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The Importance of Measuring Microbial Enzymatic Functions While Assessing  
and Predicting Long-Term Anthropogenic Perturbations<sup>1</sup>

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The enzymes associated with marine and freshwater microorganisms are the principle catalyst for a large number of chemical transformations in Nature (Morita, 1982). Many of these transformations can **only** be mediated by microorganisms since the enzymes systems required for these reactions are not found in higher organisms. Thus microbial function is the key component of both inorganic and organic nutrient cycling in these environments. In pollution **impact** research, it is therefore imperative that both the fate and effect of the pollutant be studied in terms of changes in microbial enzyme systems. This is an approach that has not generally been followed in pollution fate and effects studies.

Although there have been a large number of impact studies conducted in the past, it hasn't been until recently that microbiological variables have been considered except in a relatively superficial way. The studies that have been conducted have generally focused on shifts in microbial populations using either plate counts on specialized agar plates or have involved the isolation of bacterial strains which are subsequently analyzed for biochemical characteristics. This approach does provide limited information about microbial population shifts but this technique is inherently very selective and therefore is often of little use in determining actual shifts in in situ biological function.

The reason microbial functions have generally been ignored in impact studies can be traced to two factors. The relative importance of microbial processes in the marine environment has not been realized until recently and the techniques have not been available to measure many key microbial processes. This situation has changed dramatically in the last few years. A large number of studies have shown the importance of microorganisms in inorganic nutrient mineralization (Nixon et al., 1980; Raine & Patching, 1980;

Zeitzschel, 1980; Fisher et al., 1982), nitrogen fixation (Capone et al., 1979; Teal et al., 1979), sulfate reduction (Howarth & Teal, 1979; Sørensen et al., 1979) and carbon cycling through the **detrital** food chain (Fenchel and Jørgensen, 1977; Sibert & Naiman, 1980; Newell & Lucas, 1981; Koop et al., 1982). In short, microbial mediated processes are an important feature of **all** major cycles within marine and aquatic environments.

The techniques are now adaptable to measure most of these processes in the field. In addition, techniques are also available to measure microbial variables such as bacterial biomass (Watson et al., 1977; Fuhrman, 1981), bacterial growth rates (Meyer-Reil, 1977; Hagström et al., 1979; Karl, 1981), relative microbial heterotrophic activity (Wright & Hobbie, 1966), the in situ rate of organic nutrient utilization (Crawford et al., 1974; Wright & Shah, 1977; Bell, 1980), total respiration rates, percent respiring cells (Meyer-Reil, 1978; Zimmerman et al. 1978) and the physiological condition of the microbial community (Wiebe & Bancroft, 1975).

There have been a few studies where these and other techniques have been applied to problems of marine pollution with interesting results. A few examples are as follows. Jones & Hood (1980) observed an inhibitory effect of organophosphorous pesticides on ammonia oxidation. Ramano and Daumas (1981) reported a depression in **adenosine nucleotide "energy charge"** in seawater contaminated with sewage. Albright et al. (1972) observed altered glucose uptake kinetics in aquatic samples treated with various metal **salts**. Similar changes were also observed in an **estuarine** system by Goulter et al. (1979, 1980) and by Gillespie & Vaccaro (1981) in seawater. Gillespie & Vaccaro (1981) also observed altered glucose uptake kinetics in seawater exposed to petroleum hydrocarbons. In a related study, similar observations were made by Hodson et al. (1977). Knowles & Wishart (1977) reported that nitrogen

fixation in arctic marine sediments was inhibited by an aromatic hydrocarbon. McKinley et al. (1982) reported that petroleum hydrocarbons inhibited **lignin** mineralization in an arctic lake.

Although the above studies are but a few that have been conducted, they are representative of the types of studies that have been reported. In **all** cases, changes in microbial processes were observed in natural samples that had been exposed to a polluting agent. Unfortunately, all of these studies were quite limited in scope; i.e., few microbial processes were measured, there were few studies where changes in sediments were considered, and the duration of the experiments was relatively short. Although these studies have provided valuable information about potential impacts, this piecemeal approach **has** not produced an overall evaluation of the potential impact of **any** polluting agent on basic microbial mediated cycles in sediments. To my knowledge, **the** most comprehensive pollution effects study to date was conducted in enclosed marine waters during the **CEPEX** project (Menzel & Case, 1977).

The studies outlined below were an attempt to quantify the impact of *a* pollutant (crude oil) on the microbial activities associated with major nutrient cycling in arctic and subarctic sediments. In addition, an assessment was made concerning the potential impact of this pollutant in a perspective lease area for offshore drilling. The general approach taken and a brief summary of the results are described to illustrate the feasibility of this approach' in application to other pollution impact studies.

The procedures used in the long-term effects study and the results are reported elsewhere (**Griffiths et al., 1981, 1982b, c**). Briefly, the procedure involved mixing crude oil with **subtidal** sediments. The sediments were **placed** in containers and returned to the original collection site by scuba divers.

At various intervals, the sediments were retrieved and analyzed. The crude oil effects study was conducted using **fine-grained subtidal** sediments because microbial processes in these sediments are known to be important to the overall biological productivity in the inshore environment and because this is a possible deposition site for crude oil. The conditions for incubating the , perturbed sediments were chosen to reduce errors induced by the incubation conditions and handling prior to **and** during the analyses. The variables chosen for study were those that are related to important microbial processes in the inshore environment. The duration of the study was 1.5 years which was long enough to determine when the maximum effect occurred but was barely adequate for estimating the total duration of the effect at higher crude oil concentrations. The results of these studies clearly show that long-term effects studies may have to be conducted for several years so that the dynamics of the perturbed **system** can be accurately assessed.

