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The Bioflo reactor and the respirometer are used to carry out degradation experiments for various environmental pollutants present in propellants as well as degradation of energetic materials. The HPLC, and GC/MS, Capillary Electrophoresis, and the LC/MS/MS are used for quantification and identification of Dinitrotoluene, Nitroglycerin, Diphenylamine, Nitroglycerine and their intermediates. The purchased equipment are utilized in various phases of the projects described below. In particular, these instruments are used for propellant components, intermediate biodegradation products, and enzymes.

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FINAL TECHNICAL REPORT

ANALYTICAL INSTRUMENTATION FOR ENVIRONMENTAL RESEARCH

CONTRACT #93-NIL-359

SUBMITTED TO: *Department of the Air Force*
Air Force Office of Scientific Research (AFMC)
Bolling Air Force Base, DC

SUBMITTED BY: *Stevens Institute of Technology*
Center for Environmental Engineering
Castle Point on the Hudson
Hoboken, New Jersey 07030

FEBRUARY 25, 1995

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ANALYTICAL INSTRUMENTATION FOR ENVIRONMENTAL RESEARCH

I. INTRODUCTION

Stevens Institute of Technology was awarded a contract to establish Analytical Instrumentation for Environmental Research under Department of the Air Force, Air Force Office of Scientific Research, contract number 93-NIL-359. Under this contract the Stevens Center for Environmental Engineering (CEE) acquired and setup analytical instruments and bioreactors to support various research activities funded and supported by Department of Defense (DOD) and other agencies.

Stevens acquired and installed the following instruments at the James C. Nicoll Environmental Laboratory (JNEL) which is the main facility for CEE research.

1. Capillary Electrophoresis Unit
2. Gas Chromatograph
3. Liquid Chromatograph/Mass Spectrometer
4. Gas Chromatograph/Mass Spectrometer
5. Bench Top Fermentor
6. MX3 Autosampler
7. Bioremediation O_2/CO_2 Respirometer
8. Chromatographic software

The equipment have been installed and are operational for the last six to twelve months. The equipment are supporting chemical analysis services needed for several DoD funded environmental engineering research activities. A detailed list of these purchases is provided in Table I of this report.

TABLE I

Equipment Budget and Purchase Cost

I. INSTRUMENTS:

	Item	Vendor	Proposed Cost	Purchase Cost	P.O Number	Itemized Cost
1	**Capillary Electrophoresis**		**$21,480.00**	**$30,963.26**		
		Perkin Elmer	CE Model-270HT		29833	$30,000.00
		CMS	CE stds. & buffers		31684	$963.26
2	**Gas Chromatograph**		**$38,750.00**	**$39,929.71**		
		Varian	GC - Star 3700 CX		29865	$38,706.50
		Varian	GC supplies		31904	$163.00
		Altech	GC Installation Supplies		32498	$584.35
		CMS	GC Supplies		32003	$475.86
3	**Liquid Chromatography Mass Spectrometer**		**$199,000.00**	**$225,510.72**		
		Fisons Instruments	GC/MS & LC/MS		30860	$192,000.00
		Varian	LC pump for LC/MS		31941	$7,879.10
		Alltech associate	LC/MS Installation Supplies		34799	$695.50
		Alltech	LC/MS pump fittings		34857	$12.00
		Conney Safety Products	LC/MS noise protection		34959	$124.86
		Harward Syringe Pump	LC/MS pump		33981	$1,195.00
		Varian	LC system with diode array		29865	$19,500.00
		R. S. Crum & Co	LC fittings		34861	$189.00
		Compressed Gas	Liq N2 & Ar		34609	$131.99
		Curtis Matheson	LC/MS Supplies		32502	$611.69
		Neslab Instrument	LC/MS coolflow		32514	$1,710.00
		Global Computer	Computer Supplies for GC/MS & LC		33981	$511.58
			Instruments Bench Installation			$50.00
		Computer Service	Hard Drive & Tape Backup Upgrade			$900.00
4	**Gas Chromatograph Mass Spectrometer**		**$72,510.00**	**$60,709.17**		
		Fisons Instruments	GC/MS MD 800		30860	$58,000.00
		Curtis Matheson	GC/MS Installation supplies		31903	$859.00
		Curtis Matheson	GC/MS installation Supplies		32116	$748.61
		Chromocol	GC/MS Installation Test Supplies		32516	$80.00

