Technical Publication

Title: On-line Monitoring of Nutrients and Microbiological Contaminants In Controlled Plant Growth Environments

ASA Publication Number: 9

Presented at: 1991 Food Industry
Environmental Conference
Atlanta, Georgia
November 11-13, 1991

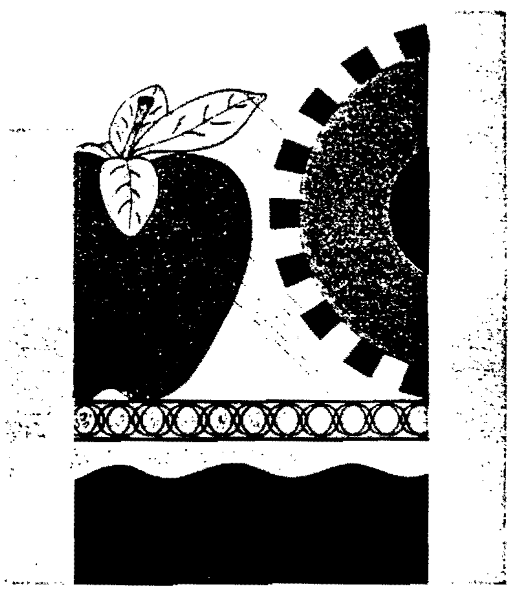
Note: This document was originally published by Biotronics Technologies, Inc. in conjunction with the ChemScan Process Analyzer technology base, now owned by Applied Spectrometry Associates, Inc. Please direct all inquiries and correspondence to:

Applied Spectrometry Associates, Inc.
W226 N555G Eastmound Drive
Waukesha, WI 53186

Phone: (414) 650-2280
FAX: (414) 650-2285

1991 Food Industry Environmental Conference

November 11-13, 1991
Atlanta, Georgia



GTRI

Georgia Tech Research Institute
Georgia Institute of Technology
A Unit of the University System of Georgia

ON-LINE MONITORING OF NUTRIENTS AND MICROBIOLOGICAL CONTAMINANTS IN CONTROLLED PLANT GROWTH ENVIRONMENTS

Kenneth J. Schlager, Ph.D.,
Kathryn M. Schloegel,
Maureen Owens,
and
Bernard J. Beemster
Biotronics Technologies, Inc.
W226 N555B Eastmound Drive
Waukesha, WI 53186
(414) 896-2650

ABSTRACT

One approach to the problem of groundwater and surface water contamination from agricultural chemicals is the cultivation of plants in systems that are designed to permit the collection and recycling of nutrients through a controlled environment. Several recent NASA projects have focused on the technology developments needed for this concept to succeed, including the continuous on-line analysis of nitrates, phosphates, sulfates, trace metals and other nutrients using absorption and emission spectroscopy and the continuous on-line monitoring of microbiological contaminants in nutrient solutions using fluorescence analysis techniques. The success of these monitoring techniques for space application offer many potential advantages for use in water conservation, waste treatment and environmental protection applications back on earth.

CELSS GOALS AND OBJECTIVES

The Controlled Ecological Life Support System (CELSS) is a long range interdisciplinary program under NASA sponsorship to assemble the knowledge to design, construct and operate a self-contained bioregenerative life support system. NASA's objective is to provide a nutritionally stable and reliable environment for a permanent human presence in space, but the research related to the accomplishment of these space ecology objectives have important immediate application to earthbound ecological problems.

Major CELSS Subsystems

Research supported by the Kennedy Space Center will provide hardware, control systems and techniques for three major CELSS subsystems:

Biomass Production Chamber. A biomass production chamber (BPC) will incorporate technology to grow plants under controlled conditions. The majority of current efforts are devoted to higher plants such as wheat, soybeans, sweet potatoes, lettuce, rice and sugar beets. Plants are now being grown by a nutrient film technique in plant growth trays contained within sealed chambers that have rigidly controlled artificial atmospheres. Several such chambers are currently in operation at the Kennedy Space Center and at the University of Wisconsin.

Food Processing Modules. Food processing modules are being developed to extract edible content from all plant materials. This area includes microorganism based systems and technologies to convert inedible biomass into edible biomass.

Waste Management Modules. Waste management modules will recover and recycle all solids, liquids and gases necessary to support life in the controlled environment.

All of these subsystems require analytical monitoring of critical operational and environmental parameters. This requirement includes continuous real time analysis of nutrient solution chemical content and nutrient solution microbial content in the biomass production chamber. Analysis of microbial populations will also be necessary for control of the waste management and food processing subsystems.

NUTRIENT MONITORING RESEARCH AND TECHNOLOGY

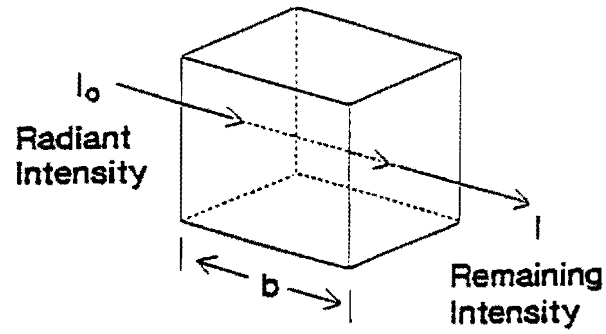
Absorption Spectrometry for Nutrient Chemical Analysis

The Need for Nutrient Analysis. The nutrient solutions used in hydroponic plant growth systems are chemically complex mixtures that contain all of the water borne elements necessary for optimum plant growth. These include nitrogen, phosphorous, potassium and several trace metals. The nutrient solutions are continuously recirculated through special trays used to support the plant and allow contact between the nutrient solution and the plant roots. While the solution circulates, the nutrients are absorbed by the plants at greater or lesser rates depending upon the specific plant and stage of growth. This absorption results in a nutrient imbalance in the solution that must be corrected through replenishment of the individual nutrient components.

