utilize different types of sample exposures such as the photoacoustic spectroscopy. The secret lies in the array processor computers that can perform Fourier transformations so rapidly that interference spectroscopy can be possible.

The FT/IR has several advantages over conventional IR spectroscopy.

1. The Fellgett advantage results from the fact that the entire spectrum passes through the sample during the entire analytical interval. The spectrum is generated by the interference between one portion of a split beam that is "retarded" in that it is reflected from a vibrating mirror. In this way the entire interferogram is allowed to impinge on the sample throughout the entire analytical interval. Conventional spectrophotometers create a beam that scans the sample with a series of wavelenghts. In these instruments the signal from each wavelength interval occurs for only a small portion of the analytical interval whereas the noise continues to be generated throughout the entire analytical interval. In the FT/IR both the signal and the noise occupy the entire analytical interval. Under the conditions of continuous analysis by the entire spectrum, during the time a signal of amplitude n is generated, the random noise of $n^{1/2}$ is also generated. This means that quadrupling the number of scans doubles the signal to noise ratio. This is the Fellgett advantage.

2. In the throughput or Jacquinot advantage the FT/IR utilizes the whole beam. Conventional spectrophotometers focus a narrow slit of light on the diffracting engine to create the analytical beam. Thus only a small portion of the light entering the monochromator is utilized in the analytical beam. The high throughput of the FT/IR means that a smaller beam can be utilized and thus a smaller portion of the sample can be analyzed. The beam of the Nicola 60 SX in our laboratory is 3 mm in diameter.

The combination of the Fellgett and Jacquinot advantages in the FT/IR gives an increase in the signal to noise ratio in the mid-IR spectral range of 2.4 orders of magnitude (Griffiths, 1975, 1983).

3. The signal generated by the FT/IR interferogram is actually produced in the time domain. To transform the signal into the frequency space in which spectra are usually perceived requires a Fourier transformation. Although this step prevented the utilization of interference spectroscopy until the advent of modern computers, it is actually an advantage. Since data described in Fourier space are in general expressed as successive approximations in terms of sines and phase (cosines) which are mathematically well behaved, simple mathematical manipulations on data in Fourier space can be utilized to correct for baseline shifts, overlapping, apodization, etc. that would require complex mathematical manipulations in frequency space if they were even possible.

4. For the interferograms to be transformed from Fourier to frequency domains the signal is digitized. This digitization is achieved using equal intervals of optical path difference using the sinusoidal interferogram from a laser beam focused on another part of the mirrors. The interferogram is digitized once per wavelength of the laser interferogram at the zero crossing which gives an extraordinary spectral resolution. Our instrument provides a spectral resolution that is continuously variable between 100 cm^{-1} and 0.25 cm^{-1}. The best conventional IR spectrometers formerly gave resolutions of about 5 cm^{-1}. The extremely high resolution greatly increases the information content of the

spectra. Unfortunately most of the standard spectral compilations have been made with conventional IR spectrophotometers and are only crude approximations of what can be achieved with the FT/IR instruments. The solution to this problem is just as it is in mass spectroscopy to generate reference spectra with authentic samples.

5. Since a large capacity computer is required to transform the signals this computer can also be utilized to manipulate the signals. The generation of difference spectra by electronically subtracting before and after treatments allows small differences to be detected. These small differences can be further increased by plotting derivatives of the spectra.

6. The high capacity of the array processors allow for 64,000 Fourier transforms/second. This makes possible the online monitoring of successive scans. The computer can be programmed to discard scans that are outside specific limits set by the spectroscopist. This facilitates the use of repetitive scanning that greatly increases the sensitivity of analyses.

7. The high signal to noise ratio allows measurement in the far infrared region (1000-10 cm^{-1}). The weak vibrations that involve heavy metallic atoms can now be included in the spectra.

Use of the FT/IR in microbial biofilm studies has been reviewed (Nichols *et al.* 1985).

