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A Model of the Effect of the Microbial Biomass on the Isotherm of the Fermenting Solids in Solid-State Fermentation

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Summary

We compare isotherms for soybeans and soybeans fermented with *Rhizopus oryzae*, showing that in solid-state fermentation the biomass affects the isotherm of the fermenting solids. Equations are developed to calculate, for a given overall water content of the fermenting solids, the water contents of the biomass and residual substrate, as well as the water activity. A case study, undertaken using a mathematical model of a well-mixed bioreactor, shows that if water additions are made on the basis of the assumption that fermenting solids have the same isotherm as the substrate itself, poor growth can result since the added water does not maintain the water activity at levels favorable for growth. We conclude that the effect of the microbial biomass on the isotherm of the fermenting solids must be taken into account in mathematical models of solid-state fermentation bioreactors.

Key words: corn, isotherms, mathematical modelling, *Rhizopus oryzae*, soybeans, SSF

Introduction

Solid-state fermentation (SSF) involves the growth of microorganisms on moist solid particles with a minimum of free water in the inter-particle spaces. Due to the particular environmental conditions imposed on the microorganism, this fermentation technique has the potential to produce selected microbial products better than submerged liquid fermentation (*1*). However, SSF processes studied in the laboratory are rarely scaled-up to commercial scale. One of the major barriers is the difficulty in controlling the water content and temperature of the bed in large-scale bioreactors. Over the last 15 years mathematical models have been developed with the intention of using them as tools to identify SSF bioreactor design and operating strategies that can overcome these difficulties (*2*).

The first models focused on predicting the bed temperature and many of them avoided the need to incorporate water balances by assuming that water was added continuously to keep the water activity of the fermenting solids at the optimum value for growth. However, this assumption is not appropriate for those bioreactors in which the bed remains static for long periods since it is not practical to add water uniformly to a static bed. More recent models have not only included water balances, but have also described how the growth rate of the process microorganism is affected by the amount of water in the solids (*3*). In this case, the isotherm of the solids must be known since the water balance equation

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calculates the water content of the solids, but the driving force for evaporation and the growth rate of the process microorganism are related to the water activity.

Almost all current models assume implicitly that the isotherm of the fermenting solids (*i.e.* the mixture of growing biomass and residual substrate) is equal to the isotherm of the substrate itself. The current paper shows that this is not true and develops a method to calculate the water activity of the fermenting solids for a given overall water content. It then uses a modelling case study to demonstrate that the effect of the biomass on the isotherm needs to be taken into account in mathematical models of SSF bioreactors.

Material and Methods

Microorganism and media

Spore suspensions (10^7 spores/mL) of *Rhizopus oryzae* ATCC 34612 were prepared from fresh cultures grown on potato dextrose agar at 34 °C. Starch agar contained, per 100 mL of 0.038 M phosphate buffer (pH=7): cassava starch 3 g, $(NH_4)_2SO_4$ 1 g, agar 3 g and 0.25 mL of a commercial plant fertilizer (Ouro Verde).

Preparation of soybeans and biomass

Dehulled soybeans were soaked in excess water for 24 h, drained, and then autoclaved at 121 °C for 15 min. There were three different treatments. Firstly, for the soybean isotherm, the autoclaved soybeans were used directly. Secondly, for the isotherm of the fermented solids, 1 mL of spore suspension was added to 3 g of soybeans. Thirdly, for the biomass isotherm, 0.1 mL of spore suspension was spread over the surface of starch agar plates, which were incubated at 34 °C for 68 h. At this time a thick mat of biomass had completely covered the surface of the agar; this biomass mat was peeled from the agar. Isotherms of these treated samples were then determined as described below.

