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ENGINEERING DESIGN I

Design and Implementation of Sensor Systems
for Control of a Closed-Loop Life Support System

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EXECUTIVE SUMMARY

The goal of the Fall 1989 EGM 4000 design class was to investigate the sensing and controlling needs for a Closed-Loop Life Support System (CLLSS). This semester was devoted to identifying the sensing needs of five particular areas and defining the requirements for workable sensors. This work was accomplished in conjunction with the ongoing research at National Aeronautics and Space Administration's Kennedy Space Center. The specific areas of interest this semester were atmosphere and temperature, nutrient delivery, plant health, plant propagation and support, and solids processing.

The group investigating atmosphere and temperature control focused on the temperature distribution within the growth chamber as well as the possibility for sensing other parameters such as gas concentration, pressure, and humidity.

The nutrient delivery group investigated the sensing needs for monitoring the solution level in a porous membrane material and the requirements for measuring the mass flow rate in the delivery system.

The plant health group examined the causes and symptoms of plant disease and explored the various techniques for sensing these health indicators.

The group investigating sensing needs for plant propagation and support focused on monitoring seed viability and measuring seed moisture content as well as defining the requirements for drying and storing the seeds.

The solids processing group covered the areas of harvesting, food processing, and resource recycling, with a main focus on the sensing possibilities for regulating the recycling process.

Having defined the needs and requirements for sensing in a closed-loop system, the spring semester will be devoted to design, construction, and testing of feasible sensors for these specific areas.

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I. INTRODUCTION

The students of the EGM 4000 Design Class have worked in cooperation with personnel from the National Aeronautics and Space Administration (NASA) Controlled Ecological Life Support System (CELSS) project, supported by a grant from the Universities Space Research Association (USRA). The research being conducted at the Kennedy Space Center is directed towards developing a closed-loop environment capable of sustaining plants and humans. The Fall 1989 Design class has focused primarily on the sensing and controlling needs for growing soybeans in microgravity.

Class Goals

Realizing the absence of accurate sensors in most areas of the closed-loop life support system, the focus of the fall semester was to define specific sensing needs and requirements. Once the needs were assessed, the class identified available technologies and proceeded to investigate their integration into a Closed-Loop Life Support System (CLLSS).

Class Organization

The closed-loop system was broken down into five sensing areas -- atmosphere and temperature, nutrient delivery, plant health, plant propagation, and solids processing. The members of the class were also divided into five groups according to individual interests and knowledge in the above areas. Each group then pursued their respective topics in order to accomplish the class goals.

II. ATMOSPHERE AND TEMPERATURE CONTROL
OF A
CLOSED-LOOP LIFE SUPPORT SYSTEM

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SUMMARY

In a closed-loop life support system, the atmosphere must be capable of maintaining both plants and humans for long-term missions. Because the atmosphere must be regenerated, a sensing network system must be capable of accurate, real-time data that can be integrated into a controlling system. This sensing network should monitor all areas of the atmosphere including temperature, pressure, relative humidity, gas concentration, and contaminant level.

After identifying the sensing needs of each area, investigation of the advantages and disadvantages of available sensing technologies of these areas led to the decision to pursue two areas of focus for the following semester. The two fields of interest are a temperature or gas profile of a growth chamber and a relative humidity sensor that incorporates fiber optics.

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INTRODUCTION

In a closed-loop life support system the atmosphere must be capable of maintaining both plants and humans for long-term missions. To establish an adequate microgravity environment with a regenerative atmosphere, a system for monitoring and controlling the parameters of an environment is required.

In designing a sensing and controlling system for a closed system capable of maintaining life for long-term flights, the group investigated the requirements of the chamber atmosphere, why these requirements need to be sensed and controlled, and the present technologies available.

Research has shown that properties such as pressure, airborne trace contaminants, gas concentrations, relative humidity, and particulates need to be controlled at respective concentrations; in addition, temperature must be regulated within certain ranges. Sensors employed to monitor these quantities should provide real-time output, be precise and accurate, and test nondestructively.

The microgravity atmosphere modeled should be capable of sustaining soybean plants. Various sensing technologies will be discussed for maintaining a compatible space environment.

TEMPERATURE

Temperature is one property that must be sensed in a closed-loop life support system (CLLSS). If the temperature becomes too hot or too cold, the system would dehydrate or freeze. It has been suggested that the ideal temperature range for soybean plants is 24 to 25°C, which is also adequate for human life support (Boote, 1989). There is, however, a range of 20 to 30°C in which plants are able to function without detrimental effects. It was also suggested that the lower and upper limits for temperature are 15 and 35°C, respectively. Beyond these limits the plants become severely stressed and are in danger of dying.

It has not yet been determined exactly how accurate a temperature sensor needs to be for CELSS system. Temperature variations of 1 or even 5°C may be acceptable, but there is not a definite answer to this question. The plant growth unit (PGU) under analysis at the Kennedy Space Center (KSC), which is 12 feet in diameter and 24 feet high (Garavelli, 1985), has a temperature gradient in the chamber of 0.5 to 1°C from the top of the chamber to the bottom of the chamber (Sager, 1989). Ongoing research at the University of Florida by Dr. Jeff Baker indicates that a temperature gradient of as much as 5°C from top to bottom could exist in a small chamber approximately 5 feet in height. While these temperature gradients are due mainly to the effects of gravity (convection), there may be other effects in microgravity, not yet determined, that could cause large temperature gradients in the system. With the realization of these temperature differences throughout the chamber, it becomes necessary to sense these variations in order to control them.

One problem with temperature gradients is that the measured temperature depends upon the location of the sensor. For example, the temperature near a light source or in a region of stagnant air would be greater than that measured within the leaf canopy or in fast moving air. The reason for sensing these gradients is to locate the "hot" and "cold" spots within the chamber. Knowing the location of these trouble spots would aid in minimizing the number of sensors required to accurately represent the temperatures throughout the system. Sensors would only be required in these trouble areas as opposed to scattering an array of sensors throughout the PGU. In addition, if the temperature requirements and trouble spots are known, engineers could arrange the air ducts in the system in order to minimize the necessary air flow, hence reducing power requirements. This would also assist the engineers in the task of designing the most efficient internal configuration for the PGU.

One problem associated with sensing a temperature profile is that it is heavily dependent upon the geometry of the chamber. The study of one chamber would only give valid results for that specific configuration. Another potential problem is the simulation of microgravity on earth during the initial experiments.

Sensing Technologies

Thermocouple. A thermocouple consists of two dissimilar wires that, when connected to a voltmeter in a closed circuit, generate a millivolt signal. This signal is proportional to the temperature difference between the measuring and reference junction. Some advantages of thermocouples include a broad sensing range of -300 to 1800°F and accuracy of +/- 0.33%. However, a major disadvantage is that the reference junction must be maintained at a constant temperature (Cole-Parmer, 1989).

RTD (Resistance Temperature Detection). Both RTD's and thermistors operate on the similar principles. The resistance of the material inside these devices changes as the temperature changes. RTD's and thermistors have comparable accuracies of approximately 0.1°C; however, RTD's have long term stability. Therefore, they do not need constant calibration (Cole-Palmer, 1989).

Other Methods. Other methods of temperature measurement include infrared sensors, bimetal strips and glass thermometers. Glass thermometers are not considered suitable for space applications because they can not be easily interfaced with a computer and because they contain mercury, a potentially hazardous material. Bimetal strips are not an acceptable temperature sensor for space applications due to inaccurate results (0.5% full scale or approximately 5°C). Infrared sensors use a thermopile (thermocouples in series) to detect radiation emitted from objects. The ability to sense remotely is a major advantage of infrared sensors. However, an infrared sensor is only accurate to approximately 5°C (Cole-Parmer, 1989).

PRESSURE

Pressurization of the plant growth chamber should be constantly regulated to ensure accurate performance of instruments. The human chambers should be pressurized to an average of 80% nitrogen and 20% oxygen and maintained at 760 mmHg +/-103 mmHg (Garavelli, 1985). In a plant growth chamber, sensors would be required

to monitor the partial pressures of elements transpired and absorbed in the regenerative atmosphere. For optimal growth of a soybean plant, the partial pressure of carbon dioxide should be regulated between 800 to 1000 parts per million (ppm).

Sensing Technologies

Several methods are available for sensing pressure in a terrestrial environment. Many of these devices are extremely accurate, such as manometers with an accuracy of +/- 0.2 of scale division; however these are gravity dependent. Piezoelectric transducers are accurate sensors that can be used in rapidly fluctuating air flows therefore making it useful in a plant growth chamber. Commercial sensors for monitoring partial pressures are very accurate as can be seen in an oxygen sensor with an accuracy of +/- 0.2%.

AIRBORNE CONTAMINANTS

In a plant growth chamber, atmospheric contaminants may originate from plants and material outgassing. In an environment where the atmosphere is recycled and is subjected to these contaminants, sensors should be capable of real-time readings of the occurrence of toxic gases and be able to locate the problem area.

Plant Produced

Plants are known to give off at least 200 discrete substances including hydrocarbons, aldehydes, alcohols, lactones, ethers, carbon monoxide, various organic acids as well as ethylene and terpenes that may be harmful to plants and humans. They may prevent normal plant growth if allowed to accumulate (Gitelson, *et al.*, 1975). Ethylene is a volatile gas that is produced by the plant as it matures. Atmospheric conditions, such as low relative humidity, have been found to increase the production of ethylene. In wheat tissue, water loss of 10% initiated the increased production of ethylene (Orcutt, 1987). In concentrations as low as 5 ppb, it has noticeable effects on plant physiology (Sinclair, 1988). Ethylene is responsible for growth

responses in plants such as leaf bending, leaf abscission, and stem swelling (Noggle, 1983). One positive aspect is that increased production of ethylene stimulates the ripening process in fruits and vegetables.

Material Outgassing

Outgassing is the gradual release of residual solvents or products of degradation processes. The evolution of such gases in a closed chamber can lead to corrosion and loss of crops.

When exposed to visible and ultraviolet radiation, polyvinyl-chloride (PVC) expels chlorine atoms from plastic molecules and produces chlorine gas. Chlorine gas reacts with latent moisture in the atmosphere to produce hydrochloric acid aerosols which can cause extensive crop damage and equipment corrosion if not removed.

Another source of outgassing is the aging of synthetic polymers. Through depolymerization these materials expel gaseous compounds. The aging is accelerated by exposure to light, ionizing radiation, trace contaminants, water, and oxygen. One extremely toxic compound, dichloroethyne, was found aboard the space shuttle. This substance is a by-product of a cleaning solvent, trichloroethane, used on the shuttle (Knott, 1986).

Sensing Technologies

Currently there are two methods of sensing within a regenerative atmosphere for airborne trace contaminants. Mass spectrometers and gas chromatographs are used in the analysis of environmental gas samples and can identify what molecules are present and at what concentration levels. These methods will be discussed in the following section on gas concentration.

GAS CONCENTRATION

While major components of the atmosphere are easier to detect, identifying trace gases to a high precision requires more than a simple sensor. An oxygen sensor that is accurate to 1% may or may not be accurate enough to measure trends in plant respiration. When the air is 21% oxygen 1% could vary by +/- 2100 ppm. Carbon

dioxide, making up only 0.3% of the mixture, would be off by +/- 30 ppm with an accuracy of 1%. One method to accurately determine the concentration of compounds such as ethylene and ammonia involves a gas chromatograph coupled with a mass spectrometer.

Sensing Technologies

Gas Chromatography. Gas chromatography is versatile and extremely accurate. However, a conventional laboratory column oven is too large and consumes too much power for use in space. By reducing the size of the oven and etching a spiral track on silicon, power requirements decrease and survivability increases. Another drawback is the sensitivity to vibration. Considering the magnitude of the vibration induced by a launch, it seems likely that the instrument would be damaged. Gas chromatography is not a real-time indicator, but if samples are to be taken hourly, the speed is adequate (Simpson, 1985).

Mass Spectrometry. Mass spectrometry is another highly accurate means of analysis. The mass spectrometer is compact, versatile, and efficient. In quadrupole mass spectrometry the field oscillation can be tuned to select specific masses with very few peak overlaps occurring only at low molecular weight levels. Oddly enough, the biggest disadvantage of a mass spectrometer is lack of a good vacuum source. Leakages from the spacecraft will create a gas pressure of 10^{-4} to 10^{-5} Torr on the outer hull (Simpson, 1985). The mass spectrometer will require lower pressure, however the outside atmosphere could be used as a backing pump. Incorporating the vacuum environment of space into the mass spectrometer system could reduce the size of the pump needed inside the chamber.

RELATIVE HUMIDITY

Relative humidity may be defined as the amount of water vapor a gas contains divided by the amount of water vapor the gas could hold if saturated. This ratio is often expressed as a percentage.

Control of relative humidity is important a CLLSS for a number of reasons. As humidity increases and transpirational cooling decreases, heat loss by convection from the leaves becomes an important concern. In extreme cases, actual heat damage to the plants can occur. Also, as stomatal conductance increases due to high humidity, the influence of atmospheric toxins on the plants' health becomes more of a problem (Tibbitts & Kozlowski, 1979). Preliminary CLLSS requirements indicate that the relative humidity range in the PGU should be between 60% to 70% with an accuracy of +/- 10% deviation from the set point. The humidity itself should be determined with an accuracy of approximately +/- 1% (Garavelli, 1985). A wide variety of methods are presently available to determine the relative humidity of a gas. Some of these technologies are discussed in the following sections.

Sensing Technologies

Wet/Dry Bulb Psychrometer. One of the most common methods to determine relative humidity uses a wet and dry bulb psychrometer. The dry bulb simply computes the gas temperature as it flows across the temperature sensing device. The wet bulb yields the temperature as distilled water is evaporated from a porous material surrounding the bulb. Relative humidity can be directly correlated to the difference in temperatures between the two bulbs. Thermistor based psychrometers can yield readings accurate to approximately +/- 1% in 30 seconds. Using a galvanometer, thermoelectric psychrometers have been as accurate as +/- 0.01% (Lapinski, Kostyrko, & Wlodarski, 1976). While wet and dry bulb sensors are simple, inexpensive, and based on a well established principle, they do require a minimal flow rate across the bulbs of three meters per second (Lapinski *et al.*, 1976).

Dew Point Sensor. Dew point sensors work on the principle that the amount of water vapor a gas may hold depends on the temperature of the gas. As the temperature decreases, a point is reached where the water vapor will condense on a surrounding surface. This temperature is referred to as the dew point temperature for the gas. Generally, a representative sample of the gas under analysis is filtered and precisely cooled until water vapor condenses on a mirror or ceramic tile. Changes in

capacitance of the tile or in the reflected light intensity of the mirror can signal the dew point temperature of the gas (Carr-Brion, 1986). Accuracy can range from 0.0015% to 2% depending on the type of dew point sensor used (Lapinski *et al.*, 1976). Unfortunately, the mirror based systems require regular cleaning of the silvered surfaces. Filters also require constant attention to assure a relatively contaminant free flow.

Crystal Oscillator Sensor. Crystal oscillator sensors compare the frequency oscillations of a dried portion of the gas along with a wet portion to a reference standard. A dedicated microprocessor then calculates the relative humidity of the gas. The stability and reliability of such a system is excellent, as drift-free operation of a year or more seems common. Actually, auto-zeroing is inherent in the sensor design. Response times of 60 seconds provide a real-time measurement of relative humidity necessary for the control of a CLLSS. Unfortunately crystal oscillator sensors are presently expensive, bulky, and accurate only to $\pm 5\%$ (Carr-Brion, 1986).

Ceramic Moisture Sensor. Ceramic moisture sensors consist of a thin film of ceramic material whose capacitance or resistance changes with the relative humidity of the gas surrounding it. Advantages of this system include temperature independent readings and accuracies of $\pm 1\%$. However, one problem is the limited accuracy of such a device at low relative humidity levels (Carr-Brion, 1986).

Fiber Optics. Personal communications with Ralph Prince at Kennedy Space Center revealed a possible new technology in determining the relative humidity of a gas. A fiber optic device correlating the shift in wavelength of light with respect to moisture is presently being studied by Geo-Centers, a company in Boston (Prince, 1989). This technology seems promising due to the minimal energy requirements and non-obstructive nature of sensing (Carr-Brion, 1986).

Other Methods. Several other sensors were also considered for CLLSS applications. Some of these technologies included electrolytic sensors (Carr-Brion, 1986), lithium chloride hygrometers (Carr-Brion, 1986), aluminum oxide hygrometers (Carr-Brion, 1986), silicon hygrometers (Carr-Brion, 1986), polymer humidity sensors (Carr-Brion, 1986), gravimetric hygrometers (Lapinski *et al.*, 1976), and chemical sensors (Lapinski *et al.*, 1976). Initial research into these technologies revealed reasons why they would be unsatisfactory in a CELSS. Some reasons why these methods were not considered further include gravity dependence, frequent maintenance requirements, unreliability, instability, incompatibility with certain gases, and sensitivity to dusty conditions.

PARTICULATES

The accumulation of particulates in a closed-loop life support system can be hazardous. Small particles such as nutrient solution droplets could render sensors inaccurate, or in a worst case scenario, be corrosive to the sensitive apparatus. Medium size particles such as human hair, loose seeds, and discarded flower petals may adhere to nutrient delivery tubes and through capillary action, draw moisture in a similar manner to rootlets. Large particles, such as leaves that are shed prior to harvest, can clog air ducts and make optical systems useless.

Electrostatic filters, such as the type used on the space shuttle, could easily be blocked by larger items and may not be able to function effectively under the greater exchange rates necessitated by intense heat produced by the light sources.

Presently no sensors have been developed for detecting and identifying particle types. It is known, however, that these particulates must be removed from a closed system. Currently the only real-time sensors used in the particulate removal system indicate when a filter needs refurbishing. A pressure differential of sufficient magnitude between the inlet and outlet of the particulate removal system signals contaminated filters.

CONCLUSION

Based on the information gathered this semester, our group has decided that two areas of atmosphere control show potential for design and testing. One possible focus involves the fabrication of a relative humidity sensor that incorporates fiber optics. However, we have just begun gathering the necessary data for the design.

Another promising area is temperature or gas profiling of a growth chamber. While the gradient profile changes from chamber to chamber, a simple model could yield important results as to the size of the gradients, as well as the amount of sensors required to map these gradients. The addition of airflow mixing would also yield important information as to the amount of flow necessary to produce a uniform temperature throughout the chamber. The building of a physical model will also allow verification of a computer model. Additionally, information of gas concentration gradients in a growth chamber may be obtained through this modeling process.

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III. NUTRIENT DELIVERY SYSTEM

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SUMMARY

The nutrient delivery system was examined in its entirety in order to gain a basic understanding of its operation and to identify unresolved sensing and control requirements. After identifying a number of important sensing and control needs, it was determined that sensing mass flow and monitoring the fluid level in the porous delivery media were the two areas that should be examined in detail. Next semester, emphasis will be given to the development of the most promising sensing technology that could be incorporated into a closed-loop system.

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INTRODUCTION

A major component of a CLLSS is the Plant Growth Unit (PGU). An important subcomponent of the PGU is the nutrient delivery system. At present, the National Aeronautics and Space Administration (NASA) at Kennedy Space Center (KSC) is using a porous tube nutrient delivery system. This system controls the flow of nutrients to the plants by using a microporous, hydrophilic tubular material. The plants obtain the nutrients by capillary action through the porous medium.

The nutrient delivery design team investigated some of the many areas of the nutrient delivery system for possible sensing and controlling interventions. The main areas that were of interest to the team were build up of salts, pH balance, nutrient solution temperature, ion detection, growth unit pressure, mass flow, and porous media wetness level.

This report summarizes basic needs and potential sensing technologies for the areas of salt build-up, temperature and pH balance of nutrient solution, and ion detection. A more detailed analysis in the areas of nutrient delivery growth unit pressure, mass flow, and porous media wetness level follows.