Absorption Spectrometry Fundamentals. One approach to the problem of continuous analysis of the individual chemical constituents in nutrient solutions is to monitor the absorption spectra of the solution. The absorption spectra for a mixture will be a function of the individual absorbing components in the solution and the interaction of their spectral overlaps and interferences across a certain selected wavelength range. Several of the chemical components in the nutrient solution (including nitrates and transition metals) are known to absorb light in the ultraviolet-visible wavelength range.

Single Component Chemical Analysis. The absorption of light in the ultraviolet-visible range is a result of shifts in the electronic energy of an atom or molecule caused by excitation from the appropriate wavelengths of light. Absorption spectrometry is based on Beer's Law which states that absorption through a liquid medium is a function of the absorptivity of the medium, the path length through the medium and the concentration of the absorbing components in the medium. These relationships can be restated to solve for concentration of an absorbing component if the absorption at a known path length through the liquid can be measured. Figure 1 illustrates the basic relationships defined in Beer's Law.

Absorption at a specific wavelength can be thought of as the ratio of the incident intensity value of light at a specific wavelength before passing through the liquid to the value of the remaining light intensity at that same wavelength after passing through the liquid. Measurement of absorption at several wavelengths over a range will result in an absorption "signature" for the liquid over the wavelength range, with smaller intervals between wavelengths resulting in better resolution of the absorption signature.



$$\text{Absorbance} = \log \frac{I_o}{I} = \epsilon\, b\, c$$

where

ϵ = molar absorbtivity constant
b = path length thru sample
c = concentration of absorbing substance

Figure 1. Beer's Law

Multi-Component Chemical Analysis. In the case of a solution with only one absorbing component, concentration can be related directly to the absorption measurements, often using a single (peak) wavelength. In more complex solutions, however, absorption at any one wavelength may be the result of the combined effects of several absorbing components. Each absorbing component has its own unique absorption signature that remains constant in relative shape but changes in intensity as concentration changes. If this signature and the signature of the other absorbing components can be characterized, their influence on the composite absorption signature for the solution can be calculated using pattern recognition algorithms. These algorithms, frequently referred to as "chemometric models", permit absorption spectroscopy to be used for multi-component chemical analysis.

Since specific absorbing components may contribute significantly to the absorption measured at one specific wavelength but little or nothing to the absorption at another wavelength, multicomponent chemical analysis using absorption spectrometry will usually require measurements of absorption at several different wavelengths. If the analysis is to be performed "on-line" such as on a flowing sample, special instrumentation is needed that is capable of capturing information for a range of wavelengths and for immediate interpretation using chemometric models.

Instrumentation. One approach to this problem is the use of a specially designed on-line absorption spectrometer featuring a flow through optical probe connected to a central analyzer with fiber optic cables. The central analyzer contains a xenon flash lamp light source for excitation at wavelengths from 200 to 800 nm. The analyzer also contains a fixed diffraction grating and a linear photodiode detector array for separation and measurement of light returned from the optical probe after transmission through the liquid under investigation. Array detectors permit rapid collection of absorbance information across a wide wavelength range.

Objectives of NASA Nutrient Analysis Investigation

During the first half of 1990 a CELSS instrumentation research project was conducted under NASA sponsorship. The purpose of this research was to explore the feasibility of on-line spectroscopic measurement of nutrient solutions used in the CELSS Biomass Production Chamber. Several spectroscopic alternatives were proposed for investigation, including primary absorbance, secondary absorbance, and secondary fluorometry. (Primary spectroscopy uses the natural properties of the chemicals in the solution under investigation. Secondary spectroscopy requires the use of reagents - usually immobilized chelate chemicals - to selectively and reversibly react with a target analyte to produce a complex that possesses detectable absorption or fluorescent characteristics.)

The original objectives for this research project were to identify a means of on-line measurement for eight of the sixteen nutrient parameters, although all sixteen parameters eventually were evaluated under the program.

Technical Approach

Screening. All of the analytes were initially screened in a pure water medium to determine the existence of any primary absorption spectra. Analytes that exhibited significant primary absorption spectra were then quantitatively evaluated in pure water at various concentrations to establish sensitivity properties.

Chemometric Algorithm Selection. If sensitivity investigations for an analyte indicated that it was possible to monitor the analyte within the CELSS range of interest, tests were performed in prepared solutions that contained a random background of absorbing components. Chemometric methods that are capable of predicting the concentrations of a test solution based on information from a learning set of known concentrations were evaluated to find the combination of data analysis techniques that produce the lowest average error, as illustrated in Figure 2. Finally, some analytes were tested at various concentrations in actual nutrient solutions that contain a random background of all other nutrient components to demonstrate the potential of absorption spectrometry and to verify the chemometric models selected for analytical use.

