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Maintenance of Heat and Water Balances as a Scale-up Criterion for the Production of Ethanol by *Schwanniomyces castellii* in a Solid State Fermentation System

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The scale-up of column fermenters by 6 to 410 gravimetric scale factors, from 10 g moist substrate size, has been achieved efficiently by maintaining heat and water balances in the media. The data on the patterns of ethanol production, biomass formation, the concentration of different carbohydrates, dry matter and pH values against time were of equal magnitude in 10- and 60-g size column fermenters. The reactors of 60-, 370- and 4100-g sizes also compared well in respect of O_2 consumption, CO_2 evolution and the specific growth rates in aerobic and anaerobic fermentation phases. The overall productivities of ethanol were similar in all the four column fermenters. The ability to obtain the same results in all the fermenter sizes, in spite of the increases in the diameter and the height of the columns, indicates the high potential of this simple scale-up criterion which has not been used earlier for scale-up of any fermentation process.

INTRODUCTION

In spite of a world-wide resurgence of interest in solid state fermentation (SSF) during the last decade,[1] together with many economic and practical advantages over conventional submerged fermentation (SmF)[2] and an increase in the range of products which can be made by SSF,[3] the commercial exploitation of SSF systems emerging from these recent efforts is almost non-existent.[4,5] Many reasons have been cited for this, including the lack of effective scale-up criteria.[3]

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The present work was undertaken to develop efficient criteria for scale-up with a view to facilitating the industrial exploitation of SSF. A simple and practicable scale-up criterion and its validity are reported in this communication.

Scale-up criterion

Even at laboratory scale, the productivity of the SSF system is affected by a number of parameters including (1) solid pretreatment, (2) medium composition, (3) initial pH of the medium, (4) medium autoclaving conditions, (5) form, age and ratio of inoculum, (6) agitation rate, (7) aeration and oxygen transfer, (8) temperature of the fermentating solids, (9) moisture content of the medium, and (10) humidity in the fermenter.[6] Of these parameters, the first five can easily be kept constant on a large scale, although problems may be involved in medium sterilization and the development of large inocula. These could be taken care of by use of efficient medium sterilizers[6] and by relying on the recently reported strategies for large scale development of inocula.[7] Most SSF processes do not involve agitation or are operated at low speeds to prevent damage to the fungal mycelia.[2,4] Scale-up problems may be encountered only in respect of the remaining four parameters.

Evolution of heat, which is directly related to the metabolic activity of the microorganisms,[8] is one of the well documented characteristics of SSF systems. Heat generation of 3200 kcal kg^{-1} dry matter,[9] 80 kcal kg^{-1} dry matter,[10] 14960 btu kg^{-1} dry solid[11] and an average of 600 kcal kg^{-1} solids[12] have been reported in most SSF processes. Such intense heat generation and poor thermal conductivity of the solids, led to a rapid temperature rise and steep temperature gradients at different depths in the fermenting solids.[3,13] A temperature of 47 °C at the centre of the fermenting substrate in tempeh production, 67 °C in a rectangular fermenter with an 80-mm bed depth and 60–70 °C in the centre of a composting bed have been reported.[3,9,14]

The deleterious effects of high temperature on spore germination, cell growth, product formation, sporulation and consequently on the overall productivity of the fermentation process are well known.[15] For example, an increase of about 2·5 °C in the temperature of the solids in a fermenter of 40 mm depth resulted in a doubling of the fermentation time required to attain similar enzyme titres to those in the temperature gradient-free fermenter of 20 mm depth.[3] It thus becomes essential to remove heat from the fermenting solids and various techniques such as forced aeration, water circulation through a jacket surrounding the fermenter, agitation of the solids, and covering the external surfaces of the fermenter with water soaked burlaps (canvas cloth with water absorbing capacity) have been employed.[4,6] The last two techniques can be ruled out for use on a large scale in static SSF processes. The circulation of water through a jacket is of poor efficiency in static SSF systems due to the poor thermal conductivity of the solids, although it can be employed to complement the action of the forced aeration, and burlaping is inefficient. Forced aeration is therefore the technique of choice for heat removal. The removal of up to 80% of the heat generated in the reactor using forced aeration[16] and greater efficiencies of evaporative heat removal as compared to those based on convection and conduction[17,18] have been reported.

A large quantity of air has to be forced through the solids for efficient control of temperature and this is considered more than sufficient to fulfil the demands of aeration, even if these are high. In this respect it is interesting to note that an air blower has been observed to work almost continuously in a 155-kg capacity koji room[19] during the logarithmic phase of growth of *Aspergillus niger* on wheat bran in trays with a medium depth of 5 cm. While aeration can therefore eliminate a deleterious temperature effect as well as provide oxygen to the culture, it results in a drastic reduction of the moisture content of the medium. An evaporation of about 7 g water from the medium takes place when air sufficient to remove the heat from the oxidation of 1 g starch is forced through the solids.[17,18] The forced aeration of the static packed bed reactor, involving growth of *Aspergillus oryzae* on rice, was found to result in gradients in the water content of the medium.[20] A greater loss of substrate moisture as well as humidity in the exhaust air and the consequent drying of the solids were also reported in tray fermentation.[19] The water loss at such high rates due to forced aeration constitutes a serious problem in SSF systems,[4] where the medium already has a low water activity.

