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CEER-PT-29

MICROBIAL SUCCESSION IN SLUDGE COMPOSTING

GRANT REQUEST TO UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

TERRESTRIAL ECOLOGY DIVISION  
CENTER FOR ENERGY AND ENVIRONMENT RESEARCH  
AND  
DEPARTMENT OF MICROBIOLOGY  
SCHOOL OF MEDICINE  
UNIVERSITY OF PUERTO RICO  
May, 1979



CENTER FOR ENERGY AND ENVIRONMENT RESEARCH  
UNIVERSITY OF PUERTO RICO — U.S. DEPARTMENT OF ENERGY

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May, 1979

Executive Summary:

Microbial Succession in Sludge Composting

Prepared by:

Terrestrial Ecology Division  
Center for Energy & Environ-  
ment Research  
Caparra Heights Station  
San Juan, Puerto Rico 00935

Project sites:

Center for Energy & Environ-  
ment Research  
Caparra Heights Station  
San Juan, Puerto Rico 00935

&

Department of Microbiology  
School of Medicine  
University of Puerto Rico  
Medical Sciences Campus,  
Caparra Heights Station  
San Juan, Puerto Rico 00935

Telephone:

(809) 767-0334

Total funding requested:

\$ 79, 686

Total budget period funding requested:

\$ 53,093

This project is an experimental evaluation of sewage sludge composting using bagass (cellulose-rich sugar cane processing wastes) or dried tropical grasses for bulking agent and the U.S.D.A. developed static pile thermophilic aerobic composting method for domestic waste sludges generated by Puerto Rican sewage treatment plants.

In particular, the relative populations of micro flora responsible for waste sludge degradation will be followed during aerobic composting. The project will serve as a field trial for use of f-2 bacteriophage population decline as a public health criterion for the elimination of primary and secondary microbial pathogens conventionally associated with waste sewage sludge.

The efficacy of static pile composting for elimination of parasites and parasites eggs will also be evaluated. Test results will measure kill, inactivation or elimination effectiveness of the static pile system on eggs from Schistosoma mansoni and other parasites determined to have survived the secondary contact treatment provided in Puerto Rican sewage treatment plants.

Development of this project has received the support of the University of Puerto Rico, Center for Energy and Environment Research, the School of Medicine, the Puerto Rico Aqueducts and Sewers Authority and the United States Department of Energy. The University of Puerto Rico is a non-profit institution of higher learning which has formally qualified for funding under minority instituteion support programs of the United States Federal Government.

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U.S. ENVIRONMENTAL PROTECTION AGENCY		EPA USE ONLY	
APPLICATION FOR FEDERAL ASSISTANCE		EPA PROJECT CONTROL NO.	DATE RECEIVED
		FORMER FEDERAL NO. (if any)	P.E. NUMBER
<b>PART I. GENERAL INFORMATION</b>			
<b>1. APPLICANT</b>			
4. NAME Arthur McBride Block/Ctr. Ener. Envir. Res.		d. ADDRESS (Street or P.O. Box Number, Town/City, State and Zip Code) Center for Energy & Envir. Research	
5. DEPARTMENT/DIVISION Terrestrial Ecology/ Health Impact		Div. Terrestrial Ecology Cap. Hqts Sta., Rio Piedras, PR 00935	
c. CONGRESSIONAL DISTRICT Puerto Rico		e. COUNTY Río Piedras	
2. PROJECT TITLE Microbial Succession in Sludge Composting			
3. CATALOG NUMBER AND TITLE OF EPA PROGRAM(S) TO WHICH THIS APPLICATION IS DIRECTED RD-680 Minority Institutes Research Support Program			
4. TOTAL COST FOR PROJECT PERIOD \$ 79,686		5. TOTAL BUDGET PERIOD COST OF BUDGET \$52,097	
6. TOTAL EPA SHARE REQUESTED FOR BUDGET PERIOD \$47,997			
7. PROJECT PERIOD FROM: Oct. 1, 1979 TO: Apr. 1, 1981		8. BUDGET PERIOD FROM: Oct. 1, 1979 TO: Sept. 30, 1980	
9. TYPE OF APPLICANT (Select appropriate type from instructions) Federally funded research & development center.		10. FEDERAL EMPLOYER IDENTIFICATION NUMBER U.S. Department of Energy Contract Contract No. EY-76-C-05-1833	
11. TYPE OF GRANT			
a. NEW (Was one application assistance received?) <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO		b. CONTINUATION (Current grant number) N/A	
c. RENEWAL (Prior grant number) NA		d. AMENDMENT N/A	
12. PROJECT LOCATION		b. COUNTY	
a. STATE Puerto Rico		Cupey Bajo and Río Piedras	
c. MUNICIPALITY San Juan and Trujillo Alto		d. CONGRESSIONAL DISTRICT Puerto Rico	
13. PROJECT AREA		b. COUNTY	
a. STATE Puerto Rico		Cupey Bajo and Río Piedras	
c. MUNICIPALITY San Juan and Trujillo Alto		d. CONGRESSIONAL DISTRICT Puerto Rico	
14. DOES PROJECT REQUIRE CLEARINGHOUSE NOTIFICATION IN ACCORDANCE WITH OFFICE OF MANAGEMENT AND BUDGET CIRCULAR A-95?			YES   NO   X
15. HAS AN ENVIRONMENTAL ASSESSMENT BEEN MADE FOR THIS PROJECT OR FOR A COMPREHENSIVE PLAN WHICH INCLUDES THIS PROJECT?			X
16. HAS AN ENVIRONMENTAL IMPACT STATEMENT BEEN MADE FOR THIS PROJECT OR FOR A CLASS OF PROJECTS INCLUDING THIS PROJECT?			X
17. FEDERAL AGENCY N/A		17. IS THE PROJECT COVERED BY A CURRENT FEDERAL APPROVED PLAN? N/A X	
18. DOES THE PROJECT REQUIRE THE ACQUISITION OF LAND OR THE DISPLACEMENT OF ANY PERSON FROM HIS HOME, BUSINESS OR FARM?			X
19. IS PROJECT IN A DESIGNATED FLOOD HAZARD AREA?			X
20. PROJECT MANAGER		20. TITLE	
a. NAME Arthur McBride Block		Scientist II	
c. ADDRESS (Street, City, State and Zip Code) University of Puerto Rico Center for Energy & Envir. Res.		b. ADDRESS (Street, City, State and Zip Code) Maperra Hqts Sta., Río Piedras San Juan, PR 00935	
		d. PHONE (Include area code) (809) 767-0334	
21. OFFICIAL OR AGENCY TO WHOM CHECKS ARE TO BE MAILED Mr. Ramón Muñiz Finance Officer		e. ADDRESS (Street, City, State and Zip Code) Center for Energy & Environment Res. Mayaguez A&M, Mayaguez, Puerto Rico 00708	
22. SUBMISSIONS TO OTHER FEDERAL AGENCIES NSTOFA N/A			

**PART 1 SCHEDULE A-BUDGET**

APPLICANT'S NAME: Center for Energy & Environment Research, EPA PROJECT CONTROL NO  
 A.M.C.B. Block, Caparra Hrts Sta., San Juan, PR 00025

**SECTION A-BUDGET BY SOURCE**

FUNDING SOURCE	FUNDS REQUIRED	
	BUDGET PERIOD	PROJECT PERIOD
EPA SOURCES (Total)	\$47,907	\$71,906
OTHER FEDERAL SOURCES (Total)	N/A	N/A
NON FEDERAL SOURCES (Total)	\$5,188	\$7,781
<b>TOTAL BUDGET</b>	<b>\$53,093</b>	<b>\$79,686</b>

**SECTION B-BUDGET ESTIMATES FOR BALANCE OF THE PROJECT PERIOD**

FUNDING SOURCE	ADDITIONAL SUPPORT TO COMPLETE PROJECT							
	BUDGET PERIOD							
	FROM (1)	TO	FROM (2)	TO	FROM (3)	TO	FROM (4)	TO
EPA SUPPORT	Oct./1980	Apr./1981	N/A		N/A		N/A	
	\$23,998							
OTHER FEDERAL SUPPORT	N/A		N/A		N/A		N/A	
OTHER FUNDING SOURCE	Oct./1980	Apr./1981	N/A		N/A		N/A	
	\$2595							
<b>TOTALS</b>	<b>\$26,593</b>		<b>N/A</b>		<b>N/A</b>		<b>N/A</b>	

**SECTION C-FORECASTED CASH NEEDS BY QUARTER**

FUNDS SOURCE	FIRST QUARTER	SECOND QUARTER	THIRD QUARTER	FOURTH QUARTER	TOTAL
FEDERAL	12,744	11,924	12,614	12,624	47,906
NON-FEDERAL	1,297	1,297	1,297	1,297	5,188

**SECTION D-BUDGET BY COST CATEGORY OR PROGRAM ELEMENT**

TABLE A. COST CATEGORY	TOTAL PROJECT COSTS	TOTAL BUDGET COSTS
1. PERSONNEL	41,242	27,405
2. FRINGE BENEFITS	8,009	5,200
3. TRAVEL	430	430
4. EQUIPMENT	N/A	N/A
5. SUPPLIES	1,900	1,900
6. CONTRACTUAL PERSONNEL SERVICES	6,960	3,980
7. CONSTRUCTION (See Schedule B)	N/A	N/A
8. OTHER	600	300
9. TOTAL DIRECT COSTS	59,141	39,405
10. INDIRECT COSTS	20,544	13,688
11. TOTAL	79,685	53,093
12. TOTAL REQUESTED FROM EPA	71,904	47,907
<b>TABLE B. PROGRAM ELEMENT</b>		

TOTALS

## SECTION E

## DETAILED ITEMIZATION OF DIRECT COST

1.	Personnel	Budget Period	Project Period
1.1	Project Investigator & Arthur McB. Block	20%	20%
1.1.1	Salary	4,174	6,261
1.1.2	Fringe Benefits (16% Salary)	668	1,002
1.1.3	Total Cost	4,842	7,263
1.2	Field Operations Associate & Alvin Mirabal	30%	30%
1.2.1	Salary	2,700	4,050
1.2.2	Fringe Benefits (16% Salary)	432	648
1.2.3	Total Cost	3,132	4,698
1.3	Parasitologist & Virgermina Quiñones	50%	50%
1.3.1	Salary	3,600	5,400
1.3.2	Fringe Benefits (16% Salary)	576	864
1.3.3	Total Cost	4,176	6,264
1.4	Head Microbiologist & (Nuri Rodríguez)	20%	20%
1.4.1	Salary	4,300.80	6,451.20
1.4.2	Fringe Benefits <sup>a</sup>	885.24	1,329.37
1.4.3	Total Cost	5,186.04	7,781
1.5	Microbiology Research Asst. (To be named)	100%	100%
1.5.1	Salary	7,320	10,930

<sup>a</sup> Fringe Benefits for UPR-Medical School calculated according to the following-schedule: 7% Salary for Retirement, 6.13% Salary for Social Security, 1.5% Salary for State Insurance Fund, 4% Salary up to \$6,000 for Christmas Bonus, 2% Salary up to \$4,200 for Unemployment Benefits, \$40 monthly for Medical Plan for personnel working greater than 50% time.

		Budget Period	Project Period
1.5.2	Fringe Benefits <sup>a</sup>	1,875	2,870
1.5.3	Total Cost	9,195	13,850
1.6	Graduate Student Asst. (to be named)	100%	100%
1.6.1	Salary	5,400	8,100
1.6.2	Fringe Benefits (16% Salary)	864	1,296
1.6.3	Total Cost	6,264	9,396
2.	Travel	430	430
	1 trip, San Juan, Beltsville, Md. and return, room, expenses-3 days.		
3.	Supplies		
3.1	Compost Pile Construction	500	500
	Tubing, shovels, chart paper, PVC Pipe, cement, elbows, con- nectors, sheet metal, rope, stakes		
3.2	Microbiological Assays		
3.2.1	Reagents Including Media, Indicators	600	600
3.2.2	Microscopy Materials	300	300
	Plates, Slides, Shadowing and Sectioning material		
3.2.3	Culturing Materials	500	500
4.	Contractual Services		
4.1	Consulting Engineer Rafael Cruz Pérez 16hrs. \$60/hr.	480	960
4.2	Consulting Microbiologists		
4.2.1	Mycologist (General) Yolanda Mejías 40 hrs. \$50/hr.	1,000	2,000

		Budget Period	Project Period
4.2.2	Thermophilic Organisms Specialist Terry Woodin 40 hrs. \$ 50/hr.	1,000	2,000
4.3	Compost Pile Construction  Trucking, Front-end loader rent, Operator salary	1,500	2,000
5.	Construction N/A		
6.	Other		
6.1	Publication Costs	300	600

SECTION F  
INDIRECT COSTS

	Budget Period	Project Period
Indirect Costs are based upon 40% of direct costs, as established by CEER.	\$ 13,687.60	\$ 20,544
Carry forward option-to-increase, not to exceed 5%		

## CHAPTER III

## Narrative Statement

## 1. Scientific Team

## 1.1 Principal Investigator -

Full Name: Arthur McBride Block  
Social Security No.: 143-30-5543  
Mailing Address: 65th. Infantry Sta., POB 30918  
 San Juan, P.R. 00929  
Business Address: Center for Energy & Environment Research  
 University of Puerto Rico  
 Caparra Heights Station  
 San Juan, Puerto Rico 00935  
Telephone No.: Bus. (809) 767-0334 Res. (809)  
 761-9389  
Place, Date of Birth: Newark, N.J. June 26, 1938  
Citizenship: U.S.A.  
Civil Status: Married, 2 daughters: 7 & 9 years,  
 respectively.  
Languages: English (fluent), Spanish (spoken,  
 reading, comprehension), French  
 (reading), German (reading), Russian  
 (reading-slow).  
Education:  
 High School Newark Academy; Newark, N.J. Diploma  
 1956.  
 University Cornell University; Ithaca, N.Y; AB  
 (Chemistry & Physics) 1961.  
 Advanced Degree Rutgers - The State University; New  
 Brunswick, NJ; Ph.D (Physical Chemistry)  
 1967.  
Physical Data: Height: 178 cm Weight: 77 kg.  
 General Health: good  
Experience:  
 1976 - present Scientist II, University of Puerto Rico  
 Center for Energy & Environment Research,  
 Caparra Heights Station, San Juan, P.R.  
 00935

Supervisor	Richard G. Clements
Supervision	1-2 Technicians, 1-2 junior scientists, 1-2 graduate students.
Responsibility	Program development and administration in contract research, publication of results, establishment and maintenance of GMP's in chemical methodology related to biological and environmental measurements, adaptation of analytical chemical methods for field applications, response to RFP's from U.S. Department of Energy, U.S. Environmental Protection Agency and others for research on water resources and energy conservation and development.
Research Areas	Plant physiology and biochemistry, solid waste management options - microbial treatment, aerobic lagooning and magnetic separations, trace element transport in environmental systems, detoxification of problem residues and wastes using chemical and biological methods, destabilization of solutions and suspensions using chemical aggregants, precipitants and flocculants.
1973 - 1975	Scientist I; U.S. Atomic Energy Commission, Puerto Rico Nuclear Center (GOCO Contract with the University of Puerto Rico) Caparra Heights Station, San Juan. P.R. 00935.
Supervisor	Richard G. Clements
Supervision	2 technicians. 1-3 graduate students.
Responsibility	Program development, contract research, publication of results, institution of good lab practices, development of chemical facilities for analytical and physical chemistry, maintenance of GMP in environmental radioactivity dosimetry, response to RFP's from U.S. Atomic Energy Commission, Puerto Rico Resources and others for research on nuclear power plant siting and environmental impact of human activities associated with use of radionuclides.
Research Areas	Plant physiology and invertebrate enzymology, physical organic chemistry, radiation dosimetry and background radiation measurement, radiation monitoring program analyses, trace element transport in environmental systems.