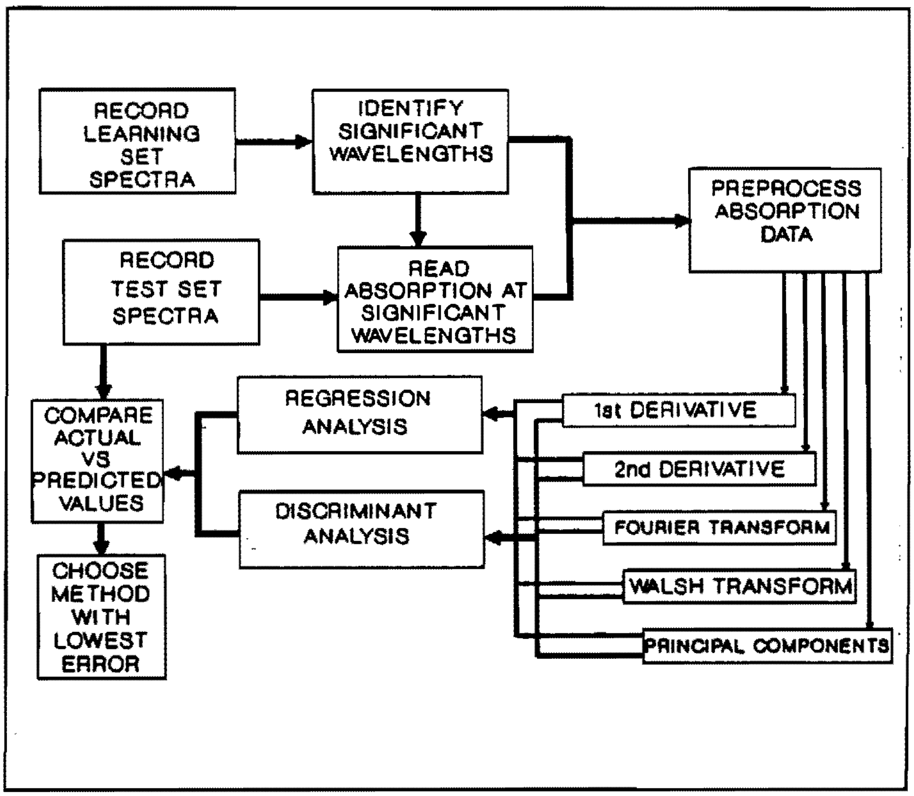


Figure 2. Chemometric Algorithm Selection

Secondary Absorbance Investigation. Analytes that failed to exhibit adequate primary absorbance characteristics were evaluated to determine if a suitable ligand chemical were available that would yield a chelant complex with adequate secondary absorbance characteristics.

Analytical Results

Of the sixteen analytical parameters investigated, fifteen were considered to have significant primary or secondary absorption spectra. These results are summarized in Table 1 below.

ALTERNATIVE TECHNIQUES FOR UV-VISIBLE ABSORPTION ANALYSIS					
ORIGINAL STUDY OBJECTIVES			EXPANDED STUDY OBJECTIVES		
	ABSORBANCE			ABSORBANCE	
ANALYTE	PRIMARY	SECOND'Y	ANALYTE	PRIMARY	SECOND'Y
Fe	YES	YES	Na	NO	YES
K	NO	YES	Cl	NO	YES
Mg	NO	YES	Mn	YES	YES
Ca	NO	YES	Cu	YES	YES
pH	NO	YES	Zn	YES	YES
NO_3	YES	NO	MoO_4	YES	YES
H_2PO_4	YES	YES	BO_3	YES	YES
SO_4	YES	YES	O_2	NO	NO

Table 1.

In addition, nitrate and iron analytes were subjected to extensive tests using various concentrations against a random background of all other nutrient components. Results from these tests demonstrated conclusively that absorption spectrometry was capable of highly accurate multicomponent chemical analysis.

Nitrate analysis was demonstrated with an error of prediction under 1.0 ppm in random nutrient solutions using a twenty member learning set with actual nitrate concentrations ranging from 10.0 to 500.0 ppm. Iron analysis was demonstrated using a twenty member learning set with actual iron values of from 0.0 to 10.0 ppm, resulting in an error of prediction under 0.03 ppm. Chemometric methods used for the analysis of nitrate and iron consist of stepwise regression using untransformed absorbance values at three wavelengths for each analyte.

EMISSION SPECTROMETRY FOR NUTRIENT CHEMICAL ANALYSIS

The need for Analytical Alternatives

Although absorption spectrometry was demonstrated to be capable of providing the means for on-line analysis of many chemical substances, some of the most important nutrient analytes need secondary methods requiring immobilization of ligands in order to produce a detectable absorption spectra. If these analytes were to be simultaneously monitored, the resulting apparatus required would be awkward and expensive.

A technology was discovered in the later stages of the NASA nutrient analysis research that offered the potential of simultaneous analysis for many chemical elements that would not otherwise be able to be detected using ultraviolet-visible absorption spectrometry. This technology was named "Liquid Atomic Emission Spectrometry (LAES)".

Basic Principles of Liquid Atomic Emission Spectrometry

Conventional Atomic Spectrometry. Several technologies are presently in use that permit analysis of elements in their atomic form. Because the elements to be analyzed are usually presented for analysis bound in molecules, energy must be applied to rupture the molecular bonds and reduce the substance to atomic form prior to analysis. The various forms of energy used to break molecular bonds differentiate several different forms of atomic spectrometry presently in use (flame, electrothermal, electric arc, electric spark, radio frequency inductive coupling).

Once a substance has been reduced to atomic form, several techniques are used to extract qualitative and quantitative information. Specific wavelengths of light can be transmitted through an energized cell and the amount of light absorbed by the atomic element under investigation can be measured (atomic absorption spectrometry). Another alternative is to measure the wavelength and intensity of light emitted from a cell that contains atomic matter following excitation of the cell with a burst of energy.

Atomic Spectrometry in Liquids. All of the above spectrometric technologies convert the material under analysis to a gaseous state or plasma prior to analysis. This state allows individual atomic elements to produce emission or absorption lines at specific wavelengths that are predictable for each element with a specific form of excitation and detection. This technique, however, requires that a sample of the substance being investigated be extracted from the source.

For on-line analysis of liquids, an analytical technique that will permit simultaneous analysis of many elements directly in the flow stream is required. Liquid Atomic Emission Spectrometry (LAES) is a new form of atomic spectrometry in which an arc or spark discharge is used to generate atomic light emission directly in the liquid medium. The light emission not only occurs directly in the liquid, but is also detected directly in the liquid using special apparatus that will permit in-situ simultaneous analysis for a spectrum of individual elements.

Instrumentation. The instrumentation required for Liquid Atomic Emission Spectrometry is similar to the instrumentation used for Ultraviolet-Visible Absorption Spectrometry. In fact, one of the principal advantages of LAES is that the same 1024 element photodiode array detector can be used for both LAES and UVAS analysis.

The LAES instrument used for experimental analysis of NASA nutrient solutions used a beaker to hold a sample of the liquid (a flow through cell will be used for on-line analysis in the future). An arc was created in the beaker using electrodes immersed in the nutrient solution and separated by an electrode gap. A fiber optic cable linked the analyzer and the beaker with the termination of the cable located in close proximity to the arc gap. This arrangement provided the energy necessary to accomplish molecular breakdown of the elements in the solution, as well as the energy necessary to stimulate atomic light emission from these elements.

