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Zymotis, A Large Scale Solid State Fermenter

Design and Evaluation

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ABSTRACT

A novel design of a large scale solid state fermenter, designated as *Zymotis*—the condensed term based on Greek word "*Zymothiras*," which means the fermenter, offers efficient control of various fermentation parameters such as temperature, moisture, and aeration of the fermenting moist solids. A large quantity of metabolic heat can be easily removed by the novel cooling system employed. The unit can be operated at different capacities simply by adding or removing the compartments. Its evaluation at different capacities for cellulase production by *Trichoderma harzianum* gave similar performance as in the parallel fermentation under optimized parameters in laboratory scale column fermenter of high efficiency. The design is entirely different from all the known fermenter designs and is of potential promise in facilitating scale up of solid state fermentation for leading to industrial exploitation and harvesting numerous socioeconomic advantages of the system.

Index Entries: *Zymotis*; large scale solid state fermenter; fermenter design; column fermenter; scale up; performance evaluation; cellulase production; *Trichoderma harzianum*.

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INTRODUCTION

Solid state fermentation (SSF), the technique involving the use of moist solid substrates in the absence of free water (*1*), offers an viable alternative to the conventional submerged fermentation (SmF) processes (*2*). A resurgence of interest has been witnessed in SSF processes in the last decade (*3,4*) owing to realization of their numerous economic advantages (*5–7*) and diverse products have been investigated (*2,8*). Inspite of the economic gain in almost all the cases (*9–13*), the commercialization of the processes arising from these efforts in the last decade is almost absent (*14*). One of the major obstacles in transferring the laboratory scale process to the industrial level is the lack of efficient designs of larger bioreactors and numerous problems in scale up (*15–17*). A number of novel and efficient laboratory scale fermenters for SSF processes have been reviewed in recent years (*1,16,17*). In contrast, the literature reports on the designs and evaluations of large scale bioreactors are extremely few (*17–20*).

A need for the design of an efficient and sufficiently larger scale bioreactor for performing scale up trials has long been recognized as the successful scale up of the process is vital for the industrial exploitation of SSF processes and for harvesting their numerous advantages of socioeconomic importance. The present communication reports the design and evaluation of a novel, large scale solid state fermenter that facilitates the scale up studies at 4–12 kg substrate dry matter (SDM) or 15–55 kg moist solid medium capacity, depending on the initial moisture content of the medium. The design of the fermenter, designated as *Zymotis*—a condensed term based on Greek word "*Zymothiras*," which means the fermenter, differs significantly from the known designs of the laboratory and large scale fermenters for SSF system. The fermenter design was patented (*21*) because to an array of novel features, but the details are being reported for the benefit of other workers and our interest in development as well as industrial exploitation of the SSF system.

MATERIALS AND METHODS

Production of Cellulases

Trichoderma harzianum CCM F-470, obtained from the Czechoslovak Collection of Microorganisms, Brno, Czechoslovakia, was used. The methodology for its maintenance and inoculum preparation in disk fermenter D2 have been reported elsewhere (*22*).

Sugar cane bagasse and wheat bran at a ratio of 80:20 (w/w) were mixed thoroughly with a mineral-salt medium (*23*) to obtain a moist medium containing 50% moisture and transferred to an aluminum tank of 55 cm diam. for sterilization-cum-solid substrate pretreatment at 121°C for 60

min. After cooling to ambient temperature, the medium was inoculated with a spore suspension at a rate of 3×10^7 spores/g SDM, and the moisture content of the moist solid medium was raised to 70% by adding sterile water. The inoculated medium was transferred in desired quantities into *Zymotis* and in 18 g moist weight quantity in column fermenter for parallel fermentation in exactly identical conditions. The design of the column fermenter and its accessories have been described elsewhere (*24*). The column fermenters used was of 20 cm height and 2.2 cm diam. The temperature of the medium was regulated by keeping the column fermenters in a temperature controlled water bath. The air was humidified by passing through water held in a tube of the size of 10 cm height and 2.2 cm diam. before its entry in the column fermenter (*24*) at a rate of 0.1 L/h/g SDM (*25*).