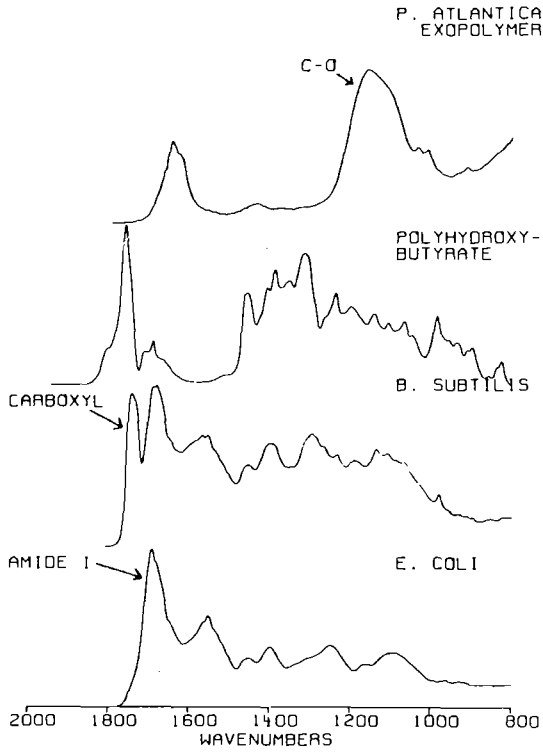

Figure 1 : Fourier transforming infrared spectra of washed and lyophilized cultures of *Pseudomonas atlantica*. poly-beta-hydroxy butyrate (alkanoate, PHA), *Bacillus subtilis*, and *Escherichia coli* (top to bottom) measured with the diffuse reflectance (DRIFT). The C-O bonds of the polysaccharides, the carbonyl of the PHA, and the amide I of the proteins are indicated.

CONCLUSIONS

The application of new analytical techniques have greatly increased the specificity and sensitivity of the destructive chemical analysis of biofilms for signature components that can provide insight into the biomass, community structure, nutritional status, and metabolic activities of the microbes and their extracellular products. The application of the non-destructive FT/IR to biofilms provides a second analytical tool that can provide real time estimates of some of these parameters. IR spectra of lyophilized biofilms measured with the diffuse reflectance cell show the presence of polysaccharide in *Pseudomonas atlantica,* and the PHA in *Bacillus subtilis* compared to *E. coli* (Figure 1).

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BELLAMY L.T., 1958. The infrared spectra of complex molecules, John Wiley and Sons, New York.

BOBBIE R.J., MORRISON S.J. and WHITE D.C., 1978. Effects of substrate biodegradability on the mass and activity of the associated estuarine microbiota. *Appl. Environ. Microbiol. 35 :* 179-184.

BOBBIE R.J. and WHITE D.C., 1980. Characterization of benthic microbiol community structure by high resolution gas chromatography of fatty acid methyl esters. *Appl. Environ. Microbiol. 39 :* 1212-1222.

BOBBIE R.J., NICKELS J.S., SMITH G.A., FAZIO S.D., FINDLAY R.H., DAVIS W.M. and WHITE D.C., 1981. Effect of light on the biomass and community structure of the estuarine detrital microbiota. *Appl. Environ. Microbiol. 42 :* 150-158.

FAZIO S.A., UHLINGER D.J., PARKER J.H. and WHITE D.C., 1982. Estimations of uronic acids as quantitative measures of extracellular polysaccharide and cell wall polymers from environmental samples. *Appl. Environ. Microbiol 43 :* 1151-1159.

FEDERLE T.W., LIVINGSTON R.J., MEETER D.A. and WHITE D.C., 1983. Modification of estuarine sedimentary microbiota by exclusion of top predators. *J. Exp. Marine Biol. Ecol. 73 :* 81-94.

FINDLAY R.H. and WHITE D.C., 1983a. The effects of feeding by the sand dollar *Mellita quinquiesperforata* on the benthic microbial community. *J. Exp. Mar. Biol. Ecol. 72 :* 25-41.

FINDLAY R.H. and WHITE D.C., 1983b. Polymeric beta-hydroxy-alkanoates from environmental samples and *Bacillus megaterium*. *Appl. Environ. Microbiol. 45 :* 71-78.

