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Scale-up strategies for packed-bed bioreactors for solid-state fermentation

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Abstract

Two approaches are compared for scale-up of solid-state fermentation processes in packed-bed bioreactors, one based on a dynamic heat transfer model, and the other based on a modified Damköhler number. A critical bed height is proposed, being the maximum bed height which can be used without undesirable temperatures being reached in the substrate bed during the fermentation. It depends on the microbial specific growth rate, as well as the superficial velocity and inlet temperature of the air. The critical heights predicted by the two approaches are almost identical, suggesting that approaches to scaling-up packed-bed bioreactors based on the modified Damköhler number may be successful. The modified Damköhler number is then used to predict how simple rules of scale-up might perform. Superficial velocities will need to increase with scale, although for practical reasons, it is not possible to increase superficial velocity in direct proportion to height indefinitely. A strategy is proposed to guide experimental programs for scaling-up of packed-bed bioreactors.

Keywords: Bioreactor design; Heat transfer; Operating variables; Packed-bed bioreactors; Scale-up strategies; Solid-state fermentation

1. Introduction

Solid-state fermentation (SSF) involves the growth of microorganisms on water-insoluble substrates in the absence of free water. This cultivation technique has potential to be used at commercial scale, but applications of SSF are limited by the lack of well-founded scale-up criteria [1]. As a result, many of the commercial processes are done at intermediate scales and involve tray fermentations which are quite labor intensive.

Scale-up problems have received detailed attention in bioreactors for submerged liquid fermentation (SLF). Rules-of-thumb have been used for many years, and more recently semifundamental models have been developed [2]. Unfortunately, this knowledge is of little use for SSF, because the limiting phenomena are different. For aerobic SLF processes the limiting step is typically the transfer of oxygen across the gas–liquid interface. In contrast, in SSF processes, growth may be limited by heat transfer, or by mass transfer of oxygen or nutrients, depending on the location in the substrate bed, the stage of the fermentation, and the design and operation of the bioreactor. As a result, no quantitative scale-up criteria are currently available for SSF systems [1]. Recently, however, models have been developed to describe heat and mass transfer processes in packed bed bioreactors [3,4], tray bioreactors [5,6] and rocking drum bioreactors [7]. These models can be used to guide scale-up processes [8] although no work has been done to date to demonstrate this.

The current work focuses on those SSF processes in which agitation is deleterious to bioreactor performance. Although the effects of agitation vary depending on the microorganism, deleterious effects have been noted in some systems. For example, in some processes for fungal spore production, agitation retards growth [9] and damages conidiophores [10], greatly reducing spore yields. In such cases, the substrate bed must be

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maintained static during the fermentation. Although tray bioreactors can be used for such processes, packed-beds are more appropriate because the forced aeration allows some control over fermentation parameters through manipulation of the flowrate and the temperature of the air used in the fermentation [4]. Therefore the current work considers only packed-bed bioreactors. In these bioreactors oxygen supply is not limiting [11,12]. Rather, due to the heat removal dynamics associated with convective cooling, which lead to a steady rise in the air and solids temperatures between the air inlet and outlet, the challenge in scale-up of packed beds is to prevent the temperature from reaching undesirably high levels near the air outlet [4].

In a static packed-bed, it is impractical to add water in a well-distributed manner to the bed during the fermentation. Therefore the bioreactor should be operated so as to minimize drying of the bed, because drying can eventually lead to the moisture content in the bed reaching values which restrict the growth of the microorganism. This necessitates the use of saturated air at the air inlet. Note that, even with saturated inlet air, evaporation still occurs because the increase in air temperature between the air inlet and outlet increases the water-holding capacity of the air. These drying considerations remove manipulation of the relative humidity of the inlet air as an operating variable for packed-bed bioreactors, leaving only the inlet air temperature and superficial velocity.

Saucedo-Castaneda et al. [3] suggested that the Peclet and Biot numbers could be used as scale-up criteria for packed-beds. Later they proposed the maintenance of heat and water balances as scale-up criteria [11]. However, these ideas were not developed quantitatively. The current work develops quantitative scale-up strategies for packed-bed bioreactors. A dynamic mathematical model of heat transfer in packed-bed bioreactors developed previously [4] is used to explore the effect of scale-up. Then a simpler approach to scale-up is developed, based on a modified Damköhler number.

2. Development of the dynamic heat transfer model

2.1. System and assumptions

The system modelled is a cylindrical packed-bed bioreactor, aerated from the bottom with moist air (Fig. 1). A moist starchy substrate is inoculated and placed in the bioreactor at time zero. During the process the substrate bed remains static. The model concentrates on the heat transfer phenomena. Equations for mass transfer have not been incorporated. Sangsurasak and Mitchell [13] described the development of a model which was identical except that it described heat transfer in both the vertical and horizontal directions. In the current work only heat transfer in the axial direction is considered because commercial scale packed-beds designed and operated as shown in Fig. 1 will have sufficiently large diameters that radial heat transfer will be negligible. Therefore radial homogeneity within the bioreactor is assumed. Sangsurasak and Mitchell [13] discussed the other assumptions in the current model and since their model described well the experimental data of Saucedo-Castaneda et al. [3] and Ghildyal et al. [14], the assumptions are accepted as reasonable and are not discussed here.

