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Pressure Pulsation in Solid-Phase Fermentation

Xiaomin Yang, Tom Huang, and George T. Tsao*

School of Chemical Engineering, Purdue University,
West Lafayette, IN 47907,
E-mail: tsaogt@ecn.purdue.edu

Abstract

Inadequate dissipation of heat generated by biologic activities has prevented the use of solid-phase fermentation in large-scale applications. This study deals with the cooling effects of pressure pulsation on solid, porous beds. Pressure pulsation also enhances evaporation of medium moisture, which is also described. Computer software has been developed for on-line control of heat accumulation and moisture loss involving automatic variation in pressure pulsation frequency and airflow direction as well as replenishment of water. *Aspergillus niger* NRRL3 was cultivated on a moist, solid medium made of wheat bran and ground corncobs to produce cellobiase. During 100 h of fermentation, the maximum temperature inside the solid bed was kept under 40°C, and the medium water content was successfully maintained between 61 and 65%, which was optimal for cell growth. Cells grew heavily on the solid-phase substrate and distributed uniformly. With good on-line control of temperature and moisture, the 12-L fermentor provided a better environment for enzyme production than 250 mL flasks did.

Index Entries: Pressure pulsation; solid-phase fermentation; *Aspergillus niger*; cellobiase.

Introduction

Solid-phase fermentation involves the growth of microbes on moist, solid substrate in the absence of free-flowing liquid *(1)*. It has been investigated for diverse new products including hydrolases, antibiotics, hormones, organic acids, ethanol, and single-cell protein *(2)*. In flasks, solid-phase fermentation is much more efficient in the production of enzymes and bio-active products than liquid submerged fermentation (fermentation in which cells of microorganisms are submerged in liquid).

*Author to whom all correspondence and reprint requests should be addressed.

Microbial cells on solid surface absorb oxygen directly from the atmosphere without hindrance by the surrounding liquid, enhancing cell growth and other metabolic activities. In large-scale solid-phase fermentation, however, there are several technical difficulties. First, solid substrates are always piled together, forming porous beds that act as good heat insulators. Heat, generated by microbes, causes temperature to increase in porous beds, and the temperature can be high enough to kill microbes. Second, oxygen supply to living cells lodged deep in the porous beds is by slow diffusion. Moist, solid substrates and mycelia often form large aggregates owing to cell growth. Metabolism in the aggregate interior is limited by oxygen supply, also reducing the overall process performance *(3)*.

Bio-reactor designs used for solid-phase fermentation include trays, packed beds, rotating drums, stirred reactors, and air-solid fluidized beds. The trays are the oldest and simplest type *(4)*. There are also newer versions with modern automation and improved air circulation. Generally, overheating and lack of oxygen in the bed interior limit the size and depth of individual trays. Packed beds are another popular design *(5)*. Forced flow of moist but unsaturated air is a common method to cool porous beds by water evaporation. For a large-scale porous bed, forced airflow will result in channeling and nonuniform distribution. Under some conditions, the temperature in the middle of the bed can be 20°C higher than the temperature of the inlet air *(6)*. A significant amount of moisture can also be carried out of the fermentor by aeration during fermentation *(7)*. The low water content reduces cell growth and production of desired product.

Pressure pulsation was explored for enzyme production in a 2-L vessel *(3)*. Porous packed beds of moist, solid substrates in an enclosed cultivation chamber were subjected to periodic pressurization and depressurization. The pressure pulsation was generated by the action of an on/off valve placed on the gas exit line of the cultivation chamber. Convective flows were created when the chamber gas was pressurized and forced into the porous bed and also when the chamber gas was depressurized to release the gas and volatile metabolites from the porous bed. This periodic convective flow into and out of the porous beds enhanced heat and mass transport significantly. Pressure pulsation also helped loosen and prevent the formation of tight cell/substrate aggregates.