Determination of isotherms

Soybeans, fermented solids and biomass were placed on supports above salt solutions within hermetically-sealed jars, which were incubated at 34 °C. The water activities of saturated and unsaturated salt solutions at this temperature were taken from the literature, being 0.990, 0.953, 0.932 and 0.913 for NaCl solutions of 0.3, 1.4, 2.0 and 2.5 molal, respectively, and 0.977, 0.902, 0.850 and 0.802 for saturated solutions of K_2SO_4, $BaCl_2$, KCl and $(NH_4)SO_4$, respectively (*4,5*). Equilibration took 21 days for samples involving soybeans and 9 days for biomass mats. Samples were then dried at 55 °C to constant mass. All points represent means of triplicates.

Modelling

A model of a well-mixed bioreactor, such as that shown in Fig. 1, was used to show the consequences of assuming that the fermenting substrate has the same isotherm as the substrate itself. A well-mixed bioreactor was selected since for demonstration purposes there is no advantage of using a more complex model to predict temperature and moisture gradients within a static bed.

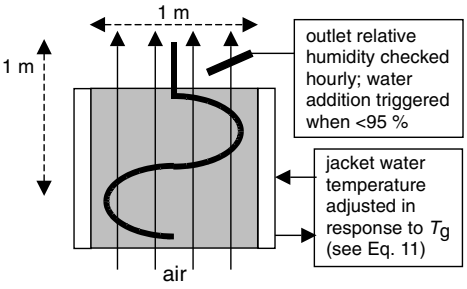

Fig. 1. The well-mixed bioreactor with key details of the operating strategy

The model has many similarities with the model of dos Santos *et al*. (*6*), however, instead of assuming that the solid and air phases are in equilibrium with one another, the model treats the air and gas phases as different subsystems, in the same manner as was done in the mathematical model of von Meien and Mitchell (*7*). Since the model is simply an adaptation of models that have been described in detail previously, the development of the model equations is not described here. Rather, Eqs. 1 to 16 of the model are listed in Table 1, while Table 2 explains the symbols and lists parameter values and initial values of variables.

To solve the model, the quantities shown in parentheses after the equation names in Table 1 were first isolated on the left hand side. The equations were then integrated numerically using the FORTRAN subroutine DRKGS, which uses a fourth-order Runge Kutta algorithm with automatic step size adjustment (*8*).

Results

Isotherms of soybeans, fermented solids and biomass

For a given water activity, the water content of soybeans fermented with *Rhizopus oryzae* (*i.e.* fermented solids) is higher than that of the soybeans themselves, indicating that the presence of the fungal biomass affects the isotherm (Fig. 2). The scatter in the results for the fermented solids is due to the fact that the biomass content within these solids varied. Presumably the equilibrium water content for a given water activity depends on the biomass content of the sample.

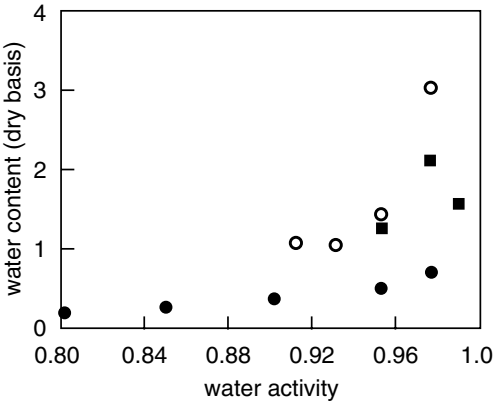

Fig. 2. Isotherms at 34 °C of (●) soybeans, (■) fermented solids (*i.e.* inoculated with *Rhizopus oryzae*) and (❍) biomass of *Rhizopus oryzae*

Table 1. Model equations

Bioreactor wall energy balance (T_b)

$$BC_{Pb}\frac{dT_b}{dt} = h_{gb}(T_g - T_b)A\varepsilon + h_{sb}(T_s - T_b)A(1-\varepsilon) - h_{bw}(T_b - T_w)A \quad /1/$$

