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Temperature Control of a Solid Substrate Cultivation Deep - Bed Reactor Using an Internal Heat Exchanger

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Notes/Citation Information

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ABSTRACT. *The solid substrate cultivation (SSC) process is often limited by rapid increases in temperature as a result of metabolic activity and poor heat transfer properties of the solid substrate. High temperatures can adversely affect microbial growth and product formation. Reduction of substrate temperatures is important for improved productivity, yet effective and efficient control of heat transfer in a deep-bed SSC reactor has proven difficult. An internal cooling water coil was incorporated into a deep-bed reactor system and evaluated for its ability to reduce substrate temperature. Three operating treatments were investigated: cooling water circulation whenever the center bed temperature was above 30°C, timed cooling water operation on 10 min on/off intervals from the start of cultivation, and timed operation on 10 min on/off intervals after the expected start of microbial exponential growth. Enzyme yield, peak temperature, and net rate of metabolic heat accumulation within the substrate bed were measured during the cultivation of Trichoderma longibrachiatum on wheat bran for xylanase production. The cooling water reduced the temperatures observed in the substrate bed by 5°C on average and was effective in decreasing the rate of heat accumulation in the bed. Within a 10 min time interval, the internal heat exchanger demonstrated a net decrease in heat accumulation at an average rate of approximately 0.5°C/min. Despite the decrease in temperatures observed with the control strategies, the xylanase yields were not statistically different from the cultivations with no temperature control.*

Keywords. Deep-bed reactor, Heat exchanger, Heat removal, Solid substrate cultivation (SSC), Temperature control, Temperature gradients, Trichoderma longibrachiatum, Xylanase.

Solid substrate cultivation (SSC) describes the growth of microorganisms on water-insoluble substrates in the absence of free water (Moo-Young et al., 1983). SSC has applications in the composting of wastes or municipal refuse, mushroom production, biofuel production, and the upgrading of staple foods and feeds. In addition, it is utilized for the production of biological control agents, antibiotics, and industrial enzymes (Rathbun and Shuler, 1983; Mudgett, 1986). The solid substrates are typically inexpensive agricultural residues such as wheat bran, corn stover, sugar cane bagasse, and other cereal grains and organic materials. Some of the advantages of using SSC over the more traditional submerged-liquid cultivation often include higher product yields, improved product recovery, minimal pretreatment of substrates, and reduced wastewater outputs (Mudgett, 1986).

Temperature is considered a critical variable in SSC because conditions higher or lower than the optimum are not favorable to growth and product synthesis (Prior et al., 1992; Ghildyal et al., 1994). One of the challenges encountered during the SSC process is the rate of heat generation and accumulation (Mudgett, 1986). Heat is generated as a result of microbial activity, and because of low substrate thermal conductivity heat cannot dissipate rapidly (Sargantanis et al., 1993). Ghildyal et al. (1993) conducted a study on the effect of temperatures at different bed depths in a rectangular deep-bed SSC system on amyloglucosidase levels produced by *Aspergillus niger* from wheat bran. The authors found that amylogucosidase production was lower with increases in substrate temperature above the optimum, and the depth of substrate affected temperature. In addition, the increased bed temperatures reduced the moisture content of the cultivation system as a result of increased evaporation. Removal of metabolic heat and reduced temperatures in deep-bed reactors during the cultivation period has been reported to be necessary for higher enzyme production (Ghildyal et al., 1993; Ghildyal et al., 1994).

Although heat and mass transfer limit deep-bed reactors, the deep-bed configuration has the potential for better process management and control than other SSC bioreactor designs (Durand et al., 1997). Achieving uniformity through environmental control is important for the design of large-scale SSC processes; however, the conventional techniques used in submerged liquid cultivations are not necessarily adaptable to SSC (Rathbun and Shuler, 1983). Three main heat transfer mechanisms contribute to metabolic heat removal within the deep-bed reactor system: conduction, convection, and evaporation. Conductive heat transfer through the wheat bran substrate is the least efficient mechanism when compared to convection and evaporation in SSC (Gutierrez-Rojas et al., 1996; Sangsursak and Mitchell, 1998).

Use of forced aeration to remove heat from the reactor system maximizes the contribution of convection and evaporation to the overall heat transfer. Ghildyal et al. (1994) conducted studies to assess the effects of aeration rates on heat transfer resistance in a deep-bed reactor (height 34.5

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cm) and on amyloglucosidase activity produced by *Aspergillus niger* grown on a wheat bran and corn flour mixture. They found that substrate temperature gradients were reduced from 0.79°C/cm to 0.13°C/cm when the bed was aerated at 5 L/min and 25 L/min, respectively, and reported a 3-fold increase in enzyme activity as a result. However, the high aeration rate of 25 L/min reduced the moisture content in the medium from 51% to 22%. A model developed by Ashley et al. (1999) investigated periodic reversal of the direction of airflow as a strategy for heat removal and discussed the characteristic heating time within a packed-bed reactor for *A. niger* on a starchy substrate. Based on their findings, it was determined that a cooling strategy would need to operate on a minimum of 10 min intervals to be effective for heat transfer in deep-bed SSC. However, it was determined that air reversal was not a good strategy for removing heat.