2.2. Growth kinetics

The growth kinetics are described empirically by the logistic equation:

$$\frac{dX}{dt} = \mu X\left(1 - \frac{X}{X_m}\right) \tag{1}$$

where X is the biomass concentration and X_m is the maximum possible biomass concentration. The specific growth rate μ (s^{-1}) is expressed empirically as a function of temperature [13]:

$$\mu = \mu_{opt} \quad T < T_{opt} \tag{2a}$$

$$\mu = \left(\frac{b + (T_{max} - T_{opt})}{(T_{max} - T_{opt})}\right)\left(\frac{\mu_{opt}(T_{max} - T)}{b + (T_{max} - T)}\right)$$

$$T_{opt} \le T \le T_{max} \tag{2b}$$

$$\mu = 0 \quad T > T_{max} \tag{2c}$$

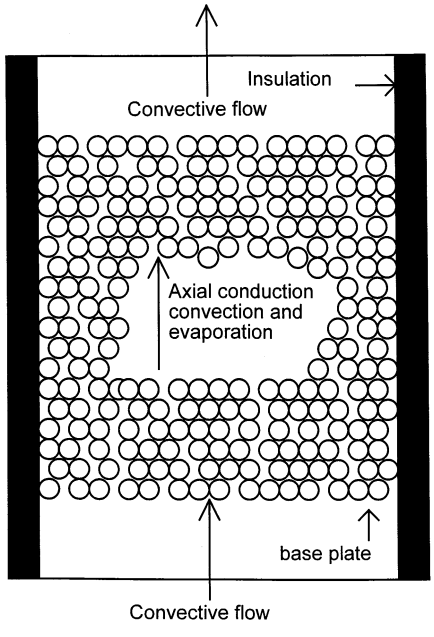

Fig. 1. Configuration and operation of a packed-bed bioreactor as modelled in this work, showing the various heat transfer processes occurring. The bioreactor is assumed to be several metres wide such that temperature gradients in the radial direction are negligible.

where μ_{opt} is the specific growth rate at the optimal temperature for growth (T_{opt}) and T_{max} is the maximum temperature at which growth can occur. The parameter b describes the sensitivity of the specific growth rate to increases in temperature [13]. Eq. (2a) is used for simplicity since the temperature never falls significantly below T_{opt}.

2.3. Energy balance

Since heat transfer to the wall is considered negligible, only axial heat transfer is considered. Eq. (3) is the macroscopic energy balance over the column, including terms for convective and evaporative heat removal, conduction in the axial direction, and the generation of heat from microbial growth:

$$\rho_b C_{pb}\left(\frac{\partial T}{\partial t}\right) + \rho_a(C_{pa} + f\lambda)V_z\left(\frac{\partial T}{\partial z}\right) = k_b\left(\frac{\partial^2 T}{\partial z^2}\right) + \rho_s(1-\varepsilon)Y\frac{dX}{dt} \quad (3)$$

where each term has the units of W m^{-3}. Air moves only in the axial direction, with a constant velocity profile across the bed. The air and the moist solid at any particular location within the bed are assumed to be in thermal equilibrium, with the air saturated with water vapor. The factor $f\lambda$ arises since the evaporation of water to keep the air saturated gives the air a higher apparent heat capacity [13]. The last term of the energy balance assumes that metabolic heat generation is directly proportional to the production of new biomass. Maintenance metabolism is ignored as are effects of microbial growth on particle size and pressure drop across the bed.

Values for density, thermal conductivity and heat capacity of the bed were calculated as weighted averages of the properties of the air and substrate within the bed. Density and thermal conductivity were volume weighted, while heat capacity was mass-weighted:

$$\rho_b = \varepsilon\cdot\rho_a + (1-\varepsilon)\rho_s \quad (4a)$$

$$k_b = \varepsilon\cdot k_a + (1-\varepsilon)k_s \quad (4b)$$

$$C_{pb} = (\varepsilon\rho_a(C_{pa} + f\cdot\lambda) + (1-\varepsilon)\rho_s C_{ps})/\rho_b \quad (4c)$$

Implicit in these equations is the assumption that the thermal properties of the microorganism are equal to those of the substrate, and that these thermal properties and the void fraction do not change with time.

2.4. Boundary and initial conditions

The boundary conditions are as follows:

$$z = 0, \quad T = T_{in} \quad (5a)$$

$$z = H, \quad \frac{\partial T}{\partial z} = 0 \quad (5b)$$

Table 1
Parameter values used in the simulations with the mathematical model[a]

Parameter	Value	Source
b	6.275	[13]
C_{pa}	1180 J kg^{-1} °C^{-1}	[15]
C_{ps}	2500 J kg^{-1} °C^{-1}	[16]
f[b]	0.00246 kg water (kg air °C)$^{-1}$	[17]
k_a	0.0206 W m^{-1} °C^{-1}	[17]
k_s	0.3 W m^{-1} °C^{-1}	[16]
T_{in}	30°C	[14]
T_o	30°C	[14]
T_{opt}	35°C	[18]
T_{max}	52°C	[13,14]
X_o	0.001 kg dry biomass (kg initial wet substrate)$^{-1}$	[3]
X_m	0.125 kg dry biomass (kg initial wet substrate)$^{-1}$	[19]
Y	8.366×10^6 J (kg dry biomass)$^{-1}$	[3,11]
ε	0.35	[20]
λ	2 414 300 J (kg water)$^{-1}$	[15]
ρ_s	700 kg m^{-3}	[3]
ρ_a	1.14 kg m^{-3}	[21]

[a] The variables μ_{opt}, V_z and H were varied for the construction of Figs. 2–4. The values of these parameters are given in the figure legends or on the figures themselves.
[b] Fitted to data between 27 and 47°C.

These boundary conditions correspond to the bottom of the bed being maintained at the inlet air temperature and the absence of external cooling at the top of the bed.

At the beginning of the fermentation both the initial temperature (T_o) and the inoculum concentration (X_o) are assumed to be constant over the whole height (H) of the bed:

$$\text{at } t = 0 \quad T = T_o \quad 0 \le z \le H \quad (6a)$$

$$\text{at } t = 0 \quad X = X_o \quad 0 \le z \le H \quad (6b)$$

2.5. Parameter values

The parameters in Table 1 were estimated from various literature sources [3,11,13–21] by Sangsurasak and Mitchell [13] for the growth of *Aspergillus niger* on wheat bran in a packed-bed bioreactor, which was the system used by Ghildyal et al. [14]. In the current work variations were made to the parameters μ_{opt}, H and V_z.

