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# PC–Based Data Acquisition for a Solid Substrate Cultivation Deep Bed Reactor

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**Abstract.** *This work describes an instrumentation and data acquisition system designed for a deep bed reactor used to cultivate Trichoderma longibrachiatum on wheat bran. The system allowed on–line measurements of substrate temperature, oxygen concentration within the reactor headspace, relative humidity and temperature of the inlet air, and inlet airflow rates while maintaining aseptic conditions and without disturbing the cultivation process. An error analysis for the instrumentation and data acquisition equipment was completed and provided insight into the reliability of the sensor readings. The collected data provided quantitative information about the reactor system dynamics which can be used to evaluate and apply environmental control schemes, gain knowledge on microbial growth characteristics, and develop and validate mathematical models describing heat and mass transfer interactions.*

***Keywords.*** *Solid substrate cultivation, Deep bed reactor, Instrumentation, Error analysis, Trichoderma longibrachiatum.*

Solid substrate cultivation (SSC) is characterized by the growth of microorganisms on water insoluble substrates in the absence of free water (Moo–Young et al., 1983). The solid substrates are typically inexpensive, unrefined agricultural residues such as wheat bran, wheat straw, rice, rice straw, and other cereal grains. Other organic substrates include sugar beet pulp, sugar cane bagasse, and wood chips. Such substrates are structurally and nutritionally complex, often providing a simple carbon and energy source for growth. Mineral and synthetic materials may also be used as solid matrices for SSC processes. These matrices often only function as supports and require nutritional supplementation to allow growth of microorganisms (Durand et al., 1996). Fungi are commonly used in many SSC applications because the conditions are very similar to those found in their natural habitat (Pandey, 1992). SSC has applications in the composting of wastes or municipal refuse, bio–fuel production, and the upgrading of staple foods and feeds (protein enrichment). In addition, it is utilized for the production of biological control agents, antibiotics, and industrial enzymes.

Environmental conditions significantly affect microbial growth and product formation in SSC. Temperature, moisture content, nutrient availability, and oxygen concentration are important factors to consider in the development of an optimal SSC production process (Prior et al., 1992). Progress in SSC implementation is impeded by limited information as to the engineering, reactor design, instrumentation, process controls, and heat and mass transfer of these systems (Durand et al., 1997; Ghildyal et al., 1993). Measurement and control of the major variables such as temperature, moisture, and oxygen is challenging because of the thermal and structural properties of the substrates and the heterogeneity of the overall system. In addition the on–line measurement devices available are not designed to operate within solid materials under aseptic biological conditions. Control of a system's environmental conditions may help regulate growth, favor the biosynthesis of desirable products, and ultimately lead to the development of optimal production schemes in SSC.

A number of research groups have worked on the development of on–line automated systems for monitoring and controlling the environmental parameters of a SSC system. The main motivation for designing such systems is that the information obtained can be used to achieve higher productivity (Saucedo–Castaneda et al., 1994). Fernandez et al. (1996) developed a semi–automatic control system for an aseptic SSC pilot scale reactor. The system allowed for on–line observation of temperature of the substrate and inlet and outlet air, relative humidity of the inlet air stream, carbon dioxide concentration in the outlet air, and the pressure drop in the reactor during the cultivation of *Gibberella fujikuroi* for the production of gibberellic acid. The measured values were used to develop a control scheme to regulate bed temperature and control bed moisture content. The instrumentation was developed for a pilot scale reactor with a 50 kg capacity. A complete description of the system was not provided. The effectiveness of the sensors used in the study was not discussed, but the measurements they provided were used extensively in temperature control schemes. The system was found to exhibit good control and maintained the substrate temperature within ±4°C of the 28°C set point. The sensors and control elements used in this study were fairly elaborate and would be difficult to implement on a small scale. Saucedo–Castaneda et al. (1994) developed a system for monitoring and controlling gases in an aerobic SSC process. The authors used polarographic and infrared oxygen

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and carbon dioxide sensors to measure the head gas concentration in real time. These sensors provided a higher sensitivity and quicker response time than a gas chromatograph, yet similarly they required pretreatment of the exit gases from the reactor. The cost of the gas analyzers used was not suitable for an inexpensive laboratory scale system. In addition to gas concentrations, the system monitored airflow rate and temperature of the substrate during the growth of *Schwanniomyces castelli* for alcohol production. The system was found to be reliable during cultivation experiments and for optimization of SSC, however the carbon dioxide sensor was not reliable for concentration measurements greater than 10%.

One difficulty in monitoring the environmental parameters of a SSC system is a lack of sensors capable of operating within the harsh environment of the reactor due to high temperatures, extreme pH levels, and/or possible caustic end products generated by the microorganism. In addition, the majority of the processes must remain aseptic and the necessary sensors are not designed to withstand sterilization methods. There is a need to develop a systematic way of creating optimal production schemes for SSC process. Very little progress has been made in this area because every process is unique and system dynamics are not well quantified. The interaction of variables is also not well understood. The aim of this work was to design an instrumentation system and to develop a working data acquisition scheme suitable for SSC studies conducted on a laboratory scale. One of the main objectives was to monitor substrate temperature, oxygen concentration within the reactor headspace, relative humidity and temperature of the inlet air, and inlet airflow rates during the cultivation of *Trichoderma longibrachiatum* on wheat bran in a deep bed bioreactor using low–cost sensors that required minimal circuitry enhancement. The system and its performance were evaluated and assessed for their utility in improving the understanding of SSC processes.

## Materials and Methods

### Instrumentation and Data Acquisition

A data acquisition system was designed to allow automated on–line measurements of important parameters for a deep bed SSC reactor. The instrumentation allowed the reactor environment to remain sterile and did not disrupt the process. The substrate temperature was measured using grounded type–K thermocouple probes and shielded thermocouple wire (KMQS–062G–6, OMEGA, Stamford, Conn.). This particular type of thermocouple could withstand the temperatures and pressures of the autoclave as well as the harsh environment within the substrate during cultivation without destroying sensor accuracy. The shielded wire helped to prevent noise from altering the sensor reading.