In order to provide an efficient and effective heat transfer system for higher productivity in SSC, a mechanism in addition to forced aeration may need to be adopted because forced aeration alters other meaningful parameters. Fernandez et al. (1996) controlled bed temperatures within ±4°C by enhancing the evaporative cooling within the system. The inlet air conditions, relative humidity, and temperature were manipulated to obtain the desired amount of heat transfer from the substrate to the moving air. Water was then added back to the substrate to compensate for water losses. Despite the observed temperature control, this strategy introduced moisture content gradients within the system, and productivity was not reported.

Temperature regulation can also be carried out by placing reactors in a water bath or by using a jacket for thermostatic water circulation. Conductive heat exchanger strategies of heat regulation may prove more promising than forced aeration because the heat capacity of water-saturated air is much less than that of cooling water, and the heat exchange will not dry the substrate (Durand et al., 1997). Deep-bed reactor studies by Saucedo-Castaneda et al. (1990), Ghildyal et al. (1994), and Sangsurasak and Mitchell (1998) involved the removal of heat by conduction through the use of a cooling water jacket; however, the performance of the water jacket was not evaluated.

Roussos et al. (1993) developed a large-scale solid substrate cultivation reactor called Zymotis. The Zymotis is an acrylic, rectangular shaped, packed-bed reactor that utilizes stainless steel, water-cooled heat exchanger plates to dissipate generated heat. Trials completed for the Zymotis involved the growth of *Trichoderma harzianum* on an 80:20 mixture of sugar cane bagasse and wheat bran. The group found the growth of *T. harzianum* to be uniform and gradients in temperature absent when compartments were spaced 5 cm apart; however, the temperature control by the circulating water was determined to be inefficient during the active growth phase. Cellulolytic enzymes were quantified for the Zymotis cultivation and a laboratory-scale column cultivation. Although the reported cellulase yields were greater for the Zymotis than for the laboratory-scale column cultivation, a statistical analysis of the data was not reported. A model developed to investigate the design and operation of the Zymotis reactor indicated that it has the potential to overcome heat transfer problems by optimizing the amount of heat transferred to the cooling water within the internal plates (Mitchell and von Meien, 2000).

The aim of this work was to introduce an internal cooling water coil into a deep-bed solid substrate cultivation reactor and to evaluate its potential as an effective heat removal strategy. The cooling coil was evaluated by assessing the heat exchanger's effect on peak substrate temperature, net rate of metabolic heat accumulation within the substrate bed, and enzyme yield during the cultivation of *Trichoderma longibrachiatum* on wheat bran for xylanase production.

MATERIALS AND METHODS
REACTOR AND EXPERIMENTAL SETUP

SSC studies were carried out in cylindrical deep-bed reactors, as shown in figure 1. Each reactor had a glass column with a diameter of 17.8 cm, height of 30.5 cm, and a working volume of approximately 5 L. The 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. There were three positions along the side of the glass columns at 3, 12, and 21 cm from the perforated plate to measure temperature of the substrate. A port was located in the cover of each reactor with a welded diffuser that extended into the reactor headspace to allow for oxygen concentration measurements. A port was also located in the bottom supports for relative humidity and temperature measurements of the inlet air. A connector on the top cover allowed for an exhaust line, and a connector on the bottom support held the inlet air line. An internal cooling water coil (copper alloy No. 122, 6 mm i.d.) was introduced into the cultivation system, in addition to aeration, with the aim of enhancing conductive heat transfer and reducing axial gradients within the substrate. The coil was constructed in a helical fashion with three turns (7.3 cm apart vertically) and an outer diameter of 8.9 cm, leaving 4.45 cm between the reactor walls and the coil surface in the radial direction.

A data acquisition system was designed to allow automated on-line measurements of important parameters for the reactors (Chinn et al., 2003). The experimental setup is provided
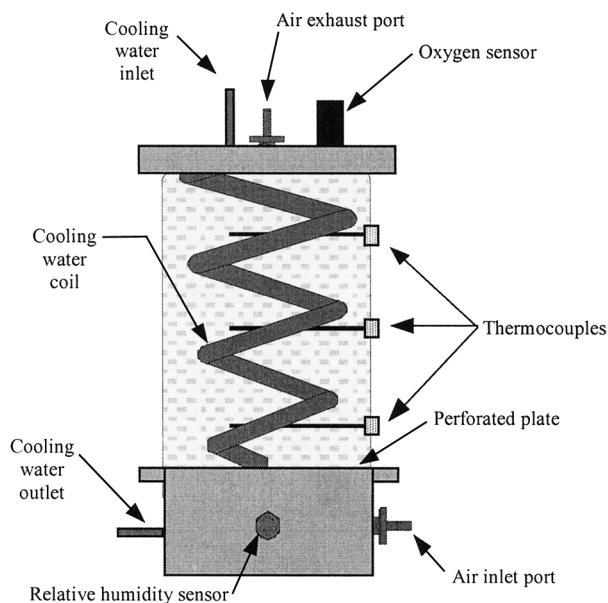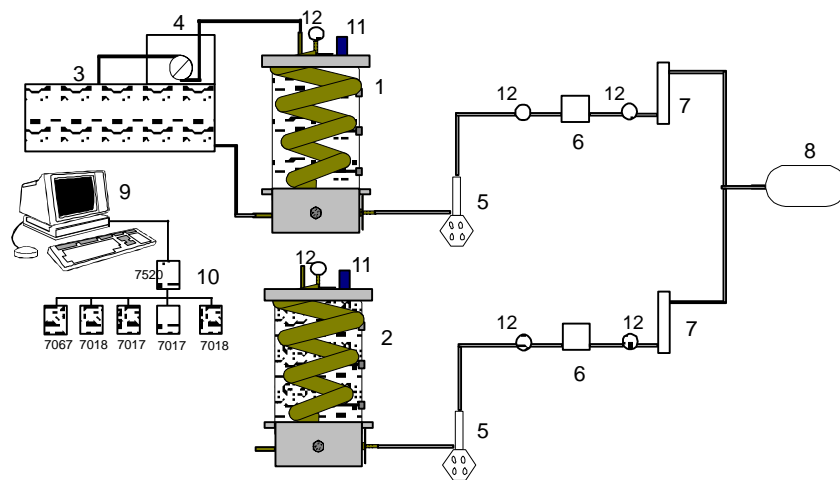

