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ROLE OF MODELLING AND MODEL SIMULATION IN FOOD INDUSTRY: A DAIRY CASE

Anton Sweere
Campina Innovation
Nieuwe Kanaal 7C
6709 PA Wageningen
The Netherlands
E-mail: a.sweere@campina.com

ABSTRACT

Modelling and model simulation are considered to be powerful tools that are widespread amongst universities, research institutes and industry. Industry? Looking to the daily practice of food industry, in this case the dairy industry, the role of models in research and development, engineering and process control is less pronounced than one would expect. In this paper the role and value of models in new product development and processing in Campina, one of the major European dairy companies will be discussed. Subjects like the type of models that are used, the limitations of modelling in dairy research, the success factors for modelling, the role of universities and research institutes, and future expectations will be treated.
The daily practice of model simulations will be illustrated by two cases:

- foam stability
- cheese milk pasteurisation

INTRODUCTION

With a turnover of about € 3.7 billion Campina is one of the major European dairy companies. Concerning consumer product focus is on European countries, with emphasis on the home markets, the Netherlands, Germany and Belgium. Concerning ingredients for food industry and pharma focus is world wide, with emphasis on Europe, USA and Japan. As Campina has its roots in the Netherlands and made important acquisitions in Germany and Belgium, R&D is concentrated in these countries.
From the past R&D in Campina has been organised de-central. However, in order to improve the efficiency of R&D currently every business sector has its own R&D center, for the white dairy sector in Wageningen, for Cheese and Butter, in Tilburg and for Industrial products in Veghel, all in the Netherlands. Locally R&D is still present in Heilbronn (Germany, white sector), Aalter (Belgium, white sector), Woerden (Netherlands, white sector) and Rijkevoort (Cream liquors). The tasks of the local R&D groups is limited to sales support, production support, value engineering and line extensions. Major product innovations are covered by the R&D centers. Recently, June 2003, the R&D center for Campina's white sector started in Wageningen. Main reasons to start this center and to start it in Wageningen are to create critical mass, to manage synergism and to be easily connected to the activties and institues of Food Valley Wageningen.

SUCCESS FACTORS OF MODELLING

Modelling and model simulations, can be important tools in new product development, processing and process control. However, compared to chemical industry, modelling in dairy industry is not as widespread as one would expect. There are a couple of reasons for this :

- critical mass : managing an R&D organisation there are a couple of tools that can be used to improve the output and effciency, e.g. project management, portfolio management, knowledge management, statistics, experimental design, TRIZ, SIT, QFD, … and modelling. Despite the fact, that one tool cannot be replaced by the other tool, they are complementary, decisions have to be made which tools to implement and to support. Each tool needs its champions, its specialists, in order to be successful in using it . This means that only organisations of a certain size can cover the total 'tool box'.
- lack of acceptors : an important factor for successful modelling is the presence of people that have a high affinity towards modelling. Food companies have a high level of people with an education in food technology. Despite the fact that also in the education of food technologists modelling has been widespread there still is an arrears compared to e.g. chemical engineering. Furthermore, looking to the Dutch situation, people choosing for a food sicence education at Wageningen University have, in general, less affinity towards mathematics, and modelling more specifically than people choosing to study at a technical university.
- complexity of food systems : it can be stated that in general food systems are far more complex than chemical systems. Food systems can be characterised by non-equilibrium multi-component systems that are not well understood. Not the unit operations but the components used and its interactions during processing make that modelling is often of limited value. Furthermore processing often knows a high degree of craftsmenship, much experience and many rules of thumb. Mathematically this often can be translated to black box models, which of course, are of limited value, but still can be quite effective in process control.

ROLE OF UNIVERSITIES AND INSTITUTES

A solution to some of the limitations can be found in hiring young people with a degree in chemical or mechanical engineering, physics or applied mathematics, but of course they are lacking food technology know-how. Another problem in an industrial R&D environment is often that people move too quickly to other positions in the organisation, thus making it difficult to organise continuity.
In the role of acceptor people cannot be replaced by external people. However, in the role of developer, supplier of models there is a chance for universities, institutes, small (high) technical companies and engineering companies. Especially in the area of CFD modelling starting companies, spin-offs from universities, arise.
Looking to Campina and the role of universities and institutes such as WCFS, TNO and NIZO Food Research play an important role in model development. Examples will be shown below. But also contacts and cooperations with the technical universities of e.g. Eindhoven and Delft either resulted in the development of models or in hiring people with experience in model development. A nice example is the PhD study of E. van Nunen on amino acid separation by membrane electrophoriesis (van Nunen, 1997) and application of process models for optimisation and trouble shooting of electrodialysis used for demineralisation of cheese whey.
On the other hand much energy has been spent and will be spent, by e.g. NIZO and WCFS, on the development of models that will hardly or never be used by dairy and food companies.

TYPE OF MODELS

Figure 1 showes a qualitative frequency distribution of models used in dairy industry, and in Campina. The question is whether real trial & error exists. Often experience, rules of thumb and simple qualitative models form the basis for this ‘ trial and error ‘.
In research and development qualitative models are extensively used, e.g. models for casein micelles, interaction of macro-molecules, behaviour of molecules at the gas-liquid-interface or water-oil-interface, fouling of membranes.

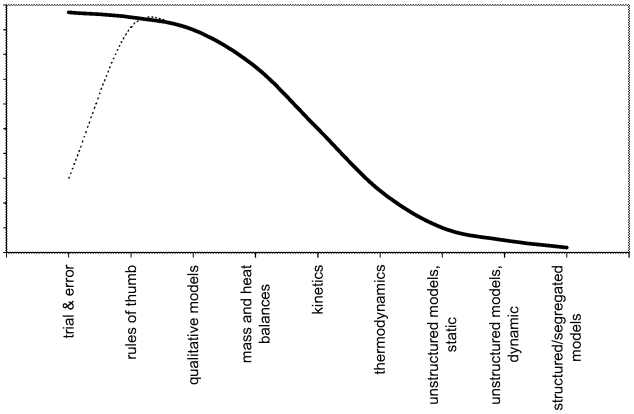

Figure 1 Type of models used

Especially in processing mass and heat balances are extensively used for evaluation of experiments and for design and engineering purposes. Most of the time only static situations are covered using Excell worksheets.
Flowsheeting is often considered as being too complex and too expensive. The threshold for hiring calculation capacity and flowsheeting expertise seems to be too high. Although this could be more effective a great deal of it can be covered by Excel sheets making flow sheeting as alternative less attractive.
Kinetic models (in combination with mass balances) are used in case of enzymatic reactions, inactivation of enzymes, denaturation of proteins, microbial growth and killing of micro-organisms. Either from a product development point of view, or shelf life, hygiene, or fouling of process equipment.
Thermodynamic models e.g. covering water activity, solubility of phosphate, sorption isotherms are applied to equilibrium conditions, which, as equilibrium will hardly be reached, only serve as an estimate.
Dynamic models, numeric techniques, CFD are hardly used without external support. The time and investment it needs to keep it operational and to be effective is just too big. Examples show, however, that when models are incorporated in joint development projects with universities and institutes they can be very successful.
On the other hand, not using models or model simulation is not the main reason for not being successful in a certain area.
In-line use of models in process control is a feature that is just starting. One of the reasons is that a great deal of the efficiency and quality improvement is already realised by changing from off-line to on-line analytical techniques, even without the automatic controls.

PROTEIN DENATURATION IN CHEESE MILK PASTEURISATION

Dutch cheese production is roughly 600.000 tons per year, using almost 6 billion litres of milk. Regarding this huge volume two factors are extremely important: fat and protein yield, and a constant quality of the cheese.
One of the parameters that affects both factors is the amount of protein that is denatured during pasteurisation and that is incorporated into cheese or lost in very fine curd.
For more then 15 years NIZO is developing models to describe the effect of heat treatment on the microbial quality of milk, on protein denaturation and on fouling of heat exchangers. A great deal of the development has been performed in co-operation with Dutch dairy companies. In the 80’s-90’s Heat Card was developed and used by several Dutch dairy companies like Campina. A couple of years ago a new software tool, called Premia has been developed and is currently effectively applied in the group Cheese and Butter of Campina (de Jong et al, 2002).
Cheese milk is pasteurised on a plate and frame heat exchanger with holding tube (figure 2 and table 1) at constant pasteurisation temperature, e.g. 73 °C and constant flow rate, e.g. 45 m^3 per hour. In practice, however, flow

rate may differ from 37 to 50 m^3/h. The effect of different flow rates on protein denaturation can be simulated with

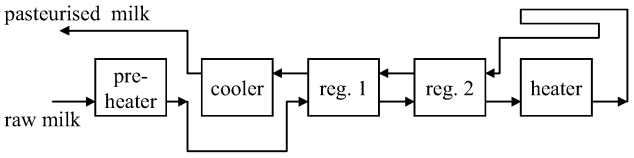

Figure 2. Layout of cheese milk pasteuriser

Table 1. Layout of 45 m^3/h cheese milk pasteuriser

Section	Volume [liter]	T-out (°C)
Pre-heater	65	21
Regenerative 1-up	157	55
Regenerative 2-up	34	65
Heater	105	Pasteurisation T
Holding tube	265	Pasteurisation T
Regenerative 1-down	34	63
Regenerative 2-down	105	29
Cooler	115	33

Premia, as a function of place in the pasteuriser and as a function of run time.
Each section of the pasteuriser can be modelled separately (figure 3) using the predefined units of Premia.

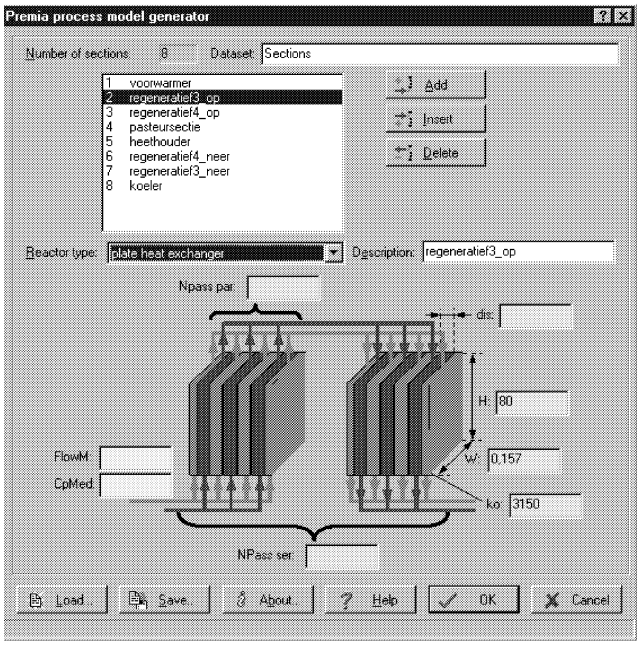

Figure 3. Example of pre-defined section of plate and frame heat exchanger in Premia

Combining this with the inactivation, denaturation and fouling kinetics of enzymes, micro-organisms and proteins yields e.g. fouling per section, degree of protein denaturation, and degree of inactivation of enzymes and micro-organisms. Figure 4 shows a typical temperature profile of a pasteuriser simulated using Premia.

In production using an average flow rate of 45 m^3/h a protein denaturation of 7.2 % is obtained. However, in

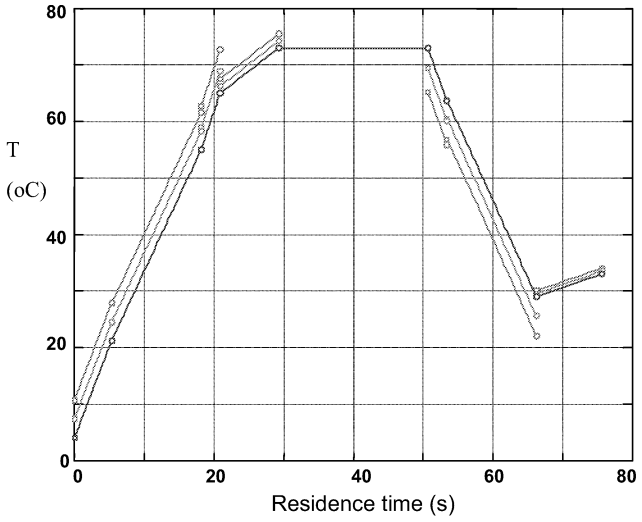

Figure 4. Temperature of heating medium, wall layer and product as a function of residence time in cheese milk pasteuriser at a run time of 6 h.

practice protein denaturation may change from 8.7 to 6.5 % when applying flow rates from 37 to 50 m^3/h, respectively (figure 5). Concerning protein yield and cheese quality a constant degree of protein denaturation is preferred. Using Premia pasteurisation temperature as a function of flow rate can be calculated giving the fact that a constant degree of protein denaturation is required. Model simulation yield a protein denaturation of 8 % at 45 m^3/h. In order to get a constant protein denaturation when flow rate differs from 37 to 50 m^3/h temperature has to change from 71,5 to 73,8 °C, respectively (figure 6).