Microorganisms require water for growth and metabolic activities and the existence, for each species, of an optimum moisture content of the medium at which the metabolic activities are maximal has been stressed recently.[21,22] The water content of the medium also has profound effects on the physico-chemical properties of the moist substrate mass and the growth characteristics of the microorganisms[22] and it becomes essential to con-

trol the water content of the medium during the course of fermentation. Methods include humidification of the air before it is charged into the fermenter, use of a slightly higher initial moisture content of the medium, humidification of the atmosphere in the fermenter at about 90–97% relative humidity, installation of humidifiers in the fermenter and intermittent addition of sterile water to the fermenting solids.[4,6] Among these, the humidification of air, before charging it into the fermenter, is the most practicable, efficient and reliable technique that is amenable to scale-up.

It is thus beneficial to combine the control of aeration and moisture content of the medium in the SSF process using humidified air. The rate of aeration by humidified air will control the temperature and the moisture content of the medium, in addition to meeting the oxygen demand of the culture. Such a policy can be developed as a scale-up criterion as follows.

The *heat balance* in the fermentation process[23] is indicated by the equation:

$$Q_{\text{metabolism}} + Q_{\text{agitation}} - Q_{\text{evaporation}} - Q_{\text{exchange}} = \alpha \frac{dT}{dt}$$

where

$Q_{\text{metabolism}}$ = heat generated due to oxidative reactions;

$Q_{\text{agitation}}$ = heat formed due to mechanical power input for agitation and air or gas sparging;

$Q_{\text{evaporation}}$ = latent heat of vaporization (this denotes the loss of heat due to evaporation of water);

Q_{exchange} = heat loss through the walls of the fermenter due to convection and conduction;

$\frac{dT}{dt}$ = variation in temperature as a function of time;

α = proportionality constant.

The *water balance* in the fermentation process can be illustrated by the equation:

$$W_{\text{replenishment}} + W_{\text{generation}} - W_{\text{consumption}} - W_{\text{evaporation}} = \beta \frac{dW}{dt}$$

where

$W_{\text{replenishment}}$ = water added to the system by forcing humidified air through the medium. Its quantity will depend on air flow rate and water content of the inlet air;

$W_{\text{generation}}$ = water generated during oxidative reactions in the medium;

$W_{\text{consumption}}$ = water consumed in biochemical reactions such as starch hydrolysis and microbial growth;

$W_{\text{evaporation}}$ = water that is evaporated from the system through exhaust air due to evaporative cooling. Its quantity will be determined by outlet flow rate and its water content;

$\frac{dW}{dt}$ = variation in the water content of the medium as a function of time;

β = proportionality constant.

When $\frac{dT}{dt}$ and $\frac{dW}{dt}$ are zero, the temperature and the moisture content of the fermenting solids will be constant at the set values at any given time of the fermentation batch. Thus, maintaining the heat and water balances assures a constant temperature and moisture content of the medium. This policy satisfies all the needs of the SSF system with respect to the four critical parameters, i.e. temperature of the fermenting solids, moisture content of the medium, humidity in the fermenter and aeration as well as oxygen transfer. Consequently, the fermentation productivity may be equal at all the stages of scale-up if heat and water balances are similar. The strategy may prove to be a simple, efficient and dependable scale-up criterion for SSF system as it assures all the critical needs of the system.

Validity evaluation

The validity studies have been carried out on the process for the production of ethanol by *Schwanniomyces castellii* in SSF involving the use of substrate absorbed onto an inert solid support in column fermenters. The selection of this process for the present studies was guided by many factors of scientific, industrial and practical importance:

(a) Considerable research and development has been undertaken in recent years to produce bioethanol from renewable biomass because of the abundant availability of starch by-products and wastes.[24] Success in terms of the economics of the production technology remains elusive.

(b) The yeast, *Saccharomyces cerevisiae*, is conventionally employed in most cases,[25] although the bacterium, *Zymomonas mobilis* has also been investigated extensively as an alternate microorganism of promise.[26] Because of the non-amylolytic nature of these organisms, the processes for bioethanol production from starchy substrates involve initially the time-consuming enzyme hydrolysis of starch to fermentable sugars. Moreover, starch liquefaction at high temperatures by bacterial alpha-amylase and subsequent saccharification by glucoamylase for about 48 h are highly cost intensive and the need for economy in the process has been stressed.[27]

(c) The time involved in the saccharification of the liquefied starch has been reduced to 4 h by adopting simultaneous saccharification by glucoamylase and fermentation by *Saccharomyces cereviseae*,[25] but the process is not economically attractive.

(d) The enzyme treatment of raw starch, as an alternative to bacterial alpha-amylase and fungal glucoamylase has been extensively evaluated for starch hydrolysis at room temperature without any cooking.[24] Although the results are promising, the process still requires an externally added enzyme.