FINDLAY R.H. and WHITE D.C., 1984. *In situ* determination of metabolic activity in aquatic environments. *Microbiological Sciences :* 90-95

FINDLAY R.H., MORIARTY D.J.W. and WHITE D.C., 1983. Improved method of determining muramic acid from environmental samples. *Geomicrobiology J. 3 :* 133-150.

FINDLAY R.H., POLLARD P.C., MORIARTY D.J.W. and 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.

GEHRON M.J. and 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. Ecol. 64 :* 145-158.

GEHRON M.J. and WHITE D.C., 1983. Sensitive assay of phospholipid glycerol in environmental samples. *J. Microbiol. methods 1 :* 23-32.

GEHRON M.J., DAVIS J.D., SMITH G.A. and WHITE D.C., 1984. Determination of the gram-positive content of soils and sediments by analysis of teichoic acid components. *J. Microbiol Methods 2 :* 165-176.

GOLDFINE H. and HAGEN P.O., 1972. Bacterial plasmalogens p. 329-350. In E. Snyder (ed.) Ether lipids: chemistry and biology. Academic Press, New-York.

GRIFFITHS P.R., 1975. Chemical infrared Fourier transform spectroscopy, Wiley-Interscience, New York.

GRIFFITHS P.R., 1983. Fourier transform infrared spectroscopy. *Science 222*. 297-302.

KING J.D., D.C. WHITE and C.W. TAYLOR. 1977. Use of lipid composition and metabolism to examine structure and activity of estuarine detrital microflora. *Appl. Environ. Microbiol. 33*: 1177-1183.

MARTZ R.F., D.L. SEBACHER, and D.C. WHITE.,1983. Biomass measurement of methane-forming bacteria in environmental samples. *J. Microbiol. Methods 1*: 53-61.

MORRISON S.J. and D.C. WHITE., 1980. Effects of grazing by estuarine gammaridean amphipods on the microbiota of allochthonous detritus. *Appl. environ. Microbiol. 40*: 659-671.

MORRISON D.J., J.D. KING, R.J. BOBBIE D, R.E. BECHTOLD and D.C WHITE., 1977. Evidence of microfloral succession on allochthonous plant litter in Apalachicola Bay, Florida, *U.S.A. Marine Biology 41*: 229-240.

NICHOLS P.D., W.R. MAYBERRY C.P. ANTWORTH and D.C. WHITE., 1984. Determination of monounsatured double bond position and geometry in the cellular fatty acids of the pathogenic bacterium *Francisella tularensis*. *J. Clin. Microbiol. 61* : 738-740.

NICHOLS P.D., J.M. MENSON J.B. GUCKERT, D.E. NIVENS and D.C. WHITE, 1985. Fourier transform-infrared spectroscopic methods for microbial ecology : analysis of bacteria, bacteria-polymer mixtures and biofilms. *J. Microbiol. Methods. 9* : 79-94.

NICKELS J.S., R.J. BOBBIE KING and D.C. WHITE, 1979. Poly-betahydroxybutyrate metabolism as a measure of unbalanced growth of the estuarine detrital microbiota. *Appl. Environ. Microbiol. 37*: 459-465.

NICKELS, J.S., R.J. BOBBIE, D.F. LOTT, R.F. MARTZ, P.H. BENSON, and D.C. WHITE, 1981a. Effect of manual brush cleaning on the biomass and community structure of the microfouling film formed on aluminum and titanium surfaces exposed to rapidly flowing seawater. *Appl. Environ. Microbiol. 41*: 1442-1453.

NICKELS J.S., R.J. BOBBIE, R.F. MARTZ, G.A. SMITH, D.C. WHITE and N.L. RICHARDS, 1981b. Effect of silicate grain shape, structure and location on the biomass and community structure of colonizing marine microbiota. *Appl. environ. Microbiol. 41*:1262-1268.

NICKELS J.S., J.H. PARKER, R.J., BOBBIE, R.F. MARTZ, D.F. LOTT, P.H. BENSON, and D.C. WHITE., 1981c. Effect of cleaning with flow-driven brushes on the biomass and community composition of the marine microfouling film on aluminium and titanium surfaces. *International Biodeterioration Bulletin. 17*: 87-94..