2.6. Computational methods

Orthogonal collocation, using Jacobi polynomials, was used to discretize the spatial coordinate [22,23] leaving a set of ordinary differential equations. A total of 23 collocation points were used, including the two end points of the column. The equations were solved using the GEAR package [24].

2.7. Derivation of a modified Damköhler number

The modified Damköhler number characterizes the relative rates of heat production and removal at the time of peak heat production. The numerator is the peak heat production rate (Q_X). Assuming logistic growth kinetics and ignoring the effect of maintenance metabolism, the maximum heat generation rate will occur when the biomass concentration is half of the maximum biomass concentration. Substituting the logistic equation for dX/dt in the term for heat generation in Eq. (3) and putting $X = 0.5X_m$ gives:

$$Q_X = 0.25\rho_s(1-\varepsilon)Y\mu_{opt}X_m \quad (7)$$

The denominator (Q_R) describes the overall energy removal by axial convection and evaporation between the air inlet and outlet. As with the mathematical model, the air is assumed to be always saturated with water, leading to the appearance of the factor $f\lambda$ as described above in relation to Eq. (3):

$$Q_R = \rho_a(C_{pa} + f\lambda)\cdot V_z\cdot(T_{out} - T_{in})/H \quad (8)$$

The units of both Q_X and Q_R are J m^{-3} s^{-1}. Taking the ratio of these two terms gives a dimensionless number, the modified Damköhler number (Da_M):

$$Da_M = \frac{0.25\rho_s(1-\varepsilon)Y\mu_{opt}X_m}{\rho_a(C_{pa} + f\lambda)V_z(T_{out} - T_{in})/H} \quad (9)$$

Note that any measurement of the peak heat production rate can be used as the numerator in Eq. (9); it is not limited to the situation with logistic growth kinetics. For example, the numerator can be obtained experimentally by determining the maximum oxygen uptake rate in a culture growing at the optimum temperature for growth, and using well-known heat yield coefficients to convert this into the peak heat production rate [25].

3. Results

Tray, packed-bed, rotating drum, stirred-bed and air–solid fluidized bed bioreactors have been developed for various SSF processes. The best way to develop a new process is to test the performance in each of these bioreactor types at laboratory scale, in order to ensure that the bioreactor type chosen for larger scale is suitable for the microorganism. However, the current work focuses specifically on the scale-up of packed-bed bioreactors. Despite this, the approach used here is relevant to the design and scale-up of all bioreactor types, as discussed later. In the current work, the focus is on preventing undesirably high temperatures from occurring within the bed. The other scale-up issue identified by Saucedo-Castaneda et al. [11], namely the issue of maintaining the water content within a desirable range, is not addressed.

3.1. Predicting the maximum temperature reached in a 1-m high bioreactor

At times, an SSF bioreactor which is already available must be evaluated for use with a new microorganism. Therefore, the model is used to predict the maximum temperature reached in a 1-m high packed-bed bioreactor, as a function of the superficial velocity of the airflow, for a range of specific growth rates (μ_{opt}) that have been observed in SSF systems (Fig. 2) [11,26]. Note that this maximum temperature occurs at the air outlet at the top of the bed.

At the lowest superficial velocity of 0.02 m s^{-1}, temperatures close to the maximum temperature for growth are reached at all values of μ_{opt}. However, the maximum temperature in the bed decreases as the superficial velocity increases, with the degree of this decrease depending on μ_{opt}. At $\mu_{opt} = 0.1$ h^{-1} the maximum temperature decreases markedly with an increase in superficial velocity to 0.05 m s^{-1}. At $\mu_{opt} = 0.5$ h^{-1} the maximum temperature decreases only slowly as superficial velocity increases.

3.2. Predicting the critical bioreactor height

At other times, a larger scale SSF bioreactor must be designed on the basis of laboratory-scale studies. For this analysis, it is assumed that it is undesirable for any part of the bioreactor to reach a temperature 5°C above T_{opt}. This will be referred to as the critical temperature. For example, such a temperature might trigger sporula-

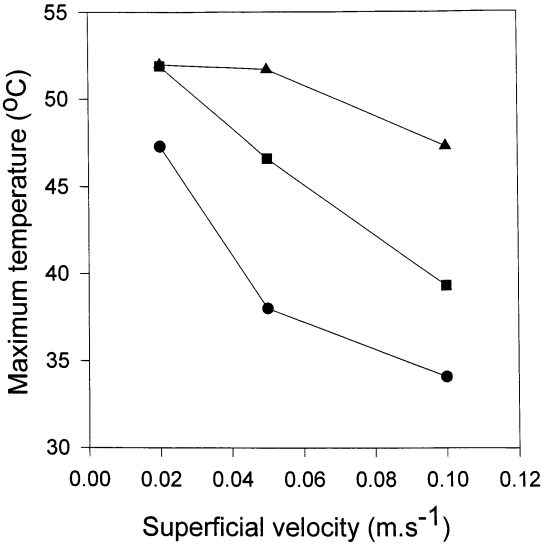

Fig. 2. The maximum temperature reached in a 1-m high packed-bed bioreactor, as a function of superficial air velocity through the bioreactor, as predicted by the mathematical model. Curves are plotted for the parameter values listed in Table 1, except that the specific growth rate at the optimal temperature (μ_{opt}) was varied: (●) 0.1 h^{-1}; (■) 0.236 h^{-1}; (▲) 0.5 h^{-1}.