TABLE I

		Tekmar	GC/MS Installation Test Supplies		32512	$370.00
		Restek	LC Supplies		31907	$232.00
		Computer Service	Data Switch for Reactor		0	$84.00
		Altex Electronics	Computer Supplies GC & LC system		32260	$335.56
5	**Bench Top Fermentor (PC ready)**		**$34,953.00**	**$38,686.00**		
		New Brunswick	MX3 Sampler, BioFlow 3000		29159	$35,156.00
		Inter-Syst Comuter	HP 500c Printer		0	$450.00
		Gateway 2000	Computer for reactor		30395	$3,080.00
6	**MX3 Autosampler/ Incubator**		**$9,900.00**	**$2,950.00**		
		CMS	Incubator		29620	$2,950.00
7	**Bioremediation O_2/CO_2 Respirometer**		**$45,000.00**	**$32,360.00**		
		N-con System	Anaerobic		29449	$31,500.00
		Comark	Bi-Nec 96F Laser Printer for Reactor		30395	$860.00
8	**Chromatography Software**		**$6,000.00**	**$10,500.00**		
		Varian	GC, LC, and data system		29865	$10,500.00
	SUBTOTAL		**$427,593.00**	**$441,608.86**		**$441,608.86**
II.	**Renovation and Installation :**					
1	**Installation of Instrumentation**		**$6,500.00**	**$9,403.03**		
					28863	$30.55
					28871	$583.10
					28878	$386.48
					27008	$980.00
					30318	$180.00
					30642	$477.50
					31899	$6,400.00
					32004	$365.40

TABLE I

2	**Installation of Temperature & humidity**	**$15,000.00**	**$4,115.90**		
				34195	$124.00
				34191	$1,089.28
				28599	$1,759.00
				34906	$1,080.00
				34570	$50.00
				UPS	$13.62
	TOTAL	**$449,093.00**	**$455,127.79**		**$455,127.79**

II. INSTRUMENT ACQUISITION

Liquid Chromatograph/Mass Spectrometer/Mass Spectrometer(LC/MS/MS)

The mass spectrometer for the LC/MS system (Quattro I) was acquired from Fisons Instruments and the LC system was acquired from Varian Instruments. The coupling of the system was performed at Stevens. The Quattro I is a state-of-the-art LC/MS system with more capabilities than the Finnigan system proposed in the contract. It is equipped with electron impact (EI), chemical ionization (CI), electrospray ionization (ESI), atmospheric pressure chemical ionization (APcI) sources, which are operable in negative and positive ion modes. This system was purchased in place of the proposed Finnigans' LC/MS system. Quattro's operating and data base software run on a PC Windows operating system unlike Finnigans LC/MS system which operates on a Digital workstation. Moreover, the Quattro is a tandem mass spectrometry system which has the added advantage of working in LC/MS/MS or GC/MS/MS modes. The Varian's Star LC 9010 system equipped with Diode Array Detector (DAD) is interfaced with the ES/MS/MS.

The acquired LC/MS system, Varian-Star LC/DAD and Fisons ES/MS/MS Quattro I, cost $23,000 more than the originally proposed system.

Gas Chromatograph/Mass Spectrometer (GC/MS)

Fisons' bench top quadrupole GC/MS system(MD-800) was acquired instead of Varian's ion-trap GC/MS system (Saturn) proposed in the contract. The ion-trap system (Saturn) is reported to generate CI types of spectra in EI mode with slightly higher carrier gas flow (>1.8 ml) or higher source temperature. This deviation would not allow to generate NIH or NBS library searchable mass spectra. This deficiency was confirmed on a Saturn ion-trap GC/MS system that is operational at Stevens. Therefore, we inspected other benchtop systems available in the market. Fisons' MD-800 is a compact benchtop system operating in Windows environment using a PC. The operating software is relatively easy to use and compatible with our operating system of the Quattro LC/MS.

