Macroscopic Modelling of Solid-State Fermentation

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Chapter 1

General introduction

GENERAL INTRODUCTION

The production of enzymes and antibiotics, together with "by-products" such as flavour compounds, is the most important contribution of fungi to modern-day life, where large quantities of enzymes and antibiotics are needed on a daily basis. Although most fungi are surface-growers by nature, production of fungal biomass and compounds has in general focussed on submerged fermentation in the western world. This is mainly due to the lack of experience in executing solid-state fermentation on an industrial scale.

Solid-state fermentation (SSF) is the general name for the fermentation of moist solid substrates in the absence of free flowing water. SSF generally involves the growth of fungi, but systems involving bacteria are also known. Common examples of the process in traditional western culture are the production of mould cheeses, the growth of mushrooms and the production of compost from organic waste.
As mentioned before, the application of SSF in western countries is limited, but in Asia SSF processes have always been dominant over submerged fermentation, and centuries-old production processes are still applied today. A well-known example of an ancient Asian food produced by SSF is soy sauce, the characteristic flavour compound of many oriental dishes. Soy sauce is prepared from a mixture of soybeans and wheat, and the ratio between the two substrates and the organism added to it determine the flavour of the sauce. In general, *Aspergillus* strains are added to the substrate to produce extracellular enzymes such as proteases and amylases. These enzymes hydrolyse proteins and starch in the substrate and release characteristic flavours. The resulting mass is called *koji* in Japanese. In the next stage of soy sauce production a salt solution is added to the substrate, and salt tolerant lactic acid bacteria and yeasts start to grow in addition to the continuing enzymatic conversions. At present, this process can be mimicked chemically, but the resulting taste is inferior to the taste of the traditional soy sauce.

Another well-known Asian SSF product is tempe. In this product, the koji itself (soy beans with *Rhizopus oligosporus*) is the end product. It is cheap, with good nutritional value and high protein content, it tastes good and it is a well-known meat substitute.

DEVELOPMENT OF MODERN SSF PROCESSES

It is only some 30 years ago that SSF production processes gained interest of scientists by its rediscovered potential in processes such as solid-waste treatment (Hesseltine, 1977b, Barrios-Gonzalez et al., 1993) and the production of secondary metabolites (Silman et al., 1980, Hesseltine, 1977a), biopesticides (Ooijkaas et al., 1998, Oostra et al., 2000) and antibiotics. The process can be carried out in simple fermentors and the products can be obtained at a relatively low cost because contrary to submerged fermentation no dehydration or filtration is needed.

In order to take full advantage of SSF possibilities, it is desirable to obtain a thorough fundamental understanding of the fermentation process. One of the major aspects in developing more insight in SSF is the difficulty of scaling up. Because no free flowing water is present, heat removal is troublesome. In many traditional processes, this problem has been solved by choosing limited dimensions for the fermentor, thus facilitating sufficient cooling by conduction. This method results in good quality products, but the procedure is very time (and hence money-) consuming. For efficient use of the process, larger fermentation volumes are needed. The most commonly used vessel is the packed bed, which is often aerated to prevent steep temperature gradients. Even though aeration is called “mild”, its effects can still be quite harsh, since the bed dries out through aeration. Fungal metabolism is highly dependent on the water content and water activity of the substrate. Another option for preventing temperature gradients in large-volume fermentations is to use mechanical mixing. The drawback of mixing is that it is very difficult to predict the actual result of a mixing period, because besides averaging the bed temperature, the bed is also damaged, which can have unexpected adverse effects. This thesis focuses on the processes occurring in aerated packed beds. It provides models that describe the temperature and moisture profiles in the bed and also deals with the characteristics of the substrate and organism used.

OUTLINE OF THIS THESIS

The aim of the research described in this thesis was to create a macroscopic model of temperature, moisture and product gradients in SSF, based on a minimum of empirical relations.

The models in this research are primarily focused on whole wheat kernels as a model substrate, and *Aspergillus oryzae* as model organism, but hemp and oats, *Aspergillus sojae* and *Coniothyrium minitans* are also described. Whole wheat kernels were chosen since these were shown to be an excellent substrate for mixed solid-state fermentations (Nagel et al, 2001), based on availability, content and rigidity.
Several types of fermentors were used during this study, ranging from very small, aerated tubes with a volume of 10 ml to a pilot-scale fermentor of 50 l.

Research on the macroscopic behaviour of solid-state fermentations concerns many aspects. One of the most basic parts is dealt with in Chapter 2, which deals with the characteristics of the substrate and its influences on fungal growth.

Chapters 3 and 4 focus on the temperature and moisture gradients that arise in an aerated packed bed with a working volume of 10 litres. Macroscopic mathematical models of the system are presented and evaluated. While Chapter 3 is a detailed study on the heat and mass transfer characteristics of SSF, Chapter 4 discusses the typical effects of evaporation on the fungal metabolism.

In Chapter 5, the model first presented in chapter 3 is extended with relations for changing bed heights and changing bed porosity, which are of importance especially in systems in which the bed shows substantial shrinkage (due to substrate usage, evaporation, hyphal networks, etc.). The extended model described in this chapter can be used to predict channelling in packed-bed fermentors.

Finally, Chapter 6 is a review on the present status of modelling SSF, focussing on the importance of various aspects of present models.

Chapter 2

Influence of wheat type and pretreatment on fungal growth in solid-state fermentation

Marisca J. Hoogschagen, Yang Zhu, Henk van As, Johannes Tramper and Arjen Rinzema

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SUMMARY

The respiration kinetics of *Aspergillus oryzae* on different varieties of whole wheat kernels were studied. Six wheat varieties were pretreated in two different ways. Five of the six substrates fermented similarly independently of the pretreatment method. However, pretreatment affected fermentation of one variety of soft wheat (Apollo). T_2 ^{1}H-NMR imaging of the water inside the kernels showed a change in water binding inside the kernels when a different pretreatment strategy was used. Differences in free sugar or amino acid content, or in kernel stiffness were not significant.

INTRODUCTION

Solid-state fermentation (SSF) is defined as the fermentation of solid substrates in the absence of free flowing water. This principle has been used in traditional food fermentation processes for centuries, especially in Asian countries, where products like Tempe and sake are produced using SSF. Recently this technique gained interest in western countries due to its potential in solid-waste treatment (Barrios-Gonzalez *et al*., 1993), the production of secondary metabolites, the production of biopesticides (Ooijkaas *et al.*, 1998; Oostra *et al.*, 2000) and the production of novel foods (Sardjono *et al.*, 1998). These new applications call for a deeper understanding of the fermentation process because little is known about the microbial physiology and metabolism under SSF conditions and process engineering aspects of SSF. For our research on fermentation kinetics, physiology and gene expression of *Aspergillus oryzae* in SSF, we want to use a representative model substrate. Therefore, we have compared the respiration kinetics of *Aspergillus oryzae* ATCC 16868 on several varieties of wheat. We used two pretreatment strategies for all types of wheat. Whole kernels were used, since it has recently been shown that these are an excellent substrate for use in mixed solid-state bioreactors (Nagel *et al.*, 2001).

In previous studies, little attention has been paid to the effects of the choice of substrate and substrate pretreatment on fermentation. Dorta *et al.,* (1994) and Liu *et al.,* (1999) compared the differences in water retention of related substrates such as rice, rice bran and rice husk. Many researchers studied the influence of water activity on fermentation of one type of substrate (Gervais 1988a,b; Huang *19*85; Liu *et al.,* 1999). Finally, comparisons have been made between different types of legumes

and seeds (Hachmeister *et al.*, 1993; Sardjono *et al.*, 1998). However, no research has focused on different varieties of the same legume or grain. Differences in growth due to pretreatment of the substrate have so far only been reported with regard to cooking procedures (Hachmeister *et al.*, 1993; Chay *et al.,* 1986), not with regard to soaking methods.

MATERIALS AND METHODS

Microorganism

Aspergillus oryzae (ATCC 16868) was cultivated on potato dextrose agar at 25 °C for 7 days. Spore suspension (± 3.7 × 10^7 spores/ml) was harvested using sterile water and was stored at –80°C until it was used for inoculation. Sterile glycerol (10%v/v) was added as a cryo protectant.

Substrate, pretreatment and fermentation

Six types of wheat of commercial origin were used: two hard varieties (Ritmo and Arnaut), and three soft varieties (Apollo, Minaret (two batches) and D1 304). All wheat was purchased from ACM, Meppel, the Netherlands, and was stored in closed plastic containers at 10°C. Two pretreatment procedures were applied: the kernels were soaked in distilled water at 50°C (4h) or 20°C (16h). Excess water was drained off after soaking. The soaked kernels were sterilized at 121°C for 1 hour and were allowed to cool down to room temperature. The total amount of water present in the kernels after pretreatment was 45% (w/w). Inoculation was carried out with 1 ml spore suspension per 100 g of soaked wheat (± 6.2 × 10^5 spores/g dry weight). The mixture, which was contained in 1 l medium flasks, was then put on a roller bank for one hour to ensure an even distribution of spores. The inoculated material was divided into 20 g portions in sterile Petri dishes (diameter 90 mm). Fermentation was carried out in a climatic incubator (VEA-Instruments, Houten, the Netherlands) at 25°C and at a relative humidity of 98%, using procedures described previously (Smits *et al.,* 1996). Samples were taken by randomly removing three Petri dishes from the incubator. Removed dishes were replaced with empty dishes to keep the local airstreams in the incubator constant.

Analysis

Water activity was measured using an electric hydrometer (Type EK 84/3H/63T, sensor type BSK-4, Novasina, Pfafficon, Switzerland).

Carbon dioxide production and oxygen consumption were measured in a setup designed by Smits *et al.,* (1998). The set-up consisted of a measurement chamber, a magnetic stirrer inside the chamber, a tube pump (Masterflex 7014, Cole Parmer, Chicago, IL), a paramagnetic O_2 analyzer (Servomex series 1100, the Netherlands) and an infrared CO_2 analyzer (Servomex Series 1400, the Netherlands). The respiration measurement took 30 minutes. All three Petri dishes were measured simultaneously.

Total carbohydrate was measured using a method derived from Dubois *et al.,* (1956). In test tubes, 500 µl sample suspension and 50 µl 20% phenol were mixed. 1000 µl H_2SO_4 (conc.) was injected in the center of the sample. After mixing and cooling, the optical density was measured at 490 nm.

Total terminal amino acids were measured using a trinitrobenzene-sulphonic acid method (TNBS). A 12 µl sample was mixed with 83 µl distilled water and 260 µl 0.1 M phosphate buffer (pH 8). After 30 seconds, 3 µl TNBS (5% TNBS in methanol) and 93 µl distilled water were added. After six minutes, the extinction was measured at 340 nm. Calibration was carried out with a glycine solution (100 mg/ l phosphate buffer (0.01M)).

Kernel stiffness was measured in a piston type pressure cell with a built-in texture analyzer. Pressure was exerted on the kernels with an aluminum weight that pressed down on a plate of 4mm thickness. The pressure sensor was fitted in the center of the lower plate.

Water distribution was tested using Nuclear Magnetic Resonance (NMR) relaxation time (T_2) imaging, which makes it possible to visualize water fractions with different binding capacities in solid particles. Experiments were carried out on a MARAN Pulsed NMR Spectrometer, Resonance Instruments Ltd., Witney, UK. Settings were as follows: (Larmor) frequency 30 MHz, pulse sequence CPMG, Tau 100 µs, number of echoes 1500, relaxation delay 2 s, number of scans 32. Measurements were performed on a single wheat kernel at room temperature. Six kernels of each pretreatment were tested.

RESULTS AND DISCUSSION

The six varieties of wheat were pretreated in two different ways, which resulted in the same water activity (0.99) and water content (0.90 ± 0.02 kg H_2O/kg dry weight) for all samples.
As can be seen from Figure 1, no significant differences were found between Ritmo, Arnaut, Minaret 1 and 2 and D1 304 wheat, irrespective of their pretreatment. This is a comforting result since it shows that the type of wheat and the pretreatment of the wheat do not affect fungal growth for these wheat varieties.

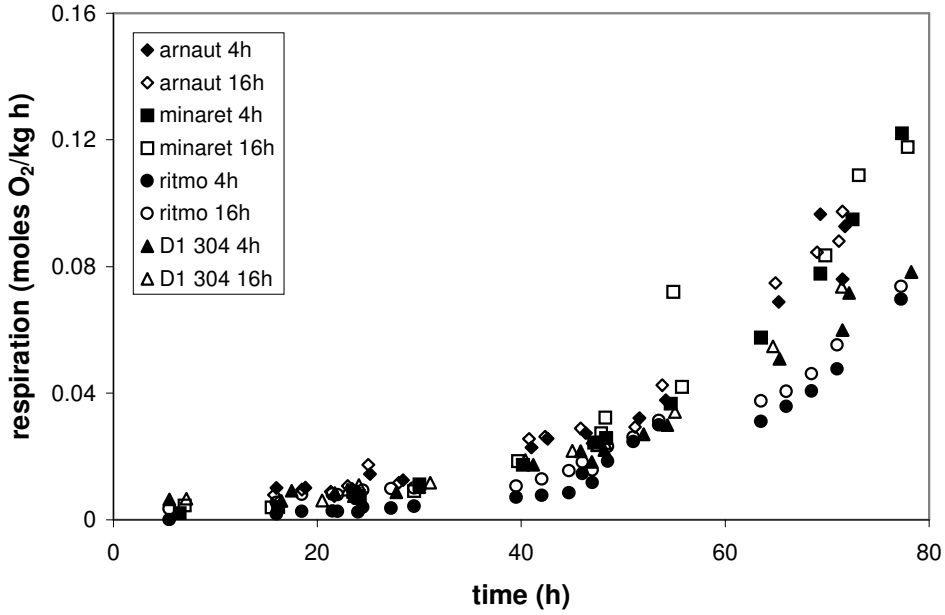

Figure 1: Respiration rate of A. oryzae growing on several types of wheat at 25°C. The wheat was pretreated in two different ways for all types. Closed symbols indicate wheat soaked 4 h at 50°C; open symbols indicate wheat soaked 16 h at 20°C.

However, when *A. oryzae* was grown on Apollo wheat, the different response to the two pretreatments was obvious. This is illustrated in Figure 2. It can be concluded from Figures 1 and 2 that the maximum respiration rate is about the same for all types of wheat except the Apollo type, which has a lower respiration for both pretreatments tested. Apollo wheat that had been soaked for 16 h at 20°C gave a higher fungal respiration rate than Apollo wheat that had been soaked at 50°C for 4 h.
To explain the observed effect of pretreatment of Apollo wheat on respiration of the fungus, four aspects were studied:

1. Effects of bacterial acidification during the soaking process
2. The presence of free sugars and amino acids (either present originally or resulting from breakdown of starch and proteins by indigenous amylase or protease activity during the soaking process). The presence of sugars and amino acids might stimulate fungal growth since these products are easier to digest than the polymers.
3. Differences in kernel stiffness. Fungal penetration might be easier when the kernels are softer; penetration facilitates the access of the fungus to substrates inside the kernels.
4. Differences in water distribution and water binding in the kernels.

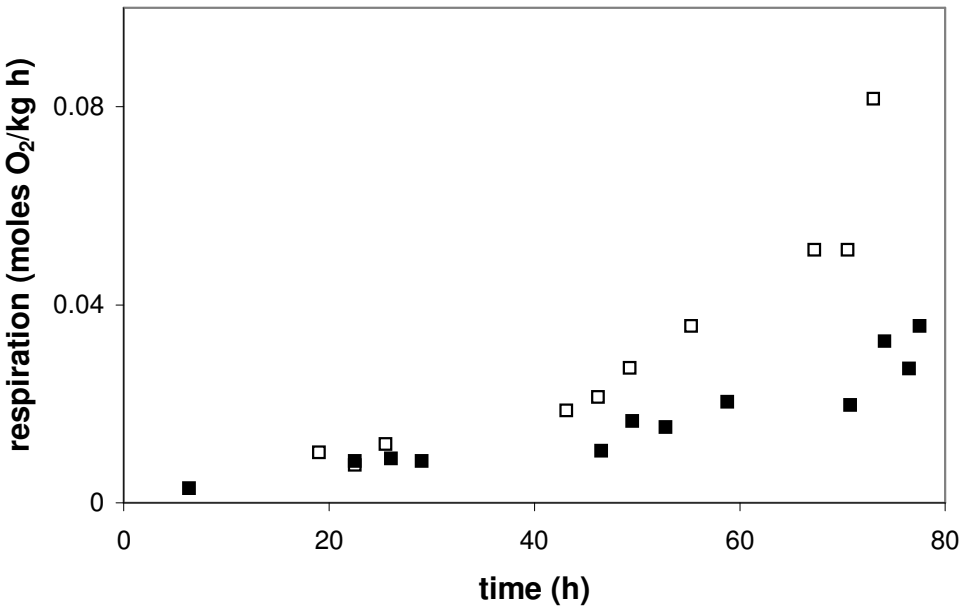

Figure 2: Respiration rate of A. oryzae growing on Apollo wheat (soft) at 25°C. Closed symbols indicate wheat soaked 4 h at 50°C; open symbols indicate wheat soaked 16 h at 20°C.

The possible effect of bacterial acidification during the soaking of wheat as described by Hachmeister *et al.,* (1993) was checked and discarded. The same decrease in pH was observed for all types of wheat and for both pretreatments (pH decreased from 6.85 to 5.66 ± 0.03).

To evaluate the enzymatic breakdown of starch and proteins in Apollo wheat during pretreatment, total reducing sugars and total free amino acids were measured. No significant difference in sugar content (average deviation 7.2%) or free amino acids concentration (about 2 µg/g wheat) was found between Apollo grains that were soaked for 4 and 16 hours.

Kernel stiffness was investigated on single Apollo wheat kernels. Each kernel was crushed with a plunger, and a pressure sensor registered the force needed to deform the kernel. No differences were found between the two pretreatments.
Water diffusion and distribution were investigated using NMR-imaging. We focused on the ratio of the different water fractions present in pretreated wheat and the mobility of these fractions. The experiment was carried out with Apollo and Ritmo wheat kernels from both pretreatments. No significant differences were found for the Ritmo kernels.

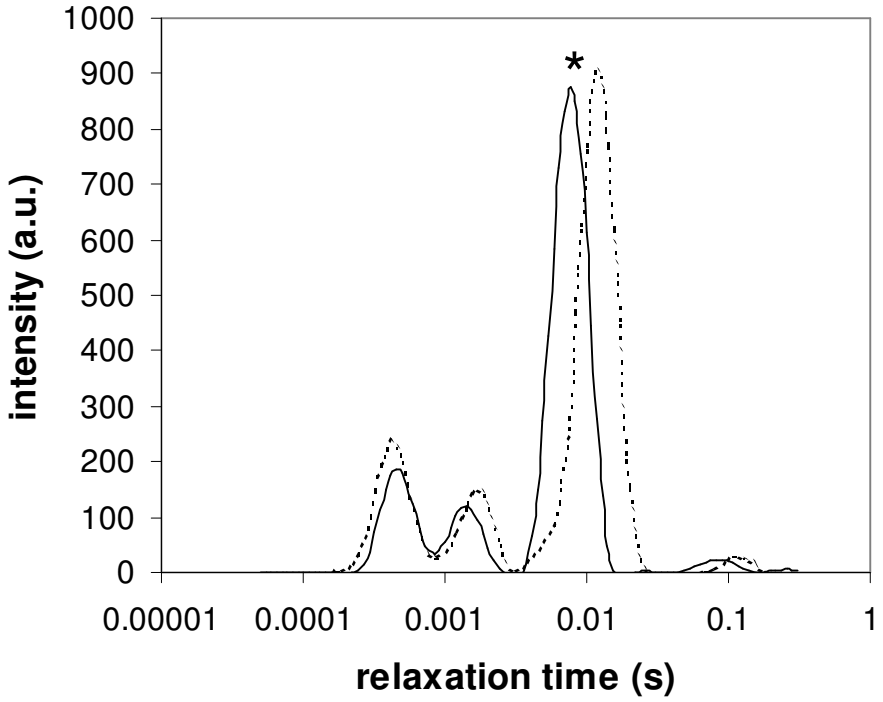

Figure 3: ^{1}H NMR T_2 response time for Apollo wheat kernels. This figure shows the average response of 6 kernels for each pretreatment.
———: soaked for 4 hours at 50°C – – –: soaked for 16 hours at 20°C
*An important difference can be seen for the most mobile fraction (with label *) in the spectrum. This fraction is shifted to the left for the wheat that was soaked at 50°C for 4 hours, which means it is less mobile than the same fraction in wheat that was soaked at room temperature.*

Figure 3 shows the relaxation time of the water in Apollo kernels. The two lines are averages of 192 measurements using 6 individual kernels. The NMR spectrum was measured 32 times for each kernel, to rule out fluctuations in the magnetic field. The deviation between the average spectrums of the 6 kernels was 1.5%. The four distinct peaks indicate the presence of four water fractions. The ratio of the four fractions differed for the two pretreatments. When read from left (least mobile fraction) to right, the peak ratios are 0, 1, 75 and 24% for the wheat that was soaked for 4 hours at 50°C and 0, 1, 61 and 38% for the wheat that was soaked for 16 hours at 20°C. The most mobile fraction, situated on the right in the spectrum (with label *), was less mobile in the wheat that was soaked 4 hours, which can been seen from the shift of this peak to the left side of the spectrum. The shift indicates that this fraction was bound more firmly in Apollo wheat after the 50°C pretreatment. This might indicate a lower local water activity for this pretreatment, which might explain the observed difference in fungal growth. 3D imaging can be used to locate the position of this fraction inside the kernel and to get more information on its influence on fungal growth.

CONCLUSION

Respiration of *A. oryzae* was studied during solid-state cultivation on six types of wheat that were either soaked for 16 hours at 20°C or for 4 hours at 50°C, prior to sterilization. We found that the wheat variety and pretreatment of wheat can have a considerable effect on the performance of solid-state fermentation. Respiration rates were comparable for five out of six substrates, independent of the pretreatment method applied. However, for one of the soft wheat types different pretreatment methods caused differences in fungal respiration. Respiration of *A. oryzae* was slower on this wheat if it had been soaked for 16 hours at 20°C. This difference could not be attributed to differences in free sugar and amino acid content, pH or kernel stiffness after soaking and sterilization. A difference in water mobility was found with NMR, which may be the cause of the difference in fungal respiration rate. One of the water fractions that could be distinguished in wheat kernels had a reduced mobility in wheat that was soaked at 50°C for 4 hours. No direct evidence that this is the cause of the observed decrease in fungal respiration rate is available yet; further research into the relation between water mobility and fungal activity in SSF is needed.

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Chapter 3

On the significance of water activity and the effect of transfer limitations in packed-bed solid-state fermentation

Marisca J. Hoogschagen, Frans J. Weber, Sebastiaan Haemers, Johannes Tramper and Arjen Rinzema

This text has been submitted for publication.

SUMMARY

Evaporation induced by forced aeration is a frequently used cooling method in large-scale solid-state fermentation (SSF). The evaporation that is on one hand beneficial for the fermentation, is on the other hand limiting microbial growth by rapidly decreasing the water activity in the fermenting bed below favourable levels. So far, the decrease in water activity has not been taken in account in a mathematical model on SSF. Besides, in previous mathematical models the assumption has always been made that evaporation was 100% effective during forced aeration and that gas and solids were in equilibrium. Here, a study on the actual effect of water activity decrease and on mass and heat transfer between the fermenting solids and the gas is presented.

Based on this work, we conclude that especially the effect of the decrease of water activity in the transfer equations makes a significant contribution to model predictions of SSF. The effect is most obvious in fermentations with high heat production rates and small axial temperature gradients over the bed. For example, in a fermentation with a heat production rate of 5000

Wm^{-3} and an axial temperature gradient of 2 K, the airflow rate needed to maintain a constant temperature would be underestimated by 31% if water activity decrease was not taken into account. In fermentations in which water content is of great importance, a model that takes into account water activity effects can give interesting insight on the fermentation process.

Improvements to the presented model can still be obtained by adding non-empirical reaction kinetics and by adding the effect of the airflow velocity more accurately.

INTRODUCTION

Solid-state fermentation (SSF) is defined as the cultivation of micro organisms (usually fungi) on solid substrates in the absence of free-flowing water, with air as the continuous phase. A major concern in SSF is the translation from laboratory-scale to industrial scale: the main difficulty in scaling-up is the removal of the metabolic heat. Due to the absence of free-flowing water and the poor conductivity of the solid substrate layer, removal of heat is troublesome and steep temperature gradients can arise in a packed bed. In order to avoid excessive temperature gradients that hamper microbial growth, it is necessary to actively cool the fermentor. Forced aeration and mixing are often applied

for this purpose. Of these methods, aeration has the advantage that it is a relatively mild procedure, which gives no shear damage, and requires limited investments on the fermentor. The cooling action of forced aeration depends largely on evaporation of water from the solid matrix.