Oxygen concentration within the reactor headspace was monitored using a galvanic oxygen sensor (MAX–250, Maxtec, Inc., Salt Lake City, Utah). This sensor was capable of measuring oxygen concentrations in which carbon dioxide was present. The sensor did not require a power supply and could be chemically and UV sterilized to reduce the introduction of contaminants. Compared to other oxygen sensors available on the market, this sensor was extremely inexpensive, did not require the reactor gases to be manipulated before use, had a length of 2 in. and diameter of 0.9 in., and could be threaded directly into the reactor cover with the sensing element exposed to the internal air of the reactor.

The relative humidity and temperature of the air entering the reactor system was monitored using a relative humidity and temperature transmitter (FT100P, Rotronic Instrument Corp., Huntington, NY). Although the sensor was not capable of operating within the harsh environment of the substrate, it worked well in the inlet air stream and could also be sterilized chemically and with UV light. A regulated DC power supply was used to operate the two–wired loop electronic circuitry of the sensor. The circuitry of the transmitter was positioned within an enclosure and was connected to the probe over a length of cable. The probe was 2 ½ in. long and had a ½–in. NPT fitting that could be threaded into the base of the reactor (Rotronic Instrument Corp., Huntington, NY).

The inlet airflow rate for the reactor was measured using a 0– to 10–L/min electronic mass flow meter (FMA 1720, OMEGA, Stamford, Conn.). This sensor required a DC power supply and had compression fittings for both inlet and outlet air lines. The internal sensing element can easily be harmed by impurities or gas crystallization so the air was filtered. The airflow into the reactor was regulated using a mechanical flow meter (RMB–51–SSV, Dwyer Instruments, Inc., Michigan City, Ind.). The filtered air was supplied by a compressor (Campbell Hausfeld VT629002AT, Grainger, Ky.) and humidified by passing the air through a sparger suspended in sterile, distilled water.

On–line data acquisition from the different sensors was accomplished using serial–based modules. The modules provided a simple and effective method of monitoring and controlling analog signals with the computer. A converter (CB7520, Measurement Computing, Corp., Middleboro, Mass.) served as a serial translator (RS232/RS485) between the module network and the computer (Gateway 2000, P5–120) and could handle up to 250 different modules. Depending on the signal of interest, a specific module was used. The variety of different modules provided a great deal of versatility in the choices of instrumentation and programming design. The eight–channel thermocouple module (CB 7018, Measurement Computing, Corp., Middleboro, Mass.) was capable of monitoring every type of thermocouple available on the market and could be configured to convert the signals into degree Celsius, Fahrenheit, or simply provide voltage. In addition, the CB 7018 module was capable of reading analog input signals at various ranges on the order of millivolts. The eight–channel analog input module (CB 7017, Measurement Computing, Corp., Middleboro, Mass.) was capable of measuring analog signals using a 16–bit A/D converter. The CB 7017 module could be configured to operate under different ranges and provide sensor outputs in voltage, percent of full scale range, or actual units, such as °C. The analog input channels on both the CB 7018 and CB 7017 modules could be configured as single ended or differential inputs. All channels for this application were set to read differential inputs. The electrical connections necessary for connecting the modules and instrumentation are provided in figures 1 and 2.

The programming for the system was completed in Microsoft® Visual Basic 5.0. This Windows programming environment was chosen because of its versatility in the application of both written code and graphical features. In

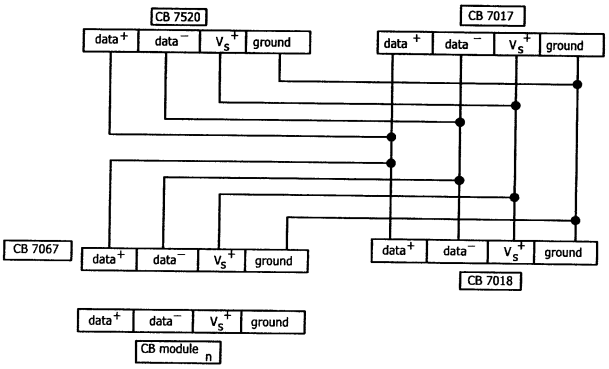


**Figure 1. Data acquisition circuit diagrams for module set up. CB 7520 connects to computer COM ports and modules are powered by 24 –V DC power supply.**

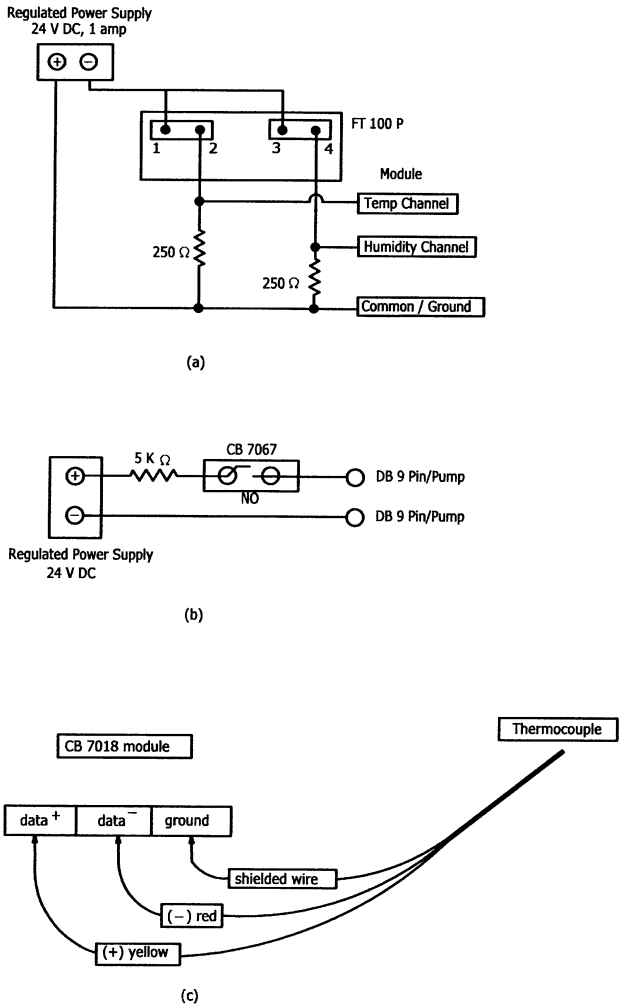


**Figure 2. Data acquisition circuit diagram for instrumentation components: a) relative humidity and temperature probe circuit, b) pump relay control, c) shielded K–type thermocouples.**

