

Process Calorimetry on Solid-state Fermentation of Vinegar Wastes in Bioreactor with Air Pressure Pulsation

L. Jian and J. C. Yang*

Room 480, Institute of Biochemical Engineering, Department of Chemical Engineering, Tsinghua University, Beijing, 100084, China

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Solid-state fermentation (SSF) of vinegar wastes saves environmental resources and helps to recover valuable material for lignocellulose purposes. The development of solid-state fermentation technology is very important for the production of cellulase and ultimately for utilization of natural cellulose. However, inadequate dissipation of heat generated by biological activities has prevented solid-state fermentation from large-scale applications. The thermal pattern of internal heat generation affects microbiology during the process. The paper deals with the process of a novel SSF bioreactor with air pressure pulsation. We demonstrate the impact of calorimetric approaches to solid-state fermentation in the laboratory, where heat dissipation calls for a thorough understanding. As a model system, cellulase production was carried out by solid-state fermentation using waste from the vinegar industry, as the substrate of *Trichoderma Koningii* AS3.4262. A comprehensive characterization of a lab-scale solid-state fermentation reactor was performed. In conclusion, air pressure pulsation significantly enhanced water evaporation and heat dissipation. With these tools we estimated caloric coefficients and mass to enthalpy ratios. Process calorimetry provided a reliable way to study the kinetics of solid-state fermentation.

Key words:

Bioreactor, calorimetry, heat dissipation, mass balance, solid-state fermentation, thermal conductivity

Introduction

Cellulase production is the most important step in the economical production of ethanol, single cell protein and other chemicals from renewable cellulosic materials. To date the production of cellulase has been widely studied in submerged culture processes, but the relatively high cost of enzyme production has hindered the industrial application of cellulose bioconversion. It has been reported that solid-state fermentation is an attractive process to produce cellulase economically due to its lower capital investment and lower operating expenses.¹ Another approach to reduce the cost of cellulase production is the use of lignocellulosic materials as substrates rather than expensive pure cellulose. In prior publications, abundant agricultural residue such as corn stover, wheat straw, rice straw, bagasse, etc. were used in cellulase production.^{2,3} Although these raw materials are cheaper, pretreatment is generally required to improve the utilization ratio of lignocellulosic materials and the cost is still considerable. In China, vinegar is an impure dilute solution of acetic acid obtained from foodstuff and wheat bran by fermentation beyond the alcohol

stage and used as a traditional condiment and preservative. A large amount of waste residue is produced in the vinegar industry and usually of no use. It often causes environmental pollution. It is an important issue to deal with the residue both for the comprehensive utilization of lignocellulosic resources and for the prevention of environmental pollution. Since most starch and protein in foodstuff and wheat bran have been consumed by acetobacter to produce acetic acid, the residue is porous and easy to degrade by cellulolytic fungi. In this work, residue without further pretreatment was used as a substrate in solid-state fermentation to produce cellulase.

This thesis project dealt with the development of novel SSF bioreactor with air pressure pulsation. Performance of SSF bioreactor with air pressure pulsation was studied by cultivating *Trichoderma koningii* in moist solid medium made of vinegar residue. This technology will rise up larger scale solid-state bioprocesses in future, but the biotechnological background remains poorly understood. Solid-state fermentation is operated with adiabatic materials, either in bioreactors, so that the temperature of the solid bed depends on heat production, which is related to microbial and biochemical activity and heat losses which are related to batch-size,

*Author for correspondence: Tel: +86-010-62785514,
E-mail: jianliu03@mails.tsinghua.edu.cn

evaporation of water, mass transfer to the gaseous phase and heat conductance of the reactor wall.⁴ In this study mass and heat-balances of solid-state fermentation processes are considered in detail, because process control and automation relies on appropriate measures of temperature. Difficulties of measurements during solid-state fermentation of particulate matter result from inhomogeneity. In the bioreactor developed in our lab, heat transfer by conductance, evaporation of water and transportation of the gas phase was either monitored directly by measurements including condensate volume or mass flow, or calculated from vapor pressure data and off-gas temperature.