The fermentation was carried out for 64 h at 28+1°C and the medium was aerated in *Zymotis* at a rate of 300:1 humidified air/h/compartment during first 12 h, whereas the aeration rate was doubled in rest of the fermentation time. The initial aeration rate works out to be 0.1 L air/h/g SDM. The initial pH of the medium was 5.6+0.1 and it was not controlled during the course of fermentation.

The enzyme was extracted from the fermented solids as per the methodology described elsewhere (*22,23*) and estimated for carboxy-methyl cullulases (CMCase) as well as filter paper activity (FPA) as per the methodology of Mandels et al. (*26*). The enzyme concentration is expressed as international unit (IU), which denotes the micromoles of glucose released/min of the reaction, whereas the enzyme titres are calculated for 1 g SDM.

RESULTS AND DISCUSSION

Fermenter Design

A rectangular box of the size of 0.50 m length×0.40 m width×0.65 m height, made up of acrylic sheets and with a working capacity of 100 L, was used as a fermenter vessel (Fig. 1; Fig. 2). An acrylic dome-shaped cover, which fits closely on the top side of the unit and prevents entry of atmospheric air into the unit, was provided to ensure closed fermentation system. It not only facilitated contamination control but also the containment of biological molecules/entities for preventing any safety hazard. The cover housed various process lines such as:

1. Inlet and outlet of water circulation circuits;
2. Exhaust gas outlets; and
3. A handle for lifting purposes.

The air entry point (1.5 cm diam. tube) was provided on the right hand side of the lateral wall of the rectangular box fermenter and was at 2 cm height from the bottom of the vessel (Fig. 1).

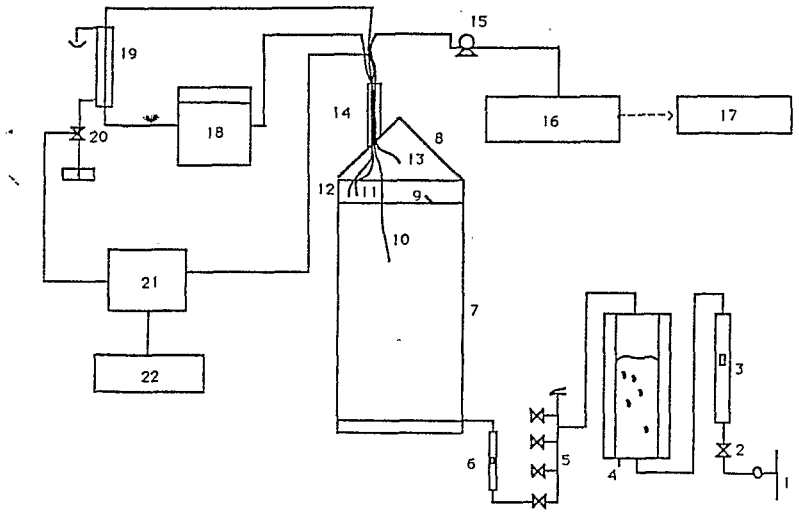

Fig. 1. Schematic diagram of *Zymotis* and the control/monitoring system. 1: air compressor. 2: air pressure regulating valve. 3: rotameter. 4: humidification column. 5: air flow valves (one for each compartment). 6: rotameter (one for each compartment). 7: fermenter vessel. 8: fermenter cover. 9: holder for heat exchanger plates. 10: thermister probe. 11: cooling water inlet. 12: cooling water outlet. 13: air outlet. 14: chimney for various service lines. 15: air pump. 16: analyzers for CO_2 and O_2. 17: recorders for CO_2 and O_2. 18: temperature regulated water bath. 19: column for mixing hot and cold water. 20: valve. 21: temperature controller. 22: temperature recorder.