addition, the programming environment provided access to powerful tools and extensive programming structure development that was directly applied to various data acquisition components and other Microsoft® software. The program was designed to perform a number of tasks and a flowchart is provided in figure 3. The program had a number of

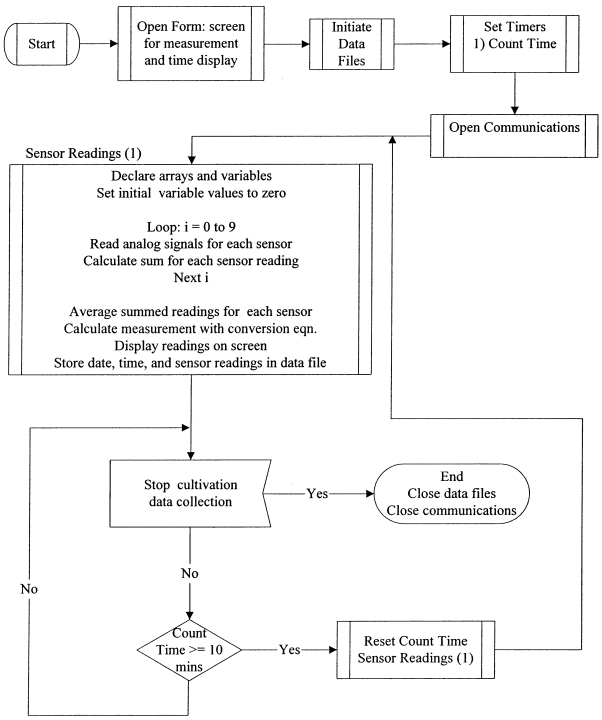


**Figure 3. Structure of the Visual Basic® program used to monitor and control the solid substrate cultivation deep bed reactor system.**

subroutines which described the loading and unloading of the form or screen, the opening and closing of communications between the computer and the module network, sensor readings and display, data storage, and timers for events. The program began with a click event of the "start" command button. The start time of the cultivation was displayed on the computer screen along with labels and textboxes for the different measurements. The data files were initiated and available for storage. The timer was set to continuously count the length of the cultivation in minutes and to display that value. The variables necessary to complete sensor readings were declared and communication was opened between the data acquisition modules and the computer. A series of 10 analog input signals from the sensors was read and a sensor average was displayed on the formatted screen. The collected data and time of collection were also stored in a file. The program ended with a click event of an "end" command button and the end time was displayed on the form and written to a storage file. Once the formatted screen was exited, the declared storage files and communication between the computer and modules were closed. The program also used driver libraries describing various functions, their inputs and outputs, and important global variables. The library information was incorporated into the program as a module.

## REACTOR

The reactors used for evaluation of the instrumentation and data acquisition system were cylindrical, deep bed reactors. A schematic of a reactor is provided in figure 4. Each reactor consisted of a glass column with a diameter of 17.8 cm, height of 30.5 cm, and a holding volume of approximately 5 L. The glass column had an aluminum bottom support and cover. A perforated plate was positioned on the bottom support to provide a base for the substrate bed.

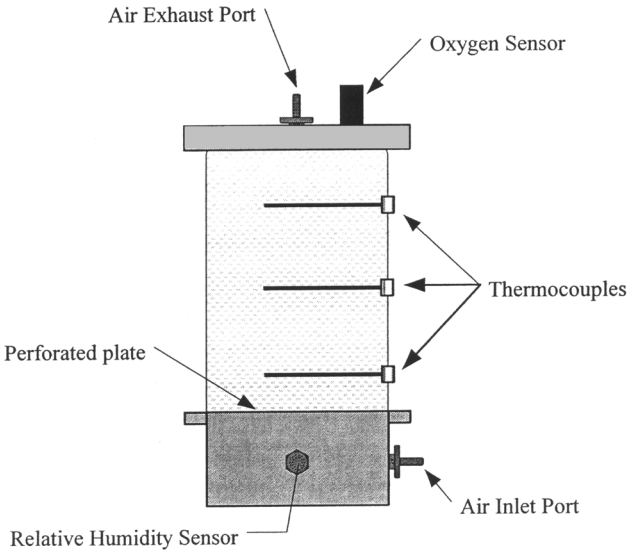


**Figure 4. Solid substrate cultivation deep bed reactor and the locations of the incorporated sensors.**

There were three ports along the side of the glass columns at 5, 14, and 23 cm above the perforated plate to allow for measurement of the substrate temperature. A threaded port was located on the cover of each reactor with a welded diffuser that extended into the reactor headspace to allow for oxygen concentration measurements. A threaded port was also located in the bottom support for relative humidity and temperature measurements of the inlet air. There was a connector on the cover for an exhaust line and a connector on the bottom support for the inlet air line. Prior to being used for cultivation, the reactors, with thermocouples attached, were autoclaved for 30 min (121°C, 19 psi).

### Organism

*Trichoderma longibrachiatum* (ATCC# 38586) was used for the SSC studies. The organism was maintained on potato dextrose agar (PDA) slants (4°C) and propagated on PDA plates (26°C, 7 days). After seven days, the plates' surfaces were covered with spores. A spore suspension was prepared by removing spores with a flame sterilized loop and placing them in 10 mL of sterile magnesium sulfate and potassium phosphate buffer. Spore counts were conducted using a hemacytometer. The final inoculum had a spore concentration of $10^{6-7}$ per mL. One mL of spore suspension was added to every 5 dry g of the substrate.