To avoid redundancy and to make a positive contribution to a CLLSS Project, the design team's efforts were focused on those areas that KSC/CELSS personnel had not addressed. In the final selection process for project emphasis, the design team chose to concentrate on development of a wetness sensor to determine the saturation level of the fluid in the porous medium of the growth unit. In order to accomplish the design team's goal, the current porous tube system will be slightly modified. This modification will simplify the development of a wetness sensor next semester.

SALT BUILD-UP

Salt accumulation on the roots and the porous plate may become a limiting factor in the performance of the nutrient delivery system. A build-up of salts, due to the evaporation of a high concentration solution, tends to decrease the availability of water

to a plant. Root permeability and plant metabolism also seem to be affected (Edminster, 1974). The ability to sense the level of salt build-up, and be able to correct the problem, would be beneficial in maintaining an efficient nutrient delivery system.

Electrical Conductivity Measurement

Salt accumulation may be detected by measuring the electrical conductivity of the nutrient solution (see Appendix A). The conductivity is determined using a standard conductance cell which measures the electrical resistance of the nutrient solution (Jones, J.B.), and indicates if the ion concentration level is sufficient. If build-up is excessive the system can be flushed with pure water.

Research will be required to determine the effects of high salt concentration on the water absorption rate and plant growth. Data may show that the effect of salt build-up is negligible over the length of the crop season. Otherwise, the level of detrimental salt accumulation should be determined for intervention purposes.

pH OF NUTRIENT SOLUTION

The pH of the nutrient solution should be monitored. The pH of the solution affects the availability of certain elements to the plant. In particular, micronutrients seem to be affected. Excessive uptake and, therefore, toxicity can result at a low pH, while elements can be precipitated from the solution at a high pH (Jones, J.B.). Research has shown that the pH of the nutrient solution should be maintained between 6.0 and 6.5. However, the pH effects on plant growth may be less critical in a flowing system as long as the pH does not fall below 5.0 or rise above 7.0 (Jones, J.B.).

pH may be controlled by various methods. In general, a pH probe is placed in the solution and sends an electric current proportional to the pH to a meter. Control systems available today can continuously monitor the pH and automatically inject an acid or base to maintain the system at a predetermined level (Cooper, 1979). Disadvantages of the pH probes currently obtainable include routine calibration requirements and maintenance.

TEMPERATURE OF NUTRIENT SOLUTION

The temperature of the nutrient solution is important since it can affect plant growth responses. Research has shown that the solution temperature should not be lower than the ambient air temperature (Cooper,1979). If the solution temperature is below ambient, plant wilting may occur. In a cool solution, roots cannot absorb sufficient amounts of water or elements. Water absorption is reduced at low temperature due to a reduction in root cell permeability and an increase in the viscosity of water (Edminster, 1974). Some effects of reduced water absorption are poor fruit set and quality, and delayed maturity. When the air temperature is low, however, a warm solution can help to maintain plant growth.

Many compatible temperature sensors are available including thermocouples and thermistors. These devices can be placed in the recirculating nutrient solution to measure deviations from an ideal temperature setting. Research has been conducted determining the optimal root zone temperature to be between 20 and 30°C.

INDIVIDUAL ION DETECTION

Individual ion concentrations need to be accurately monitored and controlled to keep the plants alive and healthy. The specific ion uptake for a healthy plant has been established. If this known data is compared to the intake of the crop, then a correlation may be made to help determine the status of the plant's health. Possible technologies to determine ion concentrations include: ion selective electrodes (ISE), thermocouple devices, and other devices measuring light scattering and photofluorescence.

Ion Selective Electrode (ISE)

An ion selective electrode is an existing technology that uses electrodes submerged in a solution to determine the amount of certain ions. Each electrode is sensitive to one particular element. When a voltmeter is attached to the ISE, the voltage output increases as the concentration of ions increases. The main advantage of an ISE is

that the data can be taken in real-time by direct sampling for most elements. Disadvantages include the need for constant calibration (usually every hour) and the possibility of contaminant interference with the instrument's accuracy (Orion, 1989).

Other Sensing Technologies

Another method that may be employed to determine ion concentrations involves thermocouple devices submerged in the solution with a heat source. The voltage change of the thermocouple can be related to the concentration of dissolved salts. This will not give individual concentrations, but may be used to determine the solute to solvent ratio.

Light scattering would involve shining light of specific wavelengths through the solution and observing the scattering effects. The amount of scattering may be related to the molecular size of the dissolved ions in the nutrient solution. Photofluorescent measurements involve adding energy to a sample of the fluid and using a spectrometer to measure any light that may be emitted by the ions.

NUTRIENT DELIVERY GROWTH UNIT PRESSURE

The purpose for using porous materials as a growth medium for plants is to enclose the nutrient solution while maintaining free access of nutrients to the roots of the plants. Using atmospheric pressure as a reference, the pressure inside should be maintained at a negative gauge pressure. This allows the atmospheric pressure, coupled with the porous material's properties, to contain the fluid within the boundary of the porous media. The upper bound of the pressure difference is determined by the need to prevent leakage across the outer surface of the conduit, which is undesirable in microgravity. The lower bound is related to the minimum surface tension that may exist between the root and the nutrient solution.

Some of the hardware involved in the control of pressure are transducers, pumps, porous conduit, feed tubes, wetness sensor, holding tanks, and a computer. Quality transducers and precise pump operations are of great concern in the control of negative

fluid pressures. Another concern is trapped gasses in the nutrient delivery system. Gas bubbles may affect steady pump operations, create local drying of the porous material, and result in erroneous pressure readings.

Porous Material Properties

A basic component of the nutrient delivery system is the porous medium in which the plants will grow. Knowledge of the porous material properties, as well as fluid properties, will aid in the selection of a growth medium. This information will also contribute to requirements for sensors that operate at, or near, the porous medium.

The properties of a porous media which are of greatest concern are pore size, capillary action, electrical resistivity, liquid permeability, weight, and durability. Porosity is defined as the percentage of void volume within a porous structure. Permeability is defined as the resistance to passage of a specific flow of fluid per unit of area (Mott Metallurgical Corporation, 1989). The impact that fluid and material properties have on the permeability of the porous media must also be considered. Specifically, fluid viscosity and density, pore size and pore size distribution, and thickness of the porous wall affect the permeability of the media. Because fluids flow from regions of high pressure to regions of low pressures, the pressure differential between the two sides of the porous media is also important in the development of fluid flow through porous structures.

Fluid Containment in Porous Media

Porous material is used to construct a conduit so that fluid may flow across the conduit's boundaries. The porous media may be ceramic, metallic, or other hydrophilic material (ceramics and metals are naturally hydrophilic). The CELSS project is presently using a tubular configuration. The porous media are hydrophilic polyethylene tubes and cylindrical ceramic filters (Dreschel, 1988). The porous media provide a foundation for air to fluid interface. With negative gauge pressures, the ambient air pressure tends to displace the fluid back into the porous media towards the origin of fluid flow. For slightly positive pressures, the fluid is displaced outward to the surface of the media. If the positive displacement of the fluid is large enough to exceed the surface tension

forces on the surface of the media, then fluid leakage will occur (Schwab, 1986). In microgravity, the excess surface tension will form fluid droplets which may break away from the surface of the media. Maintaining a negative gauge pressure will allow sufficient containment of the nutrient solution to prevent adverse water droplet formation. Maximum negative gauge pressure to support plant growth will be discussed in the section titled 'Porous Media Wetness Level'.

Negative gauge pressure can be obtained as outlined in Appendix C. Pore size also affects the pressure differences the media can support for the air/water interface (Schwab, 1986). Differential transducers are used to measure the pressure differences. Pumps are potential actuators that can control the negative fluid pressure. In conjunction, pumps and differential transducers make up a simple control scheme for fluid containment.

Need For Trapped Gas Removal

There are a number of ways air can be trapped in the nutrient delivery system. The gas may enter the system at the origin. The nutrient-injection component of the reservoir could also introduce gas, along with the nutrient. Unlike an earth-based reservoir, where the air would rise to the top of a tank, the microgravity reservoir would not readily separate. Eventually, the gas would be circulated through the system with the rest of the nutrient solution. Joints and fittings throughout the system could also allow leakage because temperature changes and different thermal expansions and contractions of materials can cause gaps. An even stronger possibility that is recognized in Thomas Dreschel's tubular delivery system is the phenomenon of local drying. William K. Schwab explains this phenomenon in his local drying hypothesis. "Local drying is thought to occur when the relative humidity outside of the tube is low enough that evaporation exceeds wicking. The dry membrane will leak air since the air no longer must displace water from the hydrophilic membrane."

Inaccurate measurements of the delivery system's state cannot be tolerated. Residual air may cause erroneous readings of pressure. It can also be asserted that non-homogenous flows (i.e. a flow that consists of part water and part air) may contribute to the degradation of pumps. More detrimental would be the condition of local drying due

to the collection of air bubbles in an area, which would create a barrier between the porous membrane and the liquid that could lead to the starvation of the root.

Solutions have been investigated for bubble removal from the simplistic gravity-functioning T-shaped bubble remover to Schwab's involved concentric bubble remover. Because they all have met with limited success, this problem requires further attention.

Peristaltic Pump

Currently, NASA has elected to use a peristaltic pump to generate fluid flow through the nutrient delivery system. The peristaltic pump is simple and durable. Furthermore, the mechanical parts are separated from the fluid. A complication associated with the pump is that it produces small pressure waves each time it moves a segment of water. Nevertheless, this pump is most promising because the advantages far outweigh the disadvantages. More work is being done to control the pressure waves (or at least minimize them) by investigating the effects of varied bearing spacing (in the driving mechanism), and varied speeds.

Pressure Sensor

Accurate, inexpensive technology already exists for the measurement of pressure. This technology involves the use of pressure transducers which incorporate diaphragms to sense fluid pressure. The deflection of the diaphragm is proportional to the applied pressure. The different kinds of pressure transducers are dependent on the method used to sense this deflection (ASHRAE Handbook, 1985). The pressure transducer considered is one that will measure the difference between fluid and cabin pressure. A diaphragm type transducer that has an insulating material to protect it from the solution is gravity independent and is accurate to within 0.3%. Generally, the power supply voltage is between 5 and 30 volts (Sensym, 1989).

MASS FLOW

Water is taken up and released by plants via transpiration. Transpiration is important because it is closely related to photosynthesis which in turn is related to growth rates and crop yield (Bennett, 1982). It is desirable to sense the rate of water uptake (i.e. the rate of transpiration) on a real-time basis since this may provide another means of monitoring crop health. It is also necessary to sense and control mass flow since an optimal nutrient level must be maintained in the delivery system's reservoir.

A crop's measured transpiration rate could be checked against a normal rate based on the current conditions. This 'normal' rate would come from a data base of optimal transpiration rates generated through experimentation. This data base would have to include the effects of factors such as relative humidity, temperature, radiation level, stage of growth, and nutrient concentration. If the measured transpiration rate exceeds limits, the controlling system would be notified and appropriate action taken.

Estimated Transpiration Rates

During peak growing times, a group of forty soybean plants may uptake from 15 to 20 liters of nutrient solution per day. This equates to 0.021 to 0.028 liters per minute (assuming a 12-hour lighting period) (Wheeler, 1989). It will probably not be necessary to sense on a per minute basis, but rather some time period that will depend on the controlling system and its ability to make effective use of the information.

Sensing Transpiration Rate

Sensing a change in the nutrient volume in the reservoir would seem to offer the most practical means of measuring transpiration rates. The underlying principle would be to measure the change in nutrient volume over a given period of time. This change in volume, with respect to a change in time, would yield the transpiration rate of the crop.

A number of factors must be considered and dealt with if the technique is to operate in a microgravity environment. Air must be removed, minimized, or held at a constant level, and not allowed to collect in any section of the delivery system. The introduction of replacement nutrients and water should be accounted for if they are introduced into the system during the testing time interval. These mass flows into the system should be accounted for or prevented during the testing period.

Sensing Techniques

Two different techniques were examined in order to sense volumetric changes in the reservoir. The first involved the use of a floating piston/cylinder assembly in which the piston would separate the nutrient solution from the atmosphere. The piston would move as the reservoir volume decreased, thus signifying a change in volume. It is expected that there will be leakage between the piston - cylinder interface.

A better approach uses a flexible bladder reservoir which is filled and expanded with the nutrient solution. A change in the internal pressure of the bladder will correspond to a change in the volume it contains (Rockwell, 1982). A pressure transducer vented to the ambient atmosphere would be employed to sense and relay this change in pressure to the controlling system (see Fig. III-1). This design has been successfully used to measure liquid levels and to store liquids on various spacecraft and is currently utilized on the Space Shuttle for water storage (Rockwell, 1982). It is a relatively simple design with few moving parts and would seem to be readily incorporated into the nutrient delivery system.

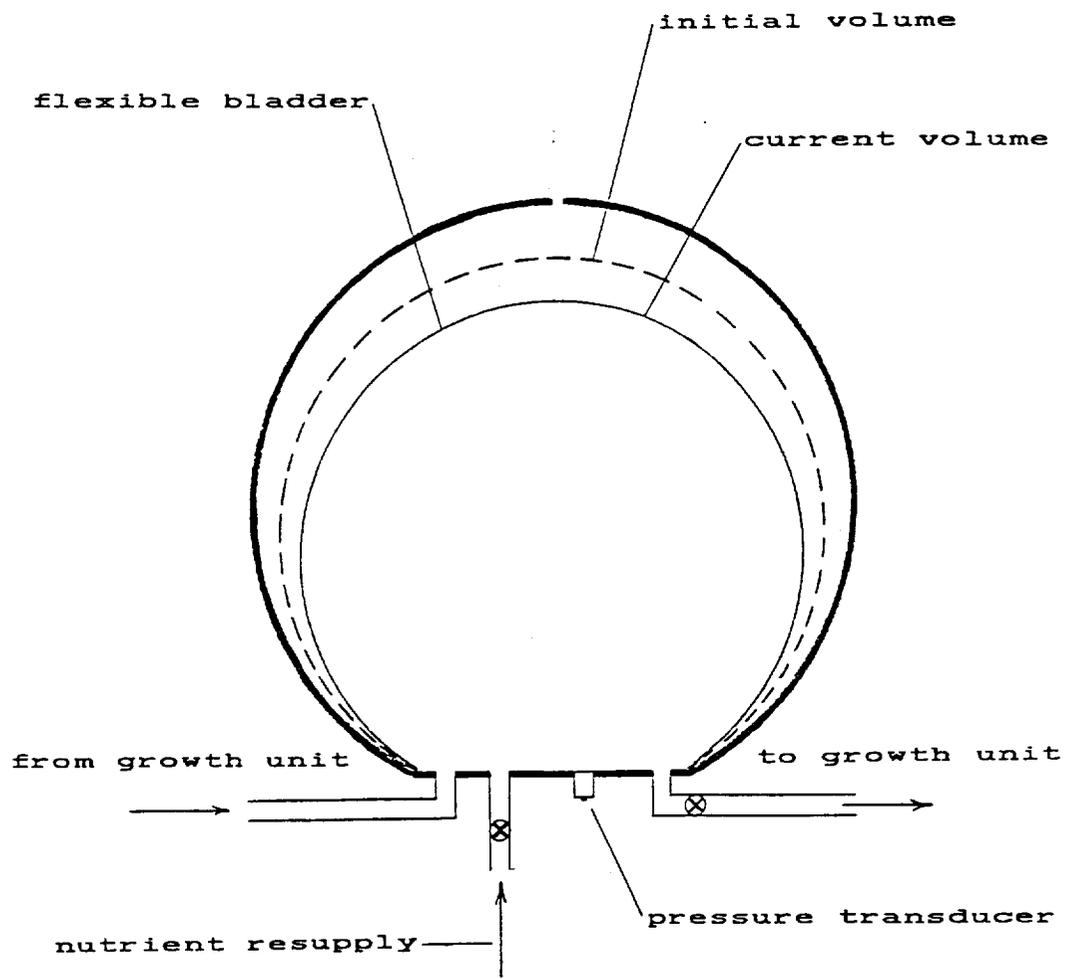


Figure III-1 FLEXIBLE BLADDER RESERVOIR

POROUS MEDIA WETNESS LEVEL

A sensing need of the nutrient delivery system involves directly monitoring the root's environment fluid potential. A wetness sensor could conceivably do this. It could sit on, or near, the porous medium (the root's environment) and continually measure its wetness (moisture content). The direct method of sensing the root's environment has advantages over a more indirect method of sensing. Sensing wetness reduces the need to predict changes in the crop's fluid requirements.

It is well understood that during the dark period of the photo-cycle, a plant's demand for nutrient solution drops drastically. Throughout the plant's life, its daily nutrient requirement is an increasing and decreasing non-linear curve. Checking the wetness sensor readout, one could ascertain if the porous medium's wetness level is appropriate. A wetness sensor could be attached to a fluid flow actuator, and in the event the porous medium becomes too dry or too soaked, the sensor will indirectly prompt an actuator to respond.

Root/Solution Interface

Proper wetness level, as a result of pressure differences, in the porous medium aids in the optimization of plant growth. This was shown in the experiment conducted by Dreschel, 1988. Although the data compiled was for wheat, it was concluded that plant total mass is affected by varying the pressure difference. Total mass for wheat was altered by a maximum of 40% with a pressure head from 41mm to 154mm (Dreschel, 1988). This change in total mass was due to the wetting angle (see Fig. III-2) of fluid at the plant's roots. The dominating forces for root wetting angles are surface tension and pressure. The percent of root wetting increases with a decrease in pressure head difference. In Schwab's wetting angle model (plant not specified) the extremes of root wetting percentages and corresponding pressure differences were 11.1% at 240cm and 50% at 7.45cm. Fig. III-2 shows the root/solution interface on a porous medium. It is desirable to maintain an ideal wetting angle, without exceeding surface tension forces, to aid in plant growth.

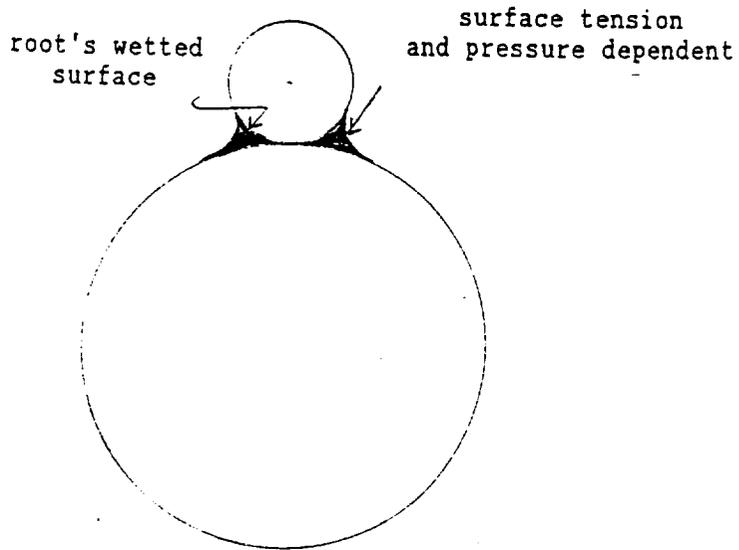


Figure III-2 ROOT/SOLUTION INTERFACE

Resistance-type Sensor

The variable resistance sensor seems to be the simplest of the three technologies studied. As seen in Fig. III-3, the sensor is composed of a constant direct current source, two test probes, and a voltage meter. In working mode, the two probes will be placed several centimeters apart touching the porous surface. The two voltage probes will tap into the surface somewhere between the current probes. Because the wetted material has electrolytic properties and is conductive, a current will be induced by the source. In essence, the porous material acts as a variable resistor completing the circuit.

It is variable since the electrolytic solution soaks the material at different concentrations depending on the pressure differential. The voltage meter, with its probes a constant distance apart, will measure different voltages at different levels of soaking of the material. Hence, the voltage reading allows insight as to what degree the material is wetted.

Three evident problems with the model should be noted. Probably what evokes most concern is the fact that the sensor is invasive. Secondly, the current running through the material may promote the ionization of some components in the solution. Thirdly, precipitation could occur, clogging the pores of the material. However, currents on the order of nanoamperes would suffice. Currents this small should not disrupt any chemical properties of the solution. Furthermore, a sensor very similar to the variable resistance sensor is employed to test the reliability of semiconductors (known as the four point probe device) (Axson, 1989).