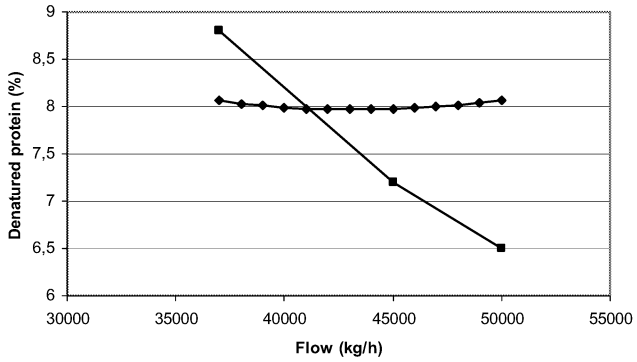

Figure 5. Measured (■, fixed temperature) and simulated (♦, variable temperature) protein denaturation as a function of flow rate.

In daily plant practice, graphs like this are being used to control temperature of cheese milk pasteurisers. Next step would be to connect Premia-like models directly to the production process in order to control the degree of protein denaturation on-line.

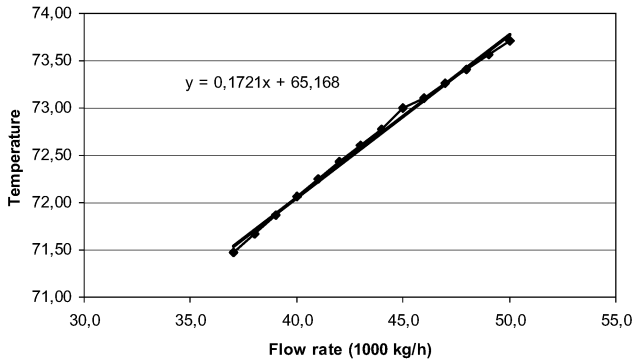

Figure 6. Pasteurisation temperature as a function of flow rate in order to obtain a constant degree of protein denaturation in cheese milk

EFFECT OF RHEOLOGICAL PROPERTIES ON FOAM STABILITY OF DESSERTS

There is a great interest of consumers and producers of dairy products in aerated products. However, due to coalescense and disproportionation aerated products are often instable. WCFS (Wageningen Centre of Food Sciences) investigated the stability of foams and emulsions and developed a model describing the effect of bulk and interfacial rheology on dissolution of air bubbles with a diameter of 1 µm to 1 mm (Kloek et al. 2001). Later on the model has been applied by Campina to develop stable aerated products.

Basis of the model is a description of the bulk and interfacial rheological properties in terms of viscosity and elasticity. As bulk and interfacial viscosity only retard disproportionation at very high values ($\eta_d > 10^5\ \sigma_d$ and bulk viscosity at values > 10^7 Pa-s), disproportionation in food products is mainly ruled by interfacial elasticity and bulk elasticity.
Due to the fact the pressure inside a bubble is bigger than the external pressure gas diffuses out of the bubble. The pressure difference due to interfacial tension is called the Laplace pressure

$$\Delta P = 2\sigma(R)/R. \qquad (1a)$$

where σ is the interfacial tension and R the radius of the of the gas bubble,

or

$$P = P_\infty + 2\sigma(R)/R = nR_gT/V \qquad (1b)$$

when assuming that the ideal gas law is valid.
With P_∞ representing the pressure of the medium, n/V the molar concentration, R_g the universal gas constant, and T the temperature.
The change of surface tension as a function of bubble radius during expansion or shrinkage can be derived from the definition of the elastic modulus, E_d

$$E_d = d\sigma / d(\ln A) = \tfrac{1}{2}R\, d\sigma/dR \qquad (2)$$

Where E_d is the elastic modulus and A the surface area.
Combining equation (1) and (2) with the diffusion equation

$$\partial c/\partial t = D\nabla^2 C \qquad (3)$$

with c = c(r,t), the gas concentration, and D the diffusion coefficient of the gas in the medium, yields, after some rearrangements, a description of the change in bubble diameter as a function of the interfacial elastic modulus.
Bulk elasticity can be incorporated in a similar way by describing pressure in the bubble at the bubble boundary (r=R) by

$$P = P_\infty + 2\sigma(R)/R - \tau_\pi = nR_gT/V \qquad (4)$$

where τ_π is the excess radial bulk stress tensor which can be described by (Fyrillas et al 2000)

$$\tau_\pi = 2G\, R_0/R + \tfrac{1}{2}G\,(R_0/R)^4 - 5/2\ G \qquad (5)$$

where G is the bulk shear modulus.
Combining interfacial elasticity and bulk elasticity in one model yields a description of bubble size as a function of the interfacial elastic modulus E_d and the bulk elastic modulus G.
Table 2 shows some order of magnitude of the moduli of various food products.
Combining these numbers with the model it can be concluded whether interfacial elasticity of bulk elasticity, or both, are responsible for stabilisation of an aerated product (figure 7): e.g. in cheese stability is realised by bulk elasticity, whereas milk shake is mainly stabilised by interfacial elastic properties. For mousse both interfacial and bulk elastic properties are important.

Table 2. Order of magnitude of bulk elasticity and interfacial elasticity moduli of food products (Kloek et al 2001).

Product	E_d (mN/m)	G (N/m^2)
Beer	20	<0.01
Milk shake	20-40	0.01-1
Mousse	40-100	1-100
Dough	100	10^3-10^4
Cheese	10^3	10^4-10^6

So, when designing an aerated product the graph can be used to define whether research should be focussed on ingredients affecting interfacial elasticity of bulk elasticity.
Whipped dairy products can be stabilised in several ways, either by high bulk elasticity, e.g. by gel formation using rennet or starch, or by high interfacial elasticity, e.g. using emulsifiers.
Aiming at a low fat, pourable, aerated dairy product a new system had to be developed. Based on the knowledge gathered by developing the model the conclusion was

drawn that stabilisation of the gas bubbles should be realised by increasing interfacial elasticity.

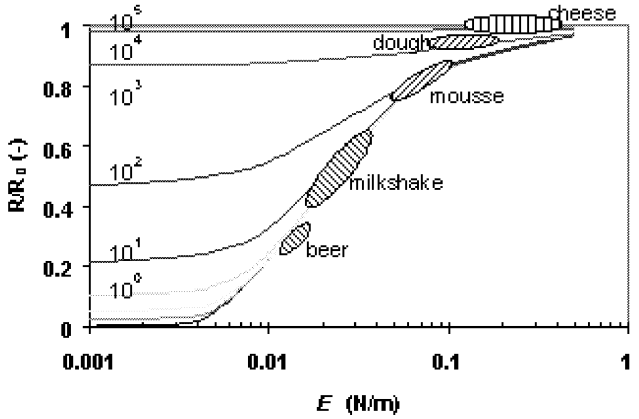

Figure 7. Relative bubble radius as a function of interfacial elastic modulus E_d and bulk elastic modulus G. The lines indicate the relative radius at which bubbles become stabilized (Kloek et al 2001)

The solution has been found in denaturation of protein located at the air-liquid-interface. Using pH induced denaturation the solution can be used in acidic dairy products like yogurt. By mixing a stabilised, acidic, aerated foam into a sweet dessert also stabilised sweet products can be made.

CONCLUSIONS

Despite the fact that modelling can be a powerful tool in food industry its application is limited due to complexity of food systems, lack of critical mass, and lack of acceptors. With support from universities and institutes, especially in developing models, clear successes can be obtained in product development as well as in engineering and process control.

REFERENCES

Jong, P. de, Verschueren, M., Vissers, M.M.M., Straatsma, J., Smit, F., Hybrid modelling for development and optimisation of food production chains including costs and food quality. In: Proceedings of the 2nd International Conference on Simulation in Food and Bio Industries (Eds. B. O'Connor, D. Thiel), ISBN 90-77039-08-2, SCS Europe (2002), p13-17

Kloek, W., van Vliet, T., and Meinders, M., 2001, Effect of bulk and interfacial rheological properties on bubble dissolution, *J. of Colloïd and Interface Science* vol 237 158-166

Nunen, C.A.P.M., van, 1997, PhD Thesis, Eindhoven, the Netherlands, Design of a large scale membrnae-electrophoresis module for separation of proteins. ISBN 90-386-0389-4

Fyrillas, M.M., Kloek, W., van Vliet, T., and Mellema, J., 2000, *Langmuir* vol 16 1014

BIOGRAPHY

ANTON SWEERE studied biochemical engineering at the Delft University of Technology, The Netherlands. After passing his PhD in 1988 he joined Campina and worked successively for DMV International bv, i.e. the food ingredient division of Campina, Campina Cheese and Butter, and Campina Innovation, the R&D center for Campinas 'white' dairy business, viz. Campina Netherlands, Campina Germany and Campina International.

ADVANCES IN THE OPTIMIZATION OF INDUSTRIAL FOOD PROCESSING

Julio R. Banga, E. Balsa-Canto, C. G. Moles, S. Garcia, O. H. Sendin, M. Rodriguez, A. A. Alonso
Process Engineering Group
IIM-CSIC
C/Eduardo Cabello 6, 36208 Vigo, Spain
e-mail: julio@iim.csic.es

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optimization, optimal control, parameter estimation.

ABSTRACT

In this contribution, we will present an overview of the state of the art regarding the model-based optimization of industrial food processing. The potential of modern optimization techniques for improving industrial processes will be discussed considering several important problem classes. Finally, we will also outline a number of research needs and probable future trends.

INTRODUCTION

Model-based approaches are increasingly applied in the food and bioprocess industries (i.e., biotechnological, pharmaceutical, environmental, etc.). In order to increase the productivity, profitability and/or efficiency of these processes, considerable research effort has been devoted to their improvement via computer aided process engineering methods. In this way, mathematical modelling, optimization and control have become fundamental tools to optimally design and operate production facilities in these sectors.

During the last decade, our group has been especially interested in robust and efficient optimization techniques which can be used, in combination with suitable models, to obtain optimal or near-optimal solutions regarding the design, operation and control of these processes . Here we will present a review of the research that our group (and others) have developed in this area.

PROBLEM CLASSES

Most models of food processing operations have an inherent dynamic nature, thus we will need to use methods designed for the optimization of dynamic systems in order to arrive to optimal decisions. There are three types of optimization problems which are especially relevant [1]:

- Optimal control: i.e. computing optimal operating policies: that is, given a process dynamic model and a set of specifications, the objective is to compute the optimal operating conditions which lead to maximum performance as measured by some predefined criteria. These problems belong to the domain of dynamic optimization (or open loop optimal control).

- Parameter estimation: or model calibration, i.e. the usual problem of finding the parameters of a nonlinear dynamic model which give the best fit to a set of experimental data. This is one of the mandatory steps in dynamic model development, but unfortunately many modelers are not aware of the dangers of applying standard optimization methods for its solution.

- Integrated process design and control: to find simultaneously the static design variables (e.g. sizes and number of units), the operating conditions (e.g. flows) and other design issues (e.g. the controllers) which minimize capital and operation costs while optimizing certain characteristics of the dynamics of the process (e.g. maximizing controllability)

Optimal Operating Policies

The computation of optimal operating policies for food processing units or full plants can be used as the kernel of a Decision Support System (DSS). Such DSS could ensure maximum quality at minimum cost while meeting safety and environmental constraints. Dynamic optimization methods can be effectively used to compute these optimal policies. However, the non-linear and highly constrained nature of food processing models can make their dynamic optimization a very challenging task.

Dynamic optimization [2], also called open loop optimal control, considers the optimization of dynamic systems in order to compute a set of time-dependent decision variables (usually called controls, u(t)), during a certain time horizon, which minimize (or maximize) a performance index (J) of the dynamic behavior of the system, subject to the following constraints:

- differential algebraic equations (DAEs) which describe the system dynamics

- equality and inequality algebraic path constraints

- bounds for the controls and/or states

The mathematical models of food processing operations have certain characteristics which will pose special difficulties for their optimization: non-linear, distributed and dynamic nature, very often involving coupled transport phenomena, plus a number of nonlinear constraints coming from safety and quality demands. Moreover, the optimal control profiles can be very rough, often exhibiting additional difficulties, like e.g. singular arcs. These characteristics can make the dynamic optimization of food processing a hard task [3].

Therefore, the adequate election of robust and efficient dynamic optimization methods is of the highest importance. It should be noted that the state of the art regarding the dynamic optimization of nonlinear systems is far from fully satisfactory: no existing method can be used automatically (i.e. in an unsupervised way) to solve any of the medium-to-high complexity problems from this domain.

Parameter Estimation

Parameter estimation, or model calibration, is a key step in the development of reliable dynamic models for food processing. Given a model structure and a set of experimental data, the objective of parameter estimation is to calibrate the model (looking for parameters which can not be measured directly) so as to reproduce the experimental results in the best possible way.

This calibration is performed by minimizing a cost function which measures the goodness of the fit, like the least squares criterion. Since most food processing models involve coupled and highly non-linear phenomena, usually described by sets of partial and ordinary differential equations, the resulting parameter estimation problem can be very challenging to solve. In particular, complex non-linearities might cause non-convexity, i.e. the optimisation problem may contain several local minima in the area of interest. Thus, traditional gradient-based methods, like Levenberg-Marquardt or Gauss-Newton, mail fail to identify the global solution of the calibration problem. Further, when these methods find a parameter set which gives a poor fit to the experimental data, the user can not be sure if the reason is due to an incorrect model, or if it is an indication of the convergence of the optimisation solver to a local solution.