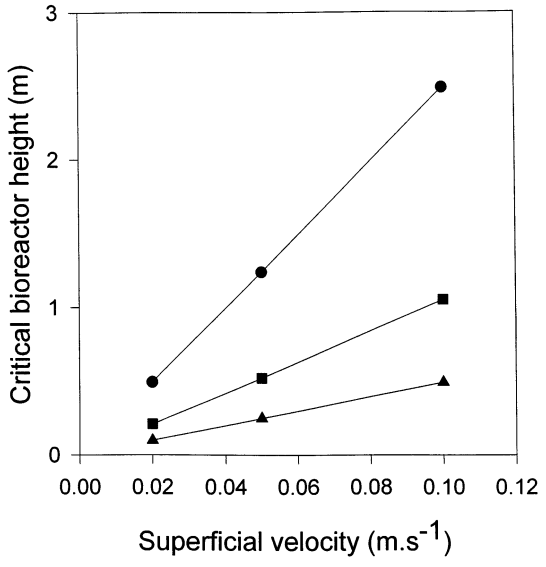

Fig. 3. Effect of superficial velocity on the maximum bed height that a packed-bed bioreactor can be without the top of the bioreactor overheating (i.e. the critical bed height), as predicted by the mathematical model. Curves are plotted for the parameter values listed in Table 1, except that the specific growth rate at the optimal temperature (μ_{opt}) was varied: (●) 0.1 h^{-1}; (■) 0.236 h^{-1}; (▲) 0.5 h^{-1}.

tion, or might have adverse effects on product formation. The critical temperature imposes a limit on the height of a packed-bed when it is operated as shown in Fig. 1: since the temperature increases steadily with height, the bed can be no higher than the height at which the critical temperature is reached during the fermentation. This height, which will be referred to as the critical height, is a key design factor which must be predicted by any scale-up method for packed-bed bioreactors operated as shown in Fig. 1.

Fig. 3 shows the predicted critical bed height as a function of superficial velocity and μ_{opt}. The critical bioreactor height is directly proportional to the superficial velocity, with the constant of proportionality depending on μ_{opt}. At $\mu_{opt} = 0.1\ h^{-1}$, the critical height increases by 2 m with an increase in the superficial velocity from 0.02 to 0.1 m s^{-1}. However, with $\mu_{opt} = 0.5\ h^{-1}$, the critical bed height only increases by 0.4 m with the same increase in superficial velocity. As a result, at high superficial velocities, significantly taller beds can be used if μ_{opt} is low. For example, with the superficial velocity of 0.1 m s^{-1}, the bed for $\mu_{opt} = 0.1\ h^{-1}$ can be 1.5 m taller than the bed for $\mu_{opt} = 0.236\ h^{-1}$.

3.3. Using dimensionless numbers as a simple tool to guide scale-up

The previous simulations have shown how a model can be used to construct operating diagrams which guide the scale-up process (Figs. 2 and 3). This section shows how the modified Damköhler number can be used to guide scale-up.

The parameters in Eq. (9) are easily determined. Physical property tables can be used for ρ_a, C_{pa}, f and λ. The substrate-dependent parameters ρ_s and ε and the growth kinetic parameters X_m and μ_{opt} can be determined experimentally. Typical values from the literature can be used for the heat yield from growth (Y) [24]. The design of the bioreactor sets H, and choice of the operating variables sets T_{in} and V_z. This leaves T_{out} as the only unknown on the right-hand side of Eq. (9).

As with the modelling approach, the modified Damköhler number can be used either to predict the performance of an existing bioreactor or to guide scale-up. To characterise an existing bioreactor, Da_M can be calculated with T_{out} set at the critical temperature. A value of Da_M greater than one indicates that at the time of peak heat generation the critical temperature will be exceeded at the outlet end of the bed. Alternatively, the Da_M number can be used to predict the maximum temperature attained in the bed for a particular set of design and operational parameters. Since the maximum temperature occurs at the top of the bed, it corresponds to T_{out}. For the temperature to be at a maximum, the rates of heat production and heat removal must be equal, and therefore the Da_M number must equal one. In this case, Eq. (9) can be rearranged to give an explicit expression for T_{out}, for which the right-hand side is known if the design and operational parameters have been chosen:

$$T_{out} = T_{in} + \frac{0.25\rho_s(1-\varepsilon)Y\mu_{opt}X_{in}}{\rho_a(C_{pa}+f\lambda)V_z/H} \quad (10)$$

For use in guiding scale-up, T_{out} can be set to the critical temperature and Eq. (10) can be rearranged to be explicit in H:

$$H = \frac{\rho_a(C_{pa}+f\lambda)V_z(T_{out}-T_{in})}{0.25\rho_s(1-\varepsilon)Y\mu_{opt}X_m} \quad (11)$$

The value calculated for H is the critical bioreactor height. As with the dynamic modelling approach described earlier, it is possible to use data obtained at small scale to predict the maximum height which should be used for a large-scale bioreactor, and to explore the effect of superficial velocity, inlet air temperature and specific growth rate on the value of this critical height. This was done with the parameters in Table 1, and the predicted critical bed heights are compared with those predicted by the dynamic modelling approach described earlier (Table 2). There is close agreement between the critical bed heights predicted by the dynamic modelling and Da_M approaches. Note that, as mentioned above for the dynamic model, the predictions based on the Da_M number are directly proportional to the superficial velocity, with the constant of proportionality depending on μ_{opt}. The

Table 2
Critical bed heights predicted by the mathematical model and the modified Damköhler number (Da_M)[a]

μ_{opt} (h^{-1})	$V_z = 0.02$ m s^{-1}		$V_z = 0.05$ m s^{-1}		$V_z = 0.1$ m s^{-1}	
	Da_M	Model	Da_M	Model	Da_M	Model
0.100	0.497	0.490	1.240	1.225	2.490	2.451
0.236	0.212	0.208	0.526	0.519	1.053	1.039
0.500	0.100	0.098	0.251	0.245	0.495	0.490

[a] Calculations were based on the parameter values listed in Table 1.

modified Damköhler number slightly underestimates the critical bed heights compared to the model, because it effectively assumes that all the biomass in the bed is growing at μ_{opt}, whereas in actual fact the biomass in regions where the temperature is above T_{opt} will grow at a lower rate. Therefore the Da_M approach leads to conservative design and operating conditions.