Gas phase energy balance (T_g)

$$G(C_{Pg} + HC_{Pv})\frac{dT_g}{dt} = F_{in}C_{Pg}(T_{in} - T_g) + h(T_s - T_g)V - h_{gb}(T_g - T_b)A\varepsilon + F_{in}C_{Pv}(H_{in}T_{in} - HT_g) \quad /2/$$

Gas phase water balance (H)

$$\frac{d(GH)}{dt} = F_{in}(H_{in} - H) + K(W - W_{sat})V \quad /3/$$

Solid phase energy balance (T_s)

$$M(C_{Pm} + WC_{Pw})\frac{dT_s}{dt} = Y_{QX}\frac{dX}{dt} - h(T_s - T_g)V - K(W - W_{sat})\lambda V - h_{sb}(T_s - T_b)(1-\varepsilon)A \quad /4/$$

Solid phase water balance (W)

$$\frac{d(MW)}{dt} = Y_{WX}\frac{dX}{dt} - K(W - W_{sat})V \quad /5/$$

Growth

$$\frac{dX}{dt} = \mu X\left(1 - \frac{X}{X_m}\right) \quad /6/$$

Consumption of dry solids

$$\frac{dM}{dt} = \left(1 - \frac{1}{Y_{XS}}\right)\frac{dX}{dt} \quad /7/$$

Water-activity-based growth dependence

$$\mu_{FW} = 1.0538\exp(-131.6a_w^3 + 94.996a_w^2 + 214.219a_w - 177.668) \quad /8/$$

Temperature-based growth dependence

$$\mu_{FT} = \frac{8.31\times10^{11}\exp\left(\frac{-70225}{R(T_s + 273)}\right)}{1 + 1.3\times10^{47}\exp\left(\frac{-283356}{R(T_s + 273)}\right)} \quad /9/$$

Specific growth rate constant

$$\mu = \mu_{opt}(\mu_{FW}\mu_{FT})^{0.5} \quad /10/$$

Temperature of water in the jacket

$$T_w = T^* - (T_s - T^*) \quad /11/$$

Solids-air mass transfer coefficient

$$K = (2.469 - 0.0177T_g)W - 0.514 + 0.00618T_g \quad /12/$$

Solids-air heat transfer coefficient

$$h = 44209.85\left(\frac{4F_{in}}{\pi B_D^2}\frac{(T_g + 273)}{0.0075P}\right)^{0.6011} \quad /13/$$

Handerson equation (inverse direction)

$$W^* = \left(-\frac{\ln(1 - a_w)}{a}\right)^{\frac{1}{b}} \quad /14/$$

Water content of the fermenting solids for a given water activity

$$W^* = (1 - X)\left(\frac{\ln(1 - a_w)}{-a}\right)^{\frac{1}{b}} + X\left(\frac{\ln(1 - a_w)}{-c}\right)^{\frac{1}{d}} \quad /15/$$