The analyzer used for the LAES experiments was identical to that used for the ultraviolet-visible absorption experiments discussed above. The fiber optic cable was connected to a spectrograph containing a diffraction grating which separates light returned to the analyzer through the cable into discrete wavelengths from 200 to 800 nm and projects these wavelengths onto a 1024 element linear photodiode detector array. Each element in the array is matched with a dedicated capacitor that can be rapidly scanned to accumulate information and convert the information into digital values. This information can then be processed by pattern recognition software contained on a 80286 computer built into the instrument. A block diagram of an instrument designed for both absorption and emission options is shown in Figure 3 below.

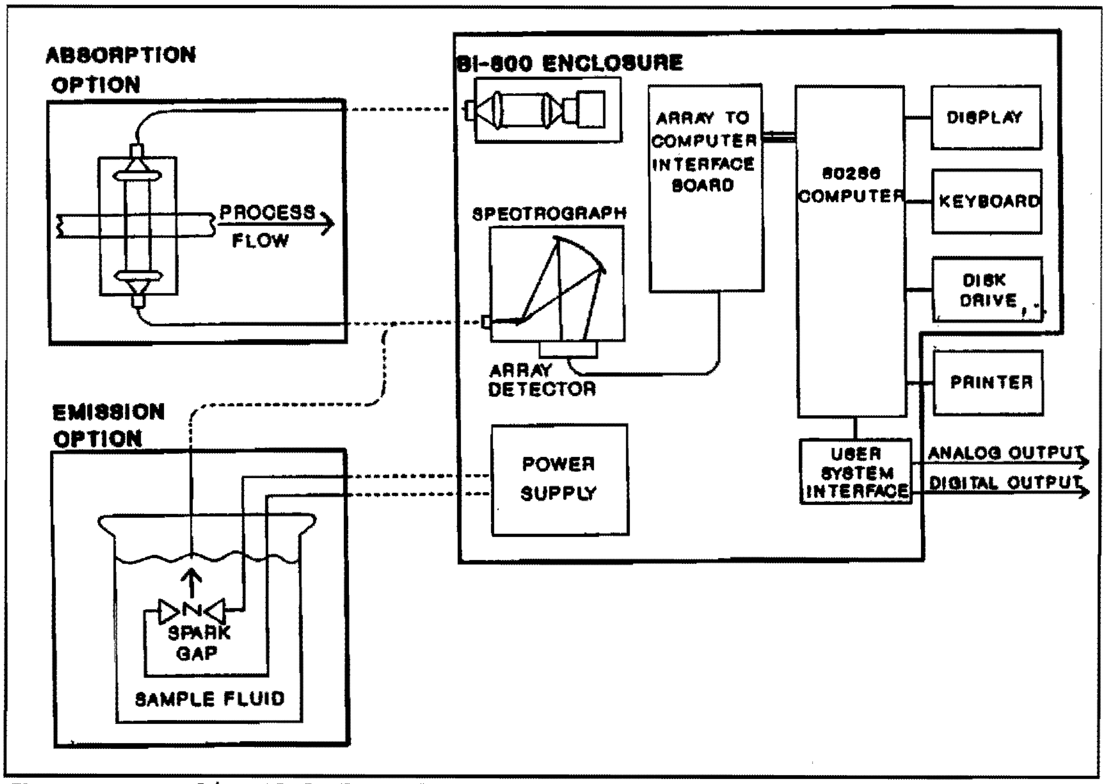


Figure 3. UVAS/LAES SPECTROMETER

Technical Approach for Nutrient Analysis

A series of experiments were conducted near the end of the research program to explore the capability of the LAES concept and the potential for use of LAES as an on-line monitoring technology. Solutions were made using distilled water and a single compound that contained an analyte of interest. Several individual test solutions were made containing known concentrations of each analyte. Each individual solution was subjected to an arc induced excitation, and the resulting light emission was captured and recorded using the instrumentation described above. Since customized pattern recognition software was not available for this new technology, a standard conventional atomic emission spectra guide was used to help identify the emission peaks observed during the experiments.

Following experimentation with the individual analyte solutions, a multicomponent nutrient solution with known components was tested and the results were recorded.

Initial Results

Individual Analyte Solutions. All of the solutions tested exhibited prominent peaks at or near the wavelengths predicted for the element under investigation when liquid atomic emission results were compared to the known emission lines from conventional atomic emission techniques. An example can be seen with sodium, shown in Figure 4, which would conventionally be expected to show peak emissions at 589 nm. The LAES experiments showed strong peaks at photodiode element number 654, which translates into a wavelength of approximately 589 nm. Furthermore, results show that the relative intensity of the peak is consistent with the relative concentration of the sodium contained in each of the test solutions. Similar results were obtained for magnesium, calcium, potassium, copper, and zinc.