### Substrate

Organic wheat bran (from a local Co–op) served as the growth substrate for the solid substrate cultivation studies. For one reactor, 1 dry kg of wheat bran was separated into three aluminum trays and adjusted to 50% w/w moisture on a wet basis with magnesium sulfate and potassium phosphate buffer. The medium was autoclaved for 30 min (121°C, 19 psi). Inoculation and loading of the autoclaved substrate were completed in the laminar flow hood using aseptic techniques. Inoculum was added to each tray and mixed thoroughly. The added inoculum brought the final moisture content of the substrate to 55% w/w wet basis. The contents of each tray were then carefully loaded into the reactor. The substrate formed a bed height of 26.5 cm when loosely loaded into a reactor.

### Operating Conditions

A diagram of the cultivation system is shown in figure 5. The reactors were placed in a constant humidity, constant temperature (CHCT) chamber and then the remaining sensors were attached. The temperature and relative humidity of the chamber were controlled and maintained at 26°C and 80%, respectively. The reactors were aerated at a rate of $3.3 \pm 0.15$ L/min (7.0 SCFH) and the cultivations were run for five days.

## Results and Discussion

### Sensor Accuracy

The reliability of a sensor reading becomes a factor of great importance when the value is directly used to make system assessments or control decisions. An error analysis was completed for the instrumentation and data acquisition equipment used. Error associated with variable measurements is a combination of the inaccuracies of both the individual sensor and the analog to digital converter used to interpret the sensor signal. The uncertainty due to the imprecision of the instrument or chain of instruments within the system and influential environmental conditions must be included in the overall inaccuracy estimates. The calculations for the overall error of the deep bed reactor system

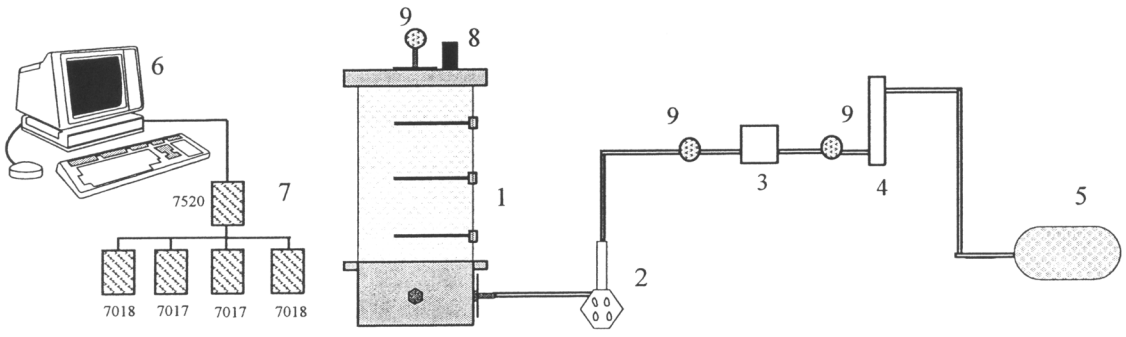


**Figure 5. Data acquisition and experimental setup. 1) SSC reactor; 2) humidifier; 3) mass flow meter; 4) airflow regulator; 5) air compressor; 6) PC computer; 7) CB modules; 8) oxygen sensor; 9) air filters.**

components are provided in Appendix A. The root sum square (rss) method was used to combine the individual uncertainties of each sensor measurement (Doebelin, 1990):

$$E_{a_{rss}} = \sqrt{\left(\Delta u_1 \frac{\partial f}{\partial u_1}\right)^2 + \left(\Delta u_2 \frac{\partial f}{\partial u_2}\right)^2 + \ldots + \left(\Delta u_n \frac{\partial f}{\partial u_n}\right)^2} \quad (1)$$

$E_{a_{rss}}$ = the overall error

f = the function desribing the desired quantity

$u_i$ = the measured independent quantities of the function f

$\Delta u_i$ = the errors in $u_i$

The accuracy of the sensors and applicable analog to digital converters used for the deep bed reactor system are tabulated in table 1. The analog to digital converter modules were calibrated according to manufacturer specifications using a calibrated digital voltmeter and power supply. The CB 7017 and CB 7018 modules produced analog measurements with ±0.01–V and ±0.05–mV errors, respectively. The absolute error of the oxygen sensor was 2% oxygen and that of the relative humidity probe was 2% RH. The airflow meter had an absolute error of 0.15 L/min. The temperature sensor component of the relative humidity/temperature probe had an absolute error of 0.32°C. For each sensor, the contribution of the sensor to the overall reading error was much greater than the contribution of the converter.

The thermocouples used to measure the substrate temperature were calibrated in a water bath at temperatures ranging from 25 to 55°C. The reference junction temperature used for the thermocouple readings was maintained at the same temperature during calibration as during operation. An ASTM thermometer provided the reference temperatures for comparison to the measured values. Fourteen measured values were determined for each thermocouple. Seven measurements were taken as the water bath temperature increased from 25 to 55°C and seven were taken as the water bath temperature decreased from 55 to 25°C. The calibration equations were determined using a linear regression analysis and applied to the final readings obtained from the converter during the experiments. The overall error of the thermocouple readings was calculated using the standard error of the regression. The uncalibrated, type–K thermocouples have a ±2.2°C error associated with them. The applied calibration limited the overall error of the thermocouple measurements to less than 0.2°C