Materials and methods

System consideration and design

Fig. 1. shows the solid-state fermentation bioreactor with pressure pulsation. The stainless steel fermentation chamber with a cover was 280 mm in height and 240 mm in diameter. A stainless steel mesh plate placed at the bottom of chamber served to support solid-state medium of 200 mm in height. The inner volume of the fermentation chamber was 12.6 L, with 9 L of solid-state medium. There were inlet ($d = 4$ mm) and outlet ($d = 10$ mm) lines at both the top and bottom of the fermentor, so that air could flow into and out of it at either position. For the convenience of presenting temperature distribution in the medium, the continuous porous bed was presented as five discrete layers, labeled as Layer One to Layer Five from top down. Temperature probes (Resistive Temperature Detectors, RTDs) were placed at the center of each layer with 40 mm in height. Five RTDs in the bioreactor were

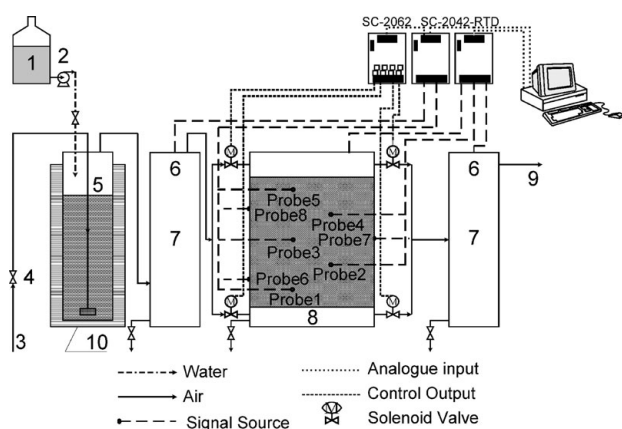


Fig. 1 – Schematic Diagram of Solid-state Fermentation Bioreactor with Pressure Pulsation

Note: 1 – Sterile water, 2 – Peristaltic pump, 3 – Air flow, 4 – Air valve, 5 – Humidifier, 6 – Humidity Sensor, 7 – Air/water separator, 8 – Fermentation Chamber (12.6 L), 9 – Air analysis, 10 – Water bath

named as Probe 1–5, according to their height (Fig. 1). All five Probes were 90 mm long. Three RTDs attached on the surface of bioreactor were named as Probe 6–8 for monitoring of the wall temperature. Through the center of the cover, a pressure transducer was installed to measure pressure in the fermentation chamber. Two temperature & humidity transducers were installed to measure the temperature and relative humidity of input and output air. All measurement output signals were connected to analogue channels of two data acquisition boards to communicate with a control computer running the control software. The control computer used digital I/O channels on the same boards to control relay switches turning on/off the valves. The On/Off actions of valves can generate six different air operation methods. Ways of air pressure pulsation are BITO (Bottom-In-Top-Out), TIBO, BIBO and TITO respectively. Two non-pulsed air flow methods were named Blow-Up and Blow-Down. A pulsation period consisted of four segments: pressurization, high-pressure holding, depressurization and low-pressure holding. Each segment had its own duration time. The air pressure pulsation period was the sum of the duration time of four segments. For example, four segments of a TITO (Top-In-Top-Out) pulsation period are top inlet on, other lets off (pressurization); all lets off (high-pressure holding); top outlet on, other lets off (depressurization) and all lets off (low-pressure holding).

Practical considerations may lead to preference for one of these operation ways. Recent researches proved that two independent strategies of operation (Top-In-Top-Out and Blow-Up) may result in representative contrast, which will be published elsewhere. So we applied both methods to a lab-scale solid-state fermentation system in this paper, which provided a comfortable platform for comparison.

Microorganism and culture media

Trichoderma koningii (AS3.4262) was from Institute of Microbe, Chinese Academy of Sciences (Beijing). The strain was activated on potato-dextrose agar containing 1.5 % agar and incubated at 30 °C for 7 days until complete sporulation. The suspension of about 10^7 spores mL^{-1} was used as an inoculum. The production of cellulase was performed in a deep trough fermentor, the thickness of the solid substrate layer was 20 cm and the room temperature was kept at 28–30 °C, air with over 90 % humidity blew through the bottom of the cultivation chamber by forced aeration.