Figure 1. Solid substrate cultivation deep-bed reactor and incorporated sensors.

Figure 2. Data acquisition and experimental setup: (1) treatment reactor, (2) control reactor, (3) water bath, (4) peristaltic pump, (5) humidifiers, (6) mass flow meters, (7) airflow regulators, (8) air compressor, (9) computer, (10) CB modules, (11) oxygen sensors, and (12) air filters.

in figure 2. The instrumentation employed allowed the reactor environment to remain sterile and did not disrupt the process. Substrate temperature was measured using type-K thermocouple probes and shielded thermocouple wire (KMQS-062G-6, Omega Engineering, Stamford, Conn.). After calibration, the overall error of the thermocouple measurements was limited to 0.2˚C. The oxygen concentration within the reactor headspace was monitored using a galvanic oxygen sensor (MAX-250, Maxtec, Inc., Kent, Wash.). 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, N.Y.). The inlet airflow rates for the reactors were measured using 0 to 10 L/min electronic mass flow meters (FMA 1720, Omega Engineering, Stamford, Conn.). 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 bubbling through a sparger suspended in sterile, distilled water. The sparger unit was placed in a constant humidity, constant temperature chamber set at 80% relative humidity and 26˚C. A peristaltic pump drive (Masterflex 7520-40, Cole-Parmer Instrument Co., Vernon Hills, Ill.) and pump head (Masterflex 7518-10, Cole-Parmer Instrument Co., Vernon Hills, Ill.) were used to move water to and from the cooling coil. The water in the water bath was maintained at 23˚C.

On-line measurements of the different sensors were performed using analog input CB COM modules (Measurement and Computing, Inc., Middleboro, Mass.) and a computer (P5-120, Gateway 2000). The programming for the system was completed in Microsoft Visual Basic 5.0. The program collected and stored average measurements from the sensors every 10 min and initiated the temperature control strategies during the cultivation process.

ORGANISM

Trichoderma longibrachiatum (ATCC No. 38586) was used for the solid substrate cultivation studies. This particular isolate of *T. longibrachiatum* is capable of producing the enzyme endo-1, 4-β-xylanase (Baker et al., 1977). The fungus was maintained on potato dextrose agar (PDA) slants

at 4˚C. Propagation of the organism for SSC was completed on PDA plates at 26˚C for seven days. After seven days, the plates' surfaces were covered with spores. A spore suspension was prepared by removing the spores with a flame-sterilized loop, placing each swipe in 10 mL of sterile magnesium sulfate and potassium phosphate buffer. Spore counts were completed with a hemacytometer (Ridder et al., 1998). The final inoculum had a spore concentration of 10^6 to 10^7 per mL. One mL of inoculum was added to every 5 dry grams of the substrate.

SOLID SUBSTRATE CULTIVATION

Prior to being used for cultivation, the reactors with thermocouples attached were autoclaved for 30 min at 121˚C and 131 kPa (19 psi). Organic wheat bran (from a local co-op) served as the growth substrate for the SSC studies. For one reactor, one dry kg of wheat bran was separated into three aluminum trays and adjusted to 50% moisture (wet basis) with magnesium sulfate and potassium phosphate buffer. The medium was covered and autoclaved for 30 min at 121˚C and 131 kPa (19 psi). Inoculation and loading of the substrate were completed aseptically. The added inoculum brought the final moisture content of the substrate to 55% (wet basis). The substrate had a bed height of 26.5 cm when loosely loaded into a reactor. The optimum SSC growth conditions for *T. longibrachiatum* in trays were 55% moisture content (wet basis) and 30˚C (Ridder et al., 1998), and the optimum propagation temperature recommended by ATCC was 26˚C. The reactors were placed in a constant humidity, constant temperature (CHCT) chamber (80% RH, 26˚C). The reactors were aerated at a rate of 3.3 L/min (7.0 SCFH), and the cultivations were run for five days.

EXTRACTION

After cultivation, the contents of each reactor were separated into three layers representing the top, middle, and bottom of the substrate bed. The layers were placed in separate 5 L containers and weighed, and 75 mL of 0.1% v/v Tween 80 solution was added to each container for every 5 dry grams of substrate. The wheat bran suspension was stirred periodically at 25˚C for a period of 30 min. Three 20 to 30 mL samples were removed from each container, filtered through Whatman No. 1 filters, and centrifuged (1342× g, 15 min). The supernatant of each sample was decanted with a clean

pipette into 2 mL plastic vials. The extracted enzyme samples were stored at -45˚C until assayed. Previous work completed in the lab confirmed that freezing in this manner was not detrimental to enzyme activity.