The cost of the MD-800 system was $60,709. This was $12,000 less than budgeted in the original proposal.

Capillary Electrophoresis(CE)

Perkin-Elmer's capillary electrophoresis unit was acquired instead of the proposed CE system from Isco, Inc. The PE/CE system, model 270HT, is equipped with an autosampler, variable UV detector, reversible power supply, and temperature programming options. These options and compact design of the system are superior to Waters, Isco, and HP systems inspected by Stevens staff.

The cost of the CE system was about $9,000 higher than budgeted in the contract.

Gas Chromatograph(GC)

The Varian GC, Model Star 3700 CX, equipped with FID, TCD, ECD was acquired. The GC system has more features (e.g. an additional detector (TCD) and automation capability) than the Varian GC system originally proposed.

The cost for the GC system was $1200 higher than budgeted in the contract.

Chromatographic Software

Varian's STAR chromatographic data integration software was acquired instead of the Justice Innovations, Inc.'s CHROM PERFECT software. The Varian STAR software can operate in a Windows environment and can control Varian's GC and HPLC systems.

The final cost for the chromatographic data system software was about $4500 higher than that proposed in the contract.

Bench Top Fermentor

The fermentor was purchased from New Brunswick scientific Instrumentation as proposed. The final cost for the fermentor was $3500.00 higher than proposed.

MX3 Autosampler & Incubator

The incubator was purchased from Curtin Matheson Scientific (CMS) as stated in the proposal. The autosampler could not be operated at low temperature as specified by the manufacturer; therefore, it was not purchased under this contract.

The final cost for the items was $7000.00 less than proposed in the contract.

Bioremediation O_2/CO_2 Respirometer

The respirometer was purchased from N-Con Systems and Comark as originally proposed.

The final cost for the respirometer was $12,700.00 less than originally proposed in the contract.

III. SUMMARY OF PROJECTS FOR WHICH THE EQUIPMENT ARE USED

The Bioflo reactor and the respirometer are used to carry out degradation experiments for various environmental pollutants present in propellants as well as degradation of energetic materials. The HPLC, the GC/MS, Capillary Electrophoresis, and the LC/MS/MS are used for quantification and identification of Dinitrotoluene, Nitroglycerin, Diphenylamine, Nitroglycerine and their intermediates. The purchased equipment are utilized in various phases of the projects described below. In particular, these instruments are used for propellant components, intermediate biodegradation products, and enzymes.

The projects for which the equipment were used are described below in Table II.

TABLE II Research Projects for Which the Equipment are Utilized

Contracting Agency	Contract Officer	Contract Number	Contract Type	Value $	Period
U.S. CERL Champaign, IL.	Dr. B. Kim Tel. (207) 352-6511	DACA88-91-D-0011 0004 & DACA88-91-D-0011 00045	Research	140,173	07/12/93 to 08/31/94
U.S. Army Picatinny Arsenal New Jersey	Mr. L. Mondello Tel. (201) 724-3104	DAAA21-93-C-1018	Research	299,661 (First Phase) 342,000 (Second Phase)	04/12/93 to 08/31/95/ 08/31/95 to 01/31/96
Hazardous Substance Management Research Center	Dr. G. Lewandowski Tel (201) 596-3573	BICM-38	Reseacrh	50,000	07/01/93 06/30/94
Hazardous Substance Management Research Center	Dr. P. B. Lederman Tel (201) 596-2457	SITE-41	Research	40,000 (First Phase) 63,000 (Second Phase)	12/01/93 to 06/30/95

1). Contract No: DACA88-91-D-0011 0004 & DACA88-91-D-0011 0005 USCERL: This contract entails the use of mixed aerobic and anaerobic bacterial cultures for the biodegradation of AHH propellant grains. The biodegradation rates of the propellant constituents namely, nitrocellulose, nitroglycerin, triacetin, cellulose acetate and the lead salts (lead hexoate and lead salicylate) are being investigated. In addition to biodegradation rates, the pertinent reactor parameters such as retention time, mixed liquor suspended solids for the aerobic systems, optimum operating temperature for the anaerobic systems, gas production, and biomass wash-out will be examined. Unstructured kinetic models (Monod) are used to estimate biodegradation parameters such as net specific growth rates, half-velocity constants, endogenous decay coefficients, and yield coefficients for each of the propellant constituents under aerobic and anaerobic conditions. These parameters will be used for the design of scale-up units. In the last phase of the study, the results of the previous stages were utilized to design aerobic and anaerobic systems for testing the biodegradation of intact propellant grains.