A total of ten numbers of stainless steel heat exchanger plates were provided along the depth of the fermenter vessel and this made nine rectangular compartments in the fermenter vessel (Fig. 3). These plates were placed in parallel to each other. Each heat exchanger plate was of the dimensions of 0.0046 m length × 0.38 m width × 0.60 m height, and these plates, therefore, fit closely in the fermenter vessel. The heat exchanger plates occupied 9.44 L vol in the fermenter vessel. Each heat exchanger plate incorporated serially placed tubular pipes, as in the radiator of automobiles or freezing gas circulation in refrigerators, for circulating cooling or heating water (Fig. 4). The inlet and outlet of water circulation points were located at the top-side of each plate (Fig. 4). It is possible to use lesser number of heat exchanger plates for achieving a higher length of the resulting compartments and for facilitating the use of higher quantity of the moist medium in each compartment. It is even possible to have the compartments of different lengths by shifting suitably the location of the heat exchanger plates so as to study the effect of different moist solid medium volumes on the extent of the product formation. Initial trials on cellulase production indicated the uniformity in growth and absence of temperature

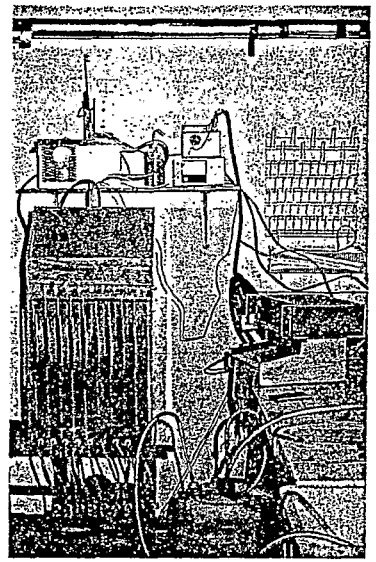

Fig. 2. *Zymotis* with the control/monitoring device.

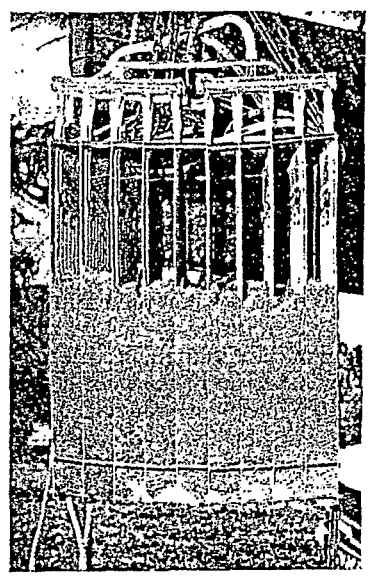

Fig. 3. Rectangular compartments (9 Nos.) of the *Zymotis* with the hook and pulley chains.

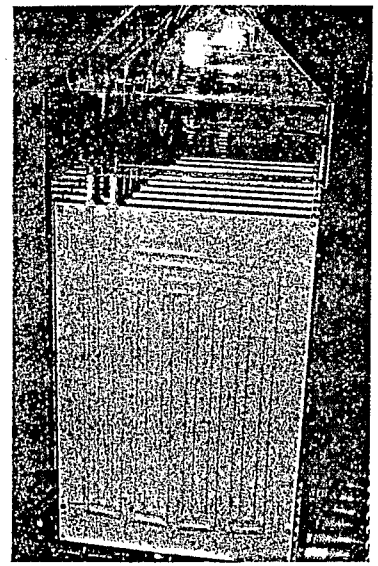

Fig. 4. The heat exchanger plate and the fashion in which the tubular pipes were placed for circulation of water.

gradients in the fermenting solids when the length of the compartment was not more than 5 cm. Thorough cleaning of the rectangular fermenter box, cover and heat exchanger plates was found to be sufficient to control contamination during fermentation. The wiping of the cleaned surfaces with 70% ethanol was also adopted as a safety measure for preventing contamination. The high inoculum ratio used in SSF processes imparts the status of dominance to the culture used. However, the prevalence of contamination was encountered when the fermentation time was higher than 72 h. This is also true in the case of larger tray fermenters (*17*). The autoclaving or steaming of the rectangular box fermenter and cover is not possible since these are made up of acrylic sheets.

Air supply to the fermenter was drawn from air compressor through pressure regulator (Fig. 1). The air was subjected to deoiling in an oil separator and sterilization by passing through a glass-wool filter. It was then humidified by passing through a column containing water and the humidified air line was then divided into 9 aeration tubes. Alternatively, the air line from the compressor can be first divided into a series of aeration tubes (9 numbers), one for each compartment of the unit so as to facilitate the studies on the effect of different aeration rates on product formation. The

air humidificator (model Nepal 2, No. KP 0010, Air Liquide, Medical, US) was used for air humidification. In such a case, it becomes necessary to provide each compartment of the fermenter unit with humidification apparatus, rotameter, and control valve. The point of air entry in the fermenting solid mass, held in each compartment, was at the centre of the bottommost portion of the compartment.