(e) The use of SSF based on fibrous and pulpy sugary or starchy substrates and *Saccharomyces cerevisiae* has been investigated by many workers, but involves practical difficulties, especially the recovery of ethanol from fermented solids.[28]

(f) The direct fermentation of starch by yeast with both amylolytic and fermentative activities has also been the topic of intense investigations in recent years.[24] However, the major problem encountered is low amylolytic enzyme production by good fermentative yeasts and *vice versa*. Mixed or associative fermentations have been proposed to overcome these problems.[24] However, the low ethanol tolerance of the amylolytic yeasts, decreased enzyme production in anaerobic conditions, lower capability to produce glucoamylase and catabolic repression of the enzyme production are the stumbling blocks.[24, 29]

(g) *Schwanniomyces* spp., especially *Schwanniomyces castellii*, have been identified as possible 'superyeasts' due to their ability to produce amylolytic enzymes and their homofermentative nature.[30] The conversion of starch to ethanol is almost complete, but the culture has low ethanol tolerance.[24] This problem can be fully overcome by continuous stripping of ethanol during the course of fermentation and this can also lead to significant savings in the cost of distillation. In spite of these attractive advantages, the process is not yet commercialized probably due to its confinement to SmF systems.[31]

(h) *Schwanniomyces castellii* has been used earlier in the SSF system but the work was confined to aerobic fermentation for the production of protein using potato or potato peel media[32] and protein enrichment of sweet potato residue.[33]

(i) Preliminary work on the production of ethanol from soluble starch by *Schwanniomyces castellii* showed a high potential for SSF systems involving continuous stripping of the ethanol formed.[34]

(j) The production of ethanol by *Schwanniomyces castellii* involves an aerobic fermentation for the production of amylases and yeast cell mass and a subsequent anaerobic fermentation for the production of ethanol.

(k) Among various bioreactor designs available for SSF processes,[4] the column fermenter is most amenable to scale-up and process control and may be coupled to a computer for on-line monitoring and control.[35] The column fermenter was also preferred for protein enrichment of sweet potato by *Schwanniomyces castellii* in a SSF system,[33] although a system based on a bread making blender was used by Rossi and Clementi.[32]

(l) The use of bagasse as a support for absorbing liquid medium has been an important development in recent years,[36] since it allows the advantages of the SSF system together with the control of media composition. It also facilitates the simple measurements of substrate, biomass and fermentation products for accurate material balance calculations and it is also possible to reuse the spent support from the previous batch for a number of cycles.

MATERIALS AND METHODS

Microorganism

Schwanniomyces castellii CBS 2863, recently identified as a strain of *Schwanniomyces occidentalis*

Table 1. Details of the column fermenters

Fermenter type	*Dimensions (cm)*		*Volume (ml)*		*Moist medium load (g)*
	Length	*Diameter*	*Total*	*Working*	
Small	20	2	63	55	10
Medium	20	4	251	221	60
Large	90	4	1130	1000	370
Larger	64	15	11300	11000	4100

Klocker,[24] was obtained from Centraal Bureau Voor Schimmelculture, Delft, Holland. It was maintained on the medium of Sills *et al.*[37] at 4 °C and subcultured every month. The inoculum was prepared, from the freshly grown culture, in the medium reported by Oteng-Gyang[38] which was agitated at 28–30 °C for 24 h. It contained about 5×10^8 cells ml^{-1} and a 10% (v/v) suspension was used to inoculate the sterilized and cooled liquid medium. The contents were mixed thoroughly to achieve a uniform distribution. All these operations were carried out under aseptic conditions.

Liquid medium preparation

The basal liquid medium that was used for absorbing onto the solid support contained: 100 g $litre^{-1}$ soluble starch (Prolabo), 1 g $litre^{-1}$ peptone (Merck, pancreatically digested casein), 1·3 g $litre^{-1}$ urea, 1 g $litre^{-1}$ yeast extract, 5 g $litre^{-1}$ KH_2PO_4, 1 g $litre^{-1}$ NaCl, one 2 g $litre^{-1}$ $MgSO_4.7H_2O$ and 1000 g $litre^{-1}$ distilled water. The pH of the medium was adjusted to 3·5 by 5 N HCl and the medium was sterilized at 121 °C for 15 min. A filter sterilized vitamin–mineral solution,[39] was added at a level of 2 ml $litre^{-1}$ to the sterilized and cooled medium before inoculation.

Bagasse support preparation

The milled sugar-cane bagasse, as obtained from a sugar-cane mill, was sieved to obtain the particle size of 0·3–0·8 mm. It was washed twice, using 10 times deionized water at each stage, to remove soluble constituents. The washed wet bagasse, held in a stainless steel (SS) sieve of 0·3 mm size at about 5 cm depth, was sterilized in an autoclave at 121 °C for 30 min. The material was allowed to cool to about 65 °C before spreading to the depth of about 2 cm in plastic trays and dried in the oven at 60 °C for about 30 h. The dried support can be stored at room temperature in closed polythene bags over a period of about 6 months without any deterioration or contamination.