Capacitor-type Sensor

The dielectric constant of water is considerably higher than that of most other materials. It is this factor that allows for easy detection of variations in moisture concentration in solids by a variable capacitance wetness sensor. Figure III-4 illustrates that the sensor consists of a basic RC circuit with a variable capacitance. The capacitor is made variable by the variable dielectric (porous medium) between its conducting surfaces. When the medium becomes more moist, the dielectric constant increases in the porous material raising the capacitance. For a specific moisture concentration the capacitor attains a unique capacitance. An oscillator can be placed in parallel to amplify the natural frequency of the circuit. Since the resistance will be known, the capacitance can be calculated. Once the capacitance has been determined, the dielectric constant remains as the last variable since the geometry of the capacitor is already specified. As stated before, the dielectric constant can be translated into the moisture level. To compliment the circuit, a frequency to voltage converter will be installed to accommodate readings from a simple voltmeter. The converter alters the

sinusoidal signal to a DC voltage of a value proportional to the frequency of the sinusoid. This DC voltage is fed to the voltmeter. Presently oscillators and frequency to voltage converters are available in the form of inexpensive integrated chips.

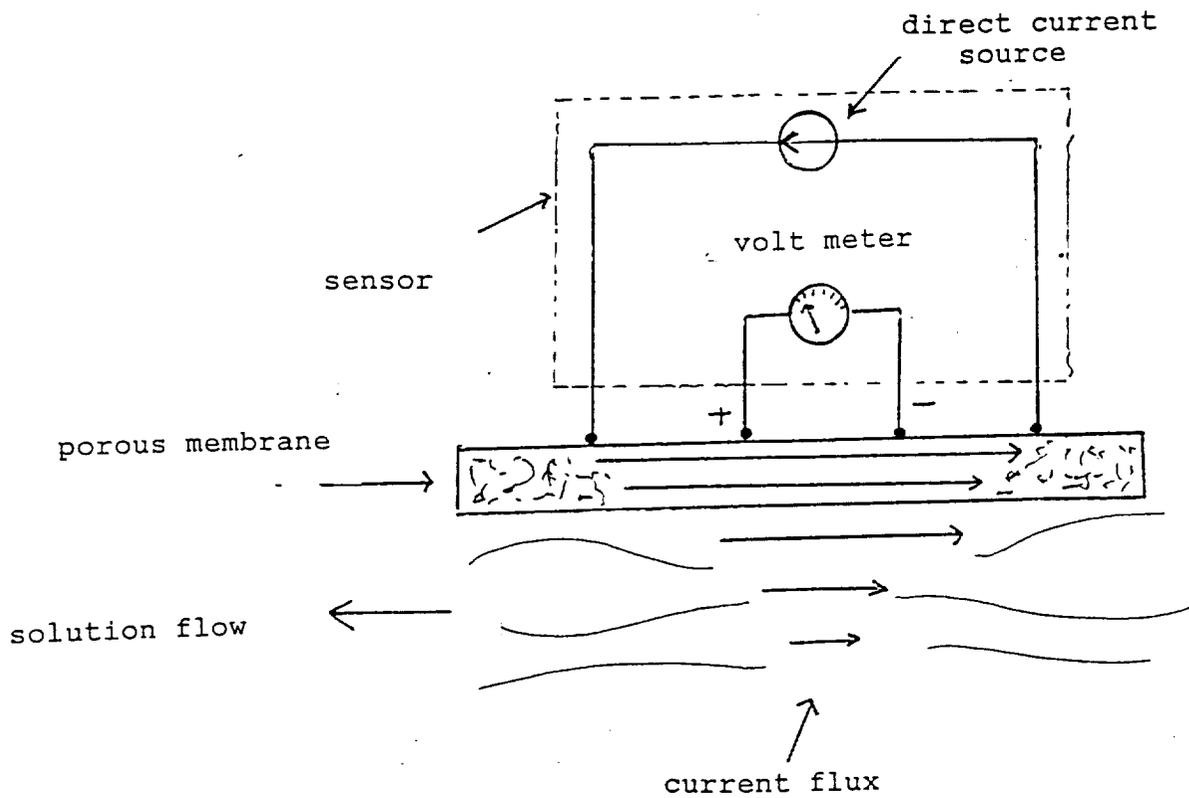


Figure III-3 VARIABLE RESISTANCE SENSOR

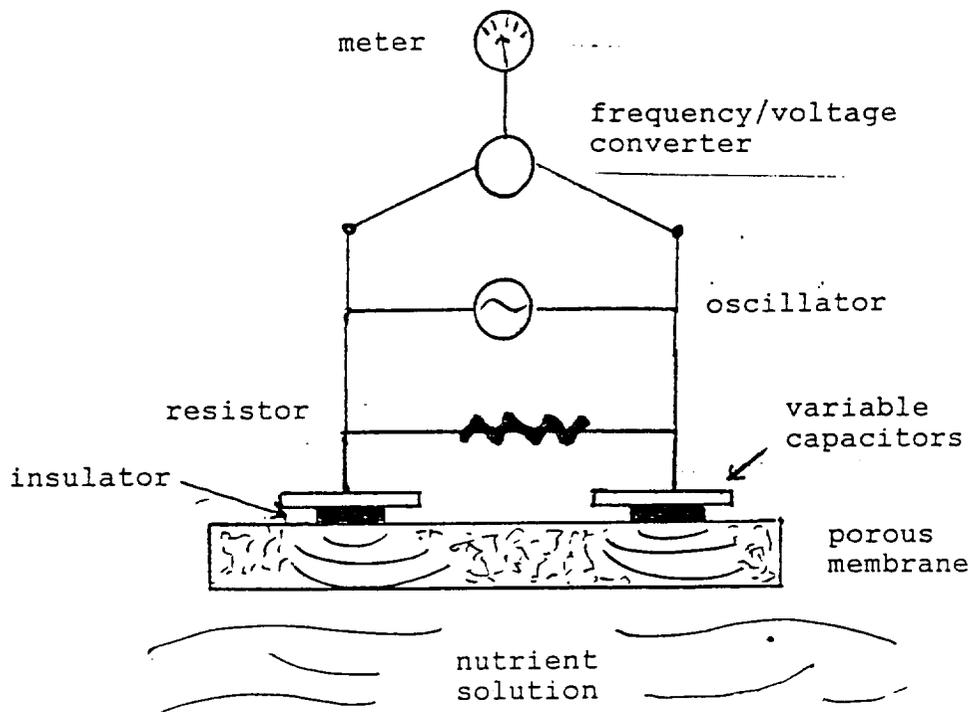


Figure III-4 VARIABLE CAPACITANCE SENSOR

The following is a list of the advantages and disadvantages of present technologies which use the variable capacitance principle to measure the moisture in solids (Carr-Brion, 1986).

The advantages in using this method are:

- 1.) It is a simple, semi-invasive technique which is readily installed.
- 2.) It is relatively inexpensive and reliable.
- 3.) In suitable applications it has a reasonably good limit of detection and repeatability.
- 4.) It can be made rugged.

The disadvantages of using this method are:

- 1.) It is bulk-density, temperature and chemical composition dependent.
- 2.) It requires electrically isolated conducting electrodes very close to the material being sensed.

Carr-Brion claims that present variable capacitance devices have a limit of detection of 0.1% moisture and repeatability of 0.2% over the range up to 30% moisture, and while poorer performance is attained at higher levels of moisture. In sensing the moisture of a porous surface in the plant chamber, environmental parameters should remain controlled, improving the limits of detection and repeatability for the sensor.

Infrared Reflectance

Liquid water is known to strongly absorb light in the infrared spectrum at specific wavelengths ranging from 0.76 to 2.95 micrometers (Carr-Brion, 1986). Consequently, different wavelengths of infrared light will reflect off a material at varying intensities based on the water content of the material. Although water vapor also absorbs the infrared, it does so at slightly shifted wavelengths, hence atmospheric water vapor does

not affect the reflectance of infrared light (Carr-Brion, 1986). A wavelength of strongly absorbed infrared light is reflected off a test surface and onto a photodetector which measures the intensity level of the reflected light (see Fig. III-5). Another wavelength, which is not strongly absorbed by water, is then reflected off the surface and its intensity level measured. The difference between the two measured intensity levels will be due primarily to water absorption of the first beam (Carr-Brion, 1986). The difference is then related to a previously calibrated graph of intensity difference verses water concentration to give the actual water concentration of the test material.

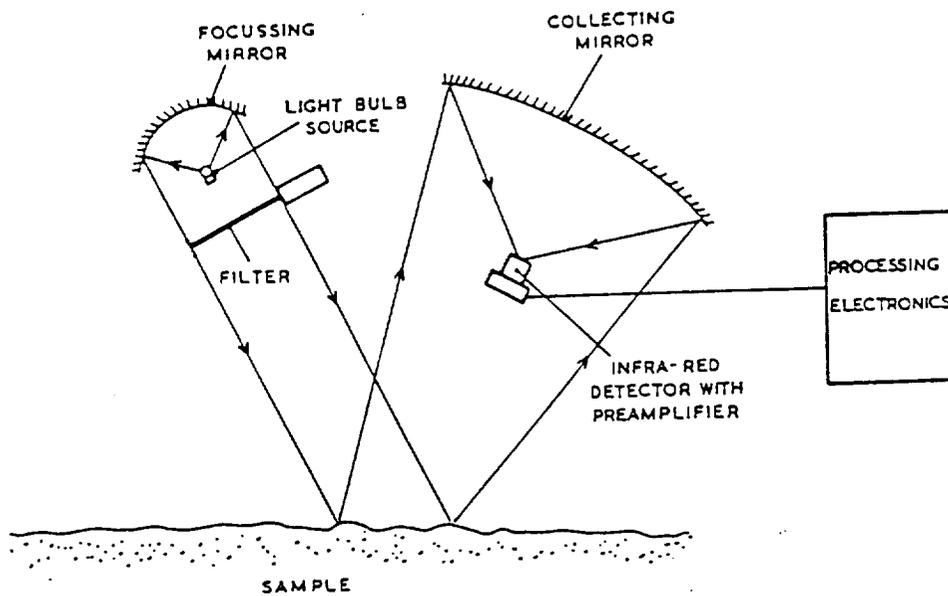


Figure III-5 ON-LINE MOISTURE MEASUREMENT IN SOLIDS
(source: Moisture Sensing and Process Control, 1986)

This is a mature technology with a number of applications currently in the field. Concentrations ranging from 0.02% to nearly 100% have been sensed with accuracy generally around $\pm 1\%$ (Carr-Brion, 1986). However, further research is needed if this sensing technology is to be incorporated into an operational nutrient delivery system.

CONCLUSION

Originally, the nutrient delivery team was assigned the task of recognizing sensing needs of a nutrient delivery system presently being studied. After reviewing technical papers on the systems and speaking to personnel involved with the CELSS project, the list of sensing needs which proved to be most crucial to the survival of the crop was established. Of the list, wetness sensing seemed most promising because it is a more direct approach to monitoring water availability to the roots of the plant. Currently, the team intends to place emphasis on the design and testing of a practical wetness sensor for the Spring term.

The wetness sensor's capabilities will be applied to a flat plate version of Thomas Dreschel's growing tube. By the middle of Spring, 1990 a working model with soybean plants growing on the plate should be developed. The sensor will be tested on several spots throughout the total growing area of the plate. Figure III-6 shows a sensor in use on a cut-away portion of the plate.

The growing plate will consist of a hydrophilic, ceramic porous covering and a solid nonporous trough. The porous top will be ceramic so as not to interfere with the electrical properties of the sensor. It should remain horizontal when influenced by gravity in order to receive an even soaking from the solution flowing in the trough. A support grill could provide support to keep the plate in a horizontal position. The trough will be made of nonporous material to limit other factors, such as leaking, from disturbing the homogenous wetting of the porous cover.

The pressure differential controls the amount of solution that can be absorbed through an area of the porous material over time; this quantity is defined as capillary flux. Currently, the flux is being measured by placing an absorbent material on the outside of the tube. Flux can be calculated by the difference between the initial and final weight over a given period of time. The pressure inside the growing plate will be varied to achieve different levels of capillary flux potential.

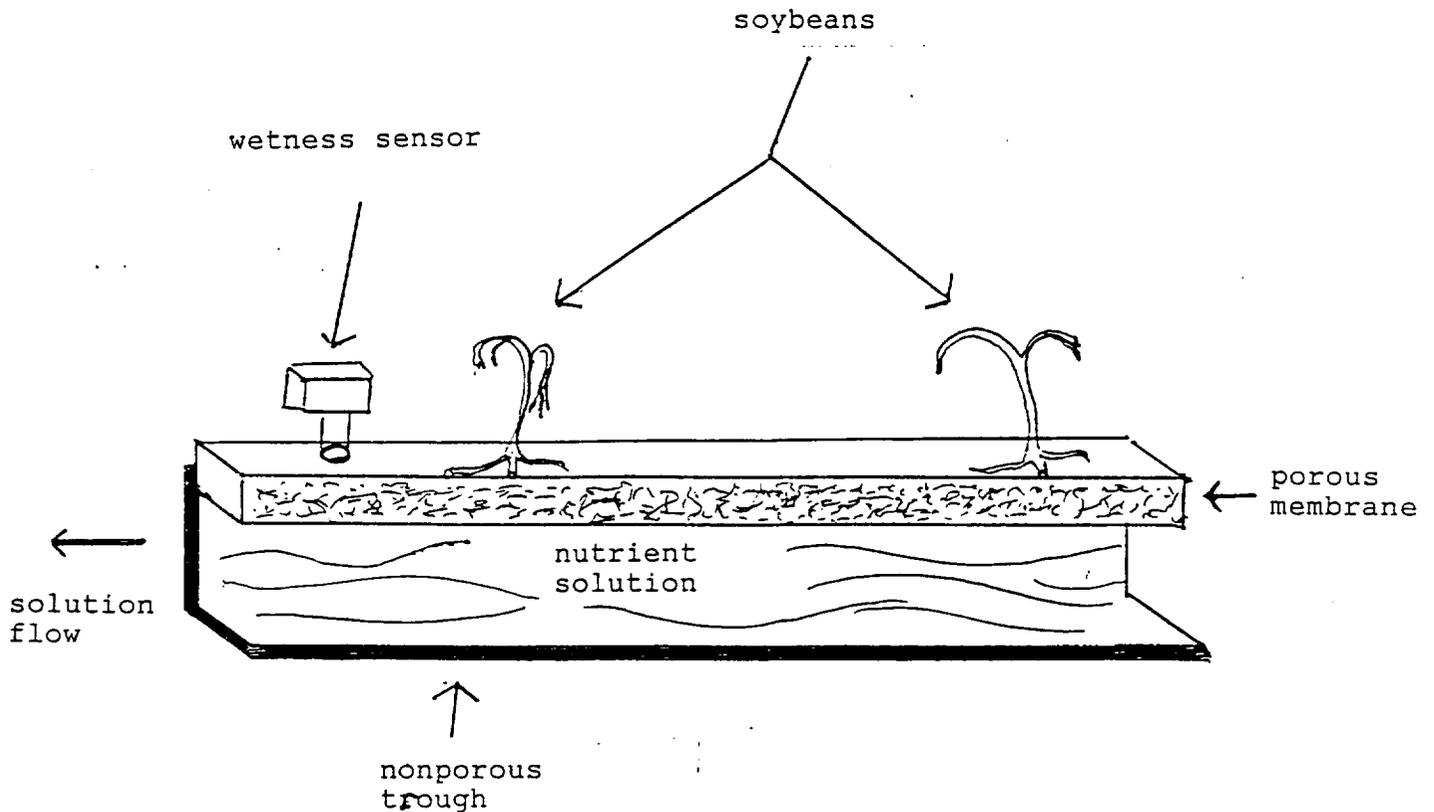


Figure III-6 WETNESS SENSOR IN ITS ENVIROMENT

It is known that an adjustment in the pressure differential across the plate system generates a change in the membrane's wetness. However, the mechanism by which this change occurs is not known, but it is believed that the plate absorbs the solution like a sponge. Moisture gradients may vary over the thickness of the plate. An understanding of the microscopic dynamics of fluid absorption through the porous media will be necessary to successfully develop a wetness sensor. Further attempts will be made to interview Dr. Sullivan (Chairman of the Physics Department, University of Florida) to gain an understanding of this area. This information is crucial because the wetness sensor functions off the dielectric properties (if the sensor is of the capacitor type) or the electrolytic properties (if the sensor is of the resistance type) of the solution. If the solution moves across the boundary of the plate as envisioned, the resistor or capacitor of the sensor may not function as expected. This concept will be investigated as related technologies are reviewed.

The feedback received from the sensor will be compared to a reference range. If the feedback drifts above the range, one can assume that the membrane is too moist and the danger of water droplets breaking free exists. If the feedback falls below the range the membrane is too dry, starving the plant of water and nutrients. These conditions could be corrected instantaneously by a computer recognizing the trouble and actuating a pump. The pressure would be increased or decreased in the growing unit, either drying or wetting the membrane as necessary. This control scheme may aid plant production by maintaining optimum saturation levels in the porous medium.

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APPENDIX A

CROP EFFECTS AND SALT CONTENT

The following table presents expected crop effects due to salt content:

Conductivity (mmhos/cm)	Total Salt Content (%)	Crop Reaction
< 2	< 0.1	Salinity effects mostly negligible
2 - 4	0.1 - 0.15	Yields of very sensitive crops may be effected
4 - 8	0.15 - 0.35	Yields of many crops restricted
8 - 16	0.35 - 0.70	Only tolerant crops yield satisfactorily
> 16	> 0.70	Only a few very tolerant crops yield satisfactorily

Source: *Diagnosis & Improvement of Saline & Alkaline Soils* (1954).
(USDA Agricultural Handbook No. 60). Washington, DC:
US Government Printing Office.