Schittkowski [4] has recently presented a thorough review of parameter estimation in dynamical systems. This author presents a detailed overview of local optimization methods (including gradient-based methods such as Gauss-Newton, sequential quadratic programming, SQP, and others) that can be used to compute parameters by a least squares fit. As Schittkowski noted, even qualified numerical algorithms can fail, or compute unacceptable answers, when using local methods. Examples of possible difficulties that can arise [4] are: convergence to a local solutions, narrow curved valleys where progress towards the solution is hard to achieve, very flat objective function in the neighbourhood of a solution, bad starting values for parameters, requiring a large number of steps, badly scaled model functions and, in particular, measurement values, or non-differentiable model functions.

Integrated Design

During the last decade, the importance of a simultaneous (integrated) design approach, considering operability together with the economic issues, has been recognized (e.g. see [5] [7] [6] and the references cited therein). It should be noted that the optimization problems arising from these formulations are very challenging. The multimodal (non-convex) nature of these problems has been highlighted by e.g. Schweiger and Floudas [7] and Bansal et al. [6], among others.

A general statement can be considered taking into account process and control superstructures which indicate the different design alternatives. These statements result in mixed integer optimal control problems (MIOCPs). A simpler case is often considered, where it is assumed that the process flowsheet is given, as well as the process specifications. Although this problem statement is obviously simpler than the above mentioned, it has been shown to be challenging enough for many optimization methods. Besides, it is a case often encountered in the real world, where many bioprocesses have well established process flowsheets, so the process and control superstructures are not an issue for the integrated design problem.

For this latter case, the objective is to simultaneously find the static variables of the process design, the operating conditions and the parameters of the controllers which optimize a combined measure of the plant economics and its controllability, subject to a set of constraints which ensure appropriate dynamic behavior and process specifications.

For many problems of interest, the simultaneous optimization of multiples objectives (e.g., product quality, operating costs, capital investment, etc.) is a more realistic and desirable approach. These problems belong to the field of multiobjective optimization [8]. Nevertheless, the associated non-linear programming problems can be very challenging to solve. Furthermore, since these objectives are frequently opposing, the optimal solution is often not unique. Thus, the purpose of multicriteria optimization is to find a set of solutions which involve optimal trade-offs between the different objectives, i.e., the set of solutions which represent the relatively best alternatives. In the field of food engineering, multi-criteria optimisation has received very little attention.

ADVANCES IN OPTIMIZATION METHODS

The classes of problems discussed above are, or can be transformed to, nonlinear programming problems subject to dynamic (usually, differential-algebraic) constraints. Their highly constrained, non-linear and sometimes non-smooth nature often causes non-convexity, thus global optimization methods are needed to find suitable solutions.

As already mentioned, the application of direct methods (i.e. control vector parameterization or complete parameterization) to optimal control problems frequently leads to nonconvex NLPs subject to nonlinear differential-algebraic constraints. Similarly, the latter also often arise in the framework of integrated design or parameter estimation problems. The more naive approach to surmount nonconvexity, i.e. multi-start local methods, fails for any mildly realistic problem. Thus, there is a clear need of robust and efficient global optimization problems in order to ensure proper solutions.

The global optimization (GO) of nonlinear dynamic systems is receiving increased attention from engineers, mathematicians and computer scientists. In the domain of deterministic GO methods, Esposito and Floudas [9] [10] have recently presented approaches to solve nonlinear optimal control (dynamic optimization) and parameter estimation problems. This is indeed a very promising and powerful approach, but the objective function and the dynamics of the system must be twice continuously differentiable, and restrictions may also apply for the type of path constraints which can be handled. Other groups [11] [12] are also making good progress in deterministic global optimization of dynamic systems, yet several issues regarding requirements and computational performance are still present. In any case, research along these lines continues and it might result in breakthrough results in the short term,

Regarding stochastic GO methods, several researches have shown that they can locate the vicinity of global solutions for nonlinear dynamic problems with relative efficiency [14] [13] [15] [3] [17] [16] [15], but the cost to pay is that global optimality can not be guaranteed. However, in many practical situations these methods can be satisfactory if they provide us with a "good enough" (often, the best available) solution in modest computation times. Furthermore, stochastic methods are usually quite simple to implement and use, and they do not require transformation of the original problem, which can be treated as a black box. Thus, they can handle problems with complicated dynamics (e.g. discontinuities [18], non-smoothness, etc.).

APPLICATION TO INDUSTRIAL PROCESSES

Several successful applications of modern optimization methods in the area of food process engineering will be reviewed. In particular, the dynamic optimization of several important operations will be discussed, emphasizing two key aspects: the need of robust yet efficient optimization methods for the robust solution of these problems, and the advantages of the computed optimal operating policies over the traditional operating conditions. The following processes will be discussed:

- Industrial thermal sterilization: widely reported as one of the most important operations in the food industry. Many authors have presented model-based computational methods to design and/or optimize this type of processes (see the reviews of e.g. Silva et al [19], Durance [20] and Banga et al [1]). In its basic form, the dynamic optimization of thermal processing problem seeks to find the heating temperature (as a time-dependent profile) which maximizes the final nutrient retention of a pre-packaged conduction-heated food subject to a constraint on the microbiological lethality. Thus, there are two conflicting demands: in order to achieve the desired minimum lethality, we must process all the regions of the food at a high enough temperature during long enough time. But, on the other side, the action of heat also destroys nutrients, and we want to minimize that undesirable effect. We will review the different approaches used to efficiently solve these problems.

- Food dehydration: although there exist many studies dedicated to the modeling (and simulation) of food dehydration, there are very few optimization studies. The pioneers in recognizing and formulating the dynamic optimization problems, taking quality as a performance index, were Karel and coworkers [21] . These researches considered the problem of maximizing product quality during air drying of a model system and of potato slabs. To solve these optimal control problems, they used both the maximum principle of Pontryagin and a modified Complex method applied to a transformed problem via control parameterization. Interestingly, they recognized the superiority of this latter approach (although less elegant than the PMP). However, the modified Complex, though more robust than gradient-based methods, is also a local method, so it might get trapped in local optima depending on the initial point chosen. We will present and discuss more recent studies which avoid getting trapped in such local solutions.

- Batch and fed-batch fermentations: these are processes of major importance not only in the food industry, but also in the biotechnological and pharmaceutical sectors. A recent review [22] reveals a very large amount of research dedicated to these interesting processes.

- Other industrial food processes: we will also review the dynamic optimization of several other processes, including microwave heating, contact cooking, freeze-drying and membrane processing.

Research Needs and Future Trends

More work is needed in a number of research avenues in order to ensure the formulation and proper solution of more realistic optimization problems at the industrial level:

- Large-scale, multi-objective optimization of food processing, considering not only unit operations but complete plants, thus providing the kernel of truly useful and plant-wide decision support systems
- Optimization under uncertainty: although modelling of uncertainty has already been applied to food processing [23], these type of models have not been used to obtain robust optimal operating policies, or to derive optimal risk-management methods
- Surrogate (reduced order) models: the key idea is that complex dynamic models can be accelerated several orders of magnitude without loosing significant information. Nonlinear model-reduction tools will play a major role in order to speed and scale up the next generation of optimization and control tools

REFERENCES

[1] Banga, J. R.; Balsa-Canto, E.; Moles, C. G.; Alonso, A. A (2003) Improving food processing using modern optimization methods. *Trends in Food Science and Technology*, 14(4):131-144.

[2] Bryson, A.E. , *Dynamic Optimization*, November 1998, Prentice Hall.

[3] Banga, J. R., A. A. Alonso, and R. P. Singh (2003) Stochastic dynamic optimization of batch and semi-continuous bioprocesses. *Biotechnol. Prog.*, 13, 326-335, 1997

[4] Schittkowski, K. *Numerical Data Fitting in Dynamical Systems*. December 2002. Kluwer Academic.

[5] Morari M. and Perkins J.D. (1994)Design for Operations. In *Foundations of Computer-Aided Process Design (FOCAPD).*

[6] Bansal V., Perkins J., Pistikopoulos E., Ross R. and Schijndel J. V. (2000) Simultaneous design and control optimization under uncertainty, *Comput. Chem. Eng. Vol. 24*, 261–281.

[7] Schweiger C.A. and Floudas A. (1997), Interaction of Design and Control: Optimization with Dyamic Models, In *Optimal Control Theory, Algorithms and Applications*, (Hager, W.W. and Pardalos, P.M., eds.), Kluwer Academic Publishers, Dordrecht.

[8] Deb, K. *Multi-objective optimization using evolutionary algorithms*. 2001, Wiley, Chichester, England.

[9] Esposito W. R. and Floudas C. A. (2000), "Deterministic Global Optimization in Nonlinear Optimal Control Problems," *J. Global Optim. Vol. 17*, 97–126.

[10] Esposito W. R. and Floudas C. A. (2000), Global Optimization for the Parameter Estimation of Differential-Algebraic Systems," *Ind. Eng. Chem. Res. Vol. 39*, 1291–1310.

[11] Singer A. B., Bok J. K.and Barton P. I. (2001), "Convex Underestimators for Variational and Optimal Control Problems," *Computer Aided Chem. Eng., Vol. 9*, 767–772.

[12] Papamichail I. and Adjiman C. S. (2002),"A Rigorous Global Optimization Algorithm for Problems with Ordinary Differential Equations," *J. Global Optimization, Vol. 24*, 1–33.

[13] Banga J. R., Alonso A. A. and Singh R. P. (1994), "Stochastic Optimal Control of Fed-Batch Bioreactors,"*Presented at the AIChE Annual Meeting*, San Francisco.

[14] Banga J. R. , Martín R. P., Gallardo J. M. and Casares J. J. (1991),"Optimization of thermal processing of conduction-heated canned foods: study of several objective functions," *J. Food Eng. Vol. 14*, 25–51.

[15] Banga J. R. and Seider W. D. (1996),"Global optimization of chemical processes using stochastic algorithms," In *"State of the Art in Global Optimization", C. A. Floudas and P. M. Pardalos (Eds.)*, Kluwer Academic Pub., Dordrecht, pages 563–583.

[15] Lopez Cruz,I.L., L. G. Van Willigenburg and G. Van Straten Efficient Differential Evolution algorithms for multimodal optimal control problems. *Applied Soft Computing*, 3 (2003) 97-122

[16] Wang F.S. and Chiou J.P. (1997), "Optimal Control and Optimal Time Location Problems of Differential-Algebraic Systems by Differential Evolution," *Ind. Eng. Chem. Res., Vol. 36*, 5348–5357.

[17] Ali M., Storey C., and Törn A. (1997), "Application of stochastic global optimization algorithms to practical problems,"*J. Optim. Theory Appl. Vol. 95*, 545–563.

[18] Barton P. I., Allgor R., Feehery W. and Galán S. (1998) Dynamic optimization in a discontinuous world, *Ind. Eng. Chem. Res. Vol. 37*, 966–981.

[19] Silva, C.L.M., Oliveira, F.A.R. and Hendrickx, M. (1993) Modelling Optimum Processing Conditions for the Sterilization of Prepackaged Foods. Food Control 4 (2), 67-78

[20] Durance, T.D. (1997) Improving Canned Food Quality with Variable Retort Temperature Processes. Trends in Food Sci. Technol. 8 (4), 113-118

[21] Karel, M. (1988) Optimizing the Heat Sensitive Materials in Concentration and Drying. In Preconcentration and Drying of Food Materials (Bruin, S., ed.), pp. 217-233, Elsevier, Amsterdam

[22] Banga, J. R., E. Balsa-Canto, C. G. Moles and A. A. Alonso (2003) Dynamic Optimization of Bioreactors - a Review. Proc. Ind. Natn. Science Academy , 69A(3-4):257-265.

[23] Nicolai, B.M., Scheerlinck, N. and DeBaerdemaeker, J. (2000) Stochastic Perturbation Analysis of Thermal Food Processes with Random Field Parameters. Transactions- American Society of Agricultural Engineers 43 (1), 131

OPTIMISATION OF HEAT TREATMENT OF MILK

Rudy de Wit and Hans Nieuwenhuijse
Corporate Research
Friesland Coberco Dairy Foods
P.O. box 87, 7400 AB Deventer
The Netherlands
E-mail: r.dewit@fcdf.nl

KEYWORDS

Uniresponse modelling, multiresponse modelling, heat treatment, milk, flavour formation.

ABSTRACT

Simulation tools are used to optimise setpoints of heat treatment processes on lab, pilot plant and production scale are used. In this paper the use of multiresponse modelling for understanding chemical reactions during heat treatment of milk will be discussed. A reaction mechanism of formation of volatile sulfur compounds is proposed. Several heat treatments of high-pasteurised milk will be simulated using the proposed reaction model.

INTRODUCTION

Obtaining competitive advantage in the market requires short time-to-market of new product introductions and reduced costs in operations. Short time-to-market of new introductions can be achieved by good design of experiments during product development projects. The design of experiments can be supported using simulation models that predict the effects of processing on the quality of the end product. However, simulation of effects can never replace experiments, but their number can be reduced.

The predictive models for simulation of heat-induced changes do not have to be based only on conversion rate models (black box models), but can also be based on more mechanistic models (grey or white box models). In this paper an example of using a mechanistic model for formation of volatile sulfur compounds in milk during heat treatment will be discussed.