Solid-state fermentation medium: 1500 g vinegar waste + 3000 ml water + 2 g $(\text{NH}_4)_3\text{PO}_4$ + 5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ + 1 g KH_2PO_4 , mix, and then steril-

ized under 121 °C steam for 30 min, and incubated with $w = 10\%$ amount of inoculation medium when cooled down.

Data processing was based on *Microsoft-Excel*. The calorimetric properties of the reactor were evaluated by controlled cooling experiments, which were performed on the reactor filled with pure hot water and continuously agitated by a 5 cm two-blade stirrer at $n_s = 60 \text{ min}^{-1}$. Calorimetric inertia was estimated from the amount of water and the weight of the bioreactor. Heat conductance to the surroundings was estimated within the operational temperature range of 295–297 K. Heat transfer to the gas phase and heat of phase transition were estimated according to the basic principles of physical chemistry. c_p data of materials and gases were taken from reports.^{4, 5}

Results and discussion

Heat-flow from the reactor to the surroundings by thermal conductivity

Thermal conductivity λ was calculated from cooling experiments of the reactor system that consisted of a certain amount of water and stainless steel (equation (1)). The driving temperature gradient was calculated from the temperature of surroundings (T_{ambient}) and the average temperature of the wall (T_{wall}) of the reactor. The change of heat content was estimated from temperatures of the average temperature inside the reactor (T_{int}). A stirrer minimized gradients inside the reactor, so T_{wall} and T_{int} were the same. Fig. 2 demonstrates the temperature varieties of the reactor walls. The steady increase of conductivity with increasing internal temperature was taken into account by a linear equation (equation (2)). Steady state heat flux to the surroundings, Φ_{exp} , was calculated by equation (3) finally, when the reactor was monitored with calorimeters. A comprehensive summary of conductivity data is given in Fig. 3. The ambient temperature was kept at 18 °C. From the regression process in Fig. 3, it can be deduced that $k_\lambda = 0.3781$, $\sigma = 1.3812$.

$$\sigma_{T_{\text{wall}}} = \frac{\sum_{i=1}^n c_{p,i} m_i dt}{(T_{\text{wall}} - T_{\text{ambient}}) dt}, \quad T_{\text{wall}} \cong T_{\text{int}} \quad (1)$$

$$\sigma_{T_{\text{wall}}} = f(T_{\text{wall}}) = \sigma_{T_0} + k_\lambda A_{\text{wall}} \quad (2)$$

$$\Phi_{\text{exp}} = \sigma_{T_{\text{wall}}} (T_{\text{ambient}} - T_{\text{wall}}) \quad (3)$$

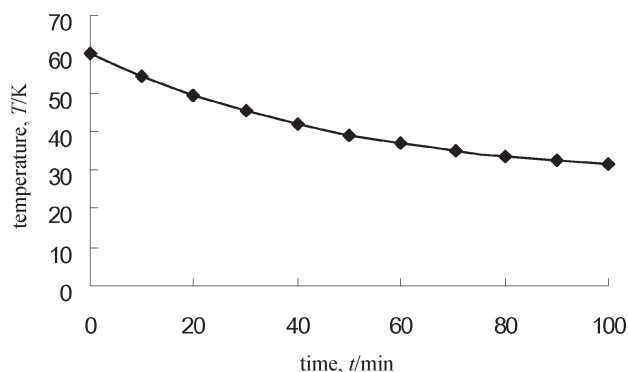


Fig. 2 – Decreasing temperature of the bioreactor filled with water. A stirrer minimized gradients inside the reactor, so T_{wall} and T_{int} were the same.

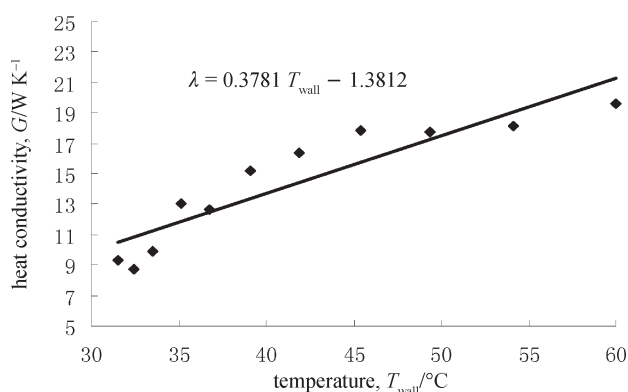


Fig. 3 – Heat conductivity plots from experiments. Regression curves were used to parameterize the linear equation on conductivity.