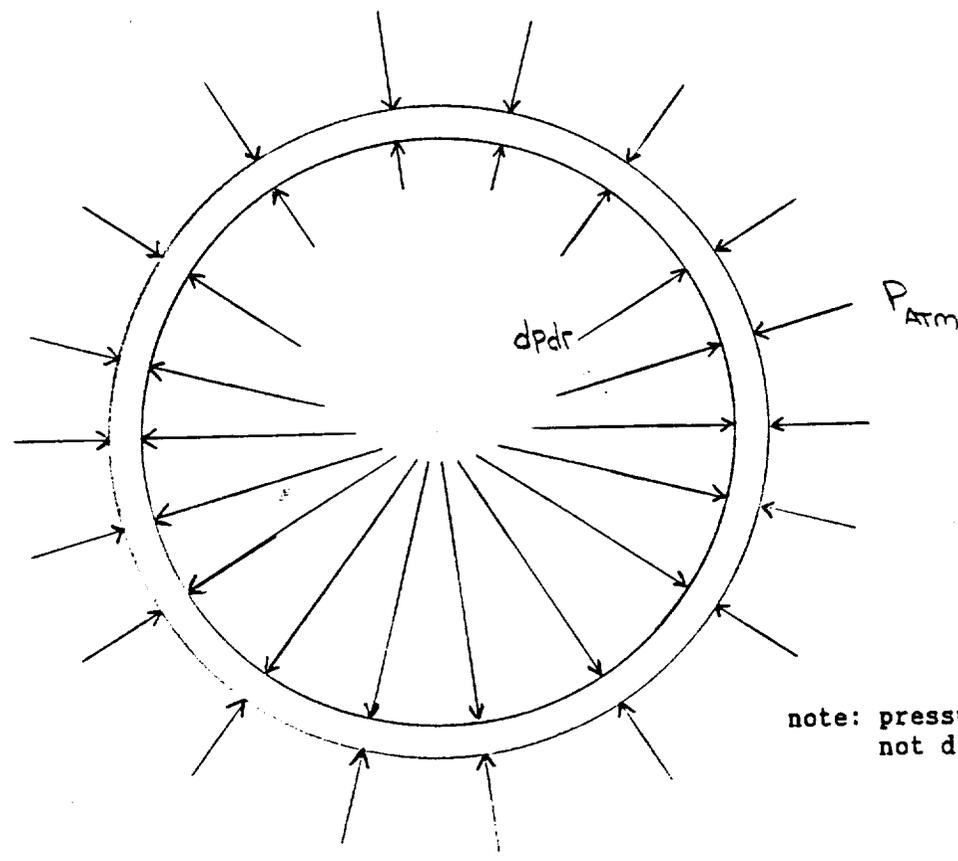
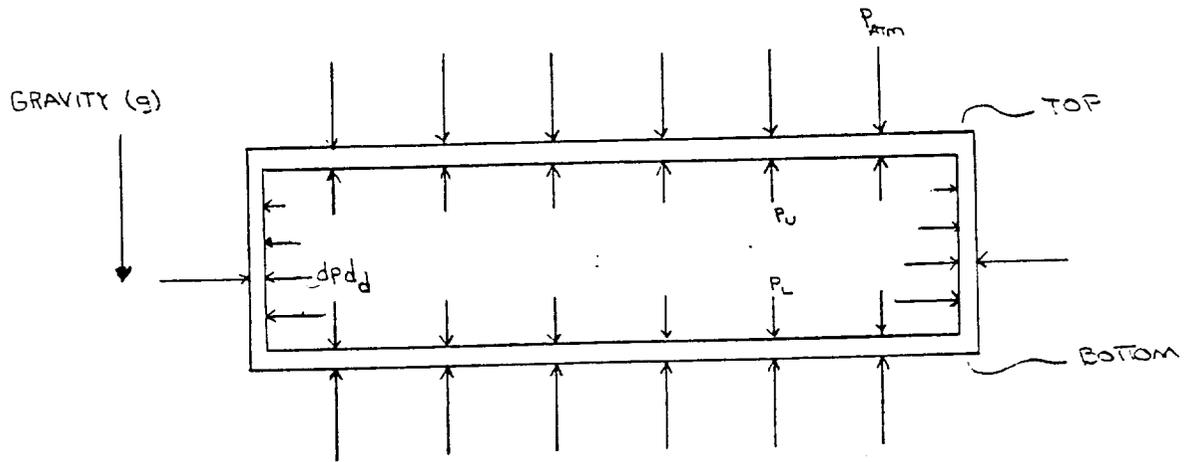
APPENDIX B

RECTANGULAR CONDUIT (POROUS PLATE)

The geometry of the growth media has been selected to be rectangular. The rectangular structure of the conduit has the advantage of minimizing the vertical change in the pressure gradient as a result of gravity. Figure III-7 shows an exaggerated view of the pressure distribution in a rectangular plate and a circular tube. It can be seen that the pressure in the tube is influenced more by the hydrostatic head than is the plate. By virtually eliminating the effects of pressure due to gravity, one can closely simulate microgravity conditions.

The dimensions of the plate will be designed to optimize the range of negative gauge pressure while retaining laminar flow. It is important to maintain laminar flow to prevent separation and to ensure that the plate does not contain any dead zones (see figure III-8). Laminar flow provides consistency in boundary layer development; it is this development that contributes to the mass flow across the porous material.

There are two ways to ensure laminar flow in a rectangular conduit that is supplied by a feed tube. A rule of thumb to ensure a laminar flow is to gradually diverge the inlet by no more than 12° (Partheniades, 1989). An alternate method would be to install fins at the inlet chamber to direct the flow. With the fins, the angle of divergence can be made up to approximately 25° . Since it is desirable to minimize the effects of gravity, the height of the plate and inlet chamber will remain the same as the diameter of the feed tube. Figure III-9 depict the physical representation of the growth plate. Note that streamlines are added to illustrate the flow through the rectangular conduit. Also, it should be noted that the angle of convergence for the exit flow has a lesser effect on the development of the laminar flow in the conduit.



note: pressure gradients not drawn to scale

Figure III-7 FLUID PRESSURE DISTRIBUTIONS IN CONDUITS

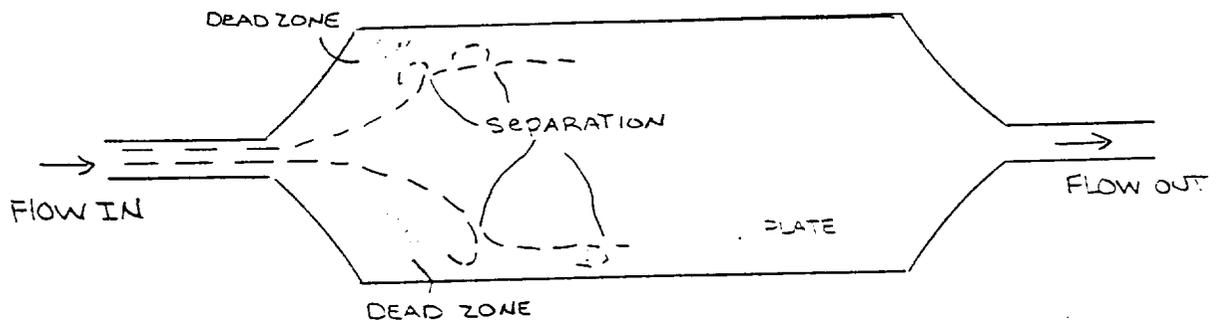
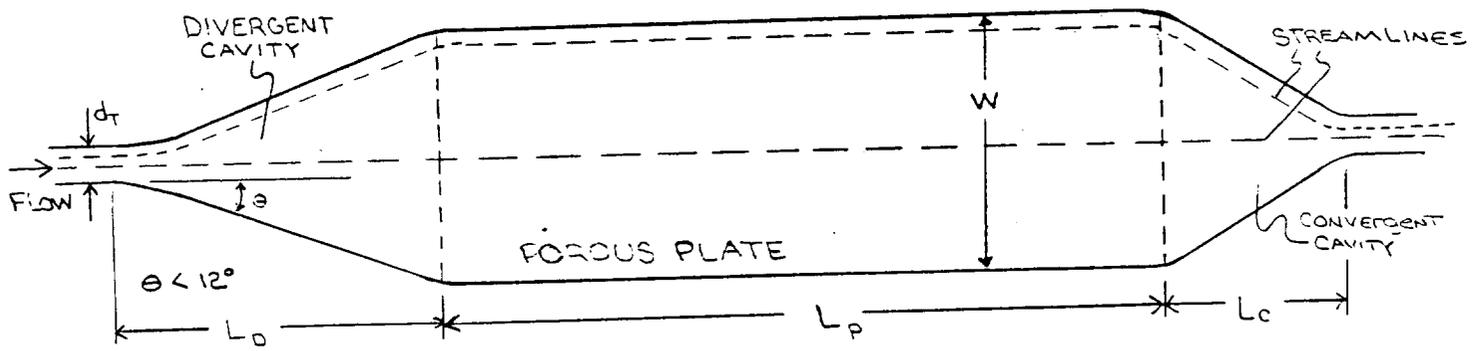
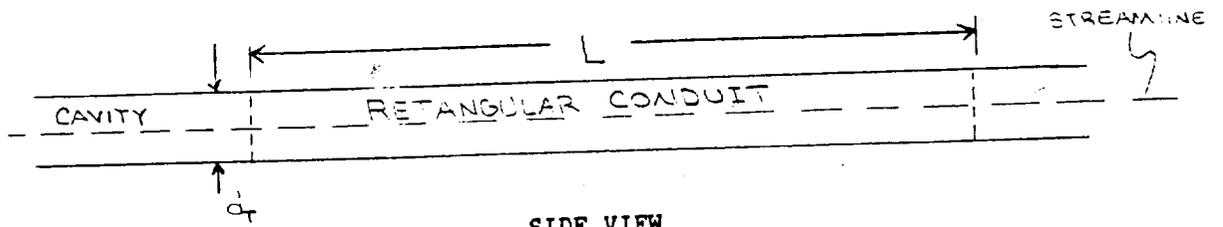


Figure III-8 TURBULENT FLOW IN RECTANGULAR CONDUIT



TOP VIEW



SIDE VIEW

Figure III-9 REPRESENTATION OF FLAT GROWTH PLATE

APPENDIX C

NEGATIVE GAUGE PRESSURE

Gauge pressure is measured relative to the absolute atmospheric pressure. In order to obtain a negative gauge pressure in the growth plate, we assume that friction forces are negligible at the boundaries in the divergent cavity and apply Bernoulli's equation along a streamline. Bernoulli's equation states that along a streamline the following relationship exists:

$$\frac{P}{\gamma} + z + \frac{V^2}{2g} = \text{constant}$$

where $P \equiv$ pressure, $\gamma \equiv$ specific weight, $z \equiv$ height, $V \equiv$ fluid velocity, $g \equiv$ gravity. Hence, to find the pressure difference between inlet and outlet the following holds true:

$$P_2 - P_1 = \gamma (z_1 - z_2) + \frac{\gamma}{2} \left(\frac{V_1^2}{2} - \frac{V_2^2}{2} \right) \quad \gamma \equiv \text{density}$$

Since there is no height difference, then $z = 0$. Hence the exit pressure is as follows:

$$P_2 = P_1 + (V_1^2 - V_2^2)(\gamma/2) \quad (1)$$

Assuming that either the inlet or outlet velocity and pressure are known, then only two unknowns remain in equation (1). If the control volume theory is used along with the continuity equation, $Q_{in} = Q_{out}$, then:

$$Q = \text{Velocity} * \text{Area}$$

$$(VA)_{in} = (VA)_{out}$$

Solve for the unknown V .

As a result of using Bernoulli and continuity equations, it is found that in order to achieve a negative gauge pressure in the rectangular conduit, the force that creates the flow must be downstream from the growth plate. That is to say, that if a pump were used to create the flow in the system, the pump must pull the fluid through the rectangular conduit, not pumped into it. By varying the pump speed the exiting velocity can be controlled to alter the pressure inside the growth plate.

Another point of consideration is the dynamic pressure change in the direction of flow. Figure III-10 shows an experimental rectangular conduit. The atmospheric pressure is constant throughout the entire outer boundary surface. Since there is no change in the atmospheric pressure, the ideal scenario for the interior plate pressure is for it to be constant. But this is not so due to the shear stresses present at the boundaries. The modified Bernoulli equation can be obtained by using the momentum equation with a differential control volume. This equation holds for incompressible constant-area flow. Note that there is a term added to include friction. So for no change in elevation, the equation is:

$$P_2 - P_1 = \frac{eV^2L}{2D_h}$$

Where $f = f_1(\text{Re}, \theta/D)$ and,

$D_h = 4 \cdot \text{cross sectional area of flow} / \text{perimeter wetted by fluid.}$

Note that Re is the Reynolds number and θ/D is a ratio of friction length to equivalent diameter.

The purpose of evaluating the dynamic pressure differential is to more accurately define the upper and lower bounds of the negative gauge pressure. With a pressure difference at the opposite ends of the rectangular conduit, it must be realized that if the upper bound on one end and the lower bound on the other are not specified, then either leakage or drying exists at one end as the pressure limit is approached. Thus, an average flow rate must be provided to avoid wetting deficiencies.

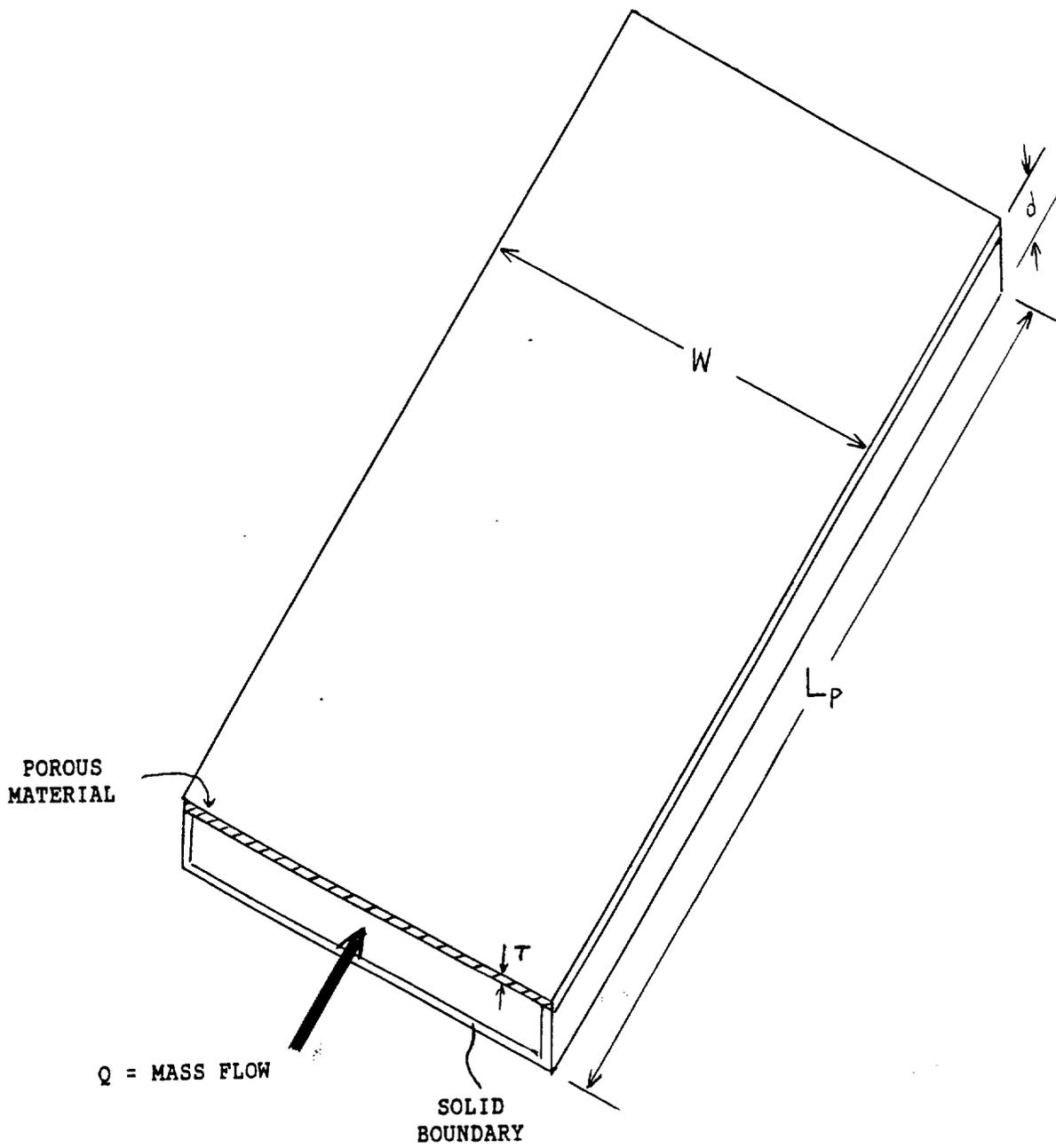


Figure III-10 RECTANGULAR CONDUIT (POROUS PLATE)

IV. PLANT HEALTH

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SUMMARY

For a Closed-Loop Life Support System (CLLSS), sensors and controllers are crucial for monitoring plant health. Using the soybean plant as a focus, it is possible to compile a list of health indicators which could be measured to determine the physical state of the plant. The most common symptoms of adverse conditions were considered as indicators for plant health sensing. Available sensing technologies were investigated to determine their ability to accurately and efficiently detect these indicators. The advantages and disadvantages of each of these technologies were examined. A viable sensing system was then proposed which, through the use of the appropriate sensors and expert systems, might be capable of sensing plant health and determining the most probable cause of any anomalies.

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INTRODUCTION

The development of a plant health sensor is essential to the success of a closed-loop system. This sensor should be capable of detecting deviations in plant health in order to initiate corrections to adverse conditions before serious damage to the plant can occur. To detect plant health, it is necessary to determine symptoms that may occur due to the environment, plant pathogens, nutrient deficiencies or toxicities. For this project, the most common indicators were used.

To sense the symptoms of plant disease, many of the available sensing technologies were studied. These technologies included both nondestructive and destructive techniques which would efficiently locate, and possibly determine, the cause of disease. Many of the advantages and disadvantages of each method were explored in order to focus on the most viable sensor or sensors. Based on the capabilities of the methods studied, it is believed that a sensing system comprised of two or more sensors would be best suited to meet the demands of a CLLSS health sensor.

This paper will discuss the factors that influence plant health and the symptoms which occur when the plant is adversely affected by its surroundings. Following this topic, the various destructive and nondestructive sensing technologies and their respective advantages and disadvantages for detecting plant health indicators will be discussed. In addition, there will be a section on expert systems and how they may be utilized to control and intervene in a sensing system. In conclusion, this paper incorporates these topics, and proposes a feasible sensing system that is capable of fulfilling the necessary requirements.

PLANT HEALTH

In a closed-loop life support system, the health of each crop is directly related to the survival of the crew. Soybeans are hardy and relatively resistant to disease and adverse conditions. However, like any plant, the soybean can be affected by pathogens such as, parasitic organisms (fungi), bacteria, and viruses, as well as imbalances in temperature, light, atmosphere, humidity, nutrients, and other aspects of the growing environment (Pandey, 1987). In a field located on earth, disease can also be caused by insects and nematodes, but this is highly unlikely in a spacecraft; thus, for this project, only the pathogens and environment will be of major concern.

Pathogens

Pathogens include bacteria, fungi, and viruses; all of which are very different in their physical make-up and how they reproduce. The manner in which they infect the soybean plant also varies: a fungi is a parasite that feeds off of the external structure of the plant, while bacteria and viruses actually enter into the plant and disrupt the internal structure. Table IV-1 summarizes some of the common pathogens and the symptoms they create.

Diseases are common in soil-based growing areas and are usually seen in wet, poorly-drained growth conditions. Therefore, this type of effect on plant health may not be a major problem in a closed-loop system. Many types of microbes have been found on spacecraft, however, plants growing in the plant growth unit have not been found to be seriously damaged by disease-causing pathogens. Most likely, the trouble with soybean health will be a result of imbalances in nutrients and environmental conditions (Strayer, 1989). A common, recognizable symptom of most bacterial or viral diseases would be a small "splotch" of dead cells on the leaf which becomes larger as the infection spreads (Kucharek, 1989).

Table IV-1.
Common Infectious Disease Agents
in Soybeans

<u>Organism</u>	<u>Symptom</u>
bacterial blight (bacteria)	small, yellow/brown lesions on leaves turn dry and black (necrosis)
bacterial pustule (bacteria)	small, pale green spots on leaves with raised centers
brown spot (fungi)	irregular, dark brown lesions on leaves
pod & stem blight (fungi)	stunted growth necrosis of stems, pods, and leaves
anthracnose (fungi)	irregularly-shaped brown areas on stems, pods, and leaves
bud blight (virus)	bud curvature, becomes brittle necrosis
bean pod mottle (virus)	green/yellow spotting on leaves decrease in water pressure

Environmental Conditions

Environmental factors such as light, temperature, humidity, carbon dioxide, and nutrients affect the development of soybeans. Deficiencies and excesses in these conditions can produce adverse reactions within the plant. Although these may sometimes be confused with those of microbial disease (Scott, 1970), the symptoms of environmental imbalances can be specific to the nature of the problem.

Light. The light intensity of the growth chamber should be kept at a level where vigorous, field-like growth will occur. A low intensity level will lead to smaller leaves and less dry weight at maturity. A high intensity level will cause bleaching of the leaves due to destruction of the chlorophyll (Langhan, 1978).

Temperature. The temperature is directly proportional to the intensity of the light and level of CO₂ in the growth chamber (Langhan, 1978). High temperatures increase water stress within the plant, as well as cause a breakdown of cell-membranes which is seen as discoloration on the leaves and stem (Sinclair, 1984).

Humidity. Relative humidity influences the transpiration rate of the plant and, therefore, the water stress (turgor pressure) within the plant. Low humidity would cause an increase in transpiration, leading to an accumulation of nutrients in the leaves and a risk of toxicity and necrosis (Langhan, 1978).