For enlarging enzyme production bioreactors and also for developing a generic method of operating solid-phase processes, systematic studies were performed to investigate the effects of pressure pulsation frequency; maximum and minimum pressure and airflow direction on heat dissipation; and moisture evaporation from fermenting moist, solid medium packed to a height of 0.19 m and a diameter of 0.24 m. Computer software has been developed to exert on-line control of the medium temperature and water content in a desired range during fermentation. Finally, production of cellobiase by *Aspergillus niger* has been used as a model system for the investigation.

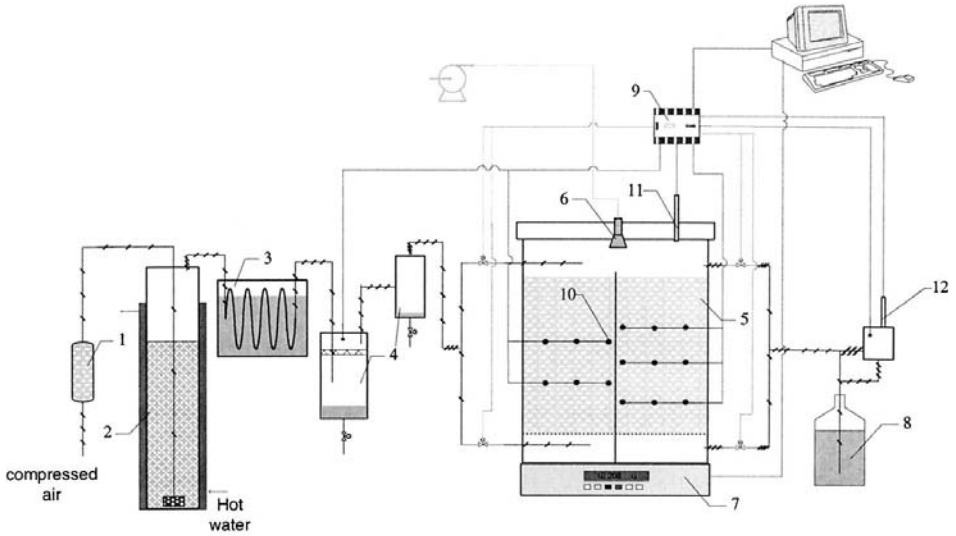

Fig. 1. Schematic diagram of experimental apparatus. 1) Air filter; 2) humidifier; 3) heat exchanger; 4) air/water separator; 5) fermentation chamber (12-L); 6) spray nozzle; 7) balance; 8) spore trap; 9) multi-function DAQ board; 10) temperature probes; 11) pressure transducer; 12)humidity sensor.

Materials and Methods

Experimental Apparatus and Software

Figure 1 is a schematic diagram of the experimental apparatus. The apparatus consisted of a humidifier, a heat exchanger, two air/water separators, a 12-L fermentation chamber, a spore trap, a bench balance, sensors, valves, pumps, and a control computer. The humidifier was a water-jacketed glass column of 12-L capacity filled to a height of 0.4 m with 10×10 mm Raschig rings and autoclaved de-ionized water. The temperature in the water jacket was 60°C. Air from the compressor was dispersed into fine bubbles through a stainless steel air stone placed at the bottom of the humidifier. The humidified warm air was first passed through a heat exchanger to be cooled down to the desired temperature and then through two air/water separators arranged in series to remove condensed water before passing into the fermentation chamber. The relative humidity of the air entering the chamber was 98 to 99%. The stainless steel fermentation chamber with a clear cover was 0.28 m in height and 0.24 m in diameter. The surface of the chamber was wrapped with insulation material to prevent heat transport from the fermentation chamber to the environment. An aluminum mesh plate placed at the bottom of the chamber served to support the 0.19-m-high solid phase medium. Autoclaved buffer was added into the chamber through a spray nozzle at the center of the cover to maintain the water content of the medium. The air from the fermentation chamber was passed through a spore trap filled with bleach to kill spores before it was released into the environment.