Models development of heat conductivity

Heat exchange between the bioreactor and its surroundings resulted from conductance as well as from heat transport by the air supply, and phase transitions like evaporation of water. Gas supply contributed to heat transfer by temperature changes of the gas phase and evaporation of water. Mass flow data were recorded as volume per time under standard conditions (273 K and 101.3 kPa). The initial gas phase (filtered outdoor air) contained 21 % O_2 , 78 % N_2 and some trace gases. Gas composition was sometimes subject to modification for random reasons. The off-gas components depend on the biological activity of the solid material and temperature. Heat transfer to the gas phase was calculated on the input gas composition according to a simplified form of equation (4). Values of heat capacities of the gaseous components are $29.12 \text{ J}\cdot\text{mol}^{-1} \text{ K}^{-1}$ (N_2) and $29.35 \text{ J}\cdot\text{mol}^{-1} \text{ K}^{-1}$ (O_2) respectively, at $T = 298.15 \text{ K}$.⁶

$$\Phi_{\text{gas}} = Q_{\text{gas}} \sum_{i=1}^n n_{\text{gas}} C_{m,p,\text{gas}_i} \cdot (T_{\text{supply-gas}} - T_{\text{off-gas}}) \quad (4)$$

Air temperature and humidity increased while the air passed through the porous bed. Heat removed by airflow with pressure pulsation consisted of two parts: convective and evaporative heat removals which were calculated using the following equations, respectively.

Relative humidity (φ) was defined as:

$$\varphi = p_w/p_{w,\text{sat}} \quad (5)$$

Pressure of saturated steam was calculated by Antoine equation:

$$p_w^{\text{sat}} = 133.322 \exp \left[18.3036 - \frac{3816.44}{(T + 273.15) - 46.13} \right] \quad (6)$$

Humidity of air was deduced from equation (7):

$$m = \frac{pV}{R(T + 273.15)} \cdot M_{\text{H}_2\text{O}} \quad (7)$$

Reorganizing equations (5) to (7), the relation of moisture of air (m), temperature (T), relative humidity (φ) and volume of air (5) was expressed by equation (8):

$$m = 2.4V \cdot \frac{\exp \left[18.3036 - \frac{3816.44}{(T + 273.15) - 46.13} \right]}{R(T + 273.15)} \cdot \varphi \quad (8)$$

Relative humidity (φ) and temperature (T) of air through inlet and outlet were acquired per second by measurement and data acquisition system and the flux of air was also known. Thus, moisture content of inlet and outlet air can be calculated by equation (8). Based on the difference between the moisture of outlet and inlet air per second, we can use the following formula to calculate total water evaporized from the packed bed during a certain period:

$$m_{\text{evaporation}} = \Sigma(m_{\text{out}} - m_{\text{in}}) \quad (9)$$

Heat transfer due to evaporation was calculated according to eq. (10).

$$\Phi_{\text{evap}} = n_{\text{evap}} \{ \Delta_{\text{evap}} H_{\text{H}_2\text{O}_{100}} + (C_{m, p_{\text{H}_2\text{O}_g}} - C_{m, p_{\text{H}_2\text{O}_1}}) (T_{\text{off-gas}} - 100) \} \quad (10)$$

The enthalpy of evaporation of water is $\Delta_{\text{evap}} H_{100} = 40.66 \text{ kJ} \cdot \text{mol}^{-1}$ and heat capacities of liquid water and water vapor are $C_{p_{\text{H}_2\text{O}_1}} = 75.24 \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$ and $C_{p_{\text{H}_2\text{O}_g}} = 33.73 \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$ at constant pressure, respectively. ⁷