FORMAL KINETIC MODELS VS MECHANISTIC KINETIC MODELS

The change of concentrations in food products due to chemical reactions can be mathematically described via at least two different approaches: formal kinetic models or mechanistic kinetic models.

Formal kinetic models are based on the change in concentration of a compound over time, which can be described as:

$$-\frac{dC}{dt} = k * C^n \qquad (1)$$

with C the concentration, t time, k the reaction rate constant and n the order of the reaction. There are two reaction orders. The first one is determined via calculation of the initial reaction rate at various concentration, this order is the reaction order with respect to concentration n_c. The second reaction order is determined via the change of concentration over time. The two reaction orders do not need to be the same (Van Boekel and Tijskens, 2001). The kinetic model according equation (1) does not give information about the mechanism of a reaction (Van Boekel and Walstra, 1995). Furthermore the reaction rate constants are only valid within the boundaries of the study, e.g. extrapolation outside the boundaries of study is not allowed (Stewart et al., 1996).

Mechanistic kinetic models are based on a proposed reaction mechanism that describes the change of concentration of key reaction compounds. Reactions in foods are usually quite complicated. In fact just studying the change of concentration of a single compound does not give information about the underlying reaction mechanism. A possible solution for better understanding of the underlying reaction mechanism is using multiresponse modelling. (Van Boekel and Tijskens, 2001). One can apply multiresponse modelling when more than one reactant is measured at the same time. Mathematically the chemical reaction mechanism (2) is described with the following coupled differential equations (3).

$$\begin{aligned} A + B &\xrightarrow{k_1} C + D \\ C &\xrightarrow{k_2} E \\ D + B &\xrightarrow{k_3} F \end{aligned} \qquad (2)$$

$$\frac{d[A]}{dt} = -k_1[A][B]$$
$$\frac{d[B]}{dt} = -k_1[A][B] - k_3[B][D]$$
$$\frac{d[C]}{dt} = k_1[A][B] - k_2[C] \quad (3)$$
$$\frac{d[D]}{dt} = k_1[A][B] - k_3[B][D]$$
$$\frac{d[E]}{dt} = k_2[C]$$
$$\frac{d[F]}{dt} = k_3[B][D]$$

The reaction rate constants are estimated by fitting the numerically solved ordinary differential equations to experimental data.

Proposing a reaction mechanism is an iterative process (figure 1). Some iteration loops are ussually necessary to fit the right model to the observed responses (van Boekel, 2000).

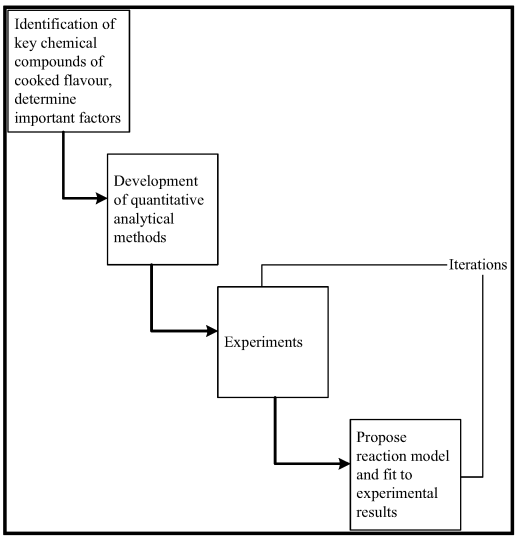

Figure 1:General iteration cycle to propose a kinetic model (after Van Boekel, 2000).

Estimation of parameters and model discrimination to determine which model should be accepted is based on Bayesian statistics (Stewart et al., 1998). It is beyond the scope of this paper to explain this method in more detail. We used the software program Athena visual workbench (www.athenavisual.com) for estimation of parameters and model discrimination.

Multiresponse modelling can be used to elucidate reaction mechanism in food systems. It has been applied to sugar reactions in milk (Berg, 1993), Maillard reaction between proteins and sugars (Brands, 2002), Maillard reactions between amino acids and sugars (Martins, 2003) and chlorophyll degradation in olives (Van Boekel, 2000).

Multiresponse modelling requires more experimental work, because more responses at one time need to be analysed (Van Boekel and Tijskens, 2001).

MODELLING OF HEAT-INDUCED FORMATION OF VOLATILE SULFUR COMPOUNDS IN MILK

Heat treatment of milk induces formation of different volatile compounds. Heated milk flavours can be devided into four notes: cooked or sulfurous, heated or rich, caramelised and burnt (Shipe et al., 1978). The change of notes is correlated to the instensity of heat treatment.

The cooked or sulfurous flavour is mainly caused by formation of volatile sulfur compounds. Hydrogen sulfide, sulfur dioxide, methanethiol, dimethylsulfide and carbon disulfide are among other volatile sulfur compounds examples of compounds that cause cooked flavour in heated milk (Steely, 1994).

Identification of key chemical compounds of cooked flavour was based on literature review. We identified hydrogen sulfide, carbonyl sulfide, methanethiol, dimethyl sulfide and dimethyldisulfide as key chemical compounds of cooked flavour. These compounds originate from protein-bound cysteine and methionine. We developed quantitative analytical methods (GC-MS, HPLC and spectrofotometric methods) to quantitate the concentrations of the key compounds in heated milk. It turned out that development of reliable reproducible analytical methods required most of the effort in this study.

The quantification of hydrogen sulfide was difficult due to the high volatality of it (figure 2). The reproducibility of the method was not good,. although a trend is visible. Therefore H_2S-concentrations were used in our parameter estimation.

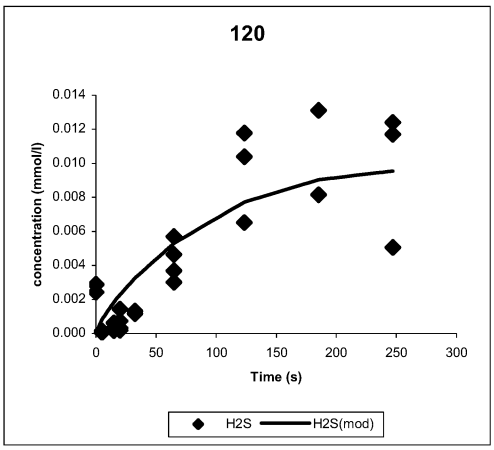

Figure 2: Formation of hydrogen sulfide during heat treatment at 120°C. Solid line represents model prediction.

The precursor of hydrogen sulfide is protein-bound cysteine. In figure 3 the concentration of masked sulfhydryl groups and free sulfhydryl groups are presented. The concentration curve of free SH-groups seemed comparable to the calculated concentration of unfolded β-lactoglobulin according to the model proposed by De Jong (1996).

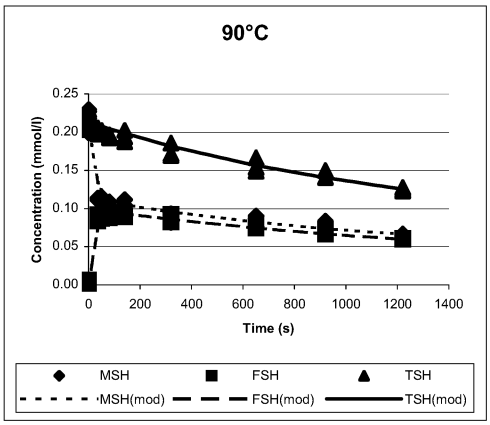

Figure 3: Masked, free and total Sulfhydryl concentration as function of heating time at 90°C. Lines represent model predictions.

Carbonyl sulfide, COS, is a intermediate compound in the reactions of volatile sulfur compound. Although the origin of COS is not yet clear to us, it is clearly formed during heat treatment of milk (figure 4).

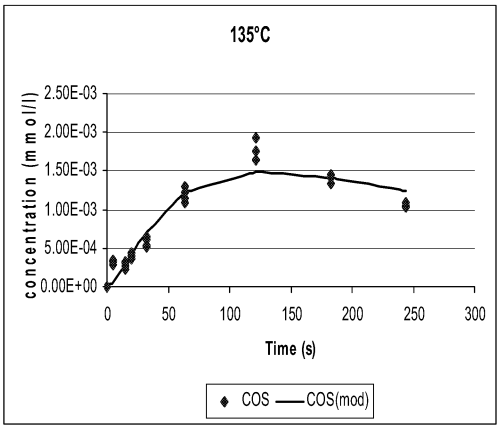

Figure 4: Formation of Carbonyl sulfide (COS) during heat treatment at 135°C. Solid line represents model prediction.

The first reaction mechanism that was proposed was based on a literature review on formation of volatile sulfur compounds (figure 5).

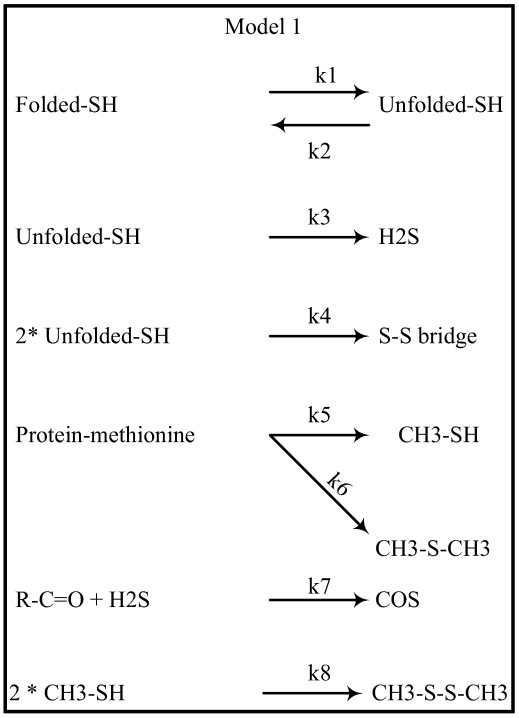

Figure 5: Reaction model of formation of volatile sulfur compounds.

Model 1 was rejected because the model did not fit the observed responses. 2 other models were fitted to the observed responses and finally the reaction scheme presented below was accepted.

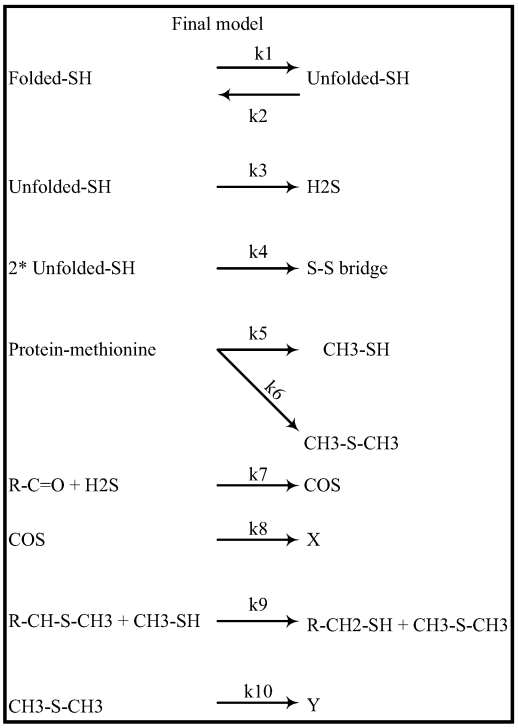

Figure 6: Reaction model 3 of formation of volatile sulfur compounds during heat treatment of milk.

The activation energies of all reaction rate constants were estimated. We observed that the reaction rate constants had different activation energies below 100°C compared to above 100°C (figure 7).

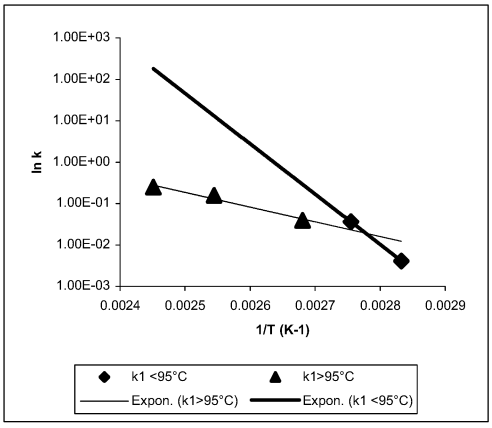

Figure 7: Arrhenius plot of reaction rate constant vs inverse Temperature. Solid line are model predictions.

Different temperature dependencies above and below 95°C are often observed in whey protein reactions in milk (Oldfield et al, 1998, Dannenberg and Kessler, 1988, De Jong, 1996 and many others).

OPTIMISATION OF HEAT TREATMENT OF MILK

Heat-treated milk can be optimised with respect to formation of formation of volatile sulfur compounds. In the next example we show a possibility to improve taste of high-pasteurised milk, while maintaining the inactivation of Bacillus cereus spores (12D-reduction). We will show the difference between direct (direct steam injection) and indirect heat treatment (figure 8 and 9) (after Bylund (1995).

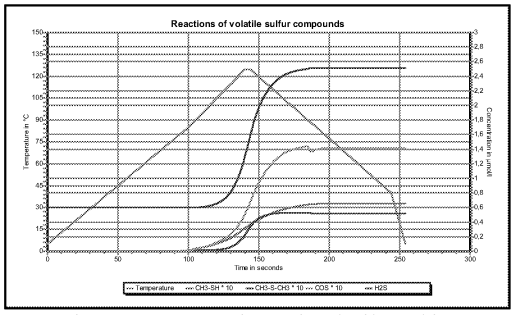

Figure 8: Formation of volatile sulfur compounds as a function of time-temperature profile of indirect heat treatment of milk at 124°C.