Mathematical models can improve our understanding of the process characteristics of SSF and can eventually be used to improve design and operation as they can help to identify bottlenecks. Mechanistic models are most helpful, since these allow extrapolation and can be more easily adapted to different substrates and organisms. Several mathematical models have already been published, but these showed inaccuracies in some aspects as discussed by Weber *et al.* (2002). Weber *et al.* (1999) extended the existing models with a water balance based on vapour and temperature equilibrium between the solids and the gas phase. In an experimental validation of this model (1999) it was shown however that relative humidity of the outlet gas was below 100% in several experiments. Weber *et al.* (2002) incorporated the measured relative humidity in their calculations, to improve the temperature predictions. They did not predict the relative humidity of the air or the water activity of the substrate, and no effect of decreasing water activity was taken into account in the model.

Transfer limitations of the substrate or effects of decreased water activity are not considered in literature on packed bed SSF, except in one paper (Von Meien and Mitchell, 2002). This paper is not conclusive, since the model has not been experimentally validated. Besides, named model has some limitations:

1) Two different desorption isotherms were used to predict gas moisture content from solids moisture content, and vice versa. Obviously, there is only one equilibrium state and therefore one isotherm should be used.
2) The mass accumulation term in the heat balance for the solids is neglected, which will give an error in the predictions.
3) The equation for the mass transfer coefficient ka gives negative values when the water content of the solids drops below 0.35 kg kg^{-1}.

The first limitation is a serious error in the model. The second limitation is less serious but makes the model less reliable (errors up to 10%). The third point limits the application range of the model.

In this paper, we present and evaluate an extended version of the model of Weber *et al.* (2002). Our aim is to overcome the need for the incorporation of the measured relative humidity. We have determined the mass and heat transfer coefficients for SSF systems and also incorporated a limitation on vapour transfer by decreased water activity. The latter is derived from sorption isotherms we measured ourselves and isotherms that were measured by Nagel *et al.* (2001). We compared the extended model with experiments and with predictions obtained with the equilibrium model, and evaluated the effect of various heat production rates and the allowed axial gradient in a packed bed fermentor on the difference between the equilibrium model of Weber *et al.* (2002) and the new model presented in this paper.

MATHEMATICAL MODEL

Because we no longer assume equilibrium between gas and solids, separate mass and enthalpy balances for both phases are required. The model consists of two enthalpy balances and four mass balances (two for water, one for substrate solids and one for fungal biomass solids), with auxiliary equations for the reaction rate and phase equilibrium.

Mass and enthalpy balances

The model describes the heat and mass transfer in a packed bed solid-state fermentation. It is based on the following assumptions:

a) Homogeneous distribution of the bed material, no channelling during fermentation, no shrinkage.
b) Axial conduction can be neglected compared to axial convection.
c) Radial heat conduction and radial transport of water can be neglected, because our pilot plant is well insulated and an industrial plant has a very large diameter.
d) There is no axial dispersion in the gas phase; we assume ideal plug flow.

The enthalpy accumulation in the solid phase is the result of respiration, heat transfer to the gas and heat transfer associated to water vapour transfer to the gas; there is no axial movement:

$$\frac{d}{dt}(SH_s)=r_{O_2}\Delta H_O-\alpha a(T_s-T_a)\ -\ ka\frac{M_w}{R}\left(a_w\frac{p_{vsat}(T_s)}{T_s}-\frac{p_{va}}{T_a}\right)(\Delta H_w\ +c_{pwv}(T_s-T_{ref}))\quad(1)$$

In this equation, the last term on the right hand side represents the enthalpy of the water vapour transported from the gas/particle interface into the gas. We assume a stagnant gas layer surrounding the solid particles, in which water vapour concentration and temperature decrease from particle to gas. The water vapour cools down while passing through this layer and the sensible heat is transferred to the air in the stagnant layer and transported further by conduction, which is included in the second term on the right hand side.

The enthalpy content of the solids, H_s, is the sum of the enthalpy contents of the substrate dry solids, the water in the substrate solids, the fungal biomass dry solids and the water in the fungal cells:

$$H_s=\left(c_{ps}+c_{pw}W_s+c_{px}X\frac{S_0}{S}+c_{pw}W_xX\frac{S_0}{S}\right)(T_s-T_{ref})\qquad(2)$$

The correction $\frac{S_0}{S}$ in the two biomass-associated terms in this equation is needed because the fungal biomass weight fraction X is expressed in kg biomass per kg initial dry substrate. When equations (1) and (2) are combined to find the change in solids temperature (T_s) the product rule has to be applied because S, W_s and X vary in time.

The gas phase consists of dry air and water vapour, and changes occur through axial flow and mass and heat transfer from the solids. There is no heat production in the gas phase. The enthalpy balance for the gas reads:

$$\frac{\partial}{\partial t}(\varepsilon\rho_a H_a)=-F_a\frac{\partial H_a}{\partial z}+\alpha a(T_s-T_a)\ +\ ka\frac{M_w}{R}(a_w\frac{p_{vsat}(T_s)}{T_s}-\frac{p_{va}}{T_a})(\Delta H_w+c_{pwv}(T_s-T_{ref}))$$

(3)

The boundary condition for equation (4) is $\frac{\partial T_a}{\partial t}\Big|_{h=0} = 0$. The chain rule needs to be applied again to find the change in gas temperature, T_a, from equation (3). The dependency of the density of dry air $\rho_a = \frac{P_{tot} - p_{va}}{RT_a} M_w$ on temperature and vapour pressure was not incorporated in the chain rule expansion since we found that this requires extra calculation time without giving a significant improvement in the accuracy of the predictions (given the limited temperature and water vapour pressure ranges in our solid-state fermentation).

The enthalpy of the moist air (H_a) is calculated using the following equation:

$$H_a = c_{pa} \cdot (T_a - T_{ref}) + W_a \cdot (\Delta H_w + c_{pwv} \cdot (T_a - T_{ref})) \qquad (4)$$

The accumulation of water in the solid substrate is the result of metabolic activity and water transfer between solids and gas:

$$\frac{d}{dt}(SW_s) = r_{O_2} Y_{wo} - W_x S_0 \frac{dX}{dt} - ka \frac{M_w}{R} \left(a_w \frac{p_{vsat}(T_s)}{T_s} - \frac{p_{va}}{T_a} \right) \qquad (5)$$

In our model, the water content of the biomass (W_x) is assumed to be constant at 2 kg of water per kg of biomass (Nagel *et al.* 2001).
The chain rule has to be applied to find the change in water content of the substrate, W_s, because the concentration of the dry substrate, S, also changes in time, according to equation (7) below. The fungal biomass production rate, X, is calculated with equation (8).

The accumulation of water in the gas phase is the result of convection and transfer. The mass balance for water in the air reads:

$$\frac{\partial}{\partial t}(\varepsilon \rho_a W_a) = -F_a \frac{\partial W_a}{\partial z} + ka \frac{M_w}{R} \left(a_w \frac{p_{vsat}(T_s)}{T_s} - \frac{p_{va}}{T_a} \right) \qquad (6)$$

The boundary condition for equation (6) is $\frac{\partial W_a}{\partial t}\Big|_{h=0} = 0$. Please note the remarks on the chain rule and changes in air density made under equation (4).

The amount of dry solid substrate will decrease only due to the metabolic activity of the fungus:

$$\frac{dS}{dt} = -Y_{so} \cdot r_{O_2} \tag{7}$$

The biomass development is calculated from the respiration rate and a yield coefficient of biomass on oxygen (Nagel *et al.* 2001):

$$\frac{dX}{dt} = \frac{Y_{xo} r_{O_2}}{S_0} \tag{8}$$

Fungal respiration kinetics

The dependence of fungal oxygen uptake rate on time was described empirically. We used Tablecurve to fit the respiration data obtained during pilot-scale fermentation of *Coniothyrium minitans* on oats and *Aspergillus oryzae* on hemp (equations (9) and (10) respectively). We chose this strategy, rather than using for instance the combination of Ratkowsky equations and logistic growth law (Weber *et al.* 2002), in order to get a proper estimate of the effect of the transfer coefficients and to minimise errors not related to mass and heat transfer in the prediction, such as decreased growth due to decreased water activity. It must be noted that the previous studies of Weber *et al.* (1999) to which we compare our predictions were also based on measured oxygen uptake rates. Of course, this manner of describing respiration is not universal but only valid for the specific fermentation for which the fit was made.

The fitted equation for *C. minitans* on oats was (R^2=0.99):

$$r_{O_2} = -5 \cdot 10^{-9} \frac{3.83 \cdot 10^{7} t^{8.94} + 7.48 \cdot 10^{34} t^{4.47} + 3.65 \cdot 10^{61} - 5.53 \cdot 10^{43} t^{5.47}}{(867 t^{4.47} + 8.46 \cdot 10^{29})^2} \tag{9}$$

The fitted equation for *A. oryzae* on hemp was (R^2=0.96):

$$r_{O_2} = 8.46 \cdot 10^{-7} + 1.64 \cdot 10^{-4} \frac{\exp\left(\frac{t - 1.14 \cdot 10^5}{6444}\right)}{\left(1 + \exp\frac{t - 1.14 \cdot 10^5}{6444}\right)^{1.06}} \quad (10)$$

The oxygen uptake rate data of *A. oryzae* on hemp and the fit obtained with equation (10) are shown in Figure 1.

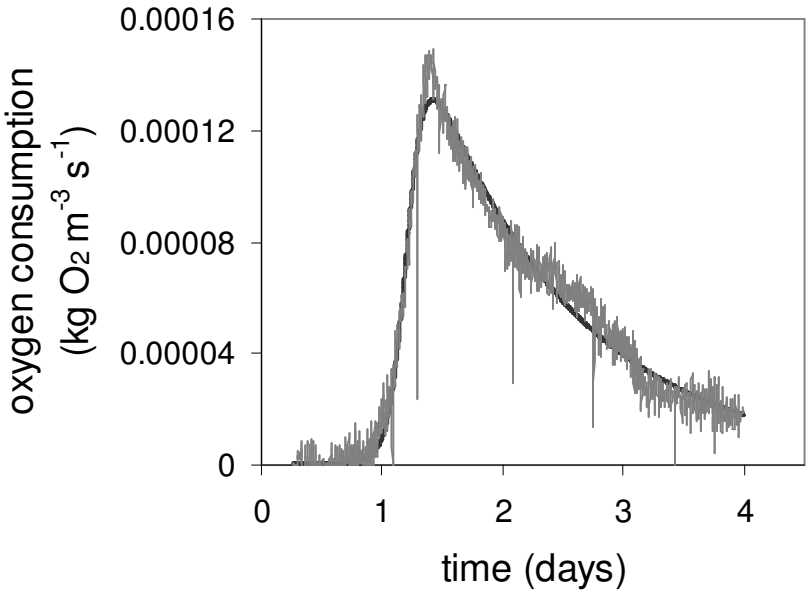

Figure 1: Measured respiration of A. oryzae on hemp (grey line) and fitted respiration (black line) from equation 9, R^2= 0.96.

Thermodynamics

The saturated water vapour pressure p_{vsat} is calculated with Antoine's law (Kaye and Laby, 1995), which is a fit of water vapour pressure data, at temperatures between 273 and 333K:

$$p_{vsat} = \exp\left(23.59 - \frac{4044.54}{T_a - 37.695}\right) \quad (11)$$

The actual water vapour pressure p_{va} is related to the weight fraction W_a as follows:

$$p_{va} = \frac{P_{tot} W_a(T_a)}{W_a(T_a) + 0.622} \tag{12}$$

The sorption isotherms for wheat, oat and hemp are:

$$a_w = \frac{1.029 W_s}{0.039 + W_s} \qquad \text{(wheat and oats; } R^2=0.92\text{)} \tag{13}$$

$$a_w = 1 - e^{-8.80646 W_s} \qquad \text{(hemp (Weber } \textit{et al.}\text{, 2002))} \tag{14}$$

Figure 2 shows the fit of equation (13) to our experimental data.

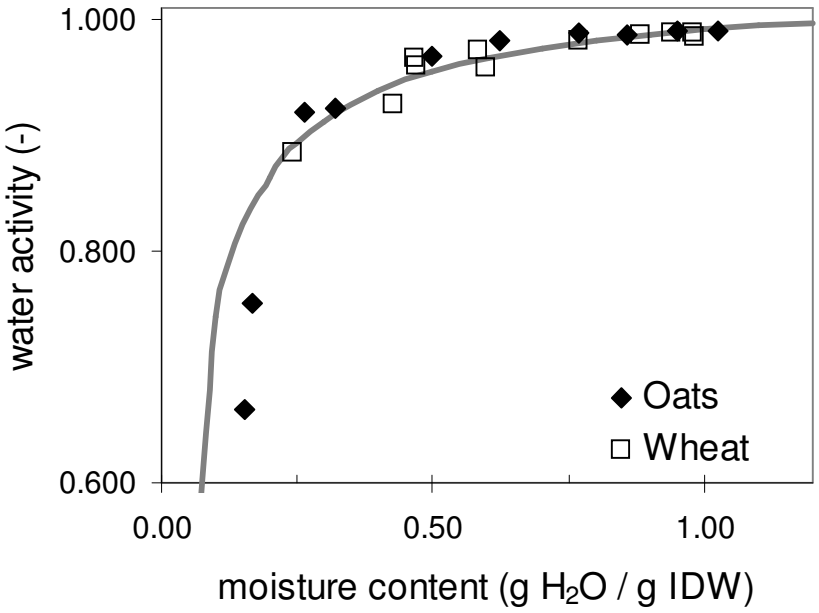

Figure 2: Measured water activity for wheat and oats of a known moisture content (symbols), and the result of equation 13 (line).

MATERIALS AND METHODS

Packed bed fermentations

The fermentation results with *Coniothyrium minitans* and *Aspergillus oryzae* were published in Weber *et al.* (2002).

Sorption isotherms

Sorption isotherms for wheat and oats were measured with the procedure used in Weber *et al.* (2002).

Cooling experiments

We used wheat, oats and hemp as substrate. The wheat and oats were obtained in a single batch from a local mill, and the hemp was obtained from Hemparade (Hempflax bv, the Netherlands).

Packed bed reactor

We used a cylindrical packed bed fermentor constructed by our own mechanical workshop for all experiments. The fermentor has an effective volume of 10 litres, can be sterilised, is well insulated and can be aerated and sampled Weber *et al.* (2002).

For the determination of the transfer coefficients αa and ka, packed bed experiments similar to those described in Weber *et al.* (2002) were carried out. The main difference was the absence of inoculum. The bed was cooled by aeration with unsaturated air (dew point 10 K lower than the initial bed temperature). The dew point of the off gas was measured, as well as the axial temperature profile in the bed. At the end of each cooling experiment samples were taken for dry weight measurements.

Parameter estimation

The transfer coefficients ka and αa were determined using the model presented above; the respiration rate was set to zero for this purpose. The sum of squared differences between measured and predicted temperatures and water vapour contents of the off-gas was minimised using a least squares algorithm (Fmins) in Matlab (Mathworks inc., version 5.3).

Numerical integration methods

In all simulations, the bed was divided into *n* slices of height *dz* in order to deal with height-related effects, with $dz = \frac{\text{bed height}}{n}$ (Figure 3). Transport between the slices is dealt with using the method of lines. We used a first order backward finite difference approximation to calculate the changes in the enthalpy of the air with regards to height ($\frac{\partial H_a}{\partial z}$) and the changes in the water content of the air with regards to height ($\frac{dW_a}{dz}$). The differential equations were solved using the ODE15S solver in Matlab (Mathworks inc., version 5.3).

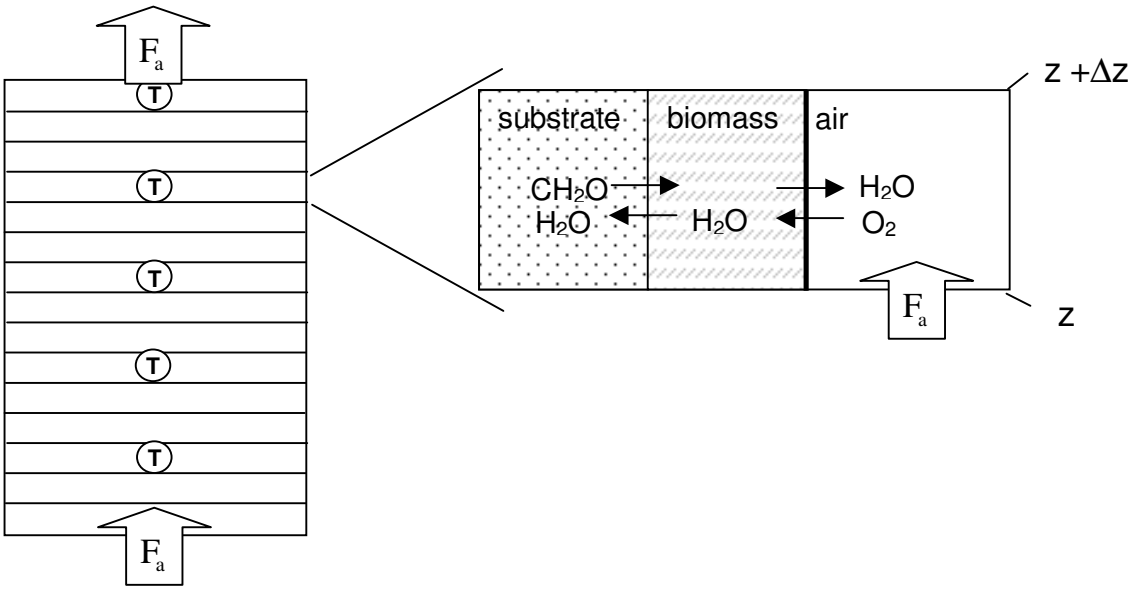

Figure 3: Schematic overview of the packed bed reactor, showing the slices with height dz for which all calculations are made and the placement of the temperature sensors.

RESULTS AND DISCUSSION

Transfer coefficients

We studied the dynamic response of several packed beds to a stepwise change in inlet gas conditions and derived transfer coefficients by fitting the mathematical model to the measured temperatures and water vapour content of the off-gas. During the first calculations, we noticed a strong conflict between ka and αa, resulting in meaningless parameter values. The predicted temperatures of the solids and the gas were virtually equal if physically realistic values for ka and αa were used, irrespective of the values of both parameters. The small temperature difference implies that convective heat transfer is not a limiting factor in the system. We therefore decided to use a fixed value for αa (comparable to known heat transfer coefficients for forced convective cooling of comparable materials such as wood chips and sliced vegetables) and to fit only ka. When the best ka values were found, our decision to choose a fixed value for αa was justified as we found that there was no effect of this parameter on the model outcome for αa-values between 720 and 42500.

The measured and simulated temperature profiles for different substrates are shown in Figures 4a-f. The fitted transfer coefficients are listed in Table 1. It can be concluded from the figures and the table that we obtained accurate coefficients for three substrates. Our ka-values for wheat and oats cannot be compared directly to the values obtained with the equations provided by Von Meien and Mitchell (2002), since they based the driving force on concentrations in the solids instead of in the gas, and used particles with a different specific surface area. Hemp has a significantly lower ka-value than wheat and oats. This cannot be due to the different water uptake capacities and water sorption isotherms, since these were taken into account in the model. However, the different shape and packing properties of hemp particles may have an effect on the mass transfer coefficient.

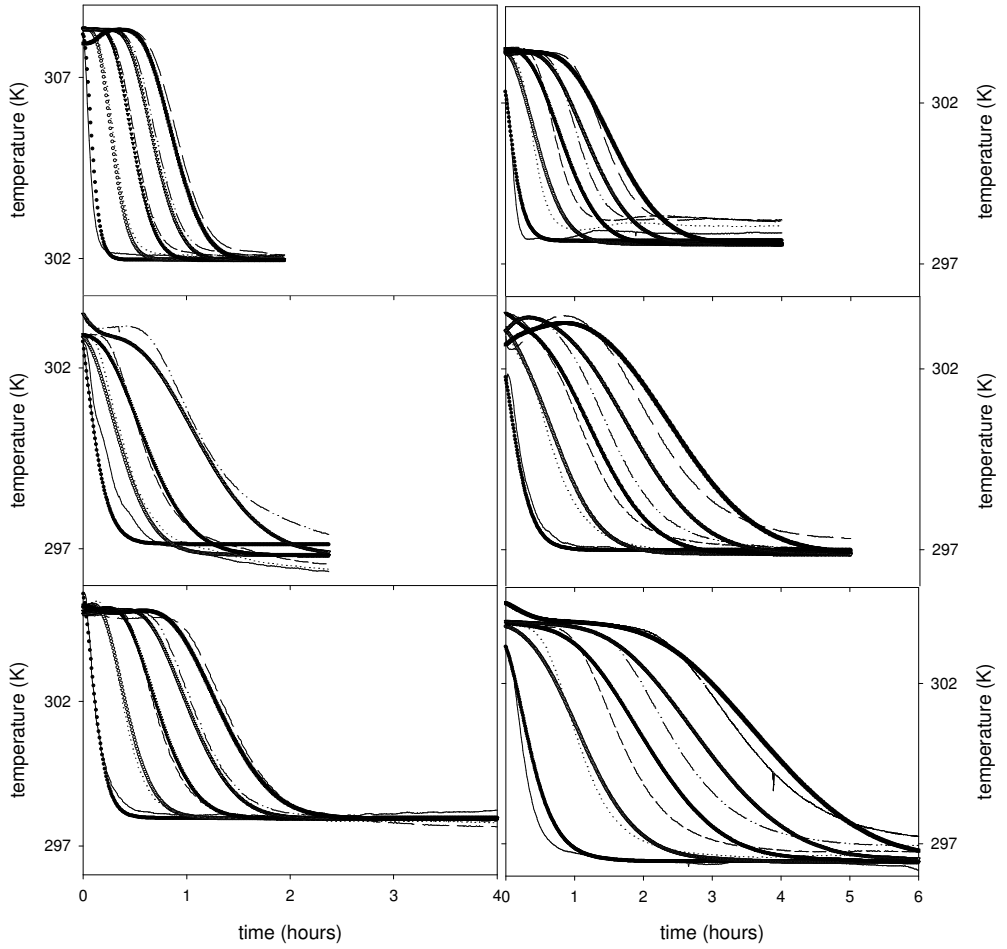

Figure 4: Experimental (thin lines) and fitted (fat lines) temperature profiles for the aerated cooling of a packed bed with the following solid substrates and air flow rates:

A	*Wheat, 0.051 kg $m^{-2}s^{-1}$*	*D*	*Wheat, 0.035 kg $m^{-2}s^{-1}$*
B	*Hemp, 0.034 kg $m^{-2}s^{-1}$*	*E*	*Coatless oats, 0.035 kg $m^{-2}s^{-1}$*
C	*Oats, 0.035 kg $m^{-2}s^{-1}$*	*F*	*Oats, 0.014 kg $m^{-2}s^{-1}$*

From left to right, the lines in each graph correspond with 5, 15, 25, 35 and 45 cm bed height.

Table 1:
The fitted ka values for oats, wheat and hemp. A fixed heat transfer coefficient αa was used for all experiments ($28.8 \cdot 10^3$ kg $m^{-2}K^{-1}$). Varying αa between 720 and 42500 did not affect the accuracy of the fits.

	Superficial airflow rate ($kg_{dry\ air}\ m^{-2}\ s^{-1}$)	*k*a (s^{-1})	R^2
Wheat	0.034	1.714	0.95
Wheat	0.051	17.64	0.98
Hemp	0.034	0.912	0.97
Oats	0.014	1.087	0.98
Oats	0.035	3.488	0.99
Coatless oats	0.035	2.265	0.98

Fermentor model
We used the fitted *ka* and αa values and the sorption isotherms of the substrates used to simulate two previously reported fermentations runs (Weber *et al.* 1999; 2002). Predictions were obtained using the appropriate equation for the respiration rate, i.e. equation (9) or (10). Predictions obtained with the model described in this paper, which we will refer to as "transfer model", were also compared to simulations with the equilibrium-based model (Weber *et al.* 1999; 2002), but contrary to published simulations Weber *et al.* (2002) we did not use the measured water vapour content in the equilibrium-based model but instead used $a_w \approx 1$ and RH=100%.
For the *Coniothyrium* fermentation (Figure 5) the temperature predicted by the transfer model is notably closer to the measured temperature. However, the equilibrium-based model shows an error of only *c.* 1.2 K at the peak temperature, which is already pretty small. The (lack of) effect of the transfer model is discussed further below. The transfer model also gives an improved prediction near the peak temperature for the *Aspergillus* fermentation (Figure 6), but there is still a deviation from *c.* 2 days onwards. The latter deviation is, however, not an error in the transfer model, since it is also present in the equilibrium model. It might be the result of changing reaction stoichiometry, perhaps due to changing maintenance requirements. At any rate, this deviation is not important for practical purposes, since most industrial scale fermentations with *Aspergillus* sp. only last 2 days.