1968-1972	Assistant Professor, Department of Chemistry, University of Puerto Rico, Rio Piedras, Puerto Rico 00931.
Responsibility	Undergraduate student instruction in physical chemistry, graduate (MS & Ph.D) student training, development of laboratory and physical chemistry study program for (ACS-approved) chemistry major, submission of research proposals, publication of original research conclusions, graduate admission committee.
Research Areas	Laser applications in light-scattering photometry, particulate size distributions in rivers, invertebrate enzymology, colloid adsorption analysis.
1967	Lecturer, Department of Chemistry, University of Puerto Rico, Rio Piedras, PR, 00931.
Responsibility	Undergraduate student instruction in physical chemistry, development of course program and laboratory facilities for undergraduate (ACS-approved) chemistry majors, submission of proposals for funding of original scientific research.
Research Areas	Laser applications in light-scattering photometry, optical instrumentation.

Other Professional Activities and Experience:

Consultant, Puerto Rico Environmental Quality Board: Project 208 - Isla, Rural non-point source pollution control. (on-going).

Member, Select Panel Workshop for Assessment of Magnetic Filtration for Problematic Aqueous Effluents in Puerto Rico (on-going).

Co-Chairman, Scientific Program Committee: Caribbean Chemical Conference IX, Dec. 8-11, 1977, San Juan, Puerto Rico.

Referee, Journal of Physical Chemistry (1976).

Proposal Reviewer, U.S. Environmental Protection Agency: Aerobic thermophilic Sewage treatment plant experimental designs (1976).

Assistant Professor, Radiological Health Program, Puerto Rico Nuclear Center (1975).

Graduate Student Researcher, Dep't. Chemistry, Putgers - The State University (Thesis: Laser Light Scattering from Uniform Spheres) (1962-1967).

Chemist, Johnson & Johnson Corp., West Research, South Brunswick, NJ: Analytical chemical methods development and research. polarographic analyses of zinc in the presence of titanium, trace elements in uncured silicon rubber lots, IR fingerprints of low molecular weight adhesive-release agents (1964).

Chemist, Merck & Co., Inc. Rahway, NJ: Analytical chemical methods development for microquantities of nucleotides used as food flavor enhancers, development of National Bureau of Standards thin layer chromatography identification tests for vitamin A esters, development of microtechniques for carotene analysis (1963).

Chemist, Fluid Chemical Corp., Newark, NJ: Quality control of batch process manufacture of soap products, aerosol perfumes and cosmetics (1961).

Society Memberships, Offices Held, Honors, Distinctions:

Chemist License No. 1604, Commonwealth of Puerto Rico.  
 Fellow, American Institute of Chemists  
 American Men & Women of Science  
 American Chemical Society; Puerto Rico Section Chairman 1978  
 Society of Microbiologists of Puerto Rico.  
 American Association for the Advancement of Science  
 Sigma Xi Society; Assoc. Member 1965, Full Member, 1973,  
 Councillor - San Juan/UPR Club 1974-1975.  
 Colgate-Palmolive Fellow 1964-1965.

Research Students:

2 Ph.Ds (biochemistry); 3 Masters (physical chemistry); 2 Undergraduates (Physical chemistry); 2 Masters (Nuclear Engineering); 3 Masters (Public health); 1 Ph.D (Physical chemistry).

Contracts and Grants:

Puerto Rico Water Resources Authority: \$120,000, Radiological survey of a nuclear power plant site.

U.S. Department of Health, Education and Welfare: \$5,000, Undergraduate physical chemistry laboratory experiment in photochemistry.

Commonwealth of Puerto Rico, Department of Internal Revenue: \$10,000, Surplus electronic equipment and parts for repair of laboratory instrumentation.

Westinghouse Gift Program, \$3,200: Instrumental grants for research.

Research Corporation, \$6,500: Quaternary structure studies of glycogen phosphorylase enzyme.

Professional References:

Dr. Waldemar Adam  
 Dept. of Chemistry  
 University of Puerto Rico  
 Rec. Rio Piedras  
 Rio Piedras, Puerto Rico 00931

Mr. Félix Santos  
 University of Puerto Rico  
 Center for Energy & Env. Research  
 Caparra Heights Station  
 San Juan, Puerto Rico 00935

Dr. Richard G. Clements  
 University of Puerto Rico  
 Center for Energy & Env. Research  
 Caparra Heights Station  
 San Juan, Puerto Rico 00935

Dr. R.S. Lamba  
 Interamerican University  
 Central Administration  
 Hato Rey Campus  
 Hato Rey, Puerto Rico 00919

Other Projects:

Research and development of vascular plant-dominated lagoons for wastewater treatment. Energy conservation aerobic treatment of municipal and industrial wastes. Magnetic separation of high BOD wastes and combined storm overflow. Rain forest energy and nitrogen budgets.

Receipt of this grant would entail redistribution of time devoted to these research areas as follows: Vascular plant lagoons - 15%, Magnetic separations - 25%, Energy conservation devices - 10%, Microbial succession (this proposal) - 20% and Forest dynamics - 30%.

Publications:

A.McB. Block, "Dispersion no linear de luz de laser en soluciones", Rev. Col. Quim. Puerto Rico 28, 10 (1969)

A.McB. Block, "Use of a 6,328 Å secondary source in differential refractometry", Appl. Optics 10, 207 (1971).

N.K. Mehta, A. Grinson and A.McB. Block, "Effects of dispersing agents on the angular dependence of light scattered from polystyrene sphere/water sols", Appl. Optics 10, 2031 (1971).

G. Stevenson, M. Colón, J.G. Concepción-García and A.McB. Block, "The cyclooctatrieneyne anion radical", J. Amer. Chem. Soc. 96, 2283 (1974).

D. Santiago, P.A. Ríos de Santiago, A.McB. Block and F. Sagardía, "Purification and properties of glycogen phosphorylase a from the muscles of blue crab (*Callinectes danae*)", Arch. Biochem. Biophys. 163, 679 (1974).

P.A. Ríos de Santiago, D. Santiago, A.McB. Block and F. Sagardía, "Kinetics of inhibition of glycogen phosphorylase a isolated from the muscle of blue crab (*Callinectes danae*)", Arch. Biochem. Biophys. 163, 688 (1974).

A.McB. Block, R.G. Clements and J.D. Parrish, "Background radiological characteristics (for Puerto Rico)", Puerto Rico Water Resources Authority Environmental Report for North Coast Nuclear Power Plant #1 (NORCO-1), USAEC Docket #50-376, 2.8 (1974).

A.McB. Block and R.G. Clements, "Preoperational monitoring program for NORCO-1 power plant", Puerto Rico Water Resources Authority Environmental Report for North Coast Nuclear Power Plant #1 (NORCO-1), USAEC Docket #50-376, 6.3 (1974).

A.McB. Block, R.G. Clements, J.D. Parrish and K. Pedersen, "Off-site radiological monitoring program (for NORCO-1 power plant)", Puerto Rico Water Resources Authority Environmental Report for North Coast Nuclear Power Plant #1 (NORCO-1) USAEC Docket #50-376, 11.6 (1974).

G.R. Stevenson, M. Colón, I. Ocasio, J.G. Concepción-García and A.McB. Block, "Electron distribution in some 1,2-disubstituted cyclooctatetraene anion radicals and dianions", J. Phys. Chem. 79, 1968 (1975).

A.McB. Block, R.G. Clements, L.I. Rosa, P. Santos, M.D. Banus, E. Hernández, R. Mosquera and K.L. Prado, "Thermoluminescence dosimetry in northwest Puerto Rico", USERDA Tech. Publ. PRNC-191 (1975).

G.R. Stevenson, A.E. Alegría and A.McB. Block, "Equilibrium studies by electron spin resonance XIII. The relationship between charge density and ion pair dissociation determined by the use of g values", J. Amer. Chem. Soc. 97, 4859 (1975).

A.McB. Block and R.G. Clements, "Structure-activity correlations for phenoxyacetic acids and indoleacetic acids used for plant growth regulation", Int. J. Quantum Chem. QES 2, 197 (1975).

A.McB. Block and L.W. Newland, "Molecular orbital calculations for 1,2,3,4,5,6-hexachlorocyclohexanes", in "Pesticides", P. Koivisto ed., Env. Qual. Safety (Suppl.) III, 569; Geo. Thieme Verlag, Stuttgart, FRG (1975).

F. Santos, A.McB. Block, R.G. Clements, L.I. Rosa and M.D. Banus, "Natural environmental radioactivity measurements in northwest Puerto Rico", Carib. J. Sci., appeared, 1978.

A.McB. Block and R.G. Clements, "Radioactivity content of soil in Barrio Islote, Arecibo, Puerto Rico", USERDA Tech. Publ. PRNC-202 (1976).

A.McB. Block and N. García, "Commentary on the analysis of mercury in soil and sediment", J. Environ. Qual. 6, 232 (1977).

A.McB. Block, R. Concepción-García and G.R. Stevenson, "Substituted benzylidene malononitrile anion radicals", J. Phys. Chem. 81, 367 (1977).

A.McB. Block, W. Bhajan, L.W. Newland & J. Estevez, "The electrochemical reduction model of anaerobic degradation of the gamma isomer of 1,2,3,4,5,6-hexachlorocyclohexane ( $\gamma$ -BHC)", J. Water Poll. Control Fed. 49, 857 (1977).

A.McB. Block, E. Cuevas and R.S. Lamba, "Auxin structure-activity relationship. Preliminary results of studies of chemical control of an ecosystem in its steady state". Int. J. Quantum Chem. QBS 4, 127 (1977).

W. Bhajan, M. Canals, R.G. Clements, J.A. Colón and A.McB. Block, "A limnological survey of the Rio Espiritu Santo drainage basin". U.S. ERDA Tech. Publ. in press (1977).

A.McB. Block, F. Santos and M.A. Gribble, "The environmental impact of artificially-produced, biologically-active radionuclides in Barrio Islote, Arecibo, Puerto Rico. Estimates of the surface soils burden of Cs-137, Ra-226 and Sr-90", Carib. J. Sci., 1978.

A.McB. Block, F.A. Santos, W.R. Bhajan, G. Goldman, "Elemental survey of the Rio Espiritu Santo river estuarine sediments", Science-Ciencia 6, 30 (1978).

A.McB. Block, "The human waste problem in rural zones of a high rainfall watershed". Proc. Sem. River Basin and Environmental Planning: Method and Inst., CEER, UPR Grad. Sch. Planning, A.I. Ch.E., (sponsors), to appear 1979.

## 1.2 Principal Microbiology Investigator

Full Name : Nuri Rodríguez de Pérez

Date of Birth: May 25, 1945

Civil Status: Married; 2 children

Address: 230 Himalaya St.  
Monterrey, Rio Piedras

Business Phone: (809) 763-6155

Education:

Undergraduate Bachelor of Science (Biology)  
Rio Piedras Campus  
University of Puerto Rico, 1966

Graduate Master of Science (Microbiology)  
Medical Sciences Campus  
University of Puerto Rico, 1971  
Thesis: Certain Aspects of Glycogen  
Metabolism in a Strain of Saccharomyces  
Cerevisiae and one of its Glycogen  
Deficient Mutants.

Doctor of Philosophy (Microbiology)  
Medical Sciences Campus  
University of Puerto Rico, 1978  
Thesis: The Effects of Serotonin,  
Cyclic Nucleotides and Calcium Ions  
on Ciliary Regeneration in Tetrahymena  
pyriformis.

Previous Positions:

1967 Research Assistant (Part-time)  
College of Pharmacy, University of P.R.

1967 - 1970 NIH trainee, under the training grant  
of the Microbiology Dept., Medical  
Sciences Campus, University of P.R.

1970 - 1978 Instructor, Dept. of Biochemistry  
Medical Sciences Campus  
University of Puerto Rico

Present Position: American Society for Microbiology  
Sociedad de Microbiólogos de Puerto Rico

Award and Honors:

1. Semifinalist for the Borden Award for students with 4.0 average in the first year of college, University of Puerto Rico, 1963.
2. Honorary Scholarship for outstanding science majors from Empresas Ferré, 1966.
3. Honorary Registration of the University of Puerto Rico, 1963-1966.
4. Member of the Tri Beta Chapter of the University of Puerto Rico (Honorary Society for Biology Majors).
5. Bachelor of Science Degree Awarded Magna Cum Laude, Dec. 1966.