Mass of water to be added to the bed

$$M_W = (W_{target} - W)M \quad /16/$$

Table 2. Values used for the simulation

	Significance	Value and unit
a	Parameter of Handerson equation for substrate	4.9888 (soybeans) 25.49780 (corn)
A	Area for heat transfer across bioreactor wall	3.14 m^2
a_{wg}*	Outlet gas humidity set point	0.95
a_{wg}	Gas phase water activity	0.99 (initial value)
a_{wgin}	Water activity of the inlet air	0.5
a_w	Fermenting solids water activity	0.99 (initial value)
a_{ws}	Water activity of the residual substrate	Eq. 17
a_{wx}	Water activity of the biomass	Eq. 18
b	Parameter of Handerson equation for substrate	0.7202 (soybeans) 1.557454 (corn)
B	Total mass of bioreactor wall	122.8 kg
B_D	Bed diameter	1.0 m
c	Parameter of Handerson equation for biomass	2.5503
C_{Pb}	Bioreactor wall heat capacity	420 J/(kg· °C)
C_{Pg}	Heat capacity of dry air	1000 J/(kg· °C)
C_{Pm}	Dry matter heat capacity	2500 J/(kg· °C)
C_{Pv}	Water vapor heat capacity	1791 J/(kg· °C)
C_{Pw}	Heat capacity of liquid water	4184 J/(kg· °C)
d	Parameter of Handerson equation for biomass	0.3596
F_{in}	Dry air flow rate	0.015 kg/s
G	Mass of dry gas in the inter-particle spaces	0.448 kg
h	Solids-air heat transfer coefficient	Eq. 13, W/(m^3· °C)
h_{bw}	Wall/jacket heat transfer coefficient	200 W/(m^2· °C)
h_{gb}	Gas/wall heat transfer coefficient	200 W/(m^2· °C)
h_{sb}	Solids/wall heat transfer coefficient	200 W/(m^2· °C)
H	Gas phase humidity	0.018 kg/kg (initial value)
H_{in}	Humidity of the inlet air	0.018 kg/kg
K	Solids-air mass transfer	Eq. 12, kg/(s·m^3)
M	Total dry solids	177.1 kg (initial value)
M_W	Mass of water to be added to the bed	Eq. 16, kg
P	Overall pressure	760 mm Hg
R	Universal gas constant	8.314 J/(mol· °C)
t	Time (independent variable)	0 h (initial value)
T_b	Bioreactor wall temperature	35 °C (initial value)
T_g	Gas phase temperature	35 °C (initial value)
T_{in}	Inlet air temperature	35 °C
T_s	Fermenting solids temperature	35 °C (initial value)
T_w	Cooling water temperature	35 °C (initial value)
T*	Set point (Eq. 11)	35 °C
V	Overall bed volume	0.785 m^3
W	Water content of the fermenting solids, dry basis	0.895 kg/kg (initial value)
W*	Calculated solids water content, dry basis	Eqs. 14 and 15, kg/kg
W_s	Water content of the residual substrate, dry basis	Eq. 20
W_{sat}	Water content that the solids need to have in order to be in equilibrium with the gas phase, dry basis	Eq. 15, kg/kg
W_{target}	Water content for the solids to be at the water activity that they had at zero time, dry basis	Eq. 14 or 15 as indicated in Table 3, kg/kg
W_x	Water content of the biomass, dry basis	Eq. 21, kg/kg
X	Biomass content of the fermenting solids	0.002 kg/kg (initial value)
X_m	Maximum biomass content of the fermenting solids	0.25 kg/kg
Y_{QX}	Heat yield from growth	8.37 MJ/kg
Y_{WX}	Water yield from growth	0.3 kg/kg
Y_{XS}	Biomass yield from substrate	0.5 kg/kg
ε	Effective bed porosity	0.5
λ	Enthalpy of water vaporization	2.414 MJ/kg
μ	Specific growth rate constant	Eq. 10, h^{-1}
μ_{FW}	Fractional specific growth rate based on water activity	Eq. 8, dimensionless
μ_{FT}	Fractional specific growth rate based on temperature	Eq. 9, dimensionless
μ_{opt}	Optimal value of the specific rate constant	0.236 h^{-1}

One possible strategy to quantify the effect of the biomass on the isotherm would be to ferment the soybeans for different times and then to measure the amount of biomass before determining the isotherm. However, this strategy is not feasible due to the impossibility of obtaining a reliable estimate of the biomass. Direct separation of the biomass from the residual beans is not possible since the mycelium is tightly attached. Several indirect methods for biomass estimation were considered but were not feasible. Firstly, protein measurements cannot be used since both the fungal biomass and the soybeans contain protein and, since the fungus degrades soybean protein, it would be impossible to determine the amount of new fungal protein from global protein measurements. Secondly, the glucosamine content of the biomass varies markedly during the growth cycle (*9*), as also occurs for the related species *R. oligosporus* (*10*), making it impossible to obtain reliable estimates of the amount of biomass from the glucosamine levels in the fermenting substrate. Given these difficulties, we determined the isotherms of soybeans and biomass separately and then used a mathematical technique (described in the next section) to estimate the water activity of a sample of fermented solids from the overall water content.