Absorbance of liquid medium on the support

The support was mixed thoroughly with the inoculated liquid medium in a sterilized beaker in the ratio of 14:86 (w:v) and an appropriate quantity was transferred to the sterilized columns of different sizes. No aseptic conditions were maintained during and beyond these operations.

Column fermenters

The details of the four different sizes of column fermenters used are presented in Table 1. The other design features of the small and medium sized fermenters are as reported elsewhere.[36] The large

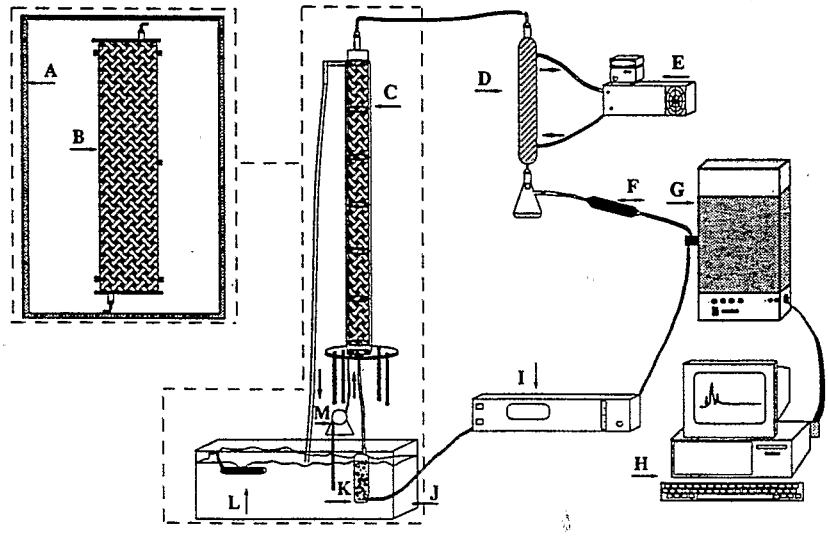

Fig. 1. Schematic diagram of the control system for large and larger column fermenters. A: temperature controlled cabinet; B: larger column fermenter with 4100 g moist medium; C: large column fermenter with 370 g moist medium; D: condenser; E: chilled water bath; F: silica gel column; G: gas chromatograph; H: computer; I: membrane pump; J: temperature controlled water bath; K: air humidification column; L: water heater; M: water pump.

column fermenter consisted of six segments, each of 15 cm length and 4 cm diameter (Fig. 1). Each segment consisted of an inner glass column, acrylic water jacket and acrylic flanges at both the ends. Four openings provided in the flanges of each segment were used to produce a continuous jacket for water circulation. The segments were held in the appropriate configuration by means of two projecting screws from the bottom side of the flange of the upper segment and corresponding holes in the upper side of the flange of the lower segment. The lateral side of the bottom flange of each segment also had four ports for inserting various probes. The segments were assembled one above the other, with two 'O' rings between two segments. When assembled, the unit had top and bottom plates which were housed in an appropriate stand. Four supporting SS rods were provided in the stand so that the segments were held in a fixed configuration by tightening winged nuts on the upper plate. The plates were provided with inlets and outlets for air and water, while the inlet air port was provided with a diffuser to avoid channelling.

The larger column fermenter of 4100 g moist medium capacity (Table 1 and Fig. 1) was provided with flanges at both the ends and SS plates to close the ends securely by means of winged nuts. An opening on each of the SS plates was used for the circulation of air or gas, while the air inlet port was provided with a diffuser. The glass column was provided with 3 and 2 ports on opposite lateral sides for inserting probes. This unit was kept in a horizontal position in a temperature-controlled cabinet during the entire course of fermentation.

Fermenter operation

The temperature was measured using thermister temperature probes (YSI, Ohio, USA). In the case of the small and medium size fermenters, each column was provided with one probe while each segment of the large fermenter contained a temperature probe. The larger unit was provided with five temperature probes. Three of these were positioned at the centre of the bed at 10, 35 and 60 cm depths. The other two probes were positioned at 3 cm distance from the wall of the column at 10 and 60 cm depths. The small, medium and larger fermenters were supplied with air in the first 10 h of fermentation, at an air flow rate of 1·4 ml min^{-1} per g wet material, but the air supply was switched off subsequently in all cases. The CO_2 produced was allowed to escape in to the atmosphere after bubbling through water in order to maintain anaerobic conditions in the case of the small and medium sizes fermenters, except in case of one of the columns (designated F) of the medium sized unit. This column F continued the fermentation up to 30 h and duplicates the fermentation in the lowermost segment of the large column fermenter.