Professionally related training:

- 1971 - Education Workshop (Dept. of Biochemistry)
- 1971 - Education Workshop (Medical Sciences Campus in Dorado).
- 1972 - Education Workshop (Medical Sciences Campus in the Holiday Inn).
- 1977 - Workshop on Anaerobic Bacteriology (Sociedad de Microbiólogos de Puerto Rico).

Publications:

1. Rodríguez, N. and F. Sagardía. Glycogen Cycle Enzymes in Normal and Glycogen Deficient Yeasts, Bact. Proc., p. 140, 1971.
2. Rodríguez, N. and F.L. Renaud. On the Possible Role of Serotonin in the Regulation of Cilia Regeneration, J. Cell Biol. 70 (No. 2, part 2) p. 94a, 1976.
3. Rodríguez N., F.L. Renaud, and R. Paoli, Cilia Regeneration: A Model System for the Study of the Function of Serotonin, J. Cell Biol. 75 (No. 2 part 2) p. 38a. 1977.

The terms of this proposal include financing of release time to the extent of 20% full-time by the Medical Sciences Campus of the University of Puerto Rico. This 20% ( 7-1/2 hours per week) would be devoted to the accomplishment of tasks associated with microbiology studies as well as supervision of personnel engaged in the study of microbiological aspects of the proposal.



1.3

**Head Field Operative (Compost Pile Staging).**

Full Name: Alvin Mirabal

Social Security: 583-68-9949

Address: Corton 308, Villa Palmeras  
Santurce, Puerto Rico 00915

Telephone: (809) 767-0334

Date and Birth Place: January 12, 1954  
Manhattan, New York, U.S.

Civil Status: Married

Citizenship: U.S.A.

Education:

High School Central High, Stop 22,  
Ponce de León Avenue  
Santurce, Puerto Rico  
August 68 - May 71

University University of Puerto Rico  
Mayaguez Campus  
August 1971 - May 1971  
B.S. Degree, Biology

Post Graduate Course:

1. Incinerator evaluation E.Q.B.
2. Opacity Evaluation (Emissions) EQB
3. Transportation of Hazardous  
Materials Seminar (F.D. of T.)
4. Air Pollution Field Enforcement E.P.A.
5. Combustion Evaluation (EPA)
6. Source Sampling for Particulate  
Pollutants (EPA)
7. Environmental Pollution Control Course,  
School Environmental Health, Medical Campus

Professional Experience:

Present Scientific Associate  
Center for Energy & Env. Research  
University of Puerto Rico  
Caparra Heights Station  
San Juan, Puerto Rico 00935

Chemical and biological measurements  
Laboratory and field work. Methodolo-  
gy analysis and development.

Jan. 1979-Jan. 1979	Natural Resources Technician II Environmental Quality Board (EQB) Office of the Governor P.O. Box 11488, Santurce, P.R. 00910
	Stack sampling, water quality analysis, inspection, maintenance of equipment. Sediment and salinity sampling. Presentation of evidence in legal proceedings.
June 1977-Jan. 1978	Same
	Environmental regulation compliance monitoring.

Society Memberships, Licenses, Distinctions:

Green Energy, Inc.  
P.R. Natural History Society  
College Association for the Protection of the Environment  
Master Scuba Diver - N.A.U.I., P.A.D.I., N.A.S.D.S. and C.M.A.S.  
Small Boat Operator  
Scientific Photographer

1.4

## Parasitologist

Full Name: Virgenmina Quiñones-GiovannettiSocial Security No.: 582-92-4138Address: Condominio De Diego 444  
Apt. 301  
Rio Piedras, Puerto Rico 00918Telephone No.: (809) 767-0321Place & Date of Birth: Ponce, Puerto Rico  
May 6, 1945Civil Status: Divorced; 4 childrenAcademic Background:High School Dr. Pila High School  
General, 1964

Catholic University Basic &amp; Sciences Studies 1964-1966

Ponce District Hospital  
Histopathology Certificate - 1968Interamerican Univ.  
Additional Studies in Natural Sciences  
(7 credits to finish BA)Center DC (San Juan Lab.)  
Special training in Parasitology  
1974-1975Professional Experience:1975 to present Center for Energy and Environment  
Research - Research Assistant IIIField collection of snail (*Marisa-  
Limnaea (Fasciola hepatica)*)Chaetogaster annelid investigation  
Liver perfusion (mouse) for schistosomiasis  
studiesTissue culture - E. coliWater analysis - biological study of phyto-  
plankton and bacteriaFecal analysis - (Ritchie modified method)  
for clinical diagnosis of schistosomiasis  
and other parasites. Quantitative & quali-  
tative analysis for parasites egg.

- 1973 - 1974 P.R. Nuclear Center (Center for Energy & Env. Research) Electron microscope techniques, ultramicroscopic section (histological) photographic.
- 1969 - 1972 University of Puerto Rico, RCM Campus Technical Research Bacteriological Analysis of ophtalmic excretions; tissue section of ocular organ.
- 1968 - 1969 Ponce District Hospital - Technician (Histopathology) Tissue section of postmortem and surgical organs, frozen tissue section and special dyes.
- 1964 - 1968 Ponce District Hospital-Assistant Technology urine & blood sample analysis.

## 1.5 Consultants

## 1.5.1 Environmental Engineer

Full Name: Rafael Cruz Pérez

Address: Violeta #153  
San Francisco, Rio Piedras, P.R. 00927

Telephone: 764-7617, 767-4542

Date of Birth: February 16, 1937

Place of Birth: Vieques, Puerto Rico

Citizenship: U.S.A.

Marital Status: Married on the 8th of October,  
1960 to María Mercedes Cassé Ballesteros

Children: María Mercedes Cruz (9/25/61)  
Marta Cruz (9/27/62)  
Carmen Ana Cruz (7/16/64)  
Margarita Cruz (7/16/68)

Languages: Spanish-Proficient  
English - Proficient

Licenses: Driver - 1960  
Graduate, Engineer 1961 (#4069)  
Chemist 1964 (#4060)  
Professional Engineer 1968 (4060)  
Certified Scuba Diver N.A.V.I.  
#332052

Education:

Elementary School 1st. Grade, Colegio La Milagrosa  
Rio Piedras, Puerto Rico

2nd. Grade to 6th. Grade, University  
of Puerto Rico Elementary School,  
Rio Piedras, P.R.

Intermediate and High School University of Puerto Rico High School,  
Rio Piedras, Puerto Rico  
Graduated in 1955

College College of Agriculture and Mechanic  
Arts, Graduated in 1961 with a Bachelor  
in Science in Chemical Engineering

Other College Studies Introductory course in Meteorology,  
University of Puerto Rico

Physical Geology, University of P.R.

Soils Engineering, University of  
Puerto Rico (CAAM)

Foundation Engineering, University  
of Puerto Rico (CAAM)

Highway Planning and Design,  
University of P.R. (CAAM)

Introduction to Surveying, University  
of Puerto Rico (CAAM)

Astronomical Surveying, University  
of Puerto Rico (CAAM)

Introduction to Marine Engineering,  
University of Puerto Rico (CAAM)  
(1969)

Coastal Engineering, University of  
Puerto Rico (CAAM) (1970)

Accounting Principles, University  
of Puerto Rico (CAAM)

Advances in Chemical Engineering,  
University of Puerto Rico (CAAM)

Special Courses  
& Training

Paint training, Pittsburg, Paints,  
San Juan, Puerto Rico

Skid Resistance Training, Bureau  
of Public Roads, Wash. D.C. (1966)

Lime Stabilization, Illinois State  
University, San Juan, P.R. (1967)

Critical Path Method, IBM, Santurce,  
Puerto Rico (1968)

Basic & Advanced Seamanship, U.S.  
C.G.A., San Juan, Puerto Rico (1970)

Introduction to Air Pollution, Control  
University of Washington, S.J. (1969)

Control of Oil and other Hazardous  
Materials, (EPA) San Juan, Puerto Rico  
(1972)

Advance Wastewater Treatment (EPA),  
San Juan, Puerto Rico (1971)

Hydrology Seminar, By Dr. V.T. Chow  
Instructor, San Juan, Puerto Rico (1969)

Engineering Aspects of Heat  
Disposal from power Generation,  
Summer Sesion, Massachusetts  
Institute of Technology, Boston,  
Mass. (1972)

River Mechanics, Summer Institute,  
Colorado State University, Ft.  
Collins, Colorado (1972)

Stack Sampling Seminar  
Research Appliance Corp.  
Philadelphia, Pa. (1975)

Professional Experience:

March 1977 to Present

President, Servicios Ambientales

Jan. 1973 to March 1977

Vice-President, Servicios Ambientales

August, 1974

Insturctor for Environmental Eng.  
RUM, University of Puerto Rico

July 1, 1972 to 1975

Member, National Air Pollution  
Control Techniques Advisory Committee,  
EPA, Washington, D.C.

Dec. 1970 to Dec. 1972

Associate Director for Air & Water  
Resources, Environmental Quality  
Board, Puerto Rico

July 1970 to Dec. 1970

Oceanographic and Marine Studies  
Consultant

July 1970 to Dec. 1970

Air Pollution Advisor for the P.R.  
Department of Health

Dec. 1969 to Feb, 1970

Technical Advisor for Natural  
Resources, Department of Public Works,  
San Juan, Puerto Rico

July 1968 to Feb. 1970

Technical Advisor for Flood Control  
and Beach Conservation-Department of  
Public Works, San Juan, Puerto Rico

Society Membership:

American Public Works Association

Smithsonian Institute

International Widllife Federation

Puerto Rico College of Chemists,  
San Juan, Puerto Rico

Puerto Rico Water Pollution Control  
Association

## National Oceanographic Foundation

Puerto Rican Institute of Chemical Engineers, San Juan, Puerto Rico  
(Several positions in Board of Directors since the creation of the Institute)

Puerto Rican Society of Engineers, San Juan, Puerto Rico  
(Secretary, Board of Trustees (1975-76))

Institute of Engineers, Architects and Surveyors, San Juan, Puerto Rico  
(President, Asbestos Housing Committee 1976-77)

(President, Nuclear Energy Commission 1975-76)

(President, Environmental Commission 1973-75)

(President, Ethics Commission, 1970-71)

Publications:

- Rafael Cruz Pérez -(1966) Drill Lime Stabilization, Research Study No. 5  
Department of Public Works (Abstracts, Highway Research Board)
- Rafael Cruz Pérez (1966) Slurry Seal Manual, Research Study No. 10 -  
Department of Public Works
- Rafael Cruz Pérez (1967) Areas Resbaladizas en Pavimientos de Carreteras  
(Skid Resistance) Research Study No. 12 - Department  
of Public Works.
- Rafael Cruz Pérez (1967) Introduction to Asphalt (a lecture) Department  
of Public Works
- Rafael Cruz Pérez &  
Luis A. Pérez (1968) Determinación de Calidad de Carpetas Asfálticas  
por Radiación (Nuclear Gauges) Research Study  
No. 11 - Department of Public Works
- Rafael Cruz Pérez,  
Luis A. Pérez and  
R. Delgado (1968) Lime Stabilization of Puerto Rico Low Grade  
Soils, Research Study No. 2 - Department of  
Public Works.
- P.A. Gelabert and  
Rafael Cruz Pérez (1977) Environmental Quality, San Juan 2,000 Municipal-  
ity of San Juan
- Rafael Cruz Pérez (1975) La Aplicación de Controles Ambientales a la In-  
dustria en el Caribe y Países en Desarrollo.



IX. STUDIES OR MAJOR PROJECTS DIRECTED

1. Ensenada Boca Vieja, Coastal Erosion Processes (1968)  
(photogrametria and oblicación)
2. Bathimetric Study at Punta Miquillos, Río Grand, P.R.  
(1970).
3. Current Study for Ocean Out-Fall at Guayama-Arroyo Coast  
(1970)
4. Oceanographic Study for Aguirre Harbor (1970)
5. Current Study for Dredging Operations at Fajardo, Puerto  
Rico (1970)
6. Current Study at Jobos, Puerto Rico (1970)
7. Air Pollution Regulation for Puerto Rico (1972)
8. Air Pollution Implementation Plan for Puerto Rico (1972)
9. Water Quality Survey at Farguera, Puerto Rico 1972
10. Water Quality Survey, Guayanilla - Tallaboa, 1972.
11. Informe de la Junta de Calidad Ambiental sobre la  
Refinería Sun Oil de Yabucoa según ordenado por la RC  
542 (Junio 1972)
12. Water Quality Survey of the Maunabo River, Puerto Rico  
(1974)
13. Marine Survey of Punta Miquillo and Ensenada Honda,  
Puerto Rico (1975)
14. Isotherm Survey of Jobos Bay, Aguirre, Puerto Rico (1975)  
unpublished
15. Marine Currents at Mona Island and Vicinity, Puerto  
Rico (1975)
16. Ponce Bay Air Quality Survey, Ponce, Puerto Rico (1975)
17. San Juan Airport Noise Survey, Isla Verde, San Juan (1975)
18. Industrial Noise Survey, Cataño, Puerto Rico (1976)
19. Río Piedras Water Quality Survey, Río Piedras, P.R. (1976)

20. Pasto Viejo Creek Water Quality Survey, Humacao, Puerto Rico (1975)
21. Río Bucarabones Water Quality Survey, Toa Alta, P.R. (1975)
22. Quabrada Finca Arroyo Lefebre Water Quality Survey, Aibonito, Puerto Rico (1976)
23. El Volcan Creek - W.Q.S., Bayamón, Puerto Rico (1975)
24. Estudio Calidad Agua Qda. Cañita, Lajas, Puerto Rico (1975)
25. Air Quality Survey, Villa Marina, Fajardo (1975)
26. Work Plan for Nickel Mines, Guanajibo, Mayaguez (1976)
27. Compliance Plan Asphalt Plant, Ponce Asphalt, Ponce (1975)
28. Compliance Plan Asphalt Plant, Ponce Asphalt, Humacao
29. Compliance Plan Asphalt Plant, Inabón Asphalt, Juana Díaz
30. Compliance Plan Asphalt Plant, Betterroads Asphalt, San Juan
31. Compliance Plan Asphalt Plant, Betterroads Asphalt, Bayamon
32. Compliance Plan Asphalt Plant, Betterroads Asphalt, Arecibo
33. Compliance Plan Asphalt Plant, Betterroads Asphalt, Añasco
34. Seminario Operadores Planta, San Juan (1976-77)
35. Sewage Treatment Plant Survey, San Vicente Development, Humacao
36. Sewage Treatment Plant Survey, Puerto Kai Development, Lofza
37. Sewage Treatment Plant Survey, El Valle Development, Lajas
38. Air Quality Control Equip, Certification, Puerto Rican Cement, San Juan Plant (1975-77)
39. Air Quality Control Equip, Certification, Puerto Rican Cement Ponce Plant (1975-76)

40. Concrete Batchers Improvement, El Valle Development, Lajas (1975)
41. Lower La Plata River Study, Dorado, Puerto Rico (1973)
42. Sabari Estates Compliance Plan for Concrete Plant, Ponce, Puerto Rico (1973)
43. V'Soske Water Quality Study, Vega Baja, Puerto Rico (1973)
44. Deep Water Port Comparative Studies, San Juan, Puerto Rico (1973) PRIDCO
45. Marine Current Survey, Caja de Muertos, Puerto Rico (1973) PRIDCO
46. Christiansted Air Quality Survey, St. Croix (1974) Barrett & Hale
47. Christiansted Noise Survey, St. Croix (1974) Barrett & Hale
48. Puerta de Tierra Noise Survey, San Juan, P.R. (1974) CRUV
49. Loíza River Estuarine Zone Water Quality Study, Loíza, Puerto Rico (1974) PFZ Properties, Inc.
50. Environmental Air Quality Compliance Plan, San Juan, Puerto Rico (1974)
51. Technical Advice Waste Treatment Plant, Barceloneta, Puerto Rico (1974) Aqueducts and Sewer Authority
52. Air Quality Survey at Union Carbide Garfita, Yabucoa
53. Water Quality Survey at Union Carbide Grafita, Yabucoa

## 1.5.2

**Mycologist**

Full Name: Yolanda Mejías

Date & Place of Birth: September 4, 1923 - Humacao, P.R.