Table 2:
Measured and modelled evaporation data (in grams) for solid-state fermentations.
A = equilibrium model of Weber et al. (2002), using 100% saturated air and water activity=1.0
B = transfer model described in this research, based on actual transfer limitations and a limiting effect of decreased water activity

System	Total air Throughput (m^3)	Measured evaporation	A RH=1	B transfer
C. minitans on oats (PBR 15, Weber *et al.* 2002)	435	3757	3498	3678
A. oryzae on hemp (PBR 22, id.)	180*	564	736	642
C. minitans on hemp (PBR 23, id.)	135	1028	1619	1263

* after 2.5 days of fermentation

The effect of the new model equations on the predictions of evaporation is shown in Table 2. For experiment 15 (Weber *et al.* 2002), the transfer model predicts more evaporation compared to the equilibrium model Weber *et al.* (2002). This is the result of the corresponding improvement in the prediction of the temperature profile, combined with the under-saturation of the air in the transfer model. For the other two experiments, the improvement in the temperature prediction was small, and the improvement in the prediction of the evaporation is the result of the mass transfer parameter and the incorporation of a_w-decrease only.

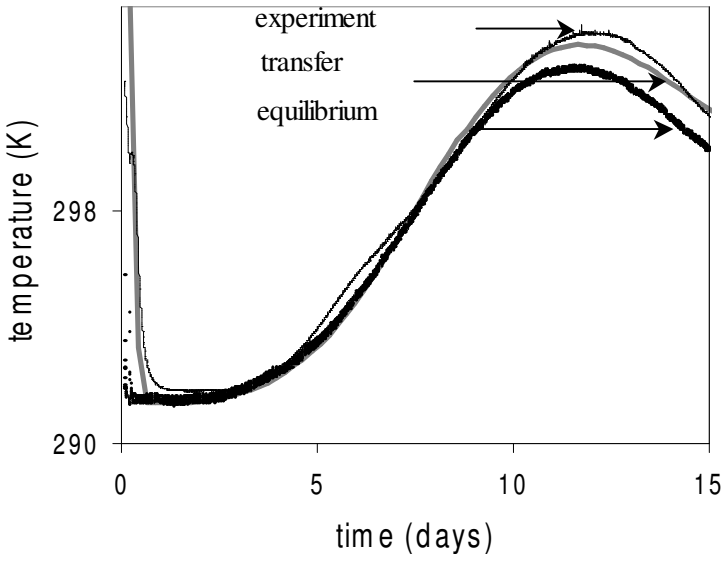

Figure 5: Simulation and experiment for C. minitans growing on oats, for the equilibrium model of Weber et al. (2002), based on the assumption of 100% saturated off gas and for the transfer model described in this paper. The airflow in this experiment was 0.014 kg $m^{-2}s^{-1}$ and the bed height was 45 cm.

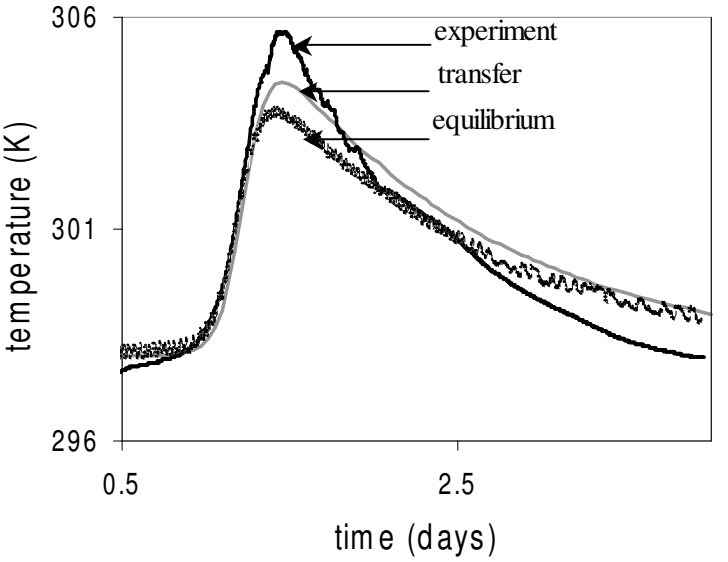

Figure 6: Simulations and experiment for A. oryzae growing on hemp. The airflow velocity in this experiment was 0.034 kg $m^{-2}s^{-1}$ and the bed was 54 cm high.

From Figures 5 and 6 and Table 2, it is clear that the incorporation of water activity dependency and heat and mass transfer limitations in the model improves the temperature predictions and the prediction of the evaporated water. However, the predictions of the equilibrium-based model are already quite accurate for the two fermentations tested, especially for temperature. Therefore, the improvement achieved with the transfer model is not very big. However, one should not forget that the fermentation experiments were not performed with stringent temperature control; instead, a large temperature variation was allowed to facilitate testing of temperature predictions. The importance of the extended model increases when the fermentor is operated under more stringent temperature control, as shown below.

Significance of water activity and transfer limitations

In a production fermentor, the axial temperature gradient is usually kept very small, by using extremely high airflow rates. We used the equilibrium-based model and the transfer model to calculate the airflow rates required to maintain a small temperature gradient in a fermentor. As we noticed that the outcome of the simulations was significantly influenced by allowing slight differences in the final temperature gradient and even more significantly by the final value of a_w we had to specify very strict values for these parameters. Water activity needed to be specified to 4 decimals to prevent measurement errors. We simulated fermentations with temperature gradients of 2.00K and 4.00K, both with a final a_w of 0.960. We assumed a constant heat production rate r_H and a bed height of 45 cm. In the simulations, the required airflow rate was determined by manual iteration.

The effect of the gas velocity on the mass transfer coefficient was incorporated in the transfer model using

$$ka = 3.488\sqrt{\frac{F_a}{0.035}} \qquad (15)$$

This equation is based on the Sherwood relation for packed beds (Beek *et al.* 1999). This model does not predict the effect of air velocity on ka found for wheat and oats very well (Table 1), but we have insufficient measurements to derive a better model. We expect that equation (15) will give predictions closer to the real situation than using one ka-value for all flow velocities and that the effect of a_w will be of more significance to the results than the effect of ka. We used our transfer coefficient for oats at 0.035 kg m^{-2} s^{-1} as a starting point, as indicated by the equation.

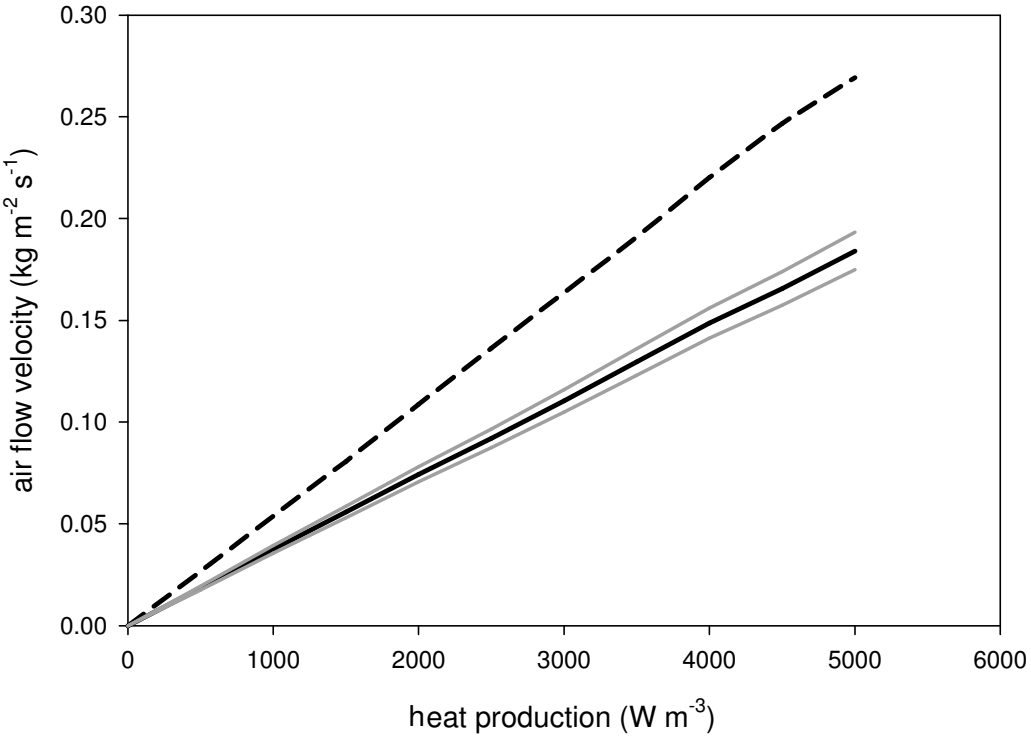

Figure 7: Simulation of the airflow velocity needed to maintain the temperature gradient over a 45 cm packed bed of oats at 2K with heat production Q. The initial temperature of the simulation was 307.0 K and the final temperature 309.0, with a final water activity of 0.960. The dashed line indicates the simulation result obtained with the new transfer model, the solid line is the result obtained with the equilibrium model of Weber et al. (2002). The grey lines indicate 5% deviation from the equilibrium model.

The results of the simulation for a temperature gradient of 2.00K are shown in Figure 7. A heat production rate of *c.* 500 W/m^3 corresponds to fermentations with slow-growing fungi such as *Coniothyrium minitans*, and *c.* 5000 W/m^3 corresponds to fermentations with fast-growing fungi like *Aspergillus oryzae* (Oostra *et al.* 2000; Nagel *et al.* 2000).

Figure 7 shows a difference of approximately 45% in the required airflow velocities for all heat production rates. The significance of the limitations in the transfer model becomes smaller when we allow a larger axial temperature gradient over the bed. The shown effect was less strong for a higher temperature gradient of 4.00K (approximately 20% difference in the required airflow velocities).

When water activity was maintained a constant value of 1.0, the effect of the transfer model decreased to 3% for the simulation with a temperature gradient of 2.00K, which indicates the important contribution of decreasing water activity in the model.

The transfer model yields *c.* 4% lower water vapour content (kg water vapour per kg dry air) than the equilibrium model. When the aeration rate and water vapour content at each data point are combined, we find *c.* 9% more dehydration in the equilibrium model. It must be noted that this extra dehydration is coupled to water activity. Had we chosen another final water activity, then the extra dehydration would be different, since the effect of changing water activity in the model is reflected in the mass balance for water in the solids.

CONCLUSION

The transfer model is an interesting addition to SSF modelling that gives insight in the physical phenomenon of evaporative cooling. It can be useful for systems with a high heat production rate and a narrow temperature optimum. In such systems, the airflow needed to maintain a small temperature gradient over the bed can be highly underestimated if the effect of decreased water activity is neglected. In addition, the effect of the decreasing water activity in the transfer model is also important for the insight into the water content of the substrate. The combined effect of a decreasing water activity and the higher aeration rate needed to maintain the desired temperature yielded 9% deviation in the total evaporation (= # kg water removed) in the system for our simulations. The exact deviation between the two models will vary with the water

activity that is reached, since water activity is present in the mass balances of the transfer model and therefore influences the desorption of water from the substrate. For systems in which water activity is crucial, it is advisable to obtain accurate sorption isotherms to get a reliable insight in the changes throughout the bed.

DISCUSSION

Two further improvements are suggested for the transfer model:

1. Incorporation of kinetic models instead of fits of oxygen uptake data
2. Incorporation of models that predict the effect of gas velocity on transfer rate.

The transfer model functions well when the measured oxygen consumption is used to describe fungal kinetics, but the incorporation of a kinetic model would improve the general applicability of the transfer model. However, the kinetic models available so far are not validated for systems with low water activities and are therefore only useful for systems in which evaporation of water has little effect on the water activity of the substrate.

ACKNOWLEDGEMENT

The authors kindly thank Ron Doezé for his assistance in the determination of the transfer coefficients.

VARIABLES

a_w	Water activity (-)
F_a	Superficial gas velocity (kg dry air·$(m^2 s)^{-1}$)
H_a	Enthalpy of moist air (J·(kg moist air)$^{-1}$)
H_s	Enthalpy of the solid phase (J·$(m^3$ solids$)^{-1}$)
k	Mass transfer coefficient (m s^{-1})
n	Number of slices (-)
p_{vsat}	Saturated water vapour pressure (Pa)
p_{va}	Water vapour pressure (Pa)
r_{O_2}	Respiration rate (kg O_2·$(m^3$ fermentor s$)^{-1}$)
S	Concentration solids (kg·$(m^3$ fermentor$)^{-1}$)
S_o	Initial concentration solids (kg·$(m^3$ fermentor$)^{-1}$)
t	Time (s)
T_a	Temperature of the air (K)

$T_{a,in}$ Temperature of the air at the inlet of the fermentor (K)
T_s Temperature of the solids (K)
W_a Water content of the air (kg water·(kg dry air)$^{-1}$)
$W_{a,in}$ Water content of the air at inlet of reactor (kg water·(kg dry air)$^{-1}$)
W_s Water content of substrate (kg water·(kg substrate)$^{-1}$)
X Concentration of biomass (kg biomass·(kg dry solid substrate)$^{-1}$)
z Height (m)
α Heat transfer coefficient (J·(m^2 sK)$^{-1}$)
ρ_a Density of dry air (kg air·(m^3 air)$^{-1}$)

CONSTANTS

a	720	Specific area of exchange (m^2 area·(m^3 particle)$^{-1}$)
c_{pa}	1005	Specific heat dry air (J kg^{-1} K^{-1})
c_{ps}	2300	Specific heat solid substrate (J kg^{-1} K^{-1})
c_{pw}	4185	Specific heat water (J kg^{-1} K^{-1})
c_{pwv}	1857	Specific heat water vapour (J kg^{-1} K^{-1})
c_{px}	1256	Specific heat biomass (J kg^{-1} K^{-1})
M_w	0.018	Molecular weight of water (kg mole^{-1})
P_{tot}	$1.01 \cdot 10^5$	Atmospheric pressure (Pa)
R	8.3144	Gas constant (J mole^{-1}K^{-1})
T_{ref}	273.15	Reference temperature (K)
$W_{s,start}$	1.35	Initial water content oats (kg H_2O·(kg dry substrate)$^{-1}$)
$W_{s,start}$	4.5	Initial water content hemp (kg H_2O·(kg dry substrate)$^{-1}$)
W_x	2	Water content biomass (kg H_2O·(kg dry biomass)$^{-1}$) (Nagel *et al.* 2001)
Y_{so}	1.95	Yield substrate from oxygen, cereals (kg kg^{-1}) (Weber *et al.* 2002)
Y_{so}	0	Yield substrate from oxygen, hemp (kg kg^{-1}) (Nagel *et al.* 2001)
Y_{wo}	0.68	Yield water from oxygen (kg kg^{-1}), *A.oryzae* (Nagel *et al.* 2001)
Y_{wo}	0.53	Yield water from oxygen (kg kg^{-1}), *C.minitans* (Weber *et al.* 2002)
Y_{xo}	0.88	Yield biomass on oxygen, *A. oryzae* (kg kg^{-1}) (Nagel *et al.* 2001)
Y_{xo}	0.75	Yield biomass on oxygen, *C. minitans* (kg kg^{-1}) (Ooijkaas *et al.* 2000)
ΔH_O	$1.4 \cdot 10^7$	Reaction enthalpy (J kg O_2^{-1}) (Cooney *et al.* 1968)

ΔH_w	$2.5 \cdot 10^6$	Evaporation enthalpy water at 273K (J kg^{-1}) (Perry *et al.* 1984)
ε	0.32	Void volume fraction oats and wheat (-)
ε	0.4	Void volume fraction hemp (-)

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Chapter 4

Water activity effects in packed-bed solid-state fermentation

Marisca J. Hoogschagen, Sebastiaan Haemers, Johannes Tramper and Arjen Rinzema

SUMMARY

Packed-bed solid-state fermentations need to be cooled by aeration to avoid overheating. The evaporation of water contributes substantially to the cooling. The resulting decrease in water activity can lead to fungal growth limitation. The quantitative effect of water activity in solid-state fermentations has so far only been studied "in vitro", neglecting the effects of solutes and time. This study presents an "in vivo" approach for studying the effect of decreased water activity on SSF. The approach is based on comparing a fermentation that is steadily dried out with the results of a fermentation that was carried out at optimal water activity. The results of the experiments described in this paper show that the set-up needs some refinements, because the system already shows a decreased fungal response at an early stage in the fermentation process, when the water activity is still optimal. Two possible explanations for this behaviour are discussed, namely intraparticle gradients and evaporative cooling resulting in wet-bulb temperatures. Wet-bulb temperatures in the bottom region of the fermentation are the most likely explanation for the observed fungal behaviour.

INTRODUCTION

Solid-state fermentation (SSF) is a process in which a micro organism, usually a fungus, grows on a solid substrate in the absence of free flowing water. Water activity (a_w, the fraction of the actual vapour pressure at a certain temperature over the maximum vapour pressure at that temperature) is, besides temperature, an important parameter in SSF.

In general, the initial water content of the solid substrate is limited (around 1 kg (kg wet material)$^{-1}$). Because SSF is often carried out using aeration as the main cooling method, water will evaporate from the solids and the water activity of the substrate may at some stage drop to unfavourable levels. This will lower the activity of the micro organism and lead to inefficient fermentation.
Gervais *et al.* (2003) suggested minimising the decrease in a_w by adjusting the relative humidity of the incoming air during fermentation. In this approach, the effect of forced aeration was misunderstood: the air will heat up while going through the bed, resulting in a decreased saturation level and subsequently evaporation and in axial vapour pressure and temperature gradients (Weber *et al.*, 2002). In fact, it is difficult to prevent an a_w decrease, especially in large-scale fermentations

where the depth of the fermentation bed makes it difficult to measure and adjust a_w inside the bed during the fermentation.

Mathematical modelling can be a valuable tool to predict the conditions inside a fermenting bed. Many studies on the description of a_w-effects in SSF use measurements of colony expansion on agar or agar-like media as indication for the fungal activity (Ramos *et al.*, 1998; Gibson *et al.*, 1994; Marín *et al.*, 1995; Baxter *et al.*, 1998). Besides the fact that agar is very different from normal SSF substrates, measurement of radial growth is a disputable strategy since it has been shown that radial growth is not directly related to hyphal extension (Gervais *et al.*, 2003). In an experimental comparison, the ratio of radial growth rate over hyphal extension rate varied between 0.70-0.96 depending on the organism and the a_w (always higher than 0.95) (Gervais *et al.*, 2003). Some groups investigated the influence of a_w on media more similar to actual SSF systems, such as wheat bran, maize grains or kernel-shaped substrates such as nutrified amberlite (Pandey *et al.*, 1994; Marín *et al.*, 1998; Gelmi *et al.*, 2002, Gervais *et al.*, 2003). Although this strategy may be expected to yield more reliable results because the substrate is a real SSF medium or a substrate similar in shape to a real substrate, a drawback is that in these studies, the water activity was reduced artificially by adding salts such as NaCl or organic solutes such as glycerol to the medium (as was the case in the studies using agar-media). These additives have an extra effect on fungal metabolism besides the reduction of water activity (Baxter *et al.*, 1998; Marín *et al.*, 1995), which makes it difficult to deduce the specific effect of the reduced water activity. Besides, the fact that the organisms were forced into an unfavourable environment from t=0 is likely to have an effect on microbial performance since proper germination may not have been possible. The measured results in the aforementioned studies can be effects on growth, effects on germination, or a combination of both. Finally, none of the studies mentioned so far checked a_w during the fermentations, which further reduces the validity of the results.

In this study, our aim was to obtain an accurate and realistic method for determining the effect of a_w-decrease during solid-state fermentation. In order to do this, we used an experimental set-up in which we could simultaneously carry out fermentations with *Aspergillus sojae* and wheat kernels at a near-constant a_w and at a decreasing a_w (obtained by respectively blowing saturated and dry air through a narrow packed bed).

The system was incubated in a water bath and the bed radius was expected to be sufficiently small to maintain constant temperature in the packed material through conduction. We measured respiration to get an indication of the fungal activity and growth rate and sampled both systems for water activity at regular intervals. One experiment gives an insight in the specific growth rate at a series of time-water activity combinations. Since fungal growth follows a sigmoid pattern, the growth rate varies in time even when water activity remains stable. The variation in time is in most studies described with the logistic law:

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

We chose to carry out experiments at several drying rates, in order to be able to determine the effect of the reduced a_w on the fungus regardless of the phase of fermentation and the amount of cells present.

Our theory for obtaining an a_w-related growth rate is to correlate the observed growth rates for the drying out experiment with the observed growth rate of the reference experiment, in order to obtain the term $g(a_w)$ which reduces the growth rate μ when a_w decreases.

When we assume that the decrease in oxygen concentration in the fermentor is linear from $f_{O_2}^{in}$ to $f_{O_2}^{out}$, the average oxygen fraction in the bioreactor becomes:

$$\left\langle f_{O_2} \right\rangle = \frac{f_{O_2}^{in} + f_{O_2}^{out}}{2} \tag{2}$$

The oxygen-mass balance over the bed reads:

$$\frac{d(\varepsilon V_b \frac{p}{RT}\left\langle f_{O_2} \right\rangle)}{dt} = (f_{O_2}^{in} - f_{O_2}^{out})F_a - r_{O_2}IDM \tag{3}$$

Assuming that ε, V_b, p, T and $f_{O_2}^{in}$ in Equation (3) are constant, the simplified oxygen balance becomes:

$$\frac{\varepsilon V_b p}{2RT}\frac{df_{O_2}^{out}}{dt} = (f_{O_2}^{in} - f_{O_2}^{out})F_a - r_{O_2}IDM \tag{4}$$

We assume that the reference experiment has a growth rate $\mu = f\left(\int_0^t r_{O2}dt\right)$, and also that the deviation of the growth rate for the reference experiment can be described as $\mu \; f\left(\int_0^t r_{O2}dt\right)^{-1} = g(a_w)$. Furthermore, we assume $\frac{r_{O2}}{\int_0^t r_{O2}dt} = \frac{r_X}{X} = \mu$. This means that the value of $g(a_w)$ can be derived from a plot of $\frac{r_{O2}}{\int_0^t r_{O2}dt}$ to $\int_0^t r_{O2}dt$ and a plot of a_w to $\int_0^t r_{O2}dt$. Figure 1 visualises this approach:

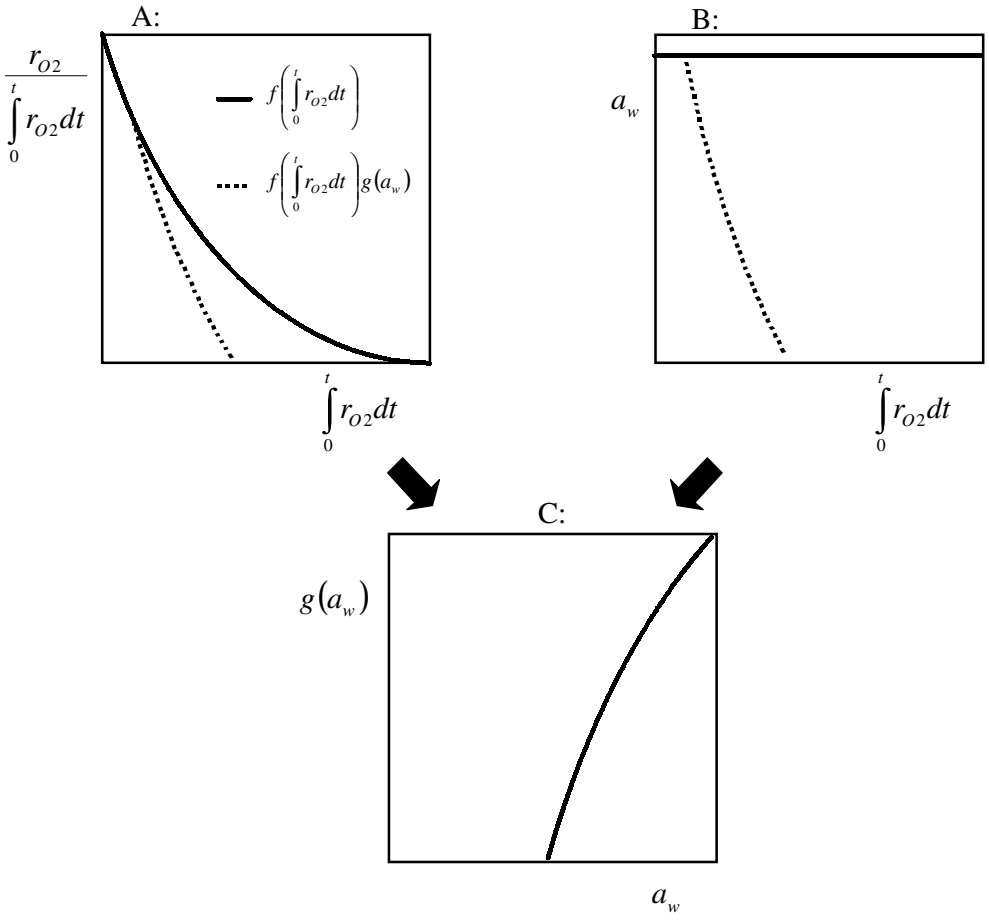


Figure 1: Visualisation of the theory suggested to determine the growth rate reduction factor $g(a_w)$. Figure A shows the correlation between the growth rate of the reference (solid line) and dried (dashed line) experiment. Figure B shows the a_w for both situations, and Figure C gives an indication of the possible profile of the factor $g(a_w)$ in relation to a_w.