All the six segments of the large fermenter, the larger fermenter and the column F of the medium sized fermenter were provided with on-line monitoring for CO_2 and O_2. The total air supply in the case of column F and the six segments of the large fermenter was 400 and 2700 ml respectively during the entire course of 30 h fermentation. In these cases, the exhaust air during the aerobic and anaerobic phases of fermentation was passed through a laboratory condenser for continuous stripping of ethanol formed during the anaerobic phase. The condenser was cooled by chilled water from the bath maintained at 2 °C. After ethanol stripping, the air or exhaust gas was dried by passing through a silica gel column for recirculation through the fermenter by means of a membrane pump. The circulation was at a rate of 20, 90 and 500–600 ml min^{-1} in medium sized column F, large and larger fermenters, respectively.

Analytical aspects

The concentration of starch, maltose, glucose and ethanol in the fermenting solids were determined by HPLC which consisted of a pump (Constametric 3200, UDC, USA), oven (Touzart-Matignon, France) and detector refractometer (PU 4026, Phillips, France) and an integration program (Chroma, Société Biosystème, France). The operating conditions were as described elsewhere.[40] Total carbohydrates were calculated by adding the values of the individual sugars determined.

CO_2 and O_2 concentrations in exhaust air were estimated with a gas chromatograph (IGC 11, Delsi, France), fitted with a thermal conductivity detector. Gases were separated using a concentric column CTRI (Alltech, USA). These were coupled to a personal computer, provided with an integration program (Chroma, Société Biosystème, France).

The dry matter was determined by the methodology of Sato *et al.*[41] For biomass determination, 36 ml of sterile distilled water (containing a drop of Tween 80) was added to 4 g of the solid medium in a sterilized beaker. The mixture was vortexed for 5 min, filtered through a 50 μm sieve to separate bagasse and the filtrate was used to determine the dry cell weight, after drying at 105 °C for 24 h.

Results were expressed on an initial dry mass (IDM) basis.

RESULTS AND DISCUSSION

Temperature and humidity profiles

The plots of temperature and moisture content of the medium during the course of fermentation in all the bioreactors are presented in Figs 2 and 3, respectively. The data indicate that the control system functioned efficiently as the temperature variation was ± 1 °C of the set value of 30 °C, although the temperature rose from 31° to 32 °C in the span of about 2 h and then fell to 31 °C in the next 2 h in larger column reactor. This increase in temperature, however, was considered to be negligible. The moisture content of the medium in the small and medium sized fermenters was fairly constant at the selected value of 77% up to about 14 h with a variation of less than $\pm 0{\cdot}5$%. Beyond 14 h, however, it ranged between 77 and 80%, probably due to ethanol formation and rapid utilization of starch in the anaerobic phase. In terms of grammes water per gramme initial dry mass (IDM), the values were constant at $3{\cdot}2 \pm 0{\cdot}1$ throughout the course of fermentation. The overall comparison of the water content of the media in all four fermenters used indicates that the water content was nearly equal at the start and end of fermentation (Table 2). The temperatures at different depths in the large and larger fermenters are depicted in Figs 4 and 5 and these show that the range was 28–30 °C in all segments of the large column fermenter. Except for a duration of about 4 h, the temperature at all depths monitored and in

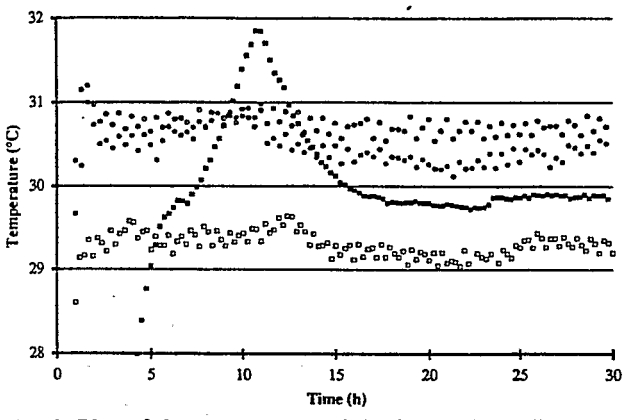

Fig. 2. Plot of the temperature of the fermenting solids against time in all the fermenters used. ○: Small fermenter; ●: medium sized fermenter; □: large fermenter; ■: larger fermenter. The values shown are an arithmetic and radial means for large and larger fermenters, respectively.

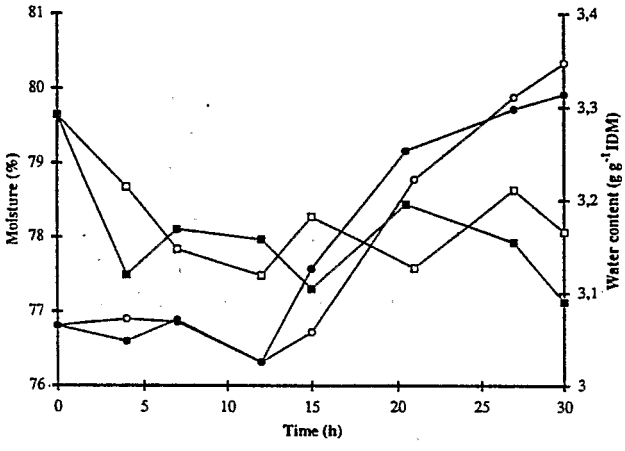

Fig. 3. Plot of moisture content of the fermenting solids and water content per gramme initial dry matter against time in small and medium sized fermenter. ●: Moisture content in small fermenter; ○: moisture content in medium sized fermenter; □: water content per gram IDM in small fermenter; ■: water content per gram IDM in medium sized fermenter.