Address: c/o Dept. of Microbiology  
School of Medicine  
University of Puerto Rico  
Medical Sciences Campus  
Caparra Heights, Río Piedras  
00935

Telephone No.: 763-6155

Education - 1944 B.S, Major in Biology, University of Puerto Rico, Rio Piedras, Puerto Rico.

1952 Microbiology for Medical Students-Dept. of Microbiology, UPR School of Medicine, San Juan, P.R.

Position Held:

1944 - 1950 Laboratory Assistant in the Department of Mycology and Dermatology School of Tropical Medicine.

1950 - 1953 Research Assistant Dept. Microbiology School of Medicine, San Juan, Puerto Rico

1958 - 1962 Teacher in Science and Biology - Colegio Espiritu Santo, Hato Rey, Puerto Rico

1962 - 1972 Laboratory Assistant in Bacteriology Dept. (Microbiology) UPR - School of Medicine, San Juan, Puerto Rico

1972 - 1977 Instructor in Microbiology - Dept. of Microbiology, San Juan, Puerto Rico.  
Lectures on Mycology to Nursing students,  
Lectures on Bacteriology for medical Technology students. Maintenance of Stock cultures on fungi and bacteria in laboratory teaching for medical students, medical technologists, nursing and dental students.

In charge of complete preparation of laboratory exercises of medical students, technologists dental students and nursing.

Writing laboratory exercises for medical students (Infections Diseases II) and dental students. Microbiology course.

Laboratory assistant for nursing students, medical students, laboratory assistant in charge of students specializing in dermatology. Preparation and assisting in courses for medical students in elective course in Mycology Maintenance library in mycology section, and of collection of audiovisual aids.

Research Activities:

- 1944 - 1950      Participation in research work with Dr. Arturo L. Carrión related to Chromoblastomycosis, Tinea negra and Dermatormycosis.
- 1962              Participation in research work with Dr. Gladys Torres-Blasini in relation to Candidiasis.
- 1963 - 1969      Participation in research work with Dr. Gladys Torres - Blasini - relation to Histoplasmosis in Puerto Rico.

Society Membership:

Sociedad de Microbiología de Puerto Rico - member and secretary since 1976.

American Society for Microbiology - member

Other Duties:

Lecturer "General Mycology and its Application to Food Microbiology" - to students toward Master's Degree in Food Microbiology - University of Puerto Rico - April, 1978.

Assistance in Workshops during 1977 - 1978

- a) Anaerobes - I and II - 1977-1978
- b) Antibiotics - 1977
- c) Nosocomial Infections - April, 1978

Lectures in Medical Mycology - for Nursing Students - 1978

Lectures in Bacteriology - for Nursing Students - 1978

Guest at the Conventions of the American Society for Microbiology 1976 - 1977 held in Atlantic City and New Orleans respectively.

## 1.5.3

## Thermophilic Fungi Specialist

Full Name: Terri Woodin

Address: c/o Dept. of Microbiology  
Catholic University  
School of Medicine  
Ponce, Puerto Rico 00731

Telephone No.: (809) 844-4150

Civil Status: Married

Citizenship: U.S.A.

Education: B.A. Alfred University, Alfred, N.Y.  
Chemistry, 1954

M.A. University of California at Davis  
Davis, California 1965

Ph.D. University of California at Davis  
Davis, California 1967

Publications: 10 publications, 3 on thermophilic  
fungi.

## 1.5.4 Parasitology Assessor

Full Name: Henry Negrón-Aponte

Soc. Sec. No.: 243-30-1614

Telephone No.: (809) 767-0321

Address: Environmental Health & Impact Div.  
Center for Energy & Environment Res.  
Caparra Heights Station  
San Juan, Puerto Rico 00935

Date & Place of Birth: San Juan, P.R.  
Sept. 2, 1920

Citizenship: U.S.A.

Education: BSCE No. Carolina State University,  
Raleigh, NC-1945  
MD University of Mexico  
Mexico City, Mexico-1957  
MPH University of Puerto Rico  
San Juan, Puerto Rico-1962

Professional Experience:

March/77 to Present Director, Division of Preventive Health, P.R. Dept. of Health.  
Consultant to CEER, University of Puerto Rico

Jan./77 to March/77 Research Scientist (epidemiology), CEER, Univ. of P.R.

July/73 to Jan./76 Resident in Anatomical Pathology, V.A. Hospital, S.J., P.R.

July/72 to June/73 Resident in Anatomical Pathology, Ponce Distric Hosp.  
Ponce, Puerto Rico

Oct./70 to July/72 Director, Cancer Detection Program, P.R. Dept. of Health

Sept./69 to Oct./70 Director of Epidemiological Control, P.R. Dept. of Health

June/62 to June/69 Research Medical Officer, USPHS, San Juan Laboratories

June/62 to Present Ad Honorem-Epidemiology instructor, UPR School of Medicine

Other Experience:

Oct. 2/78 to Nov. 3/78 Temporary consultant WHO in Sudan, Blue Nile Project

May 18 to June 74 Consultant to Mauritanian Gov't.

Oct. to Nov. 1974           and World Bank-Epidemiology  
Study of Gorgol River Project

March 1973                 Course to engineers-Epidemiology  
Maracay, Venezuela-WHO-PAHO

Publications:

- Negrón, H., 1959. "Tuberculin Sensitivity in School Children of Baja California, Mexico". Thesis, University of Mexico.
- Kagan, I., Negrón, H., Arnold J.C. and Ferguson, F.F., 1966. "A Skin Test Survey for the Prevalence of Schistosomiasis in Puerto Rico. Monograph Public Health Service Publication. No. 1525, 91 pages.
- Neff, J.M., Morris, L., González Alcover, R. Coleman, P.H., Lys, S.B., and Negrón H., 1967. "Dengue Fever in a Puerto Rican Community". A.J. Epid. V-86 162-184.
- W.R. Jobin, H. Negrón, and E.H. Michelson, 1976. "Schistosomiasis in the Gorgol Valley of Mauritania". AJTMH V 25 587-594.
- Negrón Aponte, H., Jobin, W.R., 1977. Guidelines for Spacing and Timing of Samples to Detect Populations of Schistosoma mansoni cercariae in the Field". Int. J. Parasit., V 7 123-126.
- Negrón Aponte, H., Ramos Morales, P., and Jobin, W.R., 1978. "Field Trial in Ceiba Norte of Epidemiological Tests for Operational Evaluation of Schistosomiasis Control in Puerto Rico". Bol. Med. Asoc. PR. V 70 p. 298.
- Negrón Aponte, H. Jobin, W.R., 1979. "Schistosomiasis Control in Puerto Rico'25 Years of Operational Experience". Accepted for May issue AJTMH



## 2 Objectives of this Project

### 2.1 Principal Objective

The objective of this project is the determination of ecological succession of micro-organisms during thermophilic digestive detoxification of waste sewage sludge. The digestion procedure to be investigated is the relatively low-energy, forced draft static pile method as developed and currently used by U.S. Department of Agriculture (USDA) Agricultural Research Service (ARS) in Beltsville, Maryland<sup>1-4</sup>. Several different bulking agents and processes for composting are currently under active consideration by the Puerto Rico Aqueducts and Sewers Authority (PRASA) including use of admixed solids from garbage collection and use of bagass (waste cellulosic material residue from sugar cane processing) for improvement of the carbon-to-nitrogen ratio of the waste sludge and for the conference of an easily-aerated pile structure<sup>5</sup>.

#### 2.1.1 Rationale from Planning Data

In Puerto Rico, approximately 52,813 tons of dry solid waste sludge are generated annually by 114 publicly owned sewage treatment facilities. By the year 2000, nearly 200,000 tons of dry sludge are expected to be generated<sup>6</sup>. Waste sewage sludge is currently disposed of using landfill (2%), land application near the plant (84%) and deposition on surface waters (14%)<sup>6</sup>. The latter 2 alternatives can create foci for the transmission of diseases and the last alternative: dispersal in surface waters of rivers, lakes or ocean will soon be severely curtailed under recently promulgated U.S. Environmental Protection Agency (EPA) regulations<sup>7</sup>.

Land spreading for de-watering is not a particularly sanitary method for decontamination of waste sludge because of the possibility of survival of parasite eggs, when sludge is taken prematurely by farmers for use in soil improvement regimes. Prolonged drought coupled with high winds can also create an airborne pathogen problem. Thus it is clear why PRASA is highly interested in relatively inexpensive ways of decontaminating large amounts of waste sludge. In fact, PRASA will aid projects associated with tertiary treatment and waste sludge management by making available facilities in or near functioning sewage treatment plants (Appendix I).

The School of Medicine (RCM) of the University of Puerto Rico (UPR) has traditionally assumed an active role in delineating actual and potential public health problems<sup>8</sup> and members of the staff of the Department of Microbiology including the Chairperson have expressed interest in developing a research project directed at the special problems of digestion or composting which obtain in Puerto Rico (Appendix II).

#### 2.1.2 Details of the Principal Objective

Specifically, a study is proposed to elucidate and summarize which micro-organisms are present in raw waste sewage sludge; to estimate the effective lifetime of each under well-characterized moisture and oxygen regimes during forced air static pile composting; to identify new thermophilic and/or thermotolerant organisms which succeed the primary pathogens and to determine which of these secondary organisms are potentially pathogenic.

## 2.2 Secondary Objectives

There are at least 3 secondary objectives of this project. Firstly, research capability for field microbiologists is to be promoted using persons at the Bachelor or Masters level of education in biology and/or chemistry.

Public awareness of the desirability of composting and its relatively non-polluting function are another objective of this project. The composting staging area would be open to the public for the realization of this objective.

Finally, the project will provide a necessary field test of f-2 bacteriophage titration as an indicator of primary pathogen removal in static pile composting.

## 3. Results and/or Benefits Expected

### 3.1 Principal Benefits

The primary benefits to be expected from the determination of ecological succession of micro-organisms during composting is a description of disease potentiating micro-organisms production during the digestion process. Location of large scale staging areas for composting large quantities of waste sludge may depend upon which secondary thermophilic and/or thermotolerant micro-organisms seem most prevalent during the relatively higher temperature periods. This project will give explicit results concerning the production of such micro-organisms.

Although organisms succession has been studied and pathogen thermal tolerance models have been proposed<sup>10</sup> for the USDA static pile system at Beltsville, the process

used in Beltsville is likely to be modified before its application in Puerto Rico. Bulking of compost pile with wood chips as in the USDA method may not be very practical in Puerto Rico, which is now actively trying to reforest deserted agricultural land and to reclaim barren land scarred by erosion<sup>11</sup>. Use of solids from garbage compaction processes, bagass and grass cuttings all seem to be more likely candidates for use as bulking agents in Puerto Rico. The plentiful, well-managed supply of bagass along with municipal garbage seem like the best alternatives for use here. Bagass with a very high cellulose content is softer than woodchips and is likely to give very different compost pile characteristics than wood chip. It is probable that gross similarities to the micro-organism succession observed by Burge et. al. (1978)<sup>9</sup> will be observed using the techniques relevant to local conditions but in the absence of an experimental test, this cannot be assumed. A principal benefit expected from this project is a test of the generality of temperature versus time profiles for static pile digestion.

Of very great importance to the people of Puerto Rico is the public health menace of parasite infections, most particularly schistosomiasis (bilharziasis)<sup>12</sup>. The eggs of Schistosoma mansoni blood fluke have been known to survive both primary and anaerobic secondary sewage treatment<sup>13,14</sup>. Indeed, heavy chlorination of all treatment plant effluents has been recommended<sup>15</sup> to cope with possible re-introduction of parasite populations in a water bodies believed free of the parasite. If it can be shown that the composting

process kills Schistosoma mansoni eggs with high efficiency another alternative for the de-infestation of waste sewage sludge would be available for use by public health officials in elimination of transmission foci.

Although schistosomiasis is the parasite most feared and most difficult to eradicate, hookworm-Necator americanus is also still present in Puerto Rico<sup>16</sup>; a survey of first graders in public schools carried out in 1966 revealed that 12% of the children were infected with this parasite<sup>17</sup>. The Puerto Rican public would benefit greatly if engineer/planners could depend on thermophilic composting to eliminate the threat of transmission of this parasite.