ASSAY

The assay procedure completed on the extracted enzymes was developed by Megazyme (Bray, Ireland). Xylanase activity was determined using the standard curve published by Megazyme for pure xylanases produced by *T. longibrachiatum*, which provides a conversion from absorbance to Somogyi milliunits of activity per assay. One Somogyi unit is defined as the amount of enzyme that will liberate reducing equivalents equal to 1 mg of glucose in 30 min at 40˚C (Somogyi, 1938). The units/gram of the original preparation were calculated as follows:

$$\frac{units}{dry\ gram} = \frac{milliunits}{0.5\ mL} \times \frac{1}{1000} \times 15 \times 2 \times dilution \qquad (1)$$

where

$\frac{1}{1000}$ = conversion from milliunits to units

15 = initial extraction volume (15 mL per gram dry solid)

2 = conversion from volume assayed (0.5 mL) to 1 mL of extract

dilution = further dilutions of initial extract.

This Megazyme assay has proven more reliable than the DNS reducing sugar assay because it is not limited by the lack of sensitivity and nonlinearity introduced when assaying materials that contain high levels of background reducing sugars (Megazyme, Bray, Ireland).

EXPERIMENTAL DESIGN AND ANALYSIS

Three different temperature control strategies were applied to the SSC of *T. longibrachiatum* in the deep-bed reactor using the internal cooling coil, and their effects on reactor performance and product yield were assessed. The experiments were conducted using a completely randomized design with a one-way treatment structure. Randomization of the treatment replications was completed using a random number table. The three treatments were completed with two replications, and a paired control was completed with each replication. The control reactor contained the same inoculum as the treatment reactor and used aeration as a heat removal mechanism without cooling water operation (normal operation). The three treatments included the following:

Treatment 1 — Cooling water operation based on feedback temperature control, using 30˚C as the minimum temperature threshold for operating the cooling water.

Treatment 2 — Cooling water operation for 10 min on/off cycles from the start of cultivation.

Treatment 3 — Cooling water operation for 10 min on/off cycles after the start of expected microbial exponential growth.

Based on data collected from preliminary studies, the exponential growth phase was defined by time greater than 1100 min and the bottom layer temperature greater than 28˚C. The growth status was evaluated every 10 min. Analyses of the cultivation runs were completed using the PROC GLM function of SAS. The least significant difference procedure and least squares means procedures were used to evaluate comparisons between treatments and between treatments and their respective controls. All comparisons were investigated within the three layers of the substrate bed (top, middle, and bottom). The tests of significance were evaluated using a level of significance (α) of 0.10.

RESPONSE VARIABLES

Enzyme activities were obtained for the top, middle, and bottom layers of the substrate bed in each reactor by averaging the values calculated for each dilution of three different samples from each layer. The temperature measurements in the substrate bed allowed other temperature-related variables to be determined for the top, middle, and bottom layers. The peak temperatures within the layers for each treatment were determined by selecting the highest temperature reached during the course of the cultivation. The net rate of metabolic heat accumulation (˚C/min) was determined for two stages in the cultivation period using the temperature profiles for the three layers. The first stage included selected data points from the fastest temperature rise during the active growth phase before the peak temperature. Similarly, the second stage included data points from the fastest temperature drop after the peak temperature was reached. A linear regression was performed on each of the selected groups of data points to obtain representative rates (slopes) and standard deviations.

RESULTS AND DISCUSSION

Figure 3 shows representative temperature profiles for the three different treatments. The temperature profiles for the top, middle, and bottom layers of the substrate for treatment 1 are shown in figure 3a. The profiles follow typical microbial growth patterns. The effectiveness of the cooling water can be seen in the middle layer temperature profile of treatment 1 (fig. 3a). One would expect the temperatures of the middle layer to fall in between the top and bottom layer temperatures. Yet when the cooling water is in operation, the temperatures in the middle layer were less than both the top and bottom portions of the reactor. The increased effectiveness of the cooling water coil in the middle of the substrate can be attributed to the physical design of the coil. The number of coil turns in the middle portion of the bed was greater than in the bottom and top layers by a factor of 2. The increased heat exchanger surface area available for heat transfer in the middle of the bed allowed more metabolic heat to be removed from the substrate.
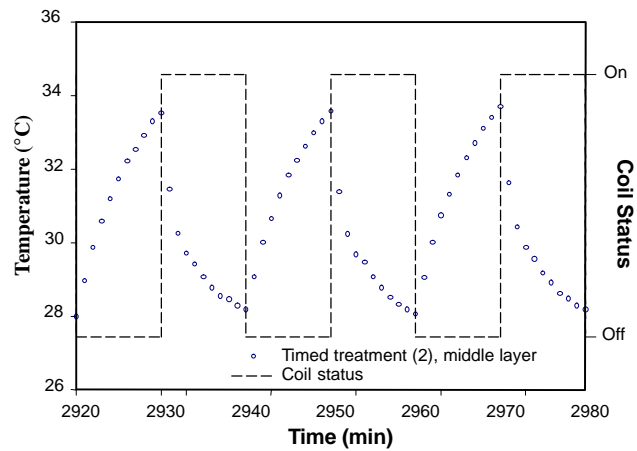
The temperature profiles for the top, middle, and bottom layers of the substrate for treatment 2 are shown in figure 3b. The cooling water was operated for 10 min time intervals starting at the beginning of the cultivation. The temperature profile for the middle layer of figure 3b appears to be two individual profiles. In fact, what is occurring is that the operation of the coil is having a larger impact on the substrate temperature in the middle of the bed. Just before the coil goes on for 10 min, the temperature reading is at a higher level. Once it goes off, the observed temperature, as a result of the cooling water, is reduced by up to 6˚C, depending on the time during the cultivation. This is discussed in greater detail below.