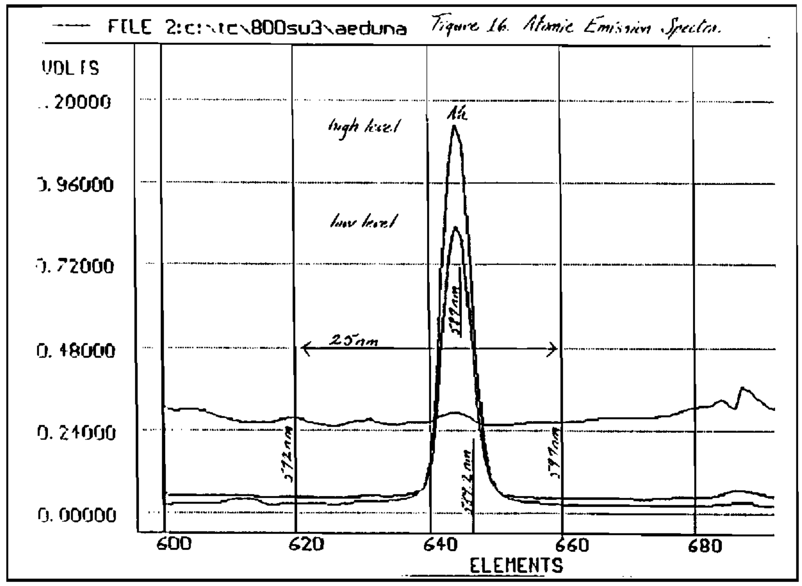


Figure 4. Sodium Emission Spectra

Nutrient Solutions. Additional experiments were performed with a multicomponent nutrient solution in order to illustrate the ability of the LAES technique to simultaneously capture multiple element emission peaks. Although some peaks were not identified, the emission information detected was able to be matched with expected peaks for calcium, copper, zinc, hydrogen, sulfur, magnesium, molybdenum, manganese, potassium and oxygen. No attempt was made to quantify these elements due to the lack of sufficient information to establish adequate calibration models.

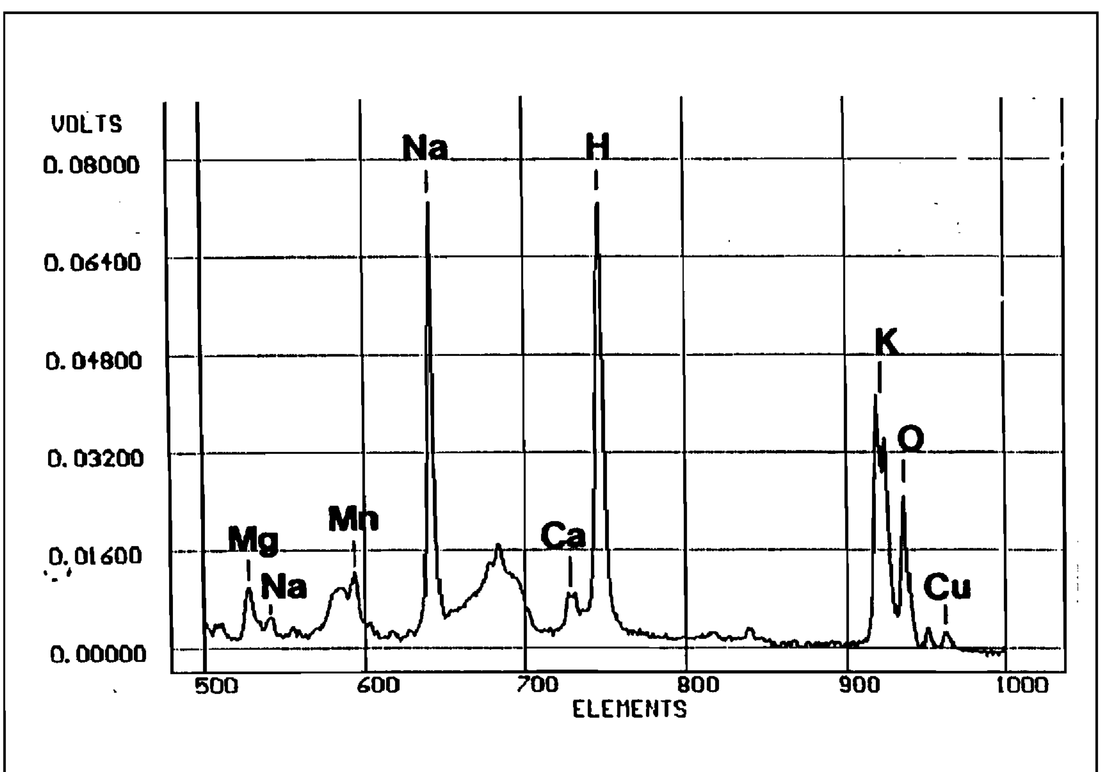


Figure 5. Liquid Atomic Emission Spectra

FLUORESCENT SPECTROMETRY FOR MICROBIOLOGICAL MONITORING

Purposes for Microbial Monitoring

Certain forms of bacteria and fungi can limit the effectiveness of the biomass production systems used in the CELSS. Because of this, BPC nutrient solutions will need to be monitored to measure total microbiological populations and also for the presence of individual nuisance species of bacteria or fungi at concentrations that are harmful to plant growth. Another CELSS microbiological monitoring need includes drinking water which must be monitored to assure that it is safe for human consumption. Several CELSS bioreactors using biological processes will require a means of rapid or continuous analysis of total microbiological populations and individual microbial species for control purposes.

Conventional analysis techniques such as culturing and microscopic assays are not able to provide the real time information needed for close control of biological processes and water supplies. Conventional optical monitoring techniques, such as optical density analysis or cell mass analysis are not able to distinguish between living and dead cells, a distinct disadvantage in a "closed loop" system, where process solutions are continuously recirculated. None of these conventional analysis techniques are able to provide on-line microbial identification.

Because of the need to provide both automation of microbial monitoring tasks and close control of microbiological parameters in process liquids, NASA sponsored an additional instrumentation research project during the first half of 1991 to evaluate fluorescence as a feasible technology for on-line microbiological analysis.

Principles of Microbial Fluorescence

Luminescence. Fluorescence is a special case of the more general phenomenon of luminescence. Luminescence is the emission of light by matter following absorption of some form of energy. The types of luminescence are classified according to the mode of energy input causing the luminescence: fluorescence and phosphorescence (light), chemiluminescence (chemical), electroluminescence (electrical energy), etc. In all cases, absorption of some form of energy by the luminescent matter precedes the emission of light. In the case of microbiological analysis, we are concerned with the light as the energy source.

Rapid Decay Time for Fluorescence. Absorption of light excites electrons in the molecular structure of matter. In most materials, this light energy is converted to heat with no emission of light. If the matter is fluorescent, light is emitted but decays rapidly (nanoseconds) after the excitation source is removed. If the emitted light persists for longer periods (micro or milliseconds) after the excitation source is removed, the phenomenon is known as phosphorescence.