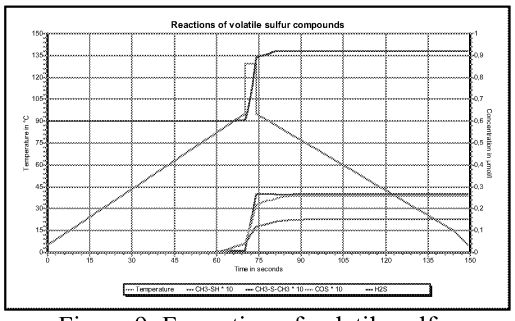

Figure 9: Formation of volatile sulfur compounds as a function of time-temperature profile of direct heat treatment of milk at 129°C.

The maximum heating temperature of direct heated milk in this example was 5°C higher than the indirect heated milk in order to reach a 12D reduction of Bacilluse cereus spores. The formation of volatile sulfur compounds in direct heated is far less than in indirect heated milk. Hence the indirect heated milk will have a much intenser cooked flavour than the direct heated milk.

CONCLUSIONS

Chemical reactions in food systems like milk are complicated. We proposed a model of reactions of volatile sulfur containing compounds using multiresponse modelling.

We showed that heat treatment processes can be optimised using simulation models with respect to formation of volatile sulfur compounds.

REFERENCES

Berg, H.E., 1993. "Reactions of lactose during heat treatment of milk: a quantitative study". PhD-thesis, Wageningen Agricultural University, Wageningen

Brands, C.M.J., 2002. "Kinetic modelling of the Maillard reaction between proteins and sugars." PhD-thesis, Wageningen University, Wageningen.

Bylund, G., 1995. "Dairy processing handbook" Tetra Pak Processing AB, Lund Sweden

Dannenberg, F. and Kesslerm, H.G., 1988. "Reaction kinetics of the denaturation of whey proteins in milk." *Journal of Food Science* 53, 258-263.

De Jong, P., 1996. "Modelling and optimisation of thermal treatments in the dairy industry." PHd-thesis, TU Delft, Delft.

Martins, S.I.F.S, 2003. "Unravelling the Maillard reaction network by multiresponse kinetic modelling." PhD-thesis, Wageningen University, Wageningen.

Oldfield, D.J., Singh, H., Taylor, M.W. and Pearce, K.N., 1998. "Kinetics of denaturation and aggregation of whey proteins in skim milk heated in an Ultra-high temperature (UHT) pilot plant." *International Dairy Journal* 8, 311-318.

Shipe, W.F., Bassette, R., Deane, D.D., Dunkley, W.L., Hammond, E.G., Harper, W.J., Kleyn, D.H., Morgan, M.E., Nelson, J.H., and Scanlan, R.A., 1978. "Off-flavors of milk: Nomencalture, Standards and bibliography." *Journal of Dairy Science* 61, 855-869.

Steely, J.S., 1994. "Chemiluminescence detection of sulfur compounds in cooked milk." In *Sulfur compounds in foods* C.J. Mussinan and M.E. Keelan (eds), Washington DC, 22-35

Stewart, W.E., Hensen, T.L. and Box, G.E.P., 1996. "Model discrimination and criticism with single response data." *AIChE Journal* 42, 3055-3062.

Stewart, W.E., Shon, Y., Box, G.E.P., 1998. "Discrimination and goodness of fit of multiresponse mechanistic models." *AIChE Journal* 44, 1404-1412.

Van Boekel, M.A.J.S. and Tijskens, L.M.M., 2001. "Kinetic modelling". In *Food process modelling,* L.M.M. Tijskens, M.L.A.T.M. Hertog and B.M. Nicolaï (eds) Cambridge, 35-59.

Van Boekel, M.A.J.S. and Walstra, P., 1995. "Use of kinetics in studying heat-induced changes in foods." In *Heat-induced changes in Milk 2nd edition* P.F. Fox (ed), Brussels, 22-50.

Van Boekel, M.A.J.S., 2000. "Kinetic modelling in food science: a case study on chlorophyll degradation in olives." *Journal of the Science of Food and Agriculture* 80, 3-9.

PROCESS MODELLING AND OPTIMISATION IN ICI FOR APPLICATIONS IN THE FOOD SECTOR

by

Tahir Malik
ICI Strategic Technology Group
Wilton Centre
Redcar TS10 4RF
U.K.

Chris Winkel
Quest International
P.O. Box 2
1400 CA Bussum
Netherlands

KEYWORDS

Dynamic Process Modelling, Parameter Estimation, Optimisation.

ABSTRACT

There are many examples of the use of process modelling and process optimisation in ICI, a specialty chemicals manufacturing company, with a significant product portfolio for supply to the foods sector. ICI's core business is organised into four international businesses (Quest Foods and Fragrances, National Starch, Uniqema and ICI Paints) each with a range of applications in process modelling and other types of modelling. This paper will give an overview of the process modelling applications, particularly with reference to applications to products for the foods sector that have been carried out by collaboration between ICI's corporate Strategic Technology Group (STG) and the international businesses.

Process Modelling is applied at a range of time and length scales and includes:

1. Thermodynamics and physical properties modelling.

2. Equation based modelling for capturing mechanistic (steady or dynamic) models that can be used for (steady, dynamic or mixed steady/ dynamic data) parameter estimation, optimisation, model discrimination or model based experimental design.

3. Fluid dynamics modelling based both on detailed finite volume discretisation in CFD methods and based on chemical engineering correlations.

4. Stochastic discrete event modelling for production capacity and supply chain dynamics, identification of key bottlenecks and their shifting behaviour and the impact of, dependence on and utilisation of key resources.

The paper will present examples of current interest in some of these categories.

SIMULATION OF PROCESSES AND PRODUCTS

ON THE USE OF CFD MODELLING IN FLUIDISED BED REACTOR DESIGN

Frédéric Depypere[a,b], Jan G. Pieters[b] and Koen Dewettinck[a]
[a] Food Technology and Engineering
[b] Biosystems Engineering
Ghent University
Coupure Links 653
B-9000 Ghent, Belgium
E-mail: Frederic.Depypere@UGent.be

KEYWORDS
Computational Fluid Dynamics, Pressure Drop, Woven Wire Plate, Fluidisation, Glatt GPCG-1, Air Distribution.

ABSTRACT

Recognised for its excellent mixing capabilities and its optimal heat and mass exchange, fluid bed technology is being increasingly applied in the food industry where drying, coating, agglomeration and/or mixing processes are involved. The primary factor influencing fluidised bed processing, is the air flow and its distribution. Numerical modelling techniques such as Computational Fluid Dynamics (CFD) provide an important means to investigate air flow distribution through specific equipment. In this paper, CFD simulation results of the air flow in a Glatt GPCG-1 lab-scale fluid bed in which stainless steel woven wire mesh distributors were used, are presented, together with the results of verification experiments. As an unequal air flow inside the plenum was found to occur, CFD was used as a design tool to investigate reactor configuration changes in order to obtain a more homogeneous air flow towards the distributor.

INTRODUCTION

One of the main reasons for the success of the fluidised bed in the food industry is its ability to perform a number of unit operations such as mixing, drying, coating and granulating, within the same piece of equipment, either separately or sequentially (Depypere et al. 2003). The unique features of the fluid bed – excellent mixing capacity and high heat and mass transfer rates – are highly dependent on the quality of fluidisation resulting from the bubble characteristics of the fluidising gas, which to a large extent depend on the distributor design (Senadeera et al. 2000). In order to understand the fluidisation hydrodynamics of a fluid bed operation, it is essential to assess how air flow is distributed through the equipment. This paper reports on the use of Computational Fluid Dynamics (CFD) as a numerical tool to enlarge this understanding. Whereas CFD software codes were originally developed as a tool for predicting air flow movement over planes and cars, it is equally appropriate to use CFD for the prediction of air flow and related phenomena inside items of food processing equipment such as a fluid bed (Scott and Richardson 1997).
The main objective of this research was to investigate the effect of the air distributor and the upstream air supply system on the air flow in a top-spray fluidised bed reactor. For this purpose, CFD simulations were performed and the modelling results were compared with laboratory experiments. Where appropriate, possibilities for reactor design alterations in order to improve air flow conditions were numerically investigated.

MATERIALS AND METHODS

CFD Model of the Fluid Bed

To simulate fluid flow and heat transfer problems, the mass conservation or continuity equation, the momentum conservation or Navier-Stokes transport equations and the energy conservation equation are numerically solved. Using CFD, this set of partial differential equations is written in a discretised algebraic form which can be solved to obtain data for the flow field variables in discrete points in space and/or time (Anderson 1995). The computational scheme used in most commercial CFD packages is the finite volume method (Wang and Sun 2003). This control-volume-based technique consists of: (1) division of the domain into discrete control volumes using a computational grid (mesh), (2) integration of the governing equations on the individual control volumes in the construction of algebraic equations for discrete dependent variables, (3) linearisation of the discretised equations, and (4) solution of the resultant linear equation system.
Prior to the CFD calculations, the geometry was defined and a grid was generated using Gambit 2.0.4 (Fluent Inc., Lebanon, U.S.), the pre-processor and mesh generator for the CFD solver. Fig. 1 displays the 3-D meshed fluidised bed domain as a realistic representation of the lab-scale Glatt GPCG-1 unit under research.

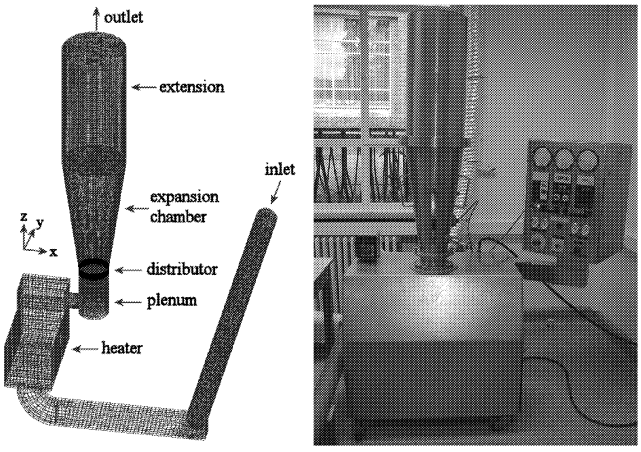

Figure 1. Gambit 3-D Meshed Domain (left) for the Glatt GPCG-1 Fluid Bed Reactor (right)

Sequentially, the air inlet, the connection ducts comprising the heating chamber, the air plenum, the distributor, the tapered expansion chamber, the cylindrical extension and the air outlet can be distinguished.

For all cases studied in this paper, a hybrid hexahedral-tetrahedral grid of 200,000-240,000 elements was generated. At locations where the largest gradients were expected to occur, mesh refinement was applied: boundary layers in the vicinity of the distributor and an increased number of nodes near the walls. The mesh quality was evaluated using the EquiAngle Skew (Q_{EAS}) criterion which is a normalised measure of the element skewness. For all generated 3-D meshes, the Q_{EAS} of at least 90% of the control volumes was lower than or equal to 0.4. Therefore, the overall mesh quality could be considered very good. The complete domain was conceived as a fluid (air) zone. After meshing the domain, the grid was imported into Fluent 6.0.20 (Fluent Inc., Lebanon, U.S.), the CFD solver used in this study.

Before solving and post-processing the results, the boundary conditions and the fluid and solids properties had to be specified. The air inlet and air outlet were modelled as a pressure inlet and a pressure outlet, respectively. The domain walls were modelled as stationary boundaries at which the no-slip condition was applied.

Using Fluent, the air distributor was modelled as an internal boundary using the porous media model in which an empirically determined flow resistance in a 3-D domain zone is defined. Therefore, porous media are modelled by the addition of a momentum sink in the standard Navier-Stokes equations, contributing to the pressure gradient in the porous cell. The momentum sink term is composed of a Darcy term describing the viscous loss and an inertial loss term, originating from the orifice equation:

$$S_i = -\left(\sum_{j=1}^{3} D_{ij} \mu u_j + \sum_{j=1}^{3} C_{ij} \frac{1}{2} \rho |u_j| u_j \right)$$

where S_i is the momentum source term for the i-th (x, y or z) momentum equation, u is the mean superficial air velocity normal to the distributor, μ is the fluid dynamic viscosity, ρ is the fluid density and D and C are matrices, representing the impermeability and the inertial resistance, respectively, in the three coordinate directions.

In this study, the use of two different woven wire stainless steel Robusta distributors (Spörl) in the Glatt GPCG-1 device was investigated. Table 1 shows the distributor characteristics, together with previously determined values for the impermeability and inertial resistance in the main air flow direction (Z). Using the porous media model enabled to also specify values for D and C in both the X- and Y-direction. From previous research (data not shown), these values were found to be at least 10 times the ones in the Z-direction. In Table 1, the number of wires per inch (wpi) in the one direction is denoted as warp mesh, while weft mesh indicates the number of wires per inch in the orthogonal direction. The filter fineness indicates the diameter of the largest, hard spherical particles which can pass through the distributor under stationary flow conditions.