**Table 1. Sensor and analog to digital converter accuracy for the solid substrate cultivation reactor system.**

| Converter | Associated Error |
|---|---|
| CB 7017 | ±0.01 V |
| CB 7018 | ±0.05 mV |

| Sensor | Associated Error | Overall Error | Error Contributed by Converter at Max. Reading (%) |
|---|---|---|---|
| Airflow meter | ±0.15 L/min<br>±0.08 V | ±0.15 L/min<br>±0.08 V | ±0.2 |
| Oxygen | ±2.0%<br>±1.06 mV | ±2.0%<br>±1.06 mV | ±0.09 |
| Relative humidity | ±2.0%<br>±0.08 V | ±2.0%<br>±0.08 V | ±0.2 |
| Temperature | ±0.30°C<br>±0.032 V | ±0.32°C<br>±0.034 V | ±0.2 |

| Thermocouple | Overall Error (°C) |
|---|---|
| Bottom | ±0.19 |
| Middle | ±0.14 |
| High | ±0.15 |

## System Performance

The environmental conditions and parameters monitored for the solid substrate cultivation system provided valuable insight into the overall process. Representative inlet air conditions entering the reactor are provided in figure 6. The airflow rate into the reactor is shown in figure 6a. The aeration rate was set at 3.3 L/min. The compressor introduced some variation in the airflow. The fluctuations were within 0.5 L/min of the set rate, providing a fairly stable airflow for the experiments. Figure 6b demonstrates that the humidifiers were effective in raising the relative humidity of the air entering the reactor. Over the course of the cultivation period, the air became nearly saturated, fluctuating between 97 and 100% humidity. These high levels of humidified air provided conditions for little drying to occur within the substrate bed due to aeration and helped maintain a more stable environment for growth. Minimal changes in the inlet air temperature also helped to provide a stable experimental environment for the SSC process. The temperature of the holding chamber for the reactor and humidifier was set at 26°C. The temperature of the inlet air remained fairly stable and within approximately a 1°C range of the chamber temperature setting as shown in figure 6c.

Substrate temperature measurements collected during the cultivation period present information concerning the growth stages of the microorganism, temperature gradients within the reactor, effectiveness of cooling strategies, and the quality of growth. Figure 7 shows representative temperature profiles for the top, middle, and bottom layers of the deep bed reactor. Changes in temperature are often associated with the growth of the organism. During the first 24 h, the temperatures within the bed were fairly stable and were consistent with the lag phase of microbial growth. Once the temperatures began to consistently rise the organism entered the active growth phase, where the microorganism was reproducing exponentially. Exponential growth produced a large amount of metabolic heat that increased internal substrate temperature. The temperatures eventually reached a peak and either leveled off or slightly declined. This change in the temperature profiles was an indication of the stationary phase of growth. During this stage of growth, the rate at which the organism reproduced was nearly equivalent to the rate at which cells were dying. In addition, cells simply maintained themselves, produced a number of metabolites, and began to produce spores.

The data collected for the three layers within the reactor exhibited the temperature gradients commonly observed in a deep bed reactor system (fig. 7). As expected in a reactor system employing forced aeration through the bottom of the substrate bed, the lowest temperatures occurred in the bottom layer whereas the highest temperatures appeared in the top layer. The air acted as a medium for both convective heat transfer and evaporative cooling. The dynamics of the air entering and leaving the reactor system contributed to the development of temperature gradients. The majority of the cooling occurred in the bottom of the bed because it was in contact with the cool inlet air. Convective cooling by the air

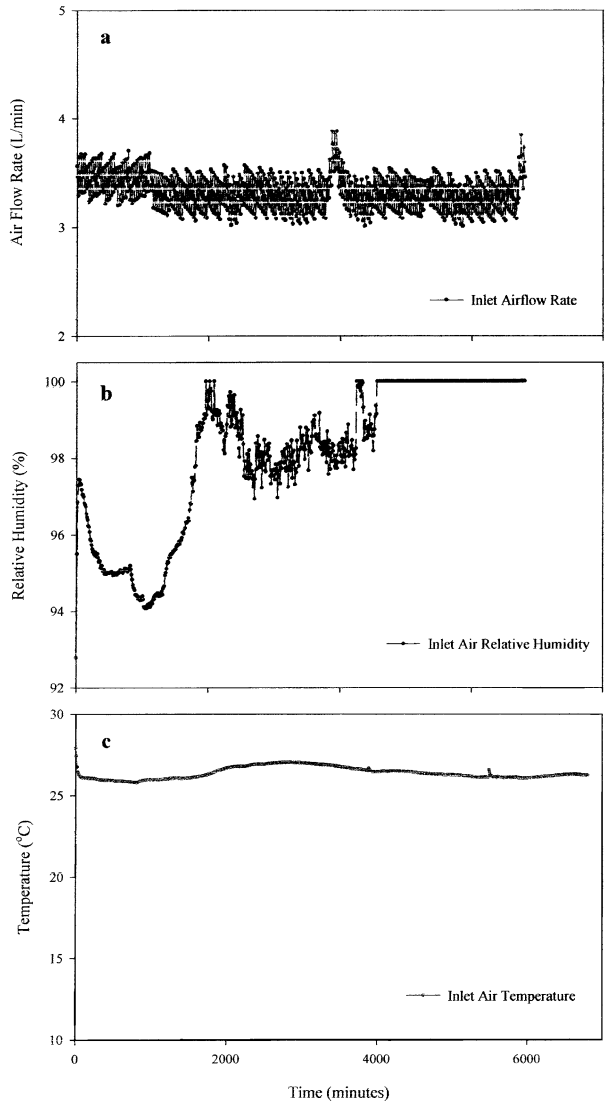


**Figure 6. Environmental conditions of the air stream entering the solid substrate cultivation reactor.**

was less effective in the upper region of the bed because of the smaller temperature differences between the preheated air from the lower regions and the substrate (Ashley et al., 1999). The ability to determine where temperature gradients exist can allow informed decisions to be made concerning the application of temperature control strategies.