The temperature of the fermenting solids was controlled by circulation of heated or chilled water through a total of ten numbers of the heat exchanger plates that formed nine compartments in the fermenter vessel. Cryostat was used for heating or cooling of the circulation water. A column for mixing hot and cold water was also provided in the water circulation circuit to minimize power input. Initial experiments indicated that the heating of circulation water was necessary in the first 10 h fermentation, in contrast to the need for chilling of the water in the subsequent fermentation period. The water circulation through the heat exchanger plates was controlled through electro-vannes to economize on the energy input. Thermisters placed at different locations in the fermenting mass controlled the opening or closing of the electro-vannes. The temperature of the fermenting mass was recorded continuously.

The control of temperature of the fermenting solids by circulation of heating or cooling water through the heat exchanger plates was found to be inefficient during the active growth phase, wherein larger quantities of metabolic heat are generated. Use of higher capacity heat exchanger plates would be able to accommodate the increased heat generation during the active growth phase. This was not attempted, since it would decrease the capacity of the fermenter to hold the moist solid medium. The increase in the rate of aeration would also be helpful in removal of heat from the fermenting solids, provided it did not affect the titers of the product formed. Therefore, the aeration rate of the solids by humidifed air was increased during this period from 0.1 to 0.2 L/h/g SDM. This did not affect the productivity of the system. The increased rate of aeration results in evaporative cooling and thereby aided in the heat dissipation from the fermenting solids during the active growth phase of the culture.

The fermenter unit was provided with on-line monitoring of CO_2 and O_2 levels in the exhaust air (Fig. 1). For this purpose, the exhaust air from the air outlet duct of the fermenter was dried in a silica gel column and fed to CO_2 and O_2 gas analyzers (Beckman, models OM-11 and LB-2, respectively, Sensor Medics, CA). An analogic-numerical interface card (Digimetrix) was employed to connect these gas analyzers to an Apple IIe computer.

The whole of the compartment system was provided with a hook in the center for lifting it out by means of pulley system (Fig. 5) for facilitating easier harvesting of fermented solids. The cake of the fermented solids can be pushed out of each compartment and collected in a suitable container for leaching of the product (*25*).

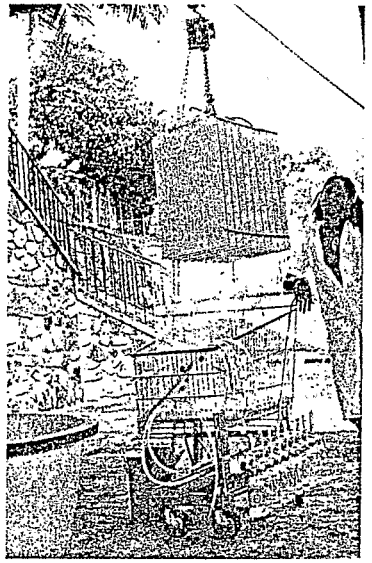

Fig. 5. The compartments of the *Zymotis*, after lifting from the fermenter vessel with the help of pulley system, for harvesting the fermented solids.

Productivity Evaluation

The results of the studies on comparative production of cellulolytic enzymes by *T. harzianum* in parallel fermentations under identical conditions in *Zymotis* at different substrate loads and 18 g moist medium sized laboratory column fermenter are depicted in Table 1. The data indicate nearly equal productivities in both the fermenters with the use of 4–12 kg SDM in *Zymotis*. In fact, the enzyme titers were marginally higher in *Zymotis* at all the substrate loads as compared to those in the parallel fermentation in the laboratory scale column fermenter, probably because of better control of cultural parameters in the former.