Table 1. Characteristics and Porous Zone Modelling Inputs for the Distributors Used in this Study

	Rob172	Rob280
Warp × weft mesh (wpi)	172 × 36	280 × 70
Filter fineness (μm)	100	55
Distributor thickness (μm)	650	390
Porous zone thickness (mm)	7	7
Z-impermeability (m^{-2})	3.40 E+08	7.54 E+08
Z-inertial resistance (m^{-1})	3,204	2,361

CFD simulations were performed using a single-precision steady-state segregated implicit solver. Flow turbulence was simulated using the "realizable" k-ε model with enhanced wall treatment, the latter being one of the available tools in Fluent to model the near-wall region. The choice for the "realizable" k-ε model (Shih et al. 1995) instead of a standard k-ε model was justified for a number of reasons. Firstly, the realizable model satisfies certain mathematical constraints on the Reynold stresses, consistent with the physics of turbulent flows. Moreover, the use of the standard k-ε model resulted in convergence problems and the occurrence of physically impossible reversed air flow at the outlet boundary.

With respect to equation discretisation, a standard scheme was used for the continuity equation while a first order upwind scheme was used for both the turbulence kinetic energy equation and the turbulence dissipation rate equation. To reduce numerical diffusion, a second order upwind scheme was selected for the discretisation of the momentum and energy equations. The relationship between velocity and pressure corrections was calculated using the SIMPLE algorithm. Default values for all under-relaxation factors were applied, except for the turbulence kinetic energy and the turbulence dissipation rate. In order to enhance convergence, the under-relaxation factors of these two turbulence quantities were lowered to a value of 0.6.

Fluid Bed Verification Experiments

A first verification experiment consisted of the determination of the velocity/pressure-drop characteristic of the distributors, placed inside the Glatt GPCG-1 fluid bed, operating under steady-state conditions. The air flow rate inside the reactor was determined using a rotating vane probe (Testo) while the pressure drop across the distributor was measured using a 10 hPa differential pressure probe (Testo) by recording the static pressure in two pressure taps, located 0.04 m upstream and downstream the distributor, respectively.

A second experiment was carried out to verify the obtained CFD air flow patterns inside the plenum and the tapered expansion chamber. A series of 23 adhesive type K thermocouples with a 7 mm metal disc termination was attached to the inner walls of these device components. Wall temperature records were registered during steady-state operation of the fluid bed. The inlet air temperature entering the plenum was set at 50°C.

RESULTS AND DISCUSSION

CFD Simulation Results

Fluent 3-D simulations were carried out for a series of different values for the reactor under-pressure, specified at the reactor outlet. Fig. 2 shows a combined contour plot of the velocity magnitude and velocity vector plot in a XZ-plane through the centre of the plenum and the expansion chamber for the GPCG-1 device in which one Rob172 and two sequential Rob280 distributor plates, respectively, were mounted. For both simulations, the pressure outlet boundary condition was set at -400 Pa.

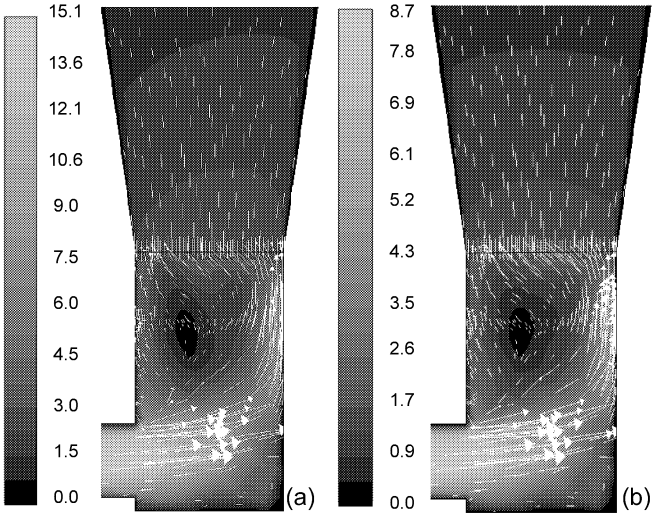

Figure 2: Fluent 3-D XZ-plane Contours of Velocity Magnitude (m/s) (Reactor Under-pressure: 400 Pa): (a) one Rob172, (b) two Rob280

From Fig. 2, it was seen that due to the left side entrance in the air plenum and the relatively small plenum height, the highest air velocities immediately beneath the distributor were found at the right side of the plenum while a recirculation zone was distinguished immediately below the left side of the distributor. From this, it was clear that upon contact with the distributor, a non-uniform air flow pattern developed inside the air plenum. Above the distributor, a decelerated upward air flow was observed.

This is an essential feature of the fluidised bed unit with a tapered expansion chamber under research: a careful selection of the air flow rate will aid to prevent particles in the fluidised bed to become elutriated or entrapped in the filter housing.

Furthermore, the simulations revealed that for low to moderate air mass flow rates, both distributors succeeded in homogenising the incoming unequal air flow. However, it was also seen that for the Rob172 distributor at moderate to high air mass flow rates, a slightly preferential air flow at the right side of the expansion chamber still remained.

Experimental Verification Results

For both distributors in this study, Fig. 3 shows the comparison between the simulated and the experimentally recorded dependence of the distributor pressure drop on the air mass flow rate in the laboratory-scale Glatt GPCG-1 fluid bed unit. Although it was seen that that the CFD simulations slightly underestimated the second order contribution of the velocity on the pressure drop across the distributor, good agreement was found between the simulation results and the experimental recordings.

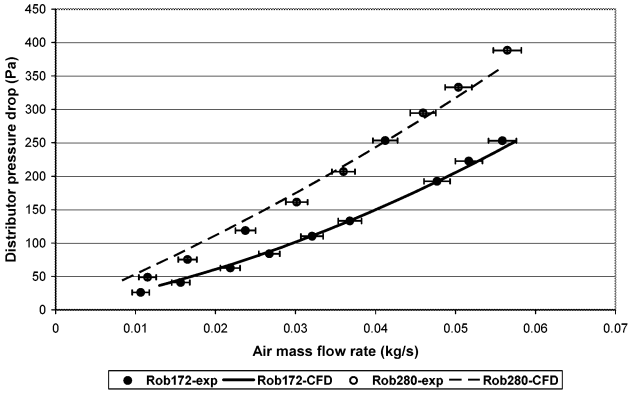

Figure 3: Pressure Drop across Both Tested Distributors versus Air Mass Flow Rate in the Glatt GPCG-1 Device: CFD versus Experimental Results

As a verification of the air flow patterns generated using CFD, Fig. 4 shows the measurement results of the steady-state inner wall temperatures in the Glatt GPCG-1 device.

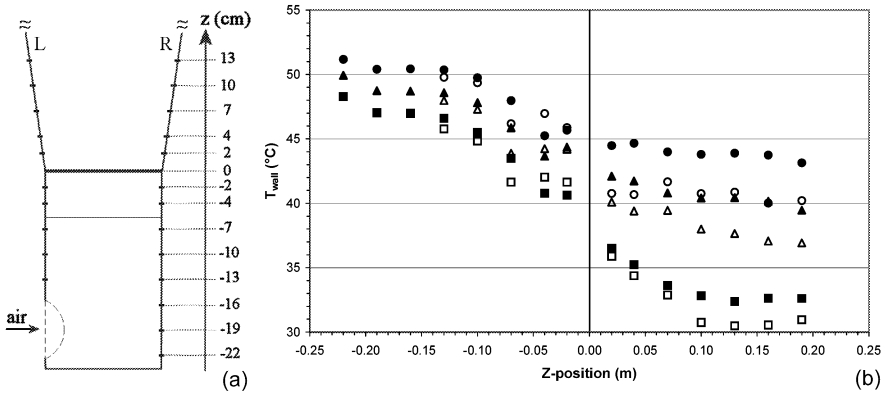

Figure 4: Wall Temperature Verification Experiment: (a) Thermocouple Positions, (b) Steady-state Inner Wall Temperature Profile at the Left (Open Symbols) and Right (Closed Symbols) Side for a Low (Squares), Moderate (Triangles) and High (Circles) Air Mass Flow Rate (One Rob172 Distributor Positioned at z = 0 cm).

Both the position of the thermocouples relative to the distributor (one Rob172, zero height) and the wall temperature profile along two sides of the plenum and the expansion chamber are shown for a low, moderate and high air mass flow rate (0.013/0.033/0.053 kg/s). As the incoming air flow was heated up to a constant temperature of 50 °C at the bottom of the plenum chamber, it was expected that the wall temperature profile along the left and right side of the plenum and the expansion chamber could be used as indicative for the main air flow behaviour.
In the plenum chamber, a gradual decrease of the inner wall temperature from the bottom to the distributor plate was observed, with only small temperature differences at opposed locations on both plenum sides. Through CFD, the occurrence of a recirculation zone in the plenum was demonstrated. By also taking into account the heat losses through the wall, the above described temperature profile on both plenum sides can be explained. Above the distributor, it was seen that the temperature profile highly depended on the air mass flow rate. The higher the latter, the slower the temperature decrease along the walls and the more the wall temperatures at opposed locations differed from each other. Higher temperature values were registered at the right side of the expansion chamber, which was in accordance with the simulated air flow pattern in Fig. 2a.

Design Alternatives

One of the principal features of CFD is its suitability to simulate the effect of changes in reactor design. Given the unequal air flow in the plenum chamber, CFD was used to evaluate a number of modifications upstream the distributor. These changes aimed at a controlled knocking down of the incoming air momentum. More specifically, comparison was made with the air flow conditions for one Rob172 distributor, as shown in Fig. 2a (mass flow rate: 0.05 kg/s). The investigated modifications consisted of three variants for which the air inlet to the plenum remained unchanged with respect to the original configuration of the Glatt GPCG-1 device and one variant for which the lateral air inlet was replaced with a central inlet at the bottom of the plenum. For a lateral air inlet to the plenum, the incorporation of a pre-distributor, a packing of equally sized ceramic balls and an air inlet tube extension, bent to the bottom of the plenum, were considered separately. The pre-distributor was placed 0.1 m below the distributor and was designed to provide half of the resistance of the latter. The pre-distributor was modelled as a porous zone boundary condition with 7 mm thickness. The ceramic balls (diameter: 0.04 m diameter, density: 1300 kg/m^3, void fraction: 0.45) packing was modelled as a porous zone with 0.08 m height, positioned 0.1 m upstream the distributor. In Fig. 5, CFD simulation results are shown for the pre-distributor, the ceramic balls packing and the central bottom plenum air inlet modification, respectively.
For the pre-distributor modification, it was found that the pre-distributor was capable of establishing a partial homogenisation of the incoming air flow, after which the distributor successfully homogenised the prehomogenised air flow. A similar result was obtained for the ceramic balls packing. Where both aforementioned modifications did not excessively increase the total reactor pressure drop, this was not the case with the downward bent inlet air tube extension. The latter modification caused the required fan power to become uneconomically high while it did not contribute to a homogenisation of the air flow.

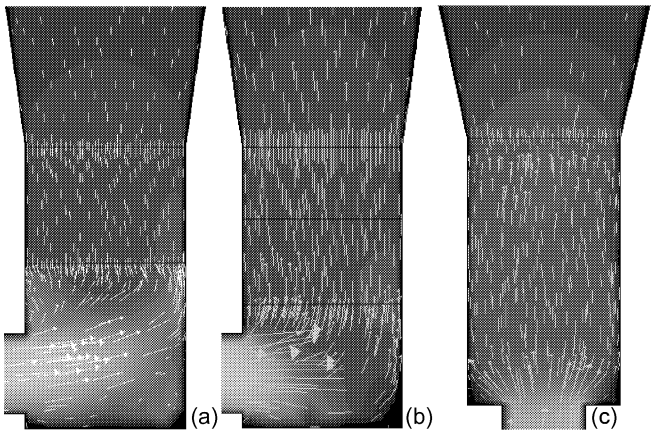

Figure 5: CFD Simulated Air Flow for a Modified Plenum Chamber Design: Cases of a (a) Pre-distributor, (b) Ceramic Balls Packing, (c) Bottom Plenum Air Inlet

When the lateral air inlet to the plenum was replaced with a central bottom inlet, the main air flow inside the plenum followed the vertical direction and a nearly homogeneous air flow contacting upon the distributor was obtained. Consequently, it was seen that the distributor easily coped with its air homogenising functionality. With respect to the findings in this section, it should off course be noticed that the above conclusions are drawn from CFD calculations and should be verified experimentally.

CONCLUSIONS

In this study, the effect of the air distributor and the upstream air supply system on the air flow in a top-spray fluidised bed reactor was investigated using a combined CFD and experimental approach. CFD simulations were performed for a laboratory-scale Glatt GPCG-1 fluidised bed coating unit in which verification experiments were conducted. The CFD modelling results for the velocity/pressure-drop characteristics of both tested distributors were found to be in good agreement with the experiment. Furthermore, the simulated air flow pattern was confirmed by inner wall temperature recordings.
CFD modelling revealed that, due to the lateral air inlet in the plenum chamber of the Glatt GPCG-1 apparatus, a non-homogeneous air flow towards the distributor was established, which posed higher demands on the distributor performance in order to obtain a fully homogenised air flow towards the expansion chamber. Using CFD as a design tool, a number of possible plenum configuration changes was investigated. With the present lateral air inlet of the plenum chamber, the inclusion of a pre-distributor or a ceramic balls packing layer was found to be appropriate without an excessive increase in fan power requirement. Evidently, the relocation of the plenum air inlet from the side to the bottom of the plenum was also found to be beneficial towards equal air flow conditions across the plenum cross-section.