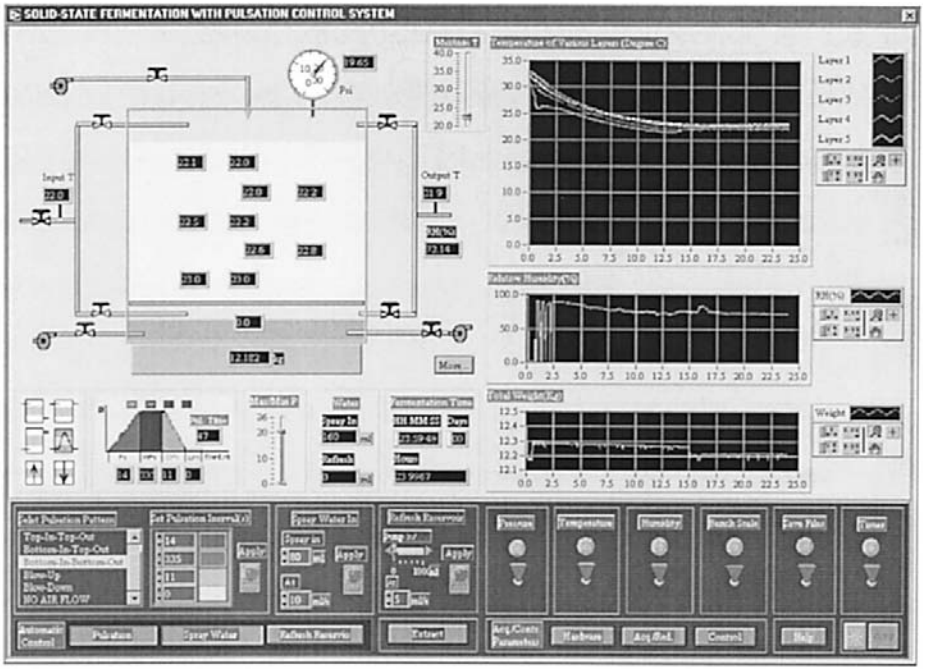

Fig. 2. Interface of control software.

A temperature probe inserted into the first air/water separator measured the input air temperature. Another temperature probe and a humidity sensor were placed in a small vessel, connected with the outlet pipe of the fermentation chamber, to measure the output air temperature and relative humidity, respectively. A set of temperature probes inserted into the chamber recorded medium temperature at various positions. A pressure transducer plugged into the chamber through the cover measured the air pressure inside the chamber. A bench balance was placed under the fermentation chamber to measure the weight change of the medium. All the measurement signals were connected with the analog channels of an National Instrument data acquisition board and serial ports to communicate with the control computer. The control computer also used the digital input/output channels on the same board to control relay switches turning on and off the valves and pumps to generate pressure pulsation, and to add water into and remove it from the chamber.

Special control software was developed to control the solid-phase fermentation system. Figure 2 shows the interface of the software consisting of display and control panels. The upper part (light area) was the display panel. Medium and air temperatures, air pressure, air relative humidity, medium weight, and valve and pump status were displayed in a schematic diagram of the fermentation chamber. Histories of the maximum temperatures in various medium layers, output air relative humidity,

and medium weight were plotted in charts, respectively. All the experimental data and control operations were saved in hard disk automatically during fermentation. The lower part of Fig. 2 (dark area) was the control panel. It provided accesses to operate all the equipment of the fermentation system such as turning on and off the measurement equipment, generating pressure pulsation with specified frequency and airflow direction, spraying water into the fermentation chamber, and extracting enzyme product when the fermentation was over. It also automatically controlled the medium temperature and water content during fermentation.

Pressure Pulsation Cycle and Direction of Aeration

A pressure pulsation cycle consisted of four intervals: pressurization, high-pressure holding, depressurization, and low-pressure holding. Average air flux was inversely proportional to the pulsation period. The direction of air passed through the solid-phase medium was changed by turning on and off valves on the inlet and exit air lines in different sequences. Four kinds of airflow directions were available in the fermentation chamber: top-in-bottom-out, bottom-in-top-out, top-in-top-out and bottom-in-bottom-out.