The temperature profiles for the top, middle, and bottom layers of the substrate for treatment 3 are shown in figure 3c. The cooling water was operable for 10 min time intervals
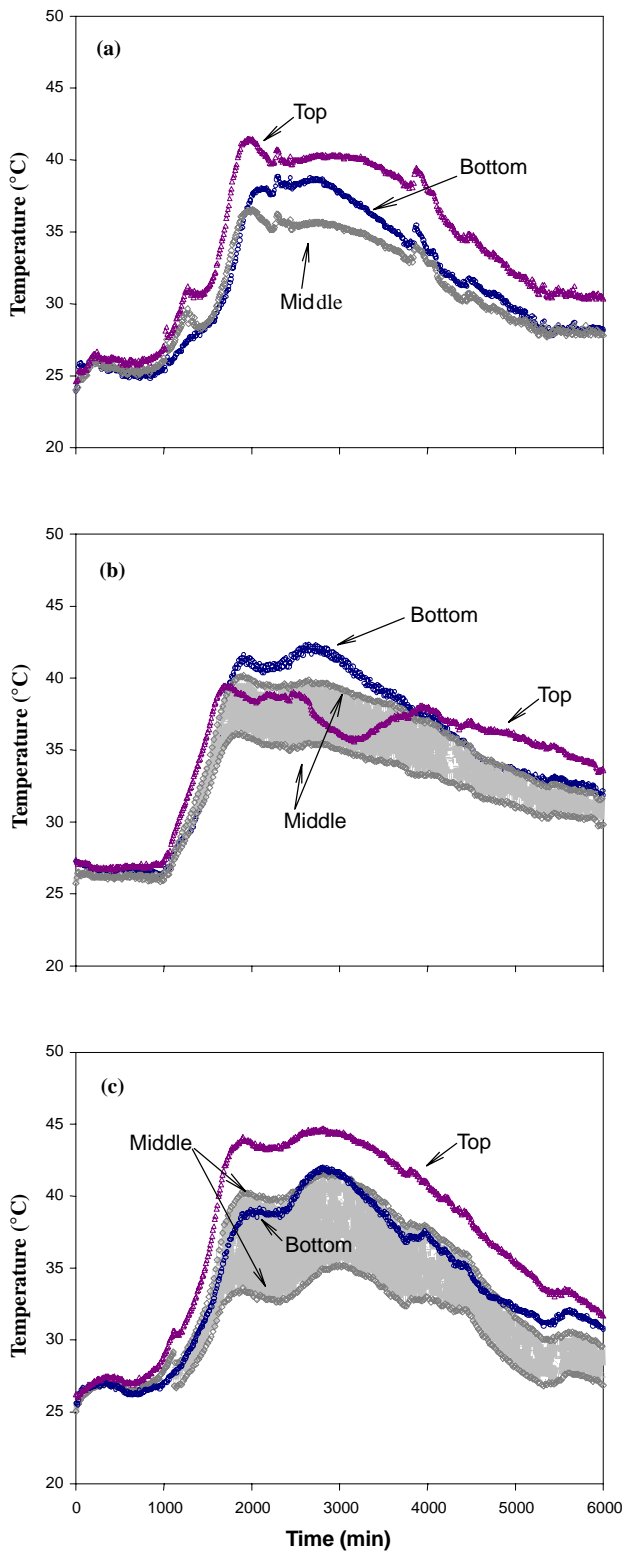
Figure 3. Representative temperature profiles for the three treatments: (a) treatment 1, (b) treatment 2, and (c) treatment 3. Profiles are shown for the top, middle, and bottom layers of the deep-bed reactor using the applied control strategies.

beginning at the start of exponential growth. The trends followed by the temperature profiles for treatment 3 were very similar to those observed for treatments 1 and 2. Operation of the cooling water started at 1110 min and, like treatment 1, introduced a short lag in the temperature increase within the


Figure 4. A segment of the middle layer temperature profile and the cooling coil status for treatment 2. The sampling interval for this experiment was 1 min.

bed. The smaller effect can be attributed to the discontinuous use of the cooling water. The middle layer temperature profile for treatment 3 shows the same patterns as treatment 2. The cooling water introduces up to an $8°C$ reduction in the middle of the substrate, depending on the time during the cultivation.