Dynamic calorimetry requires corrections for characteristics which result from heat capacity of

the sample and inertia of the instrument (equation (11)), which is characterized by heat flux to the surroundings that correlates with a driving temperature difference ΔT . Quantities of heat are not dissipated to the surroundings but converted into alterations of the temperature. In general, we restricted corrections to a term concerning inertia of the instrument. For experimental reasons, we also considered a linear decrease of the amount of solid-state fermentation sample from t_0 to t_{end} . Specific heat capacities of instrument steel and sample are $c_{p_{\text{instr}}} = 1.0 \text{ J} \cdot \text{g}^{-1} \cdot \text{K}^{-1}$ and $c_{p_{\text{sample}}} = 0.8 \text{ J} \cdot \text{g}^{-1} \cdot \text{K}^{-1}$.

$$\Phi_{\text{dyn}} = -(m_{\text{instr}} c_{p_{\text{instr}}} + m_{\text{sample}} c_{p_{\text{sample}}}) \frac{dT_{\text{instr}}}{dt} \quad (11)$$

With previous equations we were able to calculate heat fluxes within certain precision and more interestingly we calculated total heat dissipation (equation (12)).

$$\Delta_r H = \int_0^{\text{end}} (\Phi_{\text{cond}} + \Phi_{\text{evap}} + \Phi_{\text{gas}} + \Phi_{\text{dyn}}) dt \quad (12)$$

Effect of air pressure pulsation on temperature homogeneity

When two aeration methods (Blow-Up and TITO) were applied, during 72 h of fermentation time, the control software recorded temperature change on Layer One through Layer Five (20 mm, 60 mm, 100 mm, 140 mm, 180 mm in height, respectively). As shown in Fig. 4, there was a little difference between the layers in the first 12 hrs. But as time went by, fast growth of fungi led to lots of heat generation. For Blow-Up, highest peak temperature in packed bed bioreactor reached 49.5 °C (Probe 5) at 28th h. Lowest peak temperature was 36.6 °C (Probe 1) at 36th h, which made 13.1 °C temperature difference. Considering from either

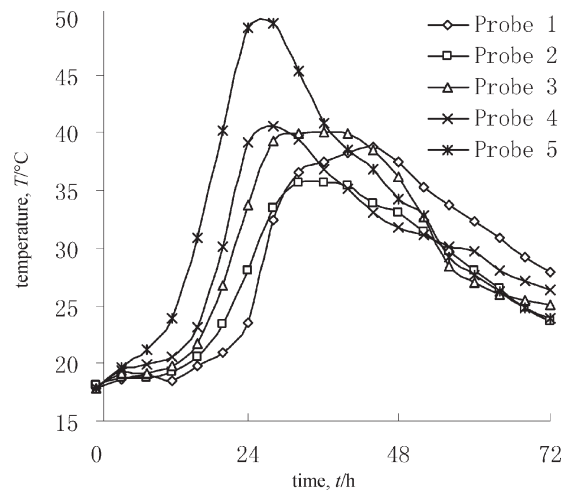


Fig. 4 – Temperature profile without pressure pulsation (Blow-Up)

highest temperature in the bed or large temperature gradient, Blow-Up (non-pulsed) could not create a homogeneous bed for fermentation.

For TITO, in 24 h: Temperatures in the bed were increasing from bottom up (Fig. 5). Highest peak temperature in the bed was 34.64 °C (Probe 3) at 32th h. The lowest peak temperature was 30.24 °C (Probe 5) at 40th h. Compared with Blow-Up, temperature gradient was much smaller, only 4.4 °C. The results showed that air pressure pulsation was a better regime for homogeneity.