A marginal reduction in the enzyme formation was observed when the substrate load in *Zymotis* was increased from 8 to 10 kg SDM (Table 1). However, the reduction was of higher magnitude when the load was further increased to 12 kg SDM, i.e., 40.02 kg moist medium. Similar reductions were also observed in 18 g moist medium capacity column fermenter that was run in parallel to *Zymotis* under similar cultural conditions, thereby indicating that these reductions are not owing to any deficiency in the performance or design of *Zymotis*. These reductions in enzyme production have been shown to be caused by insufficient transfer of heat from steam to each substrate particle during autoclaving-cum-substrate pretreatment unit operation of the process at larger scale (*27*). The autoclaving-cum-substrate pretreatment involved the heating of the moist medium to 121°C and holding it at that temperature for 60 min. It makes the substrate more amenable to microbial attack (*1,17*). The depth of the medium in the aluminum vessel during autoclaving was 10 cm in case of the medium containing 4 kg SDM, as against that of 30 cm when the moist medium contained 12 kg SDM. Each and every particle of the medium, probably, had not attained 121°C temperature or the duration for which these were at 121°C was less than 60 min during autoclaving at 30 cm depth because of heat transfer limitations, especially at the center of the mass. It will be possible to overcome these limitations by performing the autoclaving-cum-substrate pretreatment in trays with about a 10-cm bed depth (*27*), as is generally done in tray fermentation processes (*1,5,9,28*). Such possibility and the use of other autoclaving methods were not experimented on because of the lack of an appropriate facility.

Table 1
Comparative Production of Cellulolytic Enzymes by *Trichoderma harzianum* in *Zymotis* and Column Bioreactors Under Identical Conditions in Parallel Fermentation

Capacity of *Zymotis* used, kg		CM Case production IU/g SDM		FPA production IU/g SDM	
SDM	Moist medium	*Zymotis*	Column	*Zymotis*	Column
4	13.34	133.54	131.36	18.26	16.08
8	26.68	135.26	131.64	16.32	11.60
10	33.34	128.03	125.81	13.44	12.76
12	40.02	74.16	71.85	8.03	5.50

The evaluation of the fermenter assembly in respect to temperature and moisture content of the fermenting solids showed very good efficiency in a large number of trial runs. The variation in temperature was ±1°C of the set value under the operation strategies described above. The moisture content of the medium was maintained in the range of ±2% up to 48 h fermentation, but it increased subsequently and was higher by 3–5% as compared to the initial value, probably owing to the metabolic water production in the medium, lesser loss of water from the medium because of evaporative cooling, and release of water from fungal cells because of autolysis. The monitoring of CO_2 and O_2 in the exhaust air was also found to be accurate when compared with the values obtained separately by analyzing the exhaust gas in gas chromatograph.

Comparison of Design Features with Known Fermenter Designs

Widely different fermenter designs have been used in SSF processes both at laboratory and large scales (*1,17*). These can be broadly classified into five major types, i.e., tray fermenter, packed bed fermenter, tumble fermenter, stirred tubular reactor, and air-solid fluidized bed fermenter; the state of the art of each of these types has been critically reviewed recently (*17*). The damage to the mycelial cells caused by attrition and the breakage of the attachment of mycelial cells to the solid substrate particles in agitated SSF reactors are well documented (*1,17*), and hence the choice of the reactor design gets largely confined to static reactors. It is for these reasons that no agitation or mixing of the fermenting solids was considered while designing *Zymotis*. It is well known that the static reactors have some inherent limitations compared to the agitated fermenters (*17*). A combination of best design features of static and agitated reactors thus may lead to higher efficiency. Hence, such an attempt was made while designing *Zymotis*.

In the present case, *Zymotis* is grouped as a packed bed fermenter as the autoclaved, moist solid medium is packed into the rectangular acrylic box fermenter, i.e., *Zymotis*. The whole of the moist solid mass is divided into nine rectangular compartments by means of ten heat exchanger plates to facilitate efficient heat removal. These plates provide higher cooling surfaces in the moist fermenting solid mass for better heat dissipation, which is of vital importance, especially during active growth phase of the culture. It is interesting to note that the air flow rate used in the initial 12 h fermentation is so low that it cannot cause turbulent motion of the wet solid substrate particles. The spores germinate and the growth of the mycelial cells is initiated by the end of 12 h fermentation. The mycelial cells penetrate deep into the solid substrate particles, adhere to the solids, and also spread on the surfaces of the solids (*1,17*), thereby forming a more or less compact mass by 12–18 h. The compactness increases with the increase in fermentation time and even poses problems in emptying the medium from the packed bed fermenters (*1*). The compactness of the medium also prevents the turbulent motion of the moist fermenting solid substrate particles in the fermenter, in spite of doubling the aeration rate beyond 12 h fermentation time.