For a given water activity, the equilibrium water content of biomass is significantly higher than that of soybeans (Fig. 2). For example, at a water activity of 0.98, which might typically be maintained in SSF processes involving species of *Rhizopus*, the water content of biomass is four times greater than that of soybeans.

Mathematical technique for combining the isotherms of soybeans and biomass

Mathematical models of SSF bioreactors typically calculate the dry biomass content of the dry solids (X, kg/kg), the overall dry solids (M, kg) and the overall water content on a dry basis (W, kg/kg). This information can be used to determine the water activity of a sample for a given overall water content. Firstly, the Handerson equation is used to describe the isotherm of soybeans and biomass, respectively (*11*):

$$a_{ws} = 1 - \exp(-a W_s^b) \qquad /17/$$

$$a_{wx} = 1 - \exp(-c W_x^d) \qquad /18/$$

where a, b, c and d are fitting constants, W_s and W_x are the water contents on a dry basis (kg/kg) of the soybeans and biomass, respectively, and a_{ws} and a_{wx} are the water activities of the soybeans and biomass, respectively. Then, assuming that the beans and the biomass within a given sample of fermented soybeans are in equilibrium, it is possible to write:

$$a_w = a_{ws} = a_{wx} \qquad /19/$$

As a consequence, the values within the parentheses of Eqs. 17 and 18 must be equal:

$$aW_s^b = cW_x^d \qquad /20/$$

Rearrangement of Eq. 20 gives the biomass water content in terms of the soybean water content:

$$W_x = (aW_s^b/c)^{1/d} \qquad /21/$$

Finally, an equation can be written that expresses the overall water content as a function of the water contents of the soybeans and biomass:

$$W = \frac{(M - XM)W_s + XMW_x}{M} \qquad /22/$$

Substituting Eq. 21 into Eq. 22 and canceling out M gives:

$$W = (1 - X)W_s + X(aW_s^b/c)^{1/d} \qquad /23/$$

Given values of X and W, Eq. 23 can be solved for W_s. This must be done numerically (*e.g.* by the bisection method) since it is not possible to isolate W_s on the left hand side. Once W_s is determined, it can be substituted back into Eq. 17 to calculate the water activity of the fermenting solids.

The consequences of not taking the effect of biomass on the isotherm into account

Two slightly different mathematical models of a well-mixed 785-litre SSF bioreactor (see Fig. 1 and Tables 1 and 2) are used to explore the consequences of not taking into account the effect of the biomass on the isotherm of the fermenting solids: they differ with respect to the equation used to calculate the isotherm of the fermenting solids (Table 3). The equation used affects not only the time at which the outlet gas humidity is predicted to fall to the set point value (triggering a mixing event), but also the calculation of the amount of water to be added during a mixing event. Note that in the case study the intention of adding water is to bring the water activity back to the initial water activity of the solids, a_{wso}.

Model 1 takes into account the influence of the biomass on the isotherm of the fermenting solids (Table 3). For the purposes of this model-based case study, its predictions are taken as the true performance of the bioreactor when it is operated correctly. In this context, correct operation means that the water added during a mixing event is exactly the amount necessary to bring the water activity of the fermenting substrate back to a_{wso}. Model 2 is identical to Model 1 except that the calculation of the water to be added is based on the incorrect assumption that the isotherm of the fermenting solids is identical to that of the substrate itself (Table 3).