*no review*

The sediment samples were treated with various concentrations of both fresh and weathered crude oil and the chemical composition of these oils was determined before and after the incubation period by glass capillary gas chromatography-mass spectrometry (**Griffiths and Morita, 1981**). In this way, the investigators were **able to follow** the degradative process and related changes in microbial function with chemical changes in crude oil.

The results of the study showed that important microbial functions relating to inorganic nutrient mineralization and the dynamics of the **detrital** food chain were adversely affected by the presence of crude oil at concentrations that have been observed under actual spill conditions. **The** observed changes included reduced bacterial biomass production, **infaunal** burrowing activity, and total **adenylate** concentrations (**Griffiths et al., 1981**); reduced nitrogen fixation and **denitrification** rates and lower redox

potentials (Griffiths et al., 1982b); reduced activity of the enzymes that **hydrolase** structural **polysaccharides** and reduced phosphatase activity (Griffiths et al., 1982c). The transformations that were found to be affected by the presence of crude **oil** are summarized in the simplified nutrient cycling diagram given in Figure 1. The numbered transformations are those **in** which bacterial enzyme systems play a dominant role. Each reaction that was studied in detail showed that the presence of crude oil interfered with the transformation. Although transformations 1, 2, 3, 6, and 11 in Fig. 1 were not studied, **it** is very possible that these were also affected. Recent observations made with marine ammonia oxidizing bacteria in our laboratory indicate that at least one of these transformations (Fig. 1, transformation number 2) is inhibited by aromatic hydrocarbons.

Once the effects studies were completed, predictions **could** be made concerning **the** qualitative and quantitative changes **in** microbial processes that could be anticipated in the event of an oil spill. The investigators were faced with the task of determining which areas within the southeastern Bering Sea were potentially the most sensitive to crude oil perturbation. In designing the experimental protocol, it was assumed that areas which showed elevated heterotrophic activity would also be the areas where mineralization rates and microbial involvement **in** the **detrital** food chain would be the greatest. Rates of glucose and **glutamic** acid uptake and respiration and CO<sub>2</sub> production rates were measured as indicators of microbial activity (Griffiths et al., 1982a). Measurements of **phosphatase** activity were made as a relative indicator of total microbial concentrations and **arylsulfatase** activity as a relative indicator of marine bacterial numbers. Since nitrogen fixation rates were shown to **be** particularly sensitive to crude **oil** perturbation, these rates were **also** measured. These effects studies also showed that crude oil altered

the activity of a number of hydrolyses. For that reason, the activity of the following hydrolyses were also measured; **cellulase, amylase, laminarinase,** protease and **xylanase**. After three cruises in this area, it was concluded from the data that at **least** one major bay along the north coast of the Alaskan Peninsula (Port **Moller**) was potentially very sensitive to crude oil perturbation.

There was another area within this region which was of particular interest; the Saint George **Basin** (southeast of the **Pribilof** Islands). This region is a particularly important Alaskan fishery (**Bakkala**, 1981; Otto, 1981) and has been identified as an area where the **detrital** food chain is a dominant feature of the system (**Goering & Iverson**, 1981). It is generally assumed that the **high** fisheries productivity is closely tied to the **detrital** food chain. From the long-term effects studies, it would **follow** that extensive contamination of St. George Basin sediments would result in a significant reduction in this region's fisheries productivity. This **would** be due, in part, to the anticipated reduction **in** bacterial biomass production. The result of the survey showed that all of the microbial variables studied were elevated in a relatively small area within the St. George Basin; a roughly circular area with a radius of 22 km (**Griffiths et al.**, 1982a). With the information provided by both the long-term effects and Bering Sea microbial activity studies and information about probable crude **oil** trajectories and sedimentation rates, it is possible to predict the potential **impact** of crude **oil spills** in the region and to make plans to avoid contamination of the most sensitive area with respect **to** microbial processes within the St. George Basin. One of the keys to the utility of the Bering Sea survey was the information provided by the long-term effects study. Without such information, the investigators would not have known which variables **to** study

and the significance of the data relative **to** the potential impact of crude oil.

Long-term effects studies also have important application in evaluating the effects of a polluting agent after **it** has been introduced into the environment by providing information about which microbial processes are the most sensitive to the perturbing agent, which concentrations have an effect and the duration of the effect. A **field** study designed **to** evaluate the impact of a polluting event could be conducted in the following manner. Chemists would **sample** the impacted area to determine where the polluting agent is located and at what concentrations. Once this was established, the microbiologists would measure key microbial variables which were previously identified in long-term experiments. These variables would be measured both inside and outside the impacted area as defined by the chemical observations.

In conclusion, the fate and effects of any pollutant on freshwater and marine environments are going to depend to a **large** degree on the concomitant changes that take place **in** microbial enzyme systems. For the most part, these enzymes and their function have been ignored in pollution effects studies. I have described a series of studies that have been conducted on the long-term effects of crude oil on functions that are principally mediated by microbial enzymes. Some of the key features of these studies were the following: (1) measurements were made of microbial functions (enzymatic reactions) that are key components of major inorganic and organic nutrient cycles (2) the studies were of sufficient duration that both the initial impact and the rate of recovery could be estimated (3) attempts were made to determine the fate of crude oil since this is central to understanding which functions will be affected and the duration of the effect (4) the results of the long-term effects studies were used to design appropriate field surveys which could be used to assess potential impacts in other geographical areas.

It is imperative that this approach be taken *in* future pollution effects studies. Without this information, it is impossible to **fully** assess the impact of any polluting agent on biological productivity and the chemistry of the impacted area.

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## Figure Legend

Figure 1. Simplified diagram of nutrient cycling in the marine environment. The transformations that are crossed are those in which crude **oil** has an adverse effect. The numbered transformations are those that are primarily mediated by bacterial enzyme systems.