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REFERENCES

Anderson Jr., J.D. 1995. *Computational Fluid Dynamics. The Basis with Applications*. McGraw-Hill, New York.

Depypere, F.; K. Dewettinck; F. Ronsse; and J.G. Pieters. 2003. "Food Powder Microencapsulation: Principles, Problems and Opportunities." *Applied Biotechnology, Food Science and Policy* 1, No.2, 75-94.

Scott, G. and P. Richardson. 1997. "The Application of Computational Fluid Dynamics in the Food Industry." *Trends in Food Science and Technology* 8, No.4, 119-124.

Senadeera, W.; B.R. Bhandari; G. Young; and B. Wijesinghe. 2000. "Methods for Effective Fluidization of Particulate Food Materials." *Drying Technology* 18, No.7, 1537-1557.

Shih, T.H.; Liou, W.W.; Shabbir, A.; Yang, Z.; and J. Zhu. 1995. "A New k-ε Eddy Viscosity Model for High Reynolds Number Turbulent Flows." *Computers & Fluids* 24, No. 3, 227-238.

Wang, L. and D.W. Sun. 2003. "Recent Developments in Numerical Modelling of Heating and Cooling Processes in the Food Industry – a Review." *Trends in Food Science and Technology* 14, No.10, 408-423.

BIOGRAPHY

FRÉDÉRIC DEPYPERE was born in Kortrijk (Belgium) in 1978. He graduated from the Ghent University in 2001 as Bio-Engineer in Chemistry. He entered in the doctoral program at the Ghent University in the Faculty of Agricultural and Applied Biological Sciences in 2001 where he is currently Research Assistant of the Fund for Scientific Research – Flanders (Belgium) (F.W.O.-Vlaanderen). His doctoral research is entitled "Process optimisation of fluidised bed systems in the food industry using Computational Fluid Dynamics modelling" and is conducted at the Food Technology and Engineering Research Group (Department of Food Technology and Nutrition) in close cooperation with the Biosystems Engineering Research Group (Department of Agricultural Engineering). Among his research interests are food microencapsulation, gas-solid fluidisation, computational fluid dynamics modelling, powder technology and microscopical techniques.
E-mail: Frederic.Depypere@UGent.be
Webaddress 1: http://www.fte.ugent.be
Webaddress 2: http://www.BioSys.UGent.be

JAN G. PIETERS was born in Ghent (Belgium) in 1968. He graduated from the Ghent University in 1991 with a M.Sc. in Agricultural Engineering and in 1995 with a Ph.D. in Applied Biological Sciences. Since 1999, he is in charge of the Biosystems Engineering Research Group of the same university, with research topics in the fields of agricultural, environmental and food engineering.
E-mail: Jan.Pieters@UGent.be
Webaddress: http://www.BioSys.UGent.be

KOEN DEWETTINCK was born in Ghent (Belgium) in 1968. He graduated from the Ghent University in 1991 as Bio-Engineer in Chemistry. From the same university, he obtained a Ph.D. in Applied Biological Sciences in 1997. Since 1999, he is full-time docent in Food Technology and Engineering. He is head of the Laboratory of Food Technology and Engineering with research areas such as technology and functionality of dairy products and of oils and fats, powder technology, functionality of biopolymers and food authenticity. Since 2003, he is director of the Flemisch Technology Advisory Centre for the chocolate, biscuits and confectionary industries (VLAZ). Since 2004, he is director of the Flemisch Technology Advisory Centre for the vegetable processing industries (VLAG).
E-mail: Koen.Dewettinck@UGent.be
Webaddress: http://www.fte.ugent.be

MATHEMATICAL MODELLING OF DOUGH DEVELOPMENT DURING FERMENTATION PROCESS

Paolo Masi
Annalisa Romano
DSA - Facoltà di Agraria
Università di Napoli Federico II
Portici (NA), Italy
paolo.masi, anromano@unina.it

Gerardo Toraldo
Marina Marino
DIAAT - Facoltà di Agraria
Università di Napoli Federico II
Portici (NA), Italy
toraldo, mari@unina.it

KEYWORDS: fermentation kinetics, growth curve, non linear regression models, dough.

ABSTRACT

A descriptive growth model of the fermentation kinetic of wheat flour doughs was considered. The fermentation kinetic was investigated by monitoring the variation of the dough volume vs time by means of Image Analysis. This macroscopic result of leavening process is divided into three stages: the lag stage, positive acceleration stage and negative acceleration stage. The fermentation process is a nonlinear and time-dependent process; thus kinetic models describing the process should also be nonlinear and time dependent. To find the best descriptive model of the variation of dough volume versus time ($y = x(t)$), fitting results of three models were compared: Richards, Morgan-Mercer-Flodin and Gompertz models. By comparing experimental data and predicted values a good agreement is found and the present models are quite satisfactory. Therefore the modified Morgan-Mercer-Flodin model was chosen as the best descriptive model of the leavening process.

INTRODUCTION

The description of a fermentation process will always be a rough simplification of reality, since detailed picture of the various biological and physical phenomena responsible for bubbles growth during the leavening process is not fully elucidated yet. The dough leavening process involves biochemical, rheological and thermodynamic phenomena, which are nonlinear distributed-parameter processes.
From a physical point dough is a multiphase and multicomponent system mainly composed of proteins, lipids, carbohydrates, water and air. During fermentation phase, the metabolism of yeasts chemically transforms polysaccharides into carbon dioxide, ethyl alcohol and acetic and lactic acids. Carbon dioxide (CO_2), migrates toward the initial nuclei of air bubbles formed during kneading causing the dough to increase in volume giving rise to a foam-like structure (De Cindio and Correra, 1995; Autio et al., 1997).
The present study was limited to select suitable models that describe the variation of dough volume y only as a function of time t, i.e. $y= f(t)$.

MODELS, METHODOLOGY AND MATERIALS

All doughs were prepared using commercial soft wheat flour (43.75g, Barilla®: 7.5% proteins, 0.1% fat, 13.5% moisture content), water (25ml), salt (1.25g), sugar (0.5g) and yeast (Mastro Fornaio, Paneangeli®) at different quantity: 0.6 -1.1 -1.7 -2.3 -2.9 -3.4% (w/w). The leavening took place inside a leavening chamber, where both temperature and humidity were kept under control at 36 ± 1°C, 70% U.R.
Figure 1 shows the typical behavior of volume expansion on time during dough leavening given in terms of volume expansion ratio (volume at time t / volume at time 0). The loaf volume was determined by a computer assisted image analyser (Jandel Sigma Scan®Pro, 1995).
The shape is similar to the growth curve of the yeasts and is characterized by three distinct regions: (a) induction phase; (b) growth phase; (c) stationary phase (Stanier et al., 1993; Prescott, 1995). Such a behavior is accurately described in all regions by a sigmoid shaped curve.

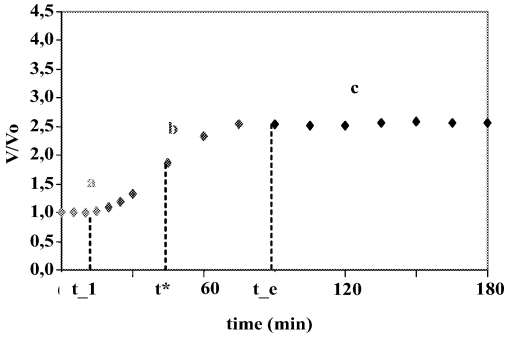

Figure 1: Volume expansion on time of doughs during leavening

Several models are available for this purpose and experimental comparison is needed to assist in the choice of the most appropriate model. The choice of a model is a not trivial task and is best made according to explicitly stated criteria. Ratkowski (1993) discussed five important points of consideration for nonlinear regression modeling:
1. parsimony: the model should contain as few parameters as possible;

2. parameterization: parameters with the best estimation properties should be used;
3. range of applicability: the data must cover the entire range described by the model;
4. stochastic specification: the error term structure must also be modeled;
5. interpretability: parameters with physical meaning are preferred.

Three of the most important sigmoidal nonlinear regression models from literature are Morgan-Mercer-Flodin, Richards and Gompertz models (Schepers, 2000). The Richards growth function has the following form:

$$y(x) = \frac{a}{(1 + e^{b-cx})^{1/d}} \tag{1}$$

The general form of the Morgan-Mercer-Flodin family function [MMF] is:

$$y(x) = \frac{(bc + ax^d)}{(b + x^d)}, \tag{2}$$

whilst the Gompertz equation is:

$$y(x) = ae^{-(b-cx)} \tag{3}$$

The independent and dependent variable are x and y, respectively.

These models contain four parameters: a, b, c, and d. For this study, after some preliminary computational tests, it is assumed that the parameter c of MMF model is equal to 1. All computations were performed by using the curve fitting system for Windows CurveExpert 1.3 (Hyams, 1995). The models show good agreement with the different experimental data set with correlation coefficient greater than 0.982.

Table 1: Comparison of the three fitting results

Yeast (%)	MMF		Richards		Gompertz	
	St. error	*Corr. coef.*	*St. error*	*Corr. coef.*	*St. error*	*Corr. coef.*
0.6	0.060	0.998	0.110	0.994	0.166	0.989
1.1	0.038	0.999	0.149	0.993	0.225	0.984
1.7	0.073	0.998	0.096	0.997	0.172	0.989
2.3	0.076	0.998	0.244	0.982	0.207	0.985
3.4	0.224	0.982	0.176	0.989	0.199	0.983

However, the overall best results, mainly in terms of standard error were actually obtained with MMF (see Table 1).

This can be justified by the mathematical features of MMF for $x=0$ $(y'(0)=0)$ which describes much better the experimental data behavior as it depicts an initial lag phase whilst the Richards and Gompertz model do not (Figure 2), unless specific modifications are considered (Fujikawa et al., 2004). In other words, MMF appears naturally adequate in describing the entire growth process.

In conclusion, MMF (with c=1) according to our experience must be preferred, since it contains just 3 parameters, fits very well to the observations, and also depicts the fermentation process more accurately than Richards and Gompertz models.

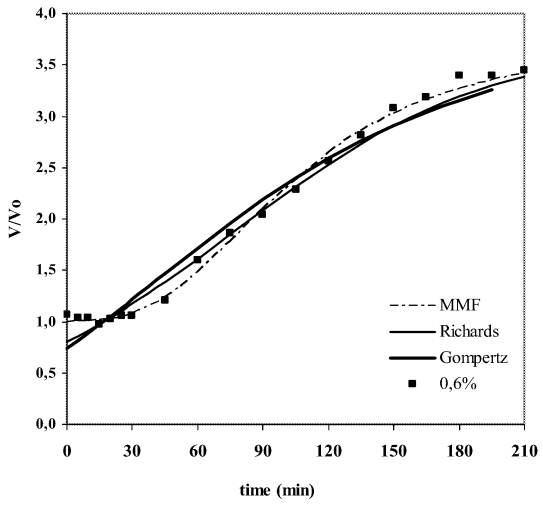

Figure 2: Observed volume loaf and fitted regression curves

Figure 3 shows MMF regression curves for different yeast contents.

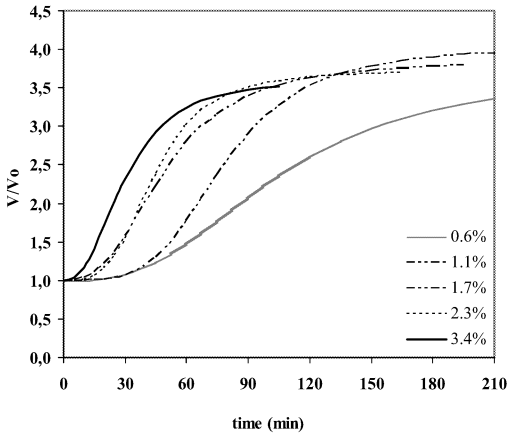

Figure 3: MMF fitting curves

The meaningful growth parameters of model, which can be expressed in terms of the curve parameters a, b, c are:

1. the inflection time t^*:

$$t^* = b^{1/d}\left[\frac{(d-1)}{(d+1)}\right]^{1/d} \tag{4}$$

2. the value s^* of the derivative at the inflection point, i.e. the maximum specific volume growth rate
3. the parameter d, which controls location of inflection point
4. the maximum relative volume expansion ratio of the loaf (a)

5. the lag time (*t_l*), numerically computed as the smallest time value in which the derivative becomes significantly greater than zero, say $y'(t\_l) > \varepsilon$ for some prefixed (small) value ε
6. the exhaustion stage (*t_e*), numerically computed as the smallest time value (after t^*) in which the derivative becomes small enough, say $y'(t\_e) < \varepsilon$.

They provide an idea about speed and intensity of the growth process. In Table 2 the parameter values obtained from the MMF fitting curves for the experimental data sets are shown.