MATERIALS AND METHODS

We used *Aspergillus sojae* and whole wheat kernels. The wheat kernels were soaked in excess water for four hours and then sterilised. The resulting water content was around 0.8 kg kg^{-1}. *A. sojae* spores were added to the kernels, and the material was mixed on a set of rollers for four hours. After mixing, the kernels were incubated overnight at the same temperature used in the actual experiment (308 K) to allow unhindered germination. Prior to distribution to the fermentation vessels the material was mixed again.

We used a custom-made column incubator (CI) (Weber *et al.* 2002, see Figure 2), in which a series of small aerated isothermal fermentations (10 ml) could be conducted simultaneously. The CI was equipped with gas analysers for online measurement of oxygen consumption and carbon dioxide formation. Since large scale packed bed fermentations are preferably operated with small temperature gradients, we chose to do fermentations at one temperature (308K) only.

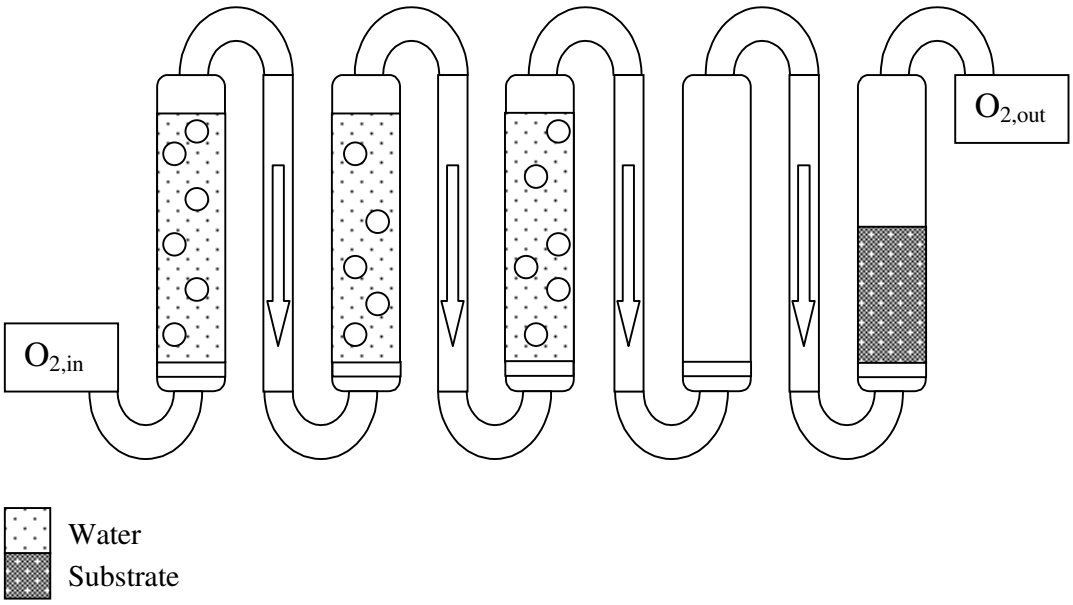


Figure 2: A schematic representation of the CI. Each experiment consists of 4 coupled tubes as depicted here: 3 for humidification of the airflow and one containing the inoculated substrate. The air is blown through sintered glass and flows from the bottom of the tube to the top for each column. An empty column is present to avoid any accumulation of water droplets in the experiment. When drying was desired, the humidification tubes were empty. All fermentations were incubated at the same temperature.

We incubated a series of fermentations at optimal conditions, obtained by aerating with water-saturated air. Simultaneously, a series of fermentations was incubated that was aerated with dry air. The airflow velocity for both systems was the same, and the oxygen concentration in the air was measured as an indication of fungal activity. All fermentations were done with a thin layer of substrate (approx. 5 cm), in order to minimise axial a_w differences in the material.

In order to obtain insight in the amount of drying, samples of both the reference and drying series were taken at certain intervals. These samples consisted of all the material from one 10-ml fermentation. Each sample was mixed prior to measurement of a_w and dry weight (Aqualab series 3, Decagon Devices, and stove at 80°C respectively).

RESULTS & DISCUSSION

We carried out several series of fermentations at different drying velocities by applying different airflow velocities. Different drying rates were used because we wanted to distinguish growth and a_w related effects as described earlier. For each series, separate reference experiments were carried out. The continuous respiration measurements from the drying and reference series were compared to determine the difference in respiration rate caused by the decreased water activity.

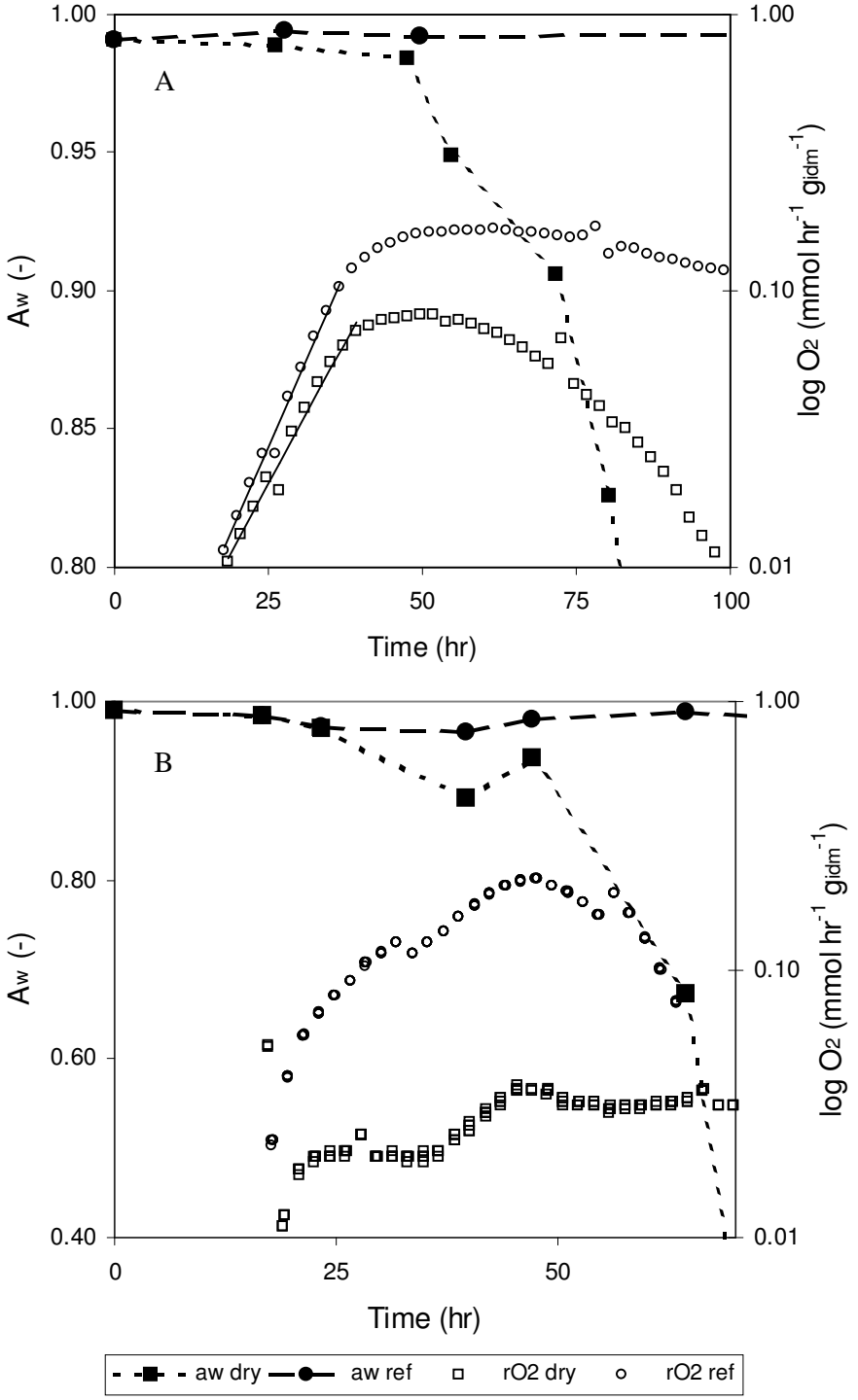
Figures 3A and B show examples of the water activities and respiration profiles. Assuming constant specific growth rate at the start of the experiment, the OUR will be proportional to the amount of fungal cells, as shown in Equations (5) to (7). Hence the slope of a log-plot of the respiration rate gives μ.

$$r_{O_2} = qX \tag{5}$$

$$X = X_0 e^{\mu t} \tag{6}$$

$$\ln(r_{O_2}) = \ln(qM_{x0}) + \mu t \tag{7}$$

As expected, the drying experiments show a reduced growth rate, and the amount of reduction depends on the rate at which the drying took place.



Figures 3 A, B: Water activity and respiration profiles of a reference (•) and a drying-out experiment (■), performed simultaneously in the CI and dried at 20 ml/min and 40 ml/min dry air respectively. For figure A, regression analysis on the respiration data showed that the growth rates for reference and dried experiment were 0.12 ± 0.01 and 0.094 ± 0.01 mmol hr^{-1} g_{idm}^{-1}. The 95% confidence intervals do not overlap.

The results obtained in our experiments show an unexpected phenomenon, as can also be seen in Figure 3. The drying experiments already show a decreased fungal activity while the measured a_w is still similar to the a_w in the reference experiment.

Two possible explanations for the observed reduction at high water activities are:

- an a_w gradient in the grains that is evened out during sampling time, but which hinders fungal growth because the fungus does not penetrate deep enough to bridge it (Figure 4)
- excessive cooling of the grain due to the use of dry air (low wet-bulb temperatures)

Both possibilities were evaluated using mathematical models.

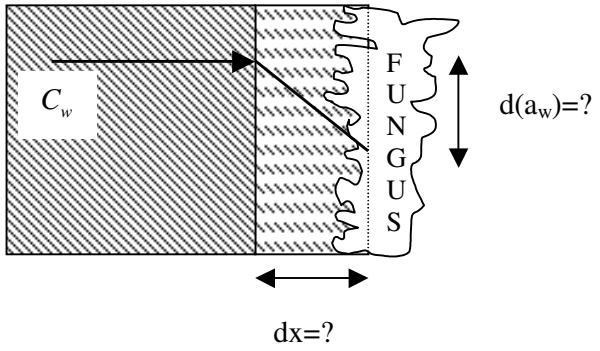


Figure 4: Schematic representation of the distribution of water in a wheat kernel, over a "dry" boundary layer with unknown depth. The fungus penetrates the kernel as it grows.

We calculated the a_w gradient in the kernels using a model developed by Nagel *et al.* (2002). This model is based on Fick's second law with a Stefan correction. The model was developed for flat plates. Based on geometrical considerations, a slab thickness equal to 1/3 of the average grain radius was used in the simulations. The water flux at the grain/air interface was calculated from the dry airflow rate, assuming the air was completely saturated at the column outlet.

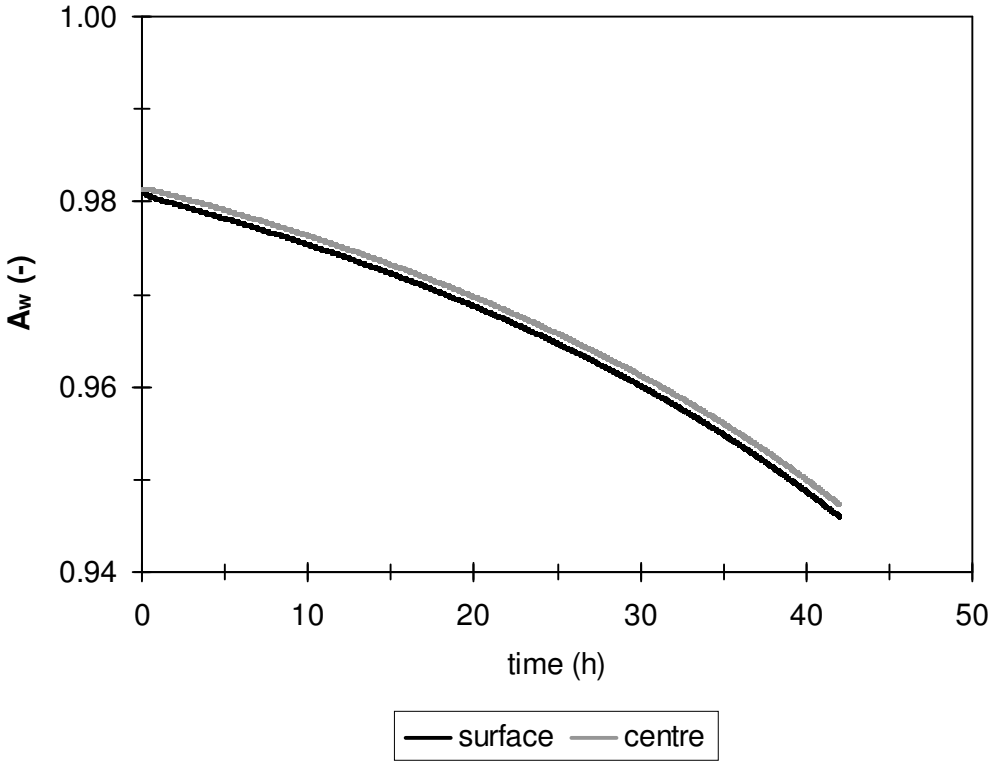


Figure 5: This figure shows the difference in water activity between the surface and the core of a wheat kernel that is continuously dried with 25 ml min^{-1} dry air. The air exits the system saturated with water vapour. The difference between the surface and core of the kernel is caused by the diffusion of water to the surface, which is modelled using a model based on Fick's second law, with a Stefan correction (Nagel et al., 2002).
The parameters used in the model are:
$W_{s,ini}$ *0.445 kg H_2O (kg initial dry matter)$^{-1}$*
d *0.001 m*
D $1.4 \cdot 10^{-10}$ $m^2 s^{-1}$
J $1.35 \cdot 10^{-6}$ $kg\, m^{-2}\, s^{-1}$

Figure 5 shows the calculated a_w profiles at the grains/air interface and in the centre of the grain. Figure 5 shows that a near-constant, very small difference in a_w between the centre and the surface of the kernel exists. It would be very hard to measure such a small difference, also because the time needed for sampling and a_w measurement (5-10 minutes) is long enough to achieve redistribution of water in the kernels. The calculated radial a_w gradient in the kernel is so small that it is unlikely that this is the cause of the observed difference in initial specific growth rate.

The second possibility was that the grain could reach unfavourable temperatures due to fast evaporative cooling with dry air. The wet-bulb temperature of the grain at the bottom of the column was estimated at 290 K (compared to 308 K for the incoming air and incubator). A simulation using simplified enthalpy and mass balances (Equations (8) to (10), adjusted from equations published in Hoogschagen *et al.* (2006)) showed that the first 5 mm of the bed is likely to have a temperature below 300 K, which would have a strong effect on the growth rate of the fungus (figure 6, unpublished results). This corresponds well with the observed decrease in average specific growth rate. Therefore, excessive cooling of the grain at the bottom of the column might explain why the respiration rate is reduced while the (measured) a_w is still equal to that of the reference experiment.

Heat balance solids:

$$0 = \alpha a \frac{1}{4} d^2 (T_a - T) - ka \frac{1}{4} d^2 \left(\frac{p_s(T)}{RT} - \frac{p_w(T_a)}{RT_a}\right) \Delta H_v + 3\lambda (T_w - T) \qquad (8)$$

Heat balance system:

$$0 = -F_a \frac{dh_a}{dz} + \frac{3\lambda}{d} \pi d (T_w - T) \qquad (9)$$

Water balance air:

$$0 = -F_a \frac{P_T}{(P_T - p_w)^2} \frac{dp_w}{dz} + ka \frac{\pi}{4} d^2 \left(\frac{p_S(T)}{RT} - \frac{p_w}{RT_a} \right) \qquad (10)$$

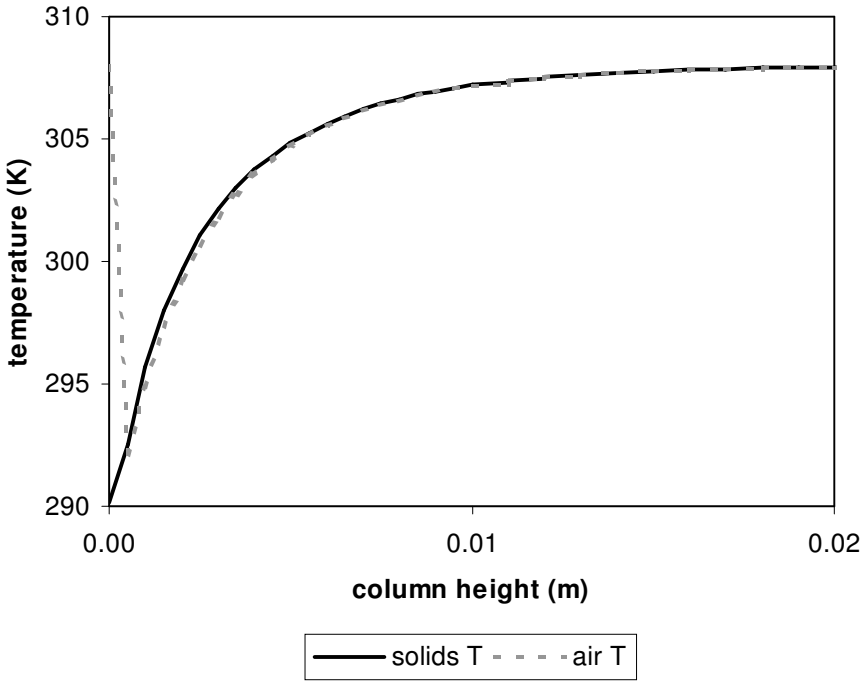


Figure 6: Calculated air and solids temperatures assuming evaporative cooling. Both the solids and air start at 308K. In the experiments described in this study, total column height was approximately 5 cm. 0.5 cm of the bed has a temperature below 303K, which means that 10% of the bed suffers from growth restricting conditions.
The parameters used to obtain these results are:

P_t 10^5 *Pa*
d *0.02 m*
λ *0.1* $Wm^{-1}K^{-1}$
a *760* m^{-1}
α *16* $Wm^{-2}K^{-1}$
k *0.01* ms^{-1}

In conclusion, the dynamic response of fungi to drying out cannot be measured with the set-up used in this study. For an accurate measurement, temperature effects need to be excluded. This can quite easily be achieved by using air with a dew point high enough to avoid excessive cooling in the drying-out experiment, but for accurate results it is important that the moisture level of the air can be kept constant throughout the experiment. When this is possible, a quantification of the dynamic response of fungi to reduced a_w can be obtained using a series of drying out regimes described in this study.

CONCLUSION

We tested a way to induce a steadily decreasing water activity in solid-state fermentation, which does not involve additives and which can be monitored throughout the fermentation process. Our system for monitoring fungal respiration at reduced water activity is in theory more similar to actual fermentation situations than those presented in previous studies. It steadily reduces the water activity by continuously drying the substrate, and the combination of several drying rates with the measured water activities and growth rates could give an indication of the fungal response to a_w levels regardless of biomass age.

However, in our system, the fungus showed a clear response to the drying out environment at an early stage, even before we could measure a difference in water activity between the drying experiment and the reference experiment. We checked if water activity gradients in the kernel existed and if the drying regime might have caused excessive cooling. We found that gradients in water activity inside the substrate particle exist, but it is unlikely that these have the observed effect on fungal growth since the difference between the surface and core is very small. A low substrate temperature due to the rate of evaporative cooling is more likely to be responsible for the reduced growth rate at the start of the drying-out fermentations. A temperature as low as 290K may occur due to the aeration with dry air. Evaporative cooling may lead to unfavourable substrate temperatures in approximately 10% of the bed volume.

The experimental set-up presented in this study is likely to yield valuable insights on the dynamic behaviour of fungal kinetics, including the formation of products such as enzymes and bio pesticides, when a reliable method is found to aerate with air of a sufficiently high dew point to avoid evaporative cooling.

SYMBOLS

a	Specific area solids/gas (m^{-1})
d	Thickness of material (m)
D	Diffusion coefficient (m^2s^{-1})
F_a	Superficial gas velocity (kg dry air$(m^2s)^{-1}$)
f_{O_2}	Oxygen fraction in air (-)
IDM	Initial dry mass (kg)
h_a	Enthalpy of the gas phase (J$(kg\ gas)^{-1}$)

J	Water flux to the air (kg m^{-2} s^{-1})
k	Mass transfer coefficient solids/gas (ms^{-1})
p_s	Vapour pressure for solids (Pa)
p_w	Vapour pressure for moist air (Pa)
P_t	Total pressure (Pa)
q	Specific O_2 uptake rate (moles O_2 [kg biomass s]$^{-1}$)
r_{O_2}	Respiration rate (moles O_2 [kg IDM]$^{-1}$ s^{-1})
R	Gas constant (8.314 J mol^{-1} K^{-1})
t	Time (s)
T	Temperature (K)
T_a	Temperature of gas phase (K)
T_w	Temperature of the reactor wall (K)
V_b	Bed volume (m^3)
$W_{s,ini}$	Initial substrate moisture content (kg initial dry matter)$^{-1}$
X	Biomass concentration (kg biomass (kg dry substrate)$^{-1}$)
X_0	Initial biomass concentration (kg biomass (kg dry substrate)$^{-1}$)
X_{max}	Maximum biomass concentration (kg biomass (kg dry substrate)$^{-1}$)
z	Bed height (m)
α	Heat transfer coefficient solids/gas (Wm^{-2} K^{-1})
ε	Interparticular porosity (m^3 m^{-3})
λ	Thermal conductivity of substrate (W m^{-1} K^{-1}); 0.1 for wheat
μ	Specific growth rate (s^{-1})

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Chapter 5

A dynamic model for shrinkage effects in packed-bed solid-state fermentation

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This text has been submitted for publication

SUMMARY

Shrinkage can have a considerable impact on solid-state fermentations; a volume loss of over 30% is not unusual. The amount and nature of the shrinkage depend on the choice of substrate material and fungus. In this paper, vertical shrinkage is studied both theoretically and experimentally. We found that a packed bed of wheat kernels inoculated with *Aspergillus sojae* can loose 25% of its bed height during fermentation. This height loss has to be taken into account during model simulations, because discrepancies between measurements and simulation are otherwise inevitable. An extended model is presented in which solid-state fermentation is described mathematically, taking into account bed shrinkage due to evaporation, fungal growth and substrate consumption. This model has proven to be a good method for predicting height loss and evaporation.

INTRODUCTION

During solid-state fermentation (SSF, the fermentation of a solid substrate in the absence of free flowing water), many processes take place simultaneously. Substrate is consumed, biomass is formed, temperatures rise and fall, water evaporates, and all of this changes the structure of the bed, often to such extent that it affects the fermentation results.

In this study, we focus on aerated packed-bed fermentations operated with fast-growing fungi on grain. In systems with non-inert substrates such as grain, the consumption of the solid substrate and the evaporation of water due to evaporative cooling will cause shrinkage of the bed, which can result in channelling, especially when a fungus with a tight hyphal network is used (Weber *et al.*, 1999; Weber *et al.*, 2002). Channelling results in inefficient cooling, which in turn leads to inefficient fermentation. In industrial scale it is hard to check if channels exist, but knowledge on the amount of channelling is valuable for understanding the situation inside the bed. It can trigger decisions for mixing, moisturising and terminating the fermentation. A mathematical model that accurately predicts shrinkage for an ideal situation with no channelling can help to predict the amount of channelling in industrial scale fermentations, because discrepancies between the bed height predicted by the model and the bed height observed in the fermentation will then be the result of channelling.

When constructing a model for shrinkage, several aspects need to be taken into account. First, the contribution of aeration to the cooling of the bed will change when the bed height decreases. Second, care has to be taken to assure that the correct model data is compared with the data obtained experimentally. This is important when bed measurements are obtained at fixed sampling points: when the bed moves down because of shrinkage, the composition of the material at the sampling point no longer changes because of metabolic activity only, but also because of the relocation of the substrate (Figure 1).