Solid-Phase Fermentation

A. niger NRRL 3 culture was kept on slants until ready for scale-up for experimental work. Previous work *(3)* showed that the favorable conditions for cellobiase production from solid substrate by *A. niger* NRRL3 were in the range of 60–65% (w of liquid/w of medium) water content and 30–40°C.

Corncob (1.08 kg) and wheat bran (0.72 g) were mixed with K_2HPO_4-KH_2PO_4 buffer (3.3 L, 0.5 *M*, pH 5.5). The mixture was autoclaved at 121°C for 30 min, allowed to cool to room temperature, and inoculated with *A. niger* seed medium (200 g). The contents were then mixed thoroughly and loaded into the fermentation chamber. A fermentation process always lasted 4 or 5 d.

Enzyme Activity

The procedure for estimating enzyme activity was reported elsewhere *(8)*. One unit of cellobiase activity is defined as the amount of cellobiase required to release 1 µmol of glucose/min at 50°C. Glucose and cellobiose concentrations were determined and quantified by high performance liquid chromatography (L-6200A; Hitachi, Tokyo, Japan) using a Bio-Rad Aminex HPX-87H ion-exclusion column (300 × 7.8 mm) (Bio-Rad, Hercules, CA) with a refractive index detector (L-3350, Hitachi). The column was eluted with dilute sulfuric acid (0.005 *M*) at a column temperature of 80°C and a flow rate of 0.8 mL/min over a 10-min period.

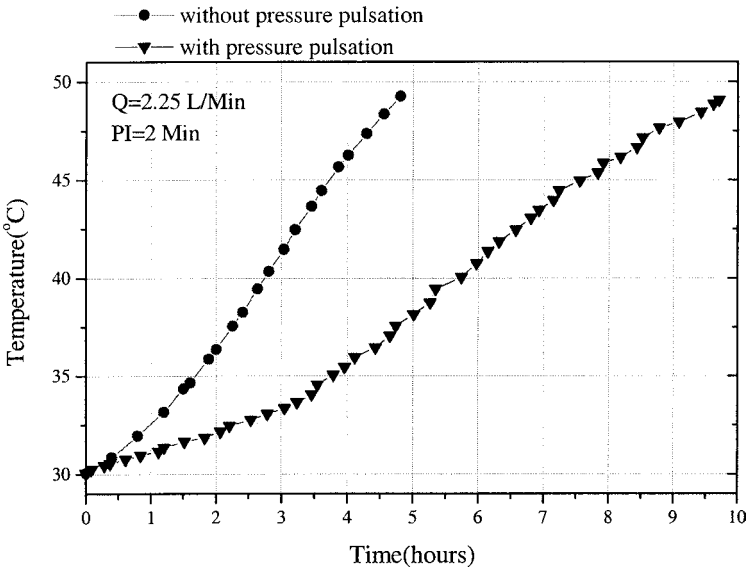

Fig. 3. Comparison of cooling effect of airflow with and without pressure pulsation. Airflow direction: top-in-bottom-out.

Results and Discussion

Comparison of Cooling Effect of Aeration With and Without Pressure Pulsation

Two experiments were conducted in the apparatus. Air flowed into the head space and then downward through the porous bed into the bottom space. The on/off action of the gas inlet and outlet valves created the pressure pulsation of the flowing gas. The gas was passed through the porous bed without pressure pulsation when both the inlet valve at the top and outlet valve at the bottom were turned on. The pressure pulsated between 0 and 15 psig with a frequency of 0.5 cycles/min. The average air flux was 2.25 L/min. The same air flux was used for the experiment without pressure pulsation.