The temperature fluctuations observed in the middle layer of the substrate were further examined to characterize the response of the cultivation system to the step changes in the cooling water operation. An additional experiment was completed for treatment 2 with an increased sampling rate. The substrate temperature was measured every minute as opposed to every 10 minutes, as shown in the principal data. A subsection of these data in relation to the operation of the cooling water is presented in figure 4. The observed values for the middle layer temperature over time indicate that the substrate temperature fluctuated, and this fluctuation was nonlinear. For example, from time 2940 to 2950 min, water was not flowing through the internal coil, and the substrate temperature began to rise. The driving force for heat exchange was the temperature difference between the middle layer, the surrounding layers (bottom and top), and the air passing through the substrate. As that temperature difference became smaller, the rate of heat transfer became smaller. As a result, the middle layer temperature dynamics were consistent with a first-order system response. From time 2950 to 2960 min, water was flowing through the internal cooling coil, and the substrate temperature dropped. The driving force was the temperature difference between the middle layer and the water within the cooling coil. Once again, as the temperature difference became smaller, the rate of cooling decreased. The cooling within the 10 min time interval was characteristic of a first-order response with τ (time constant, the time necessary to attain 64% of the total change) approximately equal to 2 min.

The dynamics of this SSC system indicated that the apparent thermal conductivity of the wheat bran was greater than previously assumed. The thermal response of the substrate was nearly immediate. This system characteristic is meaningful with regard to the design of control schemes and monitoring of the environmental parameters.

Figure 5 presents more detail on the temperature results obtained during the cultivations using treatment 1. Profiles are provided for the two replications of the top, middle, and
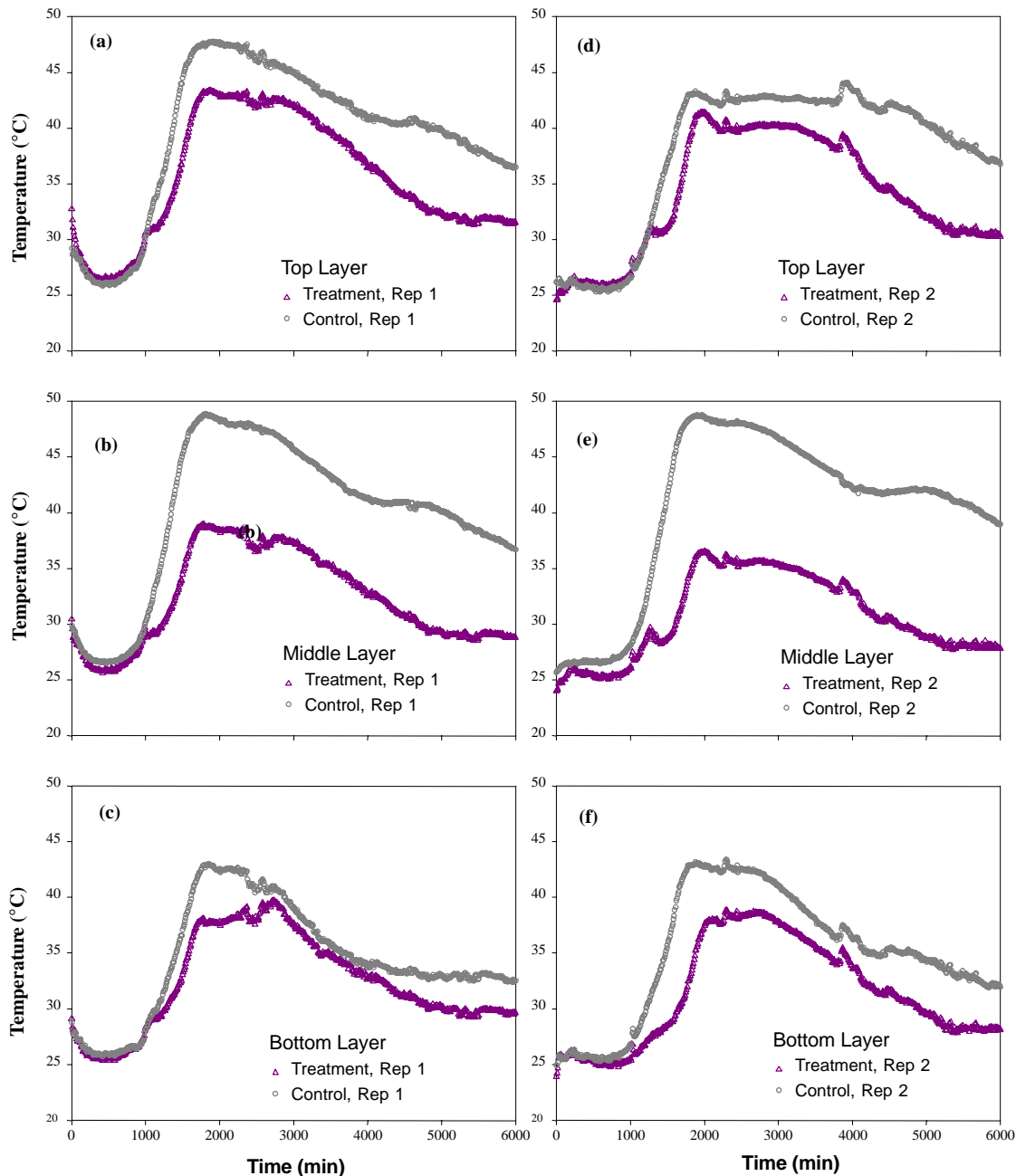
Figure 5. Temperature profiles for treatment 1. Profiles are shown for the top, middle, and bottom layers of the treatment replications and their respective controls.

bottom layers of the treatment and control reactors. Clear differences can be seen in the temperature levels reached between the treatment and the control. The treatment reactors showed reduced peak temperatures and lower temperatures throughout the entire cultivation, especially in the middle layer. Similar temperature profiles were demonstrated by treatments 2 and 3.