### 3.2 Subordinate Benefits

The island-wide average precipitation generally assumed for purposes of planning land and water use is 150 cm. (60 in.) in Puerto Rico<sup>18</sup>. Conditions which are excessively humid compared with well characterized locales such as Beltsville, MD or Bangor, ME may produce special maintenance problems in composting sewage sludge. The project as presently envisioned will be facing such conditions and problem-solving associated with high humidity operation would benefit many regions of similar precipitation regimes in continental U.S.A.

The island of Puerto Rico has a tropical climate and average 24 hr. temperatures range from 22-32°C (72-90°F). The achievement of thermophilic conditions in an aerated compost pile may be much more rapid under such conditions than those at Beltsville and, indeed the whole time frame for composting could be considerably shortened. A demons-

tration of this aspect would be an important benefit to municipalities in warmer parts of the continental USA presently considering the disposition of waste sewage sludge by the static pile method.

A subordinate benefit to the Terrestrial Ecology Division of the University of Puerto Rico Center for Energy and Environment Research (CEER) is the production of compost which would enable it to plan disposition studies on public and private lands. The impact of introduction of composted sewage sludge into tropical forests has yet to be studied. Eroded land reclamation using composted sludge has not yet been carried out in the humid tropics. Factors concerning pH stabilization of sub-soils for arid land recovery using limed sewage sludge have yet to be determined, and leaching characteristics and surface runoff under torrential conditions of a single 25 to 32 cm (10-12 in.) rainfall event have not, to our knowledge, been characterized. Production of stable compost would permit this ancillary research to be carried out.

Composting using the static pile system may also be amenable to a zero-energy input operation (no electrical costs) now under patent disclosure proceedings. This project would permit subsequent follow up using the technology under development.

#### 4 Approach

##### 4.1 Work Plan

The work plan is envisaged as ongoing in 3 distinct phases. The 3 phases are: information accumulation, compost pile construction trials and determination and/or identi-

fication of organism presence as a function of composting time.

#### 4.1.1 Information Accumulation

A literature search will be conducted first. Formal information requests on microbiology of digestions will be made from Chemical Abstracts, Biological Abstracts and Excerpta Medica. The latter reference will also be used to obtain specific medical information on pathogens. General references including modern texts will be grouped alphabetically according to author. Research articles published in journals will be assembled alphabetically and by topic. Symposium articles will be sought from authors, public libraries and the Library of Congress. In this latter case, articles will be assembled alphabetically with respect to both author and topic and a cross-reference index will be prepared. Basic large sub-divisions would classify micro-organism studies of digestion according to whether the organisms studied were virus, bacterium, fungus, protozoan or parasite.

Titles will be supplied along with author, source, date and language used. Those references adjudged to be principal or "best" sources will be abstracted critically. One of the criteria for criticality will be whether or not the article contains engineering-design data. A draft of the literature search will be made available to the granting agency for circulation for possible addition, corrections or extensions as soon as it has been prepared, with final draft preparation subject to the approval of the granting agency.

The second sub-division of the proposed program will be phased in from the beginning of the program and will overlap the literature survey.

#### 4.1.2 Compost Pile Construction

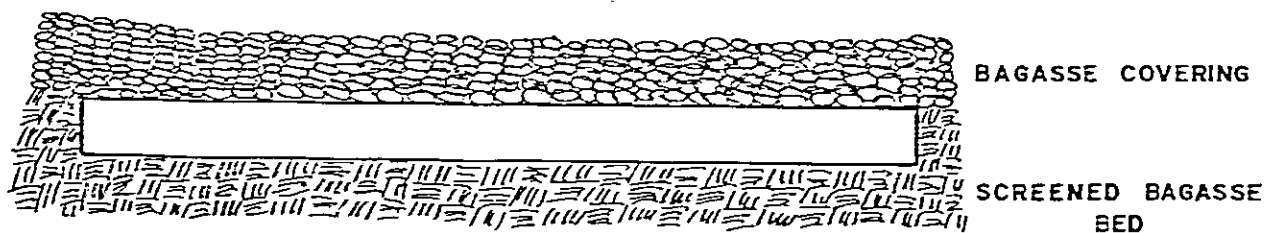
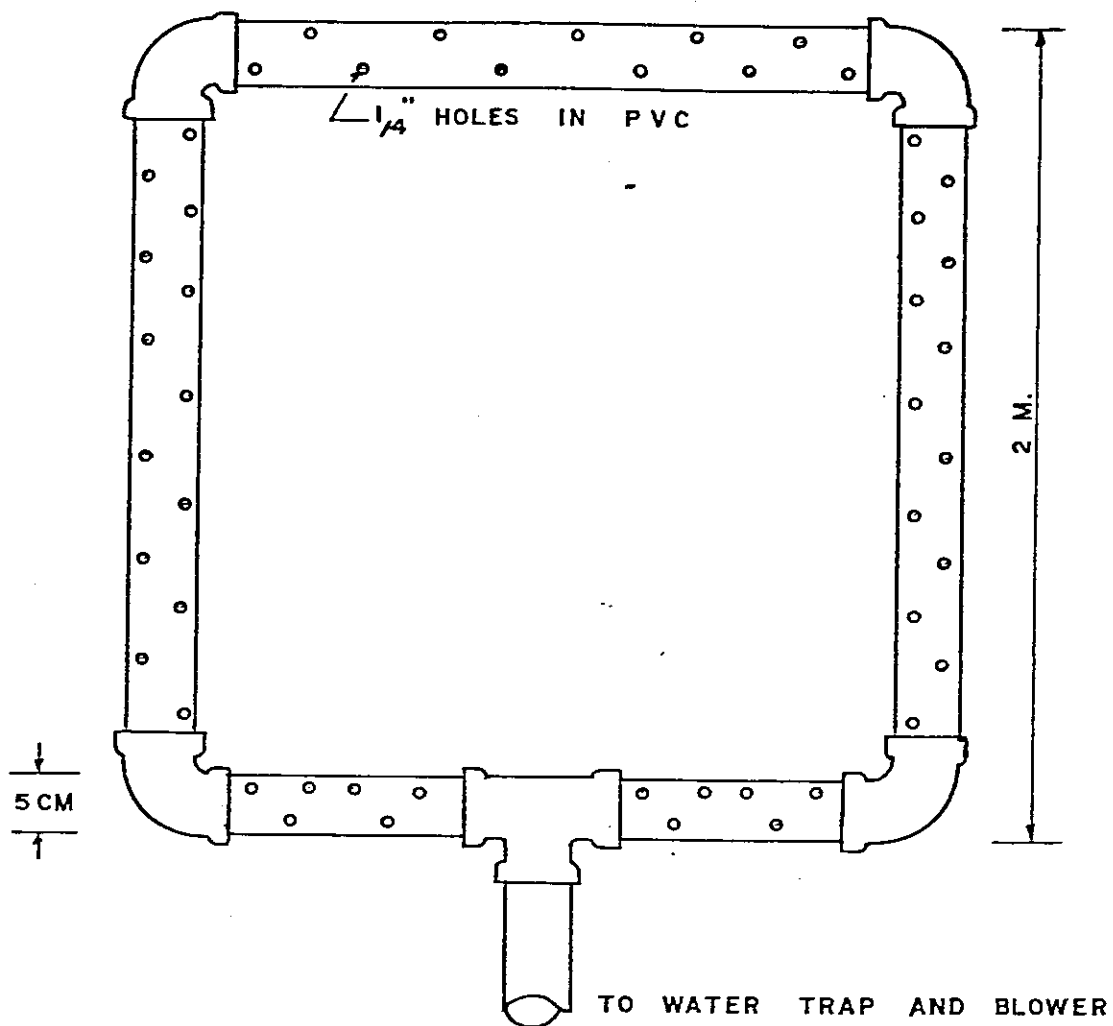
A scaled down version of procedures used by USDA will be used to construct static pile composting mounds. The dimensions of the mound will be 3x3x2m. Air indraw will be provided by a square ring of 2 inch PVC pipe with 1 1/4" holes drilled in the top, mounted on a layer of bagass with an additional layer of coarse bagass over the top of the pipe (Figure I). The ring will be connected via a T to a water trap and thence to a 1/4 or 1/3 horsepower blower in a manner providing suction to the ring. The blower will be vented into a mound of screened bagass to prevent escaping odors.

Inasmuch as the majority of the treatment plants in Puerto Rico use sludge-bed solar drying, vacuum filter cake is inappropriate as a supply for composting material. Solar dried waste sludge is a much more appropriate supply for composting carried out at the El Conquistador secondary aerobic sewage treatment plant in Carraízo, Puerto Rico; in particular, this plant has already been approved for this type of experiment by PRASA.

Mixing of the sludge with a bulking material will be accomplished using a roto-tiller and front-end loader after sampling both the sludge and the bagass for water determinations. A mixture containing a maximum of 40% by weight water will be made using data from the moisture determination. Moisture content will be determined by weighing the untreated



## TOP VIEW



## SIDE VIEW

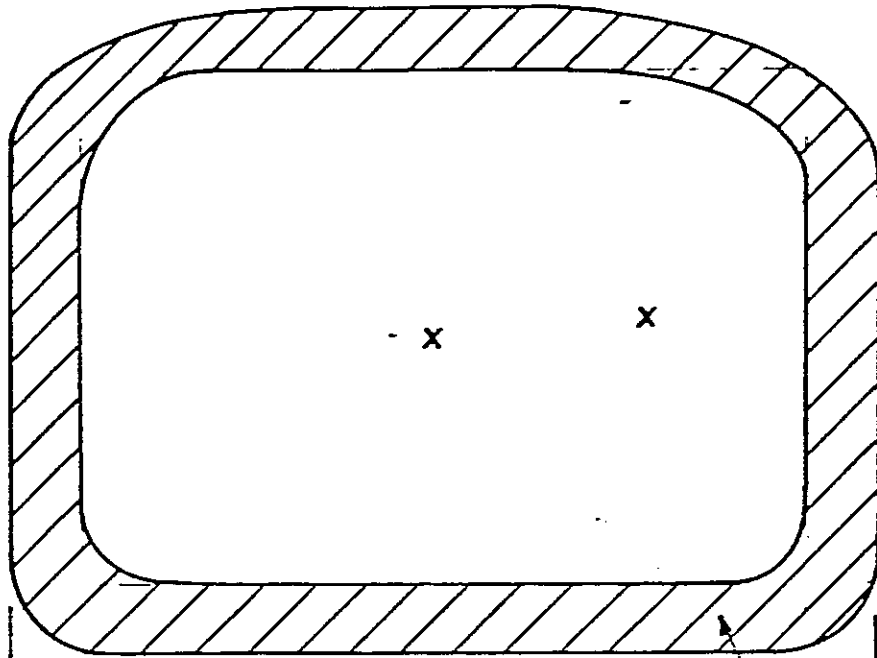
FIGURE I

sample, drying overnight at 100°C and reweighing. The moisture content calculated from these measurements assumes that all moisture and only moisture is driven off by the procedure. The pH of the mixture will be measured and adjusted with buffering to pH 6.5 using ground, agricultural grade limestone. The pile will be covered by a 30 cm. thick layer of screened bagass to entrap odors. The 2 most important parameters for deciding if the compost pile is functioning as it should are the temperature and percent oxygen in the interstitial gas in the pile.

Measurements of temperature will be carried out using conveniently spaced temperature probes situated in place as the pile is constructed. The temperature will be measured using a YSI thermal conductivity meter. The temperature probes with connection leads of 10-12 ft. sheathed by an impervious insulating layer should be sufficient for the temperature measurements. A total of 6 probes is proposed for the 3x3x2m pile, situated as illustrated in Figure II. It is believed that an optimum description of temperature versus composting time can be derived using the scheme.

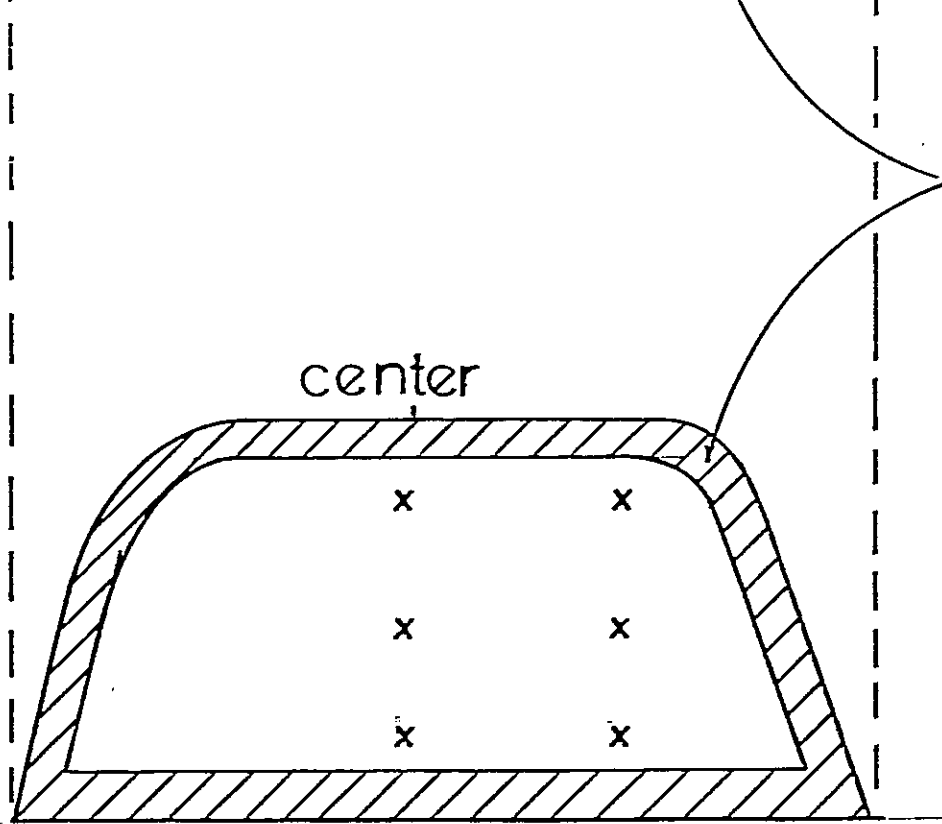
The oxygen percentage of 10-15% will be maintained in the interstitial gas, using the appropriate blower duty cycle. This cycle can be determined by in situ direct measurement of interstitial oxygen in trial compost mounds. To measure the interstitial oxygen without disruption of the mound, 4 funnels covered with screening and located inside the

# Top View Cutaway



X = Temperature probe tip

Screened Bagass Insulation



Side - View Cutaway

## FIGURE II

mound will be connected to tygon hose which will run to the outside of the pile and the tubing to each funnel will be sealed off using a Hoffman clamp. When oxygen measurement is required, the Hoffman clamp will be opened, a small portable battery powered pump will be connected to the appropriate tygon tube and the gas from the mound will be drawn through the fine nylon mesh screening over the funnel through the tube into a drying tube and subsequently into a portable Taylor Servomex Oxygen Analyzer Type OA-250. The scheme is illustrated in Figure III.

