METABOLIC RESPONSE OF ENVIRONMENTALLY ISOLATED MICROORGANISMS TO INDUSTRIAL EFFLUENTS: USE OF A NEWLY DESCRIBED CELL CULTURE ASSAY

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Prepared By: Robert N. Ferebee, Ph.D.

Academic Rank: Associate Professor

University and Department: University of Houston - Clear Lake
Department of Biological
And Environmental Sciences
Houston, Texas 77058

NASA/JSC

Directorate: Space and Life Sciences

Division: Medical Sciences

Branch: Biomedical Operations and Research

JSC Colleague: Duane L. Pierson, Ph.D.

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Contact Number:

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Robert N. Ferebee, Ph.D.
University of Houston-Clear Lake
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Duane L. Pierson, ph.D.
Biomedical Research and Operations Division

Abstract of Proposed Work Plan

An environmental application using a microtiter culture assay to measure the metabolic sensitivity of microorganisms to petrochemical effluents will be tested.

The Biomedical Operations and Research Branch at NASA JSC has recently developed a rapid and nondestructive method to measure cell growth and metabolism. Using a colorimetric procedure the uniquely modified assay allows the metabolic kinetics of prokaryotic and eukaryotic cells to be measured. Use of such an assay if adapted for the routine monitoring of waste products, process effluents, and environmentally hazardous substances may prove to be invaluable to the industrial community.

The microtiter method as described will be tested using microorganisms isolated from the Galveston Bay aquatic habitat. The microbial isolates will be identified prior to testing using the automated systems available at JSC. Sodium dodecyl sulfate (SDS), cadmium, and lead will provide control toxic chemicals. The toxicity of industrial effluent from two industrial sites will be tested.

An effort will be made to test the efficacy of this assay for measuring toxicity in a mixed culture community.

Metabolic Response of Environmentally Isolated Microorganisms to Industrial Effluents:Use of a Newly Described Cell Culture Assay.

Introduction:

Bioassays performed upon industrial process effluents continue to present problems related to time, difficulty in maintaining the animal species used, and the expense of such procedures. While this study does in no way suggest that bioassays using higer life forms be discontinued it does suggest there is a need to develop a rapid screening procedure that allows a daily monitoring of potentially toxic effluents.

Use of a recent modification of the tetrazolium reduction assay that allows simultaneous solubilization of the end product formazan indicated that the method could be used as a rapid screening assay for industrial wastes. This procedure not only allows one to detect changes in the physicochemical nature of effluents, but also provides a method to quickly screen for microorganisms that would be sensitive to toxic substances.

This research was designed to measure the effects that industrial effluents taken from three separate industrial process plants had on actively growing bacteria. The treated effluents from each of these industries are ultimately released into the Galveston Bay estuary system. Each of the industries were eager to cooperate in supplying samples for these studies.

Materials and Methods:

Test Organisms ; Nine separate bacterial genera were collected in grab samples taken form selected sites on Galveston Bay - Texas. The bacteria were identified to species using both " Vitek" (Vitek Systems Inc., Hazelwood, Mo.) and " Biolog" (Biolog Inc., Haywood, Ca.), computer assisted, automated systems. These environmental isolates were tested in the presence of control chemicals of known toxicity as well as both treated and untreated (raw) industrial effluents. Of the bacteria tested Staphylococcus hominis and Pseudomonas putida showed greatest promise as indicator species and were used in subsequent tests. These same organisms were obtained from stock culture collections (Chrisope Technologies Lake Charles,LA) ; the sensitivity of these bacteria were compared to that of the environmental isolates.

Chemicals; Chemicals for toxic controls were obtained from Mallinckrodt Chemical Works St.Louis ,Mo.. Cadmium Chloride, Mercuric Sulfate , Lead Acetate, and Sodium Dodecyl Sulfate were all tested . Cadmium chloride gave the most consistent results with all the bacteria tested and was used for subsequent testing. Chemical for the tetrazolium assay were obtained from Sigma Chemical Co., St.Louis, Mo. Tetrazolium 1-(4,5- dimethylthiazol-2-yl)- 3,5 - diphenylformazan (MTT formazan), 2-phenazine methosulfate (PMS), and t-octylphenoxypolythoxyethanol (Triton X-100) were used in the assay for bacterial growth.

Culture media were obtained from BBL Microbiological Systems (Cockeyville, Md.). Bacteria were maintained on standard methods agar while the media used for microtiter culture was R2A broth.

Tetrazolium Assays: Testing for the bacterial activity was measured by reduction of MTT spectrophotometrically at 390nm minus 650nm. Test bacteria were removed from 18 hour cultures by sterile cotton swabs and suspended in filter sterilized deionized water. Cell suspensions were adjusted to OD 590 nm = 0.6 cell numbers. This density gave $2x10^9$ cells/ml. From this suspension 25 ul were added to 50 ul of double strength R2A broth in 96 well flat bottomed microtiter culture plates. All wells except cell controls received 50 ul known toxicants or 50 ul treated or raw effluent. Indicator chemicals were finally added as follows: 15 ul of 1.2 mM MTT, 10 ul of 0.06 mM PMS, and 25 ul of 160 mM Triton X-100. All controls and tests were set as replicates of four wells.Cultures were maintained at 30C and continually monitored at 590nm - 650nm for a maximum of two hours. The microtiter cultures were read using a microtiter plate reader (Molecular Device Co. Menlo Park, CA).