Carbon Dioxide. Carbon dioxide (CO₂) greatly affects the plant growth rate. High CO₂ levels increase growth, and until levels above 2000 ppm are encountered, CO₂ will not injure the plant. CO₂ levels below 320 ppm cause the plant to slow or stop growing. Levels below 50 ppm will eventually cause the plant to die (Langhan, 1978).

Nutrients. The nutrients are extremely critical in maintaining plant health. There are 12 essential minerals that must be included in the nutrient solution. Any deficiency in one of these elements will have a significant impact on the plant development. Specific indicators may be observed for each type of mineral deficiency. These symptoms are listed in Table IV-2.

Most of the environmental conditions create a reaction within the soybean that can be seen on the surface of the plant. This is especially true of those symptoms arising from an imbalance in the nutrient solution. It was found that when a plant is deficient of a mineral, the first sign will be minor discoloration between the veins of the leaf. If the problem goes uncorrected, the "spot" will spread out to incorporate more of the surface area. Eventually, the leaf will become necrotic and may even fall off. The location of this reaction depends on whether it is a "fast" or "slow" moving nutrient. A deficiency in a mineral of the former will affect the bottom leaves of a plant first, while a deficiency in the latter type will cause these symptoms in the upper leaves (Kucharek, 1989).

Table IV-2.
Symptoms of Mineral Deficiencies

<u>Mineral</u>	<u>Symptom</u>
boron	(deficiency) yellowing or reddening of upper leaves, flowers fail to develop (excess) very toxic, stunts plant growth, crinkled, brown leaves
calcium	(deficiency) late, cup-shaped primary leaves, necrosis on the terminal bud, chlorosis
copper	(deficiency) reduces seed yield
iron	(deficiency) yellowing of leaf veins, leaf becomes white
magnesium	(deficiency) lower leaves become mottled with pale green/yellow between the veins, upper leaves display rusty specks and necrosis
manganese	(deficiency) similar to iron except veins remain green, leaves become pale and develop brown necrotic spots
molybdenum	(deficiency) reduces growth, number of pods, number of seeds, seed size, nitrogen and protein content of the seeds leaves become pale yellow, necrotic, twisted
nitrogen	(deficiency) plants become pale green and leaves become completely yellow, lose leaves, stunted growth
phosphorus	(deficiency) retards growth, spindly plant, leaves turn dark green to bluish-green
potassium	(deficiency) weak stems, irregular mottling on leaves, leaf margins become yellow and necrotic, wrinkled seeds
sulfur	(deficiency) cell walls thicken, stems become hard, thin, and elongated, small yellow-green leaves
zinc	(deficiency) stunted leaves and stems, leaves become chlorotic (yellow or light green)

SENSING TECHNOLOGIES

In studying the development of a plant health sensor, different existing technologies were considered. The main emphasis of this research has been towards various spectrographic, ultrasonic, holographic, and visual imaging techniques. These techniques may be classified into two distinct categories: destructive and nondestructive methods. Nondestructive methods are preferable. However, as will be explained later in this report, it has been concluded that certain aspects of the diagnostic analysis of the plant may need to be destructive.

Nondestructive Techniques

Holography. Holography is the production of a three-dimensional image of a given object. As shown in Figure IV-1, a hologram generator consists of a laser, a beam splitter, various lenses and mirrors, and an image receptor (i.e. film processor). To produce a hologram, the laser generates a beam that is directed via lenses and mirrors through a beam splitter. This beam is separated into the object beam and the reference beam. Through another series of lenses and mirrors, the beams are directed to their respective locations. The object beam illuminates the object, while the reference beam shines on the image receptor. The receptor, usually film, records the image. By varying the position of the object beam, a three-dimensional image may be produced (Mix, 1987).

The advantages to this imaging process include that it does not require any preparation of the specimen, nor does it require contact with the specimen. Furthermore, systems similar to the one described are already commercially available. One major disadvantage, however, lies in its resolution. In order to achieve a sharp image, particularly around the edges, it is necessary for the system and object to be perfectly motionless. The vibrations induced by the surroundings (i.e. robotics, people, air currents) could induce very poor images (Ovryn, 1987). This reduced image resolution would cause great difficulty in an accurate interpretation of the images. Consequently, a holographic system may not be adequate for CLLSS applications.

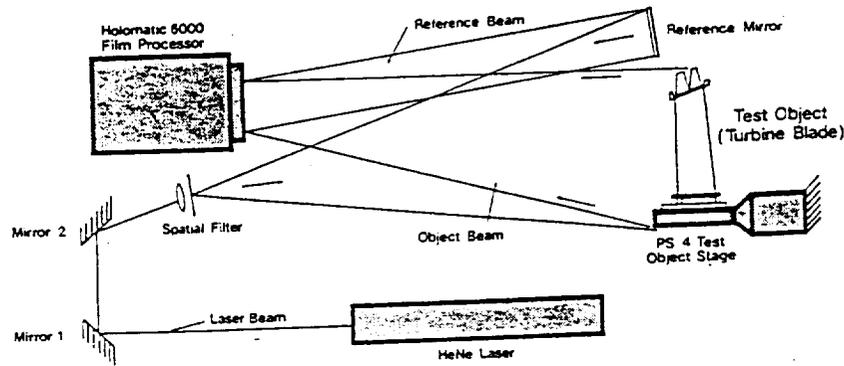


Figure IV-1. Diagram of a holographic generator.
 (Source: Mix, 1897)

Visual Imaging. Visual imaging consists of using a camera to capture a two dimensional image of an object then digitizing the image for computer analysis. This process may be used for position sensing and color sensing. The former is imperative for an automated system.

Color sensing could be used for detecting nutrient deficiencies since each nutrient deficiency causes a different pattern of discoloration in the leaves of a plant. An experiment of this nature has been conducted by Professor Gaines E. Miles (1989) of Purdue University. This experiment concluded that it is feasible to use machine vision for detecting nutrient deficiencies. The equipment consisted of a personal computer, a frame-grabber, a Panasonic WV-D5000 camera, and a bellows. By inducing certain nutrient deficiencies in the plants, Professor Gaines was able to verify that the computer would be able to sense discolorations in the leaves. However, the results also indicated difficulty in analyzing the fringes of the leaves. This problem was created by an inadequacy in differentiating between backlighting and frontlighting. Also, the Sobel operator used for detecting the edges proved to be somewhat inadequate (Gaines, 1989). In order to increase the accuracy, a multiple spectrum approach should be used. This is because different shades of colors may be composed of similar wavelengths of light. For example, white light and bright green light contain the same amount of the

maximum green light wavelength (Amery, 1989). Therefore, though some complications do exist, the concept of visual imaging is a promising approach for a CLLSS plant health sensing system.

Infrared Sensing. Infrared imaging is an alternative for visual imaging. This method has been widely used in the agricultural field for determining plant stress caused by nutrient deficiencies, diseases, and water availability (Blazquez, 1981). This process has been conducted largely through the use of remote sensing or aerial photography using either black and white or color infrared film. This technique is more sensitive than visual imaging in that it will produce a greater amount of contrast. This method involves examining the pixels of a photograph and determining the differences in shades and textures of the photographs. This photointerpretation may be accomplished either through the use of a densitometer (Blazquez & Edwards, 1984) or digitizer. To use a densitometer, a transparency of the film must be made so that each picture can be viewed by hand (Blazquez et al, 1988). By digitizing the image, film is bypassed and the image is recorded by computer in the form of an array of numbers. Each number represents a different shade of color. This form of photointerpretation may then be analyzed, using the computer and the number array.

This form of sensing has various drawbacks. Infrared imaging is affected by shadows and background noise more than a simple visual imaging system. To produce a more accurate image, color infrared film should be used; however, as false signals are produced, problems arise in the digitizing of the numerous colors. The use of filters is required to aid in the control of noise. To produce an efficient system, it may be necessary to cross reference an infrared image with a standard image in order to confirm any problems.

Nuclear Magnetic Resonance. Nuclear magnetic resonance (NMR), commonly used in medical applications, is a type of spectroscopy that can detect the shape and structure of a molecule (Partain,1983). Because the output of an NMR system is highly sensitive to changes in the chemical environment (Goetz, 1985), NMR would be an effective method for determining plant health as well as determining the probable cause of any problem. However, despite the sensing potential of this technique, NMR was found to be highly impractical for a space-bound mission. Not only is the equipment extremely large and heavy, but an NMR system also produces a large magnetic field. This might be unacceptable for a closed-loop system due to the possibility of disrupting the other electrical equipment being used in the biomass chamber.

Ultrasound. The term ultrasonic refers to sound waves which are propagated at a frequency above that which can be detected by the human ear (Brown & Goodman, 1965). The technique of ultrasonic measurement is commonly used as a sensing mechanism for measuring fluid flow rates. Ultrasound is an attractive method for sensing plant health because it offers advantages such as (Riezenman, 1989):

1. noninvasive (to the flow) measurement
2. real time data
3. inexpensive

With these characteristics, this technique appears to have potential in measuring the rate of water uptake within a plant, and therefore be a sensor for plant turgidity. However, ultrasonic measurements require a medium for propagation; thus, it may be unacceptable for a closed-loop application. Even though it is possible to send the ultrasonic signals through air (from a distance) to the plant, there is a distortion produced due to residual vibrations. To avoid this problem, it is necessary to use another medium for propagation or place the sensor in contact with the plant. Although neither of these approaches is destructive, neither appears to be acceptable for the CLLSS program because of the high risk to the plant if submerged or touched.

Optical Spectroscopy. Optical spectroscopy consists of analyzing wavelengths of light being reflected from a test object (James & Sternberg, 1969). This process allows the analysis of the color distribution over a surface (i.e. a leaf). An optical spectroscopy system consists of two components: a photospectrometer and a detector (James & Sternberg, 1969).

The photospectrometer can be designed many different ways. One of the simplest and most reliable configurations is the Wadsworth constant-deviation mounting. This mounting is shown in Figure IV-3. Light reflected from the leaf enters through the entry slit where it is directed by the lens into the prism. By selecting the angle at which the prism is set, a particular wavelength can be chosen for analysis. This selected light is directed out through the exit slit by a mirror and lens combination. The light leaving the spectrometer is that of a chosen wavelength. The prism may be rotated to select another wavelength. This process may be repeated until the entire spectrum has been analyzed (James & Sternberg, 1969).

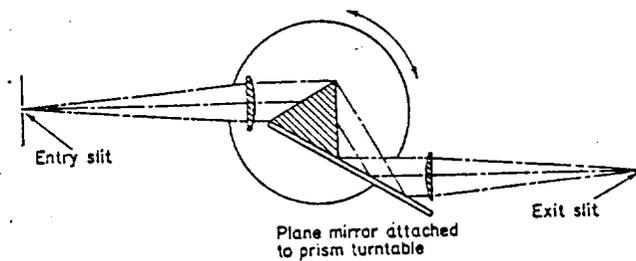


Figure IV-3. Wadsworth constant-deviation mounting of an optical spectrometer (Source: James and Sternberg, 1969).

The analysis of the exiting light is accomplished by a detector. This may be either a thermal detector or a photon detector. Thermal detectors are simply thermistors and thermocouples. The light exiting the spectrometer strikes the thermistor and creates a temperature increase. This change may be gauged to determine the intensity of that particular color. Unfortunately, these detectors have a limited range and are only used on medium and far infrared light (James & Sternberg, 1969). For near infrared, visible, and ultraviolet light, photon detectors are usually used. One type of photon detector is the photoemissive detector. Incident light coming from the exit slit of the spectrometer causes electrons to be emitted from a cathode and accelerated towards an

anode. These pulses may be counted via digital circuitry to yield a measure of the emission rate of photoelectrons. This results in a measure of the light intensity (James & Sternberg, 1969).

By measuring the light intensities of the various wavelengths, it is possible to determine the coloration of a particular area of a leaf. This could allow for the determination of a nutrient deficiency. Consequently, this is a feasible option for the CLLSS program.

Volatile Chemical Filtering. Another sensing technology consists of a filter system for collecting volatiles that are emitted by a plant. This work is currently being conducted by the United States Department of Agriculture(USDA) in Gainesville, Florida. An injured or diseased plant secretes certain gases into the surrounding atmosphere. These volatiles are collected by a filter and analyzed. Since certain diseases are associated with particular secreted chemicals, it is possible to sense plant health in this manner. From these results, corrective action may be taken to counteract a perceived disease threat.

The process of chemical filtering consists of isolating a plant in a small chamber, blowing a stream of air onto the leaves, and capturing any released volatiles in a filter. The analysis of the filter, using various gas chromatograph and spectroscopy techniques, reveal information about the health of the plant. Unfortunately, at this time, the implementation of this method on a large scale is considerably difficult. If the entire chamber is analyzed, it is possible that a single, diseased plant will not be able to produce enough volatiles to indicate that it is diseased.

However, small scale sensing, involving investigating each plant individually, will probably be extremely difficult and tedious. Besides only giving information about a single plant, it would be necessary to have a general sensing technique in order to direct the volatile chemical filtering system to the specific plant in trouble. Although this method is promising for determining specific deficiencies, there are some major technical difficulties, such as real-time data and large scale detection that must be overcome if this technique will be used to sense the health of the plants in the PGU (Manukian, 1989).

Table IV-3.
Summary of Nondestructive Techniques

	<u>Advantages</u>	<u>Disadvantages</u>
Holography	No preparation No contact Commercially available	Poor resolution
Visible	Simple equipment	Edge distortion
Imaging	Valuable data	Light overlap
NMR	3-D imaging	Large magnetic field Heavy equipment
Infrared	Good imaging sensitivity	Background noise Poor resolution
Ultrasound	Possible pressure sensing	Requires contact
Optical Spec.	Color changes Reliable Simple implementation	Possibly slow Complex data
Chemical Filtering	Nutrient specific	Not real time Difficult to isolate

Destructive Techniques

The purpose of many destructive techniques is to determine specific nutrient deficiencies within a plant. If normal ranges are known, a small sample of a plant will suffice in determining if a particular nutrient is present in adequate amounts. The basis for many spectroscopic techniques is to analyze specific elements in compounds and discover the molecular and atomic structures of different substances.

Mossbauer Spectroscopy. Mossbauer spectroscopy consists of a moving source and a gamma ray detector. This can be seen in Figure IV-4. The moving source bombards the sample which then releases detectable gamma rays. The intensity of the gamma ray irradiation may be calculated based on the velocity of the source. This technique allows for the analysis of isomer shifts, quadrupole splitting, and nuclear Zeeman splitting. This yields important chemical information about the sample. However, the sample must be in liquid form, and for best results, the test should be run at low temperatures (Chang, 1971). This technique may be feasible as an indicator for specific nutrient deficiencies.

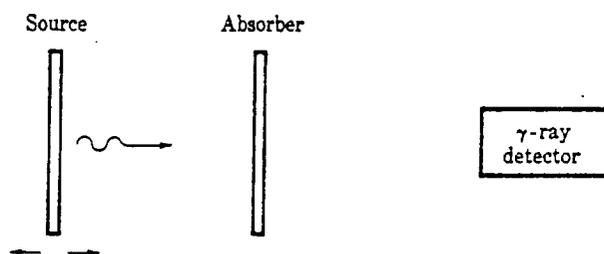


Figure IV-4. Schematic of a Mossbauer spectrometer.
(Source: Chang, 1971).

Microwave Spectroscopy. A block diagram of a microwave spectrometer is shown in Figure IV-5. It consists of a radiation source, power supply, frequency scanner, sample cell, detector, and recorder. It has two main disadvantages that make it unfavorable for a CLLSS. First, the sample must be converted into a gas. Second, the testing chamber must be extremely large, on the order of meters (Chang, 1971). Therefore, microwave spectroscopy is not considered to be a promising approach due to size limitations.

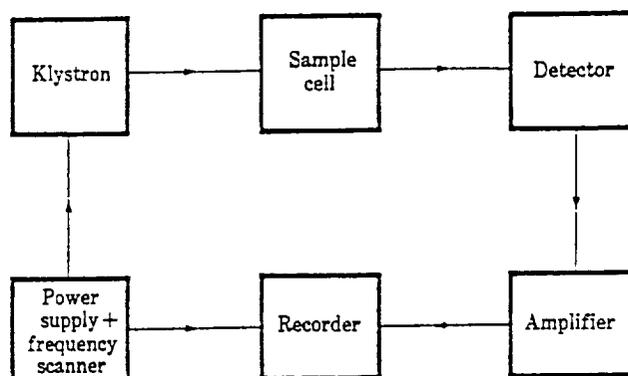


Figure IV-5. Block diagram of a microwave spectrometer.
(Source: Chang, 1971)

Flourescence Spectroscopy. Fluorescence spectrometers consist of a source, monochromator, detector, and recorder. A representation of this is shown in Figure IV-6. The source may be a tungsten or mercury lamp and the detector may be a photomultiplier. Electrons will become electronically excited when exposed to the appropriate frequency of light. Since this excited state is unstable, the energized molecule will return to its ground state by emitting a photon of light. It is possible to identify the molecules of interest through the analysis of the emitted light. Although, the sample must be liquid, it may be at room temperature during testing (Chang, 1971), and is therefore a likely candidate for further research.

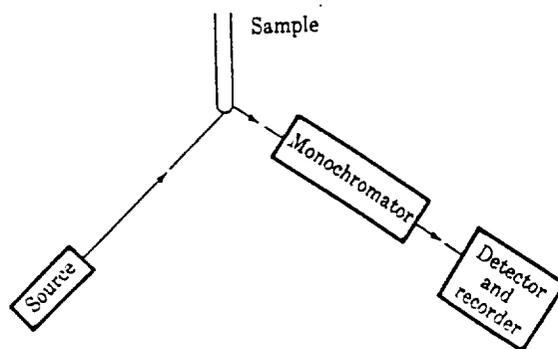


Figure IV-6. Block diagram of a fluorescence spectrometer.
(Source: Chang, 1971).

Raman Spectroscopy. Figure IV-7 depicts the block diagram of a Raman spectrometer. The spectrometer consists of a laser light source which is necessary to cut down the result time from hours or days to minutes. The other two components are a monochromator and a detector. The monochromator selects a particular light to be analyzed. The detector is usually a photomultiplier (Chang, 1971). The greatest disadvantage of this technique is the need for a laser to obtain quick results.

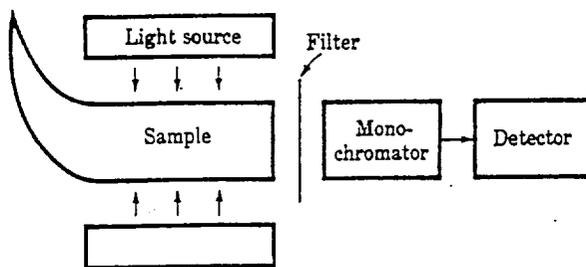


Figure IV-7. Diagram of a Raman Spectrometer.
(Source: Chang, 1971).

Photoelectron Spectroscopy. As is evident from Figure IV-8, the photoelectron spectrometer is the most complicated spectroscopy system treated in this discussion. This type of spectroscopy requires a source of ionization radiation which must be

monochromatic, and a collision chamber in which the ionization of the sample may occur. The sample inlet regulates the amount of sample entering the chamber. The electron kinetic energy analyzer measures the energy of the electrons that are ejected in the collision chamber. Other components of this device include an electron detector, usually a multiplier, and recording equipment. Vacuum pumps are used to maintain a low pressure within the system. Based on a knowledge of the ionization energy of certain elements and an accurate measure of the ionizing energy provided to the collision chamber, the elements present in the sample may be determined (Rabalais, 1977). The sample may be in either a solid, liquid, or gaseous state (Chang, 1971). Thus, this method displays promise for use in the analysis of nutrient deficiencies.