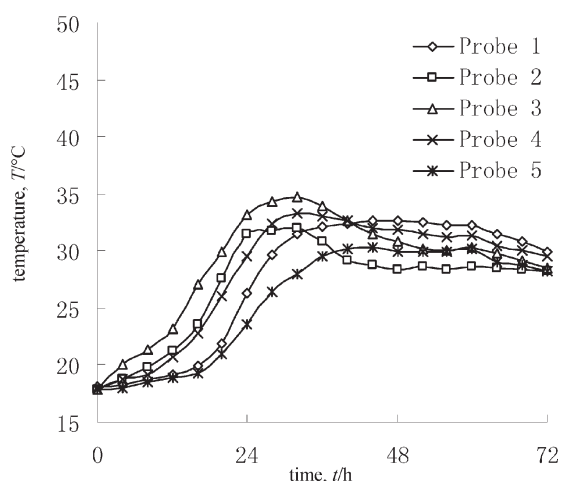


Fig. 5 – Temperature profile with pressure pulsation (TITO)

There is experimental evidence that the temperature in the whole substrate volume is equal, optimal for the definite culture. It is also necessary to prove that air pressure pulsation is a better regime for homogeneity.

Calorimetry on fermentation

During fermentation the ambient temperature was kept at 18 °C. Effect of air pressure pulsation on heat dissipation was studied ((a) and (b) in Fig. 6, Fig. 7 and Fig. 8). Fig. 6 show average wall temperature versus time pattern of two solid-state fermentation processes, on which the conduction heat was calculated. The calorimetric analysis (Fig. 7) clearly shows the power of heat flow in the total system during the process. Heat production by respiring fungi was the reason of the temperatures enhancement. Although the total average velocities of air flow in the two operating modes were the same, more gas enthalpy was available under pressure pulsation. Gradients of humidity, temperature and biodegradability along the axial dimension of the solid bed are not considered further here. Total water loss in solid medium was 479.98 g in 72 h of fermentation with air pressure pulsation, whereas

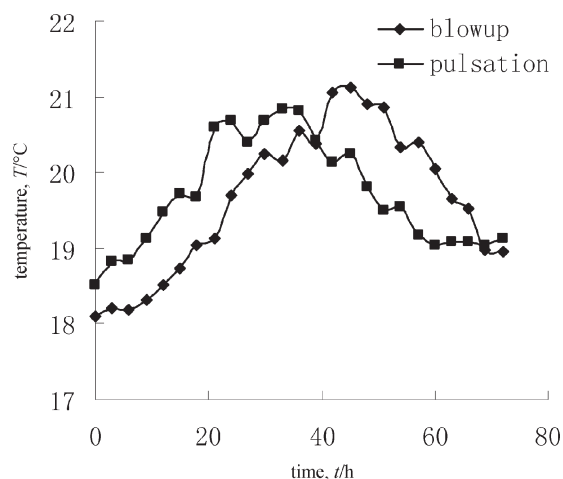


Fig. 6 – Average wall temperature versus solid-state fermentation time in the reactor

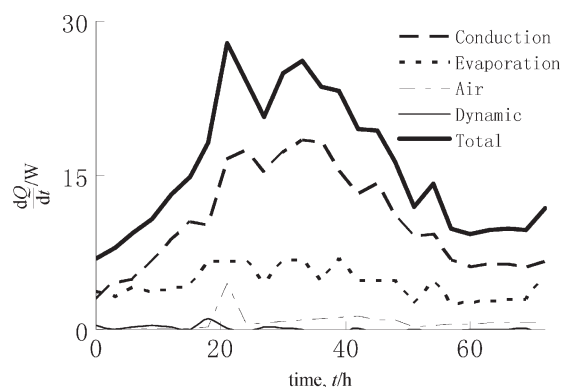
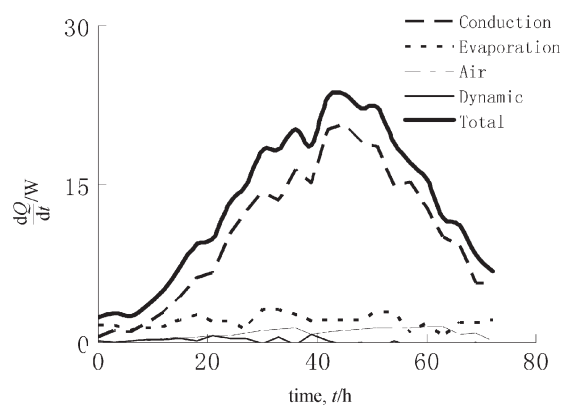