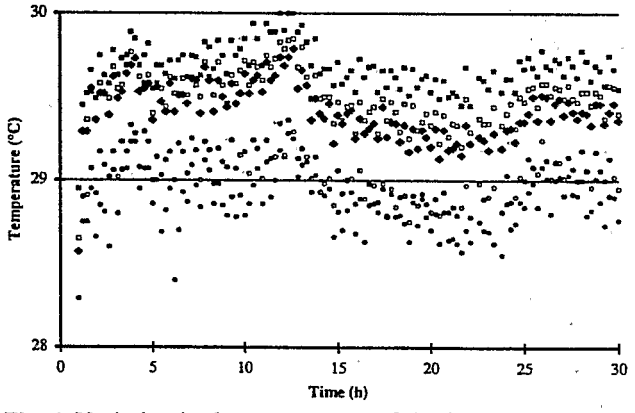

Fig. 4. Variation in the temperature of the fermenting solids at different bed depths in the large column fermenter with 370 g moist medium. ●: 15 cm; ○: 30 cm; ■: 45 cm; □: 60 cm; ◆: 75 cm.

Table 2. Overall comparison of water content in all the fermenter sizes used

Fermenter type	*Initial moisture (% w/w)*	*Initial water content (g g⁻¹ IDM)*	*Final water content (g g⁻¹ IDM)*	*Water loss (% w/w)*
Small	$76{\cdot}9 \pm 0{\cdot}5$	$3{\cdot}29 \pm 0{\cdot}02$	$3{\cdot}14 \pm 0{\cdot}01$	4·6
Medium	$76{\cdot}9 \pm 0{\cdot}5$	$3{\cdot}29 \pm 0{\cdot}02$	$3{\cdot}17 \pm 0{\cdot}01$	3·6
Large	$76{\cdot}8 \pm 0{\cdot}4$	$3{\cdot}35 \pm 0{\cdot}02$	$3{\cdot}22 \pm 0{\cdot}20$	3·9
Larger	$77{\cdot}6 \pm 1{\cdot}2$	$3{\cdot}47 \pm 0{\cdot}02$	$3{\cdot}45 \pm 0{\cdot}08$	0·6

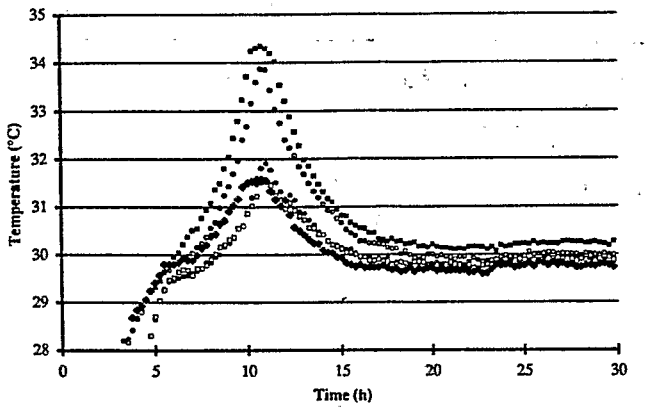

Fig. 5. Bed temperature at different positions in the larger column fermenter with 4100 g moist medium. ●: Central region at 10 cm depth; ○: central region at 35 cm depth; ■: central region at 60 cm depth; □: 3 cm away from the wall at 10 cm depth; ◆: 3 cm away from the wall at 60 cm depth.

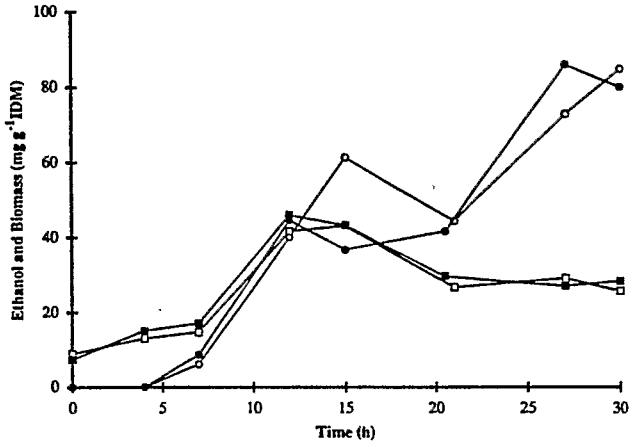

Fig. 7. Trends in biomass formation and ethanol biosynthesis in the medium at different time intervals. The solid and open symbols are for small and medium sized bioreactors, respectively. ○, ●: ethanol; □, ■: biomass.