#### 4.1.2.1 Possible Problems and Contingency Planning

The conceivable weakness in this scheme is the possible failure of a pile of the dimensions 3x3x2 m. to develop and maintain optimum thermophilic conditions of 60-70°C for at least 2 1/2 days. The original conceptual design was based on use of 1-3 "average" truck-loads of bagass as a bulking agent, each load assumed to contain 6-7 cubic yards. This quantity of bagass was calculated to be sufficient for bulking of sun-dried sludge in 3 sludge drying beds. Sludge drying beds are conventionally layered to a depth of 10-25 cm. with material sufficiently dry for composting and the 6 drying beds of El Conquistador Plant average about 8 square meters each in area. Presumably, sludge could be accumulated from all 6 beds and more bagass could be trucked to the plant if larger composting mounds were needed. This alternative would be likely to involve re-

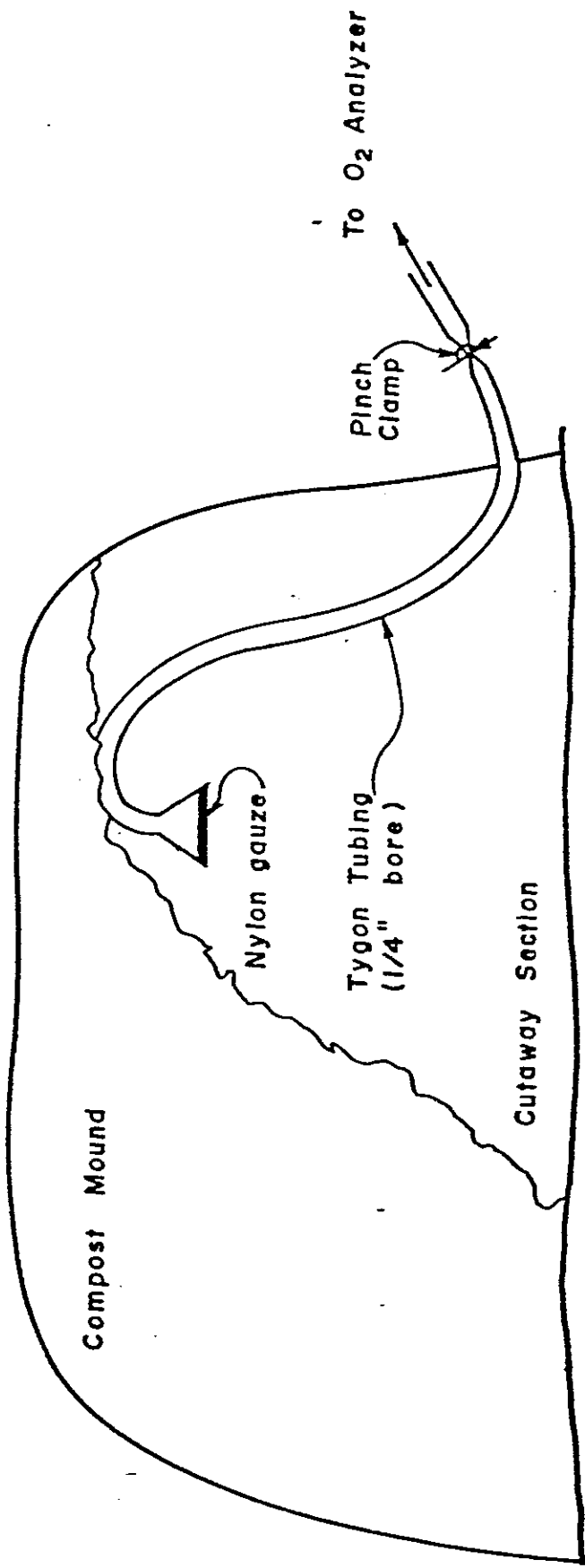


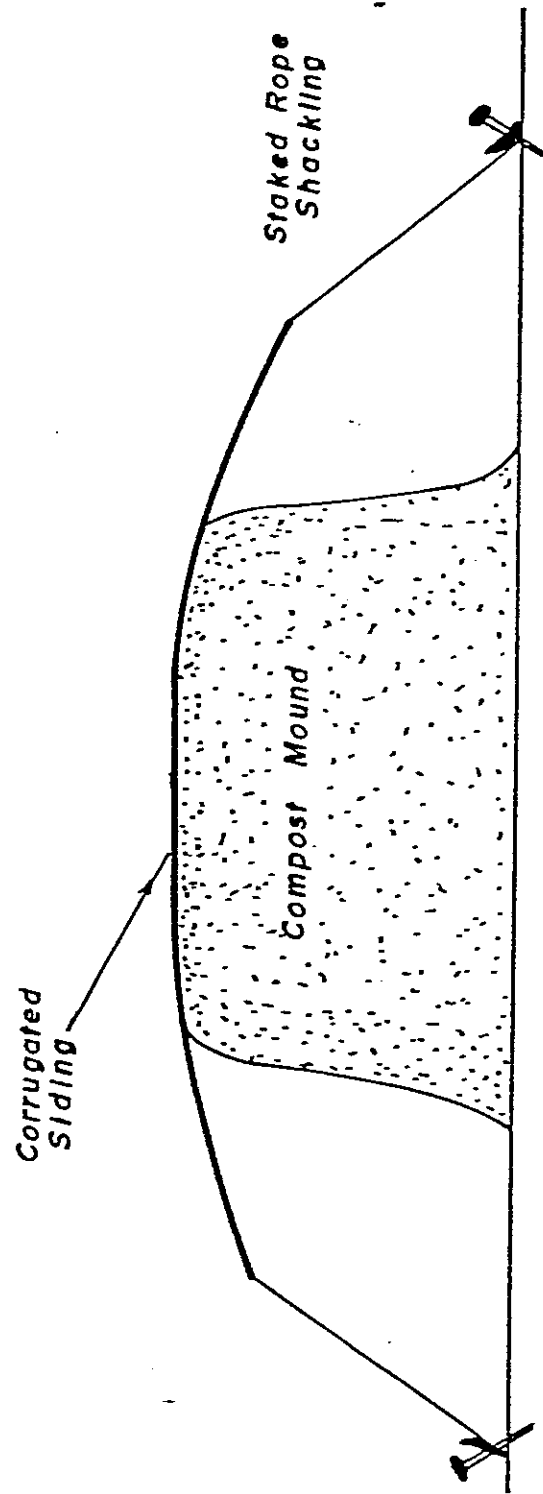
FIGURE III

design of the vacuum air-draw through larger diameter PVC pipe, but this possibility certainly must be anticipated.

It may also be necessary to protect the mounds from excessive precipitation. This protection will almost certainly be necessary during rainier months of the year such as August. To overcome this problem in a cost-effective manner one should consider heavy rainfall coupled with high winds (gusts in excess of 40 km/hr). Thus, a simple permanent open-sided shed is virtually precluded as a solution owing not only to the increased difficulties of pile construction and teardown due to the necessity of working around integral structural elements, but also owing to possibility of pile disruption from gale force winds which frequently accompany major precipitation events in Puerto Rico. Another alternative is to use corrugated metal siding over the top of the pile roped down to stake anchors in the ground. Precipitation will tend to roll off the siding and the stake anchoring should hold the siding and the pile in place reasonably well (Figure IV).

#### 4.1.3 Composting, Sampling and Sample Handling

Sampling and test procedures for determination of the detoxification of wastes present will be carried out every 3 days until a test organism titration<sup>19</sup>-bacteriophage f-2-shows that the indicator organism has declined by an order of magnitude-(10x) from what its level was at the beginning, when the pile was constructed. The f-2 bacteriophage preys upon coliform bacteria and is quite thermoto-



End-on view of mound with sliding covering

FIGURE IV

lerant, making it a natural choice upon which to base composting termination criteria<sup>10</sup>. In fact, field trials of temperature versus number of plated colonies of f-2 might be helpful in establishing this organisms as a public health standard for composting duration<sup>10</sup>.

#### 4.1.3.1 Sampling of the Compost

Small quantities of the composting pile will be withdrawn from 3 distinct places in the pile: one near the surface, one near the center and one near the bottom. Withdrawal of samples will use a non-disruptive technique which prepares samples in advance, suitably packages them and locates them at specific places in the pile for subsequent referral. Briefly, this is carried out as follows. Small sacks of the bagass/sludge mixture are prepared using nylon gauze or fine stainless steel screening and attached to long pieces of synthetic fiber string. When the pile is constructed, the sacks are located at positions at which sampling is to take place. After the appropriate time interval, a sample can be withdrawn by pulling the string gently from the pile. Some sacks will be dosed with pure cultures of the American Type Collection representative f-2 bacteriophage to a known level, so that the titration of f-2 can be carried out to check on the progress of the pile. A correlation of f-2 level with temperature in various parts of the pile will be carried out while sampling for organisms identification is made.

Only aerobic and facultative organisms will be identified inasmuch as the composting procedure, when carried out correctly, should provide an ambient sufficiently hostile to pure anaerobes that they do not survive.



#### 4.1.3.2 Culture Buildup-Virus

With the exception of bacteriophage f-2, no viral handling will be carried out, identification, culture propagation and precise population estimates being beyond the scope of the work proposed.

#### 4.1.3.3 Culture Buildup-Bacteriology and Mycology

Saprophytes and sporulating aerobic and facultative anaerobic bacteria will be cultured from each sample, buildup carried out in liquid thioglycollate medium in screw cap tubes under incubation at the appropriate compost pile temperature. Fungi will be cultured from the sample, enrichment being carried out in Sabouraud liquid medium in the presence of penicillin and streptomycin or chloramphenicol, under temperature conditions the same as those specified for bacteria.

Samples will be taken from the enriching broth and cultured on plain nutrient agar, and agar containing actidione or cycloheximide for the inhibition of fungi. Samples of fungi from the plain nutrient agar will be transferred to new plates containing penicillin, streptomycin and/or chloramphenicol for bacteria inhibition.

Identifiable colonies will be replated and the process of differentiation will be begun. In the case of bacteria, characterization with respect to Gram differentiation will be carried out. Fungi will be differentiated using lactophenol mounts or slide culture techniques. Taxonomy to at least generic level will be carried out using procedures adapted from Bergey's Manual<sup>20</sup>. Those organisms believed to

be prominent among the pile population will be identified at the species level and *Escherichia coli* and *Salmonella* (sp.) will be estimated quantitatively.

Following differentiation, fungi will be characterized at least to generic level<sup>21,22</sup>. Taxonomy at the species level will be carried out for fungi identifiable from the literature as pathogenic<sup>23</sup>. In general special attention will be paid to taxonomy of all thermophilic and thermotolerant bacteria and fungi<sup>24</sup>.

The most likely candidates for which quantification will be attempted are *Aspergillus fumigatus* and *Chaetomium thermophile*, the latter implicated as toxigenic in brine shrimp, chicken embryo and rat bioassays<sup>25</sup>.

Finally, in the case of *A. fumigatus*, establishment of a pure culture of native varieties will be attempted on a medium in which pulverized bagass is the sole carbon source. This experiment may indicate if *A. fumigatus* is as facultative toward sources of carbon in the environment as has been suggested on the basis of laboratory experiments<sup>26</sup> or if local strains of *A. fumigatus*, like certain others previously reported<sup>27</sup>, are unable to use cellulosic carbon in their metabolic processes. While this information is of considerable pedagogic value, the larger issue of compost pile staging as a function of wind direction at a locale would be a more relevant to the goals of this study. Thus if a substantial population of *A. fumigatus* appears during thermophilic conditions, recommendations concerning composting location

with respect to hospitals, allergy and respiratory illness clinics and dwellings for the elderly will be made.

#### 4.1.3.4 Parasite Identification

Initial experiments begun during the literature review will seek to determine the level of parasite eggs present in fresh and partially dried waste sludge from the El Conquistador Plant. During the experimental phase, sample bags as described above will be removed with minimum damage to the pile and examination to determine the presence of eggs of Schistosoma mansoni, Ascaris lumbricoides, Necator americanus and Fasciola hepatica will be carried out. Microscopic examination will also include protozoa.

Methods and procedures for these experiments are standard <sup>28</sup>.

#### 4.1.3.5 Possible Problems and Contingency Planning for Sampling

The most obvious contingency for which planning is necessary is a potential natural lack of pathogen in the sludge which is composted. This is usually not a problem in the case of Salmonella or E. coli. There is little problem with selective addition of f-2 Bacteriophage and this organism can be maintained under laboratory conditions. Thus, introduction of the f-2 Bacteriophage into a pile at convenient levels is definitely feasible.

Collection of eggs of S. mansoni for this purpose is not nearly as straight-forward. In general, eggs for experimentation are collected from the livers of infected animals such as rats. Fortunately, the Department of Microbiology and Parasitology of RCM does maintain its parasite specimens

using an infected colony and access to samples of eggs for implantation in the pile can be had through RCM. Alternatively, an arrangement could probably be worked out with the U.S. Center for Disease Control (CDC) which also maintains a colony in the Rio Piedras Medical Center near CEER. Experimental results could reach a very wide audience in developing countries if the information is made available through CDC.