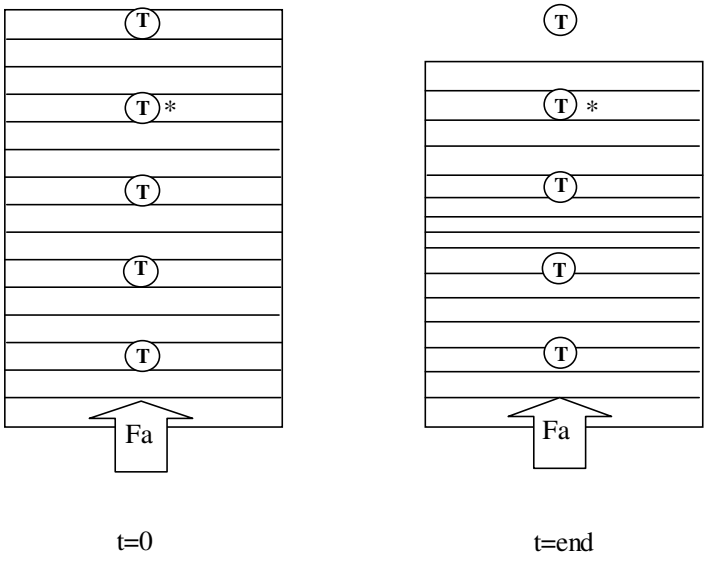

*Figure 1: Schematic overview of the shrinkage effects on validation results and of the proposed solution to the calculation of shrinkage in the bed. By allowing the separate slices to shrink independently, the model predictions can be related to the temperature measurements from the temperature sensors more accurately: the second temperature sensor from the top (marked *) was situated in the 12th slice at the start of the experiment, and in the 14th slice at the end of the experiment.*

We derived and validated a mathematical model that predicts fungal growth and shrinkage in a substrate bed. The model organism and substrate were *Aspergillus sojae* and wheat kernels. This fungus was chosen because it is a fast growing organism that forms short hyphae that don't knit the substrate particles together. Because of this characteristic, it is possible to carry out packed bed fermentations without the excessive channelling that was seen in earlier studies with *Aspergillus oryzae* (Weber *et al.*, 2002): an aerated system inoculated with *A. sojae* will still shrink, but the shrinkage is limited to the axial (vertical) direction.

THEORY

A previously reported model (Hoogschagen *et al.*, 2006) was adapted to predict axial volume decrease. The assumptions made for the incorporation of volume decrease were:

1) The bed consists of several fractions, each occupying a separate volume fraction in the bed. The fractions are: dry substrate particles, water inside the particles, biomass, water inside the biomass, air.
2) The particle packing remains constant during the experiment, i.e. the volume fraction occupied by the wet substrate particles (= substrate + absorbed water) remains the same (Figure 2), even though water evaporates and substrate is consumed.
3) Biomass only occupies the voids between the substrate particles and does not grow inside particles. The fungal growth thus results in a decreasing gas fraction.
4) No hollow spaces exist inside grain particles or in fungal mats.

The changes in substrate, water and biomass amounts are calculated using mass balances and translated to volumes using constant densities.
Model predictions were generated using the method of lines. The predictions are numerically calculated for small (50 to 100 slices in total) radial slices in the packed bed fermentor, as shown in Figure 3. In each slice we distinguish compartments for the dry substrate solids, the water absorbed in the solids, the dry fungal biomass, the water in the fungal biomass, and the air (with water vapour), with volume fractions ε_s, ε_w, ε_x, ε_{wx} and ε_a respectively.

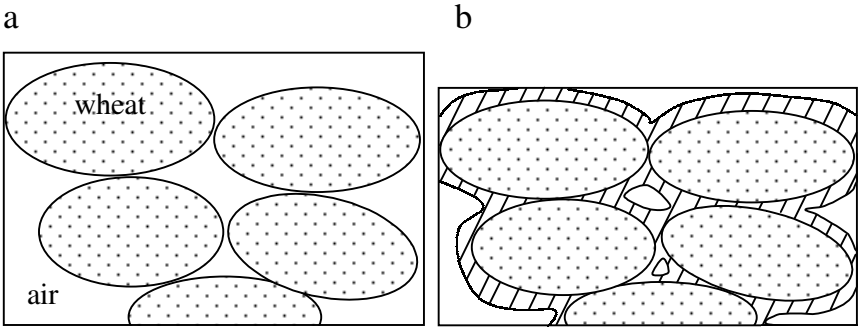

Figure 2: Illustration of the envisioned shrinkage in combination with biomass development in the pores. The volume fraction of air and biomass in picture b is the same as the volume fraction of air in picture a.

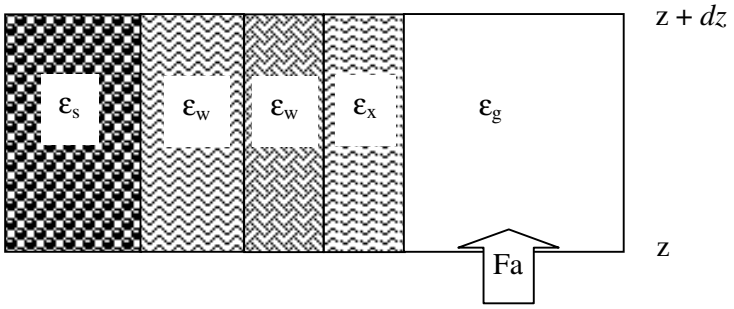

Figure 3: Schematic overview of a radial slice in the packed bed reactor, showing all fractions for which the calculations were made.

We derived mass and enthalpy balances for all compartments mentioned above. These balances were derived assuming that

1) There are no radial gradients in the bed
2) There is no axial conduction or gas dispersion
3) The fungal biomass has a constant water content similar to *Aspergillus oryzae*, being W_x =2 kg water (kg dry biomass)$^{-1}$, as reported previously (Nagel *et al.* 2001)

The only difference compared to our previous model (Hoogschagen *et al.*, 2006) is that we now take into account that the bed shrinks in vertical direction.

Because every slice in the model calculations now has its own specific volume and volume decrease, the mass balances for the dry substrate solids, the water in the solids, the dry fungal biomass, the water in the fungal biomass and the water vapour in the air have to be calculated for the actual slice height.
For biomass and water inside the biomass, the mass balances are, respectively:

$$\frac{d\varepsilon_s \rho_s dz}{dt} = r_s\, dz \tag{1}$$

$$\text{with } r_s = -r_{O_2} Y_{so} \tag{2}$$

$$\frac{\partial\varepsilon_w \rho_w dz}{\partial t} = (r_w - W_x r_x)dz - ka(a_w \frac{p_{vsat}(T_s)}{RT_s} - \frac{p_{va}}{RT_a})dz \tag{3}$$

$$\text{with } r_w = r_{O_2} Y_{wo} \tag{4}$$

$$\text{and } r_x = r_{o_2} Y_{xo} \tag{5}$$

$$\text{and } p_{vsat}(T_s) = \exp\left(23.59 - \frac{4044.54}{T_s - 37.695}\right) \tag{6}$$

$$\text{and } p_{va}(T_a) = \frac{P_{tot} W_a(T_a)}{W_a(T_a) + 0.622} \tag{7}$$

(Equation (4) to (7) from Weber *et al.*, 1999)

The water activity is determined from sorption isotherms and is related to the water content of the substrate as:

$$a_w = \frac{1.029 W_s}{0.039 + W_s} \qquad \text{(wheat and oats; } R^2\text{=0.92)} \tag{8}$$

The mass balances for the biomass and water in the biomass are:

$$\frac{\partial\varepsilon_x \rho_x dz}{\partial t} = r_x\, dz \tag{9}$$

$$\frac{d\varepsilon_{wx} \rho_w dz}{dt} = W_x r_x\, dz \tag{10}$$

The mass balance for the moist air fraction in the bed reads:

$$\frac{\partial \varepsilon_a \rho_a W_a dz}{\partial t} = -F_a \frac{\partial W_a}{\partial z} dz + kaM_w (a_w \frac{p_{vsat}(T_s)}{RT_s} - \frac{p_{va}}{RT_a}) dz \quad (11)$$

The overall volume change of a slice can be determined from the change in particle volume with the following constitutive equation:

$$\frac{d(1-\varepsilon_{ini})\left[\begin{array}{c}\text{Slice}\\ \text{volume}\end{array}\right]}{dt} = \frac{d\left[\begin{array}{c}\text{Particle}\\ \text{volume}\end{array}\right]}{dt} \quad (12)$$

In this equation, ε_{ini} is the initial gas fraction in the slice, and particle volume consists of the volume of the dry substrate together with the volume of the water inside the substrate. Because the model is based on the assumption that biomass growth takes place in the void fraction of the bed, we can use the initial gas fraction ε_{ini} throughout the model to describe the combined volume fraction of (moist) air and biomass, i.e. the non-particle volume fraction. Because the volume of the total slice decreases during the fermentation while the width of the slice remains constant, the slice height *dz* decreases independently for each slice. Throughout the entire bed, the volume changes will result in a variety of *dz* values (Figure 1).

The volume of the dry solid substrate in the slice is defined as:

$$V_s = \frac{V_{slice} S}{\rho_s} = V_{slice} \varepsilon_s \quad (13)$$

In which V_s (m^3) is the dry substrate volume, V_{slice} (m^3) the slice volume, S the dry substrate concentration (kg m^{-3} bed) and ρ_s (kg m^{-3}) the density of the dry substrate.
The change of substrate volume can be described as follows, assuming constant ρ_s:

$$\frac{\partial V_s}{\partial t} = V_{slice} \frac{d\varepsilon_s}{dt} + \varepsilon_s \frac{\partial V_{slice}}{\partial t} \quad (14)$$

The volume change of the water content of the solid substrate is derived similarly:

$$\frac{\partial V_w}{\partial t} = S V_{slice} \frac{\partial \varepsilon_w}{\partial t} + \varepsilon_w V_{slice} \frac{\partial S}{\partial t} + S \varepsilon_w \frac{\partial V_{slice}}{\partial t} \tag{15}$$

In which V_w (m^3) is the water volume.

The overall volume change of a fermenting slice can now be obtained by combining the previous equations (12), (14) and (15):

$$\frac{\partial V_{slice}}{\partial t} = \frac{\left(\frac{V_{slice}}{\rho_s} + \frac{W_s V_{slice}}{\rho_w} \right) \frac{dS}{dt} + \frac{S V_{slice}}{\rho_w} \frac{\partial W_s}{\partial t}}{(1 - \varepsilon_{ini}) - \frac{S}{\rho_s} - \frac{S W_s}{\rho_w}} \tag{16}$$

The actual air fraction ε_a ($m^3 \cdot m^{-3}$) is defined as $(1 - \frac{V_s + V_w + V_x + V_{wx}}{V_{slice}})$.

We assume ideal packing, which means that ε_a only changes when V_x and/or V_{wx} changes.

$$V_x + V_{wx} = \frac{X S V_{slice}}{\rho_x} + \frac{X S V_{slice} W_x}{\rho_w} \tag{17}$$

$$\frac{d\varepsilon_a}{dt} = -\left(\frac{V_{slice} \left(\frac{dV_x}{dt} + \frac{dV_{wx}}{dt} \right) - (V_x + V_{wx}) \frac{dV_{slice}}{dt}}{V_{slice}^2} \right) \tag{18}$$

Of course $\frac{d\varepsilon_a}{dt}$ is also taken into account in both the mass and enthalpy balances, meaning that in all cases except for cases referring to equation (12) the actual ε_a is used. The flow speed and mass transfer coefficient are also adjusted for changes in the void fraction.

Two enthalpy balances are determined over the combined solids and liquids and over the gas phase respectively. They read as follows:

$$\frac{dH_s dz}{dt} = r_{O_2}\Delta H_O dz - \alpha a(T_s - T_a)dz - kaM_w\left(a_w \frac{p_{vsat}(T_s)}{RT_s} - \frac{p_{va}}{RT_a}\right)(\Delta H_w + c_{pwv}(T_s - T_{ref}))dz \quad (19)$$

in which

$$H_s = (\varepsilon_s c_{ps}\rho_s + \varepsilon_w c_{pw}\rho_w + \varepsilon_x c_{px}\rho_x + \varepsilon_{wx} c_{pw}\rho_w)(T_{sp} - T_{ref}) \quad (20)$$

$$\frac{\partial \varepsilon_a \rho_a H_a dz}{\partial t} = -F_a \frac{\partial H_a}{\partial z}dz + \alpha a(T_s - T_a)dz + kaM_w\left(a_w \frac{p_{vsat}(T_s)}{RT_s} - \frac{p_{va}}{RT_a}\right)(\Delta H_w + c_{pwv}(T_s - T_{ref}))dz \quad (21)$$

in which

$$H_a = c_{pa}(T_a - T_{ref}) + W_a(\Delta H_w + c_{pwv}(T_a - T_{ref})) \quad (22)$$

Particle characteristics

In addition to the changes in volume and composition of the slices, the area of exchange (a) also changes in a shrinking packed bed. This affects the heat and mass transfer in the system. In the following equations we assume that the grain particles are spherical and that biomass will not take part in the evaporation and exchange of water.

$$a = \frac{6(1-\varepsilon_a)}{d_c} \quad (23)$$

In which d_c (m) is the changing characteristic diameter of a substrate particle, defined as:

$$d_c = \left(\frac{6(V_p)}{\pi}\right)^{1/3} \quad (24)$$

V_p is the particle volume at certain moisture content, derived from experimental sorption experiments. V_p changes in time per slice due to slice specific evaporation rates.

MATERIALS AND METHODS

Several experiments have been performed to find relations for the water content-volume ratio of our substrate and to validate the shrinkage predictions.

Water content-volume ratio

The relationship between moisture content and particle volume was studied in an experiment similar to an experiment of Oostra *et al.* (2000) in order to check our assumption that wet particle volume is equal to the combined volumes of the dry particle and the volume of the absorbed water. We humidified batches of wheat with a known amount of water and measured the volume, dry weight and water activity of these samples.

Validation

We performed (pilot-scale) fermentations in an insulated 15-l packed bed bioreactor (Weber *et al.*, 2002). For each fermentation, 5.00 kg of dry wheat was moistened to an initial moisture content of 0.9 kg/kg dry mass. The airflow during fermentation was 0.034 kg m^{-2} s^{-1} and the inlet air temperature and dew point were 32° and 31.5°C respectively. We measured bed temperature (at several heights), respiration (overall), dew point of the outcoming gas, pressure drop and bed height during fermentation. The fermentor was disturbed twice a day by means of hitting the walls with a rubber hammer in order to prevent channelling. The pressure over the bed was measured as an indication of channelling. Bed height was measured both before and after shaking in order to check the effect of the bed disturbance, using an optic distance measurer that was located on the (transparent) lid of the fermentor.

For the comparison of model temperature profiles with experimental temperature data obtained at fixed points in a packed-bed fermentation, it is important to match the correct data with the experiment. In order to do this accurately, dz must not be too large in the model. We chose an initial value for dz of 1 cm for comparison with experimental data obtained at heights 10 cm apart.

RESULTS

Pilot scale experiment and model validation

We performed the same fermentation three times with good reproducibility. There were no indications of radial differences in the bed either visibly or in water content of the substrate. The bed material was loose; no significant lumps of grains were present.
The fermentations showed a maximum temperature gradient of 10°C and an overall bed shrinkage of approximately 25%.

For our simulation of bed shrinkage, we wanted to exclude possible errors in the heat production in the model, and therefore we fitted the respiration of one of the 15-l packed bed experiments using the following equation:

$$r_{O_2} = 3.3\cdot10^{-6} + 2.1\cdot10^{-3}\frac{\exp\left(\frac{t-1.3\cdot10^{5}}{1.4\cdot10^{4}}\right)}{\left(1+\exp\frac{t-1.3\cdot10^{5}}{1.4\cdot10^{4}}\right)^{1.25}} \qquad (R^2=0.97) \qquad (25)$$

The fit is shown in Figure 4.

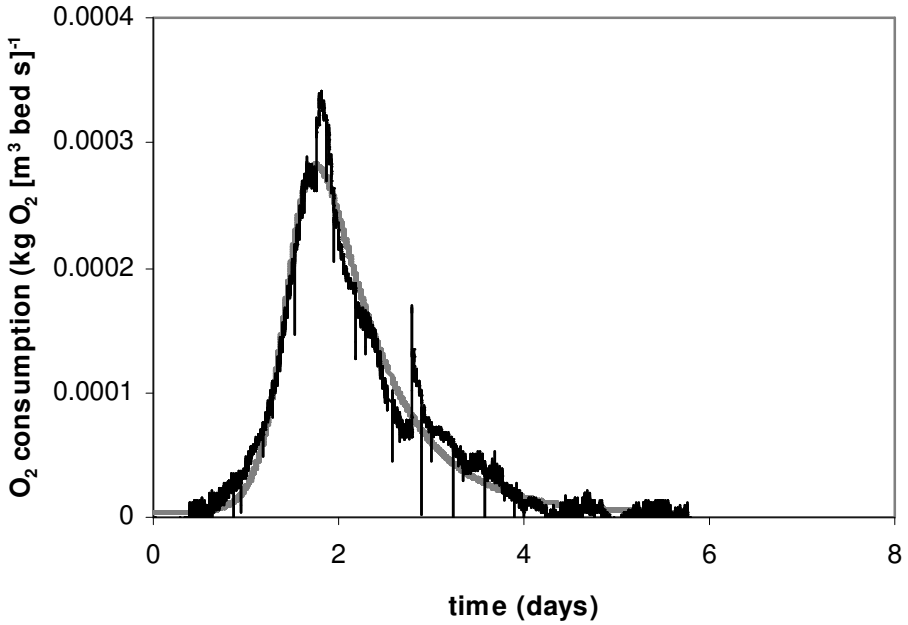

Figure 4: Measured (black line) and fitted (grey line) oxygen consumption for the validation experiment in the 15 l packed bed reactor.

In the model, the amount of respiration per slice is determined from the actual amount of bed material present in the slice, changing in time.

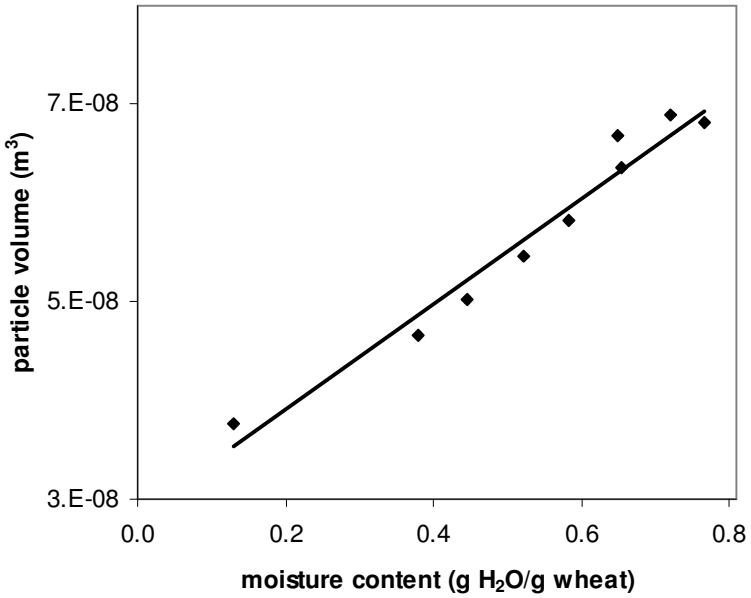

Figure 5: Particle volume versus moisture content for wheat.

Figure 5 shows the volume of grain particles in relation to their moisture content. From these data, an equation for V_p is obtained.

$$V_p = 5 \cdot 10^{-8} W_s + 3 \cdot 10^{-8} \qquad (R^2 = 0.961) \tag{26}$$

The nice linear correlation between V_p and W_s shows that our assumption about particle volume being the sum of the dry substrate volume and the absorbed water volume is acceptable.

The height and evaporation data of a pilot-scale fermentation are shown in Figures 6, 7 and 8. In these figures, model predictions obtained using the fitted respiration described above (equation (25)) are also presented.

The initial height in the model has been derived from the calculated packing volume of the amount of dry substrate particles and the volume of water in the particles at the start of the experiment. This is slightly higher than the initial measurements, due to the uneven surface of the actual bed. As can be seen in Figure 6, the model predicts the total bed shrinkage very accurately, but only when the assumption of the location of the fungus is changed to a constant void fraction, i.e. the fungus grows into the space previously occupied by substrate, and not into the void volume.

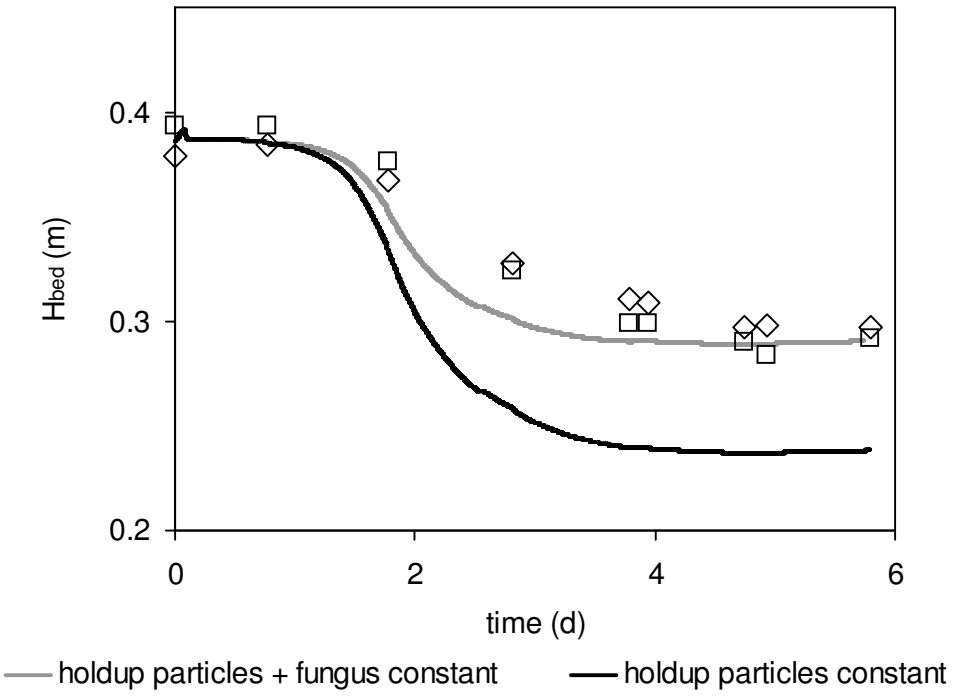

Figure 6: Measured (symbols) and predicted (line) shrinkage of a packed bed of wheat kernels fermented with Aspergillus sojae. Bed height was measured at two predefined coordinates on the bed surface.

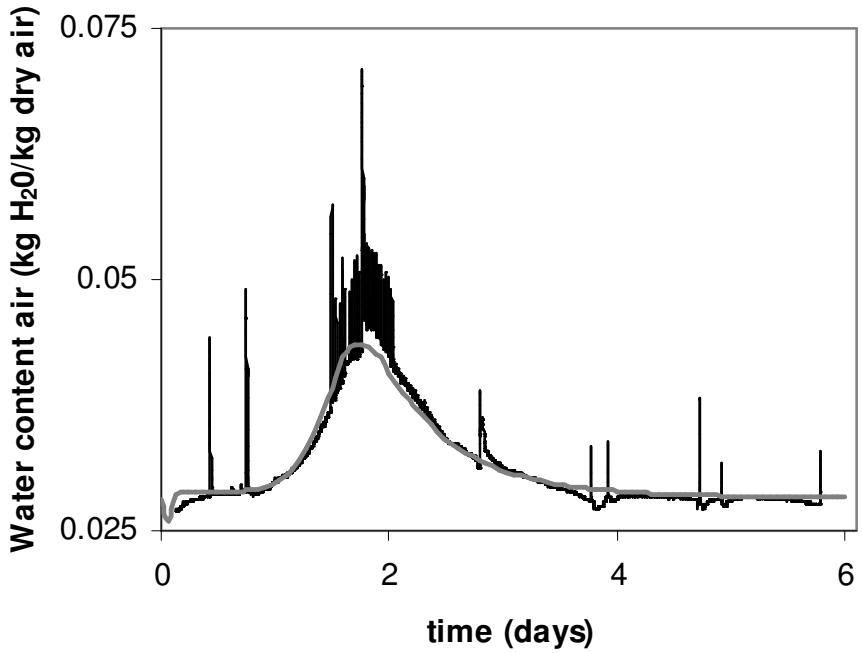

Figure 7: Measured (black) and predicted (grey) water content in the outcoming air of a 15 l aerated packed bed reactor filled with Aspergillus sojae and wheat.