To this point, the analysis of scale-up has concentrated on predicting the critical bed height. Although this is the most important consideration, the capacity of the bioreactor also depends on the bed diameter. The question therefore arises as to how the bioreactor width might be varied during the scale-up process. Since geometric similarity is commonly used in simple approaches to scaling-up bioreactors for liquid culture [2], this strategy was explored using the Da_M number. Note that for a packed-bed bioreactor scaled-up on the basis of geometric similarity the height is proportional to the cube-root of the volume, while the mass of substrate contained in the bioreactor is directly proportional to the volume.

Fig. 4 shows the predicted results of scaling-up on the basis of geometric similarity for a packed-bed bioreactor with a height to diameter ratio of 1, while maintaining a constant superficial velocity, and with the parameter values as listed in Table 1. Plots of the temperature difference between the inlet and outlet of the column are shown for three superficial velocities. As Fig. 4 shows, if the superficial velocity is constant, as the volume increases the temperature rise over the bed increases in proportion to the cube-root of the volume (i.e. the temperature rise increases in direct proportion to the height). The important point is that geometric similarity can be maintained only until the temperature at the top of the bioreactor reaches the critical temperature. Any further increase in volume achieved by an increase in height will lead to the critical temperature being exceeded at the top of the bioreactor. Therefore once the critical height is reached, further increases in volume should be achieved only by increasing the bed diameter.

The temperature of the inlet air significantly affects the critical height. If T_{in} is set at T_{opt}, which has commonly been done with packed beds, a temperature rise over the column of only 5°C would give the critical temperature at the outlet. Alternatively, T_{in} can be set several degrees below the optimum temperature for growth, but there are practical limits on how low T_{in} can be. It must be sufficiently high to support reasonable specific growth rates, since the region near the base of the column will be maintained near T_{in} by the incoming air. To this point the current work has assumed a T_{in} of 30°C, 5°C below T_{opt}, which allows the temperature rise over the column to be 10°C. The horizontal dotted lines on Fig. 4 indicate 5 and 10°C rises over the column. Doubling the allowable temperature rise from 5 to 10°C enables the bioreactor volume to be 8-fold larger. For example, with a superficial velocity of 0.1 m s^{-1}, a temperature rise of 5°C gives a critical bioreactor volume of 0.11 m^3, while a temperature rise of 10°C gives a critical bioreactor volume of

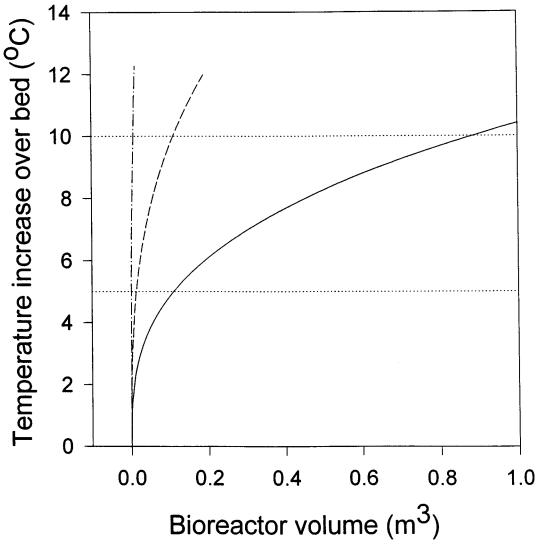

Fig. 4. Effect of bioreactor volume on the temperature increase over a packed-bed scaled-up according to geometric similarity and a constant superficial velocity, as predicted by the modified Damköhler number. Note that, since a height to diameter ratio of 1 is assumed, the height can be calculated from the volume as $H = (4V/\pi)^{0.333}$. Also note that the temperature increase plotted on the ordinate corresponds to $(T_{out} - T_{in})$ in Eq. (10). Curves are plotted for a specific growth rate of 0.236 h^{-1} and for three superficial velocities: (–·–·–) 0.02 m s^{-1}; (- - -) 0.05 m s^{-1}; (—) 0.1 m s^{-1}.

0.88 m^3. This 8-fold difference in bioreactor volume corresponds to a 2-fold greater bed height.

The Da_M number ignores the effect of T_{in} on the rate of evaporation in the bed. In any case evaporation is only indirectly related to T_{in}. Since the inlet air is always saturated, the absolute humidity of the inlet air is different for different T_{in} values. However, due to the essentially linear relationship between air temperature and saturation humidity over the temperature range of interest, the rate of evaporation depends only on the temperature gradient across the bed, and not on the absolute value of T_{in}. The indirect effect of T_{in} on evaporation arises in actual fermentations because the absolute value of T_{in} affects the spatially averaged heat production rate in the bed, through the effects of bed temperature on the specific growth rate of the microorganism. This heat production rate in turn affects the temperature gradient across the bed, which controls the evaporation rate. However, these effects are small if the value of T_{in} is reasonably close to T_{opt}, such that temperature has relatively little effect on specific growth rate. In any case, the Da_M number avoids such complexities by giving the most conservative design: it assumes that the whole of the bioreactor is at T_{opt}, and therefore is producing heat at the maximum possible rate.

The effect of scale-up on the volumetric flowrate of air required is important in guiding the design of the aeration system. If a packed-bed is scaled-up on the basis of geometric similarity and constant superficial velocity, then the volumetric flowrate required is proportional to the cross-sectional area of the bed. Until the critical height is reached, the area increases in proportion to $V^{0.667}$. After the critical height is reached and the bed is enlarged only by an increase in diameter, the area increases in direct proportion to V.