A high level of heat was produced during the second 20 h of the fermentation. Without pressure pulsation, the temperature of the medium always rose to more than 60°C, high enough to kill the cells in a short time if the heat could not be removed from the medium. As shown in Fig. 3, the maximum temperature inside the medium increased from 30 up to 49°C in only 5.07 h when the air was passed through the porous bed without pressure pulsation. With pressure pulsation, it took 9.73 h to reach the same level. The airflow with pressure pulsation was about twice as effective in removing heat from the porous bed as without pressure pulsation.

Our other experiments showed that channels between the fermentation chamber inner surface and solid medium were formed during the fermentation. These channels significantly reduced the cooling effect of

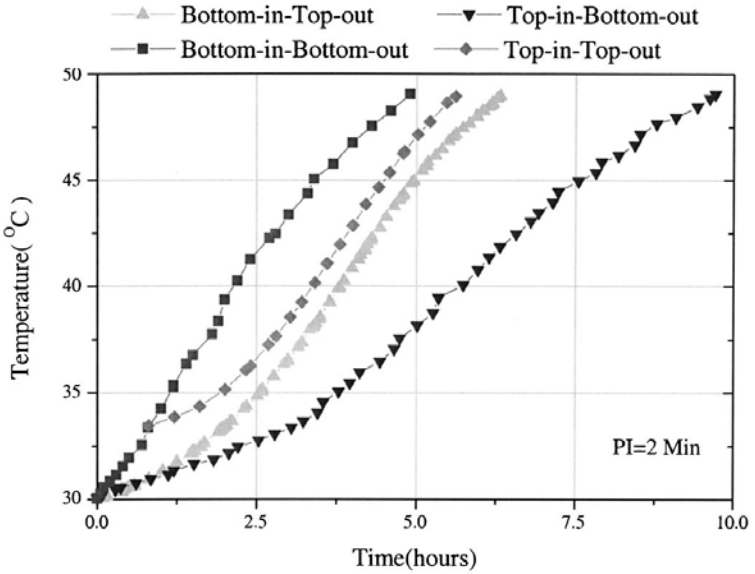

Fig. 4. Comparison of cooling effect of directions of airflow with pressure pulsation.

aeration without pressure pulsation. Little channeling problem was observed when pressure pulsation was applied to the flowing gas.

Comparison of Cooling Effect of Airflow Directions with Pressure Pulsation

A set of experiments was carried out to compare the cooling effect of the directions of airflow with pressure pulsation. The pressure pulsated also between 0 and 15 psig with a frequency of 0.5 cycles/min (see Fig. 4). The maximum temperature inside the medium increased from 30 up to 49°C in 9.73 h when airflow direction was top-in-bottom-out. However, it took only 6.32 h when air flowed in the opposite direction (bottom-in-top-out). In these experiments, the pressurization and depressurization period was 10 and 5 s, respectively, although the same amount of air was compressed into and released from the fermentation chamber. The air flux during depressurization was about twice that during pressurization. Water evaporation in depressurization also helped remove heat from the porous bed. Therefore, depressurization contributed to the heat dissipation by airflow with pressure pulsation much more than pressurization did. Head space volume of the fermentation chamber used in these experiments was about twice that of the bottom space volume. More air was passed through the porous bed during depressurization when the airflow direction was top-in-bottom-out than bottom-in-top-out. Thus, the cooling effect of airflow of top-in-bottom-out is much higher than it of bottom-in-top-out.

It took 4.94 h to reach maximum temperature when bottom-in-bottom-out was applied to the system. The flowing air moved heat down