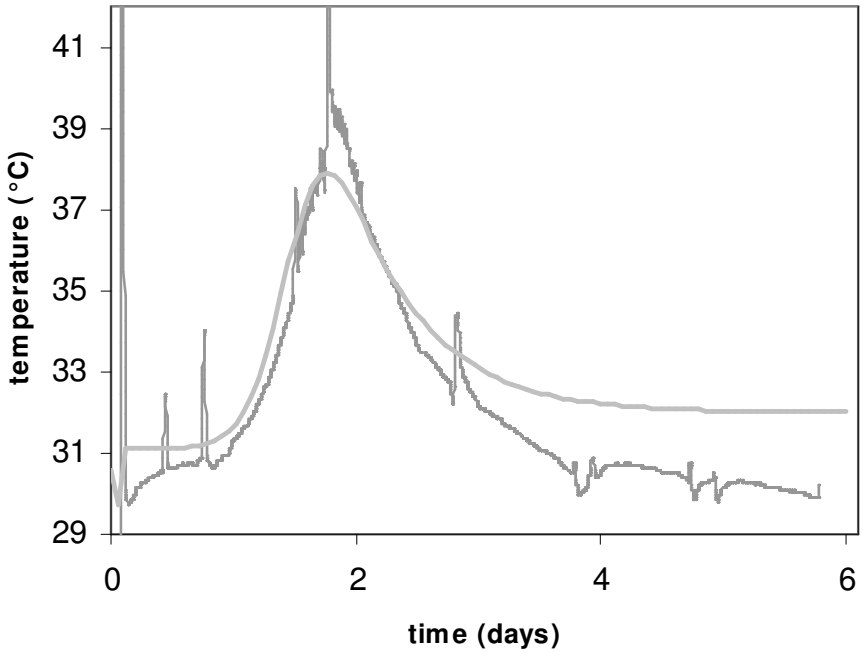

Figure 8: Modelled temperature at the top of the bed (smooth line) and measured dew point of the outcoming air (spiky line).

The evaporation from the bed has also been modelled accurately, indicating that our water balances provide a realistic description of the drying out of the bed.

The correct prediction of both bed shrinkage and evaporation is an important result for industrial application of the model: it is a useful tool to gain online insight in the status of the bed material, because deviations between the model data and the observations of bed height and the dew point of outcoming air can be considered clear indications of anomalies in the bed.

In Figure 9, the temperature profiles of the experiment and the model are compared. The predicted temperature profile was lower than the profile measured during fermentation and the temperature lines in the measurements have no resemblance with the model. Four possibilities for the deviation are:

1) Errors due to the incorporation of shrinkage
2) Errors in the heat balances
3) Inaccurate respiration kinetics
4) Corrupted temperature measurements, due to lump formation on the sensors

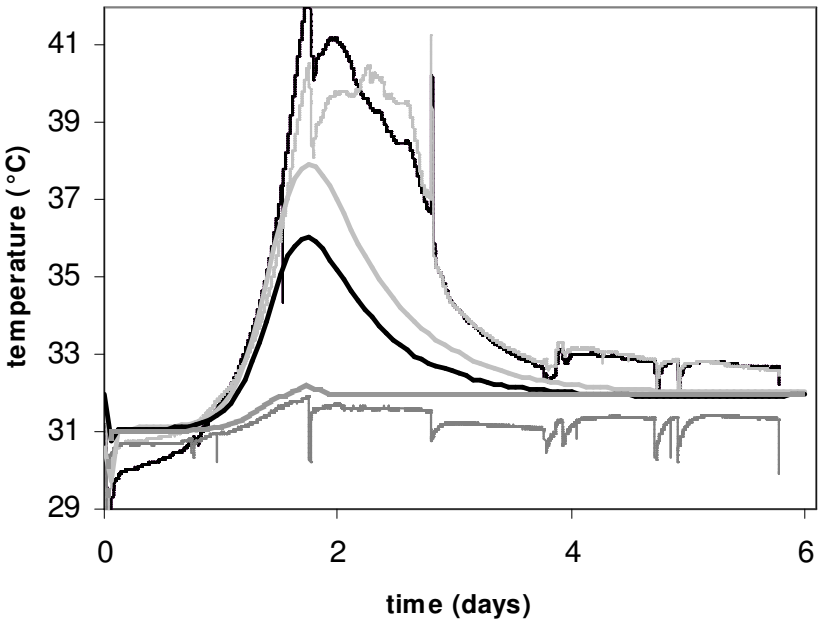

Figure 9: Measured (thin lines) and predicted (fat lines) temperatures for a 15 l packed bed fermentation of Aspergillus sojae on wheat. The prediction was obtained with the fitted respiration of the experiment (Figure 4).

The model prediction of the temperature profile did not improve on removal of the shrinkage terms, showing that there are no errors in the conversion of respiration to heat production per slice. We also checked if the model could predict the temperature profile of a packed-bed fermentation on an inert substrate (i.e. without shrinkage, experimental data from Weber *et al.*, 2002) and found an accurate temperature profile for that experiment (results not shown). These two checks indicate that the discrepancy in temperature profiles of the model and the experiment is not due to errors in our extended heat balances nor in our approach to match simulated bed height to physical height. The use of a two-step respiration fit (to improve the prediction of the steep peek in the oxygen profile) hardly improved the prediction of the model, thus ruling out the third possible cause for the results in Figure 9.

Interestingly, the model prediction of the water content in the air (W_a) (Figure 7) is very accurate, as is the correlation between the measured outgoing dew point temperature and the simulated bed temperature at the highest point (Figure 8). This information shows that the overall prediction of the occurrences in the bed was correct, despite the mismatch between the temperature profiles. The only likely explanation remaining is therefore that the bed was lumping up on the temperature sensors. Lumps of material result in local dense and poorly cooled areas around the sensors. This by itself indicates that the regular disruption of the bed was necessary because a small amount of channelling also occurs with *A. sojae*, and that we would not have had as accurate a match between our modelled and measured shrinkage without it.

The overall results of our model strongly indicate that our prediction of temperature is more accurate to the temperature development in an industrial fermentation with a large radius and no obstructions from temperature sensors than our experimental data.

DISCUSSION

Since the amount of shrinkage can be considerable in solid-state fermentations, a correct prediction of shrinkage is valuable for large-scale fermentations in which optimum fermentation is desirable. The presented model is an accurate tool for giving insight into occurrences that are difficult to check in industrial fermentations. First, the model can be used to predict bed shrinkage and to signal channelling. Besides this, the model gives accurate insight in the drying out in the bed, which can be checked during fermentation by measuring the dew point of the off-gas.
The present model is very versatile. Its development was possible because of the special characteristics of *A. sojae*, which has a short and loosely knit hyphal network. The models use however is not limited to such systems, as the combination of the predicted vertical shrinkage with the actual vertical shrinkage is an indication of the amount of radial shrinkage in the bed.
The drawback of the present model is that the predictions are dependent on the input of respiration measurements from the system it predicts. This limits the use of the model as a purely predictive tool. The development of a non-empirical description of fungal kinetics will therefore further improve the present model.

SYMBOLS

a_w	Water activity (-)
d_c	Characteristic particle diameter (m)
F_a	Superficial gas velocity (kg dry air $(m^2\ s)^{-1}$)
ka	Mass transfer coefficient · specific area of exchange (s^{-1})
H_a	Enthalpy of the gas $(J(kg\ gas)^{-1})$
H_s	Enthalpy of the solids $(J(kg\ solids)^{-1})$
M_w	Molar mass of water $(kg\ mol^{-1})$
M_x	Biomass *dry matter* content $(kg\ kg^{-1})$
p_{va}	Water vapour pressure of air (Pa)
p_{vsat}	Saturated water vapour pressure of air (Pa)
r_{o_2}	Respiration rate $(kg\ O_2\ (m^3\ fermentor\ s)^{-1})$
r_s	Substrate consumption rate $(kg\ substrate\ (m^3\ fermentor\ s)^{-1})$
r_w	Water removal rate $(kg\ H_2O\ (m^3\ fermentor\ s)^{-1})$
R	Gas constant $(8.314\ J\ (mol\ K)^{-1})$
S	Substrate load $(kg\ (m^{-3}\ bed))$
T_a	Temperature of the air (K)

T_{sp} Temperature of the solid particles (K)
V_p Particle volume (spherical) (m^3)
V_s Substrate volume (m^3)
V_{slice} Slice volume (m^3)
V_w Water volume (in substrate) (m^3)
V_x *Wet* biomass volume (m^3)
W_a Water content of the air (kg kg^{-1})
W_s Water content of the substrate (kg kg^{-1})
W_x Water content of the biomass (kg kg^{-1})
X Biomass content per kg of substrate (kg kg^{-1})
Y_{so} Yield coefficient of substrate on oxygen (kg kg^{-1})
Y_{wo} Yield coefficient of water on oxygen (kg kg^{-1})
z Bed height (m)
αa Heat transfer coefficient · specific area of exchange (J $(m^3 sK)^{-1}$)
ε_a Volume fraction of air (m^3 m^{-3})
ε_{ini} Initial volume fraction of air (m^3 m^{-3})
ε_s Volume fraction of dry substrate (m^3 m^{-3})
ε_w Volume fraction of water (m^3 m^{-3})
ε_x Volume fraction of biomass (m^3 m^{-3})
ρ_a *Dry* air density (kg m^{-3})
ρ_s *Dry* substrate density (kg m^{-3})
ρ_x Biomass density (kg m^{-3})

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Chapter 6

Modelling packed-bed solid-state fermentation: near completion?

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This text has been submitted for publication.

ABSTRACT

In the past decades, many mathematical models for solid-state fermentation processes have been published. In this paper, an overview of the present status of the models for aerated packed beds is given. The aim of this overview is to decide whether further steps are needed to improve the insight in the process. Two main areas of modelling are distinguished: microbiology and physics. With respect to the microbiological behaviour of fungi in solid-state fermentation, the modelling is not perfect yet. Temperature and water activity are difficult to study separately, since the response to both parameters varies with the stage and history of the fermentation process. The physical processes in solid-state fermentation have been modelled more successfully: the temperature development, the drying out and the shrinkage of the material can be predicted with the models available so far.

INTRODUCTION

Solid-state fermentation (SSF) is characterised by the absence of free-flowing liquid in the fermentation. A micro-organism, usually a fungus, grows on moist solid material. Several operating procedures exist, which can be categorised as tray, packed-bed and mixed fermentations. In this categorisation, tray fermentations are characterised by the least amount of control, whereas mixed fermentations have the most control mechanisms.
In SSF, big increases in substrate temperature have to be prevented because high temperature has a negative effect on fungal growth. This means that the metabolic heat released in the process has to be removed. In tray fermentations, conduction is the only way in which the temperature build-up can be compensated. Because the temperature inside the fermentation is dependent on the heat conduction capacity of the substrate, which is in general low, the bed height is limited to a couple of centimetres. In packed-bed fermentations, the bed is aerated to remove metabolic heat. Because of this, more heat can be removed from the system, and the bed height in packed-beds can be 50 cms or more. A drawback of packed-bed fermentations is that the bed will dry out because of the aeration, it is difficult to substitute the evaporated water. In mixed fermentations, such control is possible, because the bed material and any added water can be homogenised. Several modes of mixing are known, some involving rotation of the fermentation vessel and others involving rotating paddles or blades inside the fermentor. Mixing can be intermittent or continuous, and the resulting material will be loose, with

microbial growth focussing *into* the substrate material rather than *on* the substrate, as is the case in packed-beds and trays. Of the three categories, packed-bed fermentations are most popular in industry because of the low investment costs and the ease of operation.

SSF is a traditional food production process in Asia, used for the production of tempeh, , sake, soy sauce and a number of other foods. Packed-bed SSF for food fermentation came to the western world in 1914, when Takamine wanted to use mould bran to replace malt in the distillery industry (Hesseltine, 1977). The interest in the process arose mainly from the simple processing, the low energy requirement and the low investment costs.
The packed-bed fermentation procedures used for the production of various foods have been optimised on a trial-and-error basis in Asia. The desire to use SSF to produce new types of products and to increase the scale of the fermentation vessel required insight in its governing mechanisms. Therefore, scientific studies on SSF systems have been carried out in several countries around the world from the 1970's onward. At first, the studies mainly focussed on understanding the production process of traditional foods such as tempeh, but, to a lesser extent, the production of secondary metabolites such as aflatoxins (Hesseltine-1, 1977, Shotwell *et al.*, 1969) was also studied. Later, several mathematical models were derived to describe the characteristics of SSF.
In this review, we will discuss the status of SSF models for non-mixed, packed-bed systems with forced aeration, focusing on the need for improvements that still remains.

Introduction to SSF models
In packed-bed fermentations, the removal of heat is of critical importance for the fermentation result. Cooling by blowing air through the bed is effective, but also causes new limitations such as drying out and, as a result of drying out, bed shrinkage. Uncontrolled shrinkage can result in channel formation in the bed, which in turn reduces the effect of aeration. A simplified heat balance for stationary, aerated SSF is given in Figure 1. The figure shows that the heat accumulation is the result of a heat production rate (microbial growth) and several heat removal rates. In this review, the order of the processes described will follow the scheme shown in Figure 1:

1. Heat production by microbial growth
2. Heat transfer
    2.1 Conduction
    2.2 Convection
        2.2.1 Drying out
2.2.2 Channelling

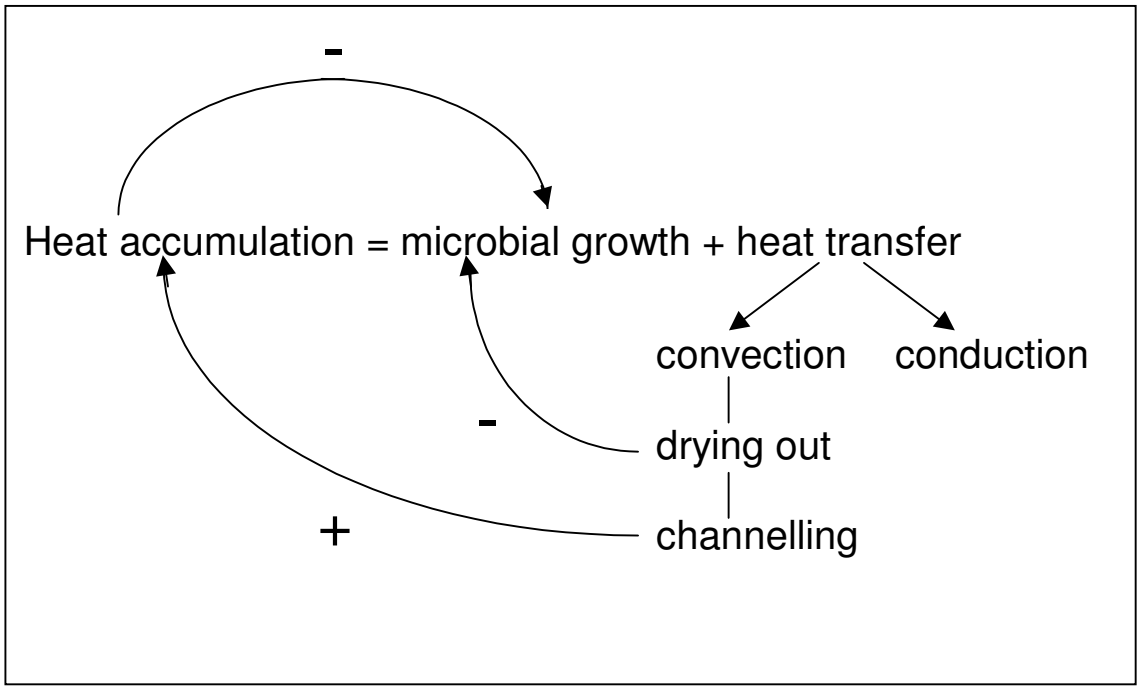

Figure 1: Schematic overview of the processes occurring in packed-bed solid-state fermentation and the corresponding effects on heat accumulation and microbial growth. The order of the subjects in this paper is based on this scheme.

1. HEAT PRODUCTION BY MICROBIAL GROWTH

Microbial growth in SSF typically follows a sigmoid curve, i.e. a lag phase followed by almost linear growth and a decrease in growth once the growth conditions (substrate availability, biomass density, etc.) reach a certain boundary. In order to describe the heat production in SSF, it is crucial to predict this sigmoid pattern accurately.
In the history of fermentor models, two categories of modelling can be distinguished with respect to the complexity in which the microbial behaviour is described:

1.1 highly detailed microbial models, yielding insight in complete metabolic routes, hyphal extension rates, enzyme secretion, etc.

1.2 grey- and black-box models, respectively describing fungal growth with a process depending on a limited number of metabolic parameters or as an empirical equation that happens to correspond with the observed behaviour.

1.1 Highly detailed microbial models

In this type of models, insight in substrate consumption and product formation is the basis of the prediction of biomass growth. For a validated approach, measurements of fungal biomass development are needed. This is problematic, because the micro-organism is in most cases growing *in* the substrate as well as *on* it, making direct measurement of biomass troublesome. This difficulty can be tackled by either preventing penetration into the substrate or by neglecting it. The researchers that choose the prevention approach use artificial substrates topped with membranes to be able to do a direct measurement of biomass (Georgiou *et al.*, 1986; Mitchell *et al.*, 1991-a; Rahardjo *et al.*, 2002). The second group is not interested in the exact location of the fungal material. They deduct biomass development from oxygen consumption or from measurements of cell components such as glucosamine in samples of the fermented bed material (Nagel *et al.*, 2001-a; Mitchell *et al.*, 2004). In the next section, we discuss both types of research simultaneously as both give the insight in fungal metabolism that is needed to predict the heat development in packed-bed fermentations.

Georgiou *et al.* (1986) modelled radial growth of a fungal colony on a solid surface with a defined substrate content. Their model consists of a physiological part and a substrate diffusion part. In the physiological part, the sigmoid growth behaviour is obtained by defining four stages in biomass growth that are each modelled separately as functions of surface substrate concentrations. The predictions obtained with the model were only checked with respect to final biomass levels obtained in measurements from another research group. Because of this, it remains unclear whether the model of Georgiou *et al.* predicts the fungal growth pattern correctly.

In 1991, Mitchell *et al.* (1991-a) also described a non-empirical microbial model based on substrate consumption. They also modelled substrate diffusion, but in a more complex form than Georgiou *et al.* (1986), because they chose starch as substrate. It was assumed that starch needed to be broken down into glucose units before being taken up by the fungus. Therefore the model was based on the production rate, diffusion and activity of glucoamylase, the hydrolysis rate of starch and the diffusivity and metabolic conversion rates of glucose. Biomass development was modelled as a function of the flux of glucose at the surface of the substrate layer. The sigmoid shape of the growth curve was obtained by the introduction of a parameter named critical biomass density, X_c. The key equations used in the study are shown in Equations (1) to (3). All symbols are explained at the end of the paper.

In the model, the transformation of glucose into biomass at the surface is assumed to follow Monod kinetics:

$$J_{G|\delta} = \frac{q_m G\big|_\delta P(X)}{K_s + G\big|_\delta} \tag{1}$$

with

$$P(X) = X + (X_c - X)H(X - X_c) \tag{2}$$

In Equation (2), $H(X - X_c)$ is zero as long as $X < X_c$ and one when $X \geq X_c$, thus limiting the flux of glucose.

$$\frac{dX}{dt} = Y_{X/G} J_{G|\delta} \tag{3}$$

Mitchell *et al.* (1991-a) used an experimental system to obtain parameters for the production-, diffusion- and activity rates of interest. In this system, the biomass (*Rhizopus oligosporus*) grew on a membrane covering a very thin (2 mm) layer of agar-based medium. Concentration profiles for starch, free glucose and glucoamylase in the substrate were measured and fitted with first order equations and Fick's law for the hydrolysis, uptake and diffusion kinetics.
The initial prediction did not match experimental biomass formation. It was improved by arbitrarily choosing new values for the glucose uptake kinetic constant (seven times higher) and the glucoamylase diffusivity (30 times lower). The authors suggested an evaluation of these results, since the two parameters adjusted were parameters that were not determined from their own data, but were extracted from an experiment in aqueous environment and literature, respectively. Although this obviously limits the model, the whole concept of modelling biomass formation based on the metabolic rate constants of substrate components is interesting. With the availability of reliable experimental data, this approach might become very accurate for a wide range of substrate concentrations.

In another study, Mitchell *et al.* (1991-b) presented a correlation model for biomass formation based on the production and diffusion rates of glucoamylase only. Glucoamylase activity is assumed to be proportional to biomass production, since no glucose accumulated in their experiments. The glucoamylase formation is modelled in three stages, namely a lag phase, a phase of linear increase and finally a phase of exponential decay. The model persistently underestimates biomass development by about 25%, apparently due to the initial glucoamylase production rate used. The fit of the glucoamylase activity in this initial period seems quite accurate, but is in fact only based on four measurements. This is probably the cause of the discrepancy in this correlation model and it is unfortunate that no more data on this approach are available.

The correlation between specific cell components, enzymes or substrate breakdown products and fungal growth is, as shown, a difficult procedure, especially because of the difficulties concerned with obtaining detailed measurements. Besides this, several researchers showed that the procedures described in the previous section incorporate some uncertainties. Biomass composition and enzyme formation were both found to vary with substrate composition and biomass age for some situations (Scotti *et al.*, 2000; Nout *et al.*, 1997, Nagel *et al.*, 2001-b).

Measurement of cell components was also found to be unreliable for determining fungal *activity*, because the presence of the components does not discriminate between living and dead cells (Scotti *et al.*, 2000; Nout *et al.*, 1997).

Overall, it can be concluded that the detailed description of metabolic processes in SSF is a very complex puzzle in which biomass activity is not only dependent on substrate availability, but also on biomass age. Many measurements are needed for accurate insight in biomass formation.

1.2 Grey- and black-box models

From the results in the previous section, it is clear that the microbial pathways in SSF are very complex and that it is difficult to accurately describe fungal growth through the measurements of substrate concentrations. Because of this, many models focusing on overall packed-bed behaviour for SSF are based on a grey- or black-box approach for the description of the microbial part of the model.

One approach that has been found very useful in empirical microbial models is the use of the logistic equation described in Equation (4). This equation incorporates a limitation of growth with the approach of the maximum possible biomass concentration, resulting in the desired sigmoid growth pattern.

$$\frac{dX}{dt} = \mu X\left(1 - \frac{X}{X_{max}}\right) \tag{4}$$

When this approach is used, the biomass growth rate μ is modelled as a function of temperature and/or water activity. McMeekin *et al.* (1993) give a comparison of three approaches to do this: polynomial fits, Arrhenius and Ratkowsky equations. Their research was focused on the prevention of microbial growth in solid foods such as fish and poultry and is therefore not specifically validated for SSF systems. The Ratkowsky approach (Equation (5)) turned out to be most useful in situations where growth is affected by the combination of low temperature and low water activity (a_w).

$$\mu(T) = \left(a_1(T - T_{min})\left\{1 - e^{a_2(T - T_{max})}\right\}\right)^2\left[a_w - a_{w,min}\right] \tag{5}$$

In SSF a combination of *high* temperature and low a_w often occurs, but since this study was focused on minimising growth instead of stimulating it, no information is given on the applicability of Equation (5) for SSF systems.

Contrary to the study of McMeekin *et al.* (1993), studies done on microbial growth models for SSF have in general focused on either temperature or water activity because modelling the response of a combined temperature and water activity change gives no insight in the contribution of the separate parameters to the fermentation results.

1.3.1 Temperature in microbial models

On industrial scale, temperature gradients can exist in a fermenting bed when insufficient control is possible. Several researchers have modelled the temperature dependence of fungal growth by combining the results of series of isothermal experiments. In the experiments, respiration was measured and correlated to fungal activity in the bed. Saucedo-Castañeda *et al.* (1994) and Weber *et al.* (2002) applied this method and used small (10 to 250 ml) aerated tubes with a radius of 1 or 2 cm, which could be operated at constant temperature. In these studies, the tubes are incubated in water baths to ensure an even temperature in the material. Each isothermal respiration profile is modelled separately using both the logistic law (Equation (4)) and linear growth law (Equation (6)). In these equations μ and m_o are considered to be the only temperature-dependent parameters. The temperature dependence of these parameters can be described using for instance Ratkowsky equations (Equations (7) and (8)). (Saucedo-Castañeda *et al.*, 1990; Weber *et al.*, 2002).

$$\frac{-r_O}{\rho_s} = \left(\frac{1}{Y_{XO}} \frac{dX}{dT} + m_o X \right) \tag{6}$$

$$\mu(T) = \left\{ a_1 (T - T_{min}) \left[1 - e^{a_2 (T - T_{max})} \right] \right\}^2 \tag{7}$$

$$m_o(T) = \left\{ b_1 (T - T_{min}) \left[1 - e^{b_2 (T - T_{max})} \right] \right\}^2 \tag{8}$$

Examples of the results of such a model are given in Figures 2A, B and C.