Figure 8 shows the top, middle, and bottom temperature profiles of a deep bed reactor that utilized an internal cooling water coil to control the bed temperatures. Cooling water (0.5 L/min, 23°C) was pumped through the coil when the average temperature of the substrate exceeded 30°C. The profiles follow similar trends to those observed in figure 7, yet the effects of the temperature control can be seen in the collected temperature data. The overall bed temperatures were reduced as were the temperature gradients within the bed. The middle bed temperature was also lower throughout the cultivation than the other temperatures. The temperature measurements provided direct information about the effects of the applied control strategy and also allowed quantitative comparisons to be made between cultivations. The effectiveness of various heat removal strategies and their influence on

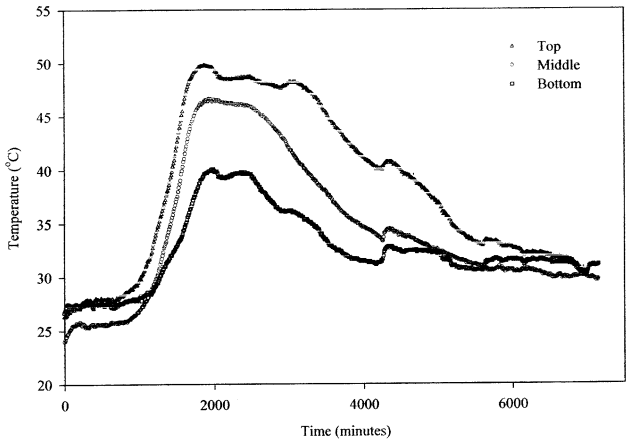


**Figure 7. Representative temperature profiles observed in the deep bed reactor, cooled by airflow at a rate of 3.3–L/min/kg dry substrate. Profiles are provided for the top, middle, and bottom layers.**

other environmental parameters can be more easily detected with the applied instrumentation.

The ability to monitor changes in the reactor system can provide indications of undesirable occurrences such as poor microbial growth, which leads to low product formation. Figure 9 shows the top and bottom temperature profiles of a deep bed reactor that experienced poor microbial growth in the upper region of the substrate bed. The bottom temperature profile observed was consistent with the typical growth phases of microorganisms. Based on the temperature changes, the organism was in a lag phase during the first 24 h. The organism then entered the active phase as the temperature quickly increased. Finally, the temperature stabilized and declined during the stationary and decline growth stages, respectively. The top temperature profile, on the other hand, had a different appearance. Although the initial stages of growth looked similar to those observed in the bottom of the bed, the top of the bed had a quick peak and then a quick decline in temperature before reaching an equilibrium with the constant humidity, constant temperature incubation chamber. This particular occurrence in the temperature evolution was an indication of the poor microbial growth observed. The reactor as a whole began the same, yet as the

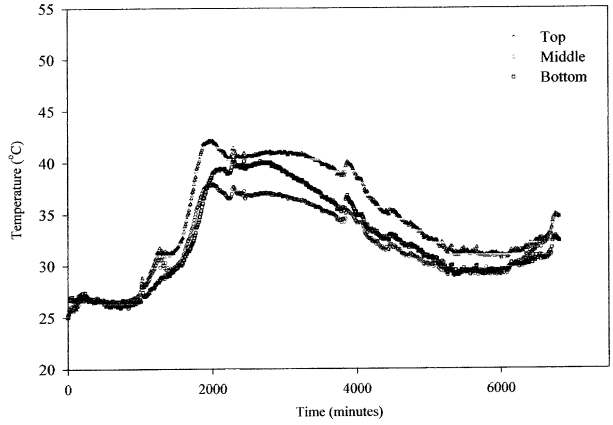


**Figure 8. Temperature profiles observed during a cultivation using forced aeration (3.3–L/min/kg dry substrate) and an internal cooling water coil operated based on average substrate temperature.**

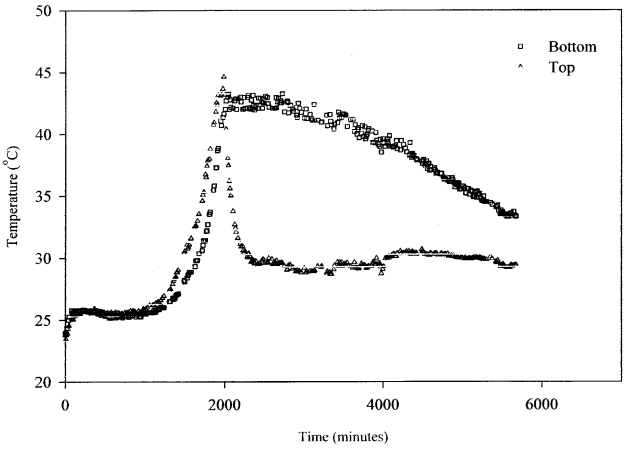


**Figure 9. Temperature profiles observed in a deep bed reactor that exhibited poor growth in the top layer, aerated at 3.3–L/min/kg dry substrate.**

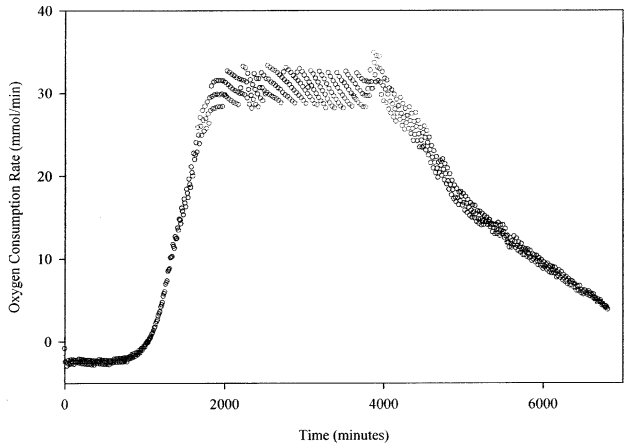


**Figure 10. Oxygen consumption rate over cultivation period of *T. longibrachiatum* in a deep bed reactor aerated at 3.3–L/min/kg dry substrate.**

cultivation continued, it was observed that the organism formed a dense mat of mycelium in the middle region of the bed. In addition to the organism utilizing the oxygen in the air in the bottom and middle portions of the bed, the dense nature of the substrate prevented oxygen from entering the top of the reactor. The significant reduction in oxygen availability limited the fungal growth in the top of the bed and the temperature dropped because little or no metabolic activity took place.