Since the critical height of the bioreactor is strongly influenced by superficial velocity, it is likely that superficial velocity would be increased rather than being held constant during scale-up, especially if quite a low superficial velocity is used in the laboratory scale studies. Eq. (10) suggests that, if V_z is increased in direct proportion to the height, the maximum temperature attained at the top of the bed will remain constant with scale, and therefore there are no apparent limits on the height of the bed. However, as discussed later, such a strategy is likely to quickly lead to the superficial velocity reaching practical or economical limits. If the superficial velocity is increased in direct proportion to height for a packed-bed which is scaled up on the basis of geometric similarity, then the required volumetric flow rate always increases in direct proportion to the volume.

4. Discussion

4.1. Comparison of the modelling and Da_M number approaches to scale-up

Although both the dynamic modelling and Da_M approaches give similar predictions, in practice the modelling approach will be more accurate if the specific growth rate varies significantly with temperature, or if significant death occurs at temperatures above the optimal temperature but below the critical temperature, because such effects can be incorporated into the model equations. Also, because the modelling approach incorporates all heat transfer mechanisms, it will be more robust than the Da_M number approach. For example, if the dominant heat removal mechanism changes with scale and the dominant mechanism at large scale is not incorporated into the Da_M number, then the Da_M number approach will not lead to successful large scale designs.

The modelling approach has other advantages. The dynamic model provides detailed predictions, being able to make predictions about the temperature at any time and position within the column. The Da_M number approach simply characterizes the overall energy balance over the column. Also, the model can describe columns of any geometry if the two-dimensional version developed by Sangsurasak and Mitchell [4] is used, whereas the Da_M number approach does not describe systems in which radial conduction is significant, such as the thin column used by Saucedo-Castaneda et al. [3]. On the other hand, the Da_M number provides a simpler approach, suitable for practitioners who do not have the resources to set up and solve models involving partial differential equations.

Both the modelling and Da_M number approaches will fail if they do not account for important phenomena. For example, in some SSF systems the structural polymers of the substrate particle are degraded by the microorganism, causing the substrate particles to shrink and the whole bed to compact [27]. Until such effects are incorporated into the Da_M number and the mathematical model, their predictions will be most accurate for those processes in which the structural polymers of the substrate are not degraded by the microorganism.

4.2. Scale-up of packed-beds

The usefulness of both the modelling and Da_M number approaches to designing packed-bed bioreactors depends on the accuracy with which the parameters and variables involved can be measured. The measurement of system parameters and variables can present difficulties in SSF, however, previous experimental work with packed beds [3,11,14] indicates that all the necessary parameters and variables can be measured

with sufficient accuracy. In fact, inaccuracies in predictions introduced by inaccuracies in measurement are likely to be smaller than those introduced by assumptions and simplifications. However, despite the many assumptions and simplifications in the model, previous work [13] showed that the model can predict bioreactor performance in different systems [3,14], giving confidence that both it and the Da_M approach represent useful tools in guiding the design and scale-up of packed bed bioreactors.

Although the analyses have been done for a particular microorganism and substrate combination, the dynamic modelling and modified Damköhler number approaches can both readily be used for other combinations as long as the appropriate parameters are available. Once they have been determined, it is possible to predict the critical height of the bed, which depends on the value of the superficial velocity chosen for full scale operation. The higher the superficial velocity, the taller the full-scale bioreactor can be, and therefore the more substrate the bioreactor can hold per unit of occupied floor space. In fact, if V_z/H is maintained constant there are no predicted limits on bed height. However, the superficial velocity cannot be increased indefinitely. Growth of fungal mycelium into the interparticle spaces causes increases in the pressure drop during the fermentation [28] and, therefore, practical limits on superficial velocity might be imposed by the maximum pressure drop through the bed with which the aeration system can cope. Furthermore, high pressure drops can promote the formation of vertical cracks in the bed, in the phenomenon of 'air-channelling'. If this happens, air flows preferentially through these cracks, meaning that the convective air flow only replenishes oxygen at the walls of the cracks. Oxygen replenishment in other regions of the bed is limited to diffusion. Even if pressure drop is not of concern, if the bed is to be static, the superficial velocity must be kept below the minimum fluidization velocity. Unfortunately, there is no information in the literature about practical limits on superficial velocities in packed-beds, and as a result it is not possible to mark these limits on Fig. 3.

4.3. General applicability of the Da_M approach to scale-up

The Da_M number proposed in the current work is specific for static packed-bed bioreactors. Other bioreactor designs, such as stirred beds, rotating drums and air–solid fluidized beds provide intermittent or continuous agitation, and can be used with microorganisms that can tolerate mixing. In its current form the Da_M number does not apply to these agitated reactors because mixing affects the heat transfer phenomena within the system. Even with packed-beds which are only intermittently agitated and therefore operate as static packed-beds for the majority of the time, the current Da_M number has only limited usefulness, because the intermittent agitation can lead to complex heat transfer behaviour [29].

Although the Da_M number itself is limited to static packed-bed bioreactors, the approach used to develop the modified Damköhler number can be extended to other SSF bioreactors and other organisms. Assuming that heat removal is the key problem, a four-step process can be used to derive an appropriate Da_M number. The first step is to identify the major heat transfer mechanisms in the bioreactor, being aware that the major mechanisms may change with scale, and to use a simplified expression to estimate the heat removal rate. Secondly, the growth kinetics of the microorganism must be determined and a simplified expression used to estimate the peak heat generation rate. Thirdly, the Da_M number is constructed by taking the ratio of the heat production and heat removal terms. Finally, with variation of the key operating and design variables, the Da_M number can be used to investigate strategies to prevent overheating. Of course if mass transfer rather than heat transfer limits bioreactor performance, the general approach is still valid. For example, a dimensionless number could be constructed as the ratio of the maximum rate of oxygen uptake to the maximum rate of oxygen supply.