NOWELL A.R.M., D. THISTLE, J.D. DAVIS, P.D. NICHOLS, M.B. TREXLER, and D.C. WHITE. 1985. The effects of bacterial mucous adhesion on the entrainment of sand. Sedimentology: in press.

PARKER F.S., 1971. Applications of infrared spectroscopy in biochemistry, biology and medicine, Plenum Press, New York.

PARKER, J.H., .A. SMITH H.L. FREDRICKSON, J.R. VESTAL, and D.C. WHITE, 1982. Sensitive assay, based on hydroxy-fatty acids from lipopolysaccharide lipid A for gram negative bacteria in sediments. *Appl. Environ. Microbiol. 44*: 1170-1177.

RIZZA B., A.N. TUCKER and D.C. WHITE, 1970. Lipids of *Bacteriodes melanogenicus*. *J. Bacteriol. 101*: 84-91.

SMITH G.A., J.S. NICKELS, W.M. DAVIS, R.F. MARTZ, R.H. FINDLAY, and D.C. WHITE, 1982a. Perturbations of the biomass, metabolic activity, and community structure of the estuarine detrital microbiota: resource partitioning by amphipod grazing. *J. Exp. Mar. Biol. Ecol. 64*: 125-143.

SMITH G.A., J.S. NICKELS, N.L. RICHARD, D.C. WHITE, 1982b. Effects of oil and gas well drilling fluids on the biomass and community structure of the microbiota that colonize sands in running seawater. *Arch, Environ. Contam. Toxicology 11*: 19-23.

SMITH R.L. and M.J. KLUG, 1981. Reduction of sulfur compounds in the sediments of a hypereutrophic lake sediment. *Appl. Environ. Microbiol. 47*: 5-11.

TUNLID A., G. ODHAM, R.H. FINDLAY and D.C. WHITE, 1984. Precision and sensitivity in the measurement of ^{15}N enrichment in D-alanine from bacterial cell walls using positive/negative ion mass spectrometry. *J. Microbiol. Methods 3*: 231-245.

UHLINGER D.J. and D.C. WHITE, 1983. Relationship between the physiological status and the formation of extracellular polysaccharide glycocalyx in *Pseudomonas atlantica*. *Appl. Environ. Microbiol. 45*: 64-70.

WHITE D.C., 1983. Analysis of microorganisms in terms of quantity and activity in natural environments. *In* microbes in their natural environments, J.H. Slater, R. Wittenbury and J. W. T. Wimpenny (editors). *Society for General Microbiology Symposium 34 :* 37-66.

WHITE D.C., A.N. TUCKER and C.C. SWEELEY, 1969. Characterization of the iso-branched sphinganines from the ceramide phospholipids of Bacteriodes melaninogenicus. *Biochim. Biophys. Acta 187.* : 527-532.

WHITE D.C., R.J. LIVINGSTON, R.J. BOBBIE and J.S. NICKELS, 1979a. Effects of surface composition, water column chemistry and time of exposure on the composition of the detrital microflora and associated macrofauna in Apalachicola Bay, Florida. *In* : Ecological Processes in Coastal and Marine Systems, R.J. Livingston, (ed). Plenum Press pp. 83-116.

WHITE D.C., W.M. DAVIS, J.S. NICKELS, J.D. KING and R.J. BOBBIE, 1979b. Determination of the sedimentary microbial biomass by extractible lipid phosphate. *Oecologia 40 :* 51-62.

WHITE D.C., R.J. BOBBIE, J.D. KING, J.S. NICKELS and P. AMOE, 1979c. Lipid anaylsis of sediments for microbial biomass and community structure. *In* : Methodology for Biomass Determinations and Microbiol Activities in sediments, ASTM STP 673, C.D. Litchfield and P.L. Seyfried, eds., American Society for testing and materials, pp 87-103.

WHITE D.C., R.J. BOBBIE, J.S. NICKELS, S.D. FAZIO and W.M. DAVIS, 1980. Nonselective biochemical methods for the determination of fungal mass and community structure in estuarine detrital microflora. *Botanica Marina 23* :239-250.