The two models are solved for the growth of *R. oryzae* on corn and on soybeans. In order to obtain the fitting parameters a, b, c and d for Eqs. 17 and 18, which are listed in Table 2, the Handerson equation is fitted to the

Table 3. Specific features of the two models[a]

To calculate	Model 1	Model 2
$W^* = W_{target}$	Eq. 15, $a_w = a_{wso}$	Eq. 14, $a_w = a_{wso}$
$W^* = W_{sat}$	Eq. 15, $a_w = a_{wg}$	Eq 15, $a_w = a_{wg}$
a_{ws} for a given W	Eq. 23 then Eq. 17	Eq. 23 then Eq. 17

[a]The symbol on the right hand side of an equal sign is substituted where the symbol on the left hand side appears in the equation in Table 2

isotherm for corn at 34 °C used by von Meien and Mitchell (*7*) and to the isotherms for soybeans and biomass at 34 °C shown in Fig. 2.

The importance of using the correct isotherm is most apparent for corn which, for a given water activity, has a lower water content than do soybeans (Fig. 3). If the correct model (Model 1) is used to estimate water requirements, then deceleration of growth due to low water activities will be avoided (Fig. 3c). However, if the amount of water added during mixing events is calculated on the incorrect assumption that the isotherm of the fermenting solids is identical to that of the substrate itself, then there will be hourly additions of 0.5 to 1.0 kg of water over the period from 22 to 100 h. These additions will maintain the water content of the fermenting solids constant (Fig. 3a), but, due to the changing properties of the fermenting solids in terms of the relative proportions of residual substrate and biomass, the water content of the solids must in fact rise in order to keep the water activity within the optimal range. The water activity therefore falls to values below 0.95, which are unfavorable for growth, and remains at these low values from 20 h onwards (Fig. 3b).

With soybeans, the predicted bioreactor performance in terms of biomass production is not significantly different between the two models over the first 50 h since there is no need for water addition during this period (Fig. 4). Note that the fermentation would in fact not be extended past 50 h since by this time the biomass has already reached 98 % of its maximum value.

Discussion and Conclusions

Our results show that fermenting solids can have an isotherm that is quite different from that of the substrate itself. Corona *et al.* (*12*) found similar results when they determined isotherms at various incubation times during the growth of *Gibberella fujikuroi* on a solid substrate consisting of wheat bran and soluble starch. As in the present study, for a given water activity, the water content of the fermenting solids was significantly higher than the water content of the substrate itself.

If the effect of the biomass on the isotherm of the fermenting substrate is not taken into account in mathematical models of SSF bioreactors, then the predictions of the models will be wrong. A model that assumes that the isotherm of fermenting solids is identical to that of the substrate not only will fail to predict the changes in the water activity that occur during the fermentation, but also will underestimate the amount of water that needs

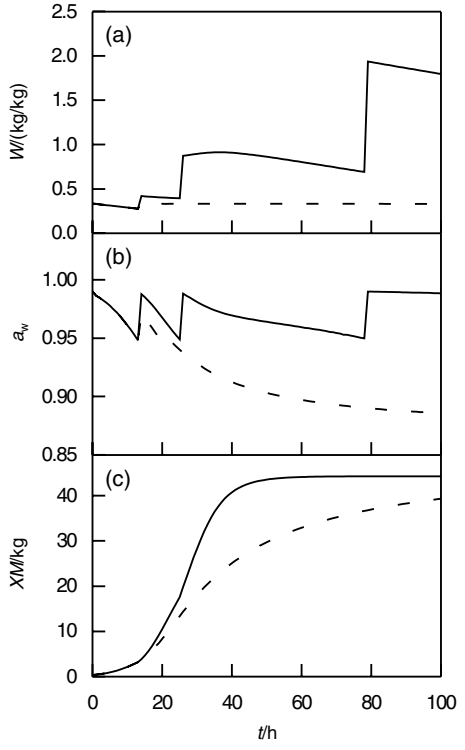

Fig. 3. Predictions made by the three models for growth of *Rhizopus oryzae* on corn. (a) Actual water content of the fermenting solids on a dry basis; (b) Water activity of the fermenting solids; (c) Total dry mass of biomass within the bioreactor. Key: (——) Bioreactor performance that would be expected if water additions were made according to Model 1, which recognizes that the biomass and substrate have different isotherms; (– – –) Bioreactor performance that would be expected if water additions were made according to Model 2, which assumes that the fermenting substrate has the same isotherm as the substrate itself