PEAK TEMPERATURE

A statistical analysis was completed to determine whether or not differences in peak temperature existed between the treatments and the controls within the three substrate layers. The results from the analysis are summarized in table 1. The treatments reduced the peak temperatures observed in the bed in comparison to normal operation (ΔT was negative for all

cases). For treatment 1, significant reductions were observed throughout the substrate bed. However, for treatments 2 and 3, the statistically significant reductions were limited to the top and middle of the bed.

Statistical comparisons between treatments, within the three individual layers were completed (table 1). The temperature-based control of treatment 1 was more effective overall than the timed control of treatments 2 and 3 in reducing bed temperatures, especially in the middle of the substrate bed. In the top substrate layer, temperature-based control was not significantly different than timed control. Within timed control methods, a time delay in the control application (treatment 3) did not reduce substrate temperatures as effectively as timed control beginning at the start of cultivation (treatment 2).

Table 1. Summary of the statistical analysis completed on the average peak temperatures observed for the top, middle, and bottom layers of the treatment and control cultivation runs.

|	Treatment 1 Average Peak Temperature (°C)			Treatment 2 Average Peak Temperature (°C)			Treatment 3 Average Peak Temperature (°C)		
	Treatment	Control	ΔT	Treatment	Control	ΔT	Treatment	Control	ΔT
Top	42.4 a,c	45.9	-3.5**	40.0 a	45.7	-5.7***	44.4 b,c	47.2	-2.8*
Middle	37.7 a	48.7	-11***	40.4 b	48.1	-7.7***	41.4 b,c	46.5	-5.1***
Bottom	39.3 a	43.2	-3.9**	42.1 b	43.6	-1.5	40.3 a,b	41.2	-0.9

The following subscripts indicate the significance level of the differences in peak temperature between the treatment and control within the specified layer:
* $P < 0.1$
** $P < 0.05$
*** $P < 0.01$

Comparisons of peak temperature were made between treatments, within the individual layers. Reading the table horizontally (within a single layer), values followed by the letters a, b, or c indicate peak temperatures that were not statistically different.
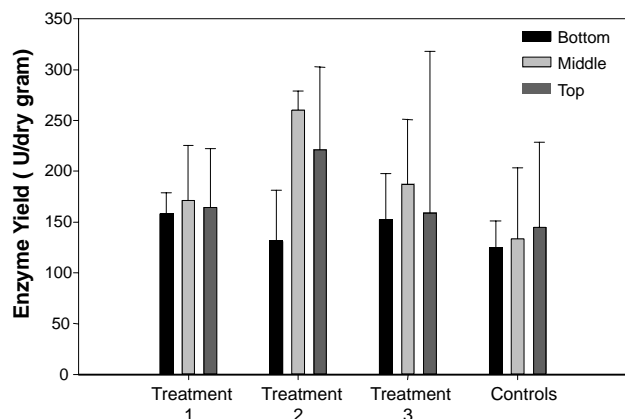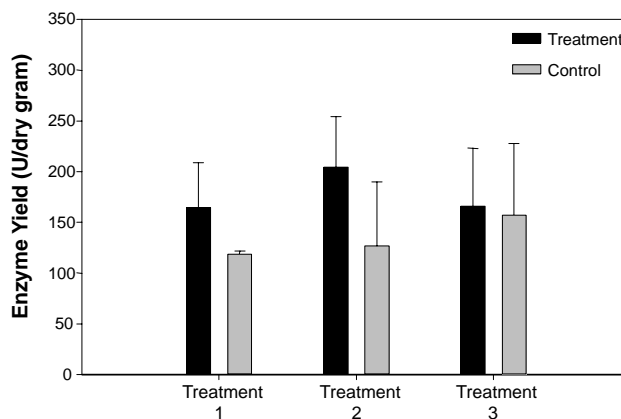

Figure 6. Mean xylanase yields for the top, middle, and bottom layers of the substrate bed for each treatment and all controls.

Figure 7. Mean xylanase yields for the entire substrate bed of each treatment and their respective controls.

ENZYME ACTIVITY

The enzyme yields for the bottom, middle, and top layers for the treatments and controls are provided in figure 6. The bottom of the bed produced the least amount of xylanase, on the average, and the middle portion of the substrate bed produced the most activity. The average xylanase yields for the entire substrate bed were determined for the treatments and their respective controls and are provided in figure 7. The differences in enzyme activity between treatment and its control were not statistically significant in any of the layers for treatments 1 and 3. The comparisons made between treatment 2 and the respective controls within the three layers showed differences in enzyme yield for the middle layer (P-value 0.022). The mean xylanase activities observed between treatments within the top, middle, and bottom layers were also not statistically significant.