4.4. Experimental programs for scale-up of packed-beds

The analyses suggest a useful experimental program for the scale-up of packed-bed bioreactors. Firstly, the laboratory scale studies to determine the growth kinetic parameters should be done in a bioreactor small enough that the kinetics of growth are not confounded by heat and mass transfer effects. An appropriate bioreactor is the 20-cm^3 packed-bed (1.8 cm diameter and 8 cm height) of Gutierrez-Rojas et al. [30], immersed in a constant temperature waterbath. A large number of such bioreactors can be operated simultaneously, with a whole bioreactor sacrificed at each sampling time. With such a thin diameter, the superficial velocity used is not a critical parameter, as long as aeration needs are met, because the contribution of conductive heat removal through the bioreactor walls minimizes axial temperature gradients [3]. However, it is desirable to have the same superficial velocity of airflow through each bioreactor.

Next, laboratory-scale studies should be done to characterize the efficiency of the heat transfer processes, and to identify operating conditions which prevent overheating. These studies should be done in a slightly larger column, such as the column used by Ghildyal et al. [14], which had a height of 34.5 cm and a diameter of 15 cm. However, rather than having a water jacket

as they used, the column walls should be insulated to mimic the situation at large scale where radial heat transfer will be negligible. Before proceeding to larger scales, a judgement needs to be made about the superficial velocity to be used at larger scale. Research is required into the pressure drops occurring in large-scale packed-beds before rational guidelines for choosing this value can be proposed.

The next step is to design a pilot-scale bioreactor with a height equal to the critical height. This critical height will be determined by the values chosen for the superficial velocity and the temperature of the inlet air. Note that if the superficial velocity is greater than that used at laboratory scale, then the scaling-up to the pilot scale reactor will not follow one of the lines on Fig. 4, but rather will move across the graph towards the right from one superficial velocity line to another. The diameter of the pilot scale bioreactor could be maintained the same as the laboratory scale bioreactor, although if there are significant wall effects on the substrate packing and air flow, then it would be better to maintain geometric similarity since this will lead to a wider pilot bioreactor, thereby decreasing the relative contribution of the wall effects. After carrying out pilot scale studies to confirm that the top of the bioreactor never exceeds the critical temperature, then the full-scale bioreactor can be built simply by maintaining the bed height and superficial velocity constant and increasing the width of the bed.

This strategy means that the pilot-scale bioreactor has the full height of the final production bioreactor, but it represents a thinner vertical column cut out of the full scale reactor. An advantage of this approach is that phenomena which depend on reactor height, such as temperature profiles and the pressure drop, can be studied at full scale in the pilot bioreactor. The main challenge in moving from a successful pilot reactor to a full-scale reactor is to ensure an even airflow through the wider bed.

4.5. Comparing with other approaches to scaling-up packed-bed bioreactors

Mathematical models have not previously been used to guide the actual scale-up of SSF bioreactors, although simple approaches to scale-up of packed-beds have been suggested, and a heat transfer model has been used in a theoretical exploration of the effects of operating variables on the operation of a small scale packed-bed [4].

The modified Damköhler number used in the present work is more useful in guiding the scale-up of packed-bed bioreactors than the Peclet and Biot numbers proposed by Saucedo-Castaneda et al. [3]. The Biot number characterizes the relative rates of radial conduction to the bioreactor wall and convection away from the wall. However, radial conduction will be a minor contributor to the overall heat removal from full-scale packed-beds operated as in Fig. 1, and therefore the Biot number has no significance. The Peclet number reflects the relative contributions of axial conduction and axial convection. However, conduction typically contributes less than 10% of the overall heat removal [31]. Also, evaporation is quite important, as demonstrated by the relative magnitudes of C_{pa} (1180 J kg^{-1} $°C^{-1}$) and $f\lambda$ (5939 J kg^{-1} $°C^{-1}$) but it is not reflected in the Peclet number at all. Therefore the Peclet number yields relatively little information.

Although they did not propose it as a scale-up criterion, Saucedo-Castaneda et al. [3] used the Damköhler III (Da_{III}) number in their energy balance over a packed-bed bioreactor. Since it is a ratio of heat production and axial convection terms, it does present itself as a potential tool to guide scale-up. In notation consistent with the current work, their Da_{III} number was:

$$Da_{III} = \frac{Q_{XO}}{(V_z \rho_a C_{pa} T_s / H)} \tag{12}$$

where Q_{XO} is the initial rate of heat production from growth, V_z is the superficial velocity of the airflow, ρ_a and C_{pa} are the density and heat capacity of the air, T_s is the temperature of the surroundings and H is the height of the bioreactor. However, this unmodified Damköhler number does not include the contribution of evaporation, which contributes about two-thirds of the heat removal [31]. Furthermore Saucedo-Castaneda et al. [3] based the heat production term (Q_{XO}) on an initial rate, whereas, in order to guide scale-up, it is more appropriate to estimate the peak heat production rate.

The approach to scale-up proposed in the current work builds directly from the concepts proposed by Saucedo-Castaneda et al. [11], who pointed out that if heat and water balance equations are written and equated to zero, and if this equality with zero can be maintained with scale, then constant temperatures and moisture contents can be maintained. After being equated to zero, their energy balance was:

$$0 = Q_X + Q_A - Q_V - Q_E \tag{13}$$

where the $Q_{subscript}$ terms represent the rates of heat generation and transfer in the system. Subscript A denotes agitation, subscript V denotes evaporation, and subscript E denotes exchange with the surroundings. In their experiments similar performance was obtained over a 410-fold increase in scale from 10 g to 4.1 kg. However, they did not explain how they used this equation in the scale-up process, and Eq.

(13) was not expanded out in terms of system parameters. While Eq. (13) is sufficient to illustrate the concept of maintaining energy balances, in itself it is not of practical use in guiding scale-up. The current work has selected the important terms in the energy balance, stated them in terms of system parameters, and demonstrated how in practical terms they can be used to guide scale-up.