It is emphasized that very few reports on design of solid state fermenters of 50 kg and higher capacities are available. The designs of large scale tray fermenters have been reported (*18,29–31*). Information is also available on the design of koji rooms with tiled walls (*29*), conveyor belt tunnel fermenters (*32*), vertical and inclined incubation cells as well as wooden cells (*33*). These later fermenters involve basically the tray fermenter type designs with improvements for facilitating mechanization and automation (*17*). The metabolic heat removal from the fermenting solids in these fermenters is solely by forcing cooling air through the medium. It thus not only requires a larger volume of air for efficient maintenance of temperature (*17*) but also results in drying of the solids caused by concomitant removal of moisture by the air (*5*). Difficulties in controlling temperature (*5,32*) and highly labor-intensive character were also stressed (*34*).

The designs of tumble fermenters based on the use of rotating drums of 55 gallon capacity (*35*), cement mixer of 70 L (*36,37*), as well as 114 L (*38*) capacities and pneumatic drums of 70 kg and higher capacities (*39*) are also available. Difficulties in scale up, injury to mycelial cells during early growth phase owing to tumbling of the medium, aggregation of the medium into balls, growth retardation owing to particle attrition, and problems in control of temperature as well as microbial contamination have been reported with the use of tumble fermenters (*33,40*). The heat removal from tumble fermenters is usually by forced aeration of the solids, since it is difficult to water jacket the fermenters because of their rotation (*17*). In one of the improved designs, the air inlet tube was branched out at several places along the whole length of the fermenter vessel for providing larger cooling surface area and the consequent better heat removal (*40*). In fact, this modification formed the base for adopting the use of heat exchanger plates in *Zymotis* for providing much larger cooling surfaces in the fermenting solids for improved efficiency.

The stirred tubular reactor is yet another type of large scale solid state fermenter that involves the use of a water jacket to remove the heat from the fermenting solids (*41–43*). The temperature control is by the combined action of the cooling water circulated through the water jacket of the fermenter and the air forced through the fermenting solids. In one case, the cooling water was also circulated through the hollow shaft of the two rows of the agitators (*41*). In addition, an internal diffusor type heat exchanger (circular chamber), in addition to an outside water jacket, was provided in a mixed layer 1.5 m^3 pilot plant fermenter (*41*). In spite of such attractive features, these types of bioreactors suffered the drawbacks of the agitated reactors and posed problems in obtaining uniform control, especially in larger units (*17*). Heat removal was stated to present unsurmountable problems, despite sophisticated internal and external cooling devices (*41,44*).

An air-solid fluidized bed fermenter of the capacity of 3.3 K1 bed volume was reported by Akao and Okamoto (*20*). It meets all the requirements of parameter control with high efficiency, but involves the use of extremely large quantity of air for causing desired level of turbulent motions of all the solid substrate particles during the whole of the fermentation period and highly advanced technological operations.

The designs of large scale packed bed fermenters were reported by Silman et al. (*44*), Sato et al. (*45*), and Laukevics et al. (*41*). The heat removal from the fermenting solids in these fermenters is by the combined action of cooling water passing through the jacket of the fermenter and the air forced through the fermenting solids. Some of the major problems

encountered in the large scale fermenters include poor heat removal, nonuniform growth, and unsatisfactory performance (*1,17*). In fact, these problems were taken into consideration while designing *Zymotis*. For example, the placing of heat exchanger plates as desired lengths in the fermenting mass was for improving the efficiency of heat removal in *Zymotis*. In addition, the difficulty in emptying the fermenter for harvesting the product forms a problematic unit operation in the packed bed fermenters of larger size (*1,17*). It is for this reason that the provision for lifting out the exchanger assembly was provided in *Zymotis* (Fig. 5) and it proved useful.

One of the most serious drawbacks of almost all the known solid state fermenter designs, except the air-solid fluidized bed fermenter, is the control of the temperature of the fermenting solids at the desired level, mainly because of the generation of a large quantity of the metabolic heat and insufficiency of the modes used for heat removal. The system used in *Zymotis* for efficient heat removal is unique and is not similar to any of those used in the known designs of the solid state fermenters. The present design is, therefore, not the case of inventing the wheel again.

