APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 1990, p. 3895–3896
0099-2240/90/123895-02$02.00/0

Vol. 56, No. 12

Sheen Screen, a Miniaturized Most-Probable-Number Method for Enumeration of Oil-Degrading Microorganisms

EDWARD J. BROWN* AND JOAN F. BRADDOCK
Water Research Center, University of Alaska Fairbanks, Fairbanks, Alaska 99775

Received 29 May 1990/Accepted 21 September 1990

Sheen Screen is a miniaturized method for enumerating oil-degrading microorganisms. The technique relies on the ability of oil-degrading microorganisms to emulsify oil when provided as a sole carbon source in 24-well tissue culture plates. Sediments that actively respire hydrocarbons have high numbers of Sheen Screen-positive microorganisms.

Survey-type assessments for hydrocarbon-degrading microorganisms in marine sediments off Alaska were suggested over a decade ago (3) when development of offshore oil deposits with concomitant oil contamination incidents seemed imminent. However, oil and gas production facilities on Alaska's outer continental shelf have not been built; thus, environmental assessment studies, including microbiology, have been limited. The EXXON *Valdez* oil spill which occurred in March of 1989 was not related to outer continental shelf oil exploration or production but did occur offshore Alaska. A rapid-response assessment program was conducted by the National Atmospheric and Oceanographic Administration shortly after the spill. Part of that program and a follow-up program conducted by the state of Alaska included most-probable-number (MPN) measurements of oil-degrading microorganisms in several hundred sediment and water samples throughout southcentral coastal Alaska.

While no technique to enumerate specific metabolic types of microorganisms in marine systems is absolute, the MPN technique can give consistent results that are appropriate for relative comparisons among sampling sites. Walker and Colwell (5) compared various methods for enumerating petroleum-degrading microorganisms, and later Roubal and Atlas (3) developed the ^{14}C-labeled hydrocarbon-spiked crude oil MPN. More recently, improved medium formulations for plate counts have been suggested (1, 2), or, when oil