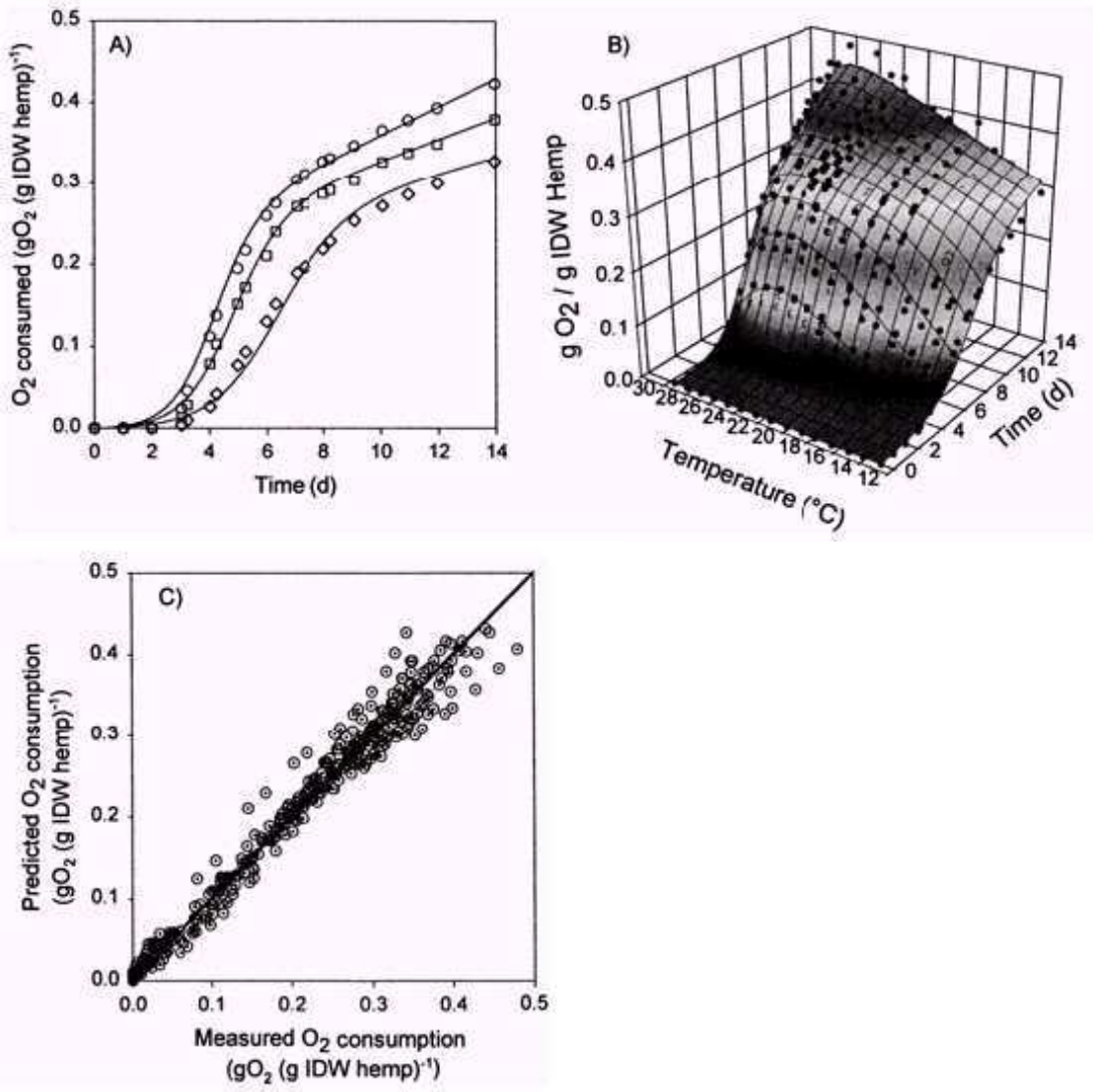

Figure 2A,B,C: Measured and fitted oxygen consumption of C. minitans growing on hemp (A) at 25.0°C, 20.2°C and 15.3°C, and (B) at all temperatures measured. (C) Parity plot of the fitted oxygen consumption against the measured consumption. Figures from Weber et al. (2002).

This approach neglects the dynamics of temperature changes. The system is assumed to instantaneously loose or gain growth speed in response to temperature changes and the history of the bed has no effect on the response. Together with other models focussing on uniform conditions, this approach was labelled "micro-scale modelling" by Mitchell *et al.* (2004), since the approach neglects the overall bioreactor performance and focuses only on the behaviour at defined and constant conditions.

Ikasari *et al.* (1998) and Dalsenter *et al.* (2005) presented a different approach to the modelling of temperature dependence in fungal growth. In these studies, the authors incorporated the effect of *changing* temperature on microbial growth. Ikasari *et al.* (1998) introduced several discrete temperature changes to cultures that had all germinated under optimal conditions. Dalsenter *et al.* (2005) improved the model description of this work (Ikasari *et al.*, 1998) by introducing the concept of a temperature-correlated growth-limiting factor F in the calculation of biomass development (Equation (9)). Because of this addition, the growth rate, μ_{max}, is a constant value in their work.

$$\frac{dX}{dt} = \mu_{max} FX\left(1 - \frac{X}{X_{max}}\right) \tag{9}$$

The growth-limiting factor F is a dimensionless number. The value of the factor is determined through temperature dependent synthesis and denaturation. The synthesis takes place according to the logistic law and the denaturation follows first-order kinetics, resulting in:

$$\frac{dF}{dt} = k_s F(1 - F^n) - k_d F \tag{10}$$

The temperature dependence of the rate coefficients for synthesis and denaturation, k_s and k_d, follows Arrhenius equations.

Dalsenter *et al.* (2005) reach a high level of accuracy with this method. For systems without accurate temperature control, the model of Dalsenter *et al.* (2005) is the best option available so far for modelling the temperature dependence of fungal activity.

1.3.2 Water activity in microbial models

Besides temperature increase, the evaporation of water is another limiting effect on fungal growth. The evaporation reduces the water activity (a_w) of the substrate. When a_w is below a fungus-specific threshold, growth slows down or stops, and this will obviously affect the production rates of enzymes and other metabolic products.

The studies of Gervais *et al.* (1988), Gibson *et al.* (1994) and Rosso and Robinson (2001) clearly show the effect of reduced a_w on fungal performance. Rosso and Robinson (2001) (equation (11)) and Gibson *et al.*. (1994) (equations (12) and (13)) presented equations to account for the loss of growth rate, here depicted as the factor $g(a_w)$. Their equations read, respectively:

$$g(a_w) = \frac{(a_w - a_w^{min})^n \cdot (a_w - a_w^{max})}{(a_w^{opt} - a_w^{min})^{n-1} \cdot \left((a_w^{opt} - a_w^{min}) \cdot (a_w - a_w^{max}) - (a_w^{opt} - a_w^{max}) \cdot \left((n-1) \cdot a_w^{opt} + a_w^{min} - n \cdot a_w\right)\right)} \quad (11)$$

$$g(a_W) = e^{C_0 + C_1 \cdot b_w + C_2 \cdot b_w^2} \quad (12)$$

$$b_w = \sqrt{1 - a_w} \quad (13)$$

A major drawback of the work on the effect of low a_w available is that the low a_w is always obtained by the addition of solutes. Often, this is combined with radial growth rate measurements on agar. This type of approach is different from actual fermentation conditions, which start at water-saturated conditions, i.e. $a_w \approx 1$. Besides, Baxter *et al.* (1998) showed that the choice of solute influences the microbial response, which further limits the comparison between such experiments and actual SSF. Still, the models for the effect of solute-induced a_w on growth can provide valuable information. The model of Gervais *et al.* (1988) is based on radial growth rate measurements. For this study, measurements were done for a_w-values ranging from 0.86 to 1. These measurements yielded insight in the relationship between radial extension and a_w, displaying an optimal a_w for radial extension of approximately 0.98. The exact value of the optimum depends on the organism studied.

Gibson *et al.* (1994) fitted an empirical 3-parameter mathematical relationship through measurements of radial growth rate and a_w for several micro-organisms. The parameters found described the radial extension profiles quite nicely for all organisms tested. In the

experimental data presented in this study (Gibson *et al.*, 1994), an optimum water activity is also found**.** The large number of organisms displaying this phenomenon makes it likely that this is to be considered a general feature of SSF.

In Hoogschagen *et al.* (2006b) a way of determining the effect of steadily decreasing a_w on fungal metabolism was presented. In this system, several small scale (10 ml) isothermal fermentations were carried out simultaneously under the same substrate conditions and in a water bath of constant temperature. After germination under optimal conditions, water activity was constantly decreased in some of the fermentations by blowing dry air through the fermenting substrate. The system was designed to obtain measurements of the effect of a_w on the growth rate of the fungus. This would enable an accurate fit of the function $g(a_w)$. It turned out that dry air was an unfortunate choice for obtaining accurate results on the effect of drying out. A decreased growth rate was observed at $a_w \approx 1$. It is likely that evaporative cooling occurred, resulting in a low temperature that could not be compensated through conduction. If measurements of a drying series at a constant bed temperature are available, the system of Hoogschagen *et al.* (2006b) can be interesting for obtaining insight in dynamic a_w effects.

Summarising, the microbial part of SSF has many aspects and several approaches for modelling it are available. Temperature development, substrate depletion and dehydration all contribute to the fermentation result. Considering the fact that industrial aerated packed-bed fermentation systems can easily be operated without temperature gradients, the challenges of modelling temperature responses may well be relevant only to the academic world and to tray fermentations. Substrate diffusion and depletion are in general too detailed for models concerning overall packed-bed behaviour. The choice for a specific approach varies with the specific interest of the researcher, the controllability of temperature gradients and the type of fermentor model that is needed.

2. HEAT TRANSFER

In order to be able to model temperature development in aerated packed-bed SSF, we have to determine the contribution of all physical processes involved. Heat removal in aerated SSF takes place through two mechanisms. Conduction is the straightforward physical process in which stationary materials transfer heat as the result of temperature gradients. In convection, at least one of the materials involved in the heat transfer is flowing. In aerated SSF, this is the moving gas phase. Its effectivity in the removal of heat is increased by the evaporation of water from the moist substrate to the gas. Aerated packed beds are in general aerated from bottom to top. It is generally agreed that for the axial direction convection is the main contributor in axial heat removal. With this assumption, the scheme depicted in Figure 1 can be written as follows for a cylindrical packed bed:

$$\underbrace{\frac{d(SH_s)}{dt}}_{\text{Heat accum.}} = \underbrace{Q}_{\text{Heat prod.}} \underbrace{-\lambda\left(\frac{dT}{dr}\frac{1}{r} + \frac{d^2T}{dr^2}\right)}_{\text{Conduction}} \underbrace{-\alpha a(T_s - T_a) - ka\frac{M_w}{R}\left(a_w\frac{p_{vsat}(T_s)}{T_s} - \frac{p_{va}}{T_a}\right)(\Delta H_w + c_{pwv}(T_s - T_{ref}))}_{\text{Convection}}$$

$$\underbrace{\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad}_{\text{Heat transfer}}$$

(14)

This equation shows the enthalpy balance for the stationary solid phase in SSF (subscript s). This phase consists of the moist substrate and the biomass growing on it. The enthalpy changes of the air (subscript *a*) can best not be incorporated in the enthalpy balance for the packed solids, as will be explained in paragraph 2.2. The heat production rate Q in Equation (14) is proportional to the microbial growth rate that has been described in the previous section. The modelling of conduction and convection are now successively discussed.

2.1 Conduction

In the first models on temperature development in packed-bed SSF, overall balances were used, taking into account only the inlet and outlet conditions. The work of Narahara *et al.* (1984) is an example of this approach. They designed a system in which evaporation in an aerated packed-bed fermentor was minimised by the promotion of conduction. The latter was achieved by using a small bed radius and varying wall temperature. Their energy balance was based on an assumed steady state with respect to temperature, which is acceptable since they succeeded in maintaining the outlet air temperature nearly constant for the major part of the fermentation. Their results show that for this system, overall balances are a good tool to predict bed temperature and bed humidity. Because of the focus on in- and outlet conditions only, the water balance neglects moisture gradients that might have occurred in the experimental bed. For the system of Narahara *et al.* (1984) this is acceptable, because they redistributed the water inside the bed by mixing it every 10 hours. In unmixed conduction-cooled systems, the radial transport of water and its effect on fungal growth may be factors of concern.
In the set-up of Narahara *et al.* (1984), the bed height was only 16 cm and the temperature difference between top and bottom of the bed was approximately 2K. When the bed height in a packed bed becomes larger, maintaining homogeneity is troublesome. The use of overall balances for heat removal is not acceptable in such systems. For the model of Narahara *et al.* (1984) this means that the driving force for conduction has to be adjusted. In the present form, it is based on the difference between the wall temperature and the outlet air temperature. In situations with a large temperature gradient over the bed, the outlet air temperature is likely to be different from the bed temperature for a major part of the bed.

In a model description for a system with spatial gradients, it will be necessary to use microbalances for distinct regions in the bed in order to get insight in the heat accumulation in each region. Saucedo-Castañeda *et al.* (1990) were the first to focus on gradients in SSF when they studied heat transfer. They set up a complete convection and conduction based model, but it turned out that their choice for a fermentor with a small radius (6 cm) made it possible to focus on the contribution of radial conductive cooling only.

In models for aerated packed-bed systems that are more similar to industrial packed-beds than the systems of Narahara *et al.* (1984) and Saucedo-Castañeda *et al.* (1990), i.e. with a large bed height and radius, radial conduction is often neglected because its contribution to the removal of heat is assumed to be negligible when aeration is also applied for heat removal. We did a check to validate this assumption.
The combined effects of radial conduction and axial convection in an aerated cylindrical bed (as described in Equation (14)) can be calculated simultaneously. We did so using a central difference approximation in the radial direction in combination with a backward approximation for the convective term in the axial direction.

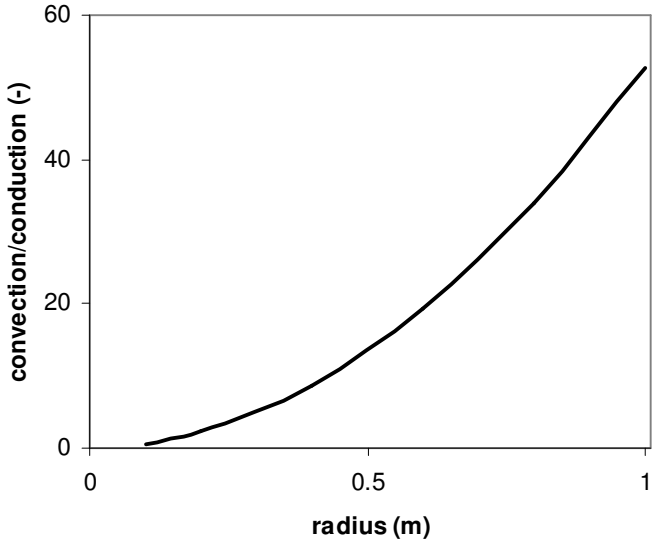

Figure 3: The relation between axial convection due to evaporative cooling and radial conductivity in SSF, calculated for a cylindrical packed bed.

Figure 3 shows the predicted contribution of the radial conduction compared to the contribution of convection in SSF. To obtain this figure, we used an oxygen consumption rate based on experimental data for the heat production part in the equation. Initially, all temperatures in the system were equal (30°C). As can be seen in the figure, convection (at a low flow velocity of 0.003 kg dry air $m^{-2}s^{-1}$, saturated with water vapour at the inlet) overtakes conduction in effectivity for beds with a radius larger than 12 cm. The ratio between the heat removal through convection and through conduction is over 50 for a 1-metre bed radius. In this calculation, the airflow velocity used is at least 10 times lower than the velocities commonly used in industrial packed-bed fermentations. At

higher velocities, the difference between the contributions to cooling of conduction and convection will be even larger. Therefore, this result justifies the choice often made in models to neglect conductive cooling in aerated packed beds of sufficiently large radius.

2.2 Convection

Saucedo-Castañeda *et al.* (1990) and Sangsurasak *et al.* (1995) presented similar models for aerated packed-bed SSF in which convection was the main contributor to heat removal. This model includes the effect of airflow velocity on growth. For simplicity, both groups approach the bed as a porous material with substrate characteristics that are the averages of the gas and particle characteristics, as can be seen in the left hand side of Equation (15).

$$\rho c_p \left[\frac{dT}{dt} + v_z \frac{dT}{dz} \right] = Q + \lambda \left(\frac{1}{r} \frac{dT}{dr} + \frac{d^2T}{dr^2} \right) + \lambda \frac{d^2T}{dz^2} \qquad (15)$$

This approach is acceptable for the heat accumulation in the bed, which indeed affects both fractions simultaneously. However, heat transported by convection can not be calculated with the average ρc_p. Two distinct phases are needed to predict the effectiveness of cooling: the gas phase has a certain, physically determined, capacity for taking up energy, and this amount of energy can be released from the solid phase. Besides this error, the increase in moisture uptake capacity of the gas phase with increasing gas temperature has been neglected and the energy involved in the evaporation of water from the substrate should have been considered in the model. For Saucedo-Castañeda *et al.* (1990), the fermentor used in the validation experiments was narrow enough to allow a large contribution of conduction to the heat removal. As such, the errors in the model were not noted in the validation experiment. For Sangsurasak *et al.* (1995), it is probably due to the absence of measurements that they did not notice the mistakes. Despite the errors, the model used by Saucedo-Castañeda *et al.* (1990) and Sangsurasak *et al.* (1995) was an important stepping-stone for further research. It describes all physical processes concerned with aerated packed-bed fermentation and introduced the concept of modelling of spatial gradients.

In more recent studies, the flow of the air through the bed *and* the water uptake by this air have both been modelled more accurately. Incorporation of air as a separate entity in the model was first described in a study of Weber *et al.* (1999). Their model featured a moving gas phase and the incorporation of a water balance. In the water balance, it was assumed that the gas was at all times saturated with water vapour. In the calculations of the actual amount of water contained by the gas, the increasing water uptake capacity of the air with increasing air temperature was incorporated. The energy involved in the evaporation of water was also included, but the possible effect of the evaporation of water from the system on the water activity of the substrate was neglected. With this model, accurate predictions of the temperature development in experimental systems were obtained. In later research by the same group, experiments were described where the relative humidity of the out coming airflow was below 100%. This contradicted the assumption of continuous saturation (Weber *et al.* 2002) and in these situations the model predictions failed to describe the actual fermentation properly. For these fermentations, Weber *et al.* (2002) assumed that heat and mass transfer relations were needed to improve the model.

On the whole, research on cooling by convection has been studied in detail. The separate phases in the packed bed have been recognised and modelled correctly. Thus, accurate insight in evaporation and in the possible heat and mass transfer limitations remains as the lead for further research.

2.2.1 Drying out

For systems with convection as the main heat removal, the correct prediction of evaporation is very important, as it is a large contributor to heat removal. As Weber *et al.* (2002) showed, it is not always accurate to assume 100% saturation of the gas phase under all conditions.

Von Meien and Mitchell (2002) presented a very detailed SSF model, incorporating additions to both the microbial and physical parts. The main goal of the model was to model mass and heat transfer between gas and solids for an intermittently mixed bioreactor. In their model, Von Meien and Mitchell (2002) dealt with all aspects mentioned so far in this review. For the microbial part, both a_w and temperature inhibition on fungal growth were taken into account. In the heat transfer part, the vapour uptake capacity of the gas phase as well as the transfer limitations between substrate and air were considered. The resulting model is a

demonstration of a thorough understanding of the connections between all processes occurring in SSF. Calculations done with the model show the result of a_w and temperature inhibition on fungal growth. The calculations also show that the gas temperature increases to match the bed temperature, but that the out-flowing gas remains unsaturated with water vapour when the water activity of the substrate starts to drop. Based on the predicted drying out and it's effect on microbial growth, mixing events were simulated that lead to the re-establishment of ideal moisture content and homogeneous bed temperature. In the model, this leads to renewed microbial activity. Unfortunately, the outcome of the simulations has not yet been validated. Some difficulties may be expected in a validation. For example, Von Meien and Mitchell (2002) used mass and heat transfer coefficients based on the drying of corn, which is quite different from the evaporation of moisture in SSF because of the much lower moisture content. In drying experiments, the main resistance for the release of water from the particles is located inside the particles, whereas in solid-state fermentation the limitation is located in the gas. Besides, the research assumes an ideal response to the mixing event, with a complete redistribution of moisture and temperature and with fungal activity fully restored. Even though a validation is missing and might reveal some flaws, the model of Von Meien and Mitchell (2002) does capture most essential aspects of SSF and provides a detailed insight in the complexity of the process.

In the model of Hoogschagen *et al.* (2006a) the combined contribution of water activity and of SSF-based mass transfer coefficients was modelled and experimentally checked. For experimental data available, the addition of a changing water activity and of transfer coefficients only yielded a small improvement in the prediction of the bed temperature, but significant improvements in the predicted relative humidity of the gas phase were observed. As was predicted also by the model of Von Meien and Mitchell (2002), the air and substrate temperature turned out to be practically identical for most of the fermentation. The vapour saturation of the outlet air was slightly lower than 100% throughout the experiment and was in fact practically similar to the calculated a_w in the bed. Because of this, it was concluded that mass transfer was not limiting in the process, and that the addition of a varying water activity is the main reason for the improvement (Hoogschagen *et al.* 2006a).

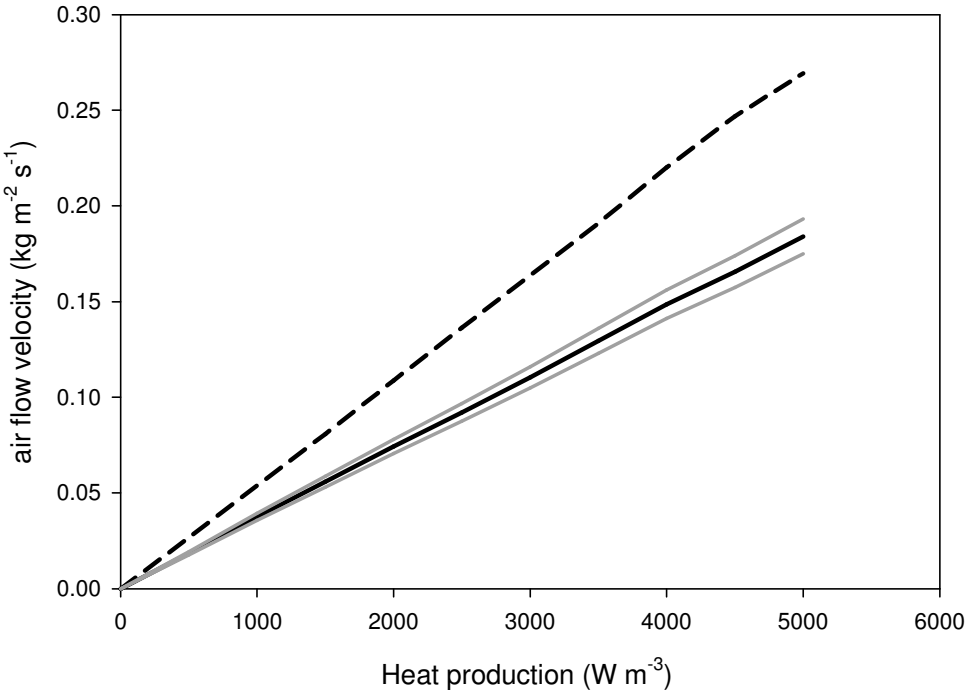

Figure 4: Simulations of the airflow velocity needed to maintain the temperature gradient over a 45 cm packed bed of oats at 2K (figure a) and 4K (figure b) with heat production Q. The initial temperature of the simulations was 307 K. The solid line indicates the simulation result obtained with the new transfer model, the dashed line is the result obtained with the equilibrium model of Weber et al. (2002). The gray lines indicate 5% deviation from the equilibrium model. (figure from Hoogschagen et al., to be submitted, 2006-a)

The effect of the addition of a_w limitation may stretch far. In Figure 4, the impact of the addition for large-scale fermentation design is demonstrated. Compared to the model of Weber *et al.* (2002), much larger airflow velocities are predicted to be necessary for the same fermentation result. Therefore, the incorporation of a varying a_w in the model is a large improvement for the design of large-scale fermentation systems.

Overall, the present models on a_w effects at the fermentor level have improved the convection-based models for aerated packed-beds. However, cooling by convection may have another disadvantageous effect on the performance of packed-bed fermentations that has not been dealt with yet in the models presented: channelling.

2.2.2 Channelling

Aerated solid-state fermentation beds consisting of non-inert substrates mainly shrink due to the evaporation of water from the substrate. Besides this, the extension of fungal hyphae may result in a substrate bed that compacts due to interparticle binding. Both changes can seriously hamper the fermentation in stationary packed beds because evaporative cooling will no longer be effective when channels appear (Weber *et al.*, 2002). Because bed shrinkage can be as much as 30 % (v/v) (Hoogschagen *et al.*, 2006c), it is obvious that this is an aspect of SSF that is of great importance.

The mass and heat balances available in most recent models on SSF provide enough detail to describe bed shrinkage when the densities of the materials are known (Figure 5) (Hoogschagen *et al.* 2006c).