2). Contract No DAAA21-93-C-1018 U.S. Army Picatinny Arsenal: This project involves the screening, identification and performance of various aerobic and anaerobic microorganisms, both pure and mixed, that degrade gun propellant components such as nitrocellulose, nitroglycerin, dibutylphthalate, dimethylamine, and dinitrotoluene. Once the microorganisms that are able to degrade these substances at satisfactory rates are selected, the beneficial enzyme systems responsible for degradation will be identified. Kinetic data will be collected and fitted to various kinetic models to determine the pertinent kinetic coefficients. Once the biodegradability of individual components has been established, intact propellant will be studied and the rates of degradation by different microbial consortia will be investigated. Combinations of physicochemical and biological treatment schemes will also be explored.

3) Contract No. BICM-38, Hazardous Substance Management Research Center: Contaminated soil together with groundwater is believed to be the best suited medium for bioremediation. Polycyclic Aromatic Hydrocarbons (PAHs) are the most prevalent environmental pollutants contaminating a number of industrial and Superfund sites. The PAHs are problematic largely due to their minimal water solubility, and strong adherence to soil. Despite their proven biodegradability, the strong adsorption to soil makes them unavailable to microorganisms. In addition, the toxicity of high PAH concentration, nutrient limitation and mass transfer problems makes them persistent in nature. Our results showed that the solubility of 2, 3, 4 and 5-ring PAHs can be increased by two to three orders of magnitude by introduction of suitable surfactants. Based on solubility enhancement, biodegradability, and microbial activity inhibition we selected a nonionic surfactant (Adsee 799) for further studies. This surfactant was found to be biodegradable and non-inhibitory up to concentrations of 1% by volume. Substantial increases in the rate of biodegradation was observed in the presence of surfactants both in aqueous solutions and soil-water slurries. The construction of the lab-scale reactor is in progress. The reactor will be used to optimize the pertinent process parameters.

4) Contract No. SITE-41 Hazardous Substance Management Research Center: Pump and treat systems installed in many contaminated sites for groundwater remediation, are expensive to operate and maintain due to the long periods of treatment required. In-situ remedial technologies for the treatment of contaminated groundwater have not, thus far, been proven effective in large scale systems. Therefore, pump and treat is the only option for the treatment of groundwater in many sites. A need for new approaches to groundwater remediation becomes more pressing as the costs of pump and treat continue to increase. A passive in-situ technology which has the potential of replacing pump and treat and it could substantially reduce the cost of groundwater remediation projects is being studied. This in-situ technology, called Trench-Bio-Sparge (TBS), relies on the principle of groundwater flow diversion under natural hydraulic gradients. The diverted flow is forced through a reactor which provides underground treatment of the groundwater. The subsurface reactor can be designed and constructed in such a way as to accommodate biological and/or physicochemical treatment of contaminated aquifers. Although the proposed technology may be applied to both organic and inorganic contaminants in unconfined aquifers, this study concentrated on the biological remediation of PAH contaminated groundwater. The benefits that can be derived from successful implementation of the TBS technology can be enormous, since long term O&M costs of pump and treat system can be avoided.

IV. OTHER CURRENT AND FUTURE USES OF INSTRUMENTS

In addition to the projects outlined above, the instruments purchased under this contract have been used for several smaller research projects funded by a variety of organizations. This research includes treatability studies for soil recovery technologies, basic research on the degradability of various organic compounds and physicochemical treatment process of industrial wastes. In addition, these instruments have been used for undergraduate and graduate student training in various courses offered at Stevens Institute of Technology. In the future we anticipate to use these instruments to support the analytical needs of the Center for Environmental Engineering in several DoD and DOE funded projects.