Performance Comparison

Similar to the case of large scale solid state fermenter designs, the availability of the reports on the comparison of the performance of laboratory and large scale solid state fermenters is scanty. In the present case, the enzyme titers achieved in *Zymotis* were higher than those in the parallel fermentation in the laboratory scale column fermenters (Table 1). Similar results were obtained in the scale up trials conducted at the Central Food Technological Research Institute, Mysore, India, for enzyme production in tray fermenters (*17*) and also at ORSTOM, Montpellier, France, in the case of ethanol production by *Schwanniomyces castelli* (*16*). Such higher efficiencies were also reported by other workers with the use of mixed layer pilot fermenter (*41*), air-solid fluidized bed fermenter (*20*), bread kneader (*46*), multi-chambered fermenter (*47*), and packed bed jar fermenter (*48*). In contrast, lower product titers in large scale solid state fermenters, as compared to laboratory scale fermenters, were reported in case of the bin fermenter (*44*), bakery kneader (*49*), tank fermenter provided with turbine agitator as well as heat exchange coils (*50*), and rotary drum fermenter (*40*). In addition, the performance of mixed layer pilot fermenter was stated to be poor compared to the SF-SSF bioreactor and stationary tray fermenter, though it performed better compared to other laboratory scale designs (*41*).

Utility of *Zymotis*

Slightly higher enzyme titers in *Zymotis* at different substrate loads, compared to those in the laboratory scale column fermenter, indicate he worth of *Zymotis* for use in SSF processes involving 4–12 kg SDM or 13–40 kg moist solid medium. The ease of operation and simplicity in design are

the attractive features. A need for such a simple and efficient design of a large scale static fermenter is always felt by the researchers involved in scale up of SSF processes. *Zymotis*, to a large extent, fulfills this need. The development of *Zymotis* thus may pave a way for efficient and easier realization of the laboratory scale data at sufficiently large scale fermentation for scale up studies, collection of engineering data for plant design, and evaluation of economy of the newer SSF processes. Thus, *Zymotis* has potential in promoting transfer of SSF technologies for industrial exploitation and leading to the harvest of numerous socioeconomic advantages of SSF system.

Research and Development Needs

It is worth mentioning that the large scale rotary drum fermenter used by Takamine (*40*) performed poorly in the beginning and, therefore, it became necessary to subject it to numerous modifications after each trial to overcome the observed lacunae and to improve productivity as well as practicability. In fact, studies reported by Takamine (*40*) represent a classic example of how a design alteration of the larger fermenter is necessary to enhance its utility. *Zymotis*, in the first few trials, functioned efficiently and no such alterations in the design were found necessary. However, some R&D needs were found to be of vital importance and these are as specified in the following paragraph.

From a bioengineering points of view, it would be interesting to determine the individual extent of heat removal by the heat exchanger system and the increased rate of aeration during the active growth phase of the culture. Similarly, the data on the sufficiency of the rate of aeration of the fermenting solids for heat dissipation and to control the temperature at the desired level, in the absence of the circulation of cooling water through the heat exchangers, will be of interest. In case such an approach is found to be sufficiently efficient, then the data on the effect of higher air flow rates on the product titers will constitute a decisive factor. It will also be worthwhile to evaluate the water jacketing of the rectangular acrylic box for imparting higher temperature control to *Zymotis*. Data on the effect of the variations in the size of air inlet opening on air transfer rates or air-solid interface area will be useful in improving the productivity of *Zymotis*. It would be interesting to know the quantum of heat that had to be added in the beginning of the fermentation to raise the temperature to the desired level, and also the heat that had to be removed in the later phase of the fermentation owing to generation of metabolic heat. Similarly, the quantification of metabolic heat generated during different phases of fermentation and the measurements of the flow as well as temperatures of the cooling/heating water will be of help in designing still larger-sized *Zymotis*. In addition, the use of properly and sufficiently autoclaved-cum-substrate pretreated medium in *Zymotis*, with higher substrate loading, is worth investigating to confirm the reasoning that the decrease in enzyme production

under such conditions was owing to the problems of insufficient heat transfer during autoclaving, especially in the medium with higher depth.

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