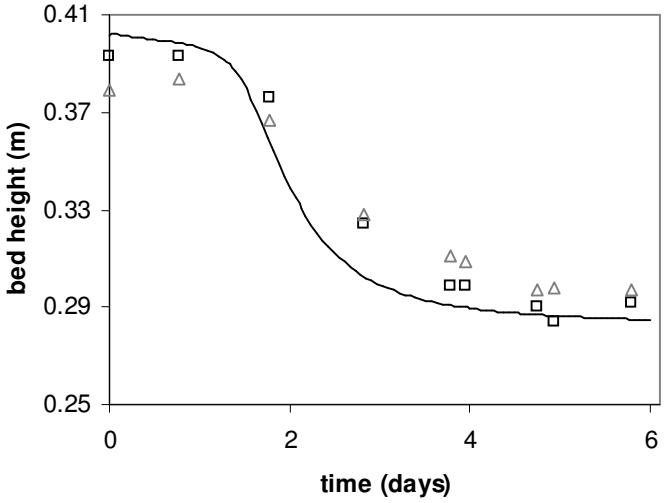

Figure 5: Measured and modeled shrinkage in a packed bed fermentation of A. sojae on wheat (Hoogschagen et al.., 2006 c)

When using microbalances, it is possible to predict the composition and volume of each cross section of the bed separately using the model of Hoogschagen *et al.* (2006c). The model yields good results when fungal biomass is assumed to occupy the space previously occupied by substrate (Hoogschagen *et al.*, 2006a b c). If the fungal biomass is assumed to penetrate the void space, thus reducing it, the model overestimates shrinkage. This seems to contradict the work of Lauckevics *et al.* (1985), who studied fungal packing density in a porous substrate layer by microscope. Based on observations, Lauckevics *et al.* (1985) stated that

the fungus grew in the voids between substrate particles only. Their model on packing densities was based on a non-branched mycelium with no interactions with neighbouring mycelia and yielded acceptable predictions of the range of fungal packing densities in SSF. The difference between the observations by Hoogschagen *et al.* (2006c) and Lauckevics *et al.* (1985) probably arises from the nature of the substrates used, since Hoogschagen *et al.* (2006c) used a substrate with variable particle volume, whereas Lauckevics *et al.* (1985) worked with a substrate with a constant particle volume, leaving the fungus no other choice than to grow in the void space.

CONCLUSION

Almost thirty years of research on models for SSF have resulted in a variety of models that all focus on a specific part of the very complex processes occurring in SSF. Only a limited number of studies attempted to integrate both the microbial and physical aspects of this fermentation process in a single model, and these models have largely remained unvalidated.

The complexity of fungal metabolism is the major reason why a model on the complete fermentation process is difficult to construct. However, the models available to date do provide enough detail to construct an overall microbial model that includes time, temperature and water activity effects and a fermentor model that deals with all spatial changes correctly. Obtaining validated quantitative data for both the microbial and physical part is an important challenge that remains.

With respect to microbiology, good experimental set-ups to measure metabolic processes in the substrate will yield enough data to supply present microbial models with the accurate rate coefficients. This will then result in the possibility to predict the production of fungal biomass accurately. For online insight in the microbial process some more work is needed, since no model on the dynamic response of fungi to water activity has been developed yet.

As far as the physical part of model descriptions is concerned, not much work remains to be done. The main aspects of stationary packed-bed fermentations can be described well with the available models. Transfer relations between gas and solids can be left out, because the main limitation to evaporative cooling is the decrease in water activity. The relations for shrinkage as a result of growth and evaporation are already useful in their present form, as long as the fungus does not knit substrate

particles together. Fungal binding is, to our knowledge, the only physical characteristic that has not been dealt with in aerated packed-bed SSF models so far.

In conclusion, most opportunities for further model improvement are in the field of microbiology. In order to devise a strategy for improvement, we advise a focus on the conditions occurring in industrial fermentations. On the whole, temperature gradients in industrial packed-bed fermentations can be kept minimal. Therefore, a focus on non-isothermal microbial models is not the best improvement for SSF models. Industry will be better served by a more thorough insight in the contribution of water activity and nutrients in isothermal situations. Very significant results can be obtained by improving the insight in the decrease of water activity. Not only the dehydration aspects need attention in this respect, since fermentation products or intermediates such as sugars also play a role in the decrease of a_w.

For situations with accurate models on the microbial part and in which decreases of water activity do not occur (e.g. with highly absorbent substrate such as hemp fibres), only a few simple measurements to monitor total evaporation and possible channelling are needed to put the available models into practice, facilitating the wide-spread use this type of fermentation deserves.

SYMBOLS

a_1 Fitted constant ($day^{0,5}\ K^{-1}$)
a_2 Fitted constant (K^{-1})
b_1 Fitted constant (kg O_2 ($day^{0,5}$ K kg biomass)$^{-1}$)
b_2 Fitted constant (K^{-1})
a specific area of exchange (m^2m^{-3})
a_w Water activity (-)
$a_{w,min}$ Minimum water activity at which fungal growth occurs(-)
$C_{0,1,2}$ Fitted constants (-)
c_p Heat capacity (J (kg K)$^{-1}$)
F Dimensionless growth limiting factor (-)
H Heaviside function (Mitchell *et al.*, 1991-a)
H_n Enthalpy of subscript n (J·(kg n)$^{-1}$)
$J_{G|_\delta}$ Flux of glucose (G) over substrate surface (δ) (kg glucose (s m^3)$^{-1}$)
K_s Saturation constant for glucose uptake (kg m^{-3})

k	Mass transfer coefficient (m s^{-1})
k_n	Synthesis (k_s) or denaturation (k_d) rate coefficient for subscript n (s^{-1})
M_n	Molecular weight of subscript n (kg $mole^{-1}$)
m_o	Maintenance coefficient (moles O_2 [kg biomass]$^{-1}$ s^{-1})
p_{va}	Water vapour pressure of air (Pa)
p_{vsat}	Saturated water vapour pressure of air (Pa)
Q	Metabolic heat production (J (kg biomass s)$^{-1}$)
q_m	Max. specific glucose uptake rate (kg glucose (kg dry biomass s)$^{-1}$)
R	Specific gas constant (J (mole K)$^{-1}$)
r	Bed radius (m)
r_o	Respiration rate (kg O_2·(m^3 fermentor s)$^{-1}$)
r_x	Biomass growth rate (kg biomass (kg dry substrate s)$^{-1}$)
S	Substrate concentration (kg substrate m^{-3} bed)
s	Solid phase (as subscript)
T_n	Temperature of subscript n (K)
T_{max}	Maximum temperature for fungal growth (K)
T_{min}	Minimum temperature for fungal growth (K)
t	Time (s)
V	Volume (m^3)
v_z	Superficial velocity in z-direction (m s^{-1})
W	Water content (kg H_20 m^{-3} bed)
w	Water (as subscript)
wv	Water vapour (as subscript)
X	Biomass concentration (kg biomass (kg dry substrate)$^{-1}$)
X_{max}	Maximum biomass concentration possible (kg biomass (kg dry substrate)$^{-1}$)
X_c	Critical biomass density (kg biomass (kg dry substrate)$^{-1}$)
x	Biomass (as subscript)
Y_{xo}	Yield of biomass over oxygen (kg biomass (kg O_2)$^{-1}$)
Y_{xg}	Yield of biomass over glucose (kg biomass (kg glucose)$^{-1}$)
z	Bed height (m)
α	Heat transfer coefficient (J(m^2 s K)$^{-1}$)
λ	Thermal conductivity (W (m K)$^{-1}$)
μ	Specific growth rate (s^{-1})
μ_{max}	Maximum specific growth rate (s^{-1})
ρ	Density (kg m^{-3})

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Samenvatting

Vaste-stoffermentatie onderscheidt zich van de meer gangbare vloeistoffermentatie door de afwezigheid van een vloeibare waterfase. Het proces wordt vooral in Azië veel gebruikt voor de productie van bekende levensmiddelen zoals tempé, sojasaus en saké. Bij vaste-stoffermentatie is sprake van een vast substraat dat voorafgaand aan de fermentatie geweekt is in een vloeistof om de groei van micro-organismen te vergemakkelijken. De inhoud van een vaste stof fermentor kan niet zo eenvoudig gemengd worden als die van een vloeistoffermentor. Hierdoor is het lastig om de tijdens de groei vrijkomende warmte onder controle te houden en om substraatgradiënten te voorkomen. Een voordeel van vaste-stoffermentatie is dat het proces goedkoop uit te voeren is, en dat de opwerking van het product eenvoudiger kan zijn dan in vloeistoffermentatie, omdat er geen sprake is van een oplosmiddel dat moet worden verwijderd. Om deze reden is vaste-stoffermentatie een interessante techniek die kostenbesparend kan zijn voor talrijke toepassingen, zoals de productie van levensmiddelen, van biopesticiden en van geur- en smaakstoffen. Het proces van vaste-stoffermentatie is veel minder uitgebreid onderzocht dan dat van vloeistoffermentatie. Dit proefschrift is een toevoeging op het redelijk schaars beschikbare onderzoeksmateriaal en geeft een aantal wiskundige modellen weer die biologische en natuurkundige processen in een belucht gepakt bed beschrijven.

In beluchte gepakte bedden wordt de warmte die tijdens de fermentatie ontstaat verwijderd door langsstromende lucht. De beluchting verwijdert de metabole warmte op twee manieren. Ten eerste neemt de lucht zelf de warmte op die in het fermentatieproces ontstaat. Ten tweede is de lucht in staat om waterdamp op te nemen uit het bed, en met deze waterdamp kan ook een aanzienlijke hoeveelheid warmte worden onttrokken. Een nadeel bij de verdamping van water uit het bed is dat op termijn de vochtgraad van het substraat zodanig kan dalen dat het micro-organisme er hinder van ondervindt.

In dit onderzoek is de groei van *Aspergillus oryzae* en *Aspergillus sojae*, twee verwante schimmelsoorten, in een belucht gepakt bed gevuld met bevochtigde tarwekorrels bestudeerd. Er is zowel aandacht besteed aan de microbiologische eigenschappen van de schimmels als aan de natuurkundige aspecten van het gebruik van het beluchte gepakte bed.

In het onderzoek naar vaste-stoffermentatie dat tot nu toe gedaan is zijn veel verschillende soorten substraat gebruikt. Voorafgaand aan het modelleerwerk werd gecontroleerd of resultaten op verschillende substraten, maar met hetzelfde organisme, met elkaar vergeleken kunnen worden. Dit werd gedaan door de groeisnelheid van *Aspergillus oryzae* op ogenschijnlijk vergelijkbare substraten, namelijk verschillende rassen graan, bij verschillende voorbehandelingsmethoden te vergelijken. Er werden aanzienlijke verschillen gevonden. Dat betekent dat het voor een goede vergelijking tussen experimenten van belang is om substraat en voorbehandeling constant te houden. Om die reden zijn de experimenten die in dit onderzoek met elkaar vergeleken worden allemaal met dezelfde batch tarwe en dezelfde voorbehandelingsmethode uitgevoerd.

Op microbiologisch gebied werd als eerste de invloed van de omgevingstemperatuur op de schimmelgroei gemeten en gemodelleerd. Het bleek lastig om een goed model te vinden om de temperatuursafhankelijkheid van de schimmelgroei mee te beschrijven. Hierdoor is in het onderzoek in dit proefschrift, dat meer gericht was op het bestuderen van natuurkundige koel- en uitdroogprincipes, besloten om in de warmtebalansen gebruik te maken van gefitte zuurstofconsumptieprofielen in plaats van modelberekeningen van de schimmelgroei. Hiermee kon de juistheid van de natuurkundige modellen beter worden gevalideerd in de fermentormodellen.

Voor het natuurkundige deel van dit proefschrift zijn experimenten met een gepakt bed van ongeveer 50 cm hoog uitgevoerd. Dit gepakte bed was geïsoleerd van de buitentemperatuur en er konden online temperatuur- en vochtmetingen in gedaan worden. Op basis van bekende natuurkundige principes zijn verschillende modellen geschreven die de omstandigheden in het gepakte bed in zowel de tijd als de ruimte (= hoogte) beschrijven. Deze modellen zijn allen gebaseerd op warmte en/of massabalansen. Zoals al eerder beschreven, werd er voor gekozen om de temperatuurrespons van de schimmel niet direct te modelleren, maar in plaats daarvan de gemeten zuurstofconsumptie te fitten met een willekeurige vergelijking. Hiermee werden mogelijke onnauw-

keurigheden in de warmteproductie vermeden en kon de aandacht gericht worden op het correct modelleren van het warmte- en massatransport.

Het eerste model in dit onderzoek is een uitbreiding op bestaande modellen, waarin de aanname dat de lucht die door het bed geblazen werd continu de maximale hoeveelheid warmte en vocht uit het bed zou verwijderen centraal stond. In de experimenten die in het kader van dit proefschrift zijn uitgevoerd, en in voorgaande literatuur, bleek dat deze aanname niet altijd juist was. De lucht die uit het bed kwam was niet in alle gevallen volledig verzadigd met waterdamp en hierdoor overschatte het model de uitdroging in het gepakte bed. Daarom werd het bestaande model uitgebreid met stof- en warmteoverdrachtscoëfficiënten en werd de invloed van een dalende wateractiviteit op de beschikbaarheid van water voor verdamping toegevoegd. Vooral de toevoeging van de invloed van de dalende wateractiviteit bleek van grote invloed te zijn op de juistheid van de modelvoorspelling van zowel de bedtemperatuur als de vochtgraad van de lucht die het bed verlaat.

Het inzicht dat lokale wateractiviteit van grote invloed is op de effectiviteit van de beluchting bracht een nieuwe uitdaging met zich mee, namelijk om voor elke positie in het bed direct de lokale respons van de schimmel op de plaatselijke wateractiviteit te modelleren. Er is een systeem ontwikkeld waarin de dynamische respons op een dalende wateractiviteit op microschaal, bij constante temperatuur, kan worden gemeten. De theorie hiervan is dat er tegelijkertijd met een experiment dat langzaam uitdroogt door er droge lucht doorheen te blazen een experiment plaatsvindt onder ideale omstandigheden, dat wil zeggen bij $a_w \approx 1$. Het idee van het systeem was, dat door de resultaten van deze twee experimenten te vergelijken kan het effect van het uitdrogen bestudeerd kon worden. Dit is een vernieuwing ten opzichte van eerder onderzoek, waarin het effect van wateractiviteit altijd in statische en kunstmatig tot stand gebrachte situaties gemeten werd. Tegen de verwachting in bleek er al een respons van de schimmel te zijn als de wateractiviteit in het uitdroogexperiment nog ongeveer 1 was, en dus geen effect zou mogen hebben op de groei. We hebben twee hypotheses onderzocht die een mogelijke verklaring zouden kunnen zijn voor dit fenomeen. Vochtgradiënten in het deeltje bleken te klein om het effect in groeisnelheid te kunnen verklaren. Het is waarschijnlijker dat er sprake is van zogenaamde natte-bol temperaturen onderin het uitdrogende materiaal. Dat wil zeggen dat er door het beluchten met de droge lucht zodanig veel energie onttrokken wordt aan de korrels dat ze kouder

worden dan de lucht. Het systeem is niet in staat dit tijdig te compenseren. Hierdoor doet naar schatting 5% van het materiaal niet mee aan de fermentatie. De experimentele omstandigheden moeten, om deze situatie te voorkomen, zodanig worden aangepast dat de natte-bol temperatuur beperkt blijft en tijdig kan worden gecompenseerd vanuit de wand van de fermentatie, die constant op de gewenste reactietemperatuur is. Wanneer er in de toekomst experimenten uitgevoerd kunnen worden met werkelijk identieke temperaturen zal dit waarschijnlijk wèl leiden tot een situatie waarin de uitdroogrespons van de schimmelgroei kan worden bestudeerd.

Tijdens de experimenten met *Aspergillus oryzae* kwam nog een probleem aan de orde dat aanleiding was voor een nieuw model: de schimmel heeft de eigenschap om het substraat te binden. Hierdoor treedt krimp op, wat nadelige gevolgen heeft voor de beluchting. In een extra toevoeging aan het model is de krimp gemodelleerd als functie van de afname van het vochtgehalte in het bed. Het model is gevalideerd met experimenten met *A. sojae*, dat dezelfde groeisnelheid heeft als *A. oryzae*, maar niet de sterk bindende eigenschappen van *A. oryzae*. Het model is een goed middel om een voorspelling te geven van de krimp die natuurkundig gezien op zou moeten treden ten gevolge van de gecombineerde effecten van schimmelgroei en beluchting in het gepakte bed. Bij afwijkingen van de realiteit ten opzichte van de modelvoorspelling kan sprake zijn van kanaalvorming in het bed, waarop vervolgens gereageerd kan worden door bijvoorbeeld kort te mengen.

De modellen die gepresenteerd worden in dit proefschrift beschrijven de belangrijkste processen van vaste-stoffermentatie in beluchte gepakte bedden. In het slothoofdstuk wordt de balans opgemaakt van het werk dat mogelijk nog verbeteringen kan aanbrengen in de huidige kennis. Aangezien vooral de microbiologische aspecten vergeleken met de natuurkundige kant minder goed gemodelleerd zijn, zal hier de grootste winst te behalen zijn voor verbeteringen in de modelbeschrijving van vaste-stoffermentatie in beluchte gepakte bedden.

Summary

Solid-state fermentation is different from the more well known process of liquid fermentation because no free flowing water is present. The technique is primarily used in Asia. Well-known products are the foods tempe, soy sauce and saké. In industrial solid-state fermentation, the substrate usually consists of loose substrate particles, although in research situations agar-like substrates are also common. Solid-state fermentations cannot be mixed as easily as liquid fermentations. Because of this, it is difficult to maintain the temperature in the fermentation at an acceptable level and to prevent differences in substrate availability throughout the solid material. An advantage of solid-state fermentation is that the process is cheap, and that products are in some cases easier to separate from the substrate than in liquid. Because of this, the technique is economically interesting. The process has not been studied as extensively as liquid fermentation. This thesis extends the available knowledge by providing several mathematical models for both biological and physical processes that occur in aerated packed beds.

In aerated packed beds, the metabolic heat that is released in the microbial process is removed by blowing air through the packed material. The effectiveness of the aeration is the result of both the heat uptake capacity of the air itself and of the evaporation of moisture to the air. In fact, the evaporation contributes more to the heat removal than the air itself. A side effect of the evaporation is that the decreasing moisture level in the substrate can become limiting for the microbial process.

In this thesis, the growth of *Aspergillus oryzae* and *Aspergillus sojae*, two related species of fungi, in an aerated packed bed of moist wheat kernels is studied. The study deals with both the microbial and physical aspects of the system.

Many different types of substrate have been used in studies on solid-state fermentation. Prior to starting the work on the mathematical models, we checked if the fermentation results of *A. oryzae* on several types of wheat matched, The check was done by matching respiration profiles for several types of wheat and two pretreatment methods. It turned out that considerable differences between the pretreatment methods can exist, which indicated the importance of using the exact same type of substrate and pretreatment in experiments that are to be compared.

No accurate model description of the microbial aspects of SSF is available yet. Because the focus of the major part of the thesis is on deriving model descriptions for the physical aspects of cooling and drying-out in aerated packed beds, it was decided that using a temperature-response model for the description of heat development would incorporate too many uncertainties in the overall packed-bed model. The heat development in the further studies presented was therefore based on fitted oxygen consumption profiles instead of on modelled microbial growth.

For the validation of the physical models in this thesis, experiments were carried out in a packed bed of approximately 50 cm height. This packed bed was insulated thermally, and offered the possibility of taking online temperature measurements and sampling the moisture content. The models that were derived to describe the changes in growth conditions in the packed bed in time and space were based on well-known physical relations. All physical models are composed of heat- and mass balances. As described above, the temperature dependence of the fungus was neglected, and the metabolic heat development was incorporated in the balances by means of fitted respiration profiles. This way, inaccuracies in the heat production in the physical model were prevented, allowing the focus on the correct description of heat and mass transfer.

The first model presented was based on an existing model, which overestimated the drying out of the solid material. This overestimation was due to the assumption of constant saturation of the gas phase with water vapour. The overestimation of the drying out meant that the assumption of vapour saturation needed to be adjusted. Heat and mass transfer coefficients were determined for the substrate involved, and besides this water activity was introduced as a factor that limited the evaporation of water from the substrate. The addition of water activity was of great influence on the model results.

The insight in the effect of local water activity on the fermentation was the onset for a study on the response of fungi to changing water activity. A system was designed that allowed the dynamic response of the fungus on decreasing water activity to be measured. The experimental set-up was based on isothermal experiments that were slowly dried out by blowing dry air through them, with simultaneous experiments carried out at $a_w \approx 1$ for comparison with the response to the drying out. Considering the fact that all studies on water activity that preceded this approach were based

on static and artificial conditions, this set-up is more similar to the actual conditions in a packed-bed fermentation.
Contrary to the expectations, the system that was dried out showed a decreased fungal growth rate when the water activity in the substrate was still the same as it was in the reference experiment. We checked two possible causes for this phenomenon. Moisture gradients in the particle were ruled out, because these were too small to be able to cause the difference in growth rate. We found that there is most likely a region of very cold substrate material due to wet-bulb cooling. Wet-bulb cooling is a phenomenon in which the evaporation of water from a system to a passing airflow allows the system to cool to temperatures below the temperature of the air. Because we used dry air in our experiment, the effect was too large to be compensated through conduction. An estimated 5% of the bed could not be fermented because of the low temperature. For a successful series of experiments, we need to obtain drying with a 100% constant bed temperature. For such a series, a good comparison of the effect of drying on fungal behaviour will be possible.

During the experiments in the packed-bed involving *Aspergillus oryzae* strong shrinkage of the packed-bed occurred because the fungus tied the substrate particles together. Because of the shrinkage, the aeration lost effectivity and the fermentation results were suboptimal. A model was designed to describe the amount of shrinkage, based on the decrease in water content in the bed. The validation of this model could not be performed with *A. oryzae*, because it was impossible to carry out controlled fermentations with this fungus. Therefore, *A. sojae* was used, which has the same growth characteristics as *A. oryzae*, except for the formation of substrate ties. The model on shrinkage offers a good prediction of the shrinkage that is expected with the combined effect of fungal growth and dehydration. If in industrial fermentations a different shrinkage pattern is observed, this is an indication that there is channel formation somewhere in the bed. This observation can be than followed by for instance a mixing event, improving the overall performance of the fermentation.

In the final chapter of this thesis, an overview of the work that could possibly offer further improvements to the present models is given. It was concluded that the modelling of the microbial aspects offer the biggest chances for success in this respect, since this aspect has of yet been modelled less accurately than the physical part.

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Tenslotte blijft er dan nog 1 iemand over die genoemd moet worden: degene die het dichtst betrokken geweest is bij het afronden van dit project: mijn echtgenoot Henk. Lieve Henk: Het is heel bijzonder dat we op dezelfde dag kunnne promoveren. In dit project ben jij het uiteindelijk geweest die het laatste zetje gegeven heeft richting “af”. Dankjewel dat je me hebt gestimuleerd om door te zetten, en ook bedankt voor je luisterend oor, goede raad en natuurlijk voor de mooie kaft die je gemaakt hebt. Maar het belangrijkste vind ik alle bijzondere, mooie en gezellige dingen die we samen gedaan hebben naast ons werk aan onze proefschriften. Ik ben heel benieuwd wat we in de toekomst allemaal nog gaan meemaken!

Marisca Zweistra-Hoogschagen, Arnhem, 20 november 2006.

Curriculum Vitae

Marisca Jolanda Hoogschagen werd in Alkmaar geboren op 21 september 1976. Na een relaxte start op kleuterschool de Roezemoes en openbare basisschool Alvitlo, beiden te Hippolytushoef, ging zij zes jaar lang door weer en wind richting RSG Wiringherlant te Wieringerwerf, waar zij in 1994 met succes het atheneum afrondde. Hierna verliet Marisca het vertrouwde Wieringen om in Wageningen bioprocestechnologie te gaan studeren aan de Landbouwuniversiteit. In september 1999 rondde zij, na het volgen van twee afstudeervakken (bij Proceskunde en bij Organische Chemie) en twee stages (bij Massey University, Nieuw Zeeland en bij het veel dichter bij huis gelegen ATO-DLO in Wageningen), haar studie af. Gelijktijdig veranderde de universiteit haar naam in Wageningen Universiteit en begon Marisca met het promotie-onderzoek waarvan dit proefschrift de afsluiting vormt. Het eerste jaar van dit onderzoek werkte Marisca bij TNO-voeding in Zeist en de laatste 3,5 jaar bij de sectie Proceskunde van Wageningen Universiteit.
Sinds 1 augustus 2004 is Marisca werkzaam bij energiebedrijf Nuon, waar zij momenteel leiding geeft aan één van de data-analyse teams van het Customer Care Center.