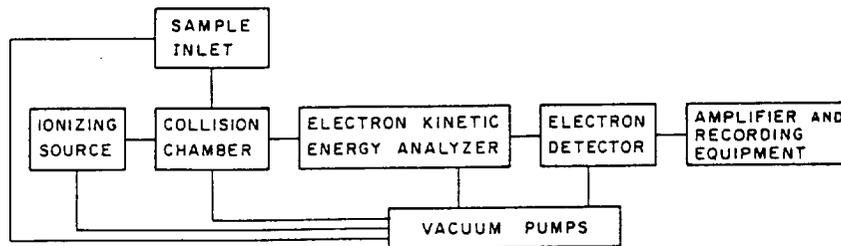


Figure IV-8. Block diagram of a photoelectron spectrometer.
(Source: Rabalais, 1977).

Absorption Spectroscopy. The set up for absorption spectroscopy is similar to that of fluorescence spectroscopy except that the monochromator is placed between the source and the sample (see Fig. IV-6). Although fluorescence and absorption spectroscopy are based upon the same concepts, fluorescence spectroscopy detects what light is emitted whereas the former detects what light is absorbed. Absorption spectroscopy does have advantages over fluorescence in that absorption spectroscopy is more sensitive (Chang, 1971). Therefore, this technique is another possibility for nutrient testing.

EXPERT SYSTEMS

In discussing expert systems, it is important to have a clear definition of an expert system's components and functions. An expert system handles real-world, complex problems requiring an expert's interpretation and solves these problems. Using a computer model of a human expert's reasoning, the system reaches the same conclusion that the human expert would reach if faced with a comparable problem (Weiss, 1984). It is these expert systems which will implement the information gathered by the sensing system to correct any factors which are adversely affecting the plant health. Also, an expert system will be necessary to increase the efficiency of the health sensing system by reducing the number of false readings.

The accumulation and codification of knowledge is one of the most important aspects of an expert system (Waterman, 1986). An expert system can only be as reliable as the knowledge supplied to it. But knowledge as a target of study is too broad and diverse; it often leads broad problems to premature solutions (Hayes-Roth, 1983). Therefore, it is important to limit the system to use extensive, high-quality specific knowledge about a narrow problem area (Waterman, 1986).

Terminology

An expert system building tool is a programming language and support package, used to construct the expert system (Waterman, 1986). The most common of these is LISP. LISP is a general-purpose programming language, chosen for most work in Artificial Intelligence because it is oriented toward symbolic computations (Weiss, 1984). The programmer can code terms such as "plant" and "health" since these terms have no pre-determined meaning in LISP. The person who does this programming is referred to as a "knowledge engineer".

Along with a programming language such as LISP, a "support environment" is necessary. A support environment is a facility associated with an expert system building tool. It helps the user to interact with the expert system by facilitating the use of debugging aids, editors, and advanced graphic devices (Waterman, 1986).

Another term often associated with expert systems is "search". It is the process of skillfully looking through the set of possible solutions in order to efficiently find the most acceptable solution (Waterman, 1986). There are several different kinds of searches, the simplest of which is a state-space search (Hayes-Roth, 1983). This search involves the formulation of a solution using states and operations. A state is a data structure that is similar to a snapshot of the problem at one stage of the solution. The operation is a change from one state to another. The object of the search is to find a sequence of operators that can be applied to an initial state until a goal state is reached (Hayes-Roth, 1983). Another type of search is the "blind search". This is the opposite of the state-space in that it starts at the end and works its way to the beginning. A blind search will eventually find a solution to a problem, but if the problem is large it could take a very long time (Hayes-Roth, 1983).

Components of an Expert System

An ideal expert system consists of many different components. One such component is a "blackboard". It records intermediate hypotheses and decisions. Most expert systems use some type of intermediate decision representation, but only a few explicitly employ a blackboard for the various types of determinations (Hayes-Roth, 1983). The "scheduler" maintains control of the potential actions awaiting execution (Hayes-Roth, 1983). It decides when and in what order to apply different pieces of knowledge (Waterman, 1986). The "interpreter" executes the action that the scheduler indicates. As the solution is being found, the "consistency enforcer" attempts to maintain a consistent representation (Hayes-Roth, 1983). The consistency enforcer handles new data to prevent disagreement with the emerging solution. The "justifier" explains the actions of the system to the user, and answers questions about why some alternatives were rejected (Hayes-Roth, 1983). The last component is the knowledge base which contains all the rules, facts, and information about the problem that may be useful in formulating a solution (Hayes-Roth, 1983).

Building an Expert System

There are five stages to building an expert system: identification, conceptualization, formalization, implementation, and testing. The problem is identified by analyzing the goals and objectives for the system through the consideration of what experts and resources are available. The problem-solving methods are described by defining the key concepts and the basic flow of information. This is called conceptualization. Formalization entails selecting a language and mapping the key concepts into a formal representation. The formalized knowledge is combined and reorganized to fit the information flow. This results in a control structure that defines an executable program. Testing involves determining the accuracy of the system and the need for revisions.

CONCLUSION

In order to accurately determine the health of a plant, it is necessary to sense on several different levels. The sensing device should be able to scan the entire crop and detect if there is any change in the health status of the plants. If there is a problem, the device should be able to locate the diseased plant(s), and perform a specific analysis in order to pinpoint the exact nature of the problem. Because these two requirements for the sensor are entirely different, the task of finding one device capable of both is rather difficult.

In light of this problem, it is necessary to consider a multi-component system containing two or more sensors in order to determine the health of the plant. The sensing devices needed to cover the various stages of detecting plant health could be divided into levels: primary, secondary, and possibly tertiary sensors.

Primary. The primary sensor should be fully automated and able to perform a general overview of the crop. The output should be real time and easily digitized for comparison in a computer data base. Infrared imaging and visual imaging are two possible sensing devices that fulfill these requirements. However, because the resolution of each techniques can be questionable, it may be more accurate to utilize them together so as to cross check the data.

Secondary. The secondary sensor would only be utilized if the primary sensor detects a problem. This stage should be able to pinpoint one specific area, and focus on the leaf or stem surfaces of the plant(s) in question. Optical spectroscopy is one possible technique that could sense and analyze the plant in this manner.

Tertiary. The tertiary level would use a sensor that could perform a detailed analysis of the plant. The goal is to determine the exact nature of the problem, whether it be a deficiency in a specific nutrient or an infection from a certain pathogen. For this, destructive tests could be used as long as only a small percentage of the plant matter is taken. The amount of destruction could be minimized by employing the secondary

system to determine specifically which portion of the plant should be removed for analysis. To avoid destroying the plant altogether, volatile chemical filtering would be another possibility.

In the future, the plant health and biological systems group will be focusing on the development and testing of a primary health sensing system. Based on a detailed analysis of plant health indicators and available sensing technologies, it was determined that the best approach would be to use a combination of infrared sensing and visual imaging for the components of the system. These systems have various drawbacks such as inaccurate digitizing of images. This problem will be addressed as well as the creation of a functioning expert system for the activation of a secondary system if the primary system reveals any problems.

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V. PLANT PROPAGATION

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INTRODUCTION

The area of plant propagation in space is relatively immature. The overall process of planting crops in a closed-loop life support system (CLLSS) in space has not been defined. Such basic areas as whether to use seeds or tissue culture, batch crops or staggered growth, are currently being investigated. Our group has chosen to concentrate on seeds exclusively, and soybeans in particular.

In any long-term mission incorporating a CLLSS, seeds will be required for each growth cycle. It is unlikely that the total mission requirements for seeds could be satisfied with a stored supply. The development of an on-board storage system, while non-trivial, should not present insurmountable difficulties. Current technology in seed storage allows for storage periods of greater than ten years with little loss of seed quality and germinability (Harrington, 1959). Even if the total mission seed requirements can be stored on board, the possibility of loss, damage, or deterioration of seed stocks must be considered. Due to the catastrophic consequences of such events, a method of seed regeneration will be necessary.

Immediate planting of fresh seed is generally desirable because seeds are highest in quality at the time they reach maturity (Delouche, 1973). If the majority of seed required for planting can be taken from harvest and planted with little or no processing, then the quantity of seeds needed for initial storage will be greatly reduced. This quantity will consist of seeds for the first planting cycle and reserve stocks for any situation in which adequate planting supply cannot be obtained from the previous harvest. In such an event, stored seed can be planted, and the stocks of stored seed can be replenished from the next harvest.

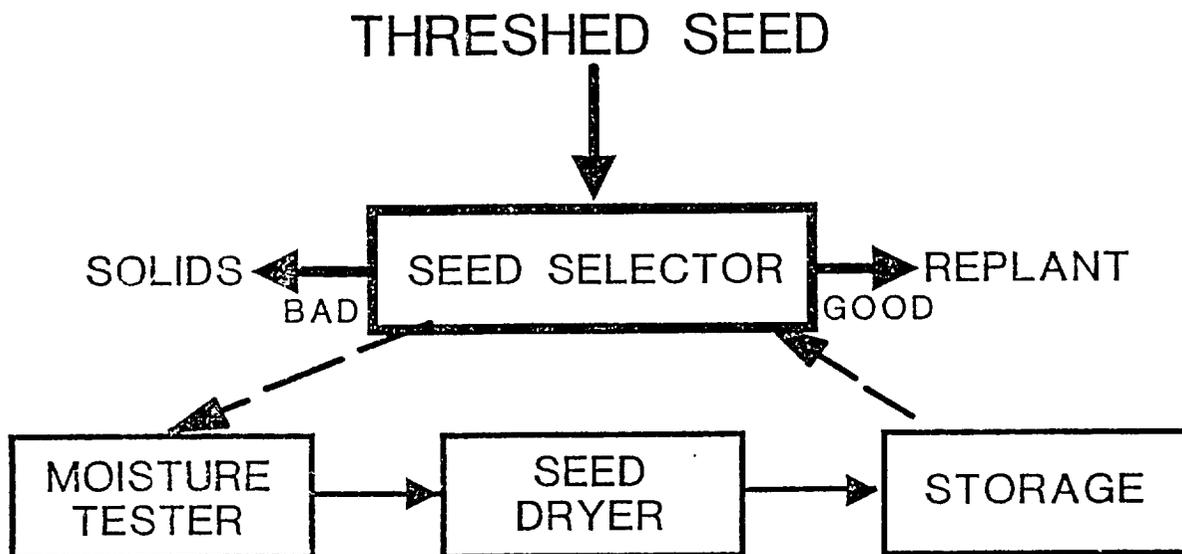
In order to store seeds, an integrated system will be needed. The seeds must be accepted from the harvester and moved without damaging. The seeds for replanting need to be selected, separated, and delivered to the planting mechanism. Seeds intended for storage also need to be selected, separated, and prepared for storage, and then placed into the storage container. Through a drying process, the moisture content of the seeds are reduced soon after harvest to prepare them for storage.

During the process of formulating a conceptual model of a planting and seed-handling system, a number of areas had characteristics that were considered to be inappropriate for the goals of the class. The problems associated with handling and transporting fragile seeds in microgravity are important and challenging; however, these problems are beyond the scope of this class.

This semester's study concentrated on sensing needs and technologies. In narrowing down the many processes involved, certain areas had to be bypassed. Variable plant spacing was found to be an already well-developed system with several proposed ideas. While the area of plant refurbishment initially offered many sensing possibilities, an overall chain of events was difficult to envision.

In modeling the soybean propagation process, an initial assumption was made that the seeds would be received at maturity, free from excess debris. Handling of the soybean is considered feasible in a microgravity environment, but not without some difficulty. After sorting the 'good' seeds from the 'bad' seeds, the most viable will either be sent for replanting or to a seed moisture detector. The less viable seed can be sent on to food processing. The seeds sent to the moisture detector will be measured for percent moisture content and integrated with a feasible seed drying system. The dryer reduces the seed moisture content to the desired level for storage of seed. The stored seed can then be used for replanting or for crew consumption in the event of seed shortages. The exact quantities needed for storage have not been determined, yet we feel storage of seeds is a necessary component in plant propagation. Once seed are planted, the plant growth unit must be monitored continuously from the time of germination to the growth of mature, seed-bearing soybean plants.

The group's investigation into soybean seed biology led us to concentrate on the areas of seed selection, seed drying, and seed storage. These areas appear challenging and useful in a CELSS application, and were the focus of our report.



SEED QUALITY TESTING

It has been well established that the performance potential of a seed progressively declines through deteriorative processes that occur over time (Delouche, 1968). During deterioration, essential biological systems and mechanisms are gradually impaired such that germination rate, resistance to environmental stress, plant growth and subsequent yield are all adversely effected. Although the process by which seeds deteriorate is not completely understood, several characteristics have been identified which can be directly related to seed quality and vigor. Since metabolic activity determines seed growth, biochemical tests which measure metabolic events in seeds associated with germination can be used to measure seed vigor (Delouche, 1974). Results of these tests will facilitate the planting of only the highest quality seeds and hopefully improve and ultimately predict yield.

The following is a review of current technologies available for rating seed quality that can be applied to a CLLSS. The standard germination test is not considered here because of the length of time required and the inability of the test to determine loss of vigor.

Requirements For A Seed Quality Tester

- 1) Nondestructive testing procedure
- 2) Adaptable to automation
- 3) Minimal handling of seed
- 4) Real-time testing
- 5) Reproducible results and 90% accuracy

Current Sensing Technologies

Tetrazolium Staining Test. The tetrazolium test (TZ) is one of the most widely used tests to quickly estimate the germination percentage of a seed lot. The TZ test is essentially a measurement of dehydrogenase enzyme activity which is a byproduct of seed cellular respiration (Hemming, 1989). The TZ test is conducted by soaking seeds in triphenyl tetrazolium chloride. This colorless solution reacts with the dehydrogenase enzyme to form a water insoluble red compound, formazan, which stains the living cells

a red color while dead cells remain colorless (Moore, 1974). The staining patterns are then evaluated to determine the viability of the seed. Originally this test was destructive to the testing sample; however, new techniques with lower concentrations of tetrazolium have not been detrimental to seed development (Hemming, 1989). The TZ test has the advantage of reliable results without requiring elaborate facilities. The main disadvantage of this test is that the results must be visually interpreted. This makes the results rather subjective in nature, therefore making reproduction of results difficult.

Conductivity Test. Another characteristic of a deteriorating seed is a poor membrane structure (Andrews, 1970). The conductivity test evaluates the permeability of the seed membrane by placing the seed in distilled water for several hours. Deteriorated seeds allow for a greater loss of electrolytes such as amino acids and organic acids. The increase of solutes in solution causes a corresponding increase in the conductivity of the solution. Good correlations have been shown between leachate conductivity and seed viability for soybeans (Tao, 1980).

The main advantages of this test are that it is nondestructive and the relatively low seed moisture levels allow significant conductivity measurements for ranges of deterioration. The only disadvantage is that this method only gives significant results for individual seeds.

Glutamic Acid Decarboxylase Activity Test (GADA). The GADA test is another sensitive measure of seed deterioration and storability. As the seed deteriorates, the activity level of enzymes, including glutamic acid decarboxylase, are measurably lower (Grabe, 1968). The test is performed by adding glutamic acid to finely ground seed. The acid then reacts with the available decarboxylase enzyme present in the seed giving off carbon dioxide as a reaction byproduct. The carbon dioxide is then used as a measurement of the decarboxylase enzyme activity. A lower GADA level indicates the amount of seed deterioration. Even though this test is destructive, its simple operation and sensitivity could make it a feasible consideration for large seed lots.

New Technology Under Development. The most promising new technology is an image processing system that is currently being developed at the University of Illinois. The system couples a video camera with an IBM AT computer which examines individual soybeans. Using the intensity of reflected light, the computer separates the seed into sectors. This system is currently able to identify four different types of fungi and can select healthy seeds with 98% accuracy (Marley, 1989).

SEED MOISTURE SENSING

A technique for performing in-situ, non-destructive seed moisture tests is desired in a CLLSS-type, fully automated process. Because seeds rapidly lose viability and thus germinability, if they are stored at higher moisture levels, an on-line moisture sensing device is important. Seed moisture sensing is separated into moisture sensor requirements and current sensor technologies, including advantages and disadvantages of each.

Moisture sensor requirements

The seed moisture sensor should meet the following general requirements:

1. Automation of device under a reliable, microelectronic-based control system
2. Simple testing method
3. Accurate, consistent and uniform information
4. Minimal handling of moist seed to avoid mechanical damage
5. Nondestructive testing
6. Testing of several commodities
7. Sensing device must "see" or penetrate a representative sample of material
8. Device should be fully integrated with a seed dryer
9. Ability to work in a batch mode

Current Moisture Sensing Technologies

Digital moisture meters. The dominant technology in commercial use for determining seed moisture content is a digital moisture meter. The Seedburo Equipment Company in Chicago, Illinois produces a Burrows Model 700 tester incorporates a push-button operation. Computer-based electronics measure the moisture content based on electrical conductivity with an automatic temperature correction. Currently, the tester is better suited to an off-line, non-automated system. To adjust for on-line use would require establishing good contact between the electrodes and the material being sensed. A built-in hopper/scale initially balances out 250 grams; then the sample can be dumped uniformly into the load cell. The electronics can be programmed for testing up to seven different types of seed. This instrument can be feasibly modified for space use, by using a simple pre-loaded cartridge cell designed to contain 250 grams of seed for testing.

Microwave attenuation. Microwave attenuation is based on the principle that water in the solid absorbs microwave radiation at certain frequency bands. One attractive feature is the use of the Schottky diode as a detector and a Gunn diode as a source. Limits of detection fall in the 0.3 to 0.5% water range with operating ranges between 1% and 70% water. Accuracies within 0.5% are achievable (Carr-Brion, 1986).

Advantages of microwave attenuation include:

1. Physical contact between sensor and material is not required
2. Nondestructive, i.e., seed is not contaminated
3. Low, safe power can be used
4. Reliable results, since microwaves pass through material, and moisture gradients or inhomogeneity in sample are eliminated

Disadvantages include:

1. Dependence on bulk-density of material analyzed
2. Calibration necessary for different materials
3. Nonmetallic path needed between source and detectors

Microwave moisture measurements are considered feasible for materials which do not vary widely in composition. On-line capabilities are promising due to the availability of solid-state microwave sources and detectors, and microprocessors for carrying out power monitoring and bulk-density compensation (Carr-Brion, 1986).

Capacitance measurement. For materials of roughly constant composition, capacitance moisture measurement is an existing technology used to determine moisture content of solids. The material flows at a constant bulk density between two metallic plates which are components of the capacitor. The capacitance value compensates for variations in temperature and bulk density of the material. The lower limit of detection is 0.1% moisture. The best operating range is up to 30% moisture with poorer performance up to about 60% (Carr-Brion, 1986).

The following are advantages in using capacitance measurement:

1. Devices are readily installed
2. Devices are inexpensive and reliable
3. Acceptable range of detection for moisture measurements

The disadvantages include:

1. Bulk-density, temperature, and chemical composition dependency
2. Conducting electrodes must be close to the material being sensed

Infrared diffuse reflectance. This is yet another technique for determining the moisture of a solid with a normal air path between the sensing head and the material analyzed. Infrared radiation is reflected from the material, but less so at wavelengths where the material is strongly absorbent. The reflected rays are focused onto a detector by a mirror or lens. Water content is calculated from the intensity of water absorption peaks using a built-in calibration graph specific to the material sensed. Concentrations of 0.02% to 100% can be determined with approximately 1% accuracy (Carr-Brion, 1986).

The following are possible reasons for using the infrared reflectance technique:

1. Non-contacting, rugged sensor head
2. Moisture can be measured in an enclosed space through an infrared transmitting window
3. Accurate and uniform measurements
4. Large application range
5. Reliable
6. Suited for determining end-point in dryers

Disadvantages of using infrared reflectance:

1. Technique involves surface determination; moisture gradients can affect results
2. More expensive

Equilibrium relative humidity. If the seed is allowed to come to equilibrium with a certain volume of gas, then the moisture content of the gas can be related to the moisture content of the seed. This technique would be hard to implement on-line because the sample must be placed in a gas enclosure which may require predrying. This method will not be acknowledged further unless an on-line application is developed in the near future.