#### 4.2 Facilities Available

Facilities ultimately to be utilized include: University of Puerto Rico (UPR) Center for Energy and Environment (CEER) Laboratories, laboratory facilities of the University of Puerto Rico School of Medicine, RCM and the PRASA El Conquistador secondary aerobic sewage treatment plant.

##### 4.2.1 Facilities at CEER

The UPR-CEER facilities will be used primarily for maintaining the field work of pile construction, monitoring and establishment of sample cages, and for the parasitology sections of the proposed experimental work. A short description of CEER's capabilities follows.

Both the Terrestrial Ecology and Environmental Health & Impact Divisions will make facilities available to this project.

The Terrestrial Ecology Division has facilities for field and laboratory investigation of environmental phenomena. Its laboratories contain equipment necessary for studies in water chemistry, general, analytical chemistry, cytology, plant physiology, radiation dosimetry, soils science and forest research. Functional equipment include: Perkin Elmer Atomic Absorption Spectrophotometer Model 303, Jarrell Ash

1.5 M Wadsworth Grating Emission Spectrometer, Perkin Elmer Gas Chromatograph Model 3920 with FID, Environmental Chamber, Dissecting and Analytical Binocular Microscopes, Incubator, Shaker, Circulating Constant Temperature Bath, Refractometer, Glass Double Distilled Water System, pH Meters, Gro-Lux Germination Trays, Bomb Calorimeter, Kjeldahl Apparatus, Spectronic 20 Spectrophotometer, Thin Layer Chromatography Outfit and Darkroom with Enlarge and Accessories. Two laboratories nominally 21 m<sup>2</sup> each and a walking freezer are used to store prepare and analyze samples.

The Environmental Health and Impact Division has facilities for field and laboratory investigations of environmental contaminants including complete chemical and microbiological laboratories for water quality analyses. The field equipment includes boats, trailer, and a mobile laboratory for lake studies on water quality. Specialized laboratory equipment of the Division include facilities for laboratory research in radiation chemistry, the use of isotopes, tropical medicine, and parasite identification immunochemistry and biochemistry.

Room 222 is currently used for water quality analyses and contains a chemical fume hood, Beckman J21B centrifuge and a Bausch and Lomb 505 recording spectrophotometer. Room 241 contains the following: Water Associates-Liquid Chromatograph, Packard 3375 Liquid Scintillation Counter with 400 channel analyzer, Picker Liquimat Beta and Gamma Counter, Perkin Elmer 141 Polarimeter, Perkin Elmer 467 Infra Red Spectrophotometer and 3 Gas Chromatography Units (Perkin Elmer, 881, Varian 120, and Perkin Elmer F-21). This equip-

ment is for sophisticated studies in organic chemistry, biochemistry, biology, and medicine. Room 242 contains a large oven, autoclave, and apparatus for distilled water to prepare high quality distilled water and watershed sterile glasswater, suitable for tissue culture studies. Room 260 contains a Hitachi electron microscope, LKB ultramicrotome, freezing microscope, vacuum evaporator, ultraviolet microscope, and other equipment. It is a suitable laboratory for any type of morphological studies at both the light and electron microscope level.

#### 4.2.2 Facilities at RCM

Facilities at RCM which would be available for bacteriological and mycological studies include a general laboratory having a floor area of 50 m<sup>2</sup> (nominal).

Special equipment available through the Department of Microbiology consist of: sterile hood, Gilford spectrophotometer, Coleman spectrophotometers, drying ovens, wet preparation facilities, vacuum pumps, water purifiers, pH meters, cell counters, preparative centrifuges, cell disintegrator, autoclave, analytical balances, high power microscopes, agar plate preparation facilities, walk-in refrigerated storage areas, incubators and a lyophilizer.

#### 4.2.3 PRASA Facilities

The PRASA facilities are located at the El Conquistador Sewage Treatment Plant, Carraízo, P.R. The plant has a treatment capacity of 0.500 MGD, and the treatment is an activated aerobic contact process. The PRASA has agreed to permit the use of land area near the waste sewage sludge

drying beds as shown in Figure v. The land area is sufficient for several compost piles run concurrently, measuring 30 m x 10 m. Electricity is available at the plant, and the plant area is surrounded by cyclone fencing, providing the minimal security necessary. Permission for use of the area has been obtained.

#### 4.3 List of Non-Federal Sponsors

Non-federal sponsors for the project consist of:  
University of Puerto Rico, Center for Energy and Environment Research.

Puerto Rico Aqueducts and Sewers Authority

University of Puerto Rico, School of Medicine

#### 4.4 Milestones or Accomplishments

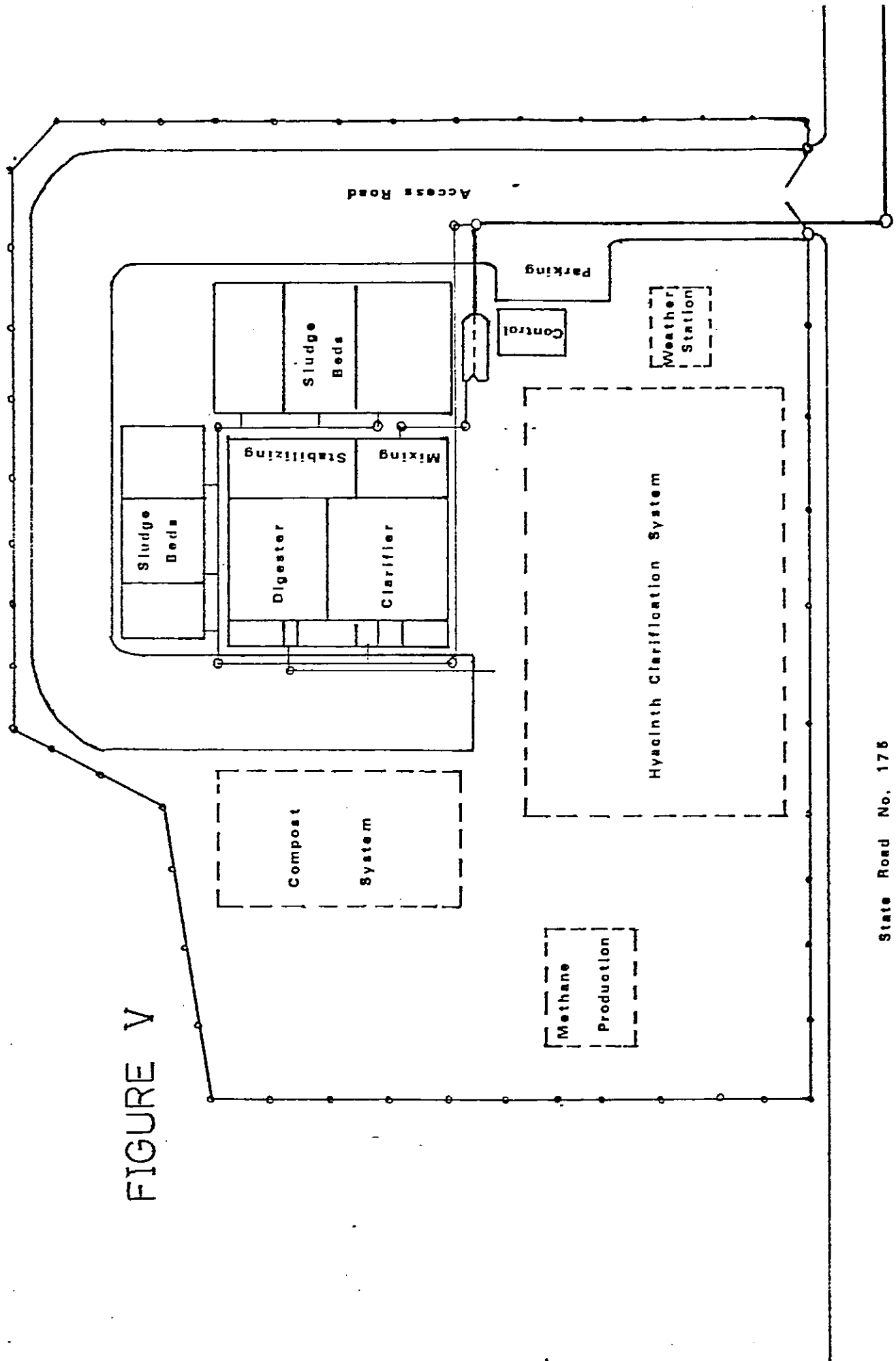
Milestones are calculated on an 18 months basis. The following bar chart summarizes the accomplishments expected and the responsibility for each.

#### 4.5 Task Responsibilities

##### 4.5.1 Critical Bibliography

The literature survey will be carried out by Arthur McB. Block, the project leaver, Nuri Rodríguez Pérez, the head microbiology investigator, Virgen Quiñones, the parasitologist and one student or aide to be named. Advice concerning best, preferred, or significant references involving fungi or health hazards will be sought from consultants: Rafael Cruz Pérez, consulting sanitary engineer, Terry Woodin, mycologist with specialty in thermophilic fungi, Yolanda Mejías, mycologist and Phillips Weil, sanitation engineer with PRASA. Advice on textual preferences in para-

FIGURE V



State Road No. 176



sitology will be sought from Henry Negrón, parasitologist and Head, Environmental Health and Impact Division-UPR, CEER.

#### 4.5.2 Compost Pile Management

Pile construction, maintenance and monitoring will be carried out by Arthur McB. Block, Alvin Mirabal and staff members of the Terrestrial Ecology Division, UPR, CEER. Sampling will be done by Alvin Mirabal, with students and/or aides to be named. Consultant input from Edward Craig and Phillips Weil will be sought for this part.

#### 4.5.3 Bacteriology, Mycologist and f-2 Bacteriophage Management

Microbiology will be under the direction of Nuri Rodríguez de Pérez and she will be helped by students and aides to be named. Consultant Yolanda Mejías will be responsible for review of: mycological techniques and/or culture procedures and consultant Terri Woodin will review procedures and techniques relevant to thermophilic organisms and particularly the fungi.

#### 4.5.4 Parasitology

Parasitology will be done by Virgen Quiñones with the direct supervision of the Head of Environmental Health and Impact Division of UPR, CEER-Henry Negrón.

#### 4.5.5 Reporting

Reporting of each section will be the ultimate responsibility of Arthur McB. Block, the project leader.

#### 4.6 Sampling, Data Collection, Procedures, Methods

Specific innovative sampling, data collection, procedure and methodology has, in general, already been covered in Section 4.1. Routine procedures may vary depending upon which parts of the experimental procedures are anticipated

BAR CHART OF ACCOMPLISHMENTS

Month  
after  
Startup

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

Literature Survey of Sludge Pathogens (Block, Rodríguez)

Pile with f-2 Diagnostic Probe Trial (Block, Rodríguez, Assistant)

Sludge Pathogen Survey (Block, Rodríguez, Quiñones)

Draft Report-Survey (Block, Rodríguez, Wall, Cruz Pérez)

EPA comments (EPA)

Final Report-Survey EPA (Block, Rodríguez, Wall, Cruz Pérez)

Compost Pile Staging (Block, Santos, Assistant)

Bacteriology, Microbiology, Identifications (Rodríguez)

Parasite Identification & Persistence Trials (Quiñones, Block)

Final Report (Block, Rodríguez, Quiñones & Cruz Pérez)

correctly, and in which parts experimental artifacts necessitate on-the-spot decisions resulting in immediate procedural modifications.

#### 4.6.1 Sampling

Sampling of temperature and interstitial pile gas has already been described as has removal of compost samples from the pile. The schedule for sampling given in section 5.1.1 will be followed, in general, for any given pile. Some 10 piles will be constructed in the interest of obtaining statistically relevant averages and testing the efficacy of criteria for pathogen removal from waste sludge based upon depletion of f-2 bacteriophage. A minimum disturbance of the pile should occur using the "prepackaged" sample concept.

##### 4.6.1.1 Sample Handling

Handling of the sample immediately after withdrawal from the pile presupposes a knowledge of the temperature of the sample. The sample will be maintained at that temperature during short term storage and cultivation. Thus portable incubators become a requisite of the sampling process.

Samples collected will be homogenized, slurried, diluted and then cultured in liquid and agar plate media appropriate for the culture of bacteria and fungi. The agar plates will be counted with respect to identifiable colonies of bacteria and fungi. Three different dilutions will be made for this purposes. Depending on the tendency for one microbe species to dominate the compost media, dilutions of the media with

water will be made so as to obtain cultures of less dominant species populations upon plating. Plating and incubation on liquid media such as that described in section 4.1 will be done at temperatures of the actual sample in the pile.

Staining and microscopic identification to the generic level will be done for bacteria, fungi, actinomycetes, etc. Species level keys will be used to identify fungi, while gram staining, gas evolution and indicator agar/pure cultures and microscopic examination will be used to identify the species of bacteria.

The f-2 bacteriophage titration will be part of the routine of sample processing.

Parasitology and identification of parasite eggs in the final product will be done on fresh compost samples sterilized in diethyl ether. Keys for identification are well described<sup>28</sup>. Rough, relatively quantitative information will be derived for single pile runs.

#### 4.6.2 Evaluation of Results

Results will be evaluated on the basis of whether or not the species distribution and relative populations charted as a function of progress of the compost toward eliminating pathogenic organisms is reproducible. The actual data to be taken is described in section 5, but since evaluation of the project results is as important or more important than the results themselves, the evaluation will be summarized here.

First, temperature vs. time curves for each temperature probe will be developed and temperature vs. interstitial oxygen will also be plotted on the same graph. Species dominance and relative distribution will be charted (i.e.

written in at the correct time of measurement) and finally, values of the  $\ln 2$  bacteriophage titration will be plotted, as a function of time. The composite graphical "picture" of a compost pile will show the progress of the composting process as a function of time. The decline of pathogen population using temperature and  $\ln 2$  bacteriophage titration values which are reproducible for secondary pathogens dominate the composting organism populations for preselected periods of time. Any of the curves can be parameterized and compared with those for other compost piles using either different bulking agents as a first step in the formulation of a generally applicable composting model. In the population of a generally applicable model, the rate of proliferation as well as on bagasse bulk as they do on wood chips, peat balls, etc. The ultimate test of the results of the project rests on the reproducibility of species distribution and compost end point parameters over the course of 10 compost pile measurements. Suggestions for future research or questions which should be raised ought to become evident from scanning the graphical representation of results described.