NET RATE OF METABOLIC HEAT ACCUMULATION

The net rates of metabolic heat accumulation before (stage 1) and after (stage 2) the peak temperature for the treatments and controls by layers are shown in table 2. The large temperature changes observed within the fluctuations of the middle layer temperature profile for treatments 2 and 3 were not considered in the determination of the net rate of heat accumulation response variable. In order to make more meaningful comparisons between treatments and controls with respect to the middle layer, the data points selected before and after the peak temperature for the timed control treatments were part of the apparent higher temperature profile. The rate (slope) determined from these data was representative of the

effective reduction and control of the two treatments. Comparisons between treatments and their respective controls were made within layers for the net rate of heat accumulation response variable before and after the peak temperature. The differences in heat accumulation rate before the peak temperature for treatment 1 and the respective controls were statistically significant in the middle layer (P-value 0.008). The stage 1 heat accumulation rate in the middle of the substrate for treatments 2 and 3 were significantly reduced in comparison to normal operation (P-values 0.002 and 0.002, respectively). The reductions in the stage 1 net rate of heat accumulation in the middle layer were consistent with the lower peak temperatures observed. The occurrence of significant differences in only the middle layer may also have been a result of the limitations in the design of the cooling water coil and the available surface area.

The stage 2 net rate of metabolic heat accumulation response variable in the substrate bed was a combined effect of slowed microbial activity and the cooling water treatment. As a result, the net rate of heat accumulation in this second stage was negative. The differences in the rate of heat accumulation following the peak temperature for treatment 1 and the respective controls were not statistically significant in the top, middle, or bottom layers (P-values 0.591, 0.857, and 0.591, respectively). For treatment 3, the stage 2 rates of heat accumulation in the top, middle, and bottom layers were also not significantly different between the treatment and respective controls (P-values 1.0, 0.86, and 0.86, respectively). The stage 2 rate of heat accumulation in the top layer of treatment 2 was significantly smaller than that of the respective control (P-value 0.0017). However, the middle and bottom

Table 2. Net rate of metabolic heat accumulation determined before the peak temperature (stage 1) and after the peak temperature (stage 2) for the top, middle, and bottom layers of the treatment and control cultivation runs.

| | | Net Rate of Metabolic Heat Accumulation, Stage 1 / Stage 2 (°C/min) | | | | | |
| | | Treatment | | | Control | | |
Treatment	Replicate	Top	Middle	Bottom	Top	Middle	Bottom
1	1	0.026 / -0.005	0.022 / -0.004	0.019 / -0.005	0.028 / -0.004	0.031 / -0.005	0.023 / -0.006
1	2	0.030 / -0.005	0.020 / -0.004	0.022 / -0.004	0.023 / -0.003	0.031 / -0.004	0.026 / -0.006
2	1	0.020 / -0.004	0.018 / -0.003	0.023 / -0.005	0.024 / -0.002	0.028 / -0.004	0.027 / -0.005
2	2	0.024 / -0.019	0.019 / -0.004	0.018 / -0.004	0.029 / -0.004	0.033 / -0.004	0.021 / -0.006
3	1	0.029 / -0.008	0.021 / -0.008	0.017 / -0.007	0.031 / -0.008	0.025 / -0.008	0.019 / -0.005
3	2	0.025 / -0.005	0.015 / -0.006	0.016 / -0.006	0.025 / -0.005	0.035 / -0.005	0.024 / -0.007

layers for treatment 2 did not show significant differences in comparison to the respective controls. The lack of differences between the treatments and normal operation with respect to the stage 2 net rate of heat accumulation indicated that the treatments did not have a significant influence on how quickly the substrate bed temperature decreased in this second stage.

Statistical comparisons between treatments for the net rate of metabolic heat accumulation for stage 1 and stage 2 were also completed within layers. The net heat accumulation rate in stage 1 was significantly smaller (P-value 0.091) for treatment 2 than for treatment 1 in the top layer (0.022°C/min and 0.028°C/min, respectively). A significant difference in the net rate of metabolic heat accumulation in stage 2 between treatments 2 and 3 was also determined for the top layer (P-value 0.085).

Treatments 2 and 3 introduced temperature fluctuations in the substrate as a result of the timed control of the cooling water. Although some researchers have found these fluctuations to be undesirable during cultivation, these fluctuations were not detrimental to product formation. Tao et al. (1997) altered the thermal environment of a packed-bed reactor by oscillating the inlet air temperature between 30°C and 50°C for an oscillating period of 1 h. The bed height utilized was 20 mm in order to maintain a homogenous growth environment. Their studies assessed the influence of temperature oscillations on cellulase formation by *Trichoderma viride* SL1 in solid substrate cultivation. Temperatures were varied both during the active growth phase and throughout the fermentation time. The study found that the oscillations had no adverse effects on cellulase productivity.

The net rate of metabolic heat accumulation for the timed heat exchanger operation treatments showed an apparent slowing of heat accumulation during stage 1 compared to the control treatments (normal operation). Closer investigation revealed that the apparent slowing of heat accumulation was the combined effect of reductions in bed temperature during heat exchanger operation and increases in bed temperature when cooling water was not flowing through the heat exchanger (fig. 4). Therefore, the apparent net rate of metabolic heat accumulation of approximately 0.02°C/min in stage 1 and -0.007°C/min in stage 2 did not reflect the instantaneous rate of temperature change during the cooling water operation. Operation of the cooling water introduced a net decrease in heat accumulation at an average rate of approximately 0.5°C/min. The 10 min time interval recommended by Ashley et al. (1999) through evaluation of the characteristic heating time of *A. niger* on a starchy substrate was used as a starting point. This may need to be adjusted depending on the substrate, organism, and reactor system of interest.

In comparison to the temperature-based control strategy, however, the timed control treatments were not as effective in minimizing the temperatures reached in the substrate bed. Further studies should assess the use of different cooling water temperatures, flow rates, and feedback control strategies on reactor performance.

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