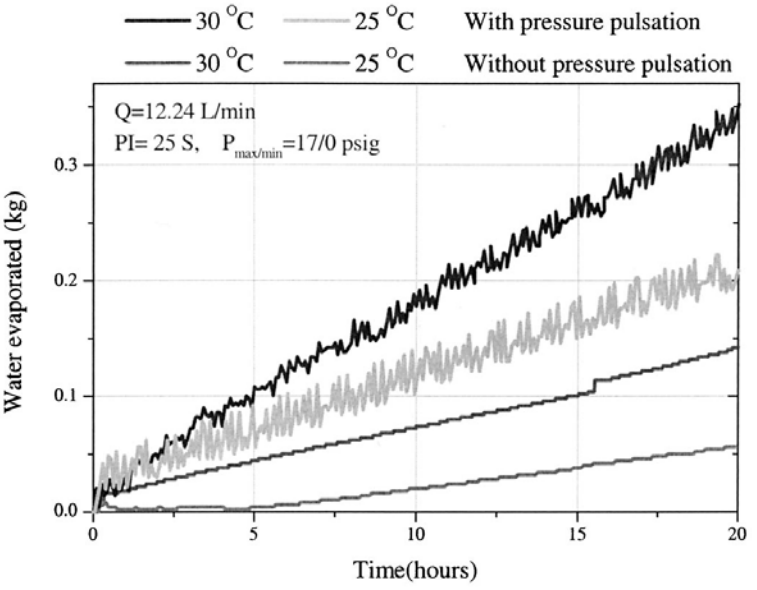

Fig. 5. Effect of temperature and airflow with pressure pulsation on evaporation of medium moisture.

and up in this case. The distribution of medium temperature was kept in a narrow range, although the maximum temperature increased in a relatively short time. Top-in-top-out also showed similar results.

On the whole, pressure pulsation, airflow directions, and void space of the head and bottom of the fermentation chamber all affected heat dissipation in the porous bed.

Effects of Temperature and Pressure on Evaporation of Medium Moisture

The medium moisture evaporated during fermentation when forced aeration was used. A copper tube was added in the fermentation chamber as a heat exchanger to keep the temperature of the medium constant. Autoclaved solid medium (4.6 kg) with 65% water content was loaded into the chamber without inoculation. Airflow with and without pressure pulsation was passed through the porous bed for 20 h (see Fig. 5) For medium at 25°C, 0.06 kg of water evaporated without pressure pulsation. Water evaporated much faster when pressure pulsation was applied to the flowing air. In the same time, 0.22 kg of water was lost.

Another simple experiment showed that the water was removed from the medium significantly in depressurization. Solid medium was filled in a 300-mL flask. The air was pressured into it to 10 psig and then released suddenly. Fog made of tiny water drops was observed inside the flask. Thus, solid medium lost moisture more significantly with pressure pulsation. Without pressure pulsation, 0.14 kg of water was lost when the

temperature was 30°C. When both higher temperature and pulsation were applied, 0.35 kg of water was removed from the solid medium. The temperature also increased the moisture loss significantly but not as much as pressure pulsation did.

On-line Control of Medium Temperature and Moisture

Airflow with pressure pulsation was effective in removing heat from the porous bed. Pressure pulsation frequency and airflow direction affected the heat-removing ability significantly. Therefore, we controlled the temperature of the medium by varying pulsation frequency and airflow direction during fermentation. Several experiments were carried out to determine control strategies. Spores of *A. niger* germinated in the first 20 h of fermentation. Only a small amount of heat was generated in this period. Therefore, top-in-top-out and bottom-in-bottom-out airflows with low pressure pulsation frequency were used. Cells grew rapidly in the second 20 h. Heat was generated quickly in this stage. This was the crucial period for temperature control. Top-in-bottom-out and bottom-in-top-out airflows with high-pressure pulsation frequency were used to cool the medium. Because aeration introduced a temperature gradient in the axial direction, airflow directions were exchanged periodically to keep the medium temperature of the medium uniform during fermentation.

Pulsation and temperature caused the medium to lose moisture during fermentation. Two techniques were used to solve this problem. In previous experiments *(8)*, enzyme production of *A. niger* did not change significantly when the water content of the medium was varied between 60 and 65%. In the present study, initial water content of the solid medium was 65%. The water content of the medium was still in a desired range even after a little water was lost by evaporation during fermentation. The second and more effective technique for controlling medium water content involved the use of a fog nozzle placed through the center of the cover of the fermentation chamber. Water was added into the chamber to keep the water content of the medium in the desired range during fermentation. The gravity and downward airflow carried the water downward into the medium although the water was sprayed on the top of the porous bed.