5 Research Project Information

## 5.1 Data and Data Treatment

5.1.1 The Data Treatment in phase I will be secondary in nature in that it will list and summarize published information relevant to pathogenic organisms in waste sludge and in compost from a number of different sources. In the other phases, primary experimental data will be collected in the field and primary laboratory data will be collected in the field and in the laboratory.

### 5.1.1 Experimental Data to be Taken

For compost staging, weight per unit volume and moisture content of bagass and waste sludge and the compost mixture will be measured. Identification of organisms present in the bagass and waste sludge will be carried out prior to mixing and after mixing but before high temperatures are achieved.

During actual composting, measurements of temperature and interstitial oxygen at 6 locations in the pile will be carried out twice daily. Prior to achievement of thermophilic operation, samples will be withdrawn from the 6 representative locations in the pile and the f-2 bacteriophage titration will be made. When the temperature of the pile reaches 55°C in any part of the pile more samples will be withdrawn, characterized with respect to organisms present and with respect to moisture content of the mixture. The f-2 bacteriophage titration will also be made. Thereafter, every 2 days samples will be pulled and characterized in a like manner until the f-2 bacteriophage has declined by 10-fold as evidenced by the titration. Approximate values for population and population changes of E. coli and Salmonella will be derived from these measurements. Growth and vigor of local strains of Aspergillus fumigatus will be examined and quantified under laboratory conditions using media in which bagass is the sole source of carbon.

### 5.1.2 Data Reduction and Display

Data will be sorted to provide temperature vs. time curves and interstitial oxygen versus time curves for all pile locations sampled. Both curves will be plotted on the

same piece of graph paper. The f-2 bacteriophage titration values versus age of the compost pile will also be plotted on the same graph and species distribution as a function of age will also be indicated perhaps by a code letter, if systematic investigation reveals highly reproducibly distributions as a function of pile age or location in the pile.

If the resultant data summaries are reproducible with respect to location in the pile and from pile to pile, the project could be considered successful from the stand point of compost process modelling. Considerable spatial and temporal variation of secondary pathogen populations would indicate the necessity of more research into compost pile parameters useful for assessing pathogen decline.

5.1.3 Test Animals and Human Trials

No experiments on animals or humans will be undertaken in this project.

5.2 Relationship to Other Projects

A draft of a comprehensive socio-economic report commissioned by President Jimmy Carter at the request of Commonwealth Authorities recommends that fallow land be reforested for development of a wood products industry in Puerto Rico. The Krebs Report Draft mentions use of deserted barren land for this purpose. A possible use for large volumes of raw sludge and composted waste sludge is as a land surface cover for pH stabilization of seriously eroded, acid soils. Reforestation would proceed much more rapidly owing to the superior water retention capacity and nutrient content of the compost land topping. At this time, concept-

ual plans for experiments showing the environmental impact of land topping are under consideration, perhaps as adjuncts of a fuels-from-biomass program under way at CEER.

The work proposed in this grant will be co-ordinated with PRASA and results will be communicated in meetings, open forums and publications to the Puerto Rico Environmental Quality Board, Puerto Rico Department of Natural Resources and professional associations with industrial information diffusion systems. Data will be made available upon request to any interested public or private group or organization.

### 5.3 Notice of Research Project:

The form: EPA 5760-1 is appended to this grant application as Appendix III.

### 5.4 Federal Water Pollution Control Act

The proposal contained herein is not a demonstration project of waste treatment. It does not involve storm and combined sewer or overflow treatment. Consequently it does not fall under Section 105 (a) of the Federal Water Pollution Control Act and no certification of approval by the State Water Pollution Control Agency - The Puerto Rico Environmental Quality Board - is necessary.

### 5.5 Clearinghouse Notification

Since the proposal is not directed toward securing funds for a demonstration project, the Puerto Rico Planning Board has not been notified and no notification of any other clearing house appears to be necessary.

### 5.6 Environmental Assessment

The grant proposal is a study or investigation, and as such, an environmental assessment is not required<sup>30</sup>.



### 5.6.1 Environmental Impact Statement

An investigative grant does not require filing of an environmental impact statement<sup>30</sup>.

### 5.7 Construction and Plant Operation Costs

No construction is planned for this project.

### 5.8 Weekly Scheduling of Construction

No construction is planned for this project.

### 5.9 Site Acquisition, Easement, Rights-of-Way Securement.

No site acquisition is planned for this project. No easement or purchase of resource rights or right-of-way securements are planned for this project.

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APPENDIX I

OCT 13 1978

Dr. Ismael Almodóvar  
Presidente  
Universidad de Puerto Rico  
G.P.O. Box 4984-G  
San Juan, Puerto Rico 00936

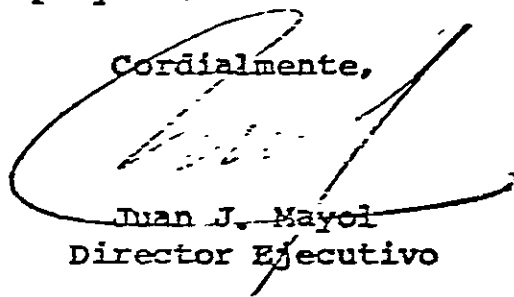
Estimado doctor Almodóvar:

Me refiero a su carta del 29 de septiembre pasado con relación al proyecto de investigación utilizando jacintos de agua para el tratamiento de aguas usadas.

Según me solicita, me place informarle que he aprobado el uso de la planta de El Conquistador para la realización de este proyecto. El Dr. Edward Craig será nuestro coordinador con ustedes y él les orientará sobre el uso de las facilidades de la planta sin que se violen las disposiciones del permiso de descarga que nos otorgó la EPA.

Les deseo mucho éxito en dicho proyecto.

Cordialmente,



Juan J. Mayol  
Director Ejecutivo

JJM:JRG:mor

cc: Dr. Edward Craig-Planificación ✓  
Ing. Rodrigo Montañes-Planificación  
Ing. Elpidio González-Subdirector Operaciones  
Ing. José R. Goitia-Aesor Técnico

OTRO CIVIL

APPENDIX II





CENTER FOR ENERGY AND ENVIRONMENT RESEARCH  
UNIVERSITY OF PUERTO RICO

Feb. 12, 1979

Dr. Gladys Torres de Blasini, Chairperson  
Department of Microbiology  
University of Puerto Rico  
School of Medicine  
Caparra Hqts., Río Piedras  
San Juan, PR 00935

Dear Dr. Torres de Blasini:

I am writing at the request of Dr. Nuri Rodríguez de Pérez of your department. I had approached Dr. Rodríguez de Pérez concerning the possibility of a jointly developed research project in an area of public health which may receive a good deal of attention in Puerto Rico over the next decade. The basis of my decision to seek a relationship with the microbiology department of the University of Puerto Rico School of Medicine was a favorable agency review of a pre-proposal which I developed and submitted to the U. S. Environmental Protection Agency (EPA) nearly 1 year ago.

Briefly, the project involves a detailed examination of microbial ecology and organism succession in a method used for the sanitary disposal of waste sludge from secondary sewage treatment plants. The method has been reviewed in a number of publications by investigators at the U.S. Department of Agriculture (USDA) (cf: Epstein et al, JWPCF 48, 68 (1976)) and the USDA is currently processing the entire output of the Blue Plains Treatment Plant in metropolitan Washington, DC using this method.

The so-called static-pile forced-draft aerobic compost process as used by the USDA at its Beltsville, MD facility has a number of advantages seen to be attractive for application in Puerto Rico.

But there are some uncertainties involved in the broad acceptance of the method here, as well. In any case, U.S. EPA concerns seem to run along the following lines. Raw sludge disposal on land can introduce disproportionately high concentrations of heavy metals which can enter the food chain leading to man via absorption in plants (cadmium uptake), or may render the land useless with respect to agricultural activity owing the phytotoxicity of certain elements (such as zinc). Prolonged ocean dumping of waste sludge can irreversibly affect fragile coastal zones through the introduction of material of high biological oxygen demand (BOD) as well as through the increase in the natural turbidity of the coastal waters. Indiscriminate utilization of waste sludge to amend nutrient deficient soils can lead to an unacceptable pathogen load in crops grown on those soils. Of more than a little importance to Puerto Rico right now are the public health aspects of land-side disposal of waste sludge because new EPA regulations will prohibit ocean dumping of sludges by 1983.

The USDA process uses wood chips mixed with the waste sludge to produce forced draft compost piles which ultimately yield a soil-like material which has better-fixed metallic content than the raw sludge; which can be limed easier to maintain problem elements insoluble after application to agricultural land; which tends to dilute the concentration of heavy elements present in raw sludge; and which has potential commercial importance for nursery or plant bedding industries. The purpose of the wood chips is to confer an easily-aerated structure on the piles and to increase the carbon to nitrogen ratio in the pile to facilitate micro-organism growth.

In Puerto Rico, wood chips could be more expensive. Garbage co-composting requires higher technology and increased energy input, making that method somewhat less desirable as an alternative. Wood chip bulking could also result in efforts which are counter-productive to the stated USDA goals of urban and rural reforestation in Puerto Rico. We have proposed use of bagass, a cellulose-rich by-product of the sugar cane processing industry because it is in fairly plentiful supply at this time and is likely to remain so even in spite of economic difficulties experienced by that industry in recent years. Unfortunately, bagass has never been used as a bulking agent under controlled conditions of static pile forced-draft composting and it is difficult to predict, a priori, if its high cellulose content might create conditions favorable to the production of secondary pathogens. The static pile system uses both mesophilic and thermophilic/thermotolerant organism populations for as long as 16 days per pile to accomplish the detoxification associated with the composting. It is not difficult to imagine that higher average temperatures and average humidity conditions present in Puerto Rico compared with temperate zones of the continental United States may produce a quite different succession of secondary organisms in composting here than has so far been observed under temperate conditions. It is still not known whether the temperatures involved would be uniform enough through the pile to kill eggs of parasites (such as *Schistosoma mansoni* and *Necator americanus*) which are usually considered tropical problems. Could the production of secondary pathogens, using unique composting conditions necessitate worker protection from "farmers lung", a disease long associated with certain thermophilic actinomycetes and spores

of several different fungi? Is location of composting facilities a problem with respect to wind currents near hospitals and homes for the elderly due to excessive production of *Aspergillus fumigatus* spores? Are any other, perhaps more toxic pathogens, produced during the USDA suggested compost procedure here on the island? These are a few of the questions this study aims to answer and which would benefit greatly from a collaborative arrangement between University of Puerto Rico Center for Energy and Environment Research (CEER) and the UPR School of Medicine (RCM):

The CEER could develop in-house capability with respect to microbiology problems, but this alternative is considered less attractive than direct formal collaboration from the standpoint of institutional arrangements and project management which could be worked out and which would obviate elaborate arrangements with consultants at each level. Since Dr. Rodríguez de Pérez has expressed some interest in the development of research in this area and since she has had experience both in bacteriology and mycology, I think she could successfully supervise both of these sections in the proposed research plan. The responsibilities which would make demands upon the time and efforts of Dr. Rodríguez de Pérez are summarized as follows.

It is hoped that permission could be obtained to devote 6-8 hours a week to the supervision and/or instruction of a graduate student or a technician or a scientist or a combination of personnel in the accomplishment of various tasks associated with laboratory development and study of cultures originating in waste sludge compost piles and collected in the field by a CEER scientist, technician, graduate student or a combination of personnel. Salaries

other than those of Dr. Rodríguez de Pérez would be provided through CEER and administrative costs involved would be sustained by CEER. Materials and supplies would be provided from the U.S. EPA funds administered by CEER. Laboratory space at both CEER and RCM would be used for completion of research work. During all phases of the research planned, Dr. Rodríguez de Pérez would be encouraged to develop ancillary interests in the project and help would be provided for submission of proposals to finance ideas or projects she might develop.

Reporting, in all cases would be directly to EPA, though copies of all reports would be made available to any interested party following submission to EPA. Publications, reports and CEER numbered technical documents would be prepared under joint authorship of all participants.

I have enclosed a copy of the EPA comments on the pre-proposal for your perusal. If a satisfactory final proposal can be written, the conventional UPR-CEER and UPR-RCM hierarchy of authorization will be sought before submission of the proposal to the appropriate agency authority.

Sincerely yours,

Arthur McB. Block

AMB: emb

enclosures

cc/ J.A. Bonnet, R.G. Clements, N. Rodríguez



DEPARTAMENTO DE MICROBIOLOGIA

March 2, 1979

1279 MAR 5 1979  
DIRECTOR'S OFFICE  
RIO PIEDRAS

Dr. Arthur McB. Block  
Center for Energy and  
Environment Research  
University of Puerto Rico  
Medical Center  
Río Piedras, Puerto Rico

Dear Doctor Block:

I am in receipt of your letter of February 12, 1979 in which you described the proposed research project on the utilization of bagass as a bulking agent of static pile forced-draft composting. I understand that this project would involve the identification of microorganisms present at any given time in the compost and thru ecological relationship and that this part of the research would be carried out under the supervision of Dr. Nuri Rodríguez de Pérez, from our Department.

I have discussed this project with her and would be willing to allow her to devote the proposed number of hours for this work. I understand that the microbiological procedures under her supervision will be carried out by the assigned personnel on the facilities made available to her in this Department, and that all necessary materials and equipment will be provided, upon her request, from the funds allotted for the above mentioned project.

I sincerely agree with you that if a solid basis for cooperation can be established between the Department of Microbiology and the Center for Energy and Environment Research, this would benefit both entities more than the development of in-house capabilities at the CEER with respect

Dr. Arthur McB. Block

-2-

March 2, 1979

to microbiological problems. It is our desire that the personnel of the Department of Microbiology would be able to cooperate with the Center of Energy and Environment Research in this and in any future project concerning microbiology that this agency would submit.

Cordially,

*Gladys Torres-Blasini, Ph. D.*

Gladys Torres-Blasini, Ph. D.  
Professor and Head

do

APPENDIX III





Appendix IV