SEED DRYING

Drying soybean for seed has been a challenge for seed producers. According to M.K. Misra, extension seed conditioning specialist at Iowa State University, the rate of drying is important. Drying too slowly can reduce seed quality, as can drying too quickly. The rate depends on the air temperature, relative humidity, initial seed moisture content, depth of seed, and airflow (Misra, 1984). Drying should also commence within a few hours after harvest and continue until completion. High moisture content seeds in a warm environment will deteriorate quickly (Delouche, 1973). Some method of sensing seed moisture content is needed so that a decision on drying parameters can be made.

Mature, fresh soybean seed has a moisture content between 28% and 30% (Delouche, 1971). The ideal moisture content for soybeans in sealed storage has not yet been conclusively determined, but may fall in the range of 4-8% (Harrington, 1959). Moisture content in this range avoids many storage problems. Some method of drying seeds to a pre-determined moisture content is needed.

Seed drying methods

All of the seed drying equipment in current production is geared towards commercial seed drying for planting seed or food processing. Drying for food processing does not require the preservation of seed life, so these dryers are generally unacceptable for this program's application. However, there are other types of dryers that can be adapted for use in a CLLSS chamber. The following descriptions of dryer types, while somewhat outdated, are still generally applicable (Harrington, 1959). Figure V-1 follows with sketches of the dryers described.

Bin dryers. This group of dryers operates by forcing air through piles of seed until the moisture level of the seed reaches equilibrium with the relative humidity of the air at a particular temperature. These have the advantage of being useful for small batches. A major disadvantage is that they dry the seed unevenly.

Column dryers. These dryers are gravity dependent, and hence unsuitable in a space environment.

Belt dryers. These dryers are usually used for high-temperature drying of grains where viability is not preserved. They are also gravity dependent.

Rotating drum dryers. In these dryers, seed are placed in a rotating drum, which drops the seeds through an air flow. Again, this method is gravity dependent.

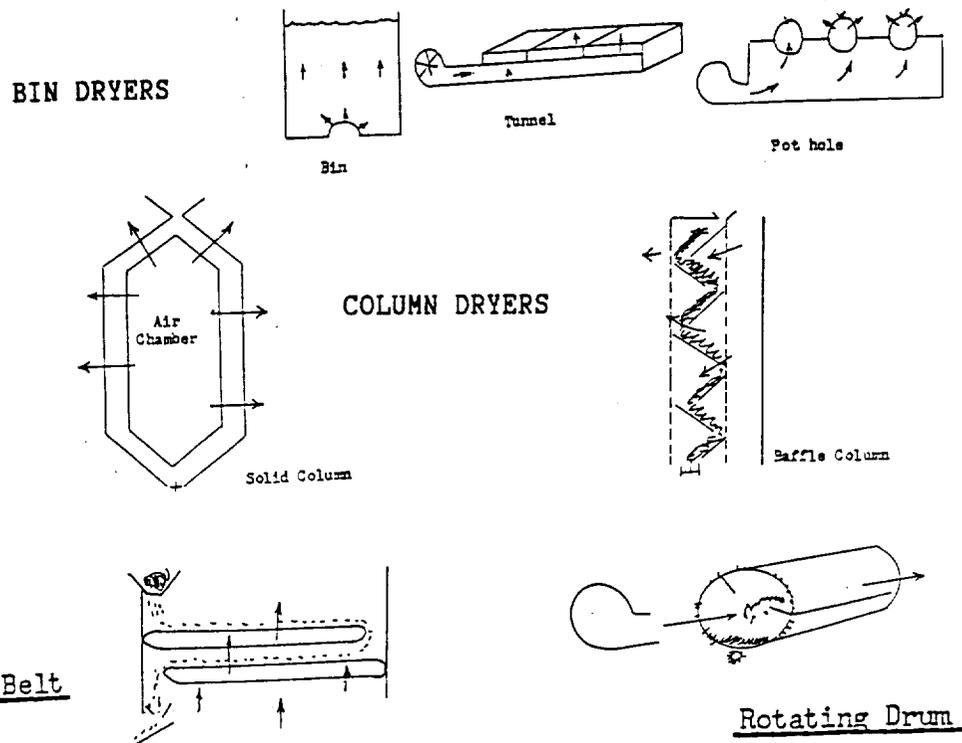


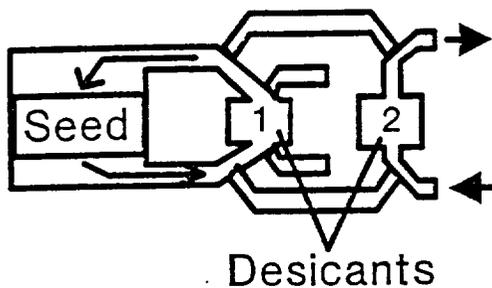
Figure V-1. a) Bin dryers. b) Column dryers. c) Belt dryer. d) Rotating drum dryer.
(Source: Harrington, 1959)

Closed circuit dryer with dehumidifier. In this dryer, the air is confined to a closed-loop and exposed to a desiccant. Since the system is closed, the air could also be heated. An advantage in using this dryer is that the desiccant is removing only the moisture in the air acquired from the seed and not the moisture inherently contained in the atmosphere. Since the system is closed, there is little chance of particulates escaping into the atmosphere. Disadvantages are that the desiccant itself must be dried in a separate cycle and the drying process is relatively slow.

Infrared dryer. This dryer appears to be a relatively new technology that could be implemented in a CLLSS. Infrared heat increases the process of moisture movement from the interior of the seed to the surrounding air. This method dries seed quickly, and causes no loss of germinability or seed quality. If the seed are in a thin layer, seed moisture can be reduced 6-7% in 15 minutes. Two disadvantages are the expense of the

dryer and the gravity dependence of the system. For a CLLSS application, a new seed movement and handling system, integrated with an infrared type dryer, may be feasible. Figure V-2 shows a sketch of both a closed-circuit dryer and an infrared dryer.

Closed Circuit Dryer



Infrared Dryer

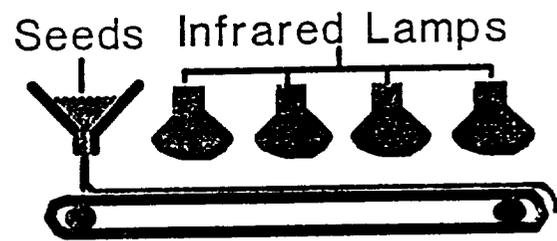


Figure V-2. a) Closed-circuit dryer with dehumidifier. b) Infrared dryer.
(Source: Harrington, 1959)

SEED STORAGE

After the seed have been dried to a moisture level between 5-14% they are ready for storage (Kozlowski, 1972). Storage of seed for long-term space missions is necessary to insure that the required quantities of edible biomass are readily available to the crew under any circumstances. Two storage methods, specifically in terms of the length of storage required, are being considered to meet this requirement. Initially, stored soybean will be brought into space for food supply until the CLLSS crops are ready for harvesting. The surplus can remain in long-term storage for emergency use. Once harvesting begins, some seed should also be set aside in short-term storage to be used for food supply or replanting in the event of low crop yield or poor seed quality.

The objective of seed storage is to maintain viability and vigor from harvesting to planting (Delouche, 1973). In an uncontrolled environment, seed will deteriorate rapidly over time; therefore, specific storage criteria should be defined according to storage needs. Two important areas of focus in seed storage are seed conditions and storage environment.

Seed Conditions

The condition of the seed before it is stored is a good indicator of its storage future. In general, seeds that store well in adverse conditions with little loss of viability are the high quality, well-dried seeds (Delouche, *et al.*, 1969). Soybean seed are hygroscopic; therefore, the moisture content of the seed is at equilibrium with the ambient relative humidity of the surrounding air. Seed high in moisture degenerate more rapidly due to a high transpiration rate; unless they are dried soon after harvesting, they may lose their capacity to germinate (Delouche, 1973). Immature damaged or disease seed are also undesirable for storage.

As previously mentioned, a distinct relationship between seed moisture content and relative humidity exists. By knowing the moisture level to which a soybean seed is dried, effective ranges of temperature and relative humidity for storage can be found.

Table 1. Equilibrium moisture content of soybean seed.*

Temperature		Relative Humidity (percent)								
°C	°F	10	20	30	40	50	60	70	80	90
5	41	5.2	6.3	6.9	7.7	8.6	10.4	12.9	16.9	22.4
15	59	4.3	5.7	6.5	7.2	8.1	10.1	12.4	16.1	21.9
25	77	3.8	5.3	6.1	6.9	7.8	9.7	12.1	15.8	21.3
35	95	3.5	4.8	5.7	6.4	7.6	9.3	11.7	15.4	20.6

*Source: Agricultural Engineer's Yearbook, 1981. The values in table 1 are for drying situations. For adsorption, the EMC value is usually less than for drying.

Sensing seed conditions. A seed selector is the sensing device that would be most suited for determining the best seeds for storage. A seed moisture testing machine would be an acceptable means to find the average equilibrium moisture content for the seed lot being stored. This value would then dictate the environmental conditions necessary to be maintained in the storage container. Other sensing areas include detecting when the seed has been dried sufficiently for storage, as well as the presence of damaged, diseased, or cracked seed.

Storage Environment

From the previous discussion it follows that controlling the environment of a seed will also contribute to the seed's storability. The two major requirements for the storage of seed are 1) to create conditions of low relative humidity, and 2) to maintain low temperatures (Kozlowski, 1972). When stored at these conditions, seeds retain a high germination percentage for several months, as shown by Figure V-3 and Figure V-4 (Burris, 1980).

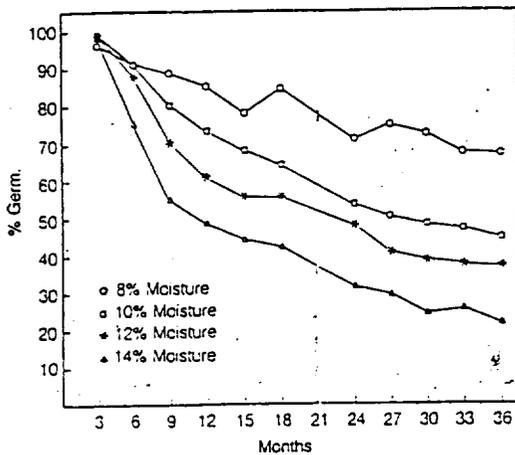


Figure V-3. Effect of moisture content on soybean seed storage (averaged across temperatures and varieties). (Source: Burris, 1980)

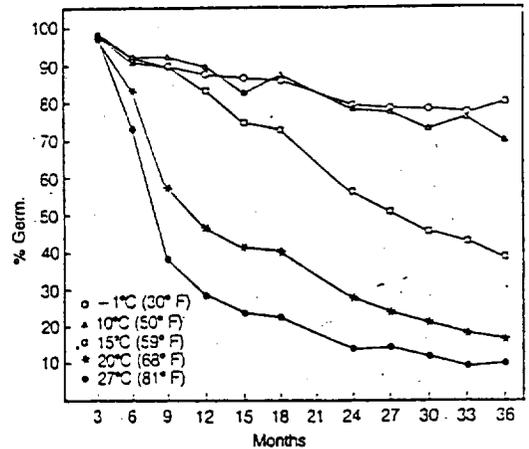


Figure V-4. Effect of temperature on soybean seed storage (averaged across moistures and varieties). (Source: Burris, 1980)

The acceptable ranges of temperature, relative humidity, and seed moisture content for safe storage of seed depend on length of time in storage. Storage periods are generally categorized as short-term, intermediate, and long term. The following table is a summary of acceptable storage data:

Table V-2. Storage Data.

Storage Period	Temperature Range	Humidity	EMC*
Short-term storage (1 to 9 months)	30°C	50%	8.0%
	20°C	60%	8.0%
Intermediate storage (9 to 18 months)	30°C	40%	7.5%
	20°C	50%	8.0%
	10°C	60%	9.0%
Long-term storage (3 to 5 years) (5 to 15 years)	10°C	45%	4-8%
	0-5°C	30-40%	

*Equilibrium moisture content
(Source: Delouche, 1973; James, 1967)

In a CLLSS, there will be a combination of both short-term and long-term storage. However, the conditions for long term storage will probably set the standard for all seed storage, as seed viability remains high for several years at these levels. Therefore, for storage in a CLLSS, relative humidity should be kept in a range of 30-45%, and temperature should remain in the range of 0-10°C to maintain the seed's dried moisture level.

Sensing needs for storage conditions. Sensing needs in this area involve sensing the temperature and relative humidity of the storage environment to insure that seed moisture content remains at the dried seed moisture level (Delouche, *et al.*, 1973). Removal of excess heat and moisture from the storage chamber is necessary for effective

storage conditioning. Several commercial technologies are available to sense these parameters. Of the two parameters, control of relative humidity is more important because it has the greater influence on the longevity of seed in storage (Delouche, et al., 1973).

Temperature control. Temperature control is usually achieved through air conditioning or refrigeration methods. However, it may be possible to utilize the cold temperatures of space for maintaining low temperatures in the storage containers.

Humidity control. Control of relative humidity may be accomplished by two types of dehumidifiers, refrigeration-type and absorption-type.

Refrigeration-type -- A refrigerant such as Freon is circulated through metal coils in the chamber. Any moisture in the chamber condenses on the coils and is removed from the system. The state of the condensate depends upon the temperature of the refrigerant. This method of moisture removal is successful at high temperatures; however, this type of humidifier loses efficiency below 70°F or 50% relative humidity. In addition, heat may be added to the atmosphere from the electric motors (Beck, 1969).

Absorption-type -- This method of humidity control is sometimes referred to as desiccant dehumidification. Moist air is drawn through a bed of desiccant, such as silica gel, that has the ability to absorb up to 40% of its total weight in moisture. The dry air is then recirculated into the storage chamber. Periodically, the desiccant bed must be dehydrated. Continuous dehumidification is achieved by rotating desiccant beds, so that one is dehumidifying the air while the other is being regenerated. Desiccant dehumidification methods provide maximum efficiency for low temperatures and are able to maintain relative humidity levels of 10% or below. However, heat is also added to the system in the dehumidification process. Some type of refrigeration may still be necessary to maintain low temperatures in the storage containers (Beck, 1969; Delouche, et al., 1973).

CONCLUSION

Next semester, the Plant propagation group will concentrate its efforts on the design, fabrication and testing of a sensing mechanism. The group is most interested in developing a seed sensor that will select healthy, viable seeds for replanting and storage, while rejecting less desirable seed to be used for crew consumption. A seed selector is an important element in the propagation of high yield soybean plants, and we feel design is within the capabilities of group.

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VI. RESOURCE RECYCLING

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SUMMARY

The solids group has concentrated on determining sensor and actuator needs for harvesting, food processing and resource recycling of soybeans in a CLLSS project. After exploring the first two areas, the decision was made that sensing needs in those areas are already well developed and therefore the group focused on resource recycling. Emphasis was further narrowed to studying pH measurement, dissolved oxygen concentration, glucose measurement and enzyme concentration of soybean biomass. Next semester, the group will test some of the ideas we have developed and hope to design a sensor that can be used in the recycling of soybean biomass.

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INTRODUCTION

This paper outlines our search to determine sensor and actuator needs in the areas of harvesting, food processing and resource recycling of soybean plants. Once these needs were identified, we proceeded to study the technologies available to implement them in microgravity with as close to 100% efficiency as possible.

Moisture content, temperature and tactile sensing of the plants and environment seemed to be the more obvious and important issues in the harvesting and food processing. The sensing technologies in these areas are already well defined, therefore the group concentrated on resource recycling.

This paper briefly outlines the requirements of harvesting, food processing and resource recycling. The four areas examined in resource recycling are pH sensing, dissolved oxygen concentration measurement, glucose measurement and enzyme concentration detection. Finally, in our conclusion we will discuss the ideas we hope to develop into a workable sensor system.

HARVESTING

Harvesting needs are generally mechanical in nature, and the sensing technologies involved are well developed. One of the areas of harvesting that requires sensing includes taking moisture measurements of the plant. It has been noted that the optimum time for harvesting of the plants is at 13 % moisture content (Aldrich, 1970). Other sensing needs include tactile sensing and shape recognition. Although these areas proved to be of interest, it was concluded that study of resource recovery would be more beneficial.

The actual harvesting of the plants might be done with a combine-type system similar to those used by farmers (Aldrich, 1970). One of the problems of a microgravity environment would be to construct a harvester that would work under these conditions. This area proves to be more of a mechanical design problem than a sensing need of the system. However, there are some sensing areas that can be further studied, such as the control and speed of the harvester, and the determination of the amount of harvesting that has already taken place.

Similarly, the actual movement of plant material is more of a mechanical design problem than a sensing need. Transportation of the harvested seeds must be done by mechanical means. The problem lies in the production and application of the forces needed to move plant material. The sensing requirements in this area include discriminating between seeds and pods in microgravity separation, and transporting solid plant material.

FOOD PROCESSING

Food processing is an essential component of a closed-loop system. Many sensing and actuating needs that are related to processing the raw soybean into some other form for human consumption were recognized. Once processing is accomplished, the problem of moving solids in a microgravity environment arises. The moisture content of the soybeans during processing, and the temperature of the environment are also important

aspects. The separation of liquids from solids may be done much like it is done on earth using various filters. The entire process requires extensive aeration and ventilation monitoring. Dust concentrations must be kept at a minimum in order to avoid explosive situations sometimes encountered in storage silos on earth. To ensure optimum nutritional value of the processed food, methods of determining fat, carbohydrate, and protein concentrations are needed. Several methods for determining protein concentrations in food solutions were briefly investigated. Since these methods are similar to those used in enzyme concentration sensors, they are described in that section. Sensing areas in food processing are not as numerous. After visiting a soybean processing facility, the decision was made that much of the present technology used could be converted for use in a space environment.

RESOURCE RECYCLING

Introduction

An integral component of a life support system will be resource recovery. The closed system should recycle all possible refuse, including matter that is normally discarded on earth. This means that anything sent to the recovery system, including inedible biomass, must be transformed into useable products.

A specific resource recovery system has not been chosen for implementation in a closed system. Many processes have been considered and each has specific disadvantages. These limitations lead us to the belief that a network of processes will be utilized. A feasible process being considered is enzymatic hydrolysis. This process uses enzymes, produced by a fungus, to decompose the inedible biomass into useable products. Since this process indicated many interesting possibilities for the design of a sensor, the group hopes to concentrate on it next semester. The remainder of this section covers our findings on this topic.

Enzymatic Hydrolysis

Within the processed soybean biomass, long chains of sugar molecules are present. These chains, called cellulose, comprise approximately 40% of the plant tissue (Wilke, 1983) and represent a possible energy supply for humans. However, humans do not possess an enzyme in their digestive system that can break down cellulose chains. Therefore, it is important in a closed-loop life support system to utilize enzymatic hydrolysis.

During the hydrolysis, an enzyme, usually cellulase, is used to break down the long chains of cellulose into glucose. The cellulase is composed of exo-glucanase, endo-glucanase, and β -glucosidase (Wilke, 1983). The endo-glucanase will break the cellulose at random bonds, while the exo-glucanase will break the bonds at the terminal ends of the chain. Finally the β -glucosidase will break the remaining disaccharides into glucose. This glucose, which is a simple sugar, can then be used by humans for nutritional needs. Do to the unpleasant taste of glucose, this sugar may be converted into a palatable sugar such as sucrose or fructose (Petrucci, 1985). If not, a high protein fungus may be grown on the glucose and used for food.

The enzymatic hydrolysis process consists of three main parts: pretreatment, enzyme production and enzymatic hydrolysis. The first step is to pretreat the inedible biomass. Pretreatment begins by grinding the inedible biomass into very small particles. The strong cellular walls and lattices which occur in cellulose can interfere with the conversion process by not allowing the enzyme to diffuse to the inner cellulose deposits. Therefore, grinding is necessary to improve the conversion process. Grinding physically breaks down the strong cellular walls and lattices of glucose, thus allowing deeper penetration of the enzyme. During pretreatment, leaching also occurs. Leaching is a process whereby the biomass is soaked in water, allowing the soluble nutrients to diffuse from the biomass into solution. This solution containing the dissolved nutrients is returned to the nutrient supply reservoir for the plant growth chamber.