Results:

Each of the environmental bacteria as well as those obtained from stock collections exhibited an expected response when toxic chemicals were added to the cultures. There was a progressive inhibition of growth as the concentrations of the chemicals were increased, (Fig 1 & 2). The cadmium chloride gave the most consistent response and was used as the toxicant control (Fig 1&2). None of the chemicals tested caused any reduction of MTT; these represented chemical controls. When the bacteria selected for indicators were tested with industrial effluents only a single sample gave a significant change in bacterial growth and MTT reduction (figure 1 & 2). The sample identified as effluent (2R) did indicate toxicity; further testing of this effluent showed a pH of 2.8 could account for the reduction in growth. When this sample was buffered to pH 7.0± 0.5 the toxicity was reversed. Staphylococcus hominis cultures were not effected by any of the effluent samples; while the Pseudomonas putida indicated changes that were minimum.

Discussion:

This new procedure for rapid reduction of tetrazolium allows for a rapid assay with good sensitivity and processing of large numbers of samples. The procedure offers a simple procedure that can easily be adapted for industrial operations. As testing continues there will probably be other microbial indicators identified that may be as good for screening as the ones used in this study. It is deemed somewhat important to choose environmental isolates that are part of the resident population.
Many of these resident organisms represent members of the natural

bioremediation/biodegradation community that ecosystems have become dependent upon for recycling, etc;. As is suggested by this study it may be beneficial to name more that a single indicator genera or even different species. Some of the preliminary results suggested that some of the chemicals will enhance the growth of certain bacteria. This was true in the case of sodium dodecyl sulfate (SDS) as a control toxicant. This type of activity indicate the eutrophic activity of many chemicals and only supports the value of this procedure in the testing of effluents.