Table 2: Parameters

Yeast (%)	a	t*	s*	t_l	t_e
0,6	3,70	89	0,018	45	158
1,1	4,01	68	0,041	32	128
1,7	3,86	37	0,048	11	117
2,3	3,72	37	0,065	14	88
3,4	3,63	23	0,070	6	77

The parameter *a* which accounts for the maximum volume expansion varies in a not monotonically manner, whist the remaining parameters shown a monotonically dependence on yeast concentration.
It is interesting to note that all the observed behaviors are congruent with the influence that yeast content exerts on physical and biological processes taking place during dough leavening (Pyler, 1988). In fact, as far as the volume expansion concerns, it results from the proper balance between gas production which involves the biological functioning of yeast and gas retention which is largely a measure of the capability of a dough to sustain local stresses due to bubble growth without collapsing.
With increasing the yeast content the gas production rate will increase and the model parameters which are all, with except the parameter *a*, related to gas formation process varies accordingly.
By contrast, the parameter *a* is affected by both gas production rate and gas retention capability and the latter will diminishes if the gas production rate is too high. As a result an optimum yeast content is expected.
A close exponential correlation was observed between yeast and growth parameters in all single-sample methods (see figg. 4-7), as one would expect by considering the kinetic nature of the gas production process.
The highest and the lowest correlation were respectively observed with the exhaustion stage and with the lag time.

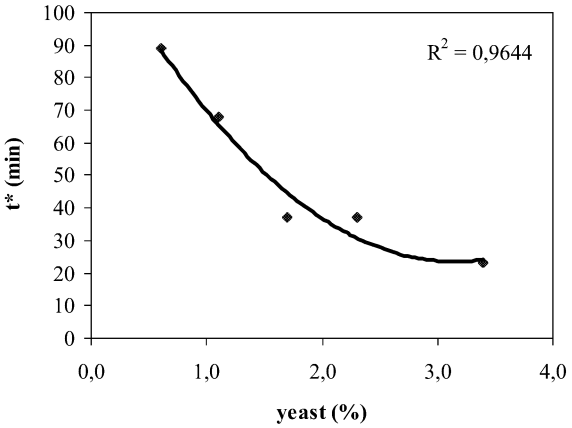

Figure 4: Regression between *yeast* and t^*

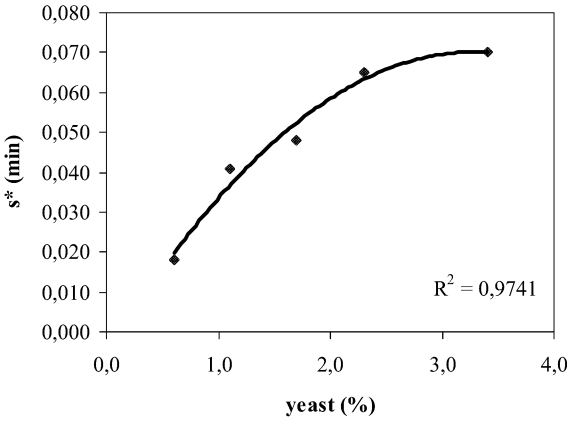

Figure 5: Regression between *yeast* and s^*

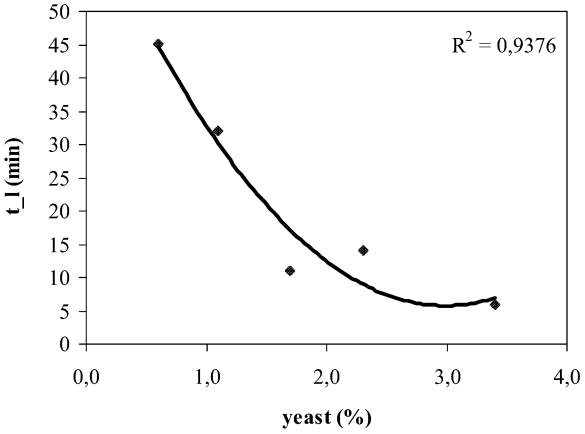

Figure 6: Regression between *yeast* and *t_l*

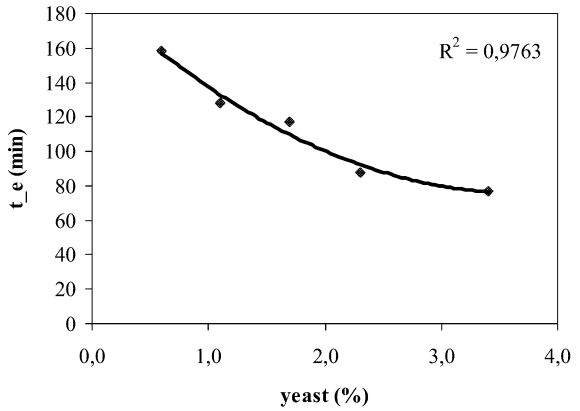

Figure 7: Regression between *yeast* and *t_e*

A preliminary analysis shows that the regression curves might have interesting predictive features. A comparison between predicted values and computed values is shown in table 3 for doughs prepared with 2.9% yeast concentration.

Table 3: Parameters

	a	t*	s*	t_l	t_e
predicted	3,77	18,8	0,09	3,4	58
computed	3,70	19,2	0,10	4,5	56
error %	1,9	1,9	10,6	24,4	3,4

CONCLUSIONS

The three-parameter Morgan-Mercer-Flodin model [MMF_M] proposed in this work appears very suitable as descriptive of the fermentation kinetic of wheat flour doughs.
Meaningful parameters such as maximun specific growth rate (*s**), lag time (*t_l*), exhaustion stage (*t_e*), the maximum relative volume expansion ratio of loaf (*a*) were estimated from the predicted curves. Moreover the parameters behavior with varying the yeast concentration is congruent with physicochemical and biological phenomena responsible for the development of the sponge structure of leavened dough. Next step in our research will be to consider in the growth model the dependency on humidity and lipids. In our preliminary study about influence of fat physical state and concentration on dough development during leavening (Romano et al., 2004), the MMF growth model can still be successfully applied.

BIBLIOGRAPHY

Autio K., Laurikainen T. 1997. Relationships between flour/dough microstructure and dough handling and baking properties. Trends Food Science & Techn., 8, 181-185.

Stanier R.Y, Wheelis M.L., Ingraham J.L. and Painter P.R. 1993. La crescita microbica. In Il mondo dei microrganismi. Ed. Zanichelli, 174-185.

De Cindio B., Correra S. 1995. Mathematical Modelling of Leavened Cereal Goods. J. of Food Engineering, 24, 379-403.

Fujikawa H., Kai A. and Morozumi S. 2004. A new logistic model for *Escherichia coli* growth at constant and dynamic temperatures. Food Micr., 21, 501-509.

Hyams D. 1995-2003. CurveExpert® version 1.3: A comprehensive curve fitting system for Windows.

JANDEL SIGMA SCAN®PRO Version 2.0 1995, Jandel Corporation.

Prescott H.K. 1995. Microbiologia. I edizione. Ed. Zanichelli, Italy, 128-129.

Pyler E.J. 1988. Physical Chemistry and Colloidal Systems. In Baking Science & Technology, Third Edition, Ed. Sosland Publishing Company, Kansans City, Missouri, 290-296.

Ratkowski D.A. 1993. Principles of nonlinear regression modeling. J. Ind. Microbiol., 12, 195-199.

Romano A., Bencivenga S., Cavella S., Toraldo G. and Masi P. 2004. The influence of lipids on dough development during mixing and fermentation process, ICEF9 – Montpellier (France) - 7-11 March, pp. 620-625.

Schepers A.W., Thibault J., Lacroix C. 2000. Comparison of simple neural networks and nonlinear regression models for descriptive modeling of Lactobacillus helveticus growth in pH-controlled batch cultures. Enzyme Micr. Tech., 26, 431-445.

BIOGRAPHY

MASI PAOLO, born in Naples in 1953, is full professor of Food Technology at the Agricoltural School, of the University of Naples Federico II. He graduated in Chemical Engineering at the Engineering school of the University of Naples and got the MSc degree in Engineering at the University of Texas at Austin. He was visiting professor at the Department of Food Engineering of the University of Massachusetts. He is Italian delegate at the Food Working Party of the European Federation of Chemical Engineers and is member of the editorial board of Journal of Texture Studies and act as referee of many journals. He is member of the Society of Rheology and Σχ Scientific Research Society and is author of more than 130 papers on several topics related to food science and technology.

BREAD CHILLING WITH WATER SPRAYING

Jean-Yves Monteau,
Jean-François Cournil,
Philippe Bransolle,
Nasser Hamdami,
Alain Le Bail
ENITIAA, UMR GEPEA (UMR CNRS 6144)
rue de la Géraudière, B.P. 82225
44322 Nantes cedex 3, France
E-mail: monteau@enitiaa-nantes.fr

KEYWORDS
Spraying, bread, chilling, simulation.

ABSTRACT

The water loss of part-baked bread during chilling is a problem in industry. The aim of this study is to apprehend the potential interest of water spraying on the chilling rate and on the reduction of water loss. A coupled heat and mass transfers model was developed and programmed using finite element method. The validation was carried out by comparing center temperature variation with experimental results. The first results given by the model in spraying show that this technique has a small influence on the chilling rate, but can prevent excessive water loss.

INTRODUCTION

Nowadays in industry, the French part-baked bread chilling after partial baking is achieved in ambient air. Consequently, loss water by evaporation can reach 3% of the total mass of the product, which is a cost for industrialists. The ideal would be to use a method which would minimize the chilling time while removing loss water. A method used in meat industry (Kuitche et al. 1996) but not in bakery is water spraying. Spraying consists in a water supply on the surface of the product so that water which evaporates is water supplied, and not the constitutive bread water.

The aim of this work is to evaluate the interest of bread chilling using spraying on chilling rate and water loss. To reach this objective, we developed a first simple model that accommodates the coupled heat and mass transfers. A simulator, based on this model, was then programmed. An experimental stage enabled thus to identify the process and to validate the model. From the use of the simulator, interesting information was obtained about the chilling of the bread when carried out in the ambient air on the one hand, and about the chilling using spraying on the other hand.

MODEL

The model represents temperature and local water content variation in a stick of French bread. Transfers are assumed to be one-dimensional in the radial direction

Diffusion Equations

Diffusion equations are the Fourier second law for the heat diffusion (1), and the Fick second law for the water diffusion (2):

$$\rho Cp\frac{\partial T}{\partial t}-\nabla\bullet\left(\lambda\nabla T\right)=0 \qquad (1)$$

$$\frac{\partial W}{\partial t}=\nabla\bullet\left(D\nabla W\right) \qquad (2)$$

Boundary Conditions

Surface heat transfer is carried out by convection, radiation and evaporation.

$$n\bullet\left(\lambda\nabla T\right)=h\left(T_a-T_s\right)+\varepsilon\sigma\left(T_a^4-T_s^4\right)+kL\left(Pv_a-Pv_s\right)$$

The water vapor pressure in ambient air and at the product surface is linked to the saturated water vapor pressure according to equations (3) and (4):

$$Pv_a=RH\ P_{sat}(T_a) \qquad (3)$$

$$Pv_s=a_w\ P_{sat}(T_s) \qquad (4)$$

The saturated water vapor pressure is given by the Antoine law:

$$P_{sat}(T)=133.3e^{18.3036-\frac{3816.44}{T-46.13}}$$

Water activity is calculated from the surface moisture according to the desorption isotherm at 25 °C, set up during previous work (Hamdami et al. 2003).

Mass transfer on surface is carried out by evaporation.

$$n \bullet (\rho_{dm} D \nabla W) = k(Pv_a - Pv_s) \quad (5)$$

In a first step, the case without spraying was considered. In a second step, the case with spraying was considered. For this second case, the condition (5) for the mass transfer on surface is replaced by (6) which means that surface water content is kept constant and equal to 1 according to the sorption isotherm used.

$$W_s = 1 \quad (6)$$

The loss water is computed by time integration of the evaporated water flow

$$p = \int_0^t 2\pi R l k(Pv_s - Pv_a) d\tau$$

Initial Conditions

Temperature and local water content are assumed constant in the whole product, and fixed to the measured values: 99 °C and 0.96 kg water/kg dm.

Thermophysical Parameters

This simulator is a first approach of the problem. This is why, thermophysical parameters are assumed no temperature dependent, and thus constant. Thermal conductivity, density, specific heat, mass diffusivity, water activity according to the product water content, and emissivity were determined during previous studies (Hamdami et al. 2003; Hamdami et al. 2004). The thermal convection coefficient was calculated using dimensionless numbers (Taine and Petit 2003). The mass convection coefficient was estimated from the experiments so that experimental and simulated water losses are equal (3.7 g). Ambient temperature and air humidity were fixed equal to those measured during experiments. All the parameters are given in Table 1.

Table 1: Thermophysical Parameters of the Model

R	0.03 m
l	0.28 m
ρ	182 kg m^{-3}
ρ_{dm}	99.4 kg m^{-3}
Cp	1930 J kg^{-1} K^{-1}
λ	0.08 W m^{-1} K^{-1}
D	6 10^{-9} m^2 s^{-1}
h	6.6 W m^{-2} K^{-1}
k	8.15 10^{-9} s m^{-1}
ε	0.9
T_a	20 °C
RH	1

SIMULATOR

The simulator was developed with Femlab 2.3, a finite element based software. A cylindrical stick of French bread, cross section perpendicular to his main axis, is considered. The mesh is represented on Figure 1.