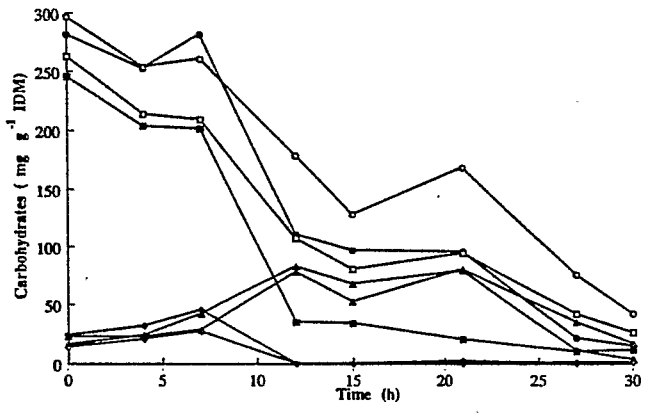

Fig. 6. Pattern of the levels of various carbohydrates in the medium at different time intervals. The solid symbols are for the small fermenter as against the open symbols for the medium sized fermenter. ○, ●: total carbohydrates; □, ■: starch; ◇, ◆: maltose; △, ▲: glucose.

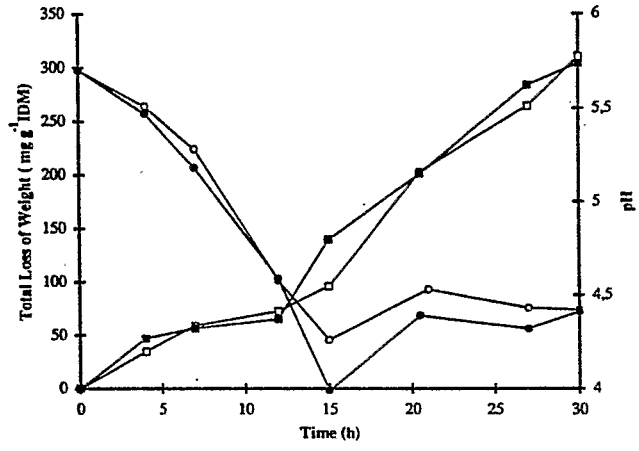

Fig. 8. Total loss of weight and pH changes with respect to time in small and medium sized fermenters. The solid and open symbols are for small and medium sized fermenters, respectively. ○, ●: pH of the medium; □, ■: total loss of weight, mg per g IDM.

the centre and near the wall of the larger column fermenter varied between 28 and 32 °C. In this fermenter, the temperature was about 30 °C during most of the anaerobic phase and the variation in temperature was confined to the initial start-up period and the stage when metabolism shifts from aerobic to anaerobic phases. Collectively, the data indicate that the maintenance of the temperature and moisture content of the media were similar in all the different sizes of the fermenters evaluated.

Comparison of small and medium sized bioreactors

The data in respect of the concentrations of total carbohydrates, starch, maltose, glucose (Fig. 6); ethanol and biomass formed (Fig. 7); pH of the medium and total loss of weight of the solids (Fig. 8) indicate nearly similar values and process productivities in both sizes of the fermenters at various time intervals.

Comparison of medium, large and larger sized bioreactors

The data on O_2 concentration in the exhaust air during the initial aerobic fermentation up to about 14 h (Fig. 9) showed nearly equal trends in all these fermenters. A slight difference in the case of the larger column fermenter was evident but this again was confined to the stage preceding the shift from aerobic to anaerobic phase. The trends with respect to CO_2 concentration in the exhaust air (Fig. 10) were also nearly similar in these reactors, although the quantity of CO_2 was slightly higher in the larger fermenter.

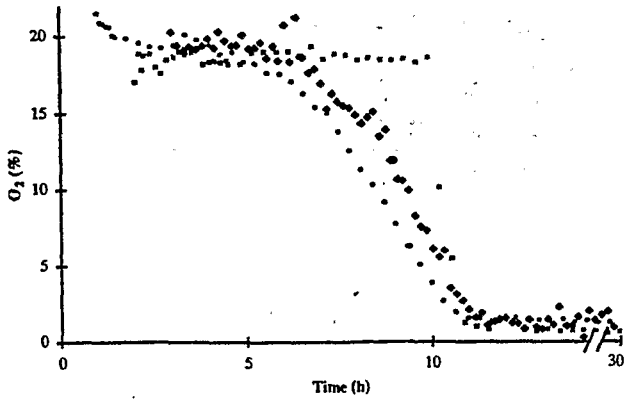

Fig. 9. Pattern of O_2 concentration in the exhaust gases from medium, large and larger fermenters with respect to time. ●: Medium sized fermenter; ◆: large column fermenter; ■: larger column fermenter.