An experiment with automatic on-line control of temperature and water content was conducted after the basic control strategy had been developed. Solid-phase medium (5.1 kg) with 65% water content was loaded into the chamber after inoculating with seed culture (0.2 kg). The height of the medium inside the chamber was 0.19 m. The fermentation was carried out for 96 h. As shown in Figs. 6 and 7, both the maximum temperature and moisture level of the medium were kept in the favorable ranges for good *A. niger* growth during fermentation. The temperature was below 40°C even during the stage of rapid cell growth. The water content of the medium was between 61 and 65% during the entire fermentation batch.

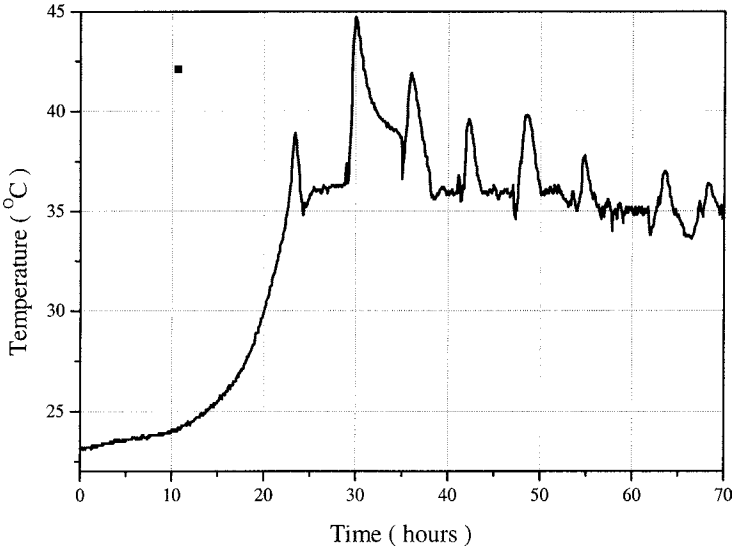

Fig. 6. Maximum temperature inside medium during fermentation with on-line control.

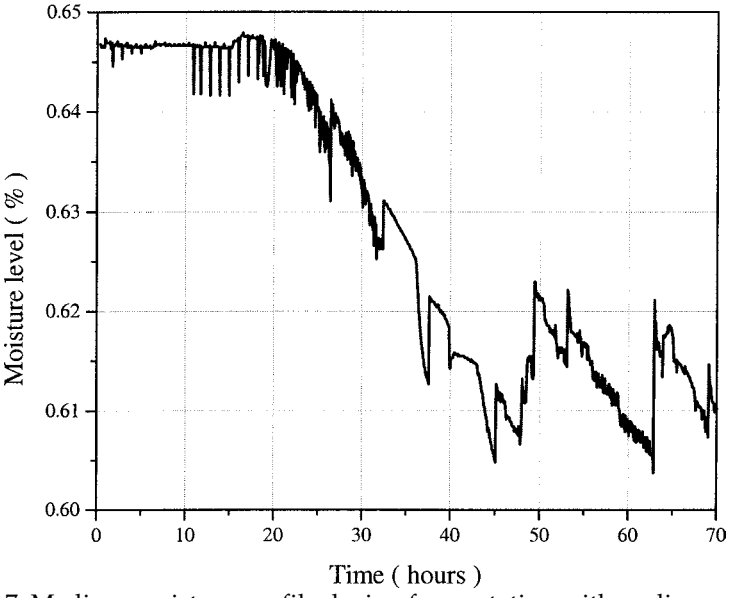

Fig. 7. Medium moisture profile during fermentation with on-line control.