TABLE 1: BACTERIAL ISOLATES COLLECTED FROM GALVESTON BAY - TEXAS

Acinetobacter calcaaceticus

Aeromonas veronii

Alcaligenes faecalis

Bacillus coagulans

Enterobacter cloacae

Pseudomonas putida

Serratia marcesens

Staphylococcus hominis

Vibrio metschnikovii

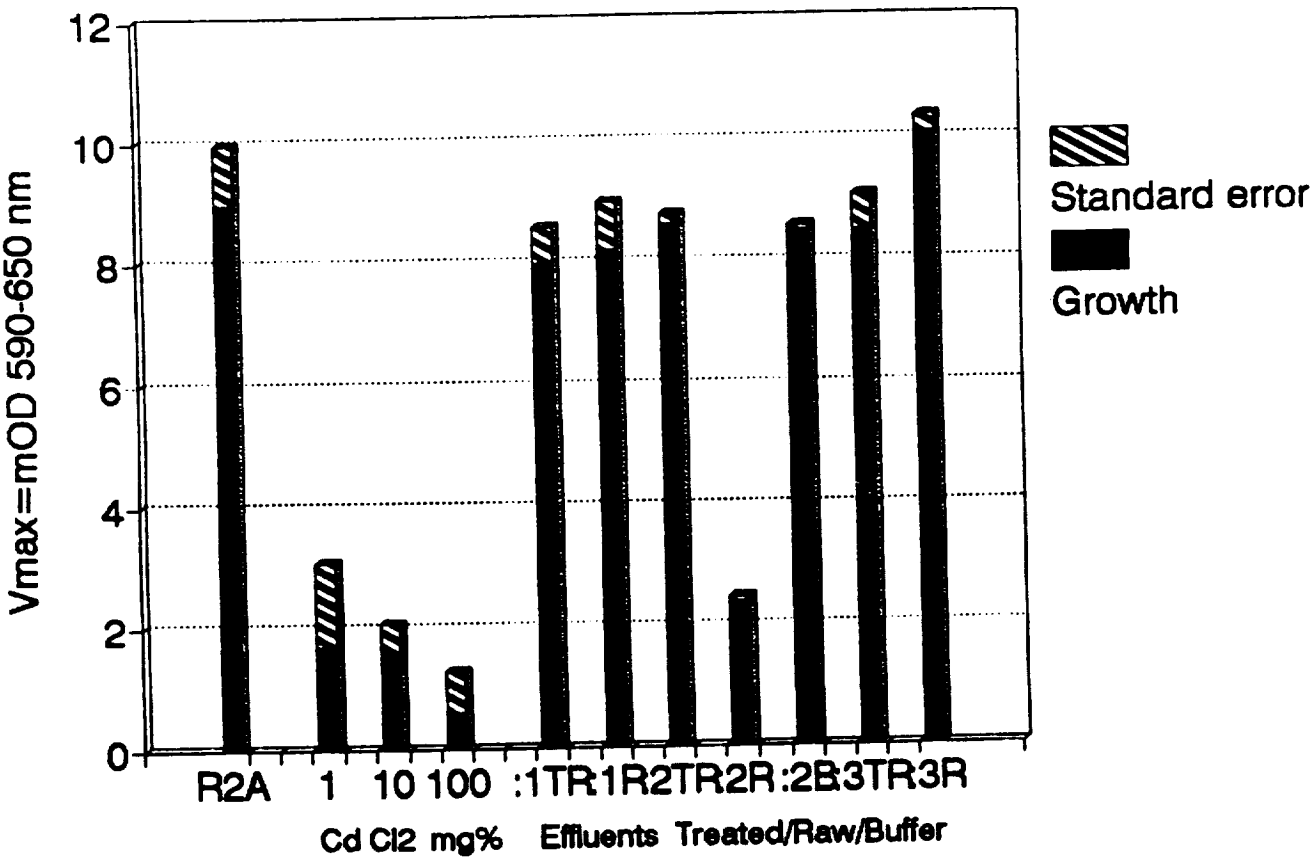

Figure1: Staphylococcus hominis
Growth in Industrial Effluent

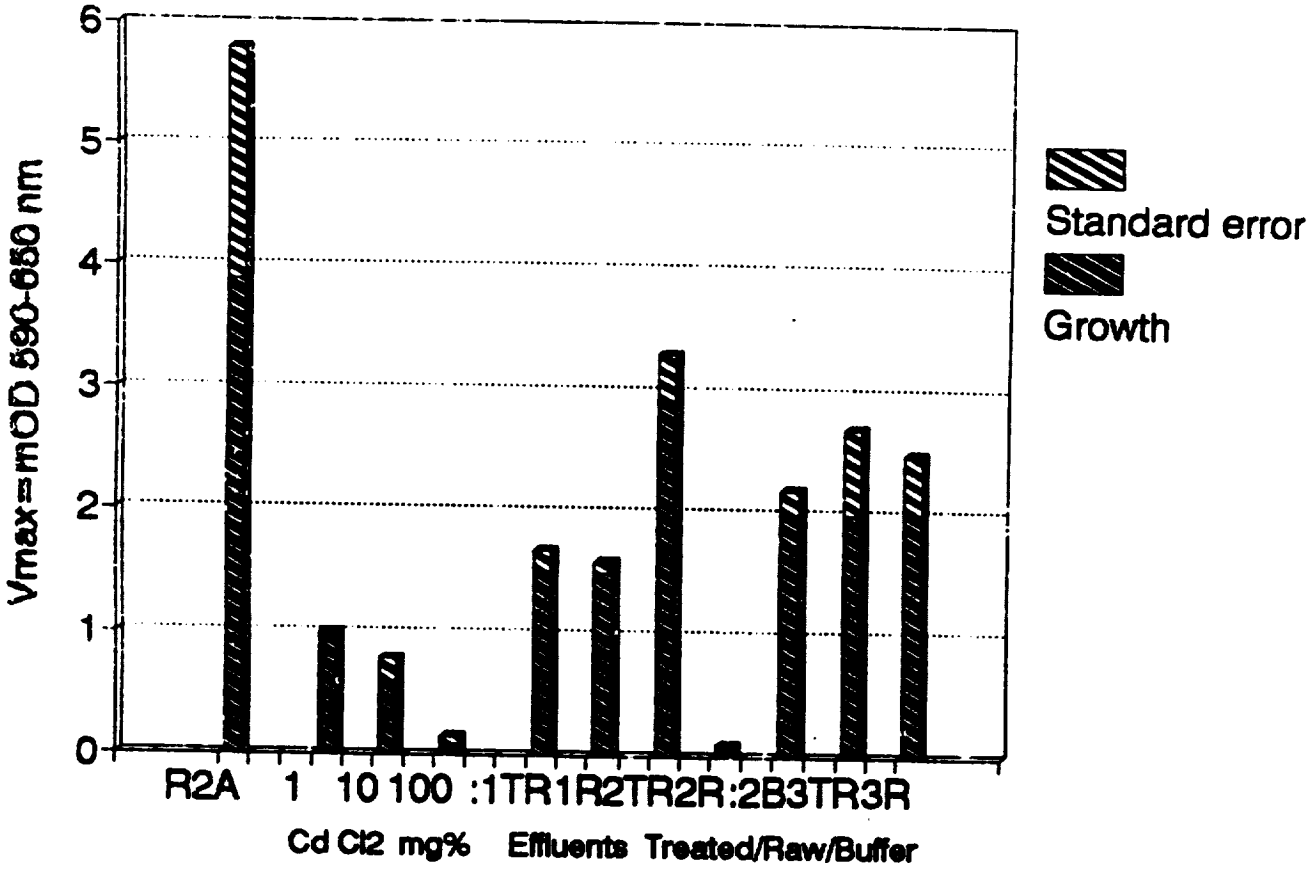

Figure2:Pseudomonas putida
Growth in Industrial Effluent

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