Absorption and Emission Spectra. Fluorescence and phosphorescence are characterized by two spectra: an absorption spectrum (as the excitation light is absorbed) and an emission spectrum. Matter that is phosphorescent will exhibit both a fluorescent and a phosphorescent spectrum, but typically only one of the spectra will be sufficiently strong to measure. Each compound that exhibits these characteristics can be identified with spectral signature that is unique for the compound.

Use of Fluorescence for Microbial Analysis. Fluorescence is well suited to microbial analysis because all living cells contain both nicotinamide adiene dinucleotides (NADPH) and adenosine triphosphate (ATP), metabolic cofactors which are natural fluorophores. Since the relative amount of these and other fluorescent substances (such as tryptophan or tyrosine) present in the cell will be different for each species, it should be possible to identify a unique fluorescent signature for each species. Even if there are a number of different species present in a solution, the combination of their individual signatures will predictably contribute to the overall fluorescent signature for the solution. Differences in decay time for each signature may also aid in detection of individual species. Pattern recognition (chemometric) techniques should be able to process fluorescent signature information such that the individual species and their respective concentrations in the solution can be identified.

Instrumentation. Two different instruments were used in the research project. A conventional laboratory luminescence spectrometer was used to characterize samples for excitation and emission spectra throughout the research project. This spectrometer measures fluorescence and phosphorescence in static samples contained in a cuvette. Sample can be analyzed using either transmissive or reflective (backscatter) techniques. The instrument uses a pulsed Xenon discharge lamp for excitation and a gated photomultiplier for detection.

A series of experiments were also performed using an on-line filter based fluorometer. This instrument has the capability of providing multiple (eight) selective excitation and emission wavebands and was used to demonstrate the feasibility of on-line analysis of biological species through detection of their fluorescent spectra. The instrument includes a Xenon arc excitation source, photomultiplier tube detector and built in microcomputer containing analytical algorithms. A optical probe is connected to the analyzer using bifurcate fiber optic bundles.

Technical Approach and Results for Microbiological Analysis

Fluorometric Indicator Characterization. Candidate fluorometric indicators of microbial activity were measured in order to characterize their spectral features. Tryptophan and tyrosine were measured as isolated substances and in combination to establish their excitation and emission spectra. An example of the spectra observed can be seen in Figure 6. Two fluorophores, NADPH and ATP are difficult to measure in isolation from living cells, but were evaluated in both fungi and bacteria even though such analysis could only provide a rough approximation of the pure biochemical compound. In addition, tryptophan was also evaluated in fungi and bacteria for comparison to results from isolated analysis.

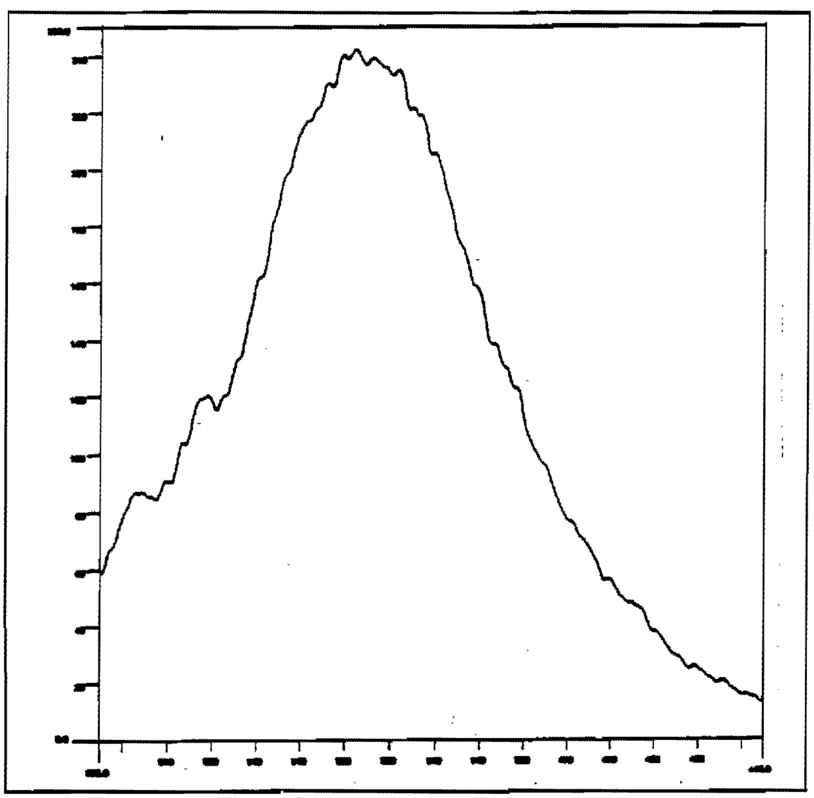


Figure 6. Tryptophan/Tyrosine Spectra

This analysis provided background information concerning sensitivities, relative intensities of spectra, and the location of possible excitation and emission sets for use in later experiments.

Metabolic State Analysis. Experiments were conducted using a facultative microbe (flavobacteria) before and after a change in metabolism from an aerobic to an anaerobic state. Since NAD serves as a cofactor in metabolic reactions, the increasing intercellular NADPH concentration in the aerobic condition should result in a greater fluorescent intensity for the microbe, compared to the intensity in the anaerobic condition.

Results from the experiment show a measurable (3.9 intensity unit) difference in fluorescent intensity between the aerobic and anaerobic states for the facultative microbe. A repeat of the experiment using an aerobic microbe (pseudomonas fluorescens) shows and enormous drop (39.0 intensity units) in fluorescent activity under anaerobic conditions, as would be expected.

On-Line Experiments With Bacteria and Fungi. Two microbes, flavobacteria and trichoderma reesei fungus were selected for experimental analysis due to their pertinence to CELSS objectives. Both microbes were individually characterized at several concentrations using the laboratory luminescence spectrometer to select optimal excitation and emission wavebands.