Before enzymatic hydrolysis can occur, it is obvious that the enzyme must be present. With the use of a fungus, in this case *Trichoderma reesei*, the enzyme can be produced. The Biomass Processing Group at NASA/KSC produces enzymes using a solution of water and ground biomass (5% by weight). A fungus will be allowed to grow

on the waste materials. In order for the fungus to gain nourishment, it must release a digestive enzyme. In this particular case, cellulase is produced. Before the fungus is allowed to grow, this digestive enzyme will be drained and used for enzymatic hydrolysis. Therefore, there are two needs for the biomass--enzyme production and enzymatic hydrolysis (Strayer, 1989).

Sensing Needs

For enzymatic hydrolysis to occur, pretreated biomass is placed in the enzymatic solution. This mixture is constantly stirred. Slowing of the glucose production indicates that all of the accessible cellulose has been converted or that the enzyme concentration has become too low. During hydrolysis, pH and temperature are important to monitor. The pH should range from 4.8 to 5.0, and the temperature should be approximately 50°C (Strayer, 1989). If either of these properties deviates from the specified range, the enzyme could become denatured and inactive.

It is necessary to monitor a number of things in order to maximize the conversion of cellulose to glucose. As mentioned, pH and temperature are important to monitor. Monitoring dissolved oxygen levels during the enzyme production phase is also important because the fungus needs oxygen to live. Other sensors which would improve efficiency are specific enzyme sensors and glucose sensors. With these, it would be possible to sense enzyme levels without wasting any enzymes. Furthermore, the glucose sensor could monitor the rate of the hydrolysis process and tell when the process was finished. Other possible sensing needs would be a fungus level sensor and a fungus health sensor.

The sensors developed would be required to work in a space environment. They would have to be gravity independent and as small and lightweight as possible. It is important that the sensors do not interfere with the process. Similarly, the sensors should be resistant to the elements of the process. For example, a sensor to measure dissolved oxygen should function accurately with fungus growing around it.

Four sensing technologies were considered. Research was conducted to see what sensors exist, what their limitations are and what modifications and developments can overcome the limitations. The four sensing units considered were:

1. pH Measurement Unit
2. Dissolved Oxygen Concentration Unit
3. Glucose Measurement Sensor
4. Enzyme Concentration Sensor

pH Measurement Unit

The pH is a measurement of acidity or alkalinity of a substance. It operates on the principle that an acid contains a greater concentration of positive hydrogen ions and a lesser concentration of negative hydroxyl ions. A common pH measuring unit used on earth is the Beckman calomel electrode shown in Figure VI-1.

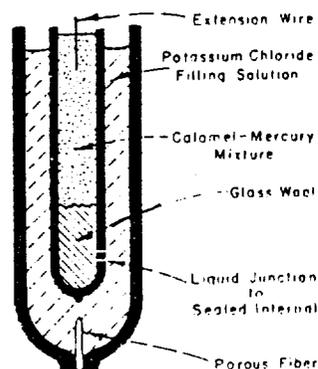


Figure VI-1. Beckman Calomel electrode.
(Source: Holzbock, 1955)

A reference electrode is located inside a glass membrane. A buffer solution of potassium chloride is maintained between the inner electrode and outer glass membrane. A porous fiber extending from the buffer solution through the glass membrane to the liquid being measured allows for the determination of the potential difference between the two solutions.

The pH requirement of the CELSS resource recycling unit is between 4.8 and 5.0 at 50°C. This requirement is easily met using existing meters. However, there are three major drawbacks to using an existing meter. First, the meter must be kept upright, or at least on its side (Cole-Parmer, 1989-1990), which is a problem that needs to be addressed in microgravity. Secondly, the buffer solution leaks out at a slow rate and needs to be replenished. Units that supply a continuous flow of filling solution are available. The rate of depletion of this solution can only be determined by actual testing, since the probe will probably be left in the solution constantly to decrease maintenance time by crew members. On earth, probes are normally not left in continuously. The last problem is the contamination of the probe by a build-up of the fungus or other debris. Submersible housings and bulb guards are available that keep debris away from most of the probe allowing accurate readings for longer stretches of time. The cost of an industrial meter with bulb guard and submersible housing is estimated at around \$270 (Cole-Parmer, 1989-1990).

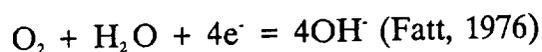
Polarographic Oxygen Sensors

The purpose of this section is to discuss the need for measuring the dissolved oxygen content during enzyme production for use in the enzymatic hydrolysis of biomass and a possible sensing technology that can do such a task.

During the production of the enzyme, the solution will need to be stirred and aerated in order to keep the dissolved oxygen level constant. The fungus' ability to keep growing and producing the enzyme can be affected by the amount of dissolved oxygen that is present. According to the experiments conducted by NASA and Dr. Richard Strayer, the usual amount of dissolved oxygen that is needed by the fungus is approximately 2 mg/liter of dissolved oxygen in the solution. If the amount of oxygen drops, the fungus can die. This demonstrates an important need of sensing. If the fungus were to die, the whole process will cease.

Oxygen Sensor. A possible technology that can be used for the measurement of dissolved oxygen is that of polarographic oxygen sensors. This type of technology has certain advantages.

The principles by which these probes work follows electrochemical theory. The idea behind this theory is that gaseous dissolved oxygen could be electrolyzed to the hydroxyl ion, the ionic component of water. The current flow during this electrolytic reaction is proportional to the concentration of gaseous dissolved oxygen. The electrochemical half-cell reaction for the reduction of oxygen is



The source of the electrons in this reaction is an external battery or some other direct current source. This source is then in turn connected to the cathode of the probe. The reduction of dissolved oxygen to hydroxyl ions at or near the surface of the cathode gives rise to an electric current if a reference or counter electrode is also in the solution. The current created between these two electrodes is then proportional to the concentration of gaseous dissolved oxygen.

It should be noted that polarographic oxygen sensors use different probes to measure the "oxygen tension" of the solution. The oxygen tension in a liquid is the pressure of oxygen in the gas phase that is or could be in equilibrium within the liquid (Fatt, 1976). From the oxygen tension, oxygen concentration can be obtained by applying Henry's Law of Gas Solubility by multiplying the oxygen tension by the Henry's Law Constant, usually stated as

$$\text{ml O}_2/\text{ml liquid} \times \text{mm Hg} \text{ (Fatt, 1976).}$$

Polarographic oxygen sensors come in many different sizes and styles. The most widely used is the Clark sensor, which is the type that may be used for these purposes. The Clark sensor is constructed with a platinum cathode and reference cathode of silver-silver chloride. These are side-by-side and both covered by the same membrane. This sensor has a useful current output, does not get poisoned easily, and is not sensitive to

the stirring effects that occur during enzyme production (Fatt, 1976). Furthermore, the Clark sensor can be the size of a hypodermic needle (Fatt, 1976). This is favorable because in a microgravity environment where space is limited, the fungus and biomass may be placed into a bladder where having a large probe would be cumbersome. Using a hypodermic-sized probe would prove to be advantageous for this case. However, at this time measurements will only be made for a system of large vats of up to 10 liters or more as done at NASA. Therefore, for experimental purposes, a larger polarographic electrode may be used. This probe is shown in Figure VI-2.

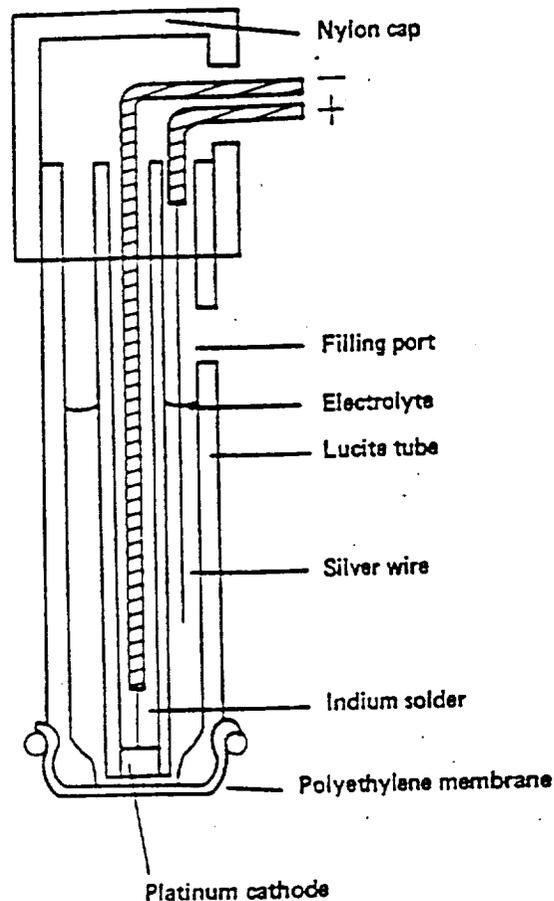


Figure VI-2. Clark Sensor.
(Source: Fatt, 1976)

The advantages of these probes include a good response time (98% in 60 sec), and a wide temperature range for operation (0° to 80°C) (Cole-Parmer, 1989-1990), which is adequate for the expected temperature of operation which is approximately 50°C (Strayer, 1989).

The price of these sensor probes ranges from approximately \$500 to \$700. With the price of the oxygen meters, which range from approximately \$500 to over \$1150, the total cost of this type of sensing would run between \$1000 to \$1850 (Cole-Parmer, 1989, 1990). The distinction between the price ranges depends on the accuracy given by each device. The accuracy for the less expensive instruments is ± 0.2 mg/l dissolved oxygen, whereas the accuracy for the more expensive instruments is ± 0.03 mg/l dissolved oxygen (Cole-Parmer, 1989-1990).

The requirements for such sensors include a reproducible current for a given oxygen tension, a reasonably rapid response to a change in oxygen tension, and finally a constant current when the oxygen tension remains constant (Fatt, 1976). The means by which these requirements can be met is through the use of simple batteries and a variable potentiometer (Fatt, 1976).

Disadvantages to these probes include the possibility of fungus growing on the probe, refilling the electrolyte solution in the probes, and changing batteries assuming batteries are used as the energy source. Since there is a possibility the probe might have fungal growth on it or be contaminated by the ground biomass, the sensor will have to be cleaned periodically. Experiments will have to be conducted in order to determine the actual rate at which cleaning should occur. Experiments will also have to be run to determine how often the electrolyte solution inside the probe needs to be refilled. Finally, the energy supply average of the batteries for the dissolved oxygen meter is approximately 1000 hours (Fatt, 1976). Therefore, these batteries will have to be changed. However, it is noticed that there will probably be a continuous voltage supply from the ships system so this problem may possibly be overlooked.

Polarographic sensing is an area in which dissolved oxygen sensing is a possibility. Since it is successful today in the world of medicine and biology, it is a technology that may be a productive means by which to measure the amount of dissolved oxygen.

Another Sensing Technique. Another possible technique that can be used to measure dissolved oxygen content is the use of spectroscopic methods. Study into this type of sensing has just begun, therefore the advantages and disadvantages of the various spectroscopic methods cannot be compared. Possible areas to be studied include photoelectron spectroscopy and fluorescence spectroscopy. These areas are promising since they can be used on liquid solutions and in the appropriate temperature range.

Glucose Detection

A possible means of determining a limiting factor of hydrolysis is in the measurement of glucose. If too much glucose is produced and is present, the enzymes ability to convert cellulose to glucose will be hampered. Therefore, it is imperative to keep a measurement of the amount of glucose that has been produced. A schematic drawing of the cellulose molecule made up of glucose molecules connected by β -1,4 linkages is shown in Figure VI-3.

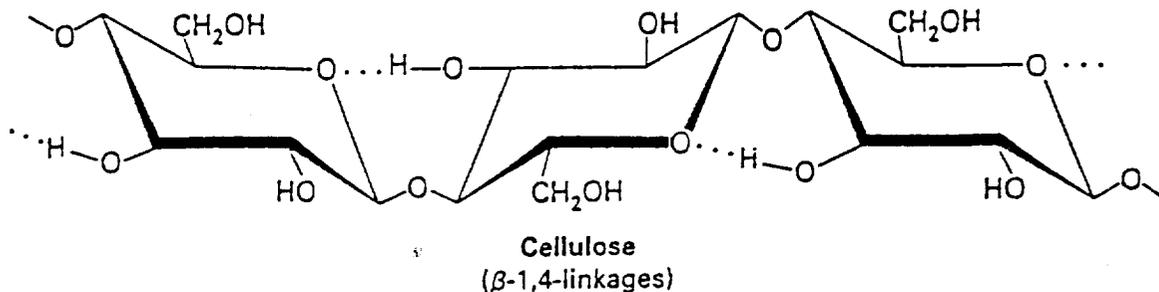


Figure VI-3. Cellulose molecule with individual glucose units.
(Source: Petrucci, 1985)

Enzymes are proteins that act as catalysts in chemical reactions. They speed up the reaction rate without affecting the end product. Because of their specificity and sensitivity, enzymes have many uses as analytical tools in clinical laboratories (Wang, 1988). An enzyme electrode combines the specificity and affinity of an enzyme for its substrate with the analytical power of electrochemical devices. Enzyme electrodes have been shown to be extremely useful for monitoring a variety of substrates, including glucose.

Enzyme-based electrochemical sensors can be divided into two groups according to their principle of operation: amperometric and potentiometric. These devices are prepared by attaching an immobilized enzyme layer to the electrode layer, which monitors changes occurring as a result of the enzymatic reaction potentiometrically or amperometrically. The glucose electrode sensor, (see Figure VI-4) developed by Updike and Hicks (Updike, 1967), represents the first reported use of an enzyme electrode.

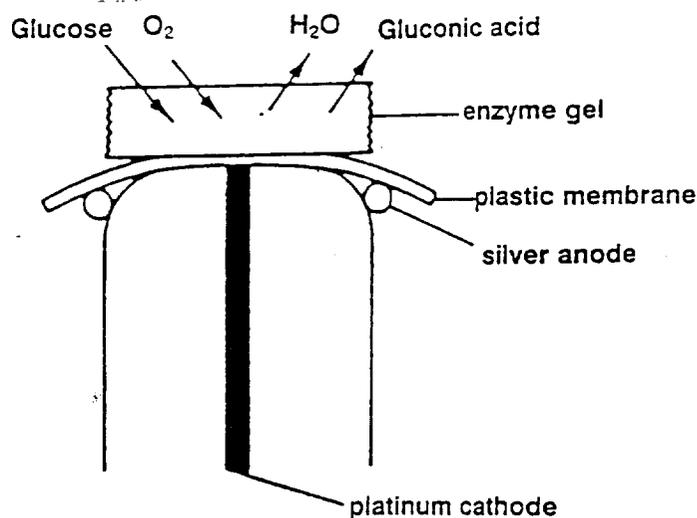
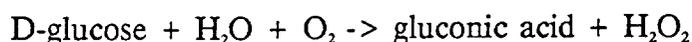


Figure VI-4. Schematic diagram of an amperometric glucose electrode.
(Source: Updike, 1967)

This electrochemical probe uses immobilized glucose oxidase and is based on the following reaction:



This consumption of oxygen or liberation of hydrogen peroxide can be monitored amperometrically with a platinum sensing probe.

Other electrochemical enzymatic probes for measuring glucose have been developed. Operation on the potentiometric principle, a glucose electrode based on an iodide ion-selective electrode, is a possibility. In this case, the hydrogen peroxide produced in the enzymatic reaction oxidizes iodide ions added to the sample solution (Nagy, 1970). Changes in the level of the iodide ion are detected at the sensor surface. This method, as with the previous method, is destructive to the sample solution. There is a great need for a closed-loop, non-destructive glucose concentration detector.

Enzyme Concentration Sensors

During enzymatic hydrolysis, it will be necessary to measure enzyme concentration in order to maximize glucose conversion and minimize conversion time and enzyme consumption. Therefore keeping the enzyme levels within a certain range will increase productivity. The present method to measure enzyme concentration is to perform an assay. An assay is a measurement of the rate at which products are produced by an enzyme using a given amount of substrate. By knowing the rate at which the reaction is taking place, it is possible to determine the amount of enzyme present. If there is a surplus of substrate, enough to keep all of the enzyme working, the rate at which the products are produced is proportional to the amount of enzyme present.

Variations in the way the rate of reaction is measured leads to the numerous methods used to measure enzyme concentration. Because there are differences in the reactions that enzymes catalyze, only certain methods will apply to each different reaction. Some of the methods used to determine the rate of reaction are the following:

1. Concentration
2. Manometric
3. Spectrophotometric
4. Electrochemical
5. Fluorescence
6. Radiochemical

Concentration Method. If the reaction that the enzyme is catalyzing produces a product or consumes a reactant which can be measured directly, it will be possible to monitor the increase in concentration of that product or the decrease in concentration of a reactant. The rate at which these are consumed or produced is the rate of the reaction.

Manometric Method. If the reaction being catalyzed produces or consumes a gas, one could measure the change in air pressure. The rate at which the air pressure changes is the rate of the process. This method has been modified by using electrodes which measure the concentration of individual gases. This electrode method is more accurate.

Spectrophotometric Method. If one or more of the products or reactants absorb light in the ultraviolet, visible, or infrared spectrum, the change in absorbance could be measured. If one of the products absorbs light and none of the reactants do, the rate of change of absorbance equals the rate of the reaction.

Electrochemical Methods.

Ion-Selective Electrodes-- For reactions which produce an acid, it is possible to measure the velocity of the process by measuring the pH with a glass electrode. But because pH affects the rate of the reaction, a "pH stat" method (Guilbault, 1976) is

used. The "pH stat" method keeps the pH of the reaction constant by adding an alkali. The rate at which the base is added is the rate of the reaction.

Potentiometry with a Small Current-- The rate at which the potential changes between two platinum electrodes polarized with a small, constant current indicates the rate of the process. For this change in potential to be noticed, it is necessary that the reactants and the products have a different redox potential. One must use care when using this type of measurement because proteins are absorbed by platinum which could decrease the sensitivity of the potentiometer.

Amperometry-- This method is similar to the potentiometry method with the exception that instead of a constant current being applied, a constant voltage potential is applied. The change in current through the solution to be analyzed is measured and is proportional to the rate of the reaction.

Fluorescence. This method is similar to the spectrophotometric method but is much more sensitive. This method has replaced the former absorbance methods and measures the rate at which a compound is being produced.

Radiochemical Method. If the reaction being catalyzed produces or consumes a radioactive substance, the rate at which the radioactive substance is produced or consumed is proportional to the amount of enzyme present.

Protein Content

One other way to measure the amount of enzyme is to measure the amount of protein in the substance. This can be done because enzymes are proteins.

The first two methods of protein measurement involve chemical reactions and are destructive to the food samples. The Lowry system involves a colorometric reaction. The percent protein can be found by measuring the color change with a spectrometer when the protein present reacts with added copper ions in alkaline solution. Finding the protein content by Biuret reaction is very similar. The protein present displaces the copper ion from a complex molecule to form a copper-protein complex with a different color and absorption intensity. Again this is a destructive testing method.

A third method is ultraviolet absorption. This method uses ultraviolet light to detect the presence of the two aromatic amino acids: tyrosine and tryptophan. These amino acids are present in some proteins. Their proportions relative to the entire protein content of the sample is very accurate if the solution contains less than 20% protein (Zaborsky, 1973). This method does not involve any chemical reaction and is not destructive to the sample. Unfortunately, since there may be other proteins in the solution or enzymes other than the one desired, this is not a very accurate way to determine the concentration of one specific enzyme.

A method of measuring the concentration of the enzyme directly has not been found. Since no specific assays to measure the enzymes occurring in the process of cellulose hydration other than β -glucosidase are known, we will have to develop an assay using the methods described above. One other possibility is to come up with a way to measure the enzyme concentration directly.

CONCLUSION

After investigation of the three topics of solid processing, the group decided to study the area of resource recycling. We feel that it might be more efficient to develop the harvesting and food processing units before designing sensors that we feel may be beneficial in these area. Therefore, the group concentrated the latter part of the semester on sensor needs for the resource recycling area. We hope to test the ideas we developed this semester and design a sensor which will monitor the process of enzymatic hydrolysis.

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