Oxygen concentrations measured over the course of the cultivation can be used to determine if adjustments are necessary to maintain a non–limiting aerobic environment. The dynamics of the oxygen concentration within a SSC system are strongly correlated to the growth of microorganisms and can be used to estimate not only the growth stages but also the growth rates (Saucedo–Castaneda et al., 1994). The rate of oxygen consumption can be calculated from the measured oxygen concentration and airflow rate at a given time during the cultivation process according to the following equation:

$$OCR = (O_2^{ambient} - O_2^{present}) \times \frac{\rho O_2}{MW_{O_2}} \times 10^3 \frac{mmol}{mol} \times Q_{air} \quad (2)$$

$O_2^{ambient}$ = the fraction of oxygen in ambient air $(0.21 \frac{L\ O_2}{L\ air})$

$O_2^{present}$ = the fraction of oxygen in air $\frac{L O_2}{L\ air}$

$\rho O_2$ = the density of oxygen $(1.43\ g\ O_2/L)$

$MW_{O_2}$ = the molecular weight of gaseous oxygen (32 g/mol)

$Q_{air}$ = the flow rate of air L/min

Figure 10 shows the rate of oxygen consumption of *T. longibrachiatum* over a cultivation period. The overall error associated with this calculation is described as a combination of errors in Appendix B. The maximum and minimum errors determined for this profile were 0.005 and 0.003 mmol/min, respectively. The majority of the inaccuracy was due to the oxygen measurement component of the oxygen consumption function. The profile in figure 10 is also indicative of the different microbial growth stages, however it provides a more reliable estimation than temperature because the quantitative values are not directly influenced by the changes in heat accumulation and transfer within the bed and control strategies such as aeration rate, cooling water jackets, and internal heat exchangers. The stages of growth were much more clearly defined. During the lag phase, the rate of oxygen uptake was small. As the consumption rates increased the organism began growing exponentially, utilizing the oxygen for metabolic activity. The beginning of the stationary phase began at approximately 1980 minutes post–inoculation and continued until approximately 4000 min. The organism's use of oxygen decreased as it entered the decline stage as indicated by the profile.

## Conclusion

The instrumentation system applied to the deep bed reactor allowed the environmental conditions to be monitored over time and the collected data provided quantitative information about the reactor system dynamics. The interactions between the variables were available for evaluation, including their potential use as indicators for specific variable control. In addition to control utility, the system provided insight into the growth characteristics of the organism of interest, greater understanding of the deep bed reactor system, and simple, quick on–line collection of data. More significantly, the data collected can be used to develop and validate mathematical models used to predict the heat and mass transfer within the deep bed reactor system.

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## Appendix A: Sensor Accuracy Calculations

### Analog To Digital Converters

CB 7017 (8 channel analog input module, 16–bit conversions)

Input range: ±5V

Accuracy: ±0.1% or better

Zero drift: ±0.03μV/Δ°C

Span drift: ±25 ppm/Δ°C

Accuracy, zero drift, and span drift all contribute to the error of the measurement. The analog to digital converters were operated at the calibration temperature, 25°C. Therefore, error contributions due to zero drift and span drift were negligible.

With a full scale range of 10V, the accuracy of the module is

0.001 × 10 = ±0.01 V

At 25°C, the absolute error due to zero drift is

0.03μV/Δ°C × 0 Δ°C = ±0 μV

At 25°C, the absolute error due to span drift is

25 ppm/Δ°C × 0 Δ°C = 0 × FSR = ±0 V

The root sum squared error of the CB 7017 module is

$$RootSSE = \sqrt{(0.01\text{V})^2 + (0\text{V})^2 + (0\text{V})^2} = \pm 0.01\text{V}$$

CB 7018 (8 channel thermocouple/analog input module, 16–bit conversions)

Input range: ±50 mV

Accuracy: ±0.05% or better

Zero drift: ±0.033 ppm/°C

Span drift: ±25 ppm/°C

Accuracy, zero drift, and span drift all contribute to the error of the sensor. The thermocouple and analog input modules were operated at the calibration temperature, 25°C. Therefore, error contributions due to zero drift and span drift were negligible.

With a full scale range of 100 mV the accuracy of the module is

100 × 0.0005 = ±0.05 mV

At 25˚C, the error due to zero drift is

0.033 ppm/Δ°C × 0 Δ°C × FSR = ±0 mV

At 25˚C, the error due to span drift is

25 ppm/Δ°C × 0 Δ°C × FSR = ±0 mV

The root sum squared error of the CB 7018 module is

$$RootSSE = \sqrt{(0.05\text{mV})^2 + (0\text{mV})^2 + (0\text{mV})^2} = \pm 0.05\text{mV}$$

### Relative Humidity/Temperature Sensor

Accuracy for relative humidity element: ±2% RH from 10 to 100% RH

Accuracy for temperature element: ±0.3°C

Error from power supply: negligible (regulated power supply)

Output range: 4 to 20 mA for 0 to 100% RH and 0 to 37.78˚C

Output voltage across a 250 Ω resistor: 1 to 5 V

Transfer function for relative humidity: 25% RH / V

Transfer function for temperature: 9.45°C / V

Relative error for relative humidity element is

$$\frac{2\%\,\text{RH}}{25\%\,\text{RH/V}} = \pm 0.08\text{V}$$

Relative error for temperature element is

$$\frac{0.3\,^{o}C}{9.45^{o}\text{C/V}} = \pm 0.032\text{V}$$

Total root sum square error for relative humidity measurements (includes AD error) is

$$RootSSE = \sqrt{(0.01\text{V})^2 + (0.08\text{V})^2} =$$

$$\pm 0.08\text{V} \rightarrow 0.08\text{V} * 25\%\,\text{RH/V} = 2\%\,\text{RH}$$