Growth of A. Niger *and Cellobiase Production with Pressure Pulsation*

The previously described successful solid-phase medium control of temperature and water content supported excellent cell growth and enzyme production by *A. niger*. Without pressure pulsation, the central portion of the porous bed was tightly packed with little growth and no spore formation. Only the portions near the air inlet and the top layer had

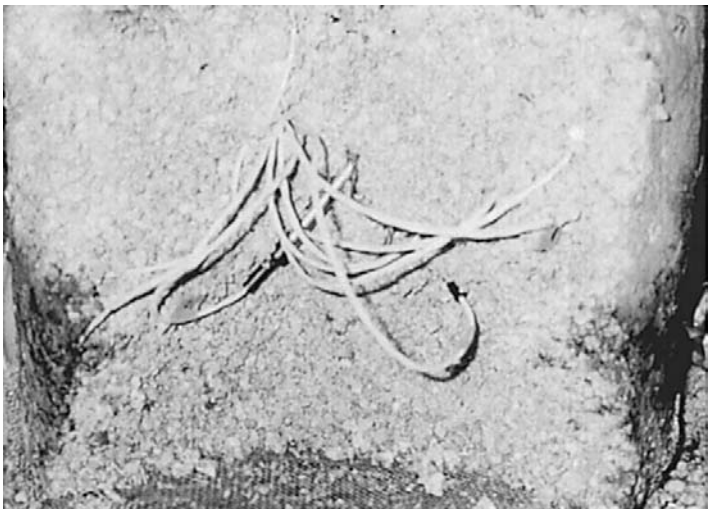

Fig. 8. Growth of *A. niger* without pressure pulsation.

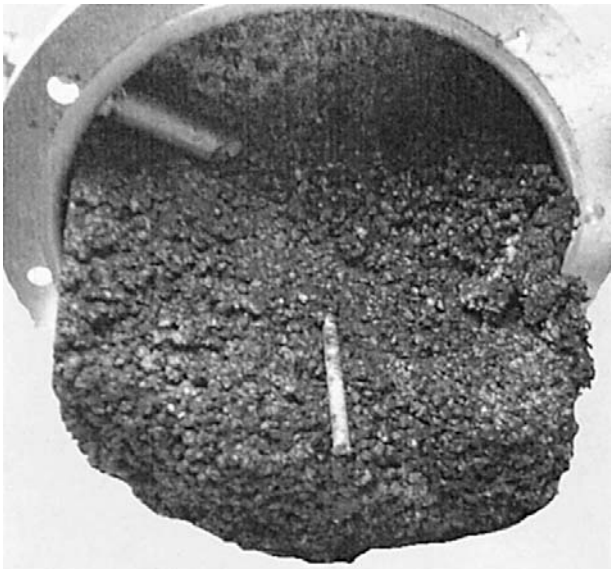

Fig. 9. Growth of *A. niger* with on-line control using pressure pulsation.

substantial white mycelial growth, and the portion next to the chamber wall had black spores, as shown in Fig. 8. With on-line control of medium temperature and water moisture, the growth was uniform and heavy throughout, and the porous bed turned totally black because of the heavy formation of the black spores by the *A. niger* culture, as shown in Fig. 9. With pressure pulsation, the whole bed was "loose," and, in fact, it was difficult to take the bed out of the chamber without portions of the bed falling off. The 12-L fermentor provided a better environment for enzyme production than 250 mL flasks did (separate publication is in preparation for the cellobiase production in solid-phase fermentation with pressure pulsation).

Conclusion

The present work showed that pressure pulsation was effective in cooling the porous bed of 0.19 m height and 0.24 m diameter. Pressure pulsation frequency, airflow direction, and head and bottom space of the fermentation chamber greatly affected heat dissipation in the porous bed. A significant amount of medium moisture evaporated when pressure pulsation was used. During 100 h of fermentation, the temperature and water content of the solid medium was kept in the desired range for the growth of *A. niger* by on-line control using pressure pulsation and replenishing water. *A. Niger* grew heavily on solid-phase substrate in the 12-L chamber.

Acknowledgement

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