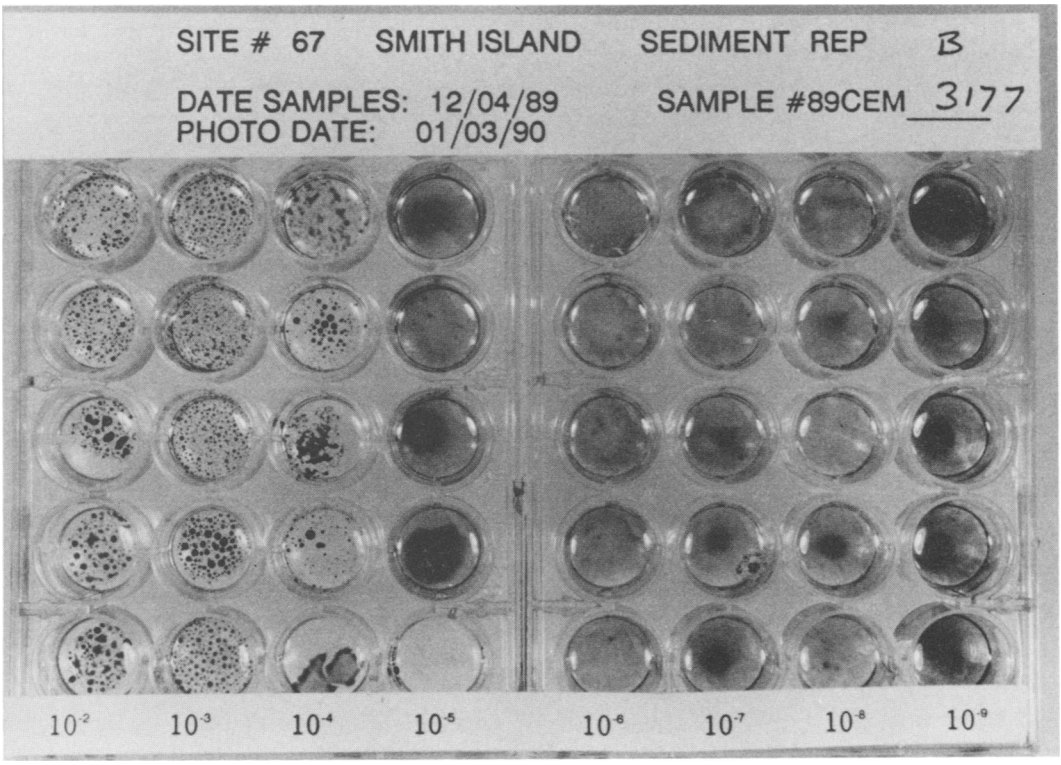

FIG. 1. Illustration of a five-tube MPN result for a marine sediment sample by the Sheen Screen method. Dilutions 10^{-2} and 10^{-3} are all positive for crude oil emulsification, four of five of the 10^{-4} dilution are scored positive, and one of five of the 10^{-5} dilution are scored positive.

* Corresponding author.

is used as a sole carbon source, turbidity in microtiter plates (2) and oxygen consumption in test tubes (4) have been used for MPN determinations of hydrocarbon-degrading microorganisms.

Faced with enumerating hydrocarbon-degrading microorganisms in several hundred samples while at sea for 59 days with only two opportunities to resupply materials, we developed the following Sheen Screen miniaturized MPN method.

The Sheen Screen procedure calls for inoculation of five 100-μl aliquots (for a five-"tube" MPN) of each serially diluted sample into sterile 24-well microtiter plates containing approximately 1.75 ml of sterile Bushnell-Hass marine mineral salts broth (Difco Laboratories) per well. Following inoculation, a sheen of sterile Prudhoe Bay crude oil is applied to each well, using a syringe fitted with a 26-gauge needle or approximately 5 μl from a positive-displacement syringe. For this study each microtiter plate was incubated at 16 ± 2°C for 3 weeks following inoculation. Wells are scored as positive when oil emulsification is clearly indicated by disruption of the oil sheen (Fig. 1). In our study, the first row (columns of six wells) was used to make the serial dilutions by placing 0.2 ml of sample into 1.8 ml of broth. The five remaining rows in each of the four columns were inoculated by repeatedly pipetting 100 μl from the dilution well with a multichannel pipetting device. Thus each plate had just enough wells for a five-tube MPN of four serial dilutions (Fig. 1). More plates can be used to extend the dilution series, or three-tube MPNs can be done for six dilutions with a single plate.

The Sheen Screen method was used along with radiorespirometric activity measurements of various hydrocarbons in Prince William Sound sediment samples (E. J. Brown, Abstr. Annu. Meet. Am. Soc. Microbiol. 1990, Special Symposium, Session 52, p. xxix). Aliquots, 10 ml, of 1:10 sediment dilutions were spiked with 100 μg of ^{14}C-labeled hexadecane, naphthalene, benzene, or phenanthrene, and five 100-μl aliquots of these and further dilutions were assayed by the Sheen Screen method. Those sediments that actively respired the added hydrocarbons had high numbers of Sheen Screen-positive microorganisms, confirming that emulsification of oil is a good indicator of hydrocarbon-degrading microorganisms.

The method can be modified to use oily substrates other than crude oil for MPN analysis as well as providing a means for enrichment and selection of microorganisms capable of growth on and surfactant production in oily substrates.

This work was supported by National Oceanographic and Atmospheric Administration contract 50-DSNC-8-00141.

Jon Lindstrom, Mike Brockman, and Tom Yeager made various useful suggestions for development and for application of this method.

LITERATURE CITED

1. **Guerin, W. F., and G. E. Jones.** 1989. Estuarine ecology of phenanthrene degrading bacteria. Estaurine Coastal Shelf Sci. **29**:115–130.
2. **Heitkamp, M. A., and C. E. Cerniglia.** 1988. Mineralization of polycyclic aromatic hydrocarbons by a bacterium isolated from sediment below an oil field. Appl. Environ. Microbiol. **54**:1612–1614.
3. **Roubal, G., and R. M. Atlas.** 1978. Distribution of hydrocarbon-utilizing microorganisms and hydrocarbon biodegradation potentials in Alaskan continental shelf areas. Appl. Environ. Microbiol. **35**:897–905.
4. **Song, H.-G., and R. Bartha.** 1990. Effects of jet fuel spills on the microbial community of soil. Appl. Environ. Microbiol. **56**:646–651.
5. **Walker, J. D., and R. R. Colwell.** 1976. Enumeration of petroleum-degrading microorganisms. Appl. Environ. Microbiol. **31**: 198–207.