Experiments were conducted using the On-Line Fluorometer, after installation of the appropriate excitation and emission filters. This instrument was calibrated using a simple two point slope-intercept model, a very unsophisticated analysis algorithm by today's chemometric analysis standards. Each microbe was individually analyzed at three concentrations in water to test the ability of on-line analysis to track changes in concentration.

Results from these experiments are reported on Table 2 below and demonstrate the ability of the instrument to closely track concentration variations for these microbes.

RESULTS OF ON-LINE TRACKING EXPERIMENT

MICROORGANISM TESTED	PREDICTED CONCENTRATION FROM ON-LINE ANALYZER	ACTUAL CONCENTRATION BASED ON DILUTIONS
Flavobacteria	25.2%	25.0%
	54.2%	50.0%
	98.6%	100.0%
Tricoderma	22.7%	25.0%
	67.0%	75.0%
	104.0%	100.0%

Table 2

Simultaneous Analysis of Bacteria and Fungi. Flavobacteria and trichoderma reesei samples were mixed in water in varying proportions to create combined samples with several mix ratios. Each combined sample was analyzed on the laboratory luminometer with three different excitation wavelengths and emission wavebands. One scan was at an excitation and emission set considered to be optimal for the bacteria, another was at the set was considered optimal for the fungi, and the third scan set was at an intermediate region. A total of 25 samples were processed, 20 as a calibration set and 5 as a test set.

This analysis, although insightful, was hampered by the long run time required (45 seconds) and the tendency of the fungi to settle, even when a magnetic stirrer was used. Analytical models employed included regression of untransformed fluorescent values and regression of fluorescent spectra principal components.

The lowest standard error of prediction (1.42%) for flavobacteria was produced using regression of fluorescence values produced by the excitation and emission set considered optimal for bacteria, as expected. The lowest standard error of prediction (5.74%) for trichoderma reesei was produced using regression of fluorescence values produced by the intermediate excitation and emission set. Remarkably, the set considered to be optimal for analysis of fungi produced higher prediction errors (12.28%) than were produced with either the bacteria set (9.86%) or the intermediate set (5.74%) for analysis of the fungus in this experiment.

Simultaneous Analysis of Two Bacteria in Nutrient Solutions. These experiments were conducted in a manner similar to the two microbe experiment above. This step introduced several complexities into the experiment. First, the two bacteria (flavobacteria and pseudomonas) have overlapping spectra to a greater extent than the two microbes previously tested. Second, the media used for the solution was a nutrient solution which contains a background of many different components, including a range of bacterial and fungal species possessing their own fluorescent characteristics that can interfere with spectra for the two bacteria of interest.

A ten member learning set was processed using regression of the fluorescence values obtained using the "optimal" excitation and emission set for bacteria. The percent errors (calculated by dividing the standard error of prediction by the mean of the learning set) were 9.64% for flavobacteria and 15.6% for pseudomonas. By way of comparison, the percent error for flavobacteria in the two microbe/pure water experiment reported above was 5.0% or nearly half of the error observed in this complex condition experiment. (Note, however, that the pattern recognition techniques in use at this point are not highly developed.)

Simultaneous Analysis of Three Microbes in Nutrient Solutions. The final set of fluorescent analysis experiments added a third microbe (trichoderma reesei) to the analysis. Forty samples were prepared in nutrient solutions, with thirty samples used as a calibration set and ten samples used as a test set. Four excitation and emission wavelength sets were used to scan each sample, each set considered to be optimal for a different analytical characteristic (bacteria, NADH, fungi, and ATP). Initial analysis of the data was performed using regression analysis of fluorescence values, although a newly developed neural network was also used to process a portion of the information.

The best regression results for all three microbes were obtained with the "optimal" bacteria excitation and emission set, which uses an excitation wavelength of 390 nm and an emission waverange of 410 nm to 610 nm. These results produced percent errors of 23.4% for flavobacteria, 10.8% for pseudomonas and 17.4% for trichoderma.

These are considered to be respectable results for this stage of research, given the fact that neither the instrumentation nor the analysis algorithms have been optimized. As an example of the improvement that may be possible, the fluorescence information that produced the above results was rerun using a newly developed genetic backpropagation neural network instead of regressions. Standard errors of prediction and percent errors for each of the microbes were substantially improved through use of the neural network. Table 3 presents a comparison of results for each analytical method.

ANALYSIS RESULTS FOR THREE MICROBES IN NUTRIENT SOLUTIONS						
	FLAVOBACTERIA		PSEUSOMONAS		TRICHODERMA	
	S.E.P.	%ERROR	S.E.P.	%ERROR	S.E.P.	%ERROR
REGRESSION OF FLUORESCENCE						
SET A	8.22	23.4	4.47	10.8	2.28	17.4
SET B	13.80	39.5	17.10	43.2	2.83	21.5
SET C	7.92	27.5	15.00	38.0	2.50	19.0
SET D	15.40	45.4	21.50	54.3	4.54	34.6
GENETIC BACKPROPOGATION NEURAL NETWORK						
SET A	6.91	15.3	3.54	8.71	1.25	9.27

DEFINITION OF TERMS

	EXCITATION WAVELENGTH	EMISSION WAVEBAND
SET A: Considered optimal for bacteria.	390 nm	410-610 nm
SET B: Considered optimal for NADPH	340 nm	360-560 nm
SET C: Considered optimal for fungi	295 nm	310-510 nm
SET D: Considered optimal for ATP	272 nm	300-500 nm

S.E.P: Standard Error of Prediction, stated here in E6 bacteria/ml or mg/ml
%ERROR: (S.E.P./Mean of Learning Set) * 100

Table 3.

Although the neural network achieved considerable improvement in the accuracy of the analysis, researchers believe that error rates of less than 5 percent of mean can be achieved. Figure 7 shows that even with an error of 8.71 percent of mean value (the lowest error rate of any of the neural networks in this study) the predicted values track verv well with actual values.