Fig. 7 – Power of the heat flow in the total system. Calorimetric data from measurements was calculated. (5-a) and (5-b) represent two processes of without \with air pulsation, respectively.

the evaporation amount was only 207.96 g under Blow-Up with same average air flow rate ($Q = 15 \text{ L min}^{-1}$). Pressure pulsation accelerated the evaporation process in moist solid substrate, with about 272.02 g more water vaporized than its absence, which was equivalent to 657.09 kJ more of heat dissipation (the latent heat of water is 2.414 kJ g^{-1}). Experimental result showed that water evaporation

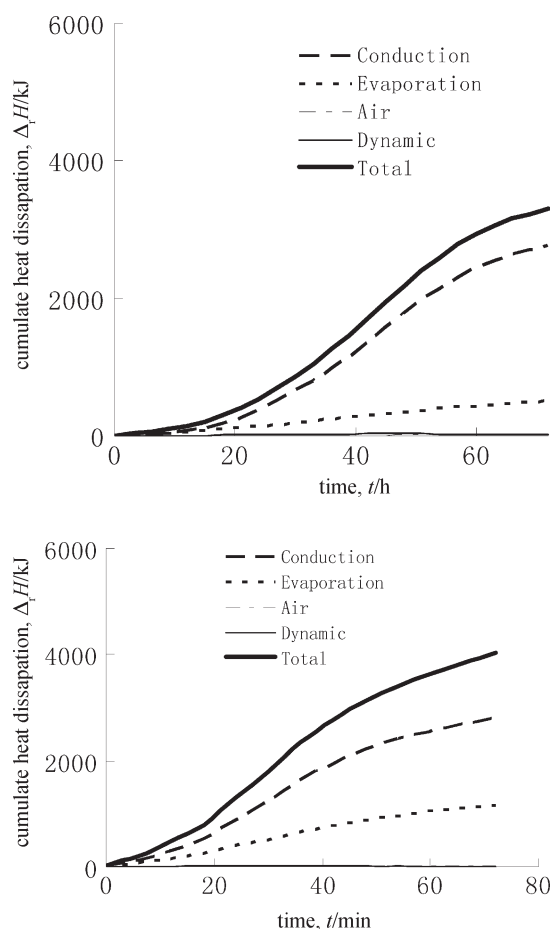


Fig. 8 – Integrated values of $\Delta_r H$ from calorimetric data. (8-a) and (8-b) represent two processes of without and with air pulsation, respectively

was enhanced. It was reported that evaporation could be accelerated in the environment with a temporal variation of air pressure.^{8,9} We can infer that in the segment of depressurization, there was more water on cellulose turning into vapor under pressure pulsation than no pulsation, resulting in more heat dissipation. Air pressure pulsation would significantly increase water evaporation, so that average temperature of the bed decreased under the effect.

Total enthalpy of reaction is summarized in Fig. 8, in which different cumulated heat of conformed the equivalence of calorimetric approaches are shown. It became evident that heat losses of more than 60 % due to conduction predominated in the air pulsation system, and evaporation of water contributed to nearly 40 %. But when it came to the no air pulsation system, heat loss of about 90 % was due to conduction, and other ways of heat losses can be ignored. Heat balances reflected dynamics and transient states during the process, which resulted from the changing metabolism of microorganisms.

The results clearly showed direct calorimetry in the laboratory systems. Thermoanalytical data

could be useful in integrating quantitative information coming from fermentation process, giving an indication of the metabolization. The composition of the vinegar residue bed, in terms of easily degradable compounds, such as dissoluble carbohydrates, and also some protein, affected the typical pattern of heat generation. The dissoluble carbohydrate and protein increased heat evolution in general, and noncrystalline cellulase, a moderately recalcitrant carbohydrate, additionally yielded delayed strong heat dissipation after more than 24 h of incubation (Fig. 7). As expected, on highly degradation processes the caloric coefficients from our experiments were in the narrow range of 30–36 h. The dynamics of the process demonstrate the demand for powerful technical devices in practice. In this stainless steel bioreactor, especially without air pulsation, the heat conduction through the wall is dominant. But when it comes to scale up, the ratio of surface to volume will be reduced, which may lead to reduced heat conduction.