The total root sum square error for temperature measurements (includes AD error) is

$$RootSSE = \sqrt{(0.01\text{V})^2 + (0.032\text{V})^2}$$

$$= \pm 0.034\text{V} \rightarrow 0.034 * 9.45^{o}\text{C/V} = 0.32^{o}C$$

OXYGEN SENSOR

Linearity: ±2% of full scale
Full scale: 0 to 100% oxygen
Output range for sensor: 0 to 53 mV
Transfer function for sensor: 1.89% $O_2$/mV

Relative error for the oxygen sensor is

$$\frac{2\%\,O_2}{1.89\%\,O_2/\,mV} = \pm 1.06 mV$$

Total root sum square error for oxygen measurements (includes AD error) is

$$RootSSE = \sqrt{(1.06mV)^2 + (0.05mV)^2} = \pm 1.06 mV$$
$$\rightarrow 1.06mV \times 1.89\%\,O_2/mV = 2.00\%\,O_2$$

AIRFLOW METER

Accuracy: ±1.5% of full scale
Temperature: coefficient: 0.15% of full scale/Δ°C
Pressure coefficient: 0.1% of full scale/Δpsi
Full scale: 0 to 5 V for 0 to 10 L/min

Accuracy, temperature coefficient, and pressure coefficient all contribute to the error of the sensor. The airflow meter was operated at the calibration temperature and pressure, 25°C and 14.7 psi. Therefore, error contributions due to the temperature and pressure coefficients were negligible.

With a full scale range of 5 V and 10 L/min, the accuracy of the flow meter is

0.015 × 5V = ±0.075 V
At 25°C, the error due to the temperature coefficient is
0.0015/Δ°C × 5V × 0 Δ°C = ±0 V
At 25°C, the error due to the pressure coefficient is
0.001/Δpsi × 5V× 0 Δpsi = ±0 V
The root sum squared error of the flow meter is

$$\text{Root SSE} = \sqrt{(0\,V)^2 + (0\,V)^2 + (0.075\,V)^2 + (0.01\,V)^2}$$

$$= \pm 0.076\,V \rightarrow 0.076\,V \times \frac{2\,L/min}{V} = 0.15\,L/min$$

## APPENDIX B: OXYGEN CONSUMPTION RATE CALCULATIONS AND ABSOLUTE ERROR

The oxygen consumption rate (mmol oxygen per min) of the microorganism can be determined from the oxygen concentration present in the reactor by the following equation

$$OCR = (O_2^{ambient} - O_2^{present}) \times \frac{\rho_{O_2}}{MW_{O_2}} \times 10^3 \frac{mmol}{mol} \times Q_{air}$$

$O_2^{ambient}$ is the fraction of oxygen in ambient air, $0.21 \frac{L\,O_2}{L\,air}$

$O_2^{present}$ is the fraction of oxygen in air $\frac{L\,O_2}{L\,air}$

$\rho_{O_2}$ is the density of oxygen, $1.43\,g\,O_2$ /L

$MW_{O_2}$ is the molecular weight of gaseous oxygen, 32 g/mol

$Q_{air}$ is the flow rate of air L/min

The root sum square error, $E_{a,rss}$, associated with the above equation can be determined by combining the component errors in the overall system such that

$$N \pm \Delta N = f(u_1 \pm \Delta u_1, u_2 \pm \Delta u_2, ....u_n \pm \Delta u_n)$$
$$= f(u_1, u_2, ....., u_n) + \Delta u_1 \frac{\partial f}{\partial u_1} + \Delta u_2 \frac{\partial f}{\partial u_2} + ...$$
$$+ \Delta u_n \frac{\partial f}{\partial u_n} + \frac{1}{2}\left[(\Delta u_1)^2 \frac{\partial^2 f}{\partial u_{12}^2} + ...\right] + ...$$

$$(\Delta u_i^2) \rightarrow negligible$$
$$= f(u_1, u_2, ....., u_n) + \Delta u_1 \frac{\partial f}{\partial u_1} + \Delta u_2 \frac{\partial f}{\partial u_2} + ... + \Delta u_n \frac{\partial f}{\partial u_n}$$

For oxygen consumption rate

$$N \pm \Delta N = f(u_1, u_2) + \Delta u_1 \frac{\partial f}{\partial u_1} + \Delta u_2 \frac{\partial f}{\partial u_2}$$
$$= f(O_2^{present}, Q_{air}) + \Delta O_2^{present} \frac{\partial f}{\partial O_2^{present}} + \Delta Q_{air} \frac{\partial f}{\partial Q_{air}}$$

$$E_{a_{rss}} = \Delta N = \sqrt{\left(\Delta u_1 \frac{\partial f}{\partial u_1}\right)^2 + \left(\Delta u_2 \frac{\partial f}{\partial u_2}\right)^2}$$

$$= \sqrt{\left(\Delta O_2^{present} \frac{\partial f}{\partial O_2^{present}}\right)^2 + \left(\Delta Q_{air} \frac{\partial f}{\partial Q_{air}}\right)^2}$$

The root sum square error is calculated as the overall error because the Δu's provided for sensor measurements are not considered to be absolute limits of error, but rather statistical bounds or uncertainties (Doebelin, 1990). The overall error for the oxygen consumption rate is

$$E_{a,rss}^{OCR} = \sqrt{\left(0.02 \frac{L\,O_2}{L\,air} \times \frac{\rho_{O_2} Q_{air}}{MW_{O_2}}\right)^2 + \left(0.15 \frac{L}{min} \times (O_2^{ambient} - O_2^{present}) \times \frac{\rho_{O_2}}{MW_{O_2}}\right)^2}$$

For the following reactor conditions:

A. $Q_{air} = 3.33 \frac{L}{min}$ and $O_2^{present} = 0.207$

$E_{a,rss}^{OCR} = .003 \frac{mmol}{min}$

B. $Q_{air} = 3.87 \frac{L}{min}$ and $O_2^{present} = 0.011$

$E_{a,rss}^{OCR} = .004 \frac{mmol}{min}$