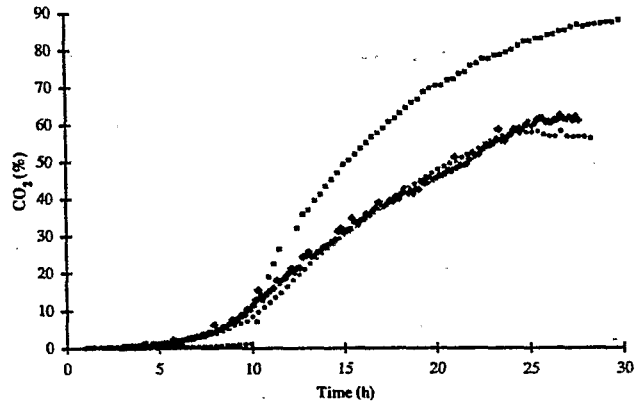

Fig. 10. Trends in the concentration of CO_2 in the exhaust gases from medium, large and larger sized fermenters against fermentation times. ●: Medium sized fermenter; ◆: large column fermenter; ■: larger column fermenter.

Overall comparison of all the fermenter sizes

The data presented in Table 3 indicate similar trends in all the sizes of fermenters used with respect to final pH, final dry matter, utilization of carbohydrates, growth rate in the aerobic phase, final biomass concentration, loss of dry solids and ethanol yields. In the larger fermenter, the data indicate that biomass formation during the aerobic phase was higher and this probably explains the higher temperature at the end of the aerobic phase (Figs 2 and 5) and the different trends with respect to O_2 as well as CO_2 concentrations in the exhaust air (Figs 9 and 10). The higher specific growth achieved also indicates that the conditions at the larger scale of fermentation were more conducive to higher productivities than at smaller scales. Similar results with respect to productivity were also reported in the case of all the scale-up trials conducted at the Central Food Technological Research Institute (CFTRI), Mysore, India.[4] The higher specific growth rate in the aerobic phase, higher final biomass formation and lower ethanol productivity in the larger fermenter as compared to those in the large bioreactor indicate that more substrate was used for biomass formation in the former. This probably resulted from the delay in the consumption of oxygen observed at 10 h in the medium, the time period when the air supply to the unit was switched off. Consequently, the shift in the metabolic state of the culture from the aerobic to the anaerobic phase was affected.

The similar trend of results with respect to a number of physico-chemical process variables and the productivities in all the sizes of the fermenters (Figs 2–10; Tables 2 and 3), in spite of the increase in the length and the diameter of the bioreactors as well as the gravimetric scale-up factor of the magnitude of 410, indicates a success in the formulation and validation of efficient and dependable scale-up criteria of maintaining equal heat and water balances in all the scale-up stages in the SSF system. The efficient maintenance of these balances was not a difficult task and was achieved by the circulation of the cooled exhaust gases after humidification. Moreover, a computer controlled integrated temperature–moisture control system has been developed successfully by other workers for SSF system in recent years.[42,43] It consists of a computer program with pre-set control values of

Table 3. Overall comparison of all the fermenter sizes used

Fermenter type	*Final pH*	*Final dry matter* (% *w/w*)	*Utilization of carbohydrates* (% *w/w*)	*μ in aerobic phase* (h^{-1})	*Final biomass concentration* (*mg* g^{-1} *IDM*)	*Loss of dry solids* (% *w/w*)	*Ethanol yield*	
							Actual (*mg* g^{-1} *IDM*)	*Theoretical* (%)
Small	4·4	18·0	94·6	ND	20·3	30·0	83·2	57·4
Medium	4·5	17·5	89·0	0·43	18·5	26·1	88·5	61·2
Large	4·3	18·1	96·3	0·43	19·7	24·6	109·3	62·5
Larger	4·4	17·1	95·0	0·46	26·2	26·9	88·1	57·2

ND = No data.

temperature and moisture as well as their maintenance by relative humidity in the system.[42] Thus the temperature control is effected by blowing air through the substrate at a constant velocity and forcing evaporation of the water to produce a cooling effect. The relative humidity of the air inlet was varied to maintain the moisture content of the solids.[42] In addition, the lost water was replaced by a cold water spray which was regulated by a computer program based on the water balance equation of the system.[43]

These computerized systems for maintaining temperature and moisture of the medium[42,43] will be more efficient in removing large quantities of heat on industrial scale fermentations. The rate of water evaporation is known to vary with the lower humidity of the inlet air and its flow rate.[42] Therefore the use of dry air and water spraying will be highly effective in the removal of a large quantity of heat by evaporation and also in maintaining the moisture content of the medium. However, this system will require that the fermenting solids are agitated intermittently, at least when water is sprayed. This may prove to be a drawback, as mixing inhibits the growth of the fungi by compacting the substrate, disrupting the mycelial attachment to the solid substrate particles and even injuring the cells.[4,43] Furthermore, the percolating liquid may clog the pores of the substrate and thereby inhibit O_2 mass transfer.[43] In this context, it is interesting to note that these problems could be effectively overcome by using a rocking bioreactor in which the substrate remains undisturbed during the rocking motions but air and moisture are distributed evenly.[43]

The scale-up criterion developed in the present studies is thus not only efficient and dependable, but is also simple and easily practicable and its development is of scientific and industrial importance. It represents the first scientific scale-up criterion for SSF systems and paves the way for extensive industrial exploitation of SSF.

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