Conclusions

In this study the natural material within a solid-state reactor is considered as a biologically active bed, so a series of process models were employed in this case to explain our results. Here the reactor with air pressure pulsation system has been improved with the aid of powerful measuring and control devices and sufficiently designed air supplies. *Trichoderma Koningii* was chosen since filamentous fungi in general are of considerable interest to workers in SSF. We focused on reactor models that comprise heat transfer of the vinegar residue bed reactor. From these experiments, we could infer that air pressure pulsation had a greater heat-carrying capacity, which took out more heat generated by bioactivities than without pressure pulsation. Thermal parameters such as temperature, heat conductivity, heat dissipation and relative humidity are used to explain details of the enthalpic characterizations of the process, and it was proved that air pressure pulsation is better regime for homogeneity. The air pressure pulsation enhanced water evaporation and much metabolizing heat was taken away, and that may contributed to heat transfer, which made a better temperature environment for fungi growth. Calorimetric data and thermometry could be available on-line at less cost so we recommend the process with air pulsation, whenever bioreactors are employed. Calorimetric evaluation could enhance the methodology of process monitoring. We aimed to conduct SSF at a scale where transport phenomena would be important, and in such way that we would be able to quantify SSF

performance. Therefore, we showed that thermal analysis can be used as reliable and suitable techniques for the assessment of the fermentation of the vinegar waste. Besides, thermal analysis proves to be a very simple and reproducible technique, which could be applied to other fermentations and composting.

ACKNOWLEDGEMENTS

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Nomenclature

A	– surface, mm ²
Φ	– heat dissipation, J s ⁻¹
G	– heat conductivity, J s ⁻¹ K ⁻¹
G	– Φ/T
λ	– λ grad T , W K ⁻¹ m ⁻¹
d	– diameter, mm
c_p	– specific heat capacity, J g ⁻¹ K ⁻¹
ambient	– conditions in the surroundings
c_m	– molar heat capacity, J mol ⁻¹ K ⁻¹
exp	– Experimentally estimated
$\Delta_r H$	– total heat dissipation, kJ
i	– incrementing index
int	– conditions internally on average
k_λ	– temperature-dependent coefficient of conductivity, W m ⁻² K ⁻¹
m	– mass, g
M	– molar mass of gases, g mol ⁻¹
M_{H_2O}	– molar mass of water, h mol ⁻¹
n	– amount of substance, mol
n_s	– stirring speed, min ⁻¹

p	– pressure, Pa
p_w	– partial pressure of vapor, Pa
p_w^{sat}	– pressure of saturated steam in a certain temperature, Pa
p_{std}	– standard atmospheric pressure, 101.3 kPa
φ	– relative humidity, %
SSF	– solid-state fermentation
T	– temperature, K
Q_{gas}	– supply flow rate of aeration, L s ⁻¹
V	– volume of air, L
wall	– conditions at the wall of a reactor
w	– mass fraction

References

1. Pandey, A, Soccol, C. R., Mitchell, D., *Process. Biochem.* **35** (2000) 1153.
2. Lechner, B. E., Papinutti, V. L., *Process. Biochem.* **41** (2006) 594.
3. Botella, C., Ory, I. de, Webb, C., Cantero, D., Blandin, o A., *Biochem. Eng. J.* **26** (2005) 100.
4. Weppen, P., *Biomass and Bioenergy.* **21** (2001) 289.
5. Bouguerra, A., Alt-Mokhtar, A., Amiri, O., Diop, M. B., *International Communications in Heat and Mass Transfer.* **28** (2001) 1065.
6. Mitchell, D. A., Krieger, N., Stuart, D. M., Pandey, A., *Process Biochem.* **35** (2000) 1211.
7. Lourenco, M. J. V., Santos, F. J. V., Ramires, M. L. V., Nieto de Castro, C. A., *The Journal of Chemical Thermodynamics* **38** (2006) 970.
8. Chen, H. Z., Xu, J., Li, Z. H., *Biochem. Eng. J.* **23** (2005) 117.
9. Sheu, W. J., Liou, N. C., *International Journal of Heat and Mass Transfer* **42** (1999) 4043.