The modified Damköhler number approach also has similarities with the work of Rodriguez Leon et al. [32]. They wrote a simple overall energy balance for SSF bioreactors with forced aeration and rearranged it to give an expression to calculate the superficial velocity required to keep the temperature of the bioreactor at a desired value:

$$V_z = \frac{R_X - hA(T_{out} - T_{in})}{1004(T_{out} - T_{in}) + \lambda(H_{out} - H_{in})} \quad (14)$$

where R_X is the overall rate of heat production in the bioreactor, h is the heat transfer coefficient for conduction through the bioreactor walls, A is the area for conductive heat transfer, and H_{in} and H_{out} are the humidities of the air at the air inlet and outlet, respectively. The value 1004 (J m^{-3} $°C^{-1}$) is the product of ρ_a and C_{pa}. As in the current work, the air was assumed to be saturated with water at both the air inlet and air outlet.

Eq. (14) is valid for any SSF bioreactor with forced aeration, it is not limited to packed-bed bioreactors. However, although Rodriguez Leon et al. [32] recognized that their equation could be used to guide the design and operation of bioreactors, they did not describe how this might be done in practice and they did not explore the implications for scale-up. In any case, their equation is less flexible than the Da_M number, because it is necessary to know the outlet temperature in order to be able to insert a value for the outlet humidity. This is no problem if the outlet temperature is chosen and Eq. (14) is used to calculate a superficial velocity. However, if the superficial velocity is set and it is necessary to calculate the outlet temperature, in the manner shown in Eq. (10) above, then H_{out} will also be unknown. This is not an insurmountable problem, because Eq. (14) can be used in a trial and error solution with the aid of humidity charts. The advantage of the Da_M number is that it avoids the need for a trial and error solution by assuming a linear relationship between the air temperature and its humidity. This linear approximation of the humidity curve, which in fact follows an exponential relationship as described by the exponential Antoine equation, is valid over the relatively small temperature ranges of 20°C experienced in SSF bioreactors. For example, linear regression of the humidity curve between 27 and 47°C gives a correlation coefficient of 0.989.

5. Conclusions

Due to the heat transfer characteristics of packed-beds, which lead to increasing temperatures with height, the bioreactor height is a key design feature for which the scale-up method must provide guidance. The critical bed height, defined as the maximum height the bed can be while avoiding overheating at the top of the bed, is determined by the growth kinetics of the organism and two operating variables, namely the temperature of the inlet air and the superficial velocity. However, there are practical limits as to how far V_z can be increased or how far T_{in} can be decreased. Further investigation is required to define these limits.

We have developed two approaches to scaling-up packed-beds to prevent overheating, one based on mathematical modelling, and the other based on a modified Damköhler number. Geometric similarity can be used to scale-up bioreactors using laboratory scale results, but only until the critical height is reached. After that, further increases in scale can only be achieved by increasing the width of the bed. This approach to scale-up of packed-bed bioreactors can be adapted for other SSF bioreactors.

6. Notation

A	area for conductive heat transfer (m^2)
b	sensitivity of growth kinetics to increase in temperature (°C)
C_{pa}	heat capacity of moist air (J kg^{-1} $°C^{-1}$)
C_{pb}	heat capacity of bed (J kg^{-1} $°C^{-1}$)
C_{ps}	heat capacity of substrate (J kg^{-1} $°C^{-1}$)
Da_{III}	Damköhler III number (dimensionless)
Da_M	modified Damköhler number (dimensionless)
f	rate at which the water-carrying capacity of air varies with temperature (kg water (kg air) $^{-1}$ $°C^{-1}$)
h	conductive heat transfer coefficient (W m^{-2} $°C^{-1}$)
H	bed height (m)
H_{in}	humidity of the inlet air (kg water (kg air)$^{-1}$)
H_{out}	humidity of the outlet air (kg water (kg air)$^{-1}$)
k_a	thermal conductivity of moist air (W m^{-1} °C $^{-1}$)
k_b	thermal conductivity of the bed (W m^{-1} °C $^{-1}$)
k_s	thermal conductivity of the substrate (W m^{-1} °C $^{-1}$)
Q_A	volumetric rate of heat transfer to the bed through agitation (W m^{-3})
Q_E	volumetric rate of heat transfer through the bioreactor walls (W m^{-3})
Q_R	volumetric rate of heat removal (W m^{-3})

Q_X volumetric rate of heat production by metabolism (W m^{-3})
Q_{XO} initial volumetric rate of heat production by metabolism (W m^{-3})
Q_V volumetric rate of heat transfer from the bed by evaporation (W m^{-3})
R_X overall rate of heat production by metabolism (W)
t fermentation time (s)
T bed temperature (°C)
T_{in} temperature of inlet air (°C)
T_{max} maximum temperature for growth (°C)
T_o initial bed temperature (°C)
T_{opt} optimum temperature for growth (°C)
T_{out} temperature of the outlet air (°C)
T_s temperature of the surroundings (°C)
V bioreactor volume (m^3)
V_z superficial velocity (m s^{-1})
X biomass concentration (kg dry biomass (kg initial wet substrate)$^{-1}$)
X_m maximum biomass concentration (kg dry biomass (kg initial wet substrate)$^{-1}$)
X_o initial biomass (kg dry biomass (kg initial wet substrate)$^{-1}$)
Y metabolic heat yield coefficient (J (kg dry biomass)$^{-1}$)
z axial position (m)

ε void fraction
λ enthalpy of vaporization of water (J kg^{-1})
μ specific growth rate (s^{-1})
μ_{opt} specific growth rate at the optimum temperature (s^{-1})
ρ_a density of moist air (kg m^{-3})
ρ_b density of bed (kg m^{-3})
ρ_s density of substrate (kg m^{-3})

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