Alternative Techniques. In addition to the fluorescent analysis experiments, a series of experiments were performed using an acoustic wave immunosensor. Although the experiments also demonstrated the potential of this technique to detect, identify and quantify pseudomonas aeruginosa, discussion of these results are outside of the topic for this presentation.

TYPE:	PSEUDOMONAS
MIN VALUE:	0.00
MAX VALUE:	89.9
MEAN:	40.67
NETWORK TYPE:	3-10-1
WAVELENGTHS NUMBERS:	3, 13, 29
TRAINING ALGORITHM:	GENETIC/BACKPROPAGATION

ERROR SUMMARY FOR:
PSEUDOMONAS

ITEM	TRAINING SET	TEST SET
AVERAGE ERROR (E6 bacteria/ml)	3.04	3.54
PERCENT ERROR (OF MEAN)	7.49	8.71
PERCENT ERROR (OF RANGE)	3.34	3.94
T-VALUE	33.16	15.0

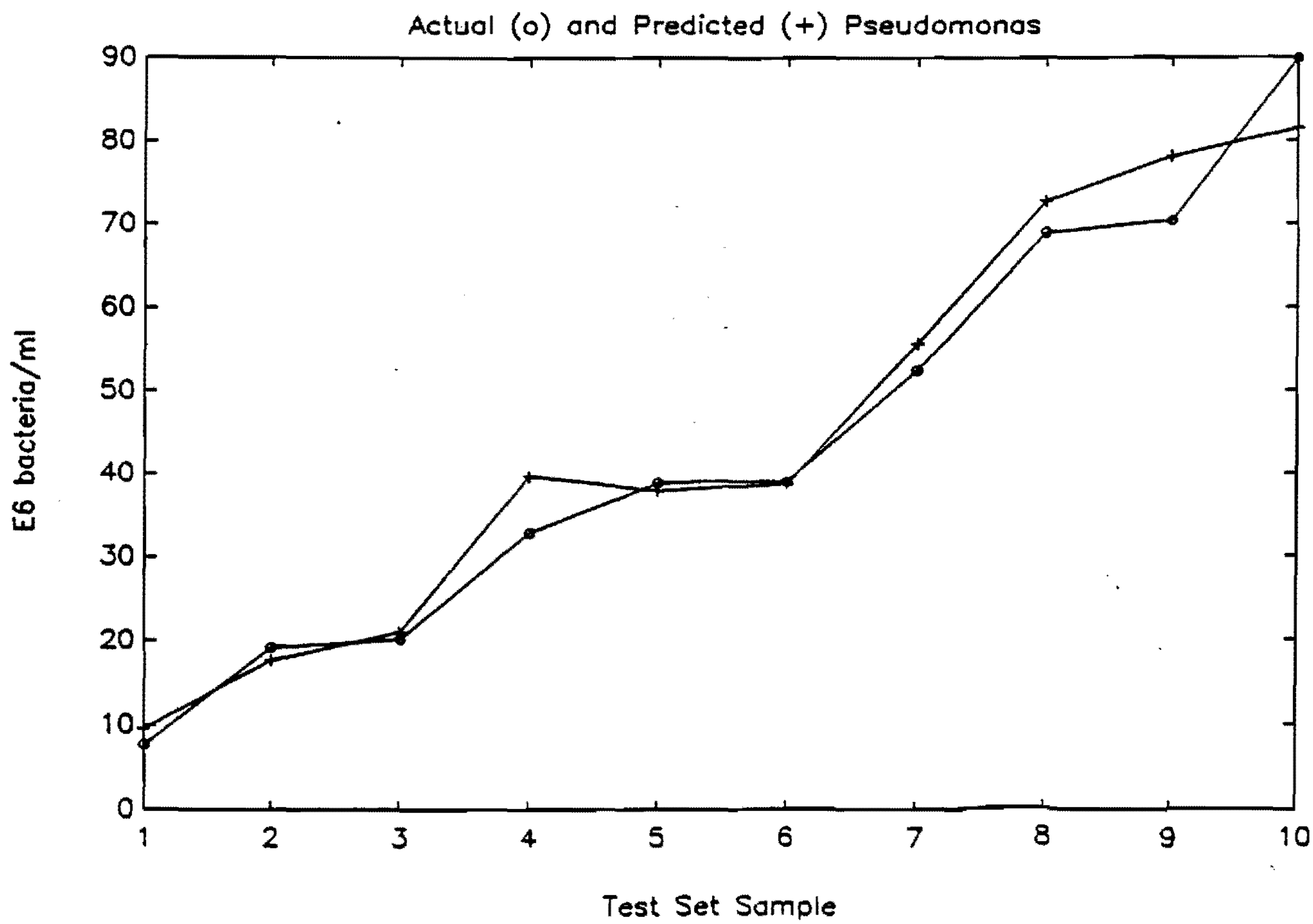


Figure 7. *Predicted and actual test set values for pseudomonas in E6 bacteria/ml.*

CONCLUSIONS

The use of spectrometric methods for on-line analysis of a wide variety of chemical and biochemical substances is an emerging technology that has been demonstrated in multicomponent nutrient solutions in conjunction with two NASA sponsored research projects.

This technology has also been evaluated by other project sponsors for the analysis of contaminants in groundwater, for monitoring the chemical content of industrial process waters, for on-line analysis of wastewaters and for several other environmental monitoring applications.

This technology may offer the potential to effectively monitor agricultural and food processing waters and wastewaters for microbial content and for critical chemical parameters, based on results to date with multicomponent nutrient solutions.

BIBLIOGRAPHY

Nelson, Wilfred H., Physical Methods fo Microorganisms Detection, Boca Raton, FL: CRC Press, 1991

Schlager, Kenneth J., Final Report: Fiber Fluorometry (Spectrometry) for On-Line Chemical Analysis of Nutrient Solutions, NASA Contract NAS10-11656, Kennedy Space Center, FL, July 27, 1990

Schlager, Kenneth J., Final Report: An On-Line Microbiological Analyzer, NASA Contract NAS10-11758, Kennedy Space Center, FL, August 12, 1991