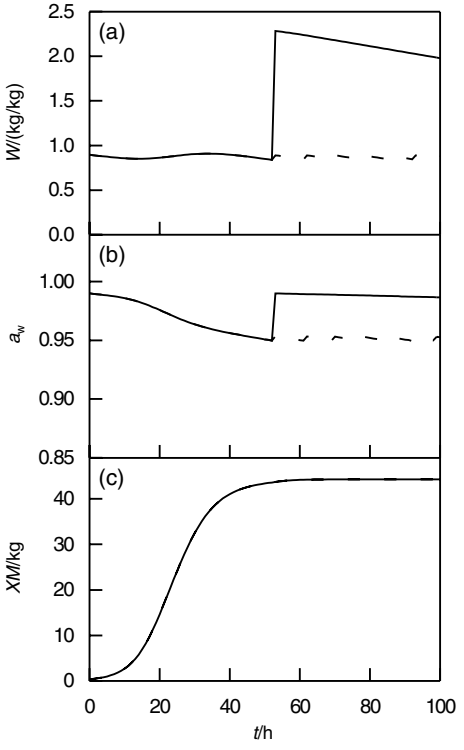

Fig. 4. Predictions made by the two models for growth of *Rhizopus oryzae* on soybeans. (a) Actual water content of the fermenting solids on a dry basis; (b) Water activity of the fermenting solids; (c) Total dry mass of biomass within the bioreactor. Key: (——) Bioreactor performance that would be expected if water additions were made according to Model 1, which recognizes that the biomass and substrate have different isotherms; (– – –) Bioreactor performance that would be expected if water additions were made according to Model 2, which assumes that the fermenting substrate has the same isotherm as the substrate itself

to be added. This may lead to unexpectedly poor growth. Problems will be more significant with solids for which the water activity of the solid varies significantly with water content, and with organisms whose growth rate is more sensitive to small changes in the water activity.

The only previous attempt to incorporate within a bioreactor model the differences between the water contents of the residual substrate and the biomass was that of Nagel *et al*. (*13*), who modelled the cultivation of *Aspergillus oryzae* on wheat grains. They used a different approach, writing separate balances for intracellular and extracellular water. Also, they did not use water activity explicitly. Rather, they used membrane filter culture to determine an empirical relationship between the intracellular and extracellular water contents. Their model assumes a constant biomass water content of 2.08 kg/kg (dry basis). However, given that the water content of the biomass can vary significantly over the range of water activities that might be expected during a fermentation, especially in the case of fungi of the genus *Rhizopus*, which grow best at high water activities, it would be preferable to take the isotherm of the biomass into account when estimating its water content. Our modelling approach does this.

The experimental approach used by Nagel *et al*. (*13*) has the advantage of characterizing the residual substrate with the presence of hydrolysis products, whereas the approach used in the current work uses the unfermented substrate. However, Nagel *et al*. (*13*) based the intracellular-extracellular water content relationship on 60-hour fermentation samples and the relationship might change as a function of biomass age and degree of substrate hydrolysis. Therefore, neither of the two approaches completely characterizes the true isotherm.

In fact, the system is far more complex than either the Nagel model or the current model recognize. A complete characterization would require determination of: (*i*) the isotherm of biomass of different ages; (*ii*) the isotherm of substrate at different degrees of hydrolysis; and (*iii*) the effect of temperature on each isotherm. However, such a detailed study may be unnecessary. The key question is whether the approach gives a description of system performance that is sufficiently accurate for it to be a useful tool in guiding water additions to the bioreactor. The case study suggests that our approach can provide such a tool. It is easy to implement, requiring the determination of only two isotherms. The mathematical approach to combining these isotherms can easily be incorporated into models of any SSF bioreactor type.

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