THE INACTIVATION OF LACTOBACILLUS PLANTARUM DURING DRYNG

Promotor: dr.ir. Klaas van 't Riet
hoogleraar in de levensmiddelenproceskunde

Lou C. Lievense

THE INACTIVATION OF LACTOBACILLUS PLANTARUM DURING DRYNG

Proefschrift
ter verkrijging van de graad van
doctor in de landbouw- en milieuwetenschappen
op gezag van de rector magnificus
dr. H.C. van der Plas
in het openbaar te verdedigen
op woensdag 5 juni 1991
des namiddags te vier uur in de aula
van de Landbouwuniversiteit te Wageningen

STELLINGEN

1. De door Zimmermann gebruikte kiemgetalbepaling om de overleving van ketenvormige micro-organismen na drogen te kwantificeren, kan leiden tot een overschatting van het aantal nog levende cellen.

   Zimmermann K (1987) Einflussparameter und mathematische Modellierung der schonenden Trocknung von Starterkulturen. Fortschr.-Ber. VDI (Reihe 14, no 36), VDI-Verlag, Düsseldorf, West Germany.

2. Relaties waarin de overleving van micro-organismen wordt gegeven als functie van het watergehalte zonder dat daarbij rekening is gehouden met waterconcentratie-profielen, dienen kritisch te worden geïnterpreteerd.

   Clementi F and Rossi J (1984) Effect of drying and storage conditions on survival of Leuconostoc oenos. Am. J. Enol. Vitic. 35: 181-186.

   Valdez GF, De Giori GS, De Riuz Holgodo AA and Oliver G (1985) Effect of drying medium on residual moisture content and viability of freeze-dried lactic acid bacteria. Appl. Env. Microbiol 49: 413-415.

3. Het indelen van te drogen biologische materialen in de door Kuts en Tutova voorgestelde categorieën heeft minder voordelen dan door deze auteurs wordt beweerd.

   Kuts PS and Tutova EG (1983) Fundamentals of drying of microbiological materials. Drying Techn. 2: 171-201.

4. Onderzoek naar het drogen van micro-organismen met optimaal activiteitsbehoud is bij uitstek geschikt om multi-disciplinair bewerkt te worden.

5. Onderwijs in het begrip 'wateractiviteit' dient vergezelt te gaan van de kanttekeningen zoals Franks die plaatst bij het gebruik van dit begrip in de praktijk.

   Franks F (1991) Water activity: a credible measure of food safety and quality? Trends Food Sci. Technol. 2: 68-72.

6. De fout van 6%, die Arnold *et al.* maken bij het berekenen van de procestijd benodigd voor een scheiding op een affiniteitskolom, is proceskundig gezien te verwaarlozen. De gebruikte berekeningswijze is echter zonder meer onjuist in het educatieve kader waarin zij wordt gepresenteerd.

   Arnold FH, Blanch HW and Wilke CR (1985) Analysis of affinity separations. II: The characterization of affinity columns by pulse techniques. Chem. Eng. J. 30: B25-B36.

7. Ontwikkelingen die de onderlinge uitwisselbaarheid van agro-industriële grondstoffen vergroten, dienen gelijke tred te houden met ontwikkelingen die de sociaal-economische situatie van producenten van deze grondstoffen verbeteren.

Ruivenkamp GTP (1989) De invoering van biotechnologie in de agro-industriële produktieketen: de overgang naar een nieuwe arbeidsorganisatie. Uitgeverij Van Arkel, Utrecht.
Bunders JFG (ed.) (1990) Biotechnology for small-scale farmers in developing countries. Analysis and assessment procedures. VU University Press, Amsterdam.

8. Het is vreemd dat velen een AIO-functie op basis van het salaris als tweede keus zien, terwijl zij tegelijkertijd het aanzienlijk slechter betaalde studentenbestaan als een nuttige investering beschouwen.

9. Een onderneming is pas 'gezond', wanneer zij het milieubelang kan en laat prevaleren boven het financieel belang.

10. Indien men bij de aanschaf van een PC overweegt een PC-gebruikerscursus te volgen, verdient het aanbeveling in het geheel geen PC aan te schaffen.

Lou C. Lievense
The inactivation of *Lactobacillus plantarum* during drying
Wageningen, 5 juni 1991

VOORWOORD

Een groot aantal mensen hebben mij de afgelopen jaren geholpen bij het werk dat in dit proefschrift staat beschreven. Ik wil er hier een aantal bij name noemen en bedanken.

Allereerst Klaas van 't Riet, Gerrit Meerdink en Moniek Verbeek die tijdens het promotie-onderzoek mij met raad en daad hebben bijgestaan. De grote lijnen of de details in het oog houdend of op een meer dan vakkundige manier zorgend voor een groot deel van de resultaten. Ik ben hen veel dank verschuldigd voor de riante werkomstandigheden, uitstekende werksfeer en voor hetgeen ik van hen geleerd heb.

Vervolgens (in ongeveer chronologische volgorde): Reinier Tilanus, Pim Vis, Jeannine Spauwen, Frans Zomers, Philip Giesbertz, Ellen Drost, Ellert Camminga, Petra Koenraad, Rogier Müller, Bernadette Kooijman, Tom Taekema, Maureen Cox en Erik Torringa. Zij hebben in het kader van een afstudeervak een grote hoeveelheid werk verzet en een wezenlijke bijdrage geleverd aan dit onderzoek of zijsporen daarvan.

Alle medewerkers van de sectie Proceskunde wil ik bedanken voor de hulp, adviezen, goede sfeer en collegialiteit, tijdens en na het werk. Rik Beeftink, Mark Smith, Liz Tudor en Marcel Zwietering voor het nauwgezet corrigeren van de artikeltjes en/of hoofdstukken, Stefan Weijers voor het schrijven van een stuk ingewikkelde programmatuur en Ad Noomen voor zijn medewerking bij het fysiologische deel van dit onderzoek.

Uiteraard ben ik ook veel dank verschuldigd aan alle mensen van Gist-brocades die betrokken waren bij dit project. Met name Piet van Egmond en Reinder Hamstra voor de discussies, raadgevingen en interface-functie, Henk Spierenburg voor het verzorgen van het entmateriaal en natuurlijk het bedrijf Gb zelf voor de niet onaanzienlijke financiële bijdrage.

Rest nog een dankwoord aan de overige medewerkers in het Biotechnion, in het bijzonder de mensen van de Centrale Dienst Biotechnion in de diverse werkplaatsen, magazijnen, administratie, fotoafdeling en bibliotheek voor hun apparaten, adviezen, hulp en vakkundige diensten.

CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

1.1 APPLICATIONS OF BACTERIAL CULTURES

Since ancient times, bacterial cultures have played an important role in the production of food and feed. The most important application of bacterial cultures in the food industry is the use as a starter culture. Lactic acid bacteria are the most frequently applied. Starter cultures are used for the production of fermented milk products in the dairy industry (Philipp 1984; Prentice and Neaves 1986), for the production of fermented sausages in the meat industry (Liepe 1983; Schillinger and Lücke 1989), for the fermentation of vegetables (Daeschel *et al.* 1987), and in the production of food grade organic acids (Lockwood 1979). In the production of beverage and bakery products the combined use of a bacterial starter culture (usually *Lactobacillus* species) and *Saccharomyces cerevisae* is traditional (Spicher 1983; Hill 1986). A detailed description of these and other applications is given by Rehm and Reed (1983ab), Lee (1989) and Hammes (1990a).

The main role of the starter culture is to ferment a substrate (usually a sugar) in the raw material to lactic acid and/or acetic acid. Relatively small amounts of other important flavour components may also be formed such as acetaldehyde, diacetyl (Marshall 1987), acetone, acetoin (Bottazzi 1983), propionic acid (Spicher 1983; Vedamathu and Washam 1983), (poly-)peptides formed by the proteolytic activity of the cultures (Philipp 1984), and other trace compounds (Olson 1990). The flavour components give the fermented product the unique taste whereas the lowered pH, due to acid production, acts as a food preservation method. Also, the change in texture of the food material, due to acid formation and proteolytic activity of the starter culture, is an eminent organoleptic aspect (Marshall 1987). In the wine production process, the fermentation of malic acid to lactic acid is of major importance (Lafon-Lafourcade 1983), whereas in the meat industry the production of nitric oxide by nitrate and nitrite reducing bacteria is essential (Liepe 1983; Hammes 1990b).

An application of bacterial cultures in agriculture is the use as a silage additive (Peppler 1983; Seale 1986). Silage can be defined as the material produced from the controlled fermentation of a crop of high moisture content (i.e. maize, grass, vegetables) and is an important winter feed for cattle in many countries. The primary objective of silage-making is to preserve the animal feed with a minimum loss of nutrients so obtaining a product of high nutritional value. Preservation is achieved through the fast lowering pH which is due to acid

fermentation by lactic acid bacteria. Silage additives containing *Lactobacillus plantarum* have been shown to be the most effective types of inoculants provided that at least 10^5 viable cells are added per gram forage (Seale 1986).

Another application is the use of bacteria as probiotics. The term 'probiotic' is normally used to describe animal feed supplements but also includes beneficial micro-organisms for human consumption. In general, an antagonistic effect toward undesirable micro-organisms, an anticarcinogenic effect, an anticholesterolemic effect, vitamin B production, reduction in lactose malabsorption, and a growth promoting effect are mentioned as the most important functions of probiotics (Fernandes *et al.* 1987; Kim 1988; Gilliland 1990). As a feed supplement for animals, probiotics are used to improve the rate of growth and the feed conversion efficiency in young animals and to reduce the incidence of enteritis and diarrhoea. Although the essential mode of action of probiotics is not fully understood, it is commonly thought that they enhance the numbers of nonpathogenic bacteria in the intestines. This may assist digestion, possibly through enzyme production and also by increased competition with populations of harmful bacteria. A review of micro-organisms that are used as probiotics for animals or humans is given by Kim (1988) and Gilliland (1990).

A number of probiotic products that contain dried bacteria are now available at retail stores. In these products, *Lactobacillus acidophilus* is a popular bacterium but also *Lactobacillus bulgaricus* or *Bifidobacterium bifidum* are used (Clements *et al.* 1981; Brennan *et al.* 1983; Fuller 1986). To be effective, a product should supply 10^8 to 10^9 physiologically normal cells per day (*L. acidophilus*; Brennan *et al.* 1983; Kim 1988). Many manufacturers claim that their products contain large numbers of viable cells, even after long storage. However, Brennan *et al.* (1983) and Gilliland (1988) found that most products contained only small numbers of viable bacteria.

From the applications mentioned above it is clear that there is great commercial interest in producing stable bacterial products that contain a large number of uninjured, viable cells. To obtain such products, several preservation methods have been used which are discussed in the next sections.

1.2 PRESERVATION OF BACTERIAL CULTURES

Modern processes require cultures with well defined properties in order to generate end products with a constant quality. Therefore, it is possible, that techniques applied originally, like sub-culturing or growth of naturally present bacteria, can no longer be used. It is then necessary to find suitable preservation methods to obtain 'ready to use' bacteria. In a preservation method such as freezing and/or drying, the bacteria are brought to an 'anabiosis state'. In this state the bacterial metabolism is reversibly converted to an extremely low activity (Beker and Rapoport 1987) and the bacteria can be preserved for a longer time.

Normally, bacteria are used in the food industry as stock cultures for the preparation of bulk starters. A disadvantage of using stock cultures is the need of a fermentation to produce the large volume that is required to inoculate the process liquid from this stock. Such a production is often accompanied by the risk of infection. It is preferable that formulations used contain enough bacteria to inoculate the process liquid directly, without the need of a fermentation ('direct to vat' cultures). This eliminates the risk of infection, and constant culture characteristics and quality can be guaranteed. Frozen and freeze-dried formulations are used for this purpose. The main advantage of using dried instead of frozen bacteria is the lower costs in transport and storage of the cultures.

In a number of cases, the main disadvantage of the dried cultures is a considerable inactivation of the culture during the drying process and the poor shelf life of the product under uncontrolled conditions. Often, the producer must add many more bacteria and so give a far higher activity than guaranteed. Under certain conditions, the inactivation during drying and storage can be too large to develop a commercial process. Other disadvantages that are mentioned are the extended lag phase which increases the required process time (Robinson 1981), and the alteration of culture characteristics when mixed cultures are dried (Stadhouders *et al.* 1969). The extended lag phase of dried bacteria cannot be shortened readily, because this problem is related to the recovery of the bacteria from the anabiosis to 'normal' state. The problem of alteration of culture characteristics can be solved by mixing dried single strain cultures to produce a formulation with the desired properties.

The large commercial interest in bacterial starter cultures explains the continuing interest in the drying of these cultures. At the moment the disadvantages of dried cultures are hindering their application. However, the advantages can overrule the disadvantages if the inactivation during drying and storage are clearly understood and reduced.

During the last century, a number of researchers investigated the drying of micro-organisms. Rogers (1914) was probably the first author to write about this subject. Most publications deal with the freeze-drying of bacteria in order to obtain stable stock cultures. Attention was also given to the convective drying of yeast (*S. cerevisiae*). Freeze-drying reviews were written by Heckly (1961, 1978ab, 1985), Bousfield and MacKenzie (1976) and Ashwood-Smith (1980). Reviews about convective drying of yeast were written by Josic (1982) and Beker and Rapoport (1987).

1.3 PRESERVATION METHODS

To produce a stable bacterial culture, a number of methods are available. First of all, one has to distinguish between typical laboratory methods and methods that can be applied on a large scale production. Typical laboratory methods include: 1) immersing a bacterial culture in oil (i.e. paraffin oil), 2) air drying of the cultures in gelatin or agar, 3) adsorption-desiccation on filter-paper or on pre-dried plugs of starch, peptone or dextran, or on sand, soil, kieselguhr, pumice stone, porcelain or silica gel (Lal *et al.* 1976; Sleesman and Leben 1978; Gherna 1981; Bousfield 1984; Leben and Sleesman 1984; Snell 1984; Barbour and Priest 1986; McEldowney

and Flechter 1988). With these methods, many bacteria can be preserved successfully, for months or even years. An essential difference between the requirements for laboratory and industrial use is that for the former the slightest survival percentage is satisfactory, as one is only interested in the possibility to obtain a fresh culture after inoculation with the stored bacteria.

For industrial use, large quantities of active bacteria are required, particularly when direct inoculation of the process liquid is applied. The above mentioned laboratory methods are not suitable for this purpose because of their complexity, the additives needed and the low survival at most of the applications. Methods which can be used for industrial preservation are: 1) sub-culturing, 2) storage of the culture in a liquid or frozen form, 3) storage of the culture in a dried form. These preservation methods will be discussed below.

Sub-culturing is a traditional method of preserving bacteria through periodic transfer to fresh media (Gherna 1981). Although this method is labour-intensive, it is still in common use in the traditional food industry. In a bakery, for example, the starter culture is often obtained from the preceding fermented dough (Spicher 1983). However, this method entails the risk of genetic instability and contamination (Barbour and Priest 1986). When sub-culturing is used in industry, often a frozen stock culture is kept in reserve.

Starter bacteria can be preserved when submersed in liquid at 4 to 8 °C for several weeks. The stability of these liquid cultures can be enhanced by adding a gelling agent, like carrageenan or alginate to keep the cells in suspension, and antioxidants and mould inhibitors. As an example, such a preparation of *L. plantarum* was stable for 9 weeks at 4 °C (Barach and Kamara 1988). At higher temperatures these liquid cultures will lose their viability rapidly. The viability after storage also depends on the composition of the suspension medium. Skim milk with glycerol is often used (Foster 1962).

Freezing is the most frequently used preservation method for starter cultures (Foster 1962; Stadhouders *et al.* 1969; Yankov and Brankova 1979; Gherna 1981; Barbour and Priest 1986). Generally, skim milk, glycerol, lactose and/or a calcium salt are used as protective additives. At temperatures above -30 °C, there is still a risk of genetic instability and loss of viability. Therefore, very low temperatures (down to -196 °C), which can increase the stability of a culture considerably, are used (Foster 1962; Gherna 1981; Robinson 1981, 1983; Philipp 1984; Gibson and Khoury 1986). To save freezing costs and to obtain a product with a high number of viable cells, cultures are usually concentrated by centrifugation (Stadhouders *et al.* 1969) or membrane filtration before freezing. The costs of transport and storage of deep-frozen cultures are a major drawback.

Freeze-drying is also a widespread preservation technique. From the freeze-drying reviews (see Introduction) is it clear that survival and stability of the cultures after freeze-drying can differ widely. A number of factors influence this survival and stability. Most of these factors are also important in the convective drying of bacteria and are discussed by Lievense and Van 't Riet (1991b). Most investigators had the sole objective to initiate growth readily on a sub-culture without optimization of the survival. Yet it appears that, providing suitable protective media and optimal process and storage conditions are applied, the survival can be high (40-80%) for a number of dehydration-resistant strains (Morichi 1974; Kilara *et al.* 1976; Porubcan and Sellars 1979; Tsetkov and Brankova 1983; Valdez *et al.* 1983ab). Therefore, freeze-drying of starter

cultures can be used as a commercial process and consequently a number of freeze-dried cultures are on the market (Robinson 1981, 1983; Philipp 1984). Nevertheless, because of the high costs and complexity of the process itself, freeze-drying is generally not considered an attractive method for preparation of large quantities.

When freeze-drying is compared with convective drying, the former has some major disadvantages. Not only is the sublimation of water more energy consuming than evaporation, but also the investment costs for a freeze-drying plant are higher (Hill 1986). Due to the vacuum required, it is quite complicated to apply freeze-drying in a continuous process. Nevertheless, it is relatively easy to operate a batch freeze-drying process aseptically, which is imperative for pharmaceutical products. From a biological viewpoint it is important to note that not only the dehydration process but also the freezing process can be responsible for a considerable inactivation (Heckly 1961, 1985). However, it is the high cost of the freeze-drying process which is the main reason for the continuing search for alternative drying methods.

1.4 PRESERVATION BY CONVECTIVE DRYING

Several drying methods can be used for the preservation of micro-organisms. Techniques that are rarely used are conductive drying methods like drum drying and vacuum drying or convective drying methods like conveyer drying and pneumatic drying (Peppler 1979; Clement and Rossi 1983; Beker and Rapoport 1987). Indeed, most applications of these techniques are restricted to yeast. Among the convective drying techniques for bacteria, only three are of real interest: spray drying, fluidized bed drying and a combination of both, spray granulation. The application of these three methods and the influence of some drying process parameters on the inactivation of the bacterial cells, will be discussed below. For a more extensive review the reader is referred to Lievense and Van 't Riet (1991ab).

1.4.1 Thermal inactivation during drying

It is generally assumed that thermal inactivation is the reason for inactivation during convective drying. One can expect that at high drying temperatures, thermal inactivation will play a substantial role. It is important to realize that, in general, the heat-resistance of bacterial cells increases with decreasing water concentrations (Brown and Melling 1971; Corry 1973). For example, Daemen (1981) found that the heat-resistance at 50 °C of *Serratia marcescens* mixed with milk powder was increased 17 times when the water concentration was decreased from 10 to 0.07 kg H_2O.(kg solids)$^{-1}$. Zimmermann (1987a) reported a two-fold increase in the heat-resistance at 50 °C of *S. cerevisiae* when the water concentration was decreased from 2.3 to 0.3 kg H_2O.(kg solids)$^{-1}$. Therefore, it is probable that the overall thermal inactivation will be determined by the temperature as well as the moisture history of the system.

In the first stage of the drying process the particle surface remains wet (constant drying rate) and due to evaporating water, the temperature will not exceed the 'wet-bulb' temperature. The thermal inactivation will be limited in this stage because the high evaporation rate and the resulting wet-bulb temperature will protect the cells from the higher air temperatures in the

dryer. At the next stage in the drying process the particle surface will become dry (falling drying rate) and the temperature will increase to maximally the inlet temperature of the dryer. In this stage the evaporative cooling is no longer available and the thermal inactivation will increase, but due to the lower water concentrations, the cells will have (generally) a higher heat-resistance.

Due to the large heat conductivity, it can be assumed that the temperature profile inside the particle can be neglected (Van der Lijn 1976, Liou 1982). During the drying process, however, the inside of the particle can still be wet, while the surface is already dry. Therefore, the cells inside the particle have a lower heat-resistance than the cells at the surface and this will influence the thermal inactivation rate.

1.4.2 Spray drying

Much research has been performed on the use of spray drying of bacteria. In view of the thermal inactivation, the main advantage mentioned is the rapid drying (Comings *et al.* 1977; Filková and Mujumdar 1987; Metwally *et al.* 1989), due to the high specific drying surface of the spray. This results in a short residence time of the bacterial cells in the spray dryer (20-40 s; Elizondo and Labuza 1974; Masters 1985) which will limit the thermal inactivation. However, in a spray drying process high inlet temperatures are necessary precisely because the specific heat of evaporation must be supplied in such a short time. This can result in a significant thermal inactivation of the cells, despite the short residence time.

Because of the evaporative cooling in the first part of the drying process, the survival of bacterial cells during spray drying is strongly correlated to the outlet temperature and not directly to the inlet temperature of the dryer. This relation is confirmed by most of the relevant literature. The highest survival is found at the lowest outlet temperatures. Substantial thermal inactivation of the cells can be avoided by choosing the correct mass ratio of drying air to liquid feed, so that at the outlet of the dryer the air will be cooled by the evaporation of water.

An advantage of the spray drying process is that the bacterial cells can be dried as a suspension, without the need of support material. The maximal concentration of the bacterial suspension is limited by the fact that the feed to the dryer must be pumpable and for the reasons of particle size mentioned above. This means that an excess of water has to be evaporated and so the spray drying process is relatively energy consuming.

As mentioned above, a proper choice of the flow rate and temperature of the inlet air and the flow rate of water that has to be evaporated (and thus the flow rate of feed pumped to the nozzle), together with particle size and size distribution, will minimize the thermal inactivation. Unfortunately, the right settings for these variables are found only by trial and error, because it is difficult to calculate them in advance. The requirement to preform such experiments together with the relatively difficult control during the drying process itself and the complicated modelling (and thus optimization of the process) can be regarded as disadvantages of the spray drying process. Furthermore, the distribution in residence time in a spray dryer is relatively large compared to a (batch) fluidized bed and a spray granulation dryer. At high drying temperatures, a distribution in residence time can cause a significant thermal inactivation.

1.4.3 Fluidized bed drying

When compared with spray drying, publications on drying bacterial starter cultures with a fluidized bed are rare. Most of the fluidized bed work is related to yeast drying. In a fluidized bed dryer the drying time can be much longer than in a spray dryer. Because the dimensions of the drying apparatus and the required ratio air flow/moisture flow to reach a certain final water concentration are no longer coupled, the residence time in the fluidized bed can be chosen freely. This also means that the air inlet temperature can be controlled without influencing the minimal obtainable water concentration after drying.

The free choice of residence time makes it possible to use relatively low air temperatures, which will help to minimize thermal inactivation. The thermal inactivation can also be minimized by controlling the product temperature, instead of the inlet temperature of the bed. For example, during the drying process the inlet air temperature can be varied from 160 °C at the start of the drying process to 30 °C at the end, while the product temperature remains at 30-35 °C (Beker and Rapoport 1987; Langejan and Khoudokormoff 1982; Hill 1987). By accurate control of temperature, an optimum between inactivation and drying time can be reached.

A drawback of the fluidized bed drying process is that only granulatable materials can be dried. Bacterial cells are not readily obtained in a granulatable form and therefore, support materials have to be used. One possibility is to mix the cells with a support material such as starch (Clementi and Rossi 1984) or wheat bran (Bera *et al.* 1988) and to extrude the paste formed. Gel-like materials are also used, such as xanthan gum, carrageenan or alginate (Taeymans *et al.* 1986; Roelans and Taeymans 1990; Divies *et al.* 1990). Unfortunately, these materials can be considered unacceptable in the (food) substance to be inoculated. In view of a spray granulation process (see below), Hill (1986) suggests using a support material which fits a particular purpose. Milk powder or lactose is mentioned for milk starters, maltodextrin for sausage starters and rye or wheat flour for bread starters. Concentration, mixing and granulation are unavoidable processes preceding the fluidized bed drying process. In these preceding steps, it is possible that inactivation of the cells may occur. However, there is no known literature about this effect.

An advantage of the fluidized bed process, compared to the spray drying process, is that it can be modelled relatively easily. The measurements and calculations practised on a laboratory or pilot plant installation can be translated to an industrial scale process. Therefore, insights in the influence of the parameters on the drying and inactivation process, obtained with the modelling and simulation, can be readily implemented.

1.4.4 Spray granulation

Spray granulation is a relatively new technique which is frequently used in the pharmaceutical industry. The terms 'fluid bed granulation' or 'fluid bed spray drying' are also used. As far as we are aware, only three authors have reported the use of this technique as a drying method for bacterial cells (Hill 1985, 1986, 1987; Zimmermann 1987a; Zimmermann and Bauer 1990; Roelans and Taeymans 1990).

Spray granulation can be defined as a particle-forming process by which a liquid feed containing solids is converted to a granular state. This is achieved by spraying the feed into a fluidized bed of already formed granules (Hovmand 1987; Uhlemann 1990). In the first stage of the process the cell suspension is sprayed on a fluidized bed consisting of, for example, a starch powder. The fine starch particles in the bed then agglomerate together with the cells to larger granules. In the second stage of the process these granules can be dried further to the desired final water concentration.

The thermal inactivation rate is determined by the moisture-temperature history of the cells which in turn, will be influenced by the size of the formed granules. The granule size can be controlled by a number of variables of which the droplet size of the spray is the most important (Hovmand 1987). Apart from granule size, two other factors should be mentioned. Firstly: the bacterial cells sprayed on the support material will dry at a fast initial rate because of water absorption by the support material. As a result, the heat-resistance of the cells will increase rapidly. Secondly: the spray granulation process has the same control potential as the fluidized bed drying process, and by optimum control, the thermal inactivation can be minimized.

In the spray granulation process the formation of granulates prior to drying is omitted, while the excellent control possibilities of fluidized bed drying still exists. Also in this process, the presence of a support material in the end product can be a disadvantage. Nevertheless, it may be clear that the combined advantages of spray drying and fluidized bed drying make the spray granulation process an attractive alternative. As with the spray drying process, a model description for spray granulation is not easily obtainable (Uhlemann 1990), however, an empirically based control is possible. For this control the remarks made for fluidized bed drying are again relevant.

1.5 MODELLING THE INACTIVATION DURING DRYING

The first step in making convective drying of bacterial starter cultures economically feasible is to preserve their activity during drying. To do this, process conditions must be adjusted during the drying process, in order to minimize inactivation. To achieve optimum operation and control, a fundamental quantitative knowledge of the process is required. The application of mathematical modelling and simulation techniques can provide such knowledge. The main objective of our research is to develop and verify such a mathematical model. The model should be able to predict the inactivation of *L. plantarum* during drying.

Other researchers have also tried to predict the inactivation of enzymes, yeasts and bacteria during drying. The most elementary approach is to determine inactivation constants and to calculate an activation energy of inactivation ($E_{a,i}$). In most cases, inactivation data obtained with spray drying experiments are used (Labuza *et al.* 1970; Elizondo and Labuza 1974; Espina and Packard 1979; Daemen 1981; Metwally *et al.* 1989; Kim and Bhowmik 1990). $E_{a,i}$ determines the temperature sensitivity of the inactivation during drying and furthermore, $E_{a,i}$ changes with water concentration. Several authors have determined this relation empirically in order to calculate the continuously changing thermal inactivation rate during drying of enzymes

(Liou 1982; Liou *et al.* 1985; Yamamoto *et al.* 1985; Meerdink and Van 't Riet 1991) and yeast (Zimmermann and Bauer 1986, 1987b; Zimmermann 1987a). In this way, the description of inactivation was coupled to the drying kinetics and so the residual activity during drying could be predicted. Theoretical studies using this approach have also been reported (Kerkhof and Schroeber 1974; Wijlhuizen *et al.* 1979; Luyben *et al.* 1982).

In the papers on modelling mentioned above, it is assumed that inactivation of biological material during drying is caused only by thermal mechanisms. However, during freeze-drying, where thermal inactivation can hardly occur, bacteria are significantly inactivated. It seems, therefore, that other inactivation mechanisms are also important. A number of papers are available that consider the inactivation of biological compounds during freezing and (freeze-)drying. In these papers inactivation by dehydration of the material is stated (Heckly 1961; Nei 1973; Orndorff and McKenzie 1973; Bousfield and MacKenzie 1976; Ashwood-Smith 1980; Josic 1982; Kuts and Tutova 1983). Dehydration inactivation was not considered in the papers on modelling. When enzymes or yeasts are dried using convective drying methods, dehydration inactivation is less important than the thermal inactivation, therefore, calculations based on thermal inactivation alone, could reasonably describe the inactivation measured. However, this approach failed to predict the inactivation of bacteria during drying. Daemen and Van der Stege (1982), Zimmermann (1987a) and Zimmermann and Bauer (1990) stated that this was due to mechanisms different from thermal inactivation.

As mentioned above, the main objective of our research project is to develop and verify a mathematical model that is able to predict the overall inactivation of *L. plantarum* during drying. Three separate goals can be distinguished:

Firstly, it is important to investigate the thermal inactivation during drying. As a consequence of the influence of temperature and water concentration on the thermal inactivation rate, the relationships between these parameters has to be investigated. With the experimental data obtained, a thermal inactivation model can be developed.

Secondly, it is necessary to develop and verify a mathematical model that is able to predict the water concentration and the temperature of the drying material. When the drying model and the thermal inactivation model are coupled, the thermal inactivation rate during drying and the overall thermal inactivation after drying can be calculated. With this approach it is possible to investigate the importance of the thermal inactivation at different drying temperatures.

Thirdly, it is possible that dehydration inactivation may also play an important role, in which case, a quantitative description of the dehydration inactivation is required. If the two inactivation processes, thermal and dehydration inactivation, have to be combined, then the prediction of the residual activity of *L. plantarum* during drying will become more complicated. Further complications may arise from the water concentration profiles inside the drying particle (Liou 1982) since the water concentration profile influences the thermal inactivation rate. Moreover, due to the dehydration inactivation it can occur that cells at the surface may well be inactivated by dehydration, whereas cells in the interior of the particle are unaffected. It is therefore necessary that water concentration profiles inside the drying particles are included in the model.

In this discussion, only those parameters of the drying process that can be important for the survival of bacteria after drying have been considered. A number of other factors will also influence the survival (Lievense and Van 't Riet 1991b). For the research described in the main part of this thesis, all these factors must be standardized. For example, the growth medium of the bacteria, the harvesting time and the washing and concentration steps must remain constant. *L. plantarum* is chosen as a model lactic acid bacterium for practical reasons such as a relative high growth rate and an easy harvesting procedure. Fluidized bed drying is used as drying method for the reasons mentioned above. *L. plantarum* cells are centrifugated and the cell pellets are mixed with potato starch to obtain a granulatable material. The paste so formed is extruded to cylindrical particles before drying.

Finally, for this research it was necessary to develop a technique to measure the survival of the *L. plantarum* cells in a standardized and reliable way. The traditional plate-count method has several major disadvantages, not least, that it is labour-intensive while the accuracy is low. Furthermore, lactic acid bacteria often occur in pairs or even in chains. This also holds for *L. plantarum*. For a proper application of the plate-count method, the bacteria in these chains have to be separated, otherwise, a dissociation of the chains before, during or after drying will lead to an overestimation of the survival. For these reasons, we have developed a technique whereby the glucose fermenting activity of *L. plantarum* cells is measured.

1.6 OUTLINE OF THIS THESIS

Chapter 2 describes the standardized technique by which the glucose fermenting activity is measured. This technique is used for measurement of the activity before and after a drying or thermal inactivation experiment.

Chapter 3 describes the drying model, which is composed of the short-cut drying theory (Liou 1982; Coumans 1987) and the heat balance of the drying material. With this model also the moisture concentration profiles inside the particles are calculated.

Chapter 4 deals with the measurement and modelling of the thermal inactivation kinetics and the coupling of these kinetics to the drying model described in Chapter 3. The thermal inactivation during drying is quantified using the coupled models.

Chapter 5 describes the measurement and implementation of the dehydration inactivation. With the overall model, in which thermal inactivation and dehydration inactivation are coupled with the drying model, the inactivation of *L. plantarum* during drying is predicted.

In Chapter 6, a general discussion of the investigations is given together with further experimental evidence of the two simultaneously occurring thermal and dehydration inactivation mechanisms during drying of *L. plantarum*.

REFERENCES

Ashwood-Smith MJ (1980) *Preservation of micro-organisms by freezing, freeze-drying and desiccation.* In: Ashwood-Smith MJ and Farrant J (eds) Low temperature in medical biology (pp. 219-252). Pitman Medical, Turnbridge Wells.

Barach JT and Kamara BJ (1988) Process for preserving acid producing bacteria and compositions produces thereby. USA Patent 4.720.460. Miles Laboratories Inc., Elkhart, Indiana, USA.

Barbour EA and Priest FG (1986) The preservation of lactobacilli: A comparison of three methods. Letters in Appl. Microbiol. 2: 69-71.

Beker MJ and Rapoport AI (1987) *Conservation of yeasts by dehydration.* In: Fiechter A (ed) Advances in Biochemical Engineering/Biotechnology, Vol.32 (pp. 127-171). Springer-Verlag, Berlin.

Bera F, Renauld H, Rikir R and Thonart P (1988) Water desorption analysis applied to *Lactobacillus plantarum* stationary bed drying. Med. Fac. Landbouw. Rijksuniv. Gent. 53: 2007-2016.

Bottazzi V (1983) *Other fermented dairy products.* In: Rehm HJ and Reed G (eds) Biotechnology, Vol.5, Food and feed production with micro-organisms (pp. 315-364). Verlag Chemie, Weinheim, Germany.

Bousfield IJ (1984) *Maintenance of industrial and marine bacteria and bacteriophages.* In: Kirsop BE and Snell JJS (ed) Maintenance of micro-organisms, a manual of laboratory practice (pp. 63-65). Academic Press, London.

Bousfield IJ and MacKenzie AR (1976) *Inactivation of bacteria by freeze-drying.* In: Skinner FA and Hugo WB (eds) Inhibition and inactivation of vegetative microbes (pp. 329-344). Academic press, London.

Brennan M, Wanismail B and Ray B (1983) Prevalence of viable *Lactobacillus acidophilus* in dried commercial products. J. Food Prot. 46: 887-892.

Brown AD and Melling J (1971) Inhibition and destruction of micro-organisms by heat. In: Hugo WB (ed) Inhibition and destruction of the microbial cell (pp. 1-37). Academic Press, London.

Clement P and Rossi J (1983) Preparation of dried baker's yeast. USA Patent 4.370.420. Societe Industrielle LeSaffre, Paris, France.

Clementi F and Rossi J (1984) Effect of drying and storage conditions on survival of *Leuconostoc oenos*. Am. J. Enol. Vitic. 35: 181-186.

Clements ML, Levine MM, Black RE, Robins-Brown RM, Cisneros LA, Drusano GL, Lanata CF and Saah AJ (1981) *Lactobacillus prophylaxis* for diarrhea due to enterotoxigenic *Escherichia coli*. Antimicrobial agents and Chemotherapy 20: 104-108.

Comings EW, Higa H, Myers JE, Koffler H and McLain HA (1977) Spray-drying of *Serratia marcescens* in the jet spray-dryer. Ind. Eng. Chem., Fundam. 16: 12-18.

Corry JEL (1973) The water relations and heat resistance of micro-organisms. Progr. Ind. Microbiol. 12: 73-108.

Coumans WJ (1987) Power law diffusion in drying processes. Ph.D. Thesis, Technical University Eindhoven, The Netherlands.

Daemen ALH (1981) The destruction of enzymes and bacteria during spray-drying of milk and whey 1. The thermoresistance of some enzymes and bacteria in milk and whey with various total solids contents. Neth. Milk Dairy J. 35: 133-144.

Daemen ALH and Van der Stege HJ (1982) The destruction of enzymes and bacteria during spray-drying of milk and whey 2. The effect of drying conditions. Neth. Milk Dairy J. 36: 211-229.

Daeschel MA, Andersson RE and Flemming HP (1987) Microbial ecology of fermenting plant materials. FEMS Micriobiol. Rev. 46: 357-367.

Divies C, Lenzi P, Beaujeu J and Herault F (1990) Procede de preparation de micro-organismes inclus dans des gels sensiblement déshydrates, gels obtenus et leur utilisation pour la préparation de boissoins fermentées. French Patent 2 633 937. Champagne Moet & Chandon, France.

Elizondo H and Labuza TP (1974) Death kinetics of yeast in spray-drying. Biotechn. Bioeng. 16: 1245-1259.

Espina F and Packard VS (1979) Survival of *Lactobacillus acidophilus* in a spray-drying process. J. Food Prot. 42: 149-152.

Fernandes CF, Shahani KM and Amer MA (1987) Therapeutic role of dietary lactobacilli and lactobacillic fermented dairy products. FEMS Microbiol. Rev. 46: 343-356.

Filková I and Mujumdar AS (1987) *Industrial spray drying systems*. In: Mujumdar AS (ed) Handbook of industrial drying (pp. 243-293). Marcel Dekker, NY.

Foster EM (1962) Culture preservation. J. Dairy Sci. 45: 1290-1294.

Fuller R (1986) Probiotics. J. Appl. Bacteriol. (Symp. Suppl.): 1S-7S.

Gherna RL (1981) *Preservation*. In: Gerhardt P (ed) Manual of methods for general bacteriology (pp. 208-217). Am. Soc. Microbiol., Washington.

Gibson LF and Khoury JT (1986) Storage and survival of bacteria by ultra-freeze. Letters Appl. Microbiol. 3: 127-129.

Gilliland SE (1988) *Probiotics: fact or fancy?* In: Durand G, Bobichon L and Florent J (eds) Proc. 8th Int. Biotechnol. Symp., Paris, Vol.2 (pp. 923-933).

Gilliland SE (1990) Health and nutritional benefits from lactic acid bacteria. FEMS Microbiol. Rev. 87: 175-188.

Hammes WP (1990a) Bacterial starter cultures in food production. Food Biotechnol. 4: 383-397.

Hammes WP, Bantleon A and Min S (1990b) Lactic acid bacteria in meat fermentation. FEMS Microbiol. Rev. 87: 165-174.

Heckly RJ (1961) *Preservation of bacteria by lyophilization*. In: Umbreit WW (ed) Advances in applied microbiology (pp. 1-76). Academic Press, NY.

Heckly RJ (1978a) *Effects of oxygen on dried organisms*. In: Crowe JH and Glegg JJ (eds) Dry Biological Systems (pp. 257-278). Academic Press, NY.

Heckly RJ (1978b) Protection kinetics of additives on survival of lyophilized bacteria. Cryobiology 15: 654-658.

Heckly RJ (1985) Principles of preserving bacteria by freeze-drying. Develop. Industrial Microbiol. 26: 379-395.

Hill FF (1985) Verfahren zum herstellen eines trockenen lagerstabilen bakterienpräparartes. European Patent 0 131 114. Chemische Werke Hüls AG, Marl, Germany.

Hill FF (1986) *Dry living micro-organisms. Products for food industry*. In: Chmiel H, Hammes WP and Bailey JE (eds) Biochemical Engineering: a challenge for interdisciplinary cooperation. Int. Congress Stuttgart sept. 1986 (pp. 199-215). VCH Publishers, NY.

Hill FF (1987) Herstellung von lagerfähigen starterkulturen in wirbelbett. Alimenta 1987 No.3: 73-80.

Hovmand S (1987) *Fluidized bed drying*. In: Mujumdar AS (ed) Handbook of industrial drying (pp. 165-225). Marcel Dekker, NY.

Josic D (1982) Optimization of process conditions for the production of active dry yeast. Lebensm.-Wiss.-Technol. 15: 5-14.

Kerkhof PJAM and Schroeber WJAH (1974) *Theoretical modelling of the drying behaviour of droplets in spray dryers*. In: Spicer A (ed) Advances in preconcentration and dehydration of food (pp. 349-397). Applied Science Publishers, London.

Kilara A, Shahani KM and Das NK (1976) Effect of cryoprotective agents on freeze-drying and storage of lactic cultures. Cultured Dairy Products J., May 1976: 8-11.

Kim HS (1988) Characterization of lactobacilli and bifidiobacteria as applied to dietry adjuncts. Cultured Dairy Products J., August 1988: 6-9.

Kim SS and Bhowmik SR (1990) Survival of lactic acid bacteria during spray drying of plain yogurt. J. Food Sci. 55: 1008-1010.

Kuts PS and Tutova EG (1983) Fundamentals of drying of microbiological materials. Drying Techn. 2: 171-201.

Labuza TP, Roux JP, Fan TS and Tannenbaum SR (1970) Engineering factors in single-cell protein production II. Spray-drying and cell viability. Biotechn. Bioeng. 12: 135-140.

Lafon-Lafourcade S (1983) *Wine and brandy.* In: Rehm HJ and Reed G (eds) Biotechnology, Vol.5, Food and feed production with micro-organisms (pp. 231-314) Verlag Chemie, Weinheim, Germany.

Lal M, Tiwari MP, Sinha RN and Ranganathan B (1976) Survival of some freeze-dried lactic cultures stored in gelatin capsules. J.Food Sci. Techn. India 13: 266-267.

Langejan A and Khoudokormoff B (1982) Active dried baker's yeast. USA Patent 4.341.871. Gist-brocades NV, Delft, The Netherlands

Leben C and Sleesman JP (1984) Preservation of plant-pathogenic bacteria on silica gel. Plant disease 66: 327.

Lee BH (1989) Biotechnological aspects of lactobacillus strains in food fermentation. In: Ghee AH, Hen NB and Kong LK (eds) Trends in food biotechnology (pp.121-128). Singapore Inst. Food Sci. Techn., Singapore.

Liepe HU (1983) *Starter cultures in meat production.* In: Rehm HJ and Reed G (eds) Biotechnology, Vol.5, Food and feed production with micro-organisms (pp. 399-424). Verlag Chemie, Weinheim.

Lievense LC and Van 't Riet K (1991a) *Convective drying of bacteria. I. The drying processes.* In: Fiechter A. (ed) Advances in Biochemical Engineering/Biotechnology. Springer-Verlag, Berlin. Submitted.

Lievense LC and Van 't Riet K (1991b) *Convective drying of bacteria. II. Factors influencing the survival.* In: Fiechter A. (ed) Advances in Biochemical Engineering/Biotechnology. Springer-Verlag, Berlin. Submitted.

Liou JK (1982) An approximate method for nonlinear diffusion applied to enzyme inactivation during drying. Ph.D. Thesis, Agricultural University Wageningen, The Netherlands.

Liou JK, Luyben KChAM and Bruin S (1985) A simplified calculation method applied to enzyme inactivation during drying. Biotechn. Bioeng. 27: 109-116.

Lockwood LB (1979) *Production of organic acids by fermentation.* In: Peppler HJ and Perlman D (eds) Microbial technology. Vol.1 Microbial processes (pp. 356-387). Academic Press, NY.

Luyben KChAM, Liou JK and Bruin S (1982) Enzyme degradation during drying. Biotechn. Bioeng. 24: 533-552.

Marshall VM (1987) Lactic acid bacteria: starters for flavours. FEMS Microbiol. Rev. 46: 327-336.

Masters K (1985) *Spray drying handbook.* George Godwin, London.

McEldowney S and Flechter M (1988) The effect of temperature and relative humidity on the survival of bacteria attached to dry solid surfaces. Letters in Appl. Microbiol. 7: 83-86.

Meerdink G and Van 't Riet K (1991) Inactivation of a thermostable α-amylase during drying. J. Food Eng. In press.

Metwally MM, Abd El-Gawad IA, El-Nockrashy SA and Ahmed KE (1989) Spray drying of lactic acid culture I. The effect of spray-drying conditions on the survival of micro-organisms. Egyptian J. Dairy Sci. 17: 35-43.

Morichi T (1974) Preservation of lactic acid bacteria by freeze-drying. Jpn. Agr. Res. Quart. 8: 171-176.

Nei T (1973) Some aspects of freezing and drying of micro-organisms on the basis of cellular water. Cryobiology 10: 403-408

Olson NF (1990) The impact of lactic acid bacteria on cheese flavour. FEMS Microbiol. Rev. 87: 131-148.

Orndorff GR and MacKenzie AP (1973) The function of the suspending medium during the freeze-drying preservation of *Escherichia coli*. Cryobiology 10: 475-487.

Peppler HJ (1979) *Production of yeast and yeast products.* In: Peppler HJ and Perlman D (eds) Microbial technology. Vol.1 Microbial processes (pp. 157-185). Academic press, NY.

Peppler HJ (1983) *Fermented feeds and feed supplements.* In: Rehm HJ and Reed G (eds) Biotechnology, Vol.5, Food and feed production with micro-organisms (pp. 599-446). Verlag Chemie, Weinheim, Germany.

Philipp S (1984) Starterkulturen in der milchverarbeitenden industrie. Lebensmitteltechnik 10/84: 518-521.

Porubcan RS and Sellars RL (1979) *Lactic starter cultures concentrates.* In: Peppler HJ and Perlman D (eds) Microbial Technology (pp. 59-92). Academic Press, London.

Prentice GA and Neaves P (1986) The role of micro-organisms in the dairy industry. J. Appl. Bacteriol. (Symp. Suppl.): 43S-57S.

Rehm HJ, Reed G and Dellweg H (eds) (1983a) *Biotechnology, Vol.3, Biomass, Micro-organisms for special applications, Microbial products I, Energy from renewable resources.* Verlag Chemie, Weinheim, Germany.

Rehm HJ and Reed G (eds) (1983b) *Biotechnology, Vol.5, Food and feed production with micro-organisms.* Verlag Chemie, Weinheim, Germany.

Robinson RK (1981) Freeze-dried starter concentrates. Dairy Ind. Int. 46: 15-21.

Robinson RK (1983) *Starter cultures for milk and meat processing.* In: Rehm HJ, Reed G and Dellweg H (eds) Biotechnology, Vol.3, Biomass, Micro-organisms for special applications, Microbial products I, Energy from renewable resources (pp. 191-202). Verlag Chemie, Weinheim, Germany

Roelans E and Taeymans D (1990) *Effect of drying conditions on survival and enzyme activity of micro-organisms.* In: Spiess WEL and Schubert H (eds) Engineering and Food, Vol.3, Advanced processes (pp. 559-569). Elseviers Applied Science, London.

Rogers LA (1914) The preparation of dried cultures. J. Infect. Diseases 14: 100-123.

Schillinger U and Lücke FK (1989) Lactic acid bacteria as protective cultures in meat products. Fleischwirtsch. 69: 1581-1585.

Seale DR (1986) Bacterial inoculants as silage additives. J. Appl. Bacteriol. (Symp. Suppl.): 9S-26S.

Sleesman JP and Leben C (1978) Preservation phytopathogenic bacteria at -70°C or with silica gel. Plant Disease Reporter 62: 910-913.

Snell JJS (1984) *General introduction to maintenance methods.* In: Kirsop BE and Snell JJS (ed) Maintenance of micro-organisms, a manual of laboratory practice (pp. 11-21). Academic Press, London.

Spicher G (1983) *Baked goods.* In: Rehm HJ and Reed G (eds) Biotechnology, Vol.5, Food and feed production with micro-organisms (pp. 1-80). Verlag Chemie, Weinheim, Germany.

Stadhouders J, Jansen LA and Hup G (1969) Preservation of starters and mass production of starter bacteria. Neth. Milk Dairy J. 23: 182-199.

Taeymans D, Roelans E and Lenges J (1986) *Comparison of different drying techniques for processing active dry yeast for use in fermentors with immobilized cells.* In: Le Maguer M and Jelen P (eds) Proc. Int. Congr. Eng. Food 4, Edmonton, Canada. Food engineering and process applications, Vol.1 (pp. 439-444). Elseviers, London.

Tsetkov TS and Brankova R (1983) Viability of micrococci and lactobacilli upon freezing and freeze-drying in the presence of different cryoprotectants. Cryobiology 20: 318-323.

Uhlemann H (1990) Kontinuierliche wirbelschicht-sprühgranulation. Chem.-Ing.-Tech. 62: 822-834.

Valdez GF, De Giori GS, De Riuz Holgodo AA and Oliver G (1983a) Protective effect of adonitol on lactic acid bacteria subjected to freeze-drying. Appl. Env. Microbiol. 45: 302-304.

Valdez GF, De Giori GS, De Riuz Holgodo AA and Oliver G (1983b) Comparative study of the efficiency of some additives in protecting lactic acid bacteria against freeze-drying. Cryobiology 20: 560-566.

Van der Lijn J (1976) Simulation of heat and mass transfer in spray drying. Ph.D. Thesis, Agricultural University Wageningen, The Netherlands.

Vedamathu ER and Washam C (1983) *Cheese.* In: Rehm HJ and Reed G (eds) Biotechnology, Vol.5, Food and feed production with micro-organisms (pp. 231-314). Verlag Chemie, Weinheim, Germany.

Wijlhuizen AE, Kerkhof PJAM and Bruin S (1979) Theoretical study of the inactivation of phosphatase during spray-drying of skim-milk. Chem. Eng. Sci. 34: 651-660.

Yamamoto S, Musahiko A, Nakodo H and Sano Y (1985) *Enzyme inactivation during drying of a single droplet.* In: Toei R and Mujumbar AS (eds) Drying '85 (pp. 330-337) Hemisphere Publishing Corporation, Washington.

Yankov YA and Brankova R (1979) Comperative studies on the survivability and activity of frozen and freeze-dried cultures of *Lactobacillus plantarum*. Proc. European Meat Research Workers 25 (Vol.2): 320-323.

Zimmermann K and Bauer W (1986) *The influence of drying conditions upon reactivation of baker's yeast.* In: Le Maguer M and Jelen P (eds) Proc. Int. Congr. Eng. Food 4, Edmonton, Canada. Food engineering and process applications, Vol.1 (pp. 425-437). Elseviers, London.

Zimmermann K (1987a) Einflussparameter und mathematische modellierung der schonenden trocknung von starterkulturen. Fortschr.-Ber. VDI (Reihe 14, no 36), VDI-Verlag, Düsseldorf, West Germany.

Zimmermann K and Bauer W (1987b) Modellierung der trocknung von hefen im wirbelschichttrockner. Chem.-Ing.-Tech. 59: 350-351.

Zimmermann K and Bauer W (1990) *Fluidized bed drying of micro-organisms on carrier material.* In: Spiess WEL and Schubert H (eds) Engineering and Food, Vol.2, Preservation processes and related techniques (pp. 666-678). Elseviers Applied Science, London.

CHAPTER 2

MEASUREMENT AND MODELLING OF THE GLUCOSE-FERMENTING ACTIVITY OF LACTOBACILLUS PLANTARUM

Lou C. Lievense[1], Klaas van 't Riet[1] and Ad Noomen[2]

1. Food- and Bioprocess Engineering Group, Department of Food Science, Wageningen Agricultural University
2. Laboratory of Dairying and Food Physics, Department of Food Science, Wageningen Agricultural University

ABSTRACT

The pH decrease in a phosphate buffer due to fermentation of glucose to lactic acid by non-growing *Lactobacillus plantarum* cells has been studied. The method used provides a rapid and reproducible way of measuring the glucose-fermenting activity of *L. plantarum*. The maximum observed velocity of pH decrease is linearly correlated with the biomass concentration and is defined as the activity of the cell suspension. With *L. plantarum*, recalculation of this arbitrary unit (ΔpH.min^{-1} per gram dry weight) to a conceivable unit of lactic acid production rate (mol.min^{-1} per gram dry weight) is possible. This recalculation is based on the titration theory of a weak base with a weak acid. The same theory together with the lactic acid production kinetics of *L. plantarum* is applied to model the entire pH-time curve.

Lievense LC, Van 't Riet K and Noomen A (1990) Measuring and modelling the glucose-fermenting activity of *Lactobacillus plantarum*. **Appl. Microbiol. Biotechnol. 32**: 669-673.

CHAPTER 2

MEASUREMENT AND MODELLING OF THE GLUCOSE-FERMENTING ACTIVITY OF LACTOBACILLUS PLANTARUM

2.1 INTRODUCTION

Lactic acid bacteria play an increasing role in many fermentation processes in food manufacture. The activity of a particular lactic acid bacterium may be expressed by its rate of (lactic-)acid production, which offers a parameter that can be used to define the effectiveness of the culture in technological processes. Many of the activity tests described in the literature are based on this concept and emanate from dairy science.

Generally, acid production is followed with time (Whitehead and Cox 1932; Elliker and Frazier 1938) or the acid concentration is determined after a fixed incubation time (Anderson and Meanwell 1942; Horrall and Elliker 1950; Pearce 1969; Stadhouders 1980a), both by means of end-point titration with a base. These methods have some disadvantages. The automation of the tests is difficult, a major drawback when large numbers of cultures have to be analysed, and also the duration (4-6h) could be a problem. The use of skimmed or whole milk as substrates in these tests may cause fluctuations in results, as mentioned by Stadhouders (1980b), and moreover the use of these media hampers modelling of the processes taking place during the test.

Junker and Liepe (1979, 1981) described the acid producing activity of a *Lactobacillus plantarum* culture in a physiological salt solution with glucose. They used an automatic titration apparatus with NaOH to keep the pH of the cell suspension at a preset value. According to the authors the results of this method are highly reproducible. The disadvantage of this method is the high cost of the titration equipment needed when series of cultures have to be analysed simultaneously.

The monitoring of the pH-time curve during fermentation of glucose to lactic acid by non-growing *L. plantarum* cells in a synthetic medium, described in this chapter, offers a rapid and reproducible method for analysis of glucose-fermenting activity. When this technique is used, the simple experimental set-up enables automated sampling and the determination of the activities of several suspensions at the same time at low cost.

For a quantitative description of the processes taking place, needed for a better insight in the procedure, the method is described along with an experimentally validated mathematical model. Furthermore, with homofermentative fermentation, the theoretical basis gives the possibility of recalculating the velocity of pH decrease into a lactic acid production rate.

2.2 THEORY

With homofermentative fermentation the pH curve follows the pattern of a weak base (phosphate)-weak acid (lactic acid) titration curve. In case of heterofermentative fermentation other acids are also formed and the pH curve is more complex. As shown later, for modelling the activity test with *L. plantarum*, the metabolism of this organism under the assay conditions may be considered as homofermentative.

For the titration of phosphate buffer with lactic acid we can derive (Willis 1981):

$$M_a = \frac{K_a[OH^-] + K_w}{K_a[OH^-]} \times$$

$$\left\{ \frac{M_b(K_{b,1}[OH^-]^2 + 2K_{b,1}K_{b,2}[OH^-] + 3K_{b,1}K_{b,2}K_{b,3})}{[OH^-]^3 + K_{b,1}[OH^-]^2 + K_{b,1}K_{b,2}[OH^-] + K_{b,1}K_{b,2}K_{b,3}} + \frac{K_w}{[OH^-]} - [OH^-] \right\} \quad (2.1)$$

where:

M_a =	lactic acid concentration in the phosphate buffer		(mol.l^{-1})
M_b =	molarity of the phosphate buffer		(mol.l^{-1})
K_a =	dissociation constant of lactic acid		(mol.l^{-1})
$K_{b,n}$ =	dissociation constants of the phosphate		(mol.l^{-1})
K_w =	dissociation constant of water ([OH$^-$].[H$^+$])		(mol^2.l^{-2})
n =	first, second or third dissociation step of phosphate		

To simplify Eq. (2.1), [OH$^-$] is used as the parameter for the acidity of the system. This value can be recalculated to [H$^+$] or pH value.

The values of the dissociation constants of lactic acid, phosphate and water (K_a, $K_{b,n}$ and K_w respectively) are important for the modelling of the activity test. These are all temperature dependent; K_a and $K_{b,n}$ also depend on the ionic strength of the solution. In the system described, the temperature was 35 °C; the ionic strength was mainly fixed by 0.01 mol.l^{-1} KH_2PO_4/K_2HPO_4 (pH 7) and 0.15 mol.l^{-1} NaCl and amounted to 0.18 mol.l^{-1}. These values allow a good approximation of the dissociation constant K_a (2.68×10^{-4} mol.l^{-1}) of the lactic acid (Hickey 1940), $K_{b,2}$ (6.84×10^{-8} mol.l^{-1}) of the phosphate (Alberty *et al.* 1951) and K_w (2.09×10^{-14} mol.l^{-1}) of the water (Weast and Astle 1979). For modelling in the pH range of the

assay, exact values of $K_{b,1}$ and $K_{b,3}$ are not important and values obtained in water at a temperature of 25 °C (Weast and Astle 1979) were taken (4.55×10^{-2} mol.l^{-1} and 1.35×10^{-12}mol.l^{-1} respectively).

It may be seen from Eq. (2.1) that at pH 7 ([OH^-] = 10^{-7}mol.l^{-1}) (lactic-)acid is already present in the measurement suspension because the first dissociation step ($K_{b,1}$) of the phosphate is taken into account in the theory. In an activity test, however, lactic acid production starts at that pH. Thus, the concentration of lactic acid formed at a certain pH during an activity test can be calculated by entering this pH value in Eq. (2.1) and subsequently decreasing the result by the theoretical concentration of lactic acid at the initial pH of the measurement suspension (pH 7).

From the pH curve recorded against time, values can be obtained for the velocity of pH decrease (dpH/dt) at any time or pH during the test. With this dpH/dt and the first derivative of Eq. (2.1), both at a particular pH, the acid forming rate (dM_a/dt) can be calculated:

$$\frac{dM_a}{dt} = \frac{dpH}{dt} \cdot \frac{dM_a}{dpH} \qquad (2.2)$$

The titration theory described above serves as a basis for the mathematical modelling of the activity test. As mentioned before, it is essential to apply this theory only to homofermentative fermentations. The facultative heterofermentative *L. plantarum* metabolism may be influenced by the oxygen concentration in the medium (Murphey and Condon 1984). Furthermore, a change in pH and lactic acid concentration may influence the rate of acid production of the cells as with decreasing glucose concentration. The influence of these factors and the assumed homofermentative fermentation were experimentally verified.

2.3 MATERIALS AND METHODS

2.3.1 Microorganism and growth conditions

Lactobacillus plantarum (P743; Netherlands Institute for Dairy Research (NIZO), Ede, The Netherlands) was grown under microaerophilic conditions in sterilized MRS medium (Gibco, Middlesex, UK) (De Man *et al.* 1960) supplemented with 10 g.l^{-1} glucose (Merck, Darmstadt, FRG) and 1 g.l^{-1} yeast extract (Oxoid, Basingstoke Hampshire, UK), at 30 °C in a pH-controlled 1.5-l laboratory fermentor (New Brunswick C10, Edison, NJ). The pH was maintained at 6.3 with 3 mol.l^{-1} NaOH (Merck). After complete consumption of the glucose, monitored by the use of glucose test sticks (Ames Diastix, Bayer Diagnostica, Mijdrecht, NL), the cells were harvested. At this point the concentration of biomass in the medium amounted to 3.8 (±0.3) g dry weight (dw).l^{-1}. After centrifugation at 16.000 *g* for 10 min (10.000 rpm, Sorvall SB-50, GSA-rotor) cell pellets were washed with AM buffer (see **2.3.2 Standard activity test**),

centrifuged again and resuspended in AM buffer to a concentration of 30 mg dw.ml^{-1}, representing 6.66 (±0.53) x 10^{10} colony-forming units.ml^{-1}. The suspension was stored at 4 °C for maximally 4 days.

2.3.2 Standard activity test

For a standard activity test, 3 ml cell suspension was added to 26 ml activity measurement buffer (referred to as AM buffer) in a 50-ml erlenmeyer flask. The AM buffer was prepared by mixing 0.01 mol.l^{-1} K_2HPO_4 (Merck) and 0.01 mol.l^{-1} KH_2PO_4 (Merck), both dissolved in a solution of 0.15 mol.l^{-1} NaCl (Merck) in demineralized water, to pH 7.0. The flask was placed in a water bath at 35 °C and stirred at constant speed (200 rpm) on a magnetic stirrer plate (Variomag HP15, Hois + Partner Labortechnik, München, FRG). This mixture was pre-incubated for 5 min and the test was initiated by addition of 1 ml glucose solution (350 g glucose.H_2O (Merck) added to 1 l AM buffer). The pH of this suspension (referred to as measurement suspension) was registered between pH 7 and pH 4 (pH electrode; Schott S61, Hofheim a. Ts., FRG). In our experimental set-up the electrode was coupled via a pre-amplifier to a calibrated data acquisition system or to a calibrated pH meter (Schott CG809) with a chart recorder.

The concentration of 0.01 mol.l^{-1} potassium phosphate for this method has been chosen as a compromise between the duration of the activity test and the buffering capacity of the AM buffer. High concentrations make it difficult to detect pH changes, whilst low concentrations result in difficulties in adjusting the pH of the AM buffer to pH 7.

The maximum velocity of pH decrease observed is defined as the activity of the cell suspension in ΔpH.min^{-1} or ΔpH.min^{-1} per gram dry weight of cells.

2.3.3 Data acquisition and analysis

A data acquisition system facilitated the recording of ten activity tests at the same time. One system coupled the electrodes via high-impedance pre-amplifiers (own design) to a data acquisition and control unit (Hewlett Packard 3421A) with a micro-computer (Hewlett Packard 86B). The data were first stored in the HP86B and later transferred to a MS-DOS microcomputer for further analysis. Another system sampled directly from the pre-amplifier to the MS-DOS machine with the help of an A/D-board (Analog Devices RTI-800, Norwood, MA) and ASYST-software (Macmillan Software Company, Rochester, NY) (Fig. 2.1).

Before sampling the systems were calibrated with calibration buffers of pH 7 and pH 4 (Merck). The mV/pH-signal was sampled at fixed time intervals (2-5 s). For further analysis the pH-curve was fitted with a third-degree polynomial (Cheney 1985). The second derivative of this polynomial gave the inflection point of the curve. The maximum velocity of pH-decrease could be calculated using the first derivative at the point of inflection.

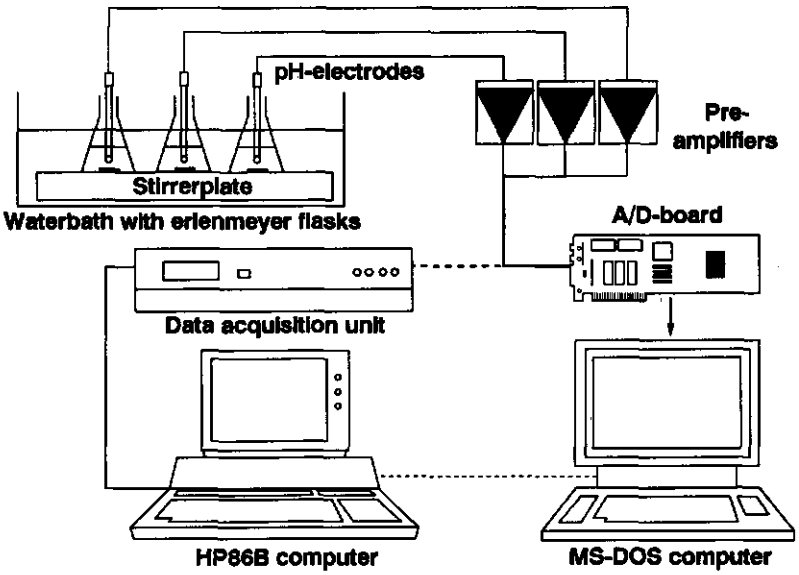

Fig. 2.1 Schematic representation of the automated measurement set-up (Lievense *et al.* 1990).

2.3.4 Titration

Consumption of 0.1 mol.l^{-1} NaOH (Merck) during fermentation at a preset pH value was registered on a chart recorder (Junker and Liepe 1979, 1981) using an automatic titration apparatus (Schott TR156).

2.3.5 Chromatography

Concentrations of acids produced and of glucose consumed were determined with an HPLC system consisting of a Biorad Aminex (Richmond, Calif) HPX-87H column at 35 °C coupled to a refractive index detector (Erma Optical Works, Tokyo, Japan). The mobile phase used was H_2SO_4 (0.005 mol.l^{-1}) in Nanopure (Barnstead water purifucation system, Boston, MA) water at a flow rate of 0.6 ml.min^{-1}. For estimation of the concentration of components during an activity test 1 ml of the measurement suspension was transferred to 9 ml Nanopure water at 80 °C. At this temperature the cells were killed and fermentation ceased.

2.4 RESULTS AND DISCUSSION

2.4.1 Activity test

In Fig. 2.2 the recorded pH-time curve during a standard activity test is shown. The maximum *dpH/dt* can be measured directly from the curve. When a data acquisition system is used, a polynomial fit of the pH-time curve can be used to compute *dpH/dt* as a function of pH or time.

According to the titration theory (Willis 1981), the maximum *dpH/dt*, as used in the definition of cell activity, occurs at a fixed pH value. This is caused by the dissociation constants of the lactic acid and of the phosphate buffer. The maximum *dpH/dt* during the activity test was always observed at pH 5.3±0.1. The deviation around pH 5.3 was probably due to the presence of acetic acid in the measurement suspension (see **2.4.5 Influence of oxygen**) and that of acid/base groups on the cell surfaces.

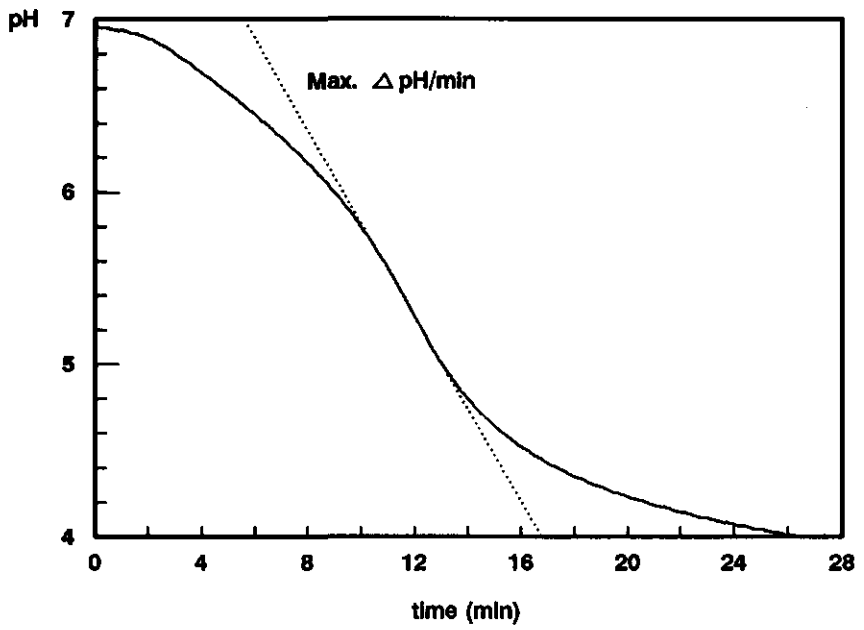

Fig. 2.2 Recorded pH-time curve in the course of an activity test under standard assay conditions.

2.4.2 Influence of cell density

Application of the method requires linearity between the maximum *dpH/dt* observed and the fermentation capacity of the cells. This linearity was checked by measurement of the activities of different concentrations of cells. In these experiments the same cell suspension was used at different cell concentrations. The results are shown in Fig. 2.3. The reproducibility of the results was good, showing a coefficient of variation of 3.8 %.

Linearity was observed up to a cell concentration of 4 g dw.l^{-1} measurement suspension. At higher concentrations a deflection was found. This may be attributed to an increasing buffering capacity of the system, superimposed on the buffer capacity of 0.01 mol.l^{-1} phosphate, with increasing cell concentration. Components in the cell wall, such as lysine in peptidoglycan (Schleifer 1987), might be responsible for this extra buffering capacity.

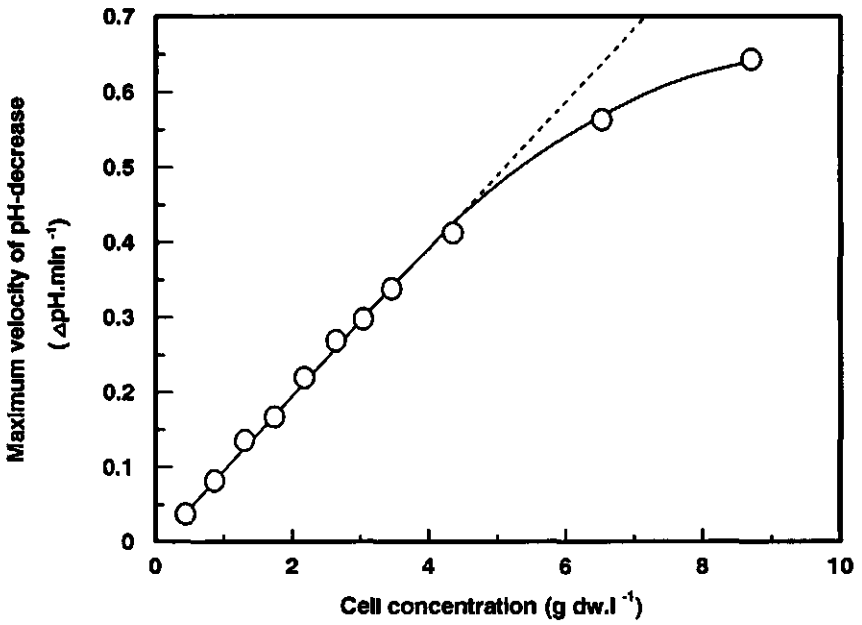

Fig. 2.3 Maximum velocity of pH decrease as a function of the concentration of *L. plantarum* cells under otherwise standard activity test conditions.

2.4.3 Influence of temperature

Activity of a suspension of cells was measured at different temperatures and increased up to 45 °C. At higher temperatures, inactivation of the fermenting system apparently becomes dominant. A standard measurement temperature of 35 °C was chosen as a compromise between the duration of the activity test and the stability of the fermenting system. At this temperature no significant inactivation of that system was observed in a time course of 2 h while a standard activity test with *L. plantarum* cells lasted about 30 min.

2.4.4 Influence of glucose concentration

The effect of different initial glucose concentrations on the initial rate of lactic acid production of cells was measured by titration with 0.1 mol.l^{-1} NaOH at pH 5.3. After an increase in acid production rate from 0.002 mol.l^{-1} to 0.02 mol.l^{-1} glucose no further influence of the glucose concentration was detected in the range 0.02-0.06 mol.l^{-1}.

The concentration of the glucose solution was 1.43 mol glucose.l^{-1}. After addition of 1 ml of this solution to 29 ml AM buffer with cell suspension the glucose concentration amounted to 0.048 mol.l^{-1}. During the activity test from pH 7 to pH 4, ±0.007 mol.l^{-1} glucose became consumed (see Fig. 2.4) resulting in an end concentration of ±0.041 mol.l^{-1} glucose. Hence, with this initial glucose concentration, no substrate limitation occurred during the activity test.

2.4.5 Influence of oxygen

Activity tests were performed in the presence of different concentrations of oxygen in the measurement suspension. After fermentation to pH 4, samples were analysed for organic acids by HPLC (see Table 2.1). Acetic acid and lactic acid were the only metabolites detected.

During aeration, a considerable amount of glucose became fermented to acetic acid. Because acetic acid is a weaker acid than lactic acid (K_a acetic acid < K_a lactic acid) the first derivative of Eq. (2.1) will be influenced such that a decreased activity will be observed.

Figure 2.4 shows the concentrations of lactic acid, acetic acid and glucose during a standard activity test. At the start of the activity test a certain amount of oxygen was present in the measurement suspension. This oxygen was rapidly consumed by the fermenting cells and a small quantity of acetic acid was formed. Later on hardly any acetic acid was formed because under the standard experimental conditions diffusion of oxygen from air into the measurement suspension was negligible.

In the experimental set-up described no special equipment was used to control the oxygen concentration in the measurement suspension. When standard conditions are used (dimension of erlenmeyer flasks, speed of agitation and temperature), fluctuations in the initial oxygen concentration will be small. For very critical estimations removal of oxygen from the measurement suspension before and during the activity test is recommended.

Table 2.1 Ratio of lactic acid to acetic acid at the end of the activity test (pH 4.0) as affected by different oxygen concentrations.

Standard activity test	Aeration with bubbling air	Deaeration with bubbling nitrogen
15.6	3.4	20.3

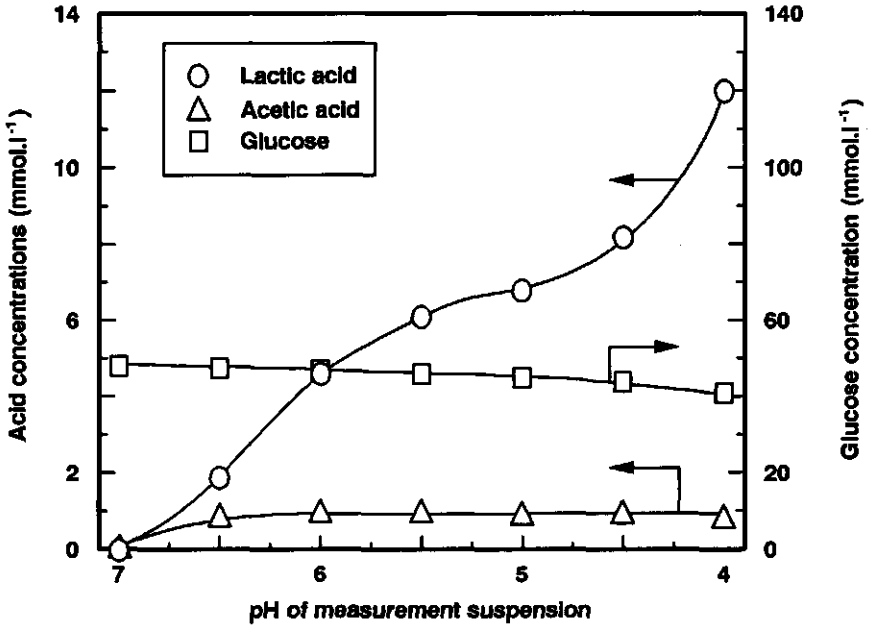

Fig. 2.4 Concentrations of lactic acid, acetic acid and glucose at different pH during the activity test under standard assay conditions.

2.4.6 Mathematical modelling of the activity test

The validity of Eq. (2.1) was verified in the course of a standard activity test. Samples of the measurement suspension were taken during the activity test at different pH values, and concentrations of lactic acid at those pH values were determined by HPLC. Figure 2.5 shows the measured data together with the theoretical curve calculated according to Eq. (2.1), corrected for the theoretical lactic acid concentration at pH 7.0.

In the standard test the activities (maximum *dpH/dt*) were found to be 3.32 (±0.23) ΔpH.min^{-1} per gram dw of cells in 30 ml measurement suspension. From this maximum *dpH/dt* around pH 5.3, the acid-forming rates can be calculated according to Eq. (2.2) using dM_a/dpH at pH 5.3 which is 2.11×10^{-3} mol.l^{-1} per pH unit. Recalculation to gram dry weight of cells yielded an acid-forming rate of 2.1×10^{-4} mol lactic acid.min^{-1} per gram dw at pH 5.3. Acid-forming rates calculated from titrations with 0.01 mol.l^{-1} NaOH at pH 5.3 agreed within 7 % with those derived from the maximum *dpH/dt* up to cell concentrations of 4 g dw.l^{-1}.

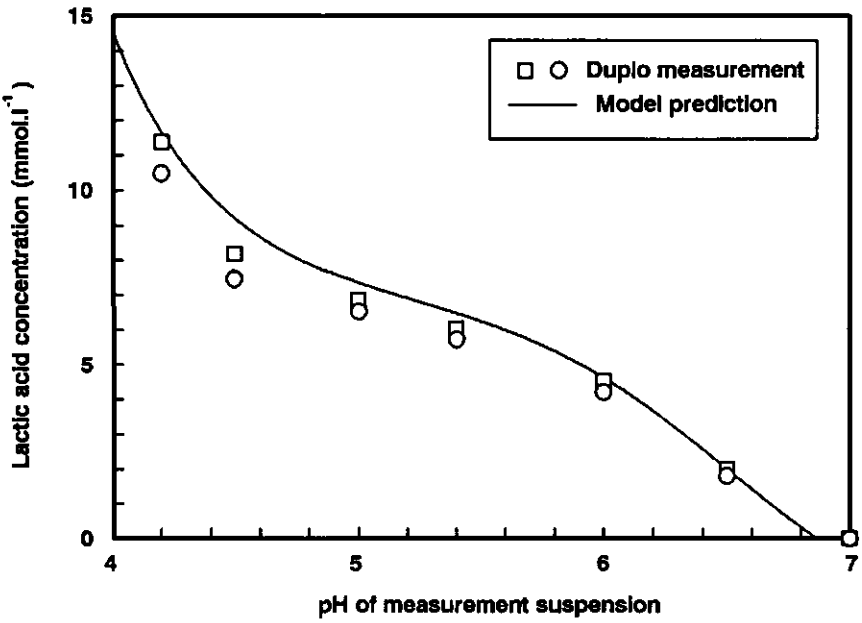

Fig. 2.5 Estimated and calculated lactic acid concentrations at different pH of the measurement suspension in the course of an activity test under standard assay conditions.

During the activity test the acid forming rate of the *L. plantarum* cells might be influenced by the change in pH of the measurement suspension as a result of the increasing lactic acid concentration. Acid forming rates were determined at different pH values and at different initial lactic acid concentrations in the medium, by titration with NaOH at preset pH values.

Figure 2.6 gives the results as a function of pH. Furthermore, a third-degree polynomial is fitted through the data points to describe this relationship. Activity of cells was only pH dependent and not influenced by concentrations of lactic acid/lactate up to 1.4×10^{-2} mol.l^{-1}, which was the maximum concentration tested. It may thus be concluded that product inhibition was negligible in the activity test because concentrations produced in that test were smaller than 1.4×10^{-2} mol.l^{-1} (see Fig. 2.4).

One of the main objectives of this work was a better insight in the test procedure based on a quantitative description. It is shown that with the described titration theory the measured maximum *dpH/dt* can be recalculated to the lactic acid production rate at pH 5.3. One step further is the modelling of the entire pH-time curve with the titration theory and the production kinetics of *L. plantarum*. The results presented here indeed show that the titration theory together with the third degree polynomial for the production kinetics gives the possibility of simulating the entire recorded pH-time curve. The measured maximum *dpH/dt* serves as the only input parameter.

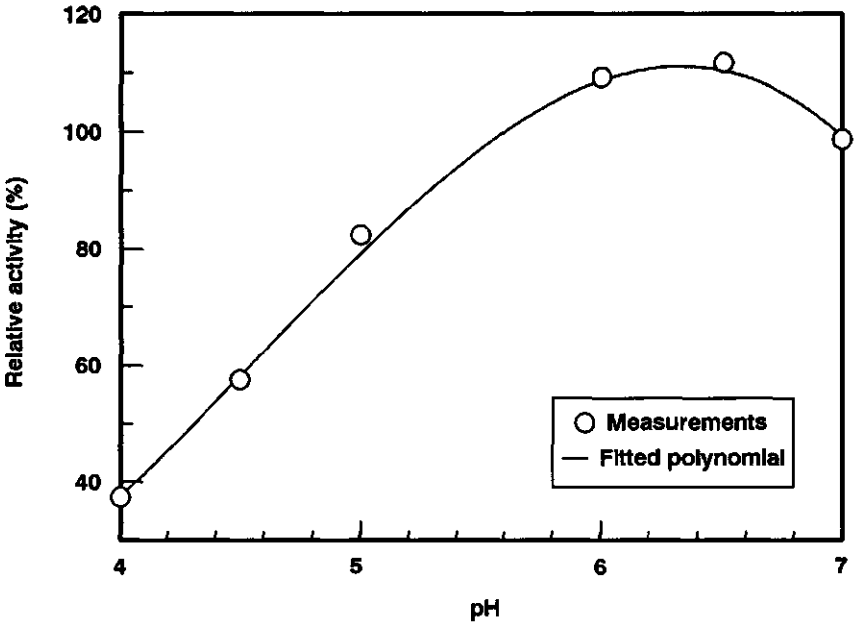

Fig. 2.6 Relative acid production rates, as measured by titration with NaOH, at different pH of the measurement suspension, fitted with a third-degree polynomial. The acid-forming rate at pH 7.0 was arbitrarily taken as 100%.

In the simulation the lactic-acid-forming rates (dM_a/dt) at the different pH values were calculated from dM_a/dt at pH 5.3, calculated from the measured maximum dpH/dt, and the polynomial from Fig. 2.6. The simulation procedure started with the dM_a/dt at the initial pH value (pH 7). This dM_a/dt was kept constant for a short period of time (3 s). After this time M_a was calculated as $M_a = M_a + 3 \cdot (dM_a/dt)$. With this increased M_a the new pH value reached was iteratively calculated by means of Eq. (2.1). At this new pH, dM_a/dt was calculated and the cycle started again.

The production kinetics at a particular pH were estimated at stationary pH. Therefore a parameter for the adaption time of the fermenting system of the cells to a change in pH had to be included to fit the real pH-time curve. An adaption time of 2 min was taken, based on the time response of the cells after addition of glucose. Because of this, in the final simulation, dM_a/dt at time t (or at the pH at time t) was taken as dM_a/dt at 't minus 2 min'.

The obtained simulated pH-time curve is shown in Fig. 2.7 along with the experimentally recorded curve. From pH 6.8 to pH 5, good agreement between the theory and experiment was observed. At lower pH the calculated values were higher than those obtained experimentally. This deviation might be due to a change in adaption time with faster pH changes and/or lower pH.

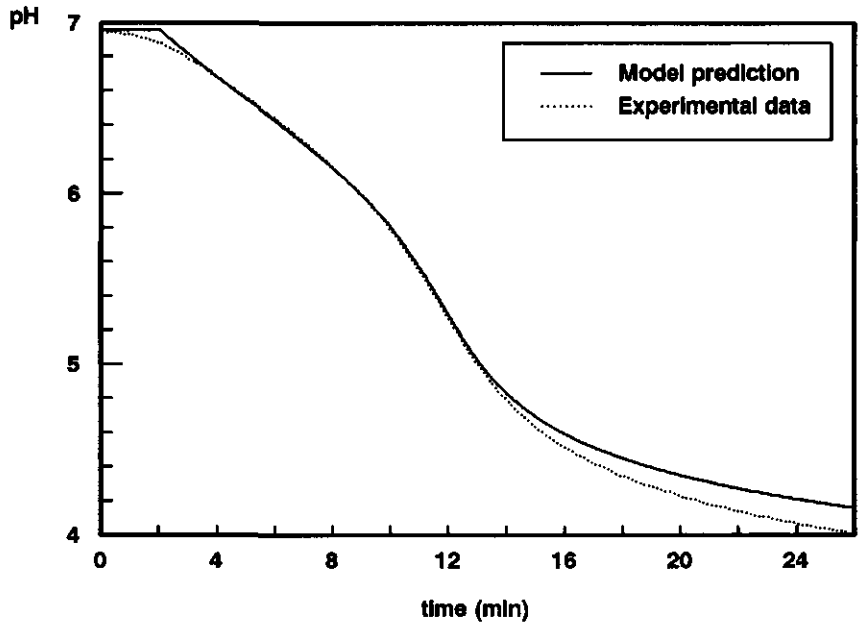

Fig. 2.7 Simulated and real pH-time curve during the time course of the activity test.

2.5 CONCLUSIONS

The method described is very adequate to measure the glucose-fermenting activities of *L. plantarum*. In principle, the method is also applicable to other lactic acid bacteria, although the described titration theory is only valid with (mainly) homolactic fermentation.

The reproducibility of the method is good; relative standard deviation never exceeded 4 %. The method is quick; with 90 mg dw young *L. plantarum* cells in 30 ml measurement suspension the test takes 30 min at maximum. The experimental set-up enables the determination of the activities of several suspensions at the same time. With the help of a data acquisition unit and a microcomputer automated sampling of in principle an infinite number of activity tests is possible.

With *L. plantarum* the correlation between the measured maximum velocity of pH-decrease and cell concentration can be considered as linear up to a concentration of 4 g $dw.l^{-1}$ measurement suspension. Within this concentration range the estimated $\Delta pH.min^{-1}$ per gram dry weight could be reliablely recalculated to mol lactic $acid.min^{-1}$ per gram dry weight. At cell concentrations higher than 4 g $dw.l^{-1}$, a significant deviation of linearity was observed and reliable recalculation with the described titration theory was no longer possible.

ACKNOWLEDGEMENTS

We would like to thank J.W.R. Tilanus for his contributions to this research and R.S. Hamstra, G. Meerdink, Prof.Dr. F.M. Rombouts and Dr. M.R. Smith for their helpful critical comments in preparing this manuscript. The financial support of Gist-brocades NV, Delft, The Netherlands is gratefully acknowledged.

REFERENCES

Alberty RA, Smith RM and Bock RM (1951) The apparent ionization constants of the adenosinephosphates and related compounds. J. Biol. Chem. 193: 425-434.

Anderson EB and Meanwell LJ (1942) The problem of bacteriophages in cheese making, Part I. Observations and investigations on slow acid production. J. Dairy Res. 13: 58-72.

Cheney W and Kincaid D (1985) Numerical Mathematics and Computing. 2nd ed., pp 362-387, Brooks/Cole Publishing Co., Monterey.

De Man JC, Rogosa M and Sharpe ME (1960) A medium for the cultivation of lactobacilli. J. Appl. Bacteriol. 23: 130-135.

Elliker PR and Frazier WC (1938) Factors affecting the activity and heat resistance of Swiss cheese starter cultures. I. Influence of time and temperature of incubation. J. Dairy Sci. 21: 801-813.

Hickey FC (1940) The ionization of lactic acid in aqueous sodium chloride solutions from 0 to 37.5°C. J. Am. Chem. Soc. 62: 2916-2919.

Horrall BE and Elliker PR (1950) An activity test for cheddar and cottage cheese starters. J. Dairy Sci. 33: 245-249.

Junker M and Liepe HU (1979) Determining the activity of starter cultures (pure cultures of lactobacilli). Fleischwirtschaft 59: 1880-1881.

Junker M and Liepe HU (1981) Influence magnitudes in determing the activity of lactobacilli starter cultures. Fleischwirtschaft 61: 1980-1981.

Lievense LC, Van 't Riet K and Noomen A (1990) *Modelling and measurement of the inactivation behaviour of Lactobacillus plantarum directed at a drying process*. In: Spiess WEL and Schubert H (eds) Engineering and Food, Vol.3, Advanced Processes (pp. 555-558). Elseviers Applied Science, London.

Murphey MG and Condon S (1984) Comparison of aerobic and anaerobic growth of *Lactobacillus plantarum* in a glucose medium. Arch. Microbiol. 138: 49-53.

Pearce LE (1969) Activity test for cheese starter cultures. N.Z.J. Dairy Technol. 4: 246-247.

Schleifer KH (1987) Recent changes in taxonomy of lactic acid bacteria. FEMS Microbiol. Rev. 46: 201-203.

Stadhouders J (1980a) Description of a method for determining the activity of cheese starters. Int. Dairy Fed. Annu. Bull. 129: 9-10.

Stadhouders J (1980b) Factors affecting the results of an activity test of mesophilic cheese starters. Int. Dairy Fed. Annu. Bull. 129: 5-8.

Weast RC and Astle MJ (eds.) (1979) Handbook of Chemistry and Physics. 59th edn. (pp. D203-D204). CRC-press, Boca Raton.

Whitehead HR and Cox GA (1932) A method for determination of vitality in starters. N.Z.J. Sci. Technol. 13: 304-309.

Willis CJ (1981) Another approach to titration curves. J. Chem. Educ. 58: 659-663.

CHAPTER 3

MEASUREMENT AND MODELLING OF THE DRYING PROCESS

Lou C. Lievense, Monique A.M. Verbeek, Gerrit Meerdink and Klaas van 't Riet

Food- and Bioprocess Engineering Group, Department of Food Science, Wageningen Agricultural University

ABSTRACT

In order to describe the inactivation of *Lactobacillus plantarum* during a fluidized bed drying process, the drying kinetics of particles with *L. plantarum* cells immobilized in potato starch were studied. The experimental drying data are described using the short-cut drying theory. The mathematical model of the drying kinetics is straightforward and can be implemented easily in an overall model which includes the inactivation of *L. plantarum* during a drying process. Furthermore, the calculation of the temperature of the drying particles in the course of the drying process is discussed.

Lievense LC, Verbeek MAM, Meerdink G and Van 't Riet K (1990) Inactivation of *Lactobacillus plantarum* during drying. I. Measurement and modelling of the drying process. **Bioseparation 1:** 149-159.

CHAPTER 3

MEASUREMENT AND MODELLING OF THE DRYING PROCESS

3.1 INTRODUCTION

Drying is an essential step in a number of processes in biotechnological product recovery. Well-known examples of drying operations are the production of enzyme granulates for application in detergents, of immobilized enzymes for biotechnical applications, of starter cultures and of bakers' yeast. In general, industrial drying applications utilize convective drying methods as they are the most economical. Convective drying can be applied to biological materials providing that the biological activity is preserved.

To optimize an industrial scale drying process for biological materials, insight into the correlation of the parameters of the drying process with the inactivation rate of the biological material is essential. The inactivation of the biological material is usually described as a function of the moisture concentration and the temperature of the drying material. These conditions continuously change during the drying process but should be known.

To describe the moisture concentration and temperature of the drying material there are a wide variety of drying models (e.g., Kerkhof and Schroeber 1974; Wijlhuizen *et al.* 1979; Luyben *et al.* 1982). Although these models are accurate, they are difficult to use because of their complexity. When fundamental understanding of the drying kinetics is not a goal as such, introduction of simplified models can be far more attractive. In this work the short-cut calculation method (Liou 1982a; Liou and Bruin 1982bc; Coumans 1987) was used.

The concentration and temperature dependency of the water diffusion coefficient, needed in the short-cut calculations, can be determined according to Luyben *et al.* (1980) and Tong and Lund (1990). Another possibility is to calculate the diffusion coefficient from the derivative of the experimentally obtained drying curve (Coumans 1987). Both methods have a disadvantage. The first method often requires separate drying experiments. The second method requires drying curves which include an extensive number of experimental data. Smoothing of these data is needed because otherwise irregularities in the derivatives will occur. To cancel out these disadvantages another method will be presented.

Although freeze-dried cultures of lactic acid bacteria are on the market, it is much more economical to use a convective drying process. However, after the convective drying of lactic acid bacteria the survival rate is usually insufficient to allow economical application. The objective of our research is to develop a mathematical model which is capable of predicting the experimentally observed inactivation of *Lactobacillus plantarum* during drying. The model is (basically) composed of the short-cut drying theory and the heat balance of the drying

material. With this model the moisture concentration profiles inside the particles and the temperature of the particles as a function of time are calculated. In Chapter 4 the equations describing the thermal inactivation of *L. plantarum* as a function of moisture concentration and temperature are added to the drying model. The coupled drying and inactivation model should provide the changing rate of thermal inactivation of the *L. plantarum* cells during drying. With this inactivation rate we should be able to quantify the thermal inactivation of *L. plantarum* during the drying process.

The objectives of this chapter are: *firstly*, the provision of a drying model, based on the short-cut calculation method, which will be used in chapter 4 to predict the inactivation during fluidized bed drying of *L. plantarum*-starch granulate; *secondly*, the comparison of the model predictions with the experimentally measured drying data; *thirdly*, the proposal of a new procedure in which the drying model parameters are fitted directly to the experimentally obtained drying curves at different drying temperatures.

3.2 THEORY

We used the short-cut drying theory to describe the drying kinetics of our system. Liou (1982a) and Liou and Bruin (1982bc) developed this theory in order to solve the complex differential equations for drying. With this method the calculations were simplified drastically. Furthermore, the short-cut approximation has an equal accuracy as the numerical models. Later the short-cut drying theory was extended to hollow and non-ideal shrinking systems and was systematically explained by Coumans (1987). In this section and in Appendix 1 the short-cut drying theory (specified to our experimental set-up and model assumptions) will be briefly considered.

The short-cut drying theory is based on the macroscopic form of the diffusion equation where all possible contributions to internal moisture transfer are lumped into one effective diffusion coefficient D_{eff}. The main assumption made in the short-cut drying theory itself, is a power law relation between the effective diffusion coefficient (D_{eff}) and the dimensionless moisture concentration (m) according to:

$$D_{eff} = D_0 m^a \tag{3.1}$$

where D_0 and a are fit parameters which are calculated from the drying curve. In addition to this, D_0 is described as a function of the temperature with an Arrhenius-type equation according to:

$$D_0 = D_{0,ref} \exp\left[-\frac{E_{a,d}}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right] \tag{3.2}$$

An important dimensionless group in the short-cut drying theory is the drying efficiency E. Drying efficiency E represents the progress of the drying process with respect to the initial moisture concentration and the final moisture concentration at infinite drying time ($0 \leq E \leq 1$; see Appendix 1).

With the short-cut method the time t (time from the start of the drying process) and the surface moisture flux j can be calculated as a function of the drying efficiency E or when a root-finding routine is implemented in the simulation programme, E can also be calculated as a function of time. These calculations are straightforward, without the need of numerically solving the nonlinear partial differential equation describing the moisture diffusion process. The drying efficiency E is also used to calculate the moisture concentration profiles inside the drying particle according to approximate relations based on known analytical solutions (Liou and Bruin 1982c).

For the calculation of the drying time and flux the drying process is divided into two sequential stages, which have their own approximate short-cut equations:

I. *The Penetration Period (PP)* in which the moisture concentration in the centre of the cylinder remains almost equal to the initial moisture concentration.

II. *The Regular Regime (RR)* during which the centre concentration significantly decreases from its initial value.

The transition between the drying stages, and the flux and drying times in both stages can be defined in terms of drying efficiency E. Liou (1982a), Liou and Bruin (1982bc) and Coumans (1987) gave these generalized short-cut drying equations for a number of cases.

In the drying model presented here, we assume that the drying granulate can be described as a non-shrinking, infinite long, massive cylinder. The high length/diameter ratio of the granulate ($\geq$ 10) justifies this assumption. Furthermore, we assume as initial condition that the moisture concentration profile inside the cylinder is uniform and as boundary condition that the surface moisture concentration is equal to zero. In fact, the last assumption includes a neglect of the constant rate period which can be rationalized by the low initial moisture concentration and the used drying conditions. Moreover, this assumption simplifies the calculations drastically and did not significantly influence the calculated drying kinetics (Coumans 1987). Accordingly we may write:

$$m = m_0 \qquad \text{for } t=0 \text{ and } 0 \leq r < R_C \tag{3.3}$$

$$m = 0 \qquad \text{for } t \geq 0 \text{ and } r = R_C \tag{3.4}$$

With these assumptions we use one particular case of the short-cut drying theory: A non-shrinking system (infinite long massive cylinder) with constant surface concentration ($m=0$).

For the calculation of the temperature of the granulate we assume a uniform temperature profile inside the cylinder because on the time scale of mass diffusion, temperature gradients will vanish relatively fast. Additionally we assume that no heat is transferred by radiation. These assumptions are reasonable as explained by Van der Lijn (1976), Liou (1982a) and Botterill

(1975). Furthermore, the heat transfer coefficient α is corrected for the effect of mass flux on the rate of heat transfer. Consequently, the heat balance over the cylinder per unit length is expressed by:

$$\pi R_c^2 \rho_d C_{p,d} \frac{dT_d}{dt} = 2\pi R_c \alpha_{eff} (T_b - T_d) - 2\pi R_c j \Delta H_{vap} \quad (3.5)$$

An important variable in Eq. (3.5) is α_{eff}, the heat transfer coefficient corrected for mass flux. This corrected heat transfer coefficient follows from the uncorrected α. The calculation of both heat transfer coefficients follows from the Nusselt-number in a fluidized bed and is described in Appendix 1.

Because of the operation conditions of the dryer it is questionable to speak of a fluidized bed drying process. In view of the large particle dimensions and high air velocities the process may be considered as a spouted bed (Bridgewater 1985). However, the low loading of the dryer prevents the occurrence of packed regions, usually present in a spouted bed. For the calculation of the Nusselt-number we considered the drying process as a fluidized bed with a bed porosity (ϵ) equal to 1 and an air stream through the bed with constant temperature (inlet temperature) and moisture concentration (equal to zero).

3.3 MATERIALS AND METHODS

3.3.1 Microorganism and growth conditions

Lactobacillus plantarum (P743; Netherlands Institute for Dairy Research (NIZO), Ede, NL) was grown and harvested as described in Chapter 2. MRS-medium (Difco, Detroit, Michigan) supplemented with 10 g.l^{-1} glucose (Merck, Darmstadt, FRG) and 1 g.l^{-1} yeast extract (Oxoid, Basingstoke, Hampshire, UK) was used as growth medium. After the first centrifugation step (10 min, 10.000 rpm, Sorvall SB-50, GSA-rotor) and washing the cells with 0.01 mol.l^{-1} potassium phosphate buffer (pH=7.0) in 0.15 mol.l^{-1} NaCl in demineralized water, the cells were centrifuged again for 10 min at 10.000 rpm. The supernatant from this centrifugation step was carefully removed.

3.3.2 Granulation

A granulate was formed for application in a fluidized bed. After the last centrifugation step the cell pellet (3.24 (±0.13) kg H_2O.(kg solids)$^{-1}$) was mixed manually with potato starch (0.064 (±0.004) kg H_2O.(kg solids)$^{-1}$) to a homogeneous *L. plantarum*-starch paste. The mixing ratio of cell pellet:potato starch was 1:0.65 (w/w). This paste was extruded using a small press (own design) with a sieve-plate having die-openings of 1 mm diameter. The strings were cut just behind the sieve-plate at a length varying from 10 to 15 mm. If necessary, the formed granulate was stored in a parafilm-sealed petri-dish at 4 °C. During handling of the paste and granulate, the moisture partially evaporated resulting in particles with an average initial moisture concentration of 0.837 (±0.040) kg H_2O.(kg solids)$^{-1}$.

3.3.3 Fluidized bed drying

The granulate was dried in a laboratory fluidized bed dryer (own design). The temperature of the in-coming air was measured with a PT-100 sensor coupled to a PID-controller (Shimaden SR24, Tokyo, Japan).

The inlet temperature was controlled by splitting a dried air stream at different ratios in a three-way valve with positioner (Research Control Valve, Badger Meter, Tulsa, Oklahoma). The positioner was set by the PID-controller. One part of the split air stream was heated. After the heater the split air streams were mixed. The diameter of the sieve-plate and the cylindrical part in the dryer was 50 mm. On the top of the cylindrical part a funnel-shaped part prevented the blow out of the granulate.

The superficial air velocity in the cylindrical part of the dryer was 5.5 $m.s^{-1}$. The loading of the fluidized bed dryer was small compared to the amount of air (max. 15 g granulate per batch) and therefore the bed porosity was considered as 1. As drying air dehumidified, compressed air was used, of which the moisture concentration was considered as zero.

3.3.4 Moisture concentration determination

The moisture concentration of the different samples was determined by weighing (Sartorius 2001 MP2, Göttingen, FRG) before and after drying in an atmospheric oven at 110 °C for 20-24h.

3.3.5 Fitting the non-isothermal drying data

The parameters which describe the diffusion process were estimated by fitting in one fit session the drying model (Eqs. (3.2) and (3.11-3.19)) directly to the whole data set, i.e. the measured drying curves at different air temperatures. A non-linear least squares fit procedure (Procedure NLIN; Secant method; SAS under VAX/VMS) was used. The experimental E was calculated from the measured X_0 and the measured $\overline{X}$ at different drying times and drying temperatures. Subsequently, the measured data were described with drying time t as the independent variable and with the experimental E as the dependent variable. Therefore, in the Penetration Period it was necessary to calculate E as function of t with a root approximation method (Newton-Raphson).

Also a description with the experimental E as the independent and t as the dependent variable was used. In that case, the root approximation could be avoided but a weighted least-squares estimation (Procedure NLIN; SAS under VAX/VMS) had to be used, because otherwise the high values for the Residual Sum of Squares in the points at the last part of the drying curve (at long times) will adverse the quality of fit in the first part of the curve. The dimensionless flux F was calculated from the experimental E and was used as the weight-factor.

3.3.6 Wet-bulb temperature

A simulation problem arises at the calculation of the drying flux at short time values and thus small efficiencies (see Appendix 1; Eq. (3.12)). In that case infinite fluxes will be calculated which give meaningless temperature drops. This is a consequence of the assumption that at $t \geq 0$ the surface moisture concentration is equal to zero (Eq. (3.4)). To avoid these temperature

drops, in the simulation programme the lower temperature limit was set by the wet-bulb temperature at the used inlet air temperature. The wet-bulb temperature was calculated iteratively (Bagnoli *et al.* 1973).

3.4 RESULTS AND DISCUSSION

The fitting procedure resulted in a description of the diffusion process at different overall moisture concentrations and temperatures with three parameters: $D_{0,ref}$, $E_{a,d}$ and an average a-value for all temperature levels. The estimates of these three parameters obtained with the root approximation, and all the other parameters used in the simulation are summarized in Appendix 2.

Figure 3.1 shows that the drying curves can be described adequately with the estimated parameters. At most drying temperatures, in the end the drying rate is slightly overestimated. This might indicate that power law diffusion does not apply strictly to the system of *L. plantarum* cells immobilized in potato starch. In the simulations the time t and the dimensionless drying flux F were calculated as a function of E, according to Eqs. (3.2) and (3.11-3.19). The air inlet temperature T_b and the measured X_0 served as input parameters for the simulation programme. Curves with no significant difference from those in Fig. 3.1 were obtained when $D_{0,ref}$, $E_{a,d}$ and a were estimated with the weighted least-squares fit procedure.

The effective diffusion coefficient D_{eff}, calculated with the estimated parameters, is shown in Fig. 3.2 as a function of moisture concentration and temperature. Both D_{eff} and $E_{a,d}$ are in agreement with literature values for food-like materials (Wijlhuizen *et al.* 1979; Luyben *et al.* 1982; Liou 1982a; Liou and Bruin 1985; Furuta *et al.* 1984; Coumans 1987). If a better fit quality is required $E_{a,d}$ can be described as a function of the moisture concentration as proposed by Luyben (1982) and Liou (1982a, 1985). The quality of fit shown in Fig. 3.1 is satisfying for the inactivation modelling as will be shown in Chapter 4.

The estimated a-value, the exponent in the power relation, indicates a nearly rectilinear dependency of D_{eff} with the moisture concentration of the granulate. Fitting the drying curves at different drying temperatures with one single a-value is required because of the fact that the short-cut drying theory is derived on the basis of this assumption.

The temperature progress curves for each drying experiment are shown in Fig. 3.3. These temperatures were obtained by solving Eq. (3.21) by a fourth-order Runge-Kutta routine (Press 1986). In the heat balance, the mass concentration ρ_S and the heat capacity $C_{p,S}$ (of the solids; Eqs. (3.32-3.34)) are approximated by literature values for native potato starch (see Appendix 2). With this procedure, the simulation procedure calculated at lower drying temperatures a decrease in temperature of the granulate in the beginning of the drying process (see insert Fig. 3.3). At higher drying temperatures the wet-bulb temperature lies above the initial temperature T_0 and an immediate increase of the granulate temperature was calculated.

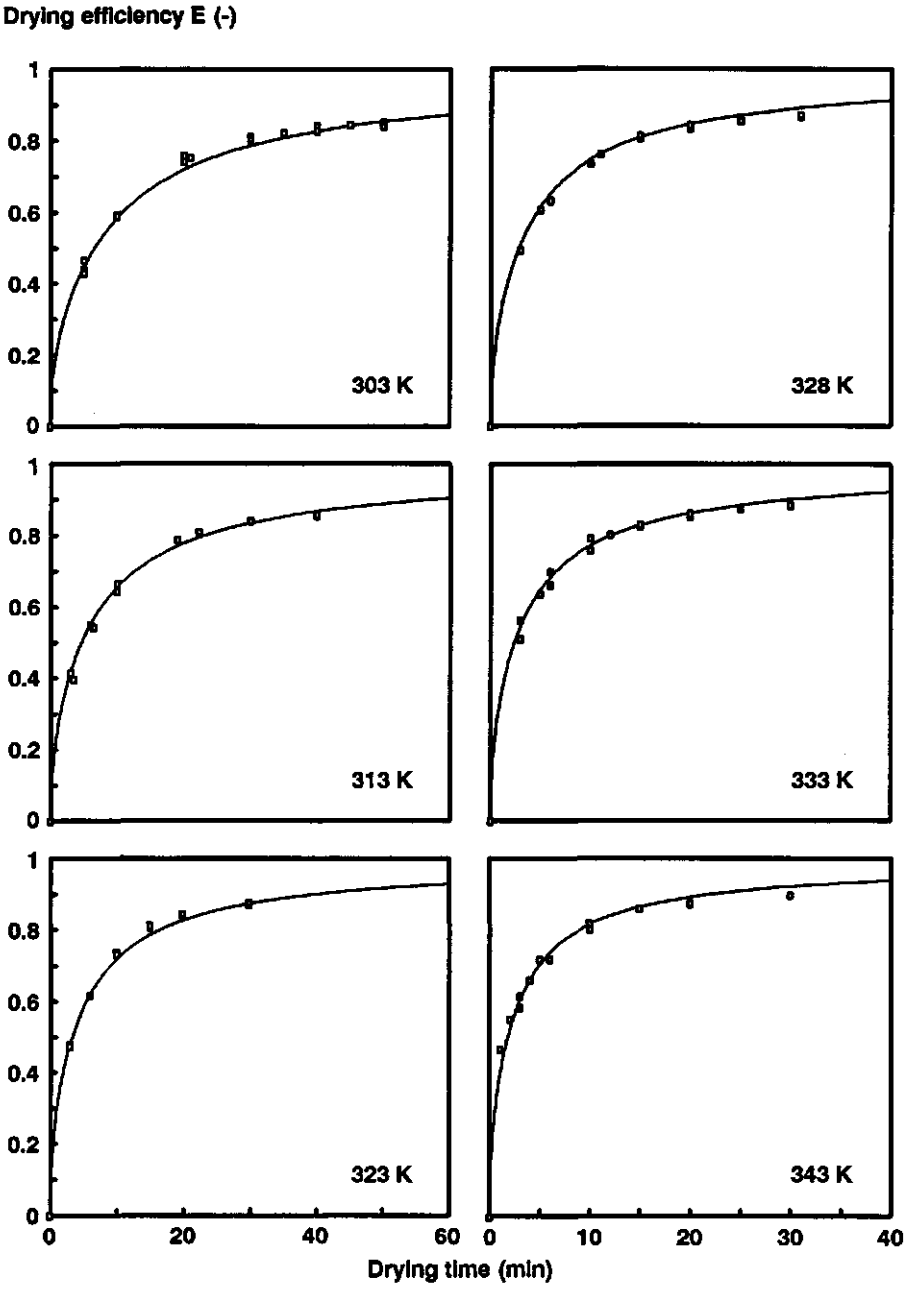

Fig. 3.1 The experimental (data points) and fitted (lines) drying curves of the *L. plantarum*-starch granulate at different air inlet temperatures of the fluidized bed.

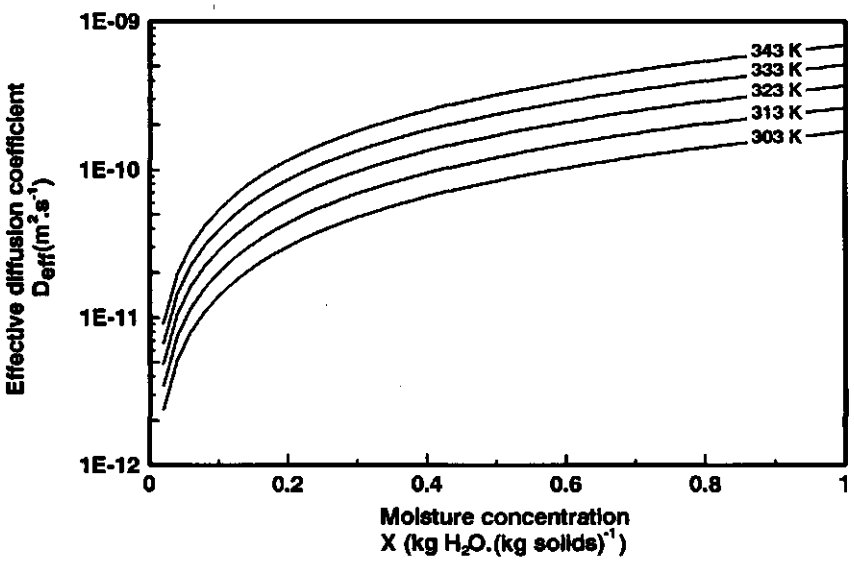

Fig. 3.2 The calculated effective diffusion coefficients (Eqs. (3.1-3.2)), resulting from the parameter estimation of a, $D_{0,ref}$ and $E_{a,d}$, given as a function of moisture concentration and temperature.

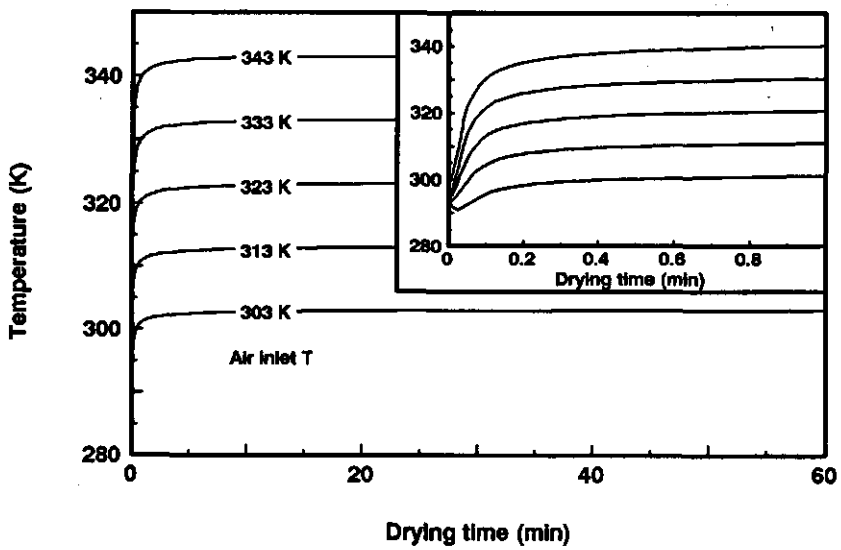

Fig. 3.3 The calculated temperature progress curves for drying at different air inlet temperatures. The insert shows the initial parts of the curves.

We could not verify the temperature progress curves experimentally. Due to the high bed porosity, neither measurement of the outlet air temperature nor the bed temperature were suitable. Furthermore, the particle dimensions were too small to incorporate a temperature sensor in the drying granulate. However, in other experimental systems, similar procedures for the calculation of the temperature of the drying material have been applied (Liou 1982a; Liou and Bruin 1985; Yamamoto *et al.* 1985). In these studies a good agreement between predicted and measured temperatures was found.

The calculated moisture concentration profiles are shown in Fig. 3.4. As can be seen from Fig. 3.4 at the start of the drying process at low drying efficiencies, the moisture concentration profiles are rapidly penetrating into the drying particle. This drying stage is called the Penetration Period. When the drying efficiency reaches the transition point (in our system at $E=0.45$) the centre concentration begins to change significantly from its initial value. From this point on, the drying process is in the Regular Regime.

In the last stage of the drying process, at overall moisture concentrations around 0.13 $kgH_2O.(kg\ solids)^{-1}$ or drying efficiencies around 0.85, we observed longitudinal cracking of the granulate. Because this cracking occurs in the last stage of the drying process (see Fig. 3.1) it cannot significantly influence the parameter estimations. Moreover, the longitudinal cracking of the granulate, which must result in an increase of the drying flux, cannot be retraced in the experimental drying curves.

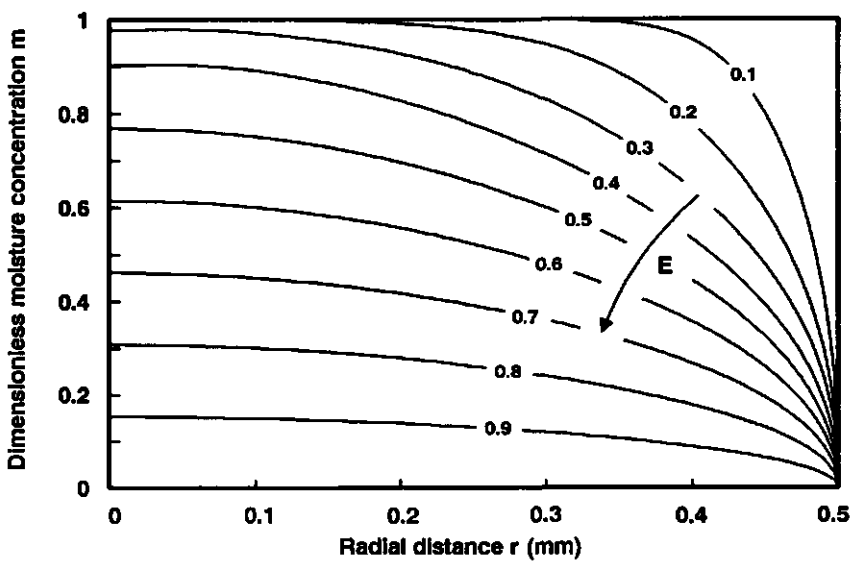

Fig. 3.4 The calculated moisture concentration profiles inside the drying cylinder as a function of the space coordinate inside the particle at different drying efficiencies and at an air inlet temperature of 30 °C.

3.5 CONCLUSIONS

The non-isothermal drying of the *L. plantarum*-starch granulate can be successfully described with the short-cut calculation method in which only three parameters account for the moisture concentration and temperature dependency of the diffusion coefficient. With the help of sophisticated fitting techniques we were able to estimate the values of these three parameters directly from the measured data.

The obtained mathematical description of the drying kinetics provides the moisture concentration and the temperature progress curves of the drying granulate. These are the two variables needed for an overall model in which the inactivation of *L. plantarum* during drying is described.

ACKNOWLEDGEMENTS

We would like to thank T. Taekema and S.R. Weijers for their contributions to this research and Dr. H.H. Beeftink, Dr. M.R. Smith and Prof.Dr. J. Tramper for their helpful critical comments in the preparation of this manuscript. The financial support of Gist-brocades NV, Delft, The Netherlands is gratefully acknowledged.

REFERENCES

Abromowitz M and Stegun I (1970) Handbook of mathematical functions. Dover Publications, New York.

Andrieu J, Gonnet E and Laurent M (1989) Thermal conductivity and diffusivity of extruded durum wheat pasta. Lebensm.-Wiss.u.-Technol. 22: 6-10.

Bagnoli E, Fuller FH, Johnson VJ and Norris RW (1973) *Psychrometry, evaporative cooling, air conditioning and refrigeration.* In: Perry RH and Chilton CH (eds) Chemical Engineers' Handbook, 5th edition (pp. 12.2-12.3). McGraw-Hill Company, New York.

Bird RB, Stewart WE and Lightfoot EN (1960) Transport phenomena (pp. 672-673). John Wiley & Sons, New York.

Botterill JSM (1975) Fluid-bed heat transfer. Academic Press, London.

Boucher DF and Alves GE (1973) *Fluid and particle dynamics.* In: Perry RH and Chilton CH (eds) Chemical Engineers' Handbook, 5th edition (pp. 5.52-5.54). McGraw-Hill Company, New York.

Bridgewater J (1985) *Spouted beds.* In: Davidson JF, Clift R and Harrison D (eds) Fluidization (pp. 201-222). Academic Press, London.

Coumans WJ (1987) Power law diffusion in drying processes. Ph.D. Thesis, Technical University Eindhoven, The Netherlands.

Furuta T, Tsujimoto S, Makino H, Okazaki M and Toei R (1984) Measurement of diffusion coefficient of water and ethanol in aqueous maltodextrin solution. J. Food Eng. 3: 169-186.

Haines V, Bizot H and Buleon A (1985) Reinvestigation of potato starch volume during the sorption process. Carbohydrate Polymers 5: 91-106.

Kerkhof PJAM and Schroeber WJAH (1974) *Theoretical modelling of the drying behaviour of droplets in spray dryers.* In: Spicer A (ed) Advances in preconcentration and dehydration of food (pp. 349-397). Applied Science Publishers, London.

Krischer O (1963) Die wissenschaftlichen Grundlachen der Trocknungstechnik. Springer-Verlag, Berlin.

Liley PE and Gambill WR (1973) *Physical and chemical data*. In: Perry RH and Chilton CH (eds) Chemical Engineers' Handbook, 5th edition (p. 3.216). McGraw-Hill Company, New York.

Liou JK (1982a) An approximate method for nonlinear diffusion applied to enzyme inactivation during drying. Ph.D. Thesis, Agricultural University Wageningen, The Netherlands.

Liou JK and Bruin S (1982b) An approximate method for the nonlinear diffusion problem with a power relation between diffusion coefficient and concentration-I. Computation of desorption times. Int. J. Heat Mass Transfer 25: 1209-1220.

Liou JK and Bruin S (1982c) An approximate method for the nonlinear diffusion problem with a power relation between diffusion coefficient and concentration-II. Computation of concentration profiles. Int. J. Heat Mass Transfer 25: 1221-1229.

Liou JK, Luyben KChAm and Bruin S (1985) A simplified calculation method applied to enzyme inactivation during drying. Biotechn. Bioeng. 27: 109-116.

Luyben KChAM, Olieman J and Bruin S (1980) *Concentration dependent diffusion coefficients derived from experimental drying curves*. In: Mujumbar AS (ed) Drying '80 (Vol.2; pp. 233-243). Hemisphere, New York.

Luyben KChAM, Liou JK and Bruin S (1982) Enzyme degradation during drying. Biotechn. Bioeng. 24: 533-552.

Martin H (1980) Wärme- und stoffübertragung in der wirbelschicht. Chem.-Ing.-Tech 52: 199-209.

Mason AE and Monchick L (1965) *Survey of the equation of state and transport properties* In: A Wexler (ed) Humidity and moisture, Vol.3, Fundamentals and standards (pp. 257-272). Reinhold Publishers, New York.

Press WH, Flannery BP, Teukolsky SA and Vetterling WT (1986) Numerical recipes. Cambridge University Press, Cambridge, USA.

Spanier J and Oldham KB (1987) An atlas of functions. Hemisphere Publishing Corporation, Washington.

Tong CH and Lund DB (1990) Effective moisture diffusivity in porous materials as a function of temperature and moisture content. Biotechnol. Prog. 6: 67-75.

Van der Lijn J (1976) Simulation of heat and mass transfer in spray drying. Ph.D. Thesis, Agricultural University Wageningen, The Netherlands.

Wasburn EW (1926) Int. Crit. Tables. National Research Council. McGraw-Hill, New York.

Wijlhuizen AE, Kerkhof PJAM and Bruin S (1979) Theoritical study of the inactivation of phosphatase during spray drying of skim-milk. Chem. Eng. Sci. 34: 651-660.

Weast RC and Astle MJ (1979) Handbook of chemistry and physics, 59th edition. CRC-Press, Boca Raton.

Yamamoto S, Agawa M, Nakano H and Sano Y (1985) *Enzyme inactivation during drying of a single droplet*. In: Toei R and Mujumdar AS (eds) Drying '85 (pp.330-337). Hemisphere Publishing Corporation, Washington.

Zimmermann K (1987) Einflussparameter und mathematische modellierung der schonenden trocknung von starterkulturen. Fortschr.-Ber. VDI (Reihe 14, no 36), VDI-Verlag, Düsseldorf, West Germany.

APPENDIX 1

In this Appendix the equations used to describe the drying kinetics of our system are given. General equations (which can be found in literature) and specific equations are used. The specific short-cut equations are based on the model assumptions (see Theory). These equations are derived from general equations and are marked with * after the equation number.

Dimensionless Groups

A few basic dimensionless groups which are used in the short-cut drying theory have to be introduced (Coumans 1987):

Ia. Local dimensionless moisture concentration m:

$$m = \frac{X - X_e}{X_0 - X_e} \tag{3.6}$$

Ib. Average dimensionless moisture concentration $\overline{m}$:

$$\overline{m} = \frac{\overline{X} - X_e}{X_0 - X_e} \tag{3.7}$$

II. Drying efficiency E:

$$E = \frac{X_0 - \overline{X}}{X_0 - X_e} = 1 - \overline{m} \tag{3.8}$$

III. Flux parameter F:

$$F = \frac{j R_c}{D_0 (X_0 - X_e) \rho_s} \tag{3.9}^*$$

IV. Dimensionless time τ:

$$\tau = \frac{D_0 t}{R_c^2} \tag{3.10}^*$$

Drying Time and Flux

The transition between the *Penetration Period (PP)* and *Regular Regime (RR)* can be defined in terms of drying efficiency (Coumans 1987):

$$E_T = \frac{1.4}{a+2} \qquad (3.11)^*$$

The flux and drying times can be calculated as a function of the drying efficiency E:

I. Penetration Period ($E<E_T$):

$$F = 2G_0\left(\frac{1}{E} - \beta\right) \qquad (3.12)^*$$

$$\tau_{PP} = \frac{-\beta E - \ln(1-\beta E)}{4G_0\beta^2} \qquad (3.13)^*$$

where

$$G_0 = \frac{2}{\pi}\left[\frac{1.42}{a+1.42}\right]^{1.98} \qquad \text{for } a \geq 0 \qquad (3.14)$$

and

$$\beta = 0.71(1.25)^a \qquad \text{for } 0 \leq a < 2 \qquad (3.15)^*$$

II. Regular Regime ($E \geq E_T$):

$$F = \frac{1}{2}\frac{Sh_d}{a+1}(1-E)^{a+1} \qquad (3.16)$$

$$\tau_{RR} = \frac{1}{Sh_d}\frac{a+1}{a}\left[\frac{1}{(1-E)^a} - \frac{1}{(1-E_T)^a}\right] \qquad (3.17)$$

where

$$Sh_d = 10.87 - 5.09\left(\frac{2}{a+2}\right) \qquad (3.18)^*$$

and where the total drying time in the Regular Regime is defined as:

$$\tau_{tot} = \tau_{PP}(E_T) + \tau_{RR} \tag{3.19}$$

Additionally, in both regimes the dimensionless drying flux can also be written as:

$$F = \frac{R_c^2}{2D_0}\frac{dE}{dt} \tag{3.20}^*$$

Because the granulate temperature T_d increases immediately to the air temperature T_b, the non-isothermal drying conditions can be ignored in the calculation of the drying kinetics. Therefore, Eq. (3.10) can be used for the calculation of the dimensional drying time t and numerical integration of Eq. (3.20) (Liou 1982a; Liou *et al.* 1985; Coumans 1987) can be avoided.

Temperature of the Granulate

When the drying efficiency E is used as a time parameter, the heat balance can be written in terms of E, by substitution of Eq. (3.9) and Eq. (3.20) into Eq. (3.5), which yields:

$$\rho_d C_{p,d}\frac{dT_d}{dE} = \frac{R_c}{D_0 F}\left\{\alpha_{eff}(T_b - T_d) - \frac{D_0(X_0 - X_e)\rho_s}{R_c} F \Delta H_{vap}\right\} \tag{3.21}$$

α_{eff} is the corrected heat transfer coefficient, according to (Bird *et al.* 1960; Van der Lijn 1976):

$$\alpha_{eff} = \alpha\frac{\gamma}{e^{\gamma} - 1} \tag{3.22}$$

with

$$\gamma = \frac{jC_{p,m,v}}{\alpha} \tag{3.23}$$

The uncorrected heat transfer coefficient α follows from the Nusselt-number in a fluidized bed. For the calculation of the Nusselt-number the length of the cylinder has to be taken into account because the Nusselt-number is defined as (Zimmermann 1987):

$$Nu = \frac{\alpha \bar{d}_p \phi_s}{\lambda_{air}} \tag{3.24}$$

where $\bar{d}_p$ is the average particle diameter, defined as the diameter of a sphere of the same volume as the particle (Boucher and Alves 1973). For a cylinder:

$$\bar{d}_p = \left[\frac{3}{2} d_c^2 L_c\right]^{\frac{1}{3}} \tag{3.25}$$

The sphericity factor ϕ_S is defined as the surface area of a sphere having the same volume as the particle divided by the surface area of the particle (Boucher and Alves 1973). For a cylinder:

$$\phi_s = \frac{1.31\, d_c^{\frac{1}{3}} L_c^{\frac{2}{3}}}{L_c + 0.5 d_c} \tag{3.26}$$

The Nusselt-number in a fluidized bed is calculated from the Nusselt-number for a single particle at the actual air velocity and porosity in the bed, according to (Martin 1980; Zimmermann 1987):

$$Nu_{FB} = Nu_p(1 + 1.5(1-\epsilon)) \tag{3.27}$$

$$Nu_p = 2 + \sqrt{Nu_{lam}^2 + Nu_{turb}^2} \tag{3.28}$$

$$Nu_{lam} = 0.664\, Pr^{\frac{1}{3}} Re^{\frac{1}{2}} \tag{3.29}$$

$$Nu_{turb} = \frac{0.037\, Re_\epsilon^{0.8} Pr}{1 + 2.44\, Re_\epsilon^{-0.1}\left(Pr^{\frac{2}{3}} - 1\right)} \tag{3.30}$$

$$Re_\epsilon = \frac{Re}{\epsilon} = \frac{u_{air} \bar{d}_p \phi_s}{\nu_{air}} \cdot \frac{1}{\epsilon} \tag{3.31}$$

The Prandtl-number of the drying air was considered as constant in the used drying temperature range (see Appendix 2). The heat capacity of the granulate is described with:

$$\rho_d C_{p,d} = \frac{C_{p,m} m_m + C_{p,s} m_s}{\pi R_c^2} \tag{3.32}$$

where m_m and m_s were described as:

$$m_m = X m_s \tag{3.33}$$

$$m_s = \rho_s V_c \tag{3.34}$$

Moisture Concentration Profiles

Because the equations used in these calculations are the same as used by Liou and Bruin (1982c) the reader is referred to this publication. To calculate the moisture concentration profiles we used polynomial and rational solution approximations for the functions $J_0(x)$, $erfc(x)$ and $K_{1/4}(x)$ used in this description (Press *et al.* 1986; Spanier and Oldham 1987; Abramowitz and Stegun 1970; respectively).

APPENDIX 2

The following variables and constants were used in the simulation programme:

I. Fitted parameter values (with T_{ref}=303.15 K):

a=1.1066	-	
$D_{0,ref}=1.5000\times10^{-10}$	$m^2.s^{-1}$	
$E_{a,d}=2.8308\times10^{4}$	$J.mol^{-1}$	

II. Physical properties of water (moisture) and air:

$C_{p,m}$=4180	$J.kg^{-1}.K^{-1}$	(Weast and Astle 1979)
$C_{p,m,v}$=2000	$J.kg^{-1}.K^{-1}$	(Washburn 1926)
Pr (air) =0.70	-	(Liley and Gambill 1973)
ΔH_{vap} (water) $=3.11\times10^{6}-2.25\times10^{3}T$	$J.kg^{-1}$	(Van der Lijn 1976)
$\lambda_{air}=4.5\times10^{-3}+7.26\times10^{-5}T$	$J.s^{-1}.m^{-1}.K^{-1}$	(Mason and Monchick 1965)
$\nu_{air}=\eta_{air}/\rho_{air}$	$m^2.s^{-1}$	
$\eta_{air}=17.19\times10^{-6}(T/273.15)^{0.76}$	$kg.m^{-1}.s^{-1}$	(Krischner 1963)
$\rho_{air}=1.2929(273.15/T)$	$kg.m^{-3}$	(Weast and Astle 1979)

III. Other constants and variables:

$C_{p,s}=5.737T-239.1$	$J.kg^{-1}.K^{-1}$	(Andrieu *et al.* 1989)
$d_C=1\times10^{-3}$	m	
$L_C=10\times10^{-3}$	m	
$R_C=0.5\times10^{-3}$	m	
T_0=293.15	K	

u_{air}=5.5	$m.s^{-1}$	
X_0=0.837 (average)	kg H_2O.(kg solids)$^{-1}$	
X_e=0	kg H_2O.(kg solids)$^{-1}$	
ϵ=1	-	
ρ (starch) =1470	$kg.m^{-3}$	(Haine *et al.* 1985)

NOMENCLATURE

a	exponent in power law	-
C_p	heat capacity at constant pressure	$J.kg^{-1}.K^{-1}$
D	diffusion coefficient	$m^2.s^{-1}$
d_c	diameter of cylinder	m
$\overline{d}_p$	average particle diameter	m
E	drying efficiency	-
$E_{a,d}$	activation energy of diffusion	$J.mol^{-1}$
$erfc(x)$	complement of error function	
F	dimensionless flux	-
G_0	help function for penetration process (Eq. (3.14))	
j	drying flux	$kg.m^{-2}s^{-1}$
$J_0(x)$	Bessel function of order 0	
$K_{1/4}(x)$	modified Bessel function of order ¼	
L_c	length of cylinder	m
m	dimensionless moisture concentration	-
m_m	mass of moisture in a particle per unit length	$kg.m^{-1}$
m_s	mass of solids in a particle per unit length	$kg.m^{-1}$
Nu	Nusselt-number	-
Pr	Prandtl-number	-
R	gas law constant	$J.mol^{-1}.K^{-1}$
r	radial distance	m
R_c	radius of cylinder	m
Re	Reynolds-number	-
Sh	Sherwood-number	-
t	time	s
T	absolute temperature	K
u_{air}	superficial velocity of drying air	$m.s^{-1}$
V_c	volume of cylinder per unit length	$m^3.m^{-1}$
X	moisture concentration	kg.(kg solids)$^{-1}$

Greek symbols

α	uncorrected heat transfer coefficient	$J.s^{-1}.m^{-2}.K^{-1}$
α_{eff}	effective heat transfer coefficient	$J.s^{-1}.m^{-2}.K^{-1}$
β	correlation parameter (Eq. (3.15))	
ϵ	porosity of bed	-
ΔH_{vap}	enthalpy change at vaporation of water	$J.kg^{-1}$
γ	help function (Eq. (3.23))	
λ_{air}	heat conductivity of air	$J.s^{-1}.m^{-1}.K^{-1}$
ν_{air}	kinematic viscosity of air	$m^2.s^{-1}$
ρ	density	$kg.m^{-3}$
ρ_S	mass concentration of solids	$kg.m^{-3}$
ϕ_S	sphericity factor	-
τ	dimensionless time	-

Subscripts

0	value at $t=0$ or at $m=1$
b	bulk of gas phase (drying air)
c	cylinder
d	dispersed phase (drying granulate)
e	equilibrium value at $t=\infty$
eff	effective
FB	Nu in fluidized bed
lam	laminair region
m	moisture
p	Nu of single particle
PP	value in Penetration Period
RR	value in Regular Regime
ref	value at reference temperature
s	solids
T	value at transition point (PP/RR)
tot	total
turb	turbulent region
v	vapour

Superscripts

$\bar{}$	average (overall) value

CHAPTER 4

MEASUREMENT AND MODELLING OF THE THERMAL INACTIVATION

Lou C. Lievense, Monique A.M. Verbeek, Gerrit Meerdink and Klaas van 't Riet

Food- and Bioprocess Engineering Group, Department of Food Science, Wageningen Agricultural University

ABSTRACT

In order to describe the inactivation of *Lactobacillus plantarum* during a fluidized bed drying process, the thermal inactivation kinetics of *L. plantarum* cells immobilized in potato starch were studied at different moisture concentrations. The measured temperature and moisture dependency of the inactivation rate was modelled with first-order kinetics. The activation energies for inactivation are in the range found by other authors. With the exception of the intermediate moisture concentration range, an increasing first-order inactivation constant with increasing moisture concentration was found. The thermal inactivation model was coupled to the drying kinetics described earlier. Although this concept was successfully applied in literature studies, the overall model significantly underestimates the measured inactivation during drying. It is concluded that the inactivation of *L. plantarum* during drying is caused by two mechanisms: thermal inactivation and inactivation due to dehydration. At moderate drying temperatures it is the dehydration that is the primary inactivation factor.

Lievense LC, Verbeek MAM, Meerdink G and Van 't Riet K (1990) Inactivation of *Lactobacillus plantarum* during drying. II. Measurement and modelling of the thermal inactivation. **Bioseparation 1:** 161-170.

CHAPTER 4

MEASUREMENT AND MODELLING OF THE THERMAL INACTIVATION

4.1 INTRODUCTION

In the past a number of workers have given attention to the mathematical modelling of inactivation of enzymes and/or microorganisms during drying. Kerkhof and Schroeber (1974) and Wijlhuizen *et al.* (1979) studied the thermal degradation of alkaline phosphatase during spray drying. Luyben *et al.* (1982) has published work on the thermal inactivation of alkaline phosphatase, catalase and lipase during drying. All of these studies were theoretical. The lack of experimental data was the main restriction to use the models for the simulation of real systems. Furthermore, in all these investigations the nonlinear partial differential equations for drying were solved by rigorous numerical modelling. Liou (1982) and Liou and Bruin (1985) were the first one to present a model which was experimentally verified. He studied the thermal inactivation of lipoxygenase immobilized in a calcium-glucose-alginate gel. The enzyme inactivation kinetics were coupled to approximate drying calculations, the so-called short-cut drying theory. Yamamoto *et al.* (1985) modelled the experimental data of the inactivation of glucose oxidase. Also in this work, the partial differential drying equations were solved numerically.

The first work on the modelling of the inactivation of microorganisms during drying came from Zimmermann (1987a) and Zimmermann and Bauer (1987b) who described the fluidized bed drying of *Saccharomyces cerevisiae*. The inactivation of intra-cellular enzymes and the yeast metabolic activity was measured and modelled. An attempt was made to model the inactivation of *Lactobacillus casei* during spraying of the culture on lactose particles in a fluidized bed dryer, without success (Zimmermann 1987a).

Essential in all these studies is that the inactivation of enzymes and/or microorganisms during drying is described with a thermal inactivation process. The rate of the thermal inactivation, in all these studies described with first-order kinetics, is given as a function of temperature and the moisture concentration of the drying biological material. This function is determined in a stationary process i.e., at constant temperature and moisture concentration. Generally, a decrease in the inactivation rate is observed with decreasing moisture concentration and, of course, with decreasing temperature.

It is assumed that during the drying process the residual biological activity is determined by the product of the continuously changing thermal inactivation rate and the drying time. Accordingly, it must be possible to optimize the residual activity after drying by minimizing the product of the thermal inactivation rate and time. In the above-mentioned studies, the inactivation of the biological material was described adequately with this approach. However, Zimmermann (1987a) found that the inactivation behaviour of *Lactobacillus casei* during drying could not be explained with thermal inactivation. It might be that beside the thermal inactivation also inactivation due to dehydration itself is relevant.

The objective of this work is to reveal quantitatively, the importance of the thermal inactivation of *Lactobacillus plantarum* during drying. We will discuss the measurement and modelling of the thermal inactivation of *L. plantarum*. This thermal inactivation model and the earlier drying model (Chapter 3) are integrated into an overall model in which the thermal inactivation of *L. plantarum* during drying is predicted. With this approach we should be able to evaluate the importance of the thermal inactivation of *L. plantarum* during drying. For the case that the measured inactivation is much larger than the predicted thermal inactivation with the overall model, the dehydration influence can be quantitatively determined.

4.2 THEORY

The thermal inactivation of *L. plantarum* may be described with first-order kinetics, given by the equation:

$$\frac{dA}{dt} = -k_i A \tag{4.1}$$

According to Arrhenius theory, the specific inactivation rate as a function of temperature may be expressed as:

$$k_i = k_\infty \exp\left(-\frac{E_{a,i}}{RT}\right) \tag{4.2}$$

The relation of the natural logarithm of the frequency factor $\ln(k_\infty)$ and the activation energy for inactivation $E_{a,i}$ with the moisture concentration X is assumed to be:

$$\ln(k_\infty) = A_1 X + B_1 \tag{4.3}$$

$$E_{a,i} = A_2 X + B_2 \tag{4.4}$$

Substitution of Eq. (4.3) and Eq. (4.4) into Eq. (4.2) yields the linear relation:

$$\ln(k_i) = \left[A_1 - \frac{A_2}{RT} \right] X + \left[B_1 - \frac{B_2}{RT} \right] \tag{4.5}$$

The results of the stationary thermal inactivation experiments showed two linear parts in the dependency of $\ln(k_i)$ on X, at low and at high moisture concentrations. A deviation from linearity was observed at intermediate moisture concentrations. Such type of curve can be described by splitting Eq. (4.5) into two linear segments with a non-linear connection. This resulted in Eq. (4.6):

$$\ln(k_i) = \left[\left(a_1 - \frac{a_2}{RT} \right) X + \left(b_1 - \frac{b_2}{RT} \right) \right] + [1 - \exp(pX^q)] \left[\left(a'_1 - \frac{a'_2}{RT} \right) X + \left(b'_1 - \frac{b'_2}{RT} \right) \right] \tag{4.6}$$

At low moisture concentrations ($\exp(pX^q) \rightarrow 1$), $\ln(k_i)$ is described with the first linear segment; at high moisture concentrations ($\exp(pX^q) \rightarrow 0$, when $p<0$), $\ln(k_i)$ is described as the linear sum of the two linear segments. It is important to realize that with this description only the thermal inactivation is considered. Hence, inactivation due to dehydration is not taken into account.

With this equation we can describe the temperature and moisture concentration dependency of the first-order inactivation constant k_i as a mathematical function without fundamental background of the thermal inactivation mechanism of *L. plantarum*. Such a description is required and satisfying for the calculation of k_i at each temperature and moisture concentration during the drying process. Equation (4.6) must be seen as a concept for the interpolation between the measured data rather than a theoretically based model to describe the thermal inactivation of *L. plantarum*. Another approach would be a purely empirical polynomial fit of the measured data. The advantage of the concept based on the Arrhenius theory, is that the obtained $E_{a,i}$-values can be compared to literature values.

4.3 MATERIALS AND METHODS

The *L. plantarum* strain, the growth conditions, the forming of the *L. plantarum*-starch particles, the fluidized bed drying of this granulate and the moisture concentration determination, were described in Chapter 3.

4.3.1 Thermal inactivation experiments

To obtain *L. plantarum* cells at different moisture concentrations, 10 g of the granulate was dried in the fluidized bed dryer at an air inlet temperature of 30 °C. At different drying times,

samples were taken from the bed. From these samples 0.5 to 2 g (depending on overall moisture concentration and inactivation temperature) was sealed into polyethylene bags under low vacuum (0.1 bar; Henkovac 1500 vacuum sealer, 's Hertogenbosch, NL). The bags were placed overnight (18-21 h) at 5 °C to equalize the moisture concentration profiles inside the granulate. Subsequently, the bags were placed in a water bath set at the desired inactivation temperatures (±0.05 °C). After a pre-incubation of 10 s to 2 min each bag was incubated for different times, varying from 20 s to 40 h. Pre- and incubation times were varied with inactivation temperature. After the incubation, the bags with granulate were cooled in melting ice and the activity of the *L. plantarum*-starch granulate was measured.

4.3.2 Activity measurement

The activities of the *L. plantarum*-starch granulate were measured as described in Chapter 2. The activity is based on the velocity of pH-decrease in a potassium-phosphate buffer due to fermentation of glucose to lactic acid by non-growing *L. plantarum* cells. The granulate was solved/rehydrated (5 min) in 35 ml potassium-phosphate buffer at room temperature. Introducing potato starch in the measurement system did neither affect the measurement of the activity nor the activity of the *L. plantarum* cells. The residual activity is defined as the ratio of activity after and at the start of the thermal inactivation or drying process.

4.3.3 Desiccator drying

In order to obtain a *L. plantarum*-starch granulate with a pre-defined moisture concentration, drying above a saturated salt solution was used. The granulates were placed above a saturated KCl-solution (a_W=0.88 at 4 °C; Young 1967) in a stainless-steel vacuum desiccator. The desiccator with granulate was stored at 4 °C for 48h to obtain equilibrium between the saturated salt solution and granulates.

4.4 RESULTS AND DISCUSSION

4.4.1 Thermal inactivation of L. plantarum

During the thermal inactivation of *L. plantarum* without pre-incubation an initial activation was observed especially in the temperature range of 35 °C to 45 °C. The occurrence of this activation is a well-known deviation from the first-order inactivation kinetics (Moats 1971; Corry 1973) and is of no importance to our modelling purposes.

Pre-incubation was needed for warming up the granulate to the desired inactivation temperature. An approximation of the required pre-incubation time or warming up time (Knudsen *et al.* 1973) can be made with the thermal conductivities (λ) of extruded starch paste with different moisture concentration (Andrieu *et al.* 1989). Even in the worst case (dry particles and inactivation temperature of 70 °C) calculated warm-up times never exceeded 10 s.

When pre-incubation was applied, only the linear part of the thermal inactivation curve was observed. This part was used to determine the first-order inactivation constant k_i by linear regression of $\ln(A/A_0)$ versus t. Figure 4.1 shows that the thermal inactivation of *L. plantarum* cells, at constant moisture concentration of the granulate, can be described by first-order kinetics. Deviations from the first-order inactivation kinetics, which have recently been reviewed by Gould (1989), were nearly absent.

In a wide range of temperatures and moisture concentrations of the granulate the thermal inactivation constant k_i was determined. Table 4.1 gives the measured values of the first-order thermal inactivation constants.

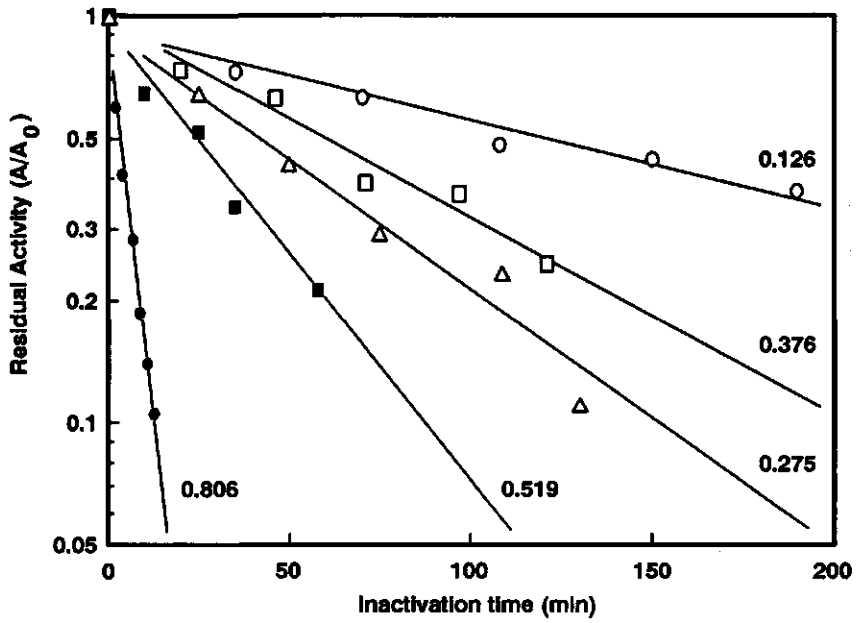

Fig. 4.1 Thermal inactivation of immobilized *L. plantarum* at 52.5 °C at moisture concentrations ranging from 0.806 to 0.126 (kg H_2O.(kg solids)$^{-1}$). Lines were obtained by linear regression of the data points at different moisture concentrations.

Table 4.1 Measured first-order inactivation constants k_i (s^{-1}) and their 90% confidence interval (±)[a] at different temperature T (°C) and moisture concentration X (kg H_2O.(kg solids)$^{-1}$).

T	X	k_i	±	T	X	k_i	±
30.0	0.887	3.50×10^{-6}[b]	2.12×10^{-6}	49.9	0.825	9.02×10^{-4}	3.69×10^{-4}
	0.786	3.33×10^{-6}[b]	9.79×10^{-7}		0.564	2.55×10^{-4}[b]	6.10×10^{-5}
	0.417	1.22×10^{-5}[b]	4.03×10^{-6}		0.370	1.25×10^{-4}	4.81×10^{-5}
	0.136	5.00×10^{-6}[b]	2.75×10^{-6}		0.270	1.72×10^{-4}	9.10×10^{-5}
					0.145	1.00×10^{-4}[b]	1.27×10^{-5}
35.0	0.869	9.36×10^{-6}	2.34×10^{-6}	52.5	0.806	2.86×10^{-3}	3.24×10^{-4}
	0.562	7.22×10^{-6}	1.95×10^{-6}		0.519	4.29×10^{-4}[b]	1.22×10^{-4}
	0.366	2.25×10^{-5}	4.51×10^{-6}		0.376	1.85×10^{-4}	3.28×10^{-5}
	0.260	1.80×10^{-5}	3.73×10^{-6}		0.275	2.50×10^{-4}	7.49×10^{-5}
	0.129	5.47×10^{-6}	8.52×10^{-7}		0.126	8.36×10^{-5}	1.70×10^{-5}
40.0	0.742	2.74×10^{-5}	6.92×10^{-6}	54.5	0.909	9.64×10^{-3}[b]	2.28×10^{-3}
	0.515	2.25×10^{-5}	6.11×10^{-6}		0.563	1.36×10^{-3}	4.27×10^{-4}
	0.309	3.13×10^{-5}	5.43×10^{-6}		0.380	3.22×10^{-4}	5.57×10^{-5}
	0.125	1.05×10^{-5}[b]	6.66×10^{-7}		0.247	3.19×10^{-4}	7.63×10^{-5}
					0.150	1.66×10^{-4}	4.63×10^{-5}
45.2	0.893	1.23×10^{-4}	3.66×10^{-5}	60.0	0.386	1.91×10^{-3}	5.41×10^{-4}
	0.520	6.40×10^{-5}	1.94×10^{-5}		0.299	1.24×10^{-3}	5.47×10^{-4}
	0.271	5.73×10^{-5}	1.09×10^{-5}		0.262	6.39×10^{-4}	1.73×10^{-4}
	0.112	2.34×10^{-5}	4.30×10^{-6}		0.176	3.98×10^{-4}	1.29×10^{-4}
					0.115	2.80×10^{-4}	1.64×10^{-4}
47.6	0.836	3.25×10^{-4}	7.79×10^{-5}	70.0	0.207	6.44×10^{-3}	1.46×10^{-3}
	0.558	1.01×10^{-4}	3.71×10^{-5}		0.167	4.81×10^{-3}	1.71×10^{-3}
	0.456	8.10×10^{-5}	2.15×10^{-5}		0.158	2.30×10^{-3}	6.14×10^{-4}
	0.283	1.01×10^{-4}	2.19×10^{-5}		0.114	1.35×10^{-3}	3.31×10^{-4}
	0.120	4.41×10^{-5}	1.18×10^{-5}				

[a] The 90% confidence interval for k_i at each temperature and moisture content follows from the linear regression (Caulcutt and Boddy 1983) of the natural logarithm of the rest activity as a function of time ($\ln(A/A_0) = k_i \cdot t + c$).

[b] k_i-value was calculated from 5 data points (all other k_i-values were calculated from 6 or 7 data points).

L. plantarum can grow up till temperatures of approximately 43 °C (Zwietering *et al.* 1991). In our system growth cannot occur but we may assume that repair of thermal injury is possible at sub-maximal temperatures only. If so, this could result in a different inactivation behaviour for temperatures for and below 43 °C. Therefore, we considered the k_i-values measured up to 40 °C separately from those measured at temperatures of 45 °C and higher. Furthermore, this approach favoured the quality of fit.

Accordingly, the thermal inactivation model (Eq. (4.6)) was fitted to the data points in Table 4.1 which resulted in two different sets of parameters for the model. A non-linear fit procedure was used to calculate the least-squares estimates of the parameters (Procedure NLIN; method Marquardt; SAS under VAX/VMS). These estimates are summarized in Table 4.2. Figure 4.2 shows the measured inactivation constants together with the model descriptions. As shown in Fig. 4.2 the dependence of $\ln(k_i)$ on the moisture concentration and temperature can be described adequately with the proposed inactivation model.

Table 4.2 The fit parameters used to describe the thermal inactivation rate as a function of temperature and moisture concentration.

Temperature range (K)[a]	a_1	a_2	b_1	b_2	a'_1	a'_2	b'_1	b'_2	p	q
$303 \leq T \leq 313$	-9.37	-39571.7	14.78	70284.2	40.55	117143.9	13.47	33570.2	-150.54	6.40
$318 \leq T \leq 343$	101.31	253129.9	34.06	119744.9	5.01	20580.3	-8.68	-22745.2	-906.58	6.09

[a] For $313 < T < 318$ interpolation between the two descriptions was used.

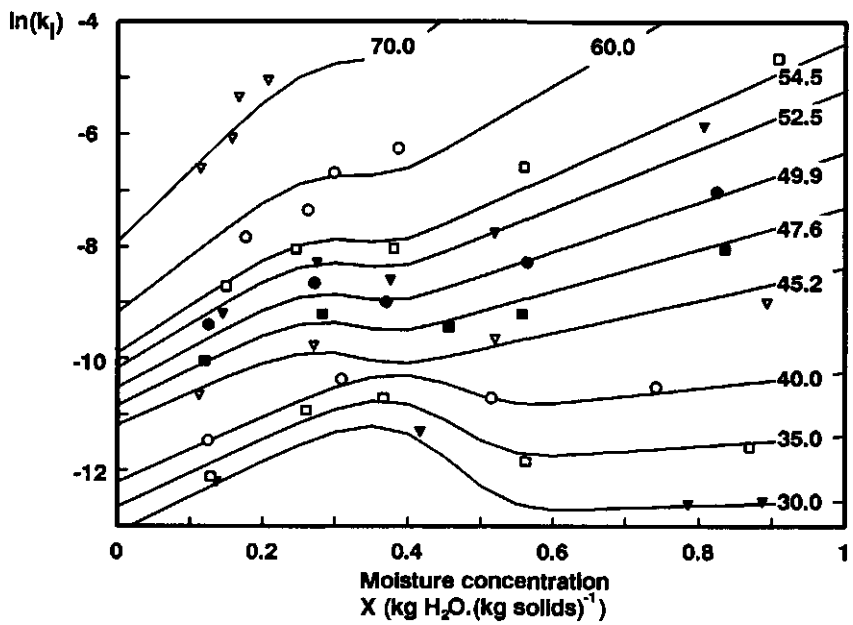

Fig. 4.2 The measured k_i-values (data points) together with the model description (lines) as a function of moisture concentration at different temperatures (°C).

Figure 4.2 shows an increase in inactivation rate with increasing moisture concentrations. Increased heat resistance in the absence of water has been generally accepted for many years (Brown and Melling 1971). Since the mechanism of heat damage under normal 'wet' conditions is incompletely understood it is difficult to decide why micro-organisms are more heat resistant in 'dry' conditions (Corry 1973). A simple explanation is that water is a reactant involved in the heat inactivation. Lowering the water concentration leads thus to decreasing heat inactivation rates. As an anomaly however, the data given in Fig. 4.2 show that in the intermediate moisture concentration range (*X*-range between 0.3-0.6 kg H_2O.(kg solids)$^{-1}$ depending on inactivation temperature), a decreasing inactivation rate with increasing moisture concentration is present. Conceivably, this could be due to a transition between two primary heat inactivation mechanisms.

In Fig. 4.3 the obtained dependency of the activation energy for inactivation $E_{a,i}$ on the moisture concentration is shown. The obtained $E_{a,i}$ values are in the same range as those found in literature for biological materials (Zimmermann 1987a; Luyben *et al.* 1982; Liou *et al.* 1985).

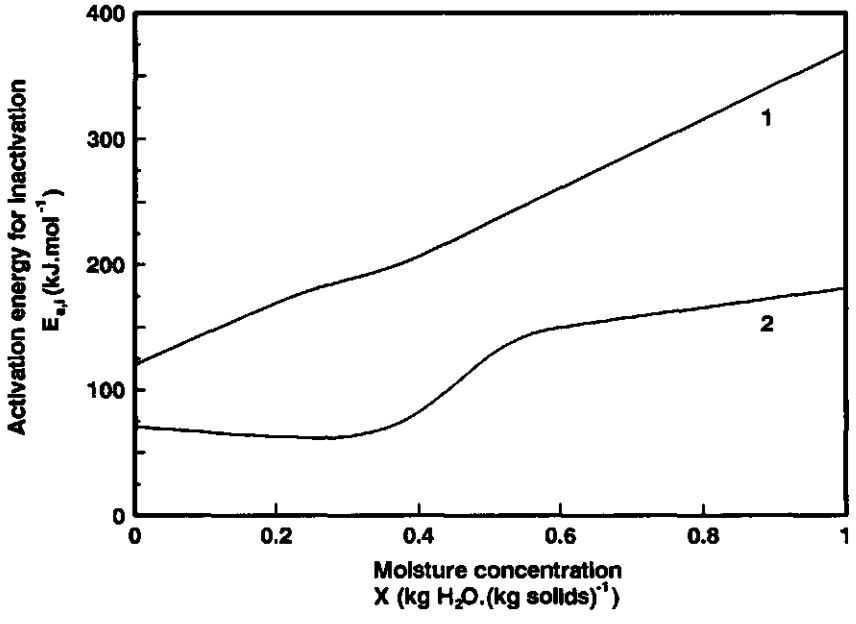

Fig. 4.3 Obtained relationships of $E_{a,i}$ with *X* in temperature range 45-70 °C (line 1) and 30-40 °C (line 2).

We were not able to measure the high inactivation rates of immobilized *L. plantarum* cells at high moisture concentrations and temperatures. Therefore, at temperatures above 55 °C the model description was extrapolated to high moisture concentrations. At low temperatures (<35 °C) the thermal inactivation becomes un-reproducible. Although first-order inactivation kinetics were still observed in this region, it was more difficult to get reproducible results than at higher temperatures. However, this does not affect our overall model because in the time course of a drying process, the thermal inactivation at these temperatures is negligible.

4.4.2 Influence of de- and rehydration on thermal inactivation rate

The thermal inactivation rates were measured in wet and dry particles. In the dry particles the moisture concentration profiles were equalized to obtain a uniform moisture concentration profile. During the equalization, the moisture concentration in the centre of the particle will decrease whereas the surface concentration will increase. This implies that the *L. plantarum* cells in the centre of a dried particle had another history than the cells on the surface of the particle. The *L. plantarum* cells in the centre of the particle had only experienced a dehydration process, the surface cells also a rehydration process.

To examine the influence of this difference, *L. plantarum*-starch particles were dried in the fluidized bed dryer at 30 °C down to moisture concentrations of 0.11 kg H_2O.(kg solids)$^{-1}$. This dried granulate was rehydrated in a vacuum desiccator above a saturated KCl-solution to a moisture concentration of 0.33 kg H_2O.(kg solids)$^{-1}$ (granulate *I*). In the same desiccator wet granulate was dried to the same moisture concentration (granulate *II*). From granulate *I* and *II* the thermal inactivation rates were measured at 52.5 °C. We found the same (within 5%) k_i-values for granulate *I* and *II* ($k_i = 1.98 \times 10^{-4}$ s^{-1}). Therefore, we can assume this de- and rehydration history of the *L. plantarum* cells did not significantly influence the thermal inactivation rate. This is a prerequisite in the used experimental set up.

4.4.3 Prediction of thermal inactivation during drying

The goal of this work was to predict the inactivation of *L. plantarum* during drying on the basis of the stationary measured thermal inactivation kinetics. Therefore, in the overall simulation program the drying model described in Chapter 3 was coupled to the inactivation model with the thermal inactivation as the primary inactivation process.

At each time step, or actually drying efficiency step, the drying time, temperature and moisture concentration of the granulate were calculated. The moisture concentration profile inside the cylinder leads to different k_i-values inside the cylinder which is important for the calculation of the thermal inactivation. Therefore, the moisture concentration profile was numerically implemented in the overall simulation as 10 different concentric shells inside the cylinder, with equal volume (see Chapter 3). In each shell the thermal inactivation of the *L. plantarum* cells is calculated according to the thermal inactivation model. This calculation is carried out with the inactivation constant k_i at the average moisture concentration in the shell and the average temperature in the time step.

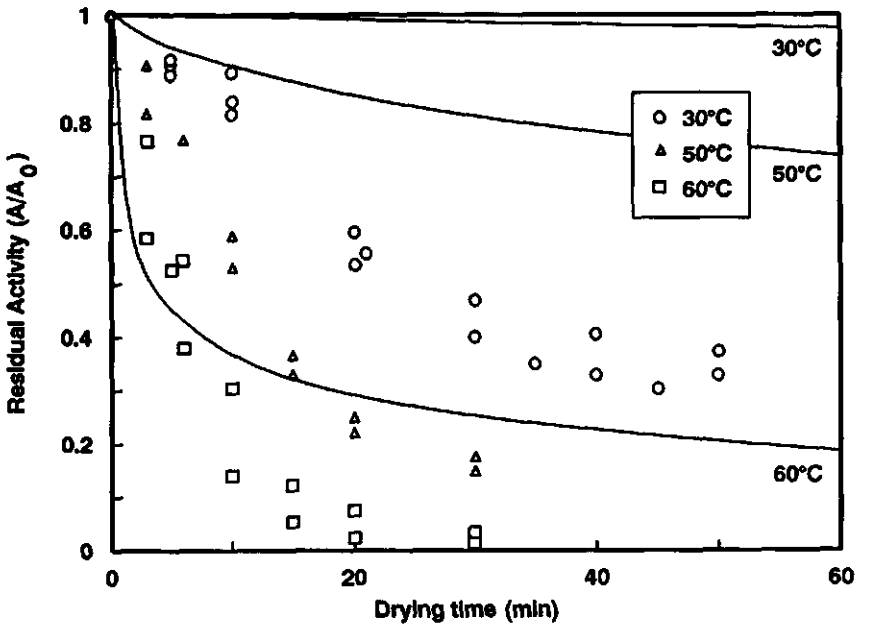

Fig. 4.4 The measured (data points) and predicted (lines) inactivation of *L. plantarum* cells during a fluidized bed drying at different air inlet temperatures.

For temperatures between 40 and 45 °C an interpolation between both sets of inactivation parameters is used. The overall residual activity is calculated as the average activity of the 10 shells. In Fig. 4.4 the simulation output is shown together with the measured inactivation at different drying times and temperatures.

As shown in Fig. 4.4 it is not possible to model the inactivation of *L. plantarum* cells during drying with thermal inactivation as the primary inactivation process. A large deviation exists between the measured and predicted inactivation curves. This is a noteworthy result since the inactivation of enzymes and yeast during drying can be reasonable described with this concept.

In the drying model we have made certain assumptions which may influence the predicted residual activity. The main assumption in the overall model is the power law relation between D_{eff} and moisture concentration (m) and the resulting moisture concentration profiles inside the cylinders (see Chapter 3). In order to examine the influence of these profiles on the predicted inactivation, simulations were made with uniform moisture concentration profiles (1 shell) equal to the average moisture concentration and with 100 shells. The influence of the number of shells was marginal at temperatures below 50 °C, due to the small values and the limited moisture concentration dependency of the inactivation constants. At temperatures above 50 °C, no significant difference was found between 10 and 100 shells. At these temperatures, the implemented single uniform moisture concentration profile gave a significant influence on the

predicted residual activities. We can conclude that a simulation with 10 shells is accurate enough. Simulation with more shells takes needless computer-time whereas simulation with a single uniform profile can provoke inaccuracies.

Variation in the heat transfer coefficient α in the heat-balance with a factor 10 or 0.1, which may be seen also as a variation in the other heat-balance constants, did not significantly affect the predicted residual activity at temperatures below 50 °C, in contrast to higher temperatures. However, even at high temperatures and high heat transfer coefficients the measured inactivation remains underestimated. Neither the moisture concentration profiles nor over- or underestimation of α can explain the large deviation between the measured and predicted residual activities.

Table 4.3 shows the time constants for the inactivation and drying processes. At temperatures < 50 °C, the time constants for the inactivation process are much more than an order of magnitude higher than the time constants for the drying process. Therefore, at these temperatures, the influence of the thermal inactivation can be ignored in the time course of a drying process.

The inactivation of enzymes can be satisfactory described with a primary thermal inactivation (Liou 1982; Liou *et al.* 1985; Yamamoto *et al.* 1985). Zimmermann (1987a) has more or less successfully described the inactivation of yeast with this concept but failed to described the inactivation during drying of *L. casei*. In his work it is not quit clear where the problems arise, but Zimmermann also mentioned that the inactivation of *L. casei* could not be described with thermal inactivation.

Table 4.3 Comparison between the time constants which are calculated from the model equations of the thermal inactivation (τ_i)[a] and the drying process (τ_d)[a].

T(°C)	τ_i (s)		τ_d (s)	ratio τ_i/τ_d	
	wet system[b]	dry system[b]		wet system	dry system
30	3.01×10^{5}	7.94×10^{4}	743	405	106
40	3.55×10^{4}	3.54×10^{4}	521	68.2	68.1
50	1.14×10^{3}	7.08×10^{3}	374	3.05	18.9
60	2.88×10^{1}[c]	8.32×10^{2}	274	0.105	3.04
70	9.31×10^{-1}[c]	1.13×10^{2}[c]	204	0.00455	0.552

[a] The time constant τ_i is defined as $1/k_i$ and τ_d is defined as the time needed to reach $E = 0.63 = 1\text{-}\exp(-1)$ in the drying process.

[b] The wet system is defined as $X = X_0$ (0.837 kg H_2O.(kg solids)$^{-1}$) and the dry system as $X = 0.3097$ kg H_2O.(kg solids)$^{-1}$ (X at $E = 0.63$).

[c] extrapolated values (other values were interpolations between measured k_i-values).

Our results show that contrary to the case of enzymes and yeast, another inactivation mechanism must be predominant. In Fig. 4.5 the residual activity of the *L. plantarum* cells are plotted against the reached drying efficiency in the granulate. In the inlet temperature range of 30 °C to 50 °C the influence of the air temperature is negligible. In this temperature range, all data can be fitted by a single curve with the drying efficiency E (or moisture concentration X; see Chapter 2) as the single variable. This shows that the residual activity is primary influenced by the moisture concentration. Above 50 °C the air temperature becomes important. This is in agreement with the time constant concept of Table 4.3.

It seems that the inactivation of *L. plantarum* during drying is caused by two different mechanisms. The first mechanism is the inactivation due to dehydration. During drying this inactivation mechanism will always manifest. The second mechanism is the thermal inactivation, which becomes important at high temperatures. With the time constants in Table 4.3 we can state: when $\tau_i > \tau_d$ the inactivation during drying is primary caused by dehydration; when $\tau_i \approx \tau_d$ both mechanisms are active and when $\tau_i < \tau_d$ the thermal inactivation mechanism predominates.

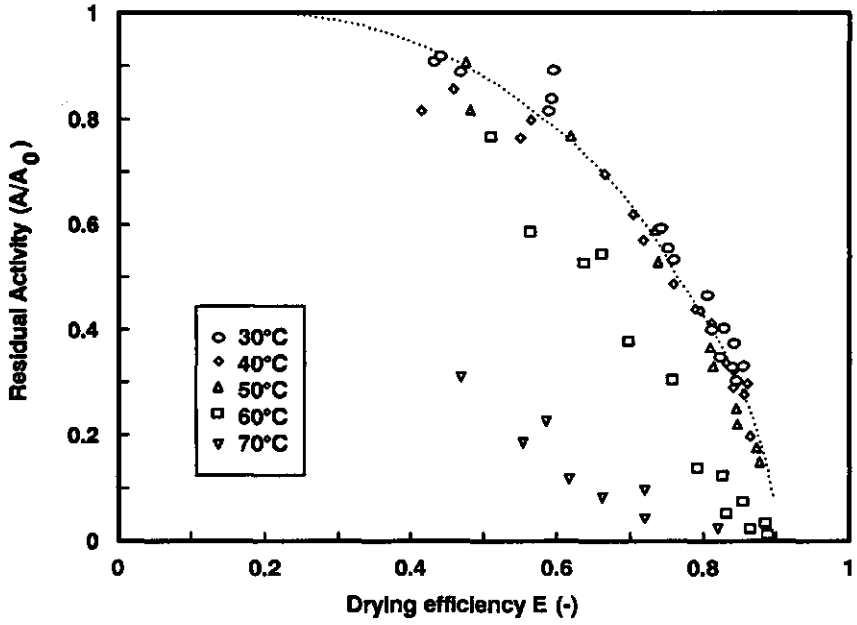

Fig. 4.5 The measured residual activity of the *L. plantarum* cells as a function of the drying efficiency at different air inlet temperatures of the fluidized bed dryer.

As mentioned before, it is interesting that the inactivation of enzymes and especially yeast during drying can be described with thermal inactivation as the only mechanism. It appears that enzymes and yeast are not primarily inactivated through dehydration, whereas for *L. plantarum* dehydration is dramatic. Why the dehydration damage is so significant with *L. plantarum* cells and not for yeast cells remains an uncertainty. Probably a difference in the sturdiness of the cell membrane and/or cell wall gives organisms with differences in drying resistance.

During drying the dehydration inactivation will be inevitable. Protective agents (i.e., Morichi 1974; Porubcan and Sellars 1974; Valdez *et al.* 1985; Prajapati *et al.* 1987) can help to minimize this inactivation. When dramatic dehydration damage can be avoided, modelling the thermal inactivation becomes applicable. With a thermal inactivation model a decision can be made between drying at higher or lower temperatures. At higher temperatures the energy consumption of the drying apparatus will be more economical whilst at low temperatures the residual activity will be optimal.

4.5 CONCLUSIONS

Previous studies (see **4.1 Introduction**) have shown that the inactivation of enzymes and yeast during drying could be described with a mechanism that is based on thermal inactivation. We have shown that this concept is not suitable for *L. plantarum*.

The inactivation of *L. plantarum* during drying is caused by two mechanisms: thermal inactivation and inactivation due to dehydration. During drying, dehydration inactivation will be inevitable whereas thermal inactivation can be minimized though drying at moderate temperatures. Hence, in a thermal drying process for *L. plantarum* low temperatures should be used (<40 °C).

ACKNOWLEDGEMENTS

We would like to thank T. Taekema and S.R. Weijers for their contributions to this research and Dr. H.H. Beeftink, Dr. M.R. Smith and Prof.Dr. J. Tramper for their helpful critical comments in the preparation of this manuscript. The financial support of Gist-brocades NV, Delft, The Netherlands is gratefully acknowledged.

REFERENCES

Andrieu J, Gonnet E and Laurent M (1989) Thermal conductivity and diffusivity of extruded durum wheat pasta. Lebensm.-Wiss.u.-Technol. 22: 6-10.

Brown MRW and Melling J (1971) *Inhibition and destruction of micro-organisms by heat.* In: Hugo WB (ed) Inhibition and destruction of the microbial cell (pp. 1-37). Academic Press, London.

Caulcutt R and Boddy R (1983) Statistics for analytical chemists (p. 82), Chapman and Hall, London.

Corry JEL (1973) The water relations and heat resistance of micro-organisms. Progr. Ind. Microbiol. 12: 73-108.

Gould GW (1989) Mechanisms of action of food preservation procedures (pp. 11-42), Elseviers Applied Science, London.

Kerkhof PJAM and Schroeber WJAH (1974) *Theoretical modelling of the drying behaviour of droplets in spray dryers.* In: Spicer A (ed) Advances in preconcentration and dehydration of food (pp. 349-397). Applied Science Publishers, London.

Knudsen JG, Bell JK, Holt AD, Hottel HC, Sarofim AF, Standiford FC, Stuhlbarg D and Uhl VW (1973) *Heat transmission.* In: Perry RH and Chilton CH (eds) Chemical Engineers' Handbook, 5th edition (p. 10.8). McGraw-Hill Company, New York.

Liou JK (1982) An approximate method for nonlinear diffusion applied to enzyme inactivation during drying. Ph.D. Thesis, Agricultural University Wageningen.

Liou JK, Luyben KChAM and Bruin S (1985) A simplified calculation method applied to enzyme inactivation during drying. Biotechn. Bioeng. 27: 109-116.

Luyben KChAM, Liou JK and Bruin S (1982) Enzyme degradation during drying. Biotechn. Bioeng. 24: 533-552.

Moats WA (1971) Kinetics of thermal death of bacteria. J.Bacteriol. 105: 165-171.

Morichi T (1974) Preservation of lactic acid bacteria by freeze-drying. Jpn. Agricul. Res. Quart. 8: 171-176.

Porubcan RS and Sellars RL (1974) Stabilized dry cultures of lactic acid-producing bacteria. Chr. Hansen's Lab. Inc. USA-Patent 3,897,307.

Prajapati JB, Shah RK and Dave JM (1987) Survival of *Lactobacillus acidophilus* in blended spray dried acidophilus preparations. Austr. J. Dairy Technol. March/June 1987: 17-21.

Valdez de GF, Giori de GS, Ruiz Holgado de AP and Oliver G (1985) Effect of drying medium on residual moisture content and viability of freeze-dried lactic acid bacteria. Appl. Env. Microbiol. 49: 413-415.

Wijlhuizen AE, Kerkhof PJAM and Bruin S (1979) Theoretical study of the inactivation of phosphatase during spray drying of skim-milk. Chem. Eng. Sci. 34: 651-660.

Yamamoto S, Agawa M, Nakano H and Sano Y (1985) *Enzyme inactivation during drying of a single droplet.* In: Toei R and Mujumdar AS (eds) Drying '85 (pp.330-337). Hemisphere Publishing Corporation, Washington.

Young JF (1967) Humidity control in the laboratory using salt solutions-a review. J. Appl. Chem. 17: 241-245.

Zimmermann K (1987a) Einflussparameter und mathematische modellierung der schonenden trocknung von starterkulturen. Fortschr.-Ber. VDI (Reihe 14 no36), VDI-Verlag, Düsseldorf.

Zimmermann K and Bauer W (1987b) Modellierung der trocknung von hefen im wirbelschichttrockner. Chem.-Ing.-Tech. 59: 350-351.

Zwietering MH, De Koos JT, De Wit JC, Hasenack BE and Van 't Riet K (1991) Modeling the bacterial growth curve as function of temperature. Appl. Env. Microbiol. Accepted.

NOMENCLATURE

A	activity of *L. plantarum* cells (see Chapter 2)	$\Delta pH.min^{-1}.g^{-1}$
A_0	initial activity of *L. plantarum* cells before inactivation or drying	$\Delta pH.min^{-1}.g^{-1}$
A_i, a_i, a'_i	parameters to describe $\ln(k_i)$ as $f(X,T)$	
B_i, b_i, b'_i	parameters to describe $\ln(k_i)$ as $f(X,T)$	
E	drying efficiency (see Chapter 3)	-
$E_{a,i}$	activation energy for inactivation	$J.mol^{-1}$
k_i	first-order thermal inactivation constant	s^{-1}
k_∞	frequency factor	s^{-1}
p,q	parameters to describe $\ln(k_i)$ as $f(X,T)$	
R	gas law constant	$J.mol^{-1}.K^{-1}$
t	time	s
T	absolute temperature	K
X	moisture concentration	kg H_2O.(kg solids)$^{-1}$
X_0	initial moisture concentration	kg H_2O.(kg solids)$^{-1}$

Greek symbols

α	(uncorrected) heat transfer coefficient	$J.s^{-1}.m^{-2}.K^{-1}$
τ_d	time constant of drying process	s
τ_i	time constant of thermal inactivation	s

CHAPTER 5

MODELLING THE OVERALL INACTIVATION

Lou C. Lievense, Monique A.M. Verbeek, Tom Taekema, Gerrit Meerdink and Klaas van 't Riet

Food- and Bioprocess Engineering Group, Department of Food Science, Wageningen Agricultural University

ABSTRACT

In this chapter the inactivation of *Lactobacillus plantarum* in a fluidized-bed drying process is modelled. The inactivation is described with two different mechanisms: thermal inactivation and dehydration inactivation. These inactivation mechanisms are described as two processes occurring simultaneously during the drying process. The thermal inactivation process has been quantitatively described before. The dehydration inactivation is quantified by fitting an arbitrary set of equations to the measured inactivation data obtained at a drying temperature of 30 °C. At this temperature the thermal inactivation can be neglected. Both inactivation models are coupled to the drying kinetics described in Chapter 3. The overall model predicts the measured inactivation of *L. plantarum* during a fluidized-bed drying process, up to a drying temperature of 55 °C. From experiments it is concluded that the influence of the drying rate on the residual activity of *L. plantarum* can be neglected and that the moisture concentration rather than the water activity is the essential parameter in dehydration inactivation.

Lievense LC, Verbeek MAM, Taekema T, Meerdink G and Van 't Riet K (1991) Modelling the inactivation of *Lactobacillus plantarum* during a drying process. **Chem. Eng. Sci.** Accepted.

CHAPTER 5

MODELLING THE OVERALL INACTIVATION

5.1 INTRODUCTION

In downstream processing of bioproducts the stabilization of the final product is an important step at the end of the bioseparation process. Drying is one of the commonly used stabilization processes. One of the applications is the stabilization of starter cultures used in industrial fermentation processes. Drying could give important advantages when compared to freezing, which is normally used to stabilize starter cultures. Particularly, the transport and storage of dried cultures is much more economical. Freeze drying is the most frequently used method in the preparation of dried cultures. Due to the high costs of this process a convective drying method is preferable, especially for large-scale production. However, it is important that the microbial activity and/or growth capacity is preserved during the drying process. To optimize the drying process, insight into the correlation of the parameters of the drying process with the survival of the micro-organisms is essential. The application of mathematical modelling and simulation techniques can provide such insight.

In the last two decades a number of workers has given their attention to the mathematical modelling of inactivation of biological materials during drying. Most work was carried out with enzymes (Kerkhof and Schroeber 1974; Wijlhuizen *et al.* 1979; Liou 1982; Luyben *et al.* 1982; Liou *et al.* 1985; Yamamoto *et al.* 1985). The first work on the modelling of the inactivation of micro-organisms (*Saccharomyces cerevisiae* and *Lactobacillus casei*) during drying came from Zimmermann (1987) and Zimmermann and Bauer (1987). Essential in all these studies is that the inactivation of enzymes and/or micro-organisms during drying is described with a thermal inactivation mechanism. The rate of the thermal inactivation was invariably described with first-order kinetics and was given as a function of temperature and moisture concentration. This function was determined in a stationary process at a given temperature and moisture concentration. Generally, a decrease in the inactivation rate is observed with decreasing moisture concentration and, of course, with decreasing temperature. In most of the above-mentioned studies this approach provided a satisfying description of the inactivation of enzymes (and yeast) during drying. However, the inactivation of *L. casei* during drying could not be described with this approach (Zimmermann 1987). Apparently, mechanisms other than thermal inactivation only are relevant in the drying of bacteria.

In Chapter 3 and 4 we have described a mathematical model to predict the thermal inactivation of *Lactobacillus plantarum* during a drying process. The *L. plantarum* cells were mixed with potato starch and granulated as cylindrical particles, which were dried in a fluidized-bed dryer. Chapter 3 considers the prediction of the overall moisture concentration, the moisture concentration profiles inside the particles and the temperature of *L. plantarum*-starch particles during the fluidized-bed drying process. This drying model is based on the short-cut drying theory (Liou 1982; Liou and Bruin 1982ab; Coumans 1987) and the heat balance of the drying material. Chapter 4 considers the description of the first-order thermal inactivation of the *L. plantarum* cells as a function of moisture concentration and temperature. In that work the thermal inactivation description is coupled to the drying model to reveal quantitatively the importance of the thermal inactivation of the *L. plantarum* cells during drying. From these studies it is quite clear that the inactivation of *L. plantarum* during drying can not be due to thermal inactivation only. It was concluded that another mechanism occurs, probably inactivation due to dehydration.

In the models mentioned above the drying rate is not taken into account. Various literature sources mention, qualitatively, the influence of the drying rate on the residual activity or survival of micro-organisms. Tutova (1982) and Kuts and Tutova (1983) mention that a high drying rate negatively affects the survival of *Rhizobium pisum* during a fluidized-bed drying process. These authors state that the drying rate must be regarded as one of the most important parameters that influence the residual activity of vegetative cultures during drying. Zimmermann (1987) mentions that the inactivation of *L. casei* can possibly be caused by a high drying rate. In the present work the relation between the drying rate and the residual activity of *L. plantarum* during a fluidized-bed process is quantitatively studied.

A fundamental question in the description of the dehydration inactivation is whether the inactivation is caused by the decreasing water activity (a_W) of the system or by the decreasing water concentration. During the drying process the drying material (a heterogeneous, multi-component system) is far from equilibrium and therefore it is questionable to speak about a_W which is an equilibrium thermodynamic function defined for a homogeneous, binary system (Franks 1982). Nevertheless, the importance of a_W as parameter in the dehydration inactivation has to be studied.

The main objective of this work is to model the inactivation of *L. plantarum* during drying. The coupling of the two inactivation mechanisms, the thermal inactivation and the inactivation due to dehydration, to an overall model is discussed. The thermal inactivation and the dehydration inactivation are considered as two simultaneous processes. The overall model should predict the residual activity of *L. plantarum* cells during a fluidized-bed drying process.

5.2 THEORY

A detailed description of the drying and thermal inactivation model was given in Chapter 3 and 4. Here, only the essential formulas and explanations are given.

An important dimensionless group in the short-cut drying theory is the drying efficiency *E*. *E* represents the progress of the drying process with respect to the initial moisture concentration X_0 and the final moisture concentration at infinite drying time X_e according to:

$$E = \frac{X_0 - \overline{X}}{X_0 - X_e} \tag{5.1}$$

where $\overline{X}$ is the overall moisture concentration of the particle. When $E=0$, $\overline{X}$ equals X_0 and when $E=1$, $\overline{X}$ equals X_e. Because *E* represents the progress of the drying process it can be used as a time parameter.

The thermal inactivation and the dehydration inactivation of *L. plantarum* are depending on the moisture concentration. Therefore, the moisture concentration profiles inside the drying granulate must be calculated. This is done by numerical division of the cylindrical granulate in concentric shells with equal volume. In Chapter 4 it was shown that a numerical division in 10 shells provides a satisfactory accuracy for the thermal inactivation calculation. The calculated profile will be numerically implemented as 10 shells, each with a moisture concentration equal to the average moisture concentration in that shell. For the calculation of the temperature of the granulate a uniform temperature profile can be assumed (Chapter 3 and 4).

As mentioned before, the overall inactivation of *L. plantarum* during drying is considered as the result of two simultaneous inactivation processes: thermal inactivation and inactivation due to dehydration. Analogously to simultaneous or parallel chemical reactions (Levenspiel 1972), the total inactivation rate can be described as:

$$\left(\frac{dA}{dt}\right)_{tot} = \left(\frac{dA}{dt}\right)_{therm} + \left(\frac{dA}{dt}\right)_{dehydr} \tag{5.2}$$

where *A* is the activity of *L. plantarum* (Chapter 2), *t* is the time from the start of the drying process and the subscripts *tot*, *therm* and *dehydr* denote the total, the thermal and the dehydration inactivation, respectively. Because in the short-cut drying theory the calculations are based on *E* rather than *t* also the derivatives in Eq. (5.2) are given as a function of *E* which yields:

$$\left(\frac{dA}{dE}\right)_{tot} = \left(\frac{dA}{dE}\right)_{therm} + \left(\frac{dA}{dE}\right)_{dehydr} \tag{5.3}$$

The thermal inactivation rate of *L. plantarum* is described with first-order kinetics (Chapter 4):

$$\left(\frac{dA}{dt}\right)_{therm} = -k_i A \tag{5.4}$$

where k_i is the first-order inactivation constant. With the dimensionless flux F, which follows from the mass balance over the drying cylinder (Chapter 3):

$$F = \frac{R_c^2}{2D_0}\frac{dE}{dt} \tag{5.5}$$

Eq. (5.4) can be transformed from time- to E-basis which yields:

$$\left(\frac{dA}{dE}\right)_{therm} = \frac{-R_c^2}{2D_0F} \cdot k_i A \tag{5.6}$$

where R_C is the radius of the cylinder (drying granulate) and D_0 a diffusion coefficient parameter (Chapter 3).

The first-order inactivation constant k_i is a function of moisture concentration and temperature and given in Chapter 4:

$$\ln(k_i) = \left[\left(a_1 - \frac{a_2}{RT}\right)X + \left(b_1 - \frac{b_2}{RT}\right)\right] + [1 - \exp(pX^q)]\left[\left(a'_1 - \frac{a'_2}{RT}\right)X + \left(b'_1 - \frac{b'_2}{RT}\right)\right] \tag{5.7}$$

where a_i, b_i, a'_i, b'_i, p and q are parameters to describe $\ln(k_i)$ as function of the moisture concentration X and absolute temperature T, and where R is the gas constant. This relation was derived from the Arrhenius equation for k_i in which the natural logarithm of the frequency factor $\ln(k_\infty)$ and the activation energy for inactivation $E_{a,i}$ were described as a linear function of X (Chapter 4).

The dehydration inactivation can be written as:

$$\left(\frac{dA}{dE}\right)_{dehydr} = \frac{dA}{dX} \cdot \frac{dX}{dE} \tag{5.8}$$

The first derivative dA/dX follows from the relationship between the residual bacterial activity and the moisture concentration. This relationship is assumed to be described by the equation set:

$$\frac{A}{A_0} = 1 \qquad \text{for } X \geq b \qquad (5.9a)$$

$$\frac{A}{A_0} = \left(\frac{a}{a-b}\right) - \left(\frac{1}{a-b}\right)X \qquad \text{for } a < X < b \qquad (5.9b)$$

$$\frac{A}{A_0} = 0 \qquad \text{for } X \leq b \qquad (5.9c)$$

The parameters *a* and *b* are obtained by fitting Eq. (5.9) to the measured residual bacterial activity versus moisture concentration during fluidized-bed drying at an air inlet temperature of 30 °C. At this temperature the thermal inactivation can be neglected (Chapter 4). The second derivative *dE/dX* can be calculated numerically in each shell.

With Eqs. (5.3-5.9) the inactivation rate in each shell can be calculated. The overall residual bacterial activity of the *L. plantarum*-starch granulate can be calculated with an straightforward averaging procedure over the 10 shells.

5.3 MATERIALS AND METHODS

The *L. plantarum* strain, growth conditions, forming of the *L. plantarum*-starch particles, fluidized-bed drying of this granulate and moisture concentration determination were described in Chapter 3 and 4.

5.3.1 Activity measurement

The activities of the *L. plantarum*-starch granulate were measured as described in Chapter 2 and 4. The activity measurement is based on the velocity of pH-decrease in a potassium-phosphate buffer due to fermentation of glucose to lactic acid by non-growing *L. plantarum* cells. Unless mentioned otherwise, 0.3-0.5 g of the granulate was rehydrated/solved (5 min) in 35 ml potassium-phosphate buffer at room temperature. The activity of the cells (in $\Delta pH.min^{-1}.g^{-1}$ dry weight) was defined as the maximum velocity observed. The residual activity is defined as the ratio of the activities after and at the start of drying.

5.3.2 Desorption isotherms

The desorption isotherms of the *L. plantarum*-starch granulate were measured with two methods. With the first method, the desorption isotherm of about 200 mg *L. plantarum*-starch granulate was measured in a McBain sorption balance at a temperature of 20.0±0.05 °C (Weldring *et al.* 1975). The equilibrium period (Van der Berg 1981) varied from 50 to 70 h for each measurement. The second method was based on the conventional technique of a vacuum desiccator with saturated aqueous salt solutions to control the water activity (Spiess and Wolf 1987). The desiccators with the *L. plantarum*-starch samples were placed in a ventilated stove

(Marius, Utrecht, NL) with a temperature of 20, 30 or 40 °C controlled at ±0.4 °C. The data for the water activity of the saturated aqueous salt solutions at the different temperatures were taken from tables of Young (1967). The dry weight of the granulate was measured after 20-24 h drying at 110 °C as described in Chapter 3.

5.3.3 Drying rates

The drying rate was varied in three different ways. Firstly, the diameter from the *L. plantarum*-starch granulate was varied from 0.5 to 4 mm. This was done by extruding the *L. plantarum*-starch paste through sieve-plates with die-openings of different diameters (Chapter 3). The granulate then was dried in a fluidized-bed dryer at 30 °C. Secondly, granulate of 1 mm diameter was dried above a saturated aqueous LiCl solution at 20 °C (a_W=0.11 at 20 °C; Young 1967) in a stainless steel vacuum desiccator. These experiments were carried out with and without vacuum to obtain a significant difference in drying rate compared to the fluidized-bed drying experiments. Thirdly, a suspension of 28.5 g *L. plantarum*-starch paste in 70.0 g potassium-phosphate buffer (0.15 mol.l^{-1} KH_2PO_4/K_2HPO_4, pH=7.0 in 0.15 mol.l^{-1} NaCl in demineralized water (Chapter 2) was spray-dried in a laboratory spray-dryer (Büchi 190, Flawil, CH) with an inlet air temperature of 90 °C. The measured air outlet temperature was 42 °C. The airflow through the column and the suspension flow to the nozzle were set at the lowest rate possible (450 l.min^{-1} (at 20 °C) and 5 ml.min^{-1} respectively). The airflow through the nozzle was set at maximum rate (13 l.min^{-1} (at 20 °C)).

5.3.4 Desiccator drying

For the desiccator drying experiments approximately 3 g of *L. plantarum*-starch granulate of 1 mm diameter was placed in a petri-disk at 5.0±0.5 °C in a stainless steel vacuum desiccator above the following saturated aqueous salt solutions (a_W-values at 5 °C from Young (1967)): LiCl, a_W=0.12; KAc, a_W=0.25; $MgCl_2.6H_2O$, a_W=0.34; KNO_2, a_W=0.52; $NaNO_2$, a_W=0.68; NaCl, a_W=0.76; KCl, a_W=0.87 and $CuSO_4.5H_2O$, a_W=0.98. The samples were dried for 48 h. Afterwards the glucose fermenting activity and moisture concentration of the samples were measured.

5.3.5 Water activity

The influence of the water activity on the residual activity of the *L. plantarum* cells was measured through a change in temperature at a certain moisture concentration. To facilitate a significant temperature shift of the granulate after drying, a fluidized-bed drying experiment was performed with an air inlet temperature of 40 °C. At this temperature, thermal inactivation of the *L. plantarum* cells can still be neglected during the experiment (Chapter 3). During the drying process samples were taken from the fluidized bed at different times. Part of the sample was rehydrated in potassium-phosphate buffer at 40 °C, immediately after taking the sample out of the fluidized bed. The cell-starch-buffer suspension was then cooled down to 20 °C. Other parts

of the same sample were cooled to 20 °C and 1 °C respectively and thereafter rehydrated at 20 °C. Afterwards the glucose fermenting activity of the suspensions and the moisture concentration of the samples were measured.

5.3.6 Fitting procedures

The parameters of the relation between actual bacterial activity and overall moisture concentration (Eq. (5.9)) and the desorption isotherms were estimated by a non-linear fitting procedure (NLIN, SAS under VAX/VMS; Marquardt and secant method respectively). The parameters *a* and *b* in Eq. (5.9) were estimated from the measured relationship between overall residual activity and overall moisture concentration. At a given overall moisture concentration or drying efficiency, the concentration profiles inside the granulate were calculated and the cylinder was numerically divided in 10 concentric shells (Chapter 3). In each shell the average moisture concentration was calculated. This procedure results in a data-set with a moisture concentration profile inside the granulate (implemented as 10 moisture concentrations) with a corresponding overall residual bacterial activity. In this fitting procedure Eq. (5.9) was evaluated in each shell of the granulate and an averaging procedure was implemented to calculate the overall residual bacterial activity. The fast temperature increase of the granulate to the inlet temperature of the drying air at the start of the drying process (Chapter 3) was neglected.

5.4 RESULTS AND DISCUSSION

5.4.1 Desorption isotherms

The desorption isotherms data are given in Fig. 5.1. The results obtained with two methods were in good agreement. The isotherm measured at 40 °C was fitted with the GAB-equation (Appendix 1). The desorption isotherms at 20 °C, 30 °C were calculated with the GAB-equation at 40 °C as a standard by means of the Clausius-Clapeyron equation, in which the net isosteric heat of sorption was fitted with a power law function of the moisture concentration. This equation can then be used to calculate the desorption isotherm at other temperatures (Appendix 1). As can be seen from Fig. 5.1, a temperature change of 20 °C of the cell-starch granulate can result in a a_W-shift up to 0.2, depending on the moisture concentration.

In the low water activity range (a_W < 0.6), the desorption isotherm at 20 °C corresponds to desorption isotherms for native potato starch (Van den Berg 1981; Buléon *et al.* 1982).

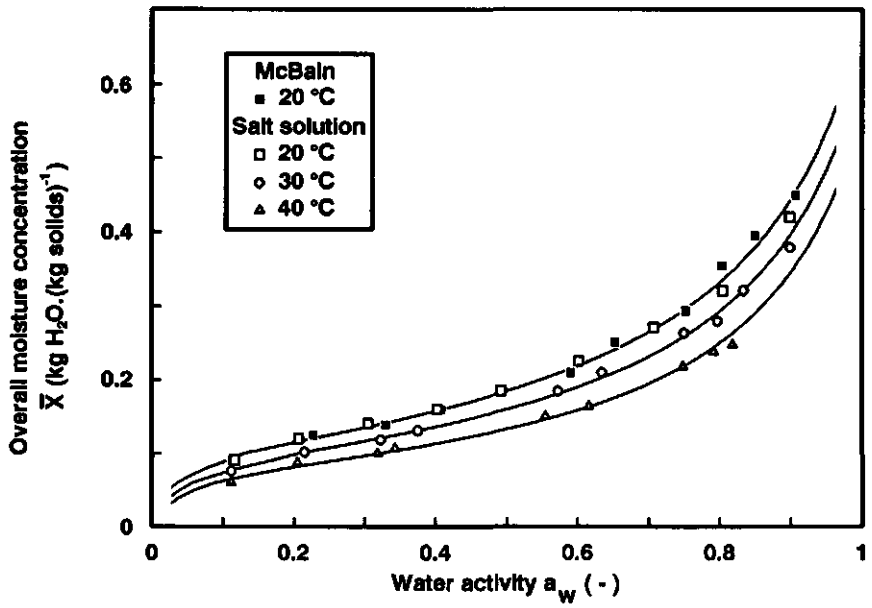

Fig. 5.1 The measured (data points) and fitted (lines) desorption isotherms at different temperatures. The desorption isotherm at 40 °C is described with the GAB-equation, at 20 °C and 30 °C derived from the 40 °C-isotherm with the Clausius-Clapeyron equation (see Appendix 1).

5.4.2 Modelling the dehydration inactivation

L. plantarum-starch granulates with different diameters were dried in a fluidized bed at 30 °C, to reveal the importance of the dehydration rate. As mentioned before, the thermal inactivation at this temperature can be neglected. The residual activity and the overall moisture concentration of each size of the granulate was measured during drying. The time constant τ of the drying process was defined as the time needed to reach a drying efficiency $E=0.63=1-\exp(-1)$ or (with $X_e=0$) $X=0.37 \cdot X_0$. As can be seen from Fig. 5.2, the variation in granulate diameter resulted in τ_d -values from 2 to 70 min. Drying above saturated aqueous LiCl solutions at 5 °C with and without vacuum gave τ_d -values of 50 min and 8 h, respectively. A reasonable approximation for the time constant for the spay-drying process can be calculated from the experimental conditions and is in the order of seconds. The temperature inactivation in all these experiments may be neglected. This also holds for the spray-drying experiments, where the temperature of the *L. plantarum*-starch powder, with a residence time of several seconds, can not significantly exceed the measured outlet temperature of the dryer (43 °C).

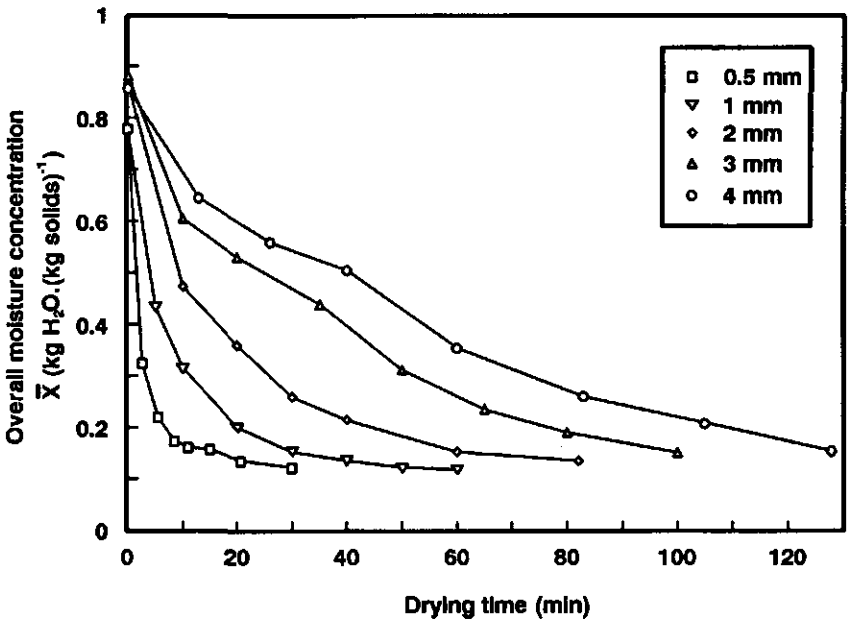

Fig. 5.2 Drying curves of *L. plantarum*-starch granulate of different diameter dried in a fluidized-bed dryer at 30 °C inlet temperature.

The influence of the different dehydration rates on the residual activity as a function of overall moisture concentration is shown in Fig. 5.3. While τ_d -values range from several seconds to several hours, no significant influence of the drying rate was found. Even the results of the spray-drying experiments are in the same range.

For the fluidized-bed drying experiments, similar moisture concentration profiles inside the particles will exist. For spray-drying and drying above a saturated LiCl-solution the moisture concentration profiles will be different. Therefore, the data obtained with the latter experiments cannot be compared directly with the data from the other experiments. However, no significant effect of these different profiles nor of the drying rate can be detected within the experimental accuracy.

From these experiments it is concluded that the residual activity of *L. plantarum* depends only on the final moisture concentration and not on the dehydration rate. Therefore, in the modelling of the inactivation of *L. plantarum* during a fluidized-bed drying process, the dehydration rate can be neglected.

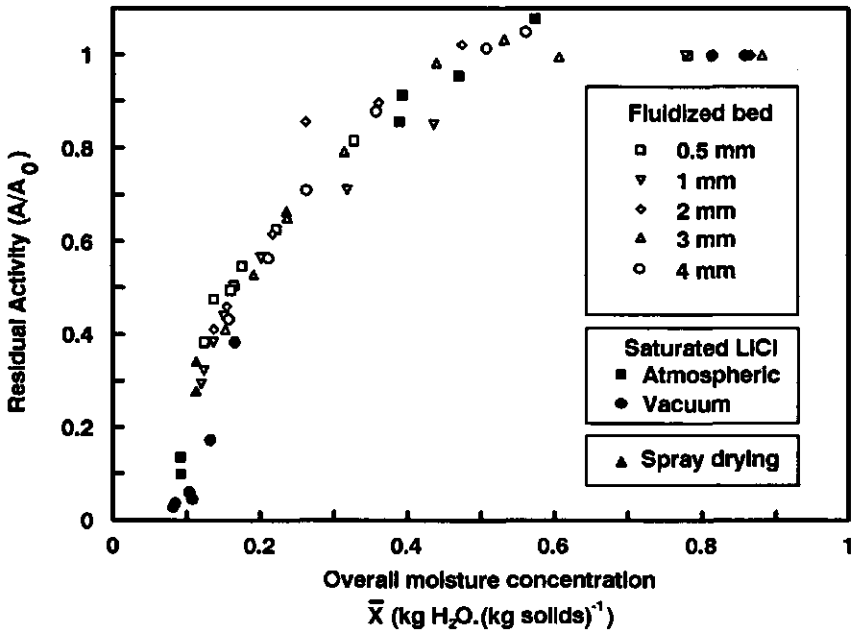

Fig. 5.3 The residual activities as a function of the overall moisture concentration obtained during drying with different drying rates.

From the measured relationship between residual activity and overall moisture concentration obtained with the fluidized-bed drying experiments (Fig. 5.3) the parameters *a* and *b* in Eq. (5.9) were estimated with the fitting procedure. The thus obtained relationship between local moisture concentration and actual residual bacterial activity is shown as the solid line in Fig. 5.4.

The relationship in Fig. 5.4 was verified in separate drying experiments. The residual activities of the *L. plantarum* cells after drying the granulate to a uniform moisture concentration profile were measured. These uniform moisture concentration profiles were obtained by desiccator drying until equilibrium above saturated aqueous salt solutions. Equilibrium was assumed after 48 h as no further decrease in weight was measured after this time. The bacterial activity of the undried granulate at 5 °C was constant for 5 days. Because of this, inactivation of the *L. plantarum* cells through other mechanisms than dehydration can be ignored in this experiment. Because the humidity in the drying chamber, and therefore the equilibrium moisture concentration X_e of the granulate, are fixed by the salt solution, uniform moisture concentration profiles were achieved. The residual activities as a function of moisture concentration are given in Fig. 5.4. A good agreement between the fitted line, obtained from fluidized-bed experiments and the experimental data was found.

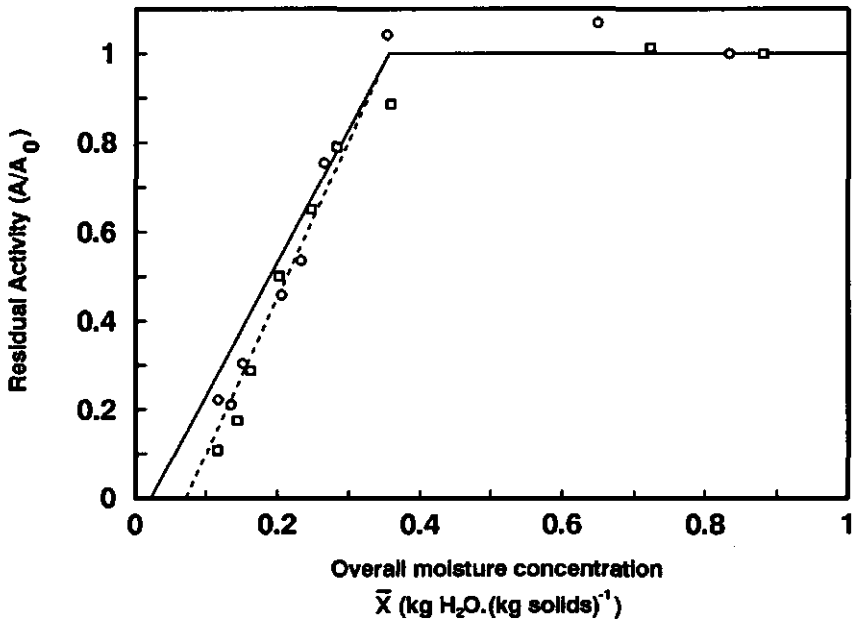

Fig. 5.4 The relationship between the residual activity and the local moisture concentration obtained with the fitting procedure applied on the fluidized-bed drying experiments (solid line) and applied on the separate drying experiments above different saturated aqueous salt solutions (dashed line). The data-points shows the (duplo) results of the separate drying experiments.

Besides the model of Eq. (5.9), several other models for this relation have been used, such as sigmoid or polynomial curves. The fitting procedure was able to estimate parameters for these models in such a way that the final description predicts the measured data with similar accuracy as Eq. (5.9). Equation (5.9) was preferred because it is the simplest model.

Obviously, the values of the parameters a and b in Eq. (5.9) are very important for the predicted residual activity. These parameters can also be estimated from data on the residual activity versus moisture concentration, which were obtained from the separate drying experiments above saturated aqueous salt solutions. This results in an estimate for $a = 0.071$ and $b = 0.355$ (dashed line in Fig. 5.4). When these values for a and b are used in the overall model to predict residual activity versus X in the same way as done for Fig. 5.7, the inactivation is slightly overestimated. At an inlet temperature of 30 °C, this overestimation in the residual activity ranges from 0.01 at $X = 0.5$ kg H_2O.(kg solids)$^{-1}$ to 0.11 at $X = 0.1$ kg H_2O.(kg solids)$^{-1}$. The scattering of the data points obtained with the separate drying experiments is in the same range. Therefore, further calculations are done with the a and b values estimated from a direct fit to the measured fluidized-bed drying data.

The values of *a* and *b* obtained from the fluidized-bed experiments were estimated under the condition that the moisture concentration profile inside the granulate was as calculated with the short-cut theory. But, due to an interval of time between the sampling of the granulate and the measurement of the residual activity (1-10 min), a certain equalization of the profile can occur. A characteristic diffusion time defined as $(R_C)^2/D_{eff}$ equals approximately 40 min at X=0.6 kg H_2O.(kg solids)$^{-1}$ and 400 min at X=0.1 kg H_2O.(kg solids)$^{-1}$ (for a ø1mm granulate at 30 °C with $D_{eff}=10^{-10}$ and 10^{-11} $m^2.s^{-1}$ respectively; Chapter 3). Therefore, equalization can partially occur in the fore-mentioned interval. During this equalization, the moisture concentration in the centre of the particle will decrease whereas the surface concentration will increase. Because of this, the *L. plantarum* cells in the centre of the particle will be further inactivated whereas the cells with a moisture concentration below the average moisture concentration, directly after drying, retain the same residual activity. However, an influence of such an interval of time on the residual activity was never observed experimentally. Apparently, the used experimental set-up is not sensitive enough to retrace such a behaviour. Simulations showed that a full equalization should be detectable experimentally. However, the time constant of the equalization process and the interval of time needed, explain that full equalization will not occur. The influence of profile equalization was therefore neglected.

When the water activity is considered as the key parameter in the dehydration inactivation, the same fitting procedure as described above can be applied. In that case, instead of 10 different moisture concentrations describing the moisture concentration profile, 10 different water activities describing the water activity profile inside the granulate are used. Because the granulate after drying is cooled down to 20 °C before the residual activity is measured, these water activities were calculated from the desorption isotherm at 20 °C. When the residual bacterial activity as a function of the local water activity was fitted with the arbitrary function:

$$\frac{A}{A_0} = a \cdot (a_w)^b \tag{5.10}$$

this resulted an a nearly linear dependency of A/A_0 on a_w (a=1 and b=0.9). When this relation is functional, a decreasing residual bacterial activity will be found by decreasing the water activity at a certain moisture concentration by lowering the temperature. However, lowering the water activity by cooling did not affect the residual activity, as can be seen in Table 5.1. The measured bacterial activities differ within 3% which is in the accuracy range of the method (Chapter 2).

Table 5.1 Influence of cooling on residual activity after drying *L. plantarum*-starch granulate (ø 2 mm) in a fluidized-bed dryer at 40 °C.

Drying time (min)	X kg H_2O. (kg solids)$^{-1}$	a_w-shift each 20 °C[a]	Residual Activity after cooling down to		
			40 °C	20 °C	1 °C
0	0.87	0	1	1	1
10	0.46	0.055	0.94	0.93	0.91
30	0.18	0.183	0.54	0.52	0.54
50	0.13	0.210	0.36	0.35	0.35
80	0.10	0.196	0.27	0.28	0.28

[a] based on the fitted sorption isotherms in Fig. 5.1

From these results it can be concluded that the moisture concentration is the essential parameter in the dehydration process and not the water activity. Although this conclusion is important from a fundamental point of view, it is not essential in the modelling of the inactivation of *L. plantarum* during drying. In a practical situation, and also in our experimental set-up, after drying the granulate is first cooled down to room temperature before rehydration and activity measurement. Therefore, if the water activity was the key parameter, it would always be determined by approximately the same desorption isotherm, namely the isotherm at room temperature. Because of this, no difference would be found by modelling the dehydration inactivation as a function of water activity or as function of moisture concentration.

5.4.3 Modelling the overall inactivation during drying

With the foregoing theory we are able to predict the residual activity of *L. plantarum* during a fluidized-bed drying process. With the drying model (Chapter 3) we can calculate the temperature T, the time from the start of the drying process t, the moisture concentration profiles inside the cylinders and the dimensionless flux F as a function of E. In each of the 10 shells inside the cylinder the average moisture concentration X is calculated. With T and X the thermal inactivation constant k_i in each shell is calculated (Chapter 4).

At this point, all the variables needed for evaluation of the thermal inactivation are known. The dehydration inactivation is determined from X and Eq. (5.9). In the simulation programme these calculations are executed at each E-step together with a numerical integration of Eq. (5.3) with a fourth-order Runge-Kutta routine (Press *et al.* 1986) in each shell in the cylinder. A block diagram of the complete simulation model is given in Fig. 5.5. The diagram describes the drying kinetics (left block), the thermal inactivation (middle block), the dehydration inactivation (right block) and the coupling of these descriptions (middle and right block) in order to predict the residual activity of *L. plantarum* during drying ($(A/A_0)_{overall}$). Each block is described in detail in the chapters mentioned.

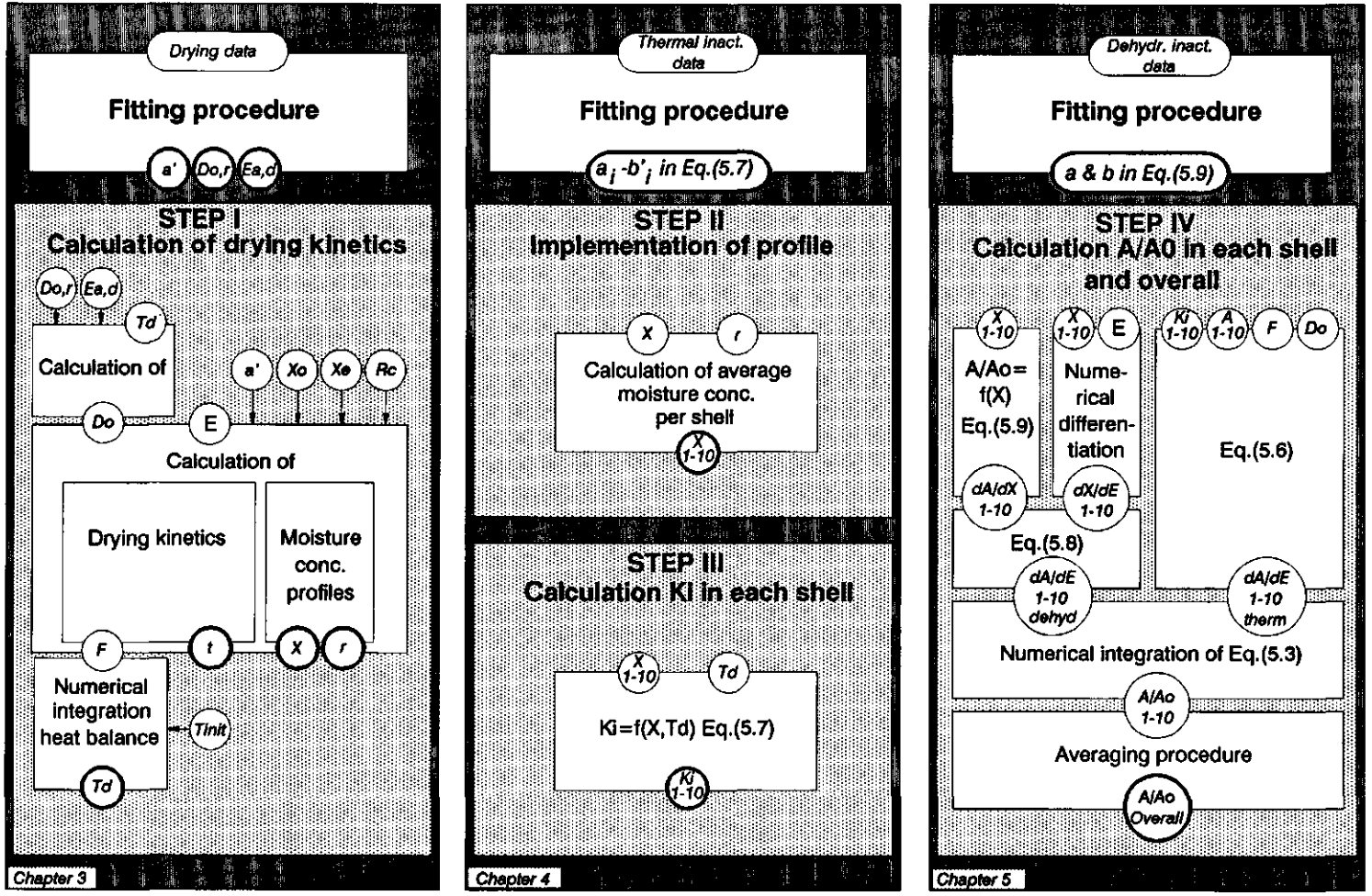

Fig. 5.5 A diagram of the complete simulation model. The variables in the upper and lower edge of each block show the primary input and output of a certain action, respectively. The most essential input constants are denoted with arrows. The primary output of each step is thick encircled.

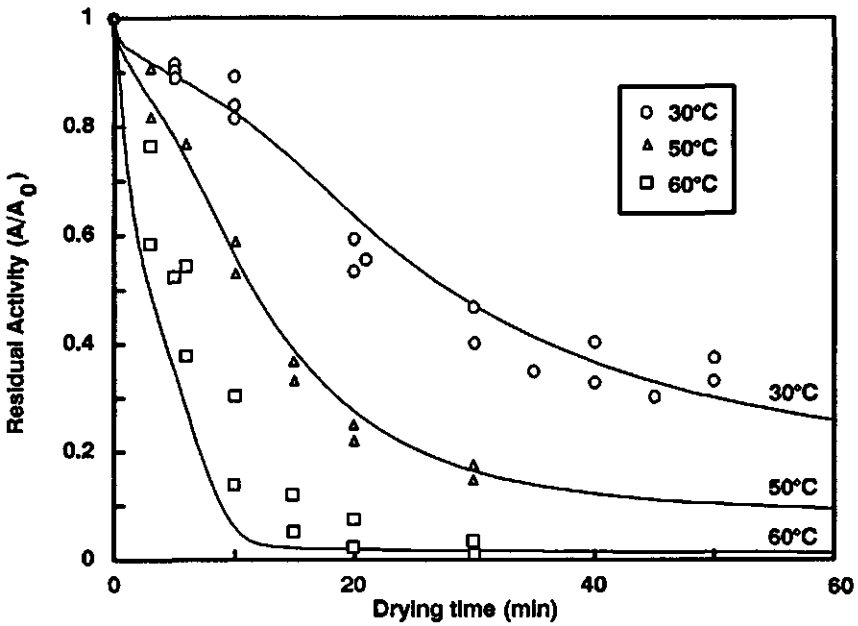

Fig. 5.6 The predicted overall (lines) and the measured (data points) residual activity during fluidized-bed drying of *L. plantarum*-starch granulate (ø 1 mm) at different inlet temperatures.

Fig. 5.6 gives a simulation output in time for three inlet temperatures of the fluidized bed. The most important simulation parameters can be found in Chapter 3 and the parameters of the dehydration inactivation model are given in Appendix 1. As can be seen from Fig. 5.6, a good prediction of the inactivation of *L. plantarum* during a fluidized-bed drying process is possible with this concept. At higher temperatures (>55 °C) the inactivation is slightly overestimated.

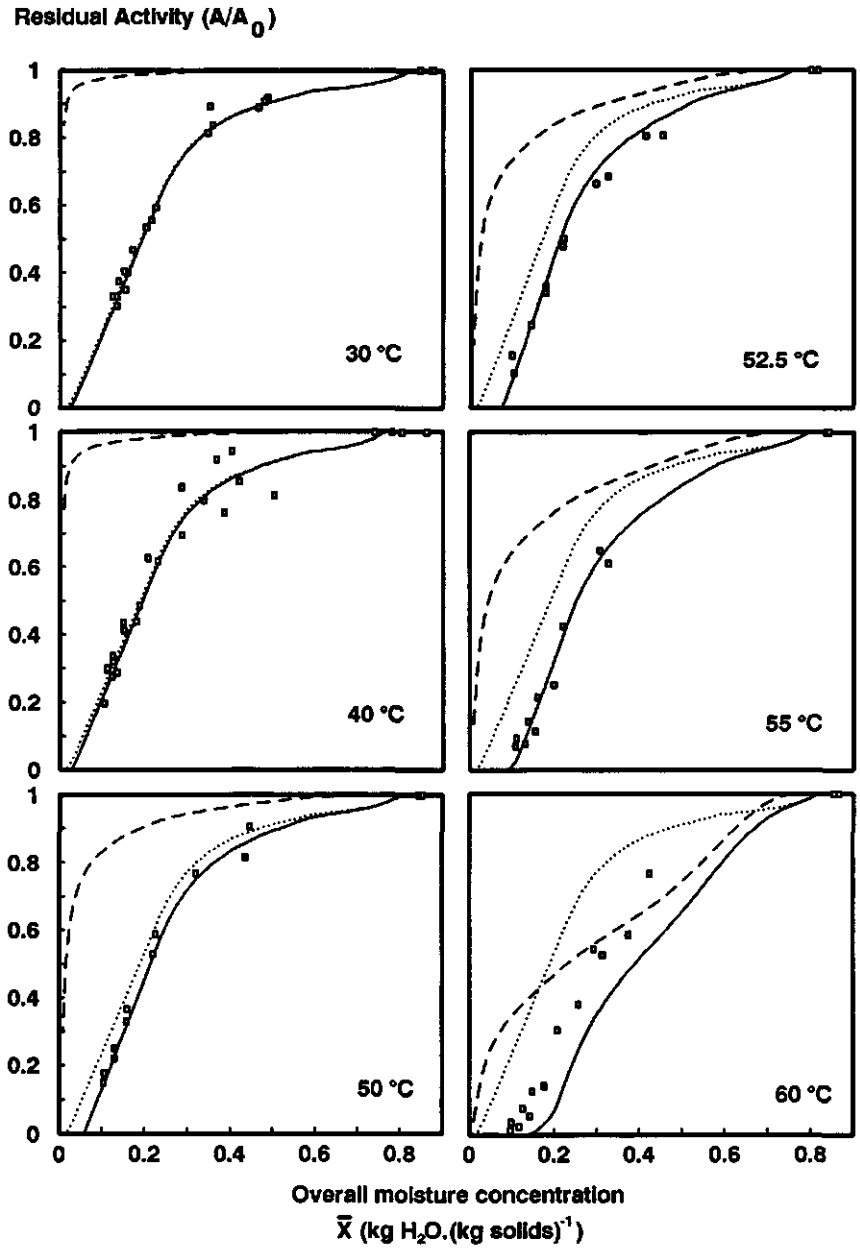

Fig. 5.7 The predicted overall (solid lines), thermal (dashed lines) and dehydration (dotted lines) inactivation as a function of overall moisture concentration together with the experimental data obtained by drying *L. plantarum*-starch granulate (ø 1 mm) at different temperatures.

Fig. 5.7 shows the residual activity as a function of overall moisture concentration at different air inlet temperatures of the fluidized bed. From Fig. 5.7 it is again clear that the inactivation is overestimated at higher temperatures. This overestimation can have several reasons. It is possible that the value of heat transfer coefficient α, which is calculated from the Nusselt-number in the fluidized bed and which is used in the calculation of the temperature of the granulate (Chapter 3), is too high. This will result in an overestimation of the temperature of the granulate, resulting in an overestimation of the thermal inactivation rate. This will be particularly manifest at higher drying temperatures and thus higher thermal inactivation rates.

Fig. 5.8 gives the influence of the variation of the heat transfer coefficient α, ranging from 5 to 0.2 times the originally calculated value (Chapter 3) for three different inlet temperatures of the fluidized bed. As can be seen from Fig. 5.8, this variation in α at 30 °C does not influence the predicted residual bacterial activity. At a temperature of 55 °C, the best prediction of the residual activity is made with the initially calculated α, whereas at 70 °C a reduction of α by a factor 0.4 forces the predicted residual activity through the measured points. Overestimation of α can explain the difference in predicted and measured residual activity at 70 °C. However, when this overestimation also holds true for a drying temperature of 55 °C, the predicted inactivation at this temperature is slightly underestimated.

Another, more obvious, explanation is that the first-order inactivation constants k_i at temperatures above 55 °C are overestimated. At these temperatures the k_i-values at $X > 0.4$ kg H_2O.(kg solids)$^{-1}$ were extrapolated from the measured data whereas at temperatures below 55 °C an interpolation between the data was used. We were not able to measure the high inactivation rates at temperatures above 55 °C and moisture concentrations above 0.4 kg H_2O.(kg solids)$^{-1}$ (Chapter 4).

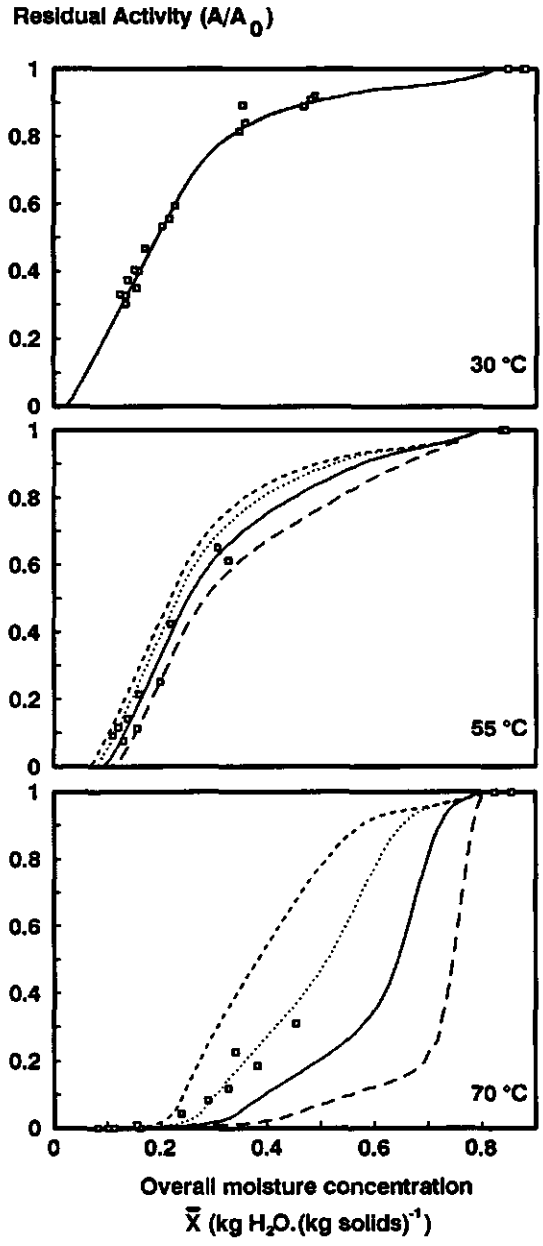

Fig. 5.8 The parameter sensitivity of the overall simulation model towards the heat transfer coefficient α with a simulation at different inlet temperatures of the fluidized bed. The data points show the experimental obtained values (ø 1 mm granulate). Variation in α with a factor 0.2 (dashed lines), 0.4 (dotted lines), 1 (no variation) (solid lines) and 5 (long dashed lines).

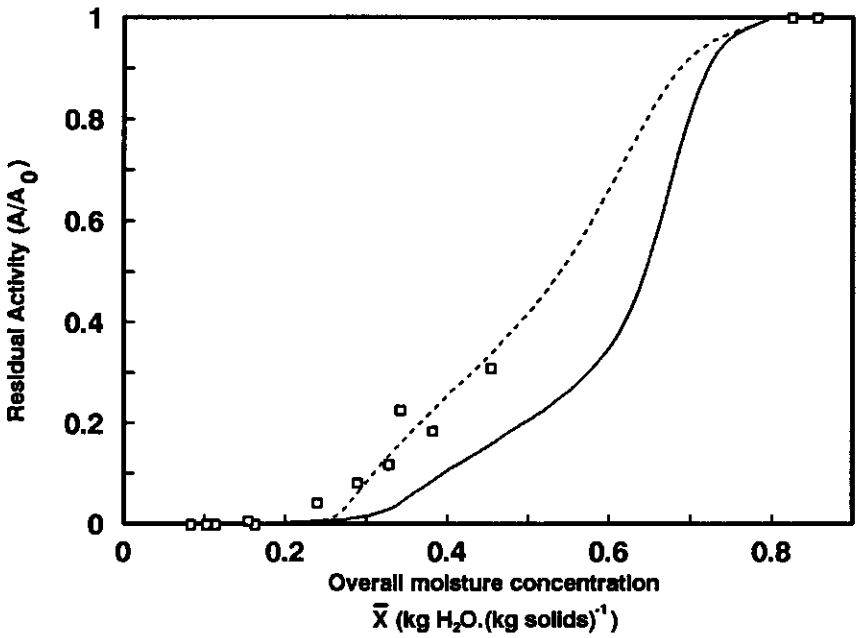

Fig. 5.9 The influence of variation in parameter a'_2 of the thermal inactivation model (Eq. (5.7)) on the predicted residual activity as a function of overall moisture concentration at a inlet temperature of the fluidized bed of 70 °C. The data points show the experimental obtained values (ø 1 mm granulate). Variation in a'_2 with factor 1.28 (dashed line) and 1 (no variation) (solid line).

To reveal the importance of the thermal inactivation at higher drying temperatures, the influence of variation of parameter a'_2 in the description of the first-order inactivation constant k_i (Eq. (5.7)) is shown in Fig 9. Variation of this parameter with a factor 1.28 will force the predicted inactivation line at a drying temperature of 70 °C through the measured points. This variation in a'_2 corresponds with a variation in k_i ranging from a factor 1.7 at X = 0.35 kg H_2O.(kg solids)$^{-1}$ (beginning of extrapolation to higher moisture concentrations) to a factor 5.4 at $X = X_0$ = 0.85 kg H_2O.(kg solids)$^{-1}$ (end of extrapolation). This required correction of k_i is acceptable if the extrapolation of the thermal inactivation description is kept in mind.

An alternative reason for the observed overestimation at higher temperatures could be an alteration in the dehydration inactivation model at these temperatures. However, this cannot be verified experimentally and is not likely considering the above-mentioned observations.

From these studies we can conclude that at moderate temperatures ($\leq$55 °C) the overall inactivation prediction is mainly influenced by the parameters *a* and *b* of dehydration description (Eq. (5.9)). At higher drying temperatures, the thermal inactivation becomes important and the parameters of the thermal inactivation description (Eq. (5.7)) and the heat balance (Chapter 3 and 4) also significantly influence the predicted residual activity. However, prediction of the residual activity at drying temperatures above 50 °C is of minor practical importance because of the high thermal inactivation rates and thus low residual bacterial activities.

5.5 CONCLUSIONS

The inactivation of *L. plantarum* during a fluidized-bed drying process can be described with two coupled inactivation mechanisms: thermal inactivation and inactivation due to dehydration. Our experiments show that the dehydration inactivation of *L. plantarum* depends on the reached moisture concentration only and is independent of the drying rate. It is clear that the dehydration inactivation is the most important inactivation mechanism at drying temperatures lower than 50 °C. Furthermore, the dehydration inactivation is related to the moisture concentration and not to the water activity of the system. To avoid thermal inactivation superimposed on the dehydration inactivation, low drying temperatures are recommended.

ACKNOWLEDGEMENTS

We would like to thank I. Wolters for his technical assistance by measuring the desorption isotherms and Dr. H.H. Beeftink and Dr. M.R. Smith for their helpful critical comments in the preparation of this manuscript. The financial support of Gist-brocades NV, Delft, The Netherlands, is gratefully acknowledged.

REFERENCES

Buléon A, Bizot H, Delage MM and Multon JL (1982) Evolution of Crystallinity and specific gravity of potato starch versus water ad- and desorption. Starch/Stärke 34: 361-366.

Coumans WJ (1987) Power law diffusion in drying processes. Ph.D. Thesis, Technical University Eindhoven, The Netherlands.

Franks F (1982) Water activity as a measure of biological viability and quality control. Cereal Foods World 27: 403-407.

Kapsalis JG (1987) *Influences of hysteresis and temperature on moisture sorption isotherms.* In: Rockland LB and Beuchat LR (eds) Water activity: Theory and applications to food (pp. 203-207). Marcel Dekker, New York.

Kerkhof PJAM and Schroeber WJAH (1974) *Theoretical modelling of the drying behaviour of droplets in spray dryers.* In: Spicer A (ed) Advances in preconcentration and dehydration of food (pp. 349-397). Applied Science Publishers, London.

Kuts PS and Tutova EG (1983) Fundamentals of drying of microbiological materials. Drying Techn. 2: 171-201.

Levenspiel O (1972) Chemical reaction engineering. John Wiley & Sons, New York.

Liou JK (1982) An approximate method for nonlinear diffusion applied to enzyme inactivation during drying. Ph.D. Thesis, Agricultural University Wageningen.

Liou JK and Bruin S (1982a) An approximate method for the nonlinear diffusion problem with a power relation between diffusion coefficient and concentration - I. Computation of drying times. Int. J. Heat Mass Transfer 25: 1209-1220.

Liou JK and Bruin S (1982b) An approximate method for the nonlinear diffusion problem with a power relation between diffusion coefficient and concentration - II. Computation of concentration profiles. Int. J. Heat Mass Transfer 25: 1221-1229.

Liou JK, Luyben KChAM and Bruin S (1985) A simplified calculation method applied to enzyme inactivation during drying. Biotechn. Bioeng. 27: 109-116.

Luyben KChAM, Liou JK and Bruin S (1982) Enzyme degradation during drying. Biotechn. Bioeng. 24: 533-552.

Press WH, Flannery BP, Teukolsky SA and Vetterling WT (1986) Numerical recipes. Cambridge University Press, Cambridge, USA.

Spiess WEL and Wolf W (1987) *Critical Evaluation of methods to determine moisture sorption isotherms.* In: Rockland LB and Beuchat LR (eds) Water activity: Theory and applications to food (pp. 224-229). Marcel Dekker, New York.

Tutova EG (1982) *Heat engineering principles of drying microbiological materials.* In: Ashworth JC (ed) Proceedings of the third international drying symposium. Vol. 2 (pp. 239-251). Drying Research Limited, Wolverhampton, UK.

Van den Berg C and Leniger HA (1978) The water activity of foods. Wageningen Agricultural University Papers, 78-15, Agricultural University Wageningen, The Netherlands.

Van den Berg C (1981) Vapour sorption equilibria and other water-starch interactions; a physico-chemical approach. Ph.D. Thesis, Agricultural University Wageningen, The Netherlands.

Weldring JAG, Wolters I and Van den Berg C (1975) Apparatus for the accurate determination of the isotherms for water vapour sorption in foods. Wageningen Agricultural University Papers, 75-18, Wageningen Agricultural University, The Netherlands.

Wijlhuizen AE, Kerkhof PJAM and Bruin S (1979) Theoretical study of the inactivation of phosphatase during spray-drying of skim-milk. Chem. Eng. Sci. 34: 651-660.

Yamamoto S, Musahiko A, Nakodo H and Sano Y (1985) *Enzyme inactivation during drying of a single droplet.* In: Toei R and Mujumbar AS (eds) Drying '85 (pp. 330-337) Hemisphere Publishing Corporation, Washington.

Young JF (1967) Humidity control in the laboratory using salt solutions-A review. J. Appl. Chem. 17: 241-245.

Zimmermann K (1987) Einflussparameter und mathematische modellierung der schonenden trocknung von starterkulturen. Fortschr.-Ber. VDI (Reihe 14 no36), VDI-Verlag, Düsseldorf.

Zimmermann K and Bauer W (1987) Modellierung der trocknung von hefen im wirbelschichttrockner. Chem.-Ing.-Tech. 59: 350-351.

APPENDIX 1

Parameters desorption isotherms
The desorption isotherm at 313 K (Kapsalis 1987):

$$(a_w)_{313} = \frac{2+(W_m/X-1)C-([2+(W_m/X-1)C]^2-4(1-C))^{0.5}}{2K(1-C)} \qquad (5.11)$$

with: $W_m = 7.94\times10^{-2}$ kg H_2O.(kg solids)$^{-1}$
$C = 2.80\times10^{1}$
$K = 8.59\times10^{-1}$

For other temperatures:

$$\ln\frac{(a_w)_{313}}{(a_w)_T} = \frac{\Delta H}{R}\left[\frac{1}{313}-\frac{1}{T}\right] \qquad (5.12)$$

with (Van den Berg and Leniger 1978):

$$\Delta H = \beta_1 X^{\beta_2} \quad \text{for } X \geq 0.1 \text{ kg } H_2O.(\text{kg solids})^{-1} \qquad (5.13a)$$

$$\Delta H = \beta_1 0.1^{\beta_2} \quad \text{for } X < 0.1 \text{ kg } H_2O.(\text{kg solids})^{-1} \qquad (5.13b)$$

with $\beta_1 = -5.53\times10^{2}$
$\beta_2 = -1.79$

Parameters dehydration inactivation model (Eq. (5.9))
$a = 0.022$
$b = 0.356$

For a list of the additional simulation parameters the reader is referred to Chapter 3.

NOMENCLATURE

A	activity of *L. plantarum* cells (Chapter 2 and 4)(in simulation $0 \leq A \leq 1$ and dimensionless)	$\Delta pH.min^{-1}.g^{-1}$
A_0	activity of *L. plantarum* cells at start of drying process (in simulation $A_0 = 1$ and dimensionless)	$\Delta pH.min^{-1}.g^{-1}$
a	parameter to describe the dehydration inactivation (Eq. (5.9))	
a'	exponent in power law diffusion	-
a_i, a'_i	parameters to describe the thermal inactivation (Eq. (5.7))	
a_w	water activity	-
b	parameter to describe the dehydration inactivation (Eq. (5.9))	
b_i, b'_i	parameters to describe the thermal inactivation (Eq. (5.7))	
D_0	short-cut diffusion coefficient parameter (Chapter 3)	$m^2.s^{-1}$
$D_{0,r}$	diffusion coefficient fit parameter	$m^2.s^{-1}$
E	drying efficiency	-
$E_{a,d}$	activation energy of diffusion	$J.mol^{-1}$
$E_{a,i}$	activation energy of inactivation	$J.mol^{-1}$
F	dimensionless flux	-
k_i	first-order thermal inactivation constant	s^{-1}
k_∞	frequency factor	s^{-1}
p,q	parameters to describe the thermal inactivation (Eq. (5.7))	
R_C	radius of cylinder (drying granulate)	m
r	radial distance	m
t	time	s
T	absolute temperature	K
T_d	temperature dispersed phase (drying granulate)	K
T_{init}	initial temperature	K
X	local moisture concentration	kg H_2O.(kg solids)$^{-1}$
$\overline{X}$	average (overall) moisture concentration	kg H_2O.(kg solids)$^{-1}$
X_0	initial moisture concentration	kg H_2O.(kg solids)$^{-1}$
X_e	equilibrium moisture concentration at infinite drying time	kg H_2O.(kg solids)$^{-1}$

Greek symbols

α	heat transfer coefficient	$J.s^{-1}.m^{-2}.K^{-1}$
β_1,β_2	fit parameters in desorption isotherm	-
ΔH	isosteric net heat of sorption	$J.mol^{-1}$
τ_d	time constant of the drying process	s

Subscripts

dehydr	dehydration inactivation
therm	thermal inactivation
tot	total inactivation

CHAPTER 6

GENERAL DISCUSSION

In the previous chapters it is shown that inactivation of *Lactobacillus plantarum* during fluidized bed drying is caused by two mechanisms: thermal and dehydration inactivation. In this chapter the consequences of these inactivation mechanisms, methods which influence dehydration inactivation and data relating to the physiological mechanism behind dehydration inactivation are discussed.

6.1 THERMAL AND DEHYDRATION INACTIVATION MECHANISMS

To minimize thermal and dehydration inactivation it is important to understand the physiological mechanisms involved in these inactivation processes. In the literature, there is an extended speculation concerning possible inactivation mechanisms. These are summarized in the next sections and are followed by experimental evidence of two different mechanisms for thermal and dehydration inactivation.

6.1.1 Thermal inactivation mechanisms

Allwood and Russel (1970), Corry (1973), Tomlins and Ordal (1976) and, more recently, Gould (1989) have written reviews on the mechanisms of thermal inactivation of vegetative bacterial cells. From these reviews it is clear that thermal inactivation is still not fully understood. It is generally assumed that there are four main primary sites for thermal damage that can cause injury or death. These sites are **1)** DNA, **2)** RNA (including ribosomes (rRNA)), **3)** proteins (enzymes), and **4)** cell membrane. It is of note that, if the cell wall is also added (Allwood and Russel 1970), then most of the essential bacterial cell components are named.

It is clear that most, if not all cell components can be subjected to thermal damage. It is therefore extremely difficult to detect a single key factor that causes cell injury or death and most likely, a multiplicity of effects are involved. DNA (Gould 1989) and (r)RNA (Allwood and Russel 1970) are considered the most probable targets for heat inactivation. Gould (1989) stated that when less critical components (proteins, cell membrane and cell wall) are not damaged, then DNA or (r)RNA damage will not lead to lethal injury. According to Gould, a combination of damage to critical and less critical components will lead to cell death.

6.1.2 Dehydration inactivation mechanisms

Mechanisms of dehydration inactivation are mainly summarized in reviews on freeze-drying and yeast drying (see Chapter 1). It is not always clear if inactivation during convective drying is due solely to dehydration mechanisms, because thermal inactivation can occur simultaneously. Also during freeze-drying two processes, freezing and drying, are occuring which can both influence the inactivation state.

As with thermal inactivation, dehydration inactivation may affect a number of different cellular components. A number of publications reported damage to DNA/RNA, intracellular proteins and the cell wall. However, cytoplasmatic membrane damage is generally considered as the main mechanism of dehydration damage (Lievense and Van 't Riet 1991). Crowe *et al.* (1987, 1990) proposed and reviewed a general theory about membrane damage due to dehydration.

Several authors have reported leakage of cellular components (cations, nucleotides, enzymes, proteins, amino acids or 'UV-absorbing materials') out of (freeze-)dried cells during rehydration (Wagman 1960; Webb 1961; Heckly 1985; Brennan *et al.* 1986; Beker and Rapoport 1987). It can be expected that damage to the cell membrane will lead to leakage of intracellular components. Yet it appears that the release of 'UV-absorbing materials' is not quantitatively related to inactivation. Brennan *et al.* (1986) reported that membrane damage after freeze-drying of *Lactobacillus acidophilus* was not severe enough to release significant amounts of β-galactosidase, while the release of other UV-absorbing materials was observed. Wagman (1960) found a significantly higher release of UV-absorbing materials from freeze-dried *Escherichia coli* and *Serratia marcescens* cells when compared to undried cells. Release was strongly dependent on the rehydration medium used but was not related to the measured survival. Webb (1961) reported that the release of UV-absorbing materials from *E. coli* cells with a high survival (71%) after freeze-drying, was three times higher than the release from cells with a low survival (1%). Morichi *et al.* (1967) found that the materials released from rehydrated freeze-dried *L. bulgaricus* mainly consisted of RNA-related substances but also in this work, no correlation was found between survival and the extent of leakage.

6.1.3 Experimental evidence for membrane damage

It can be concluded that the two mechanisms, thermal inactivation and dehydration inactivation, have been widely studied but are not yet fully understood. Every cell component can be influenced by high temperatures or low water concentrations. After drying, alterations of the cell membrane are nearly always observed. It is likely, that damage to the cell membrane is the key factor in the dehydration inactivation of *L. plantarum*.

One objective of this research project was to investigate the mechanism of dehydration damage. We attempted to quantify dehydration damage by the measurement of leakage of intracellular components out of the cells after dehydration/rehydration. Lactate dehydrogenase activity (Vassault 1983), total protein concentration (Bradford 1976) and UV-absorbance (Brennan *et al.* 1986) of the rehydration buffer were measured. As found by other authors (see above) with these techniques, it was impossible to obtain a reproducible and quantitative relation between either the measured quantity and the residual activity or the final moisture

concentration. With the help of a newly developed technique (Lammers and Noomen 1990) a distinction could be made between dehydration and thermally inactivated *L. plantarum* cells. The method is based on hydrolysis of DNA by DNase.

The hypothesis behind this method is that inactivated dried cells have damaged cell membranes and/or cell walls which will be permeable for DNase. When these damaged cells are incubated with DNase then hydrolysis of the intracellular DNA will occur. In cells with an undamaged cell membrane, the intracellular DNA remains unaffected. The hydrolysed DNA will diffuse into the medium. The amount of hydrolysed DNA in the medium after a certain incubation time of the *L. plantarum* cells with DNase, quantifies the amount of cells with a damaged cell membrane/cell wall. The principle of this process is summarized in Fig. 6.1.

Reproducible results could not be obtained when the above method was applied to the *L. plantarum*-starch paste dried in the fluidized bed dryer. This is probably due to interaction of starch with either the cells in the dried *L. plantarum*-starch paste or with DNA and/or DNase. The results reported in this section were obtained using cell suspensions without starch which were dried above saturated aqueous salt solutions. In all the experiments reported, with the exception of the activity measurements, a buffer solution according to Jenness and Koops (1962) (JK-buffer) was used instead of the KPi-buffer (Chapter 2).

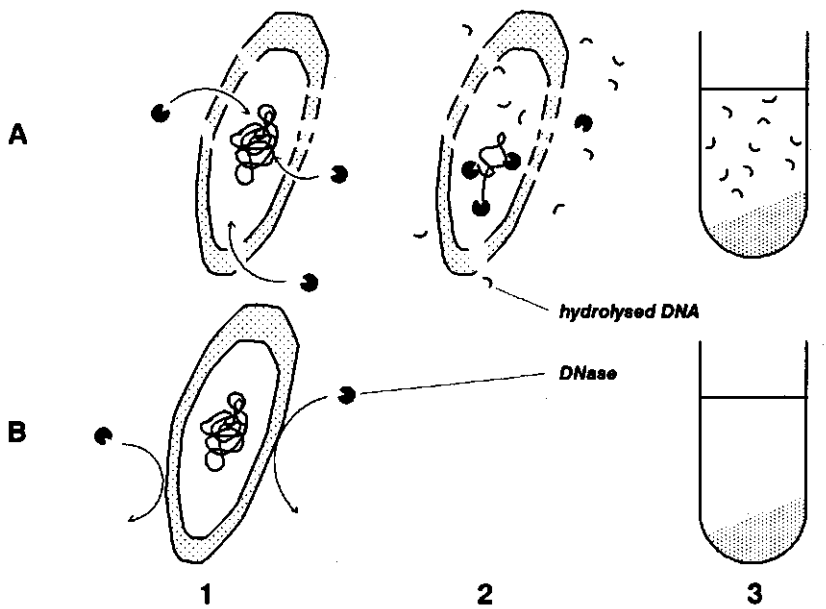

Fig. 6.1 The principle of the DNA/DNase method for the measurement of cells damaged by dehydration. A: cells with cell membrane/cell wall damage. B: cells without cell membrane/cell wall damage. 1: after addition of DNase. 2: during incubation. 3: after centrifugation.

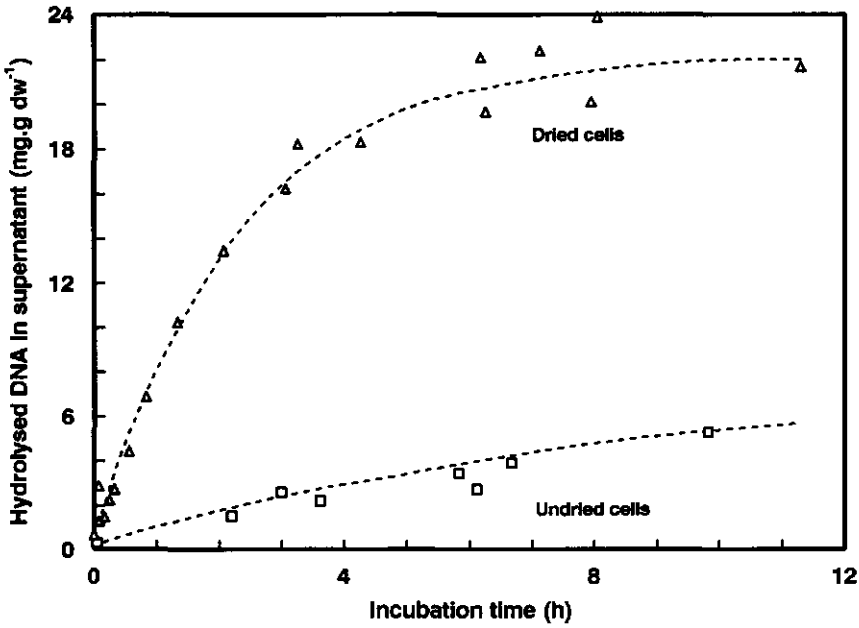

Fig. 6.2 The release of hydrolysed DNA by a dried and undried cell suspension in JK-buffer at 37 °C. The cell suspension (±85 mg biomass dry weight (dw).ml^{-1}) was vacuum-dried above a saturated aqueous LiCl solution (40h; 4 °C; Chapter 5) to 0.077 kg H_2O.(kg solids)$^{-1}$. Before incubation with DNase the cell suspension was rehydrated and/or diluted to 3-4 mg dw.ml^{-1}. The DNase concentration (DNase I, Grade II, Boehringer Mannheim) amounted to ± 80 mg DNase.(g dw)$^{-1}$. After DNase-incubation, the cell suspension was centrifugated (10 min, 11.500 rpm; Heraeus Biofuge A) and the (hydrolysed) DNA concentration in the supernatant was measured according to Burton (1956) and Herbert *et al.* (1971). The amount of (hydrolysed) DNA in the supernatant was calculated with a calibration curve obtained with calf-thymus DNA (Serva).

In Fig. 6.2 the results of incubation of a dried and an undried *L. plantarum* cell suspension with DNase are given. It is clear that dried cells release significantly more DNA in the presence of DNase than undried cells. However, it is noteworthy that undried cells also release DNA. It is likely that this release is either due to damage to the cell membrane during incubation with DNase, or to activity of endogenous DNase. Furthermore, it can be seen that within the incubation time used, no asymptotic maximum value is reached for released hydrolysed DNA. In subsequent experiments described in this chapter, a DNase incubation time of 6 h was used.

In order to validate the DNA/DNase method, the amount of DNA in the supernatant and in the cell pellet after DNase-incubation and centrifugation was measured. The sum of these amounts was compared with the amount of DNA in the total suspension after cell lysis. The cell suspensions were vacuum-dried above different saturated aqueous salt solutions.

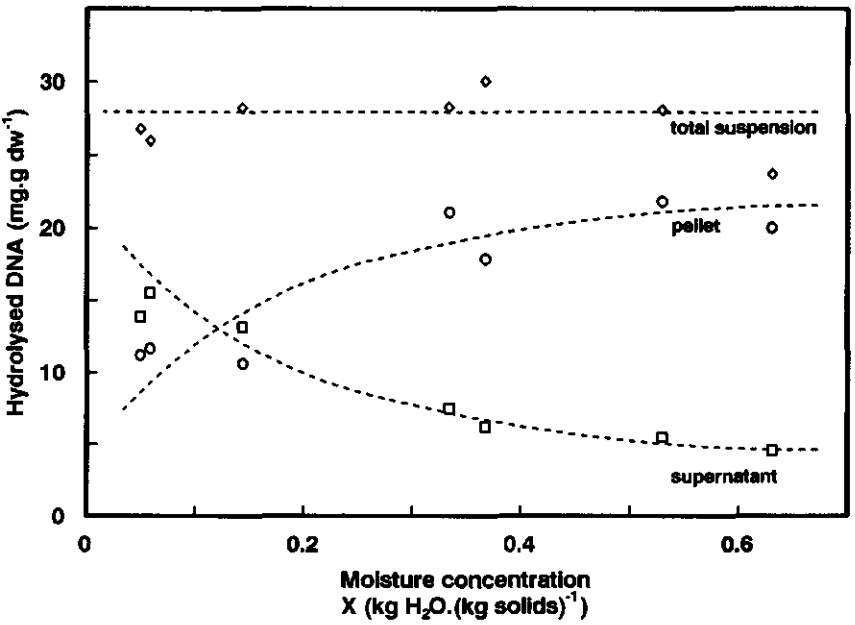

Fig. 6.3 The amount of hydrolysed DNA in supernatant and cell pellet after 6 h of incubation with DNase and the amount of hydrolysed DNA in the total cell suspension as a function of the final water concentration. Cell suspensions were vacuum-dried (40 h; 4 °C) above P_2O_5 and above saturated aqueous solutions (Chapter 5) of LiCl, $MgCl_2$, $CuCl_2.2H_2O$, NaCl, $(NH_4)_2SO_4$ and KCl to moisture concentrations of 0.050, 0.059, 0.143, 0.334, 0.368, 0.530 and 0.631 kg H_2O.(kg solids)$^{-1}$, respectively. The total amount of DNA in the cell suspension and the amount of DNA in the pellets after centrifugation were measured after cell lysis: After dilution to ± 1 mg dw.(ml)$^{-1}$, the suspensions were incubated (60 min, 37 °C) with lysozym (130 mg.(g dw)$^{-1}$; Boehringer Mannheim) followed by incubation with NaOH (0.3 M, 20 min, 37 °C), neutralization with HCl and incubation with DNase (20 min, 37 °C). Further experimental conditions are given in Fig. 6.2.

In Fig. 6.3 the amount of hydrolysed DNA measured (after 6 h incubation with DNase) is given as a function of the final water concentration after drying. It is clear that the amount of DNA in the supernatant after drying and DNase incubation increases with decreasing water concentration. Furthermore, it can be seen that the amount of DNA in the cell pellet decreases, whereas the amount of hydrolysed DNA in the supernatant increases. According to the hypothesis, this increase should be due to the increase in hydrolysed DNA in the medium, indicating that the cell membrane/cell wall is damaged during dehydration. The balance between (hydrolysed) DNA content of the supernatant, the cell pellet and the total cell suspension in these experiments indicates that the DNA/DNase-method is reasonably accurate. However, fluctuations in the results limit a precise quantitative application.

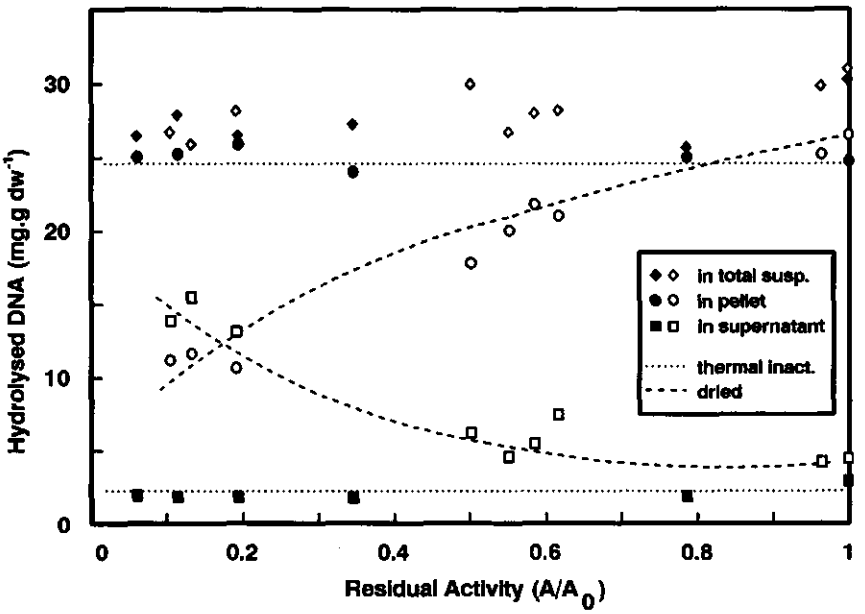

Fig. 6.4 The amount of hydrolysed DNA in supernatant and cell pellet after 6 h of incubation with DNase after vacuum-drying (open symbols) and thermal inactivation (closed symbols) as a function of the residual activity. Cells with a residual activity of 1 indicates untreated cells. Cells with a residual activity of 0.96 were vacuum-dried (40 h; 4 °C) above a saturated aqueous salt solution of $CuSO_4.5H_2O$ to a moisture concentration of 5.54 kg H_2O.(kg solids)$^{-1}$. Other dried cells were treated as described at Fig. 6.3. Thermal inactivation was carried out with cell suspensions with a concentration of 30 mg dw.ml^{-1}. This suspension was incubated for 0, 5, 10, 15, 20 and 31 s at 60 °C. After this incubation one part of the suspension was used for the activity measurement (Chapter 2) and one part was used for the DNA/DNase analysis (diluted to 3-4 mg dw.ml^{-1}). Further experimental conditions are given in Fig. 6.2 and Fig. 6.3.

In Fig. 6.4 a relation is shown between the release of hydrolysed DNA (after 6 h incubation with DNase) and residual activity of the *L. plantarum* cells. The activity of the cell suspension was lowered in two ways. Firstly, by vacuum-drying above different saturated aqueous salt solutions at 4 °C (Legend Fig. 6.3), in which case, inactivation will be caused solely by dehydration. Secondly, by thermal inactivation of the *L. plantarum* cell suspensions at 60 °C for different time values. It is clear that the two inactivation mechanisms act differently on the cell. The dehydration inactivation causes cell membrane/cell damage: the amount of released DNA increases with decreasing residual activity. However, when the cells are thermally inactivated this behaviour is not observed: the amount of released DNA is constant while the residual activity decreases. It can be concluded, therefore, that thermal inactivation (at 60 °C) does not cause membrane damage while dehydration inactivation does. This conclusion is an important support for the existence of the two inactivation mechanisms during drying as described in previous chapters.

As mentioned above, during drying of the cells at 4 °C, dehydration inactivation will be the only inactivation mechanism. It can be expected, therefore, that at a residual activity of (for example) 20%, a final (100-20=) 80% of the total DNA-pool will be hydrolysed and will diffuse into the medium. After complete lysis of the *L. plantarum* cells (Legend Fig. 6.3), the total hydrolysed DNA amount is measured at ± 30 mg DNA.(g dw)$^{-1}$. Therefore, 80% of damaged cells must result in approximately 24 mg DNA.(g dw)$^{-1}$ in the supernatant. Fig. 6.4 shows that this value is in fact lower. From Fig. 6.2 it is clear that after 6 h of incubation of the cells with DNase an asymptotic value is not reached. This may mean that not all the DNA in the damaged cells has been hydrolysed and/or that not all the hydrolysed DNA has diffused into the medium. It is noteworthy that after even longer incubation times, no asymptotic value is reached (Fig. 6.2). Furthermore, undamaged cells do release some DNA. These observations indicate that a quantitative relation between the amount of hydrolysed DNA in the supernatant and the amount of damaged cells can not be given, altough a qualitative relation certainly exists. These observations are the basis for our further research.

6.2 INFLUENCING THE DEHYDRATION INACTIVATION

As mentioned in Chapter 1, a number of factors, besides that of the drying process, can be important for the inactivation of bacteria during drying. During the experiments described in the previous chapters, conditions and procedures were standardized to remove the influence of these factors. For example, the inoculation material, the growth conditions and the harvesting time (described in Chapter 2), and the washing steps, concentration steps and the granulation process (described in Chapter 3) were standardized. The rehydration process after drying (Chapter 4) was also standardized. This was necessary in order to obtain reproducible results.

Apart from the research described in this thesis, several studies were carried out which consider the influence of variations in the process steps before and after drying. Of these variations, the influence of growth conditions was the most extensively studied. The main findings of this study are reported in this section.

6.2.1 Bacterial growth curve

In Fig. 6.5 a growth curve is given of *L. plantarum*, together with the glucose and lactate concentrations in the growth medium.

Under standard conditions, *L. plantarum* cells were harvested at the moment when the glucose was consumed. This was measured with glucose test sticks (Chapter 2). The results obtained with this method correspond with the glucose concentration measured with HPLC. The harvesting time was normally 18 (±0.5) h after inoculation. From Fig. 6.5 it is apparent that this time corresponds with a point in the early stationary growth phase.

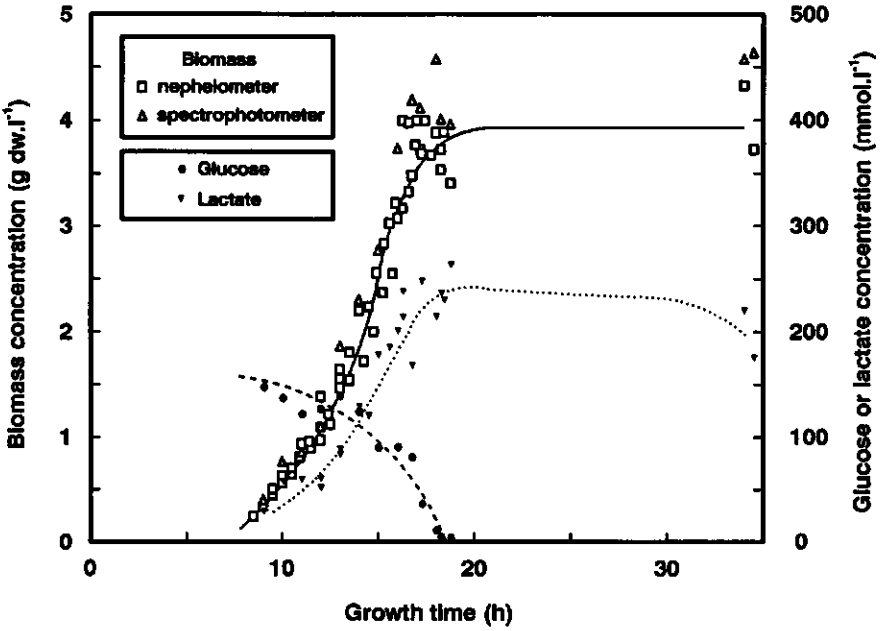

Fig. 6.5 Growth curve of *L. plantarum* (solid line) with glucose concentration (dashed line) and lactate concentration (dotted line). Curves were obtained in a 1.5-l laboratory fermentor under standard growth conditions (Chapter 2). Supplemented MRS-medium (Difco) was used as growth medium (Chapter 3). Biomass concentrations were estimated with a nephelometer (Unigalvo 20, Evans Electroselenium Ltd, Essex, UK) and a spectrophotometer (660nm; LKB Biochrom 4050, Cambridge, UK), both with sterilized growth medium as reference. Glucose and lactate concentration were determined with HPLC (Chapter 2).

6.2.2 Variation in harvesting time

To discover the importance of harvesting time, cells were dried that were harvested before and after the standard time of 18 h. The residual activity of these different cells is given as a function of moisture concentration in Fig. 6.6.

Firstly, it is remarkable that the residual activities of *L. plantarum* cells measured in this experiment, harvested under normal conditions (18h), are significantly larger than the values reported in the previous chapters. For comparison, the overall model prediction (30 °C; Fig. 5.7) is plotted as a dotted line in Fig. 6.6. Because of the large volumes required for these experiments, another fermentor than that reported in Chapter 2 was used. It is probable, that different conditions (such as sterilization conditions) causing changes in the growth medium, could have influenced the dehydration resistance. Furthermore, another MRS-medium batch and/or changes in the inoculation material may have influenced the results.

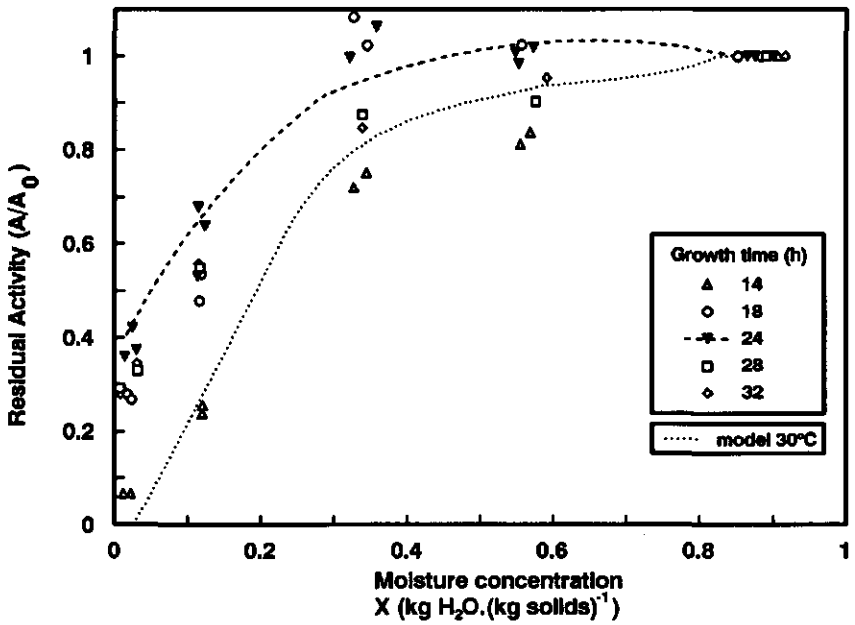

Fig. 6.6 The residual activities as a function of the overall moisture concentration at different harvesting times. The dotted line indicates the model prediction (30 °C). Three different harvesting times were obtained with one batch culture (3-l laboratory fermentor, Biotec FL103, Stockholm, Sweden). Samples with moisture concentrations below 0.1 kg H_2O.(kg solids)$^{-1}$ were obtained by vacuum-drying (subsequent to fluidized bed drying) of the granulates (ø 2mm) above P_2O_5 in a stainless steel desiccator (Chapter 4) at 4 °C for 5 days. Further experimental conditions were as described in Chapter 3.

An important conclusion from these observations is that the harvesting time influences the dehydration resistance significantly. Cells harvested in the exponential growth phase (14 h) are less resistant to dehydration than cells harvested in the early or late stationary growth phase. It seems that an optimum exists, which is located after the early stationary growth phase. Cells with a harvesting time of 24 h have a higher survival rate than cells with a harvesting time of 18, 28 or 32 h.

These results also indicate that the values of the parameters *a* and *b* of the dehydration inactivation model (Eq. (5.9)) depend strongly on the harvesting time and on other, yet unknown, factors. For example, when *a* and *b* are estimated with the 18 h harvesting data reported in Fig. 6.6, significantly lower values of *a* and *b* are obtained than are reported in Chapter 5. Furthermore, the residual activity at the low moisture concentrations obtained with additional drying above P_2O_5 indicates that the value of *a* may even be negative. The values of *a* and *b* in Chapter 5 are therefore only valid under the strictly controlled experimental conditions as were used for their estimation.

It can be concluded that for optimization of the residual activity of *L. plantarum* after drying, the influence of harvesting time and other factors have to be examined. For a review concerning possible factors that can influence the dehydration resistance, the reader is referred to Lievense and Van 't Riet (1991).

6.3 DRYING PROCESS CONDITIONS

6.3.1 Dehydration inactivation

From this thesis it is clear that during drying of *L. plantarum* thermal and dehydration inactivation play an important role. It seems that dehydration inactivation is less important during drying of enzymes and yeast than during drying of bacteria. Indeed, a model which includes only thermal inactivation may be sufficient for a prediction of the residual activity of enzymes and yeast (see Chapter 1). It is likely, that for enzymes and yeasts, dehydration inactivation occurs at moisture concentrations lower than those at which biological activity is already preserved. Because the drying process has usually stopped before these moisture concentrations are reached, dehydration inactivation is not observed.

Josic (1982) and Kuts and Tutova (1983) described a form of dehydration inactivation during drying of yeast and bacteria. They used the term 'critical water concentration', below which the cell was inactivated. It is most likely, that dehydration inactivation will not occur at a single water concentration but within a water concentration range (Chapter 5). This may be due to biological variations in the cell population. Josic, and Kuts and Tutova mentioned that bacterial cells should not be dried to a water concentration lower than that of the lower inflection point of the sorption isotherm. This statement is based on the assumption that below this point, the structural 'bound' water will be removed, causing severe damage to cell proteins and the cell membrane. In Chapter 5 it is shown that the dehydration inactivation for *L. plantarum* immobilized in potato starch, already starts at a water concentration of 0.35 kg H_2O.(kg solids)$^{-1}$. The lower inflection point of the sorption isotherm is located at a much lower value (Fig. 5.1). The calculations in Chapter 5 provide a more straightforward insight than the use of a lower inflection point on the sorption isotherm and the water concentration profile inside the particle is also taken into account.

It can be assumed that because of the large heat conductivity, the temperature profile inside the particle can be neglected (Chapter 3). However, this is certainly not the case for moisture concentration profiles. As mentioned in Chapter 1, it is possible that cells at the surface are already inactivated by dehydration, whereas cells in the interior of the particle are still unaffected. As a result of this, relationships in which the water concentration profiles are not taken into account, such as those described by Valdez *et al.* (1985) and Divies *et al.* (1990), must be considered critically. This is especially important in the early stage of the drying process were water concentration profiles may be steep.

6.3.2 Influence of drying rate

Although little experimental evidence regarding the effects of the drying rate on inactivation of bacteria exists, there is much speculation. One can expect that if bacteria are exposed to adverse conditions (high temperatures) or detrimental reactions (oxidation, acid production), then a high drying rate which shortens the drying process is favourable (Labuza *et al.* 1970; Comings *et al.* 1977; Espina and Packard 1979). A side effect of an initially high drying rate can be the formation of a dry shell at the surface of the drying particle, which will decrease the overall drying rate. This will render the moisture-temperature history of the cells inside the particle uncontrollable (Josic 1982). Only a few authors have discussed the influence of the drying rate itself. Krallish *et al.* (1986) found that low drying rates gave high NADH-dehydrogenase levels in yeast. These high NADH-dehydrogenase levels resulted in higher ATP concentrations in the cells which enhance the recovery during rehydration. Furthermore, it was reported by Marino *et al.* (1989) and Pearce *et al.* (1989), that yeast cells could synthesize trehalose not only during unfavourable growth conditions, but also during drying. Of course, neither the NADH-dehydrogenase concentration nor the trehalose concentration can be increased instantaneously. This may explain the low survival rate when yeast is spray dried. Bullock and Lightbrown (1947) and Zimmermann (1987) believed that inactivation of bacteria during drying was probably due to the rapid removal of water. Kuts and Tutova (1983) mentioned that the rate of drying must be regarded as one of the most important factors that influences the survival. Kuts and Tutova carried out fluidized bed drying experiments with *Rhizobium pisum* and reported that a two-fold decrease in drying rate in the constant rate period gave a five-fold higher survival. However, drying temperatures were not reported and because the initial drying rate was varied by initial water concentration, the moisture-temperature history of the cells could also have influenced the survival. Nevertheless, the authors concluded from this experiment that for drying of bacterial cells, high drying rate methods have to be avoided. In Chapter 5 it is shown that large differences in drying rate, achieved by drying above saturated aqueous salt solutions, in a fluidized bed and in a laboratory spray dryer, did not affect the inactivation of *Lactobacillus plantarum*. At lower temperatures (<50 °C) the residual activity was only determined by the water concentration reached. However, in view of these sparse results from isolated experiments, no general conclusion about the influence of the drying rate itself can be drawn and further research on this subject is necessary. If the dehydration rate is found to be important, then the drying process must be chosen accordingly.

6.3.3 Choosing a drying process

When one is interested in the production of a dried bacterial starter concentrate at low cost, convective drying is unavoidable. One of the three methods mentioned in Chapter 1 may be chosen. In Table 6.1 a summary is given of the references we have found in which quantitative survival data are reported for the convective drying of bacteria. Due to dehydration inactivation, the survival data have to be compared in relation to the final water concentration. The latter is preferably described in unambiguous terms. In Table 6.1 the unit 'kg H_2O.(kg solids)$^{-1}$' is used. When authors used the expression '%-moisture' this was considered as 'kg H_2O.(kg total)$^{-1}$×100%'. It is important to realize that comparison is only meaningful, when the same

bacteria are dried and rehydrated under the same conditions. In Fig. 6.7 the survival data of the lactic acid bacteria given in Table 6.1, are plotted as a function of the final water concentration. In this figure no clear correlation can be seen between survival and water concentration. Large variations in drying conditions and other factors diminish this correlation. Nevertheless, Fig. 6.7 does reveal some trends.

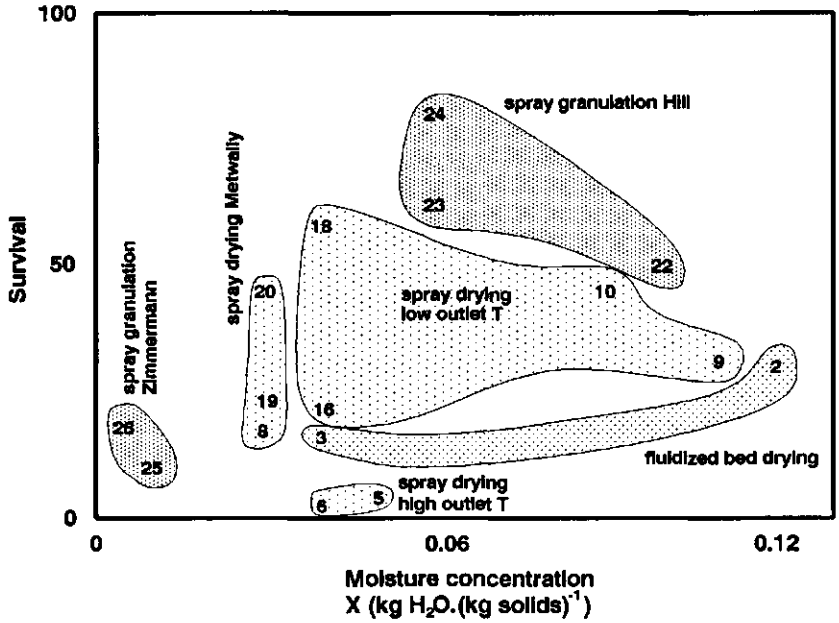

Fig. 6.7 An overview of the results found with lactic acid bacteria dried with different methods (the numbers refer to Table 6.1).

Table 6.1 Convective drying of bacteria

Drying method		Micro-organism	Drying conditions	Medium-Protectant /Support	Residual moisture concentration (kg H_2O. (kg solids)$^{-1}$)[a]	Survival (%)[a]	Reference
Fluidized bed	1	*Leuconostoc oenos*	in:30-40°C 25-75'	water/ soluble starch	0.1-0.4	1-10	Clementi and Rossi 1984
	2	*Lactobacillus plantarum*	in:30°C 60'	potassium phosphate buffer/ native potato starch	0.12	30	This thesis
	3	*Lactobacillus plantarum*	in:30-40°C 240'	culture medium/ wheat bran	0.04	17-19	Bera *et al.* 1988
	4	*Saccharomyces cerevisiae*	in:120-35°C product:35°C 8'	sorbitan monostearate/ no support	0.07	75-91	Langejan and Khoudokormoff 1982
Spray drying	5	*Lactobacillus acidophilus*	in:170°C out:75-80°C	NFMS	0.04-0.06	0.3-8	Espina and Packard 1979
	6	*Lactobacillus acidophilus*	in:170-180°C out:75-90°C	skim milk	0.03-0.05	0.4-3.5	Prajapati *et al.* 1987
	7	*Lactobacillus acidophilus*	in:140°C out:70°C	skim milk	-	13-18	Kozachuk *et al.* 1984
	8	*Lactobacillus bulgaricus*	in:190-200°C out:71°C	-	0.03	19.5	Metwally *et al.* 1989
	9	*Lactobacillus plantarum*	in:90°C out:42°C	native potato starch	0.11	31	This thesis
	10	Mixed starter culture[b]	in:70°C out:42°C	neutralized culture medium	0.09	45	Stadhouders *et al.* 1969
	11	*Saccharomyces cerevisiae*	in:190°C out:60°C	glycerol	-	0.02	Taeymans *et al.* 1986
	12	*Saccharomyces cerevisiae*	in:180°C out:60°C	water	0.09	1.6	Labuza *et al.* 1970
	13	*Serratia marcescens*	in:110°C out:65°C	-	0.04	43	Freeman *et al.* 1964
	14	*Serratia marcescens*	in:260°C out:60°C	dextrin and ascorbic acid	0.05	86[c]	Comings *et al.* 1977
	15	*Serratia marcescens*	in: < 100°C out:50°C	skim milk	0.06	2	Daemen 1981, 1982
	16	*Streptococcus cremoris*	in:80°C out:-	NFMS	0.04	18	Foster 1962
	17	*Streptococcus lactis*	in:60-180°C out:-	peptone and NaCl	-	< 1	Bullock and Lightbrown 1947
	18	*Streptococcus lactis*	in:80°C out:-	NFMS	0.04	53-63	Foster 1962
	19	*Streptococcus lactis*	in:190-200°C out:71°C	-	0.03	22	Metwally *et al.* 1989
	20	*Streptococcus thermophilus*	in:190-200°C out:71°C	-	0.03	45	Metwally *et al.* 1989

Table 6.1 (continued)

Spray granulation	21	*Lactobacillus acidophilus*	in: - bed:45-50°C 45'total	MRS-medium/ milk powder	-	24	Roelans and Taeymans 1990
	22	*Lactobacillus brevis*	in:- bed: 30°C 15'spraying + 30'drying	culture medium/ wheat flour	0.1	50	Hill 1985
	23	*Lactobacillus brevis*	-	-/ wheat flour	0.06	62	Hill 1986
	24	*Lactobacillus brevis*	in:60-40°C bed:20-35°C 10'spraying + 20'drying	-/ rye flour	0.06	80	Hill 1987
	25	*Lactobacillus casei*	in: 78°C bed: - 40'spraying in 65'total	MRS-medium/ lactose	0.01	7	Zimmermann 1987
	26	*Lactobacillus casei*	in: 30-48°C bed:25-39°C 150'spraying in 160'total	MRS-medium/ lactose	0.005	18	Zimmermann and Bauer 1990

[a] Approximate values, especially those obtained by estimation from data in figures

[b] Mixed starter culture including *Streptococcus diacetilactis*

[c] Highest survival reported; average survival 48%

in = inlet air temperature

out = outlet air temperature

- = not mentioned

NFMS = reconstituted Non Fat Milk Solids

The most promising results (Hill 1985, 1986, 1987) were obtained when a spray granulation process was used. Zimmermann (1987) and Zimmermann and Bauer (1990) used the same process and found a significantly lower survival. However, the reported water concentrations are also very low. Apart from the results of Metwally *et al.* (1989) who used intermediate outlet temperatures (around 70 °C), the results of spray drying with a high outlet air temperature (above 75 °C) show considerably lower values than the results with low outlet temperatures (below 50 °C). Also, Metwally *et al.* (1989) found a significantly lower survival at higher outlet temperatures. The fluidized bed survival data are located in the intermediate range. Points no. 2 and 9 in Fig. 6.7 indicate the survival of *L.plantarum* (reported in Chapter 5) after fluidized bed and spray drying, respectively. This survival is low when compared with the results of other authors. However, it must be recognized that these survival numbers were found without any optimization. The survival of *L.plantarum* can be increased significantly by prolonging the growth time (Fig. 6.6) and/or addition of protective additives and variation of other factors (Lievense and Van 't Riet 1991).

In the literature, survival data vary widely both within and between the three drying methods and a rational choice based on Table 6.1 or Fig. 6.7 can not be made. Unfortunately, it is not possible to give a straightforward recommendation for one of the three aforementioned processes. The most important advantage of spray drying is that no support material is needed. Alternatively, fluidized bed and spray granulation drying offer the freedom of choosing the drying time and good control possibilities. From the perspective of survival, when the difference in control possibility is eliminated by advanced spray drying techniques, the choice between the three convective drying processes becomes trivial. With optimal control, a high thermal inactivation can be avoided. In some cases, and depending on the application, the other (dis-)advantages may be important. It is likely that combined processes can help to optimize survival. However, dehydration inactivation of the cells is inevitable during drying and furthermore, the dehydration inactivation will not be influenced by the choice of the drying process because it is determined by other factors which are discussed by Lievense and Van 't Riet (1991).

6.4 CONCLUSIONS

The activity measurement method, based on the glucose-fermenting activity of *L.plantarum*, is very suitable for defining the residual activity after drying. In principle, the method is also applicable to other lactic acid bacteria, although the titration theory described is only valid with (mainly) homolactic fermentation.

Through systematic modelling, it is found that during drying *L.plantarum* is inactivated by two mechanisms: thermal inactivation and dehydration inactivation. Coupling of the mathematical descriptions of these mechanisms to a drying model, allows a prediction of the inactivation of *L.plantarum* during drying to be made.

Experiments show that the dehydration inactivation of *L.plantarum* depends only on the moisture concentration reached and is independent of the drying rate. It seems that dehydration inactivation is related to the water concentration and not to the water activity of the system. Furthermore, it is shown that dehydration inactivation, unlike thermal inactivation, causes damage to the cell membrane/cell wall.

During drying, dehydration inactivation of the cells is inevitable. Application of modelling techniques for the optimization of *L.plantarum* survival after drying, is only meaningful if the dehydration inactivation can be minimized. This may be achieved by adjustment of the growth conditions and addition of protective additives.

An important task that remains, is to investigate the mechanisms by which survival after drying is influenced. Interdisciplinary research is needed to answer such fundamental questions. With the knowledge obtained, thermal and dehydration inactivation can then be minimized further.

ACKNOWLEDGEMENTS

The author likes to thank the following people for their contribution to this chapter: Dr. A. Noomen, W.L. Lammers, E. Drost, E. Camminga and R. Müller (Laboratory of Dairying and Food Physics) for the cooperation in initiating the research on the DNA/DNase-method and M.A.M. Verbeek for the results presented in Fig. 6.1-6.4. P.M.F.J Koenraad and F.H.A Zomers for the data in Fig. 6.5 and M. Cox for the data in Fig. 6.6. The financial support of Gist-brocades NV, Delft, The Netherlands is gratefully acknowledged.

REFERENCES

Allwood MC and Russel AD (1970) Mechanisms of thermal injury in nonsporulating bacteria. Adv. Appl. Microbiol. 12: 89-119.

Beker MJ and Rapoport AI (1987) *Conservation of yeasts by dehydration*. In: Fiechter A (ed) Advances in Biochemical Engineering/Biotechnology, Vol.32 (pp. 127-171). Springer-Verlag, Berlin.

Bera F, Renauld H, Rikir R and Thonart P (1988) Water desorption analysis applied to *Lactobacillus plantarum* stationary bed drying. Med. Fac. Landbouw. Rijksuniv. Gent. 53: 2007-2016.

Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-254.

Brennan M, Wanismail B, Johnson MC and Ray B (1986) Cellular damage in dried *Lactobacillus acidophilus*. J. Food Prot. 49: 47-53.

Bullock K and Lightbrown JW (1947) The inactivation of enzymes and micro-organisms in oil and powders II. The effects of drying on the viability of bacteria and their thermolability in powders. Q.J. Pharm. Pharmacol. 20: 312-328.

Burton K (1956) A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem. J. 62: 315-323.

Clementi F and Rossi J (1984) Effect of drying and storage conditions on survival of Leuconostoc oenos. Am. J. Enol. Vitic. 35: 181-186.

Comings EW, Higa H, Myers JE, Koffler H and McLain HA (1977) Spray-drying of Serratia marcescens in the jet spray-dryer. Ind. Eng. Chem., Fundam. 16: 12-18.

Corry JEL (1973) The water relations and heat resistance of microorganisms. Progr. Ind. Microbiol. 12: 73-108.

Crowe JH, Crowe LM, Carpenter JF and Aurell Wistrom C (1987) Stabilization of dry phospholipid bilayers and proteins by sugars. Biochem. J. 242: 1-10.

Crowe JH, Carpenter JF, Crowe LM and Anchordoguy TJ (1990) Are freezing and dehydration similar stress vectors? A comparison of modes of interaction of stabilizing solutes with biomolecules. Cryobiology 27: 219-231.

Daemen ALH (1981) The destruction of enzymes and bacteria during spray-drying of milk and whey 1. The thermoresistance of some enzymes and bacteria in milk and whey with various total solids contents. Neth. Milk Dairy J. 35: 133-144.

Daemen ALH and Van der Stege HJ (1982) The destruction of enzymes and bacteria during spray-drying of milk and whey 2. The effect of drying conditions. Neth. Milk Dairy J. 36: 211-229.

Divies C, Lenzi P, Beaujeu J and Herault F (1990) Procede de preparation de micro-organismes inclus dans des gels sensiblement déshydrates, gels obtenus et leur utilisation pour la préparation de boissoins fermentées. French Patent 2 633 937. Champagne Moet & Chandon, France.

Espina F and Packard VS (1979) Survival of *Lactobacillus acidophilus* in a spray-drying process. J. Food Prot. 42: 149-152.

Foster EM (1962) Culture preservation. J. Dairy Sci. 45: 1290-1294.

Freeman RR, Tschernitz JL and Marshall WR (1964) Design of a laboratory spray dryer for biological materials. Biotechn. Bioeng. 6: 473-490.

Gould GW (1989) *Heat-induced injury and inactivation.* In: Gould GW (ed) Mechanisms of action of food preservation procedures (pp.11-42). Elseviers Science Publ., Essex, UK.

Heckly RJ (1985) Principles of preserving bacteria by freeze-drying. Develop. Industrial Microbiol. 26: 379-395.

Herbert D, Phipps PJ and Strange RE (1971) *Chemical analysis of microbial cells.* In: Norris JR and Ribbons DW (eds) Methods in Microbiology, Vol. 5B (pp. 209-344). Academic Press, London.

Hill FF (1985) Verfahren zum herstellen eines trockenen lagerstabilen bakterienpräparartes. European Patent 0 131 114. Chemische Werke Hüls AG, Marl, Germany.

Hill FF (1986) *Dry living microorganisms. Products for food industry.* In: Chmiel H, Hammes WP and Bailey JE (eds) Biochemical Engineering: a challenge for interdisciplinary cooperation. Int. Congress Stuttgart sept. 1986 (pp. 199-215). VCH Publishers, NY.

Hill FF (1987) Herstellung von lagerfähigen starterkulturen in wirbelbett. Alimenta 1987 No.3: 73-80.

Jenness R and Koops J (1962) Preparation and properties of a salt solution which simulates milk ultrafiltrate. Neth. Milk Dairy J. 16: 153-164.

Josic D (1982) Optimization of process conditions for the production of active dry yeast. Lebensm.-Wiss.-Technol. 15: 5-14.

Kozachuk DN, Globa LI, Gordienko AS and Krasnobrizhii NY (1984) Separation of *Lactobacterium acidophilum* from the culture liquid. Microbiology: A translation of Mikrobiologiya 52: 336-340.

Krallish IL, Damberga BE and Beker MJ (1986) The effect of yeast dehydration on the activity of a number of enzymes of cell energetic metabolism. Appl. Microbiol. Biotechnol 23: 482-486.

Kuts PS and Tutova EG (1983) Fundamentals of drying of microbiological materials. Drying Techn. 2: 171-201.

Labuza TP, Roux JP, Fan TS and Tannenbaum SR (1970) Engineering factors in single-cell protein production II. Spray-drying and cell viability. Biotechn. Bioeng. 12: 135-140.

Lammers WL and Noomen A (1990) Release of DNA as an indicator for lysis of *Lactococcus lactis ssp. cremoris*. FEMS Microbiol. Rev. 87: P114.

Langejan A and Khoudokormoff B (1982) Active dried baker's yeast. USA Patent 4.341.871. Gist-brocades NV, Delft, The Netherlands

Lievense LC and Van 't Riet K (1991) *Convective drying of bacteria. II. Factors influencing the survival.* In: Fiechter A. (ed) Advances in Biochemical Engineering/Biotechnology. Springer-Verlag, Berlin. Submitted.

Marino C, Curto M, Bruno R and Rinaudo MT (1989) Trehalose synthase and trehalase behaviour in yeast cells in anhydrobiosis and hydrobiosis. Int. J. Biochem. 21: 1369-1375.

Metwally MM, Abd El-Gawad IA, El-Nockrashy SA and Ahmed KE (1989) Spray drying of lactic acid culture I. The effect of spray-drying conditions on the survival of microorganisms. Egyptian J. Dairy Sci. 17: 35-43.

Morichi T, Irie R, Yano N and Kembo H (1967) Death of freeze-dried *Lactobacillus bulgaricus* during rehydration. Agr. Biol. Chem. 31: 137-141.

Pearce DA, Rose AH and Wright IP (1989) *Trehalose and trehalase in active dried yeast.* Yeast 5: s453-s457 (Special issue: Proc. 7th Int. Symp. on Yeast).

Prajapati JB, Shah RK and Dave JM (1987) Survival of *Lactobacillus acidophilus* in blended spray dried acidophilus preparations. Austr. J. Dairy Technol. March/June 1987: 17-21.

Roelans E and Taeymans D (1990) *Effect of drying conditions on survival and enzyme activity of microorganisms.* In: Spiess WEL and Schubert H (eds) Engineering and Food, Vol.3, Advanced processes (pp. 559-569). Elseviers Applied Science, London.

Stadhouders J, Jansen LA and Hup G (1969) Preservation of starters and mass production of starter bacteria. Neth. Milk Dairy J. 23: 182-199.

Taeymans D, Roelans E and Lenges J (1986) *Comparison of different drying techniques for processing active dry yeast for use in fermentors with immobilized cells.* In: Le Maguer M and Jelen P (eds) Proc. Int. Congr. Eng. Food 4, Edmonton, Canada. Food engineering and process applications, Vol.1 (pp. 439-444). Elseviers, London.

Tomlins RI and Ordal ZJ (1976) *Thermal injury and inactivation in vegetative bacteria.* In: Skinner FA and Hugo WB (ed) Inhibition and inactivation of vegetative microbes (pp.153-190). Academic Press, London

Valdez GF, De Giori GS, De Riuz Holgodo AA and Oliver G (1985) Effect of drying medium on residual moisture content and viability of freeze-dried lactic acid bacteria. Appl. Env. Microbiol 49: 413-415.

Vassault A (1983) *Lactate dehydrogenase analysis.* In: Bergmeyer HU (ed) Methods of Enzymatic Analysis, Vol. 3 (pp. 118-126). Verlag Chemie, Weinheim.

Wagman J (1960) Evidence of cytoplasmic membrane injury in the drying of bacteria. J. Bacteriology 80: 558-564.

Webb SJ (1961) Factors affecting the viability of air-borne bacteria. Can. J. Microbiol. 7: 621-632.

Zimmermann K (1987) Einflussparameter und mathematische modellierung der schonenden trocknung von starterkulturen. Fortschr.-Ber. VDI (Reihe 14, no 36), VDI-Verlag, Düsseldorf, West Germany.

Zimmermann K and Bauer W (1990) *Fluidized bed drying of microorganisms on carrier material.* In: Spiess WEL and Schubert H (eds) Engineering and Food, Vol.2, Preservation processes and related techniques (pp. 666-678). Elseviers Applied Science, London.

SUMMARY

Cultures of lactic acid bacteria play an important role in the production of food and feed. The most important application is the use as a starter culture. The main role of a starter culture is to ferment a sugar in the raw material to lactic acid and/or acetic acid. In the food industry, the formation of small amounts of other flavour components can also be important. The flavour components give the fermented product the unique taste whereas the lowered pH, due to acid production, also acts as a food preservation method.

It is preferable, that formulations used contain enough bacteria to inoculate the process material directly. Frozen as well as freeze-dried formulations are used for this purpose. The main advantage of (freeze-)dried over frozen bacteria is the lower transport and storage costs of the cultures. For industrial use, large quantities of active bacteria are required. Freeze-drying is generally not considered an attractive method for the preparation of these large quantities, due to its high cost and inherent complexity. Application of convective drying methods, such as spray drying, fluidized bed drying and spray granulation, is much more economical. However, the main disadvantage of the convective drying processes is that a considerable part of the bacteria is inactivated during drying.

To optimize an industrial scale convective drying process for lactic acid bacteria, it is essential that an insight into the correlation of parameters of the drying process with the inactivation rate of the bacteria is developed. The main objective of this study was to develop and verify a mathematical model that is able to predict the inactivation of bacteria during drying. *Lactobacillus plantarum* was used as a model lactic acid bacterium and fluidized bed drying was used as the drying method. With an appropriate mathematical model, more insight can be obtained about those parameters of the drying process that are important for the inactivation of *L. plantarum*.

To define a criterion to quantify survival of *L. plantarum* after drying, the pH decrease in a phosphate buffer due to fermentation of glucose to lactic acid by non-growing *L. plantarum* cells was studied. The method used offers a rapid and reproducible means of measuring the glucose-fermenting activity of *L. plantarum*. The maximum observed velocity of pH decrease is defined as the activity of the cell suspension. The residual activity is defined as the ratio of the activity after and at the start of the drying process.

In order to describe the inactivation of *L. plantarum* during a fluidized bed drying process, the drying kinetics of particles with *L. plantarum* cells immobilized in potato starch was studied. Experimental drying data were described using the short-cut drying theory. This theory was also used for the calculation of moisture concentration profiles inside the particles. Temperature of the drying particles in the course of the drying process was calculated with a heat balance in which the heat transfer coefficient was derived from the Nusselt-number in a fluidized bed. The mathematical model of the drying kinetics is straightforward and can be implemented easily in an overall model which includes the inactivation of *L. plantarum* during drying.

To describe the thermal inactivation of *L. plantarum* during drying, the thermal inactivation kinetics of *L. plantarum* cells immobilized in potato starch were measured at different moisture concentrations. The measured temperature and moisture dependency of the inactivation rate was modelled with first-order kinetics. The thermal inactivation model was coupled to the drying kinetics model. Although this concept has been successfully applied in literature studies, the model significantly underestimates the measured inactivation during drying.

It was concluded that inactivation of *L. plantarum* during drying is caused by two mechanisms: thermal inactivation and inactivation due to dehydration. In the final simulation model these inactivation mechanisms were described as two processes occurring simultaneously during drying. The dehydration inactivation was quantified by fitting an arbitrary set of equations to the measured inactivation data obtained at a drying temperature at which the thermal inactivation can be neglected. Both inactivation models were coupled to the drying kinetics model. The overall model predicts the measured inactivation of *L. plantarum* during a fluidized-bed drying process, up to a drying temperature of 55 °C.

From experiments, it was concluded that the influence of the drying rate on the residual activity of *L. plantarum* can be neglected and that the moisture concentration rather than the water activity is the essential parameter in dehydration inactivation. With the help of a newly developed DNA/DNase method (in cooperation with the Laboratory of Dairying and Food Physics), it was shown that dehydration, unlike thermal inactivation, causes damage to the cell membrane/cell wall. In order to optimize the residual activity after drying, the influence of the harvesting time on the dehydration resistance of *L. plantarum* was studied. Cells harvested after the early stationary growth phase showed the highest dehydration resistance.

It was concluded that during drying dehydration-inactivation of cells is inevitable. This dehydration-inactivation will not be influenced by the choice of the drying process but will be determined by other factors.

The research project was financially supported by Gist-brocades NV, Delft, The Netherlands.

SAMENVATTING

Melkzuurbacteriën spelen een belangrijke rol in de produktie van voedsel voor mens en dier. De meest bekende toepassing is het gebruik als startercultuur. De belangrijkste taak van een startercultuur is het fermenteren van een suiker in de grondstof tot melkzuur en/of azijnzuur. In de levensmiddelenindustrie is vaak ook de vorming van (andere) smaakstoffen belangrijk. De smaakstoffen geven het gefermenteerde produkt een unieke smaak, terwijl de door zuurproduktie verlaagde pH tevens conserverend werkt.

Bij voorkeur worden er preparaten gebruikt die veel bacteriën bevatten, zodat directe aanenting van de procesgrondstof mogelijk is. Hiervoor worden zowel diepgevroren als gevriesdroogde preparaten toegepast. Een groot voordeel van een gedroogde cultuur ten opzichte van een diepgevroren cultuur is dat de transport- en opslagkosten aanzienlijk lager zijn. Voor industriële processen zijn grote hoeveelheden aktieve bacteriën nodig. Vanwege de hoge kosten en complexiteit is vriesdrogen voor de produktie van deze hoeveelheden minder geschikt. De toepassing van convectieve droogmethoden, zoals sproeidrogen, fluïdebed drogen en sproeigranulatie, is economisch veel aantrekkelijker. Een groot nadeel van convectieve droogmethoden is echter dat een aanzienlijk deel van de bacteriën tijdens drogen inaktiveert.

Om optimalisatie van een industrieel droogproces voor melkzuurbacteriën mogelijk te maken, is inzicht in de parameters van het droogproces die de inaktivering beïnvloeden essentieel. Het belangrijkste doel van dit onderzoek was het ontwikkelen en verifiëren van een mathematisch model dat de inaktivering van bacteriën tijdens drogen kan voorspellen. *Lactobacillus plantarum* werd gekozen als model-melkzuurbacterie en als droogproces werd fluïdebed drogen gebruikt. Met een adequaat model is het mogelijk meer inzicht te verkrijgen in de parameters van het droogproces die de inaktivering beïnvloeden.

Om een criterium voor de overleving van *L. plantarum* na drogen te definiëren, werd de pH-daling in een fosfaat-buffer als gevolg van de fermentatie van glucose tot melkzuur door niet-groeiende *L. plantarum* cellen bestudeerd. De gebruikte methode bleek een snelle en reproduceerbare manier om de glucose fermenterende aktiviteit van *L. plantarum* te meten. De maximale snelheid van pH-daling is gedefinieerd als de aktiviteit van de celsuspensie. De restaktiviteit is gedefinieerd als het quotiënt van de aktiviteit voor en na het droogproces.

Om de voorspelling van de inaktivering van *L. plantarum* tijdens een fluïdebed droogproces mogelijk te maken, werd de droogkinetiek van deeltjes, bestaande uit *L. plantarum* cellen en aardappelzetmeel, bestudeerd. De verkregen experimentele data werden beschreven met de kortsluit-rekenmethode. Deze methode werd ook gebruikt om de waterconcentratie-profielen te berekenen. De temperatuur van de deeltjes tijdens het droogproces werd berekend met een warmtebalans waarin de warmte-overdrachtscoëfficient werd afgeleid vanuit het Nusselt-getal in een fluïdebed. Het mathematische droogmodel van de droogkinetiek is ongecompliceerd en kan eenvoudig geïmplementeerd worden in het uiteindelijke model dat ook de inaktiveringskinetiek bevat.

Om de thermische inaktivering van *L. plantarum* tijdens drogen te kunnen beschrijven werd de thermische inaktiveringskinetiek van *L. plantarum* cellen gemengd met aardappelzetmeel bestudeerd bij verschillende waterconcentraties. De gemeten temperatuurs- en waterconcentratie-afhankelijkheid van de inaktiveringssnelheid werd gemodelleerd met eerste-orde kinetiek. Het thermisch inaktiveringsmodel werd gekoppeld aan het droogmodel. Hoewel dit concept eerder succesvol in de literatuur is beschreven, bleek het gekoppelde model de inaktivering van *L. plantarum* tijdens drogen aanzienlijk te onderschatten.

Hieruit werd geconcludeerd dat de inaktivering van *L. plantarum* tijdens drogen werd veroorzaakt door twee mechanismen: thermische inaktivering en inaktivering door ontwatering. In het uiteindelijke simulatiemodel werden deze inaktiveringsmechanismen beschreven als twee processen die gelijktijdig tijdens drogen kunnen optreden. De ontwaterings-inaktivering werd gekwantificeerd door het fitten van een set van willekeurige vergelijkingen aan de inaktiverings-data verkregen bij een temperatuur waar thermische inaktivering kan worden verwaarloosd. Beide inaktiverings modellen werden gekoppeld aan het droogmodel. Het uiteindelijke model geeft een goede voorspelling van de inaktivering van *L. plantarum* tijdens een fluïdebed droogproces tot een droogtemperatuur van 55 °C.

Uit experimenten werd geconcludeerd dat de invloed van de droogsnelheid verwaarloosd kan worden en dat niet de wateraktiviteit maar de waterconcentratie de essentiële parameter is bij de ontwaterings-inaktivering. Met behulp van een nieuw ontwikkelde DNA/DNase-techniek (in samenwerking met de sectie Zuivel en Levensmiddelennatuurkunde) werd aangetoond dat ontwatering, in tegenstelling tot thermische inaktivering, schade veroorzaakt aan de celmembraan/celwand. Om de restactiviteit na drogen te optimaliseren werd de invloed van het oogsttijdstip op de ontwaterings-inaktivering bestudeerd. Het bleek dat cellen die geoogst werden na het begin van de stationaire groeifase de grootste resistentie tegen ontwatering vertonen.

Geconcludeerd werd dat bij drogen ontwaterings-inaktivering onvermijdelijk is en dat deze inaktivering niet wordt beïnvloed door de keuze van het droogproces maar door tal van andere factoren.

Het onderzoek werd financieel ondersteund door Gist-brocades NV te Delft.

CURRICULUM VITAE

De auteur van dit proefschrift werd geboren op 27 februari 1962 te Westkapelle. In 1980 behaalde hij het HAVO- en in 1982 het VWO-diploma aan de Christelijke Scholengemeenschap Walcheren te Middelburg. In 1982 begon hij met een studie Moleculaire Wetenschappen aan de Landbouwuniversiteit te Wageningen. In 1986 studeerde hij daar af. Als vervolg van deze studie startte hij in december 1986 een vierjarig, door Gist-brocades NV medegefinancieerd, promotie onderzoek bij de sectie Proceskunde. Dit onderzoek heeft geleid tot dit proefschrift. Vanaf februari 1991 werkt hij bij de sectie Bioprocessing van het Unilever Research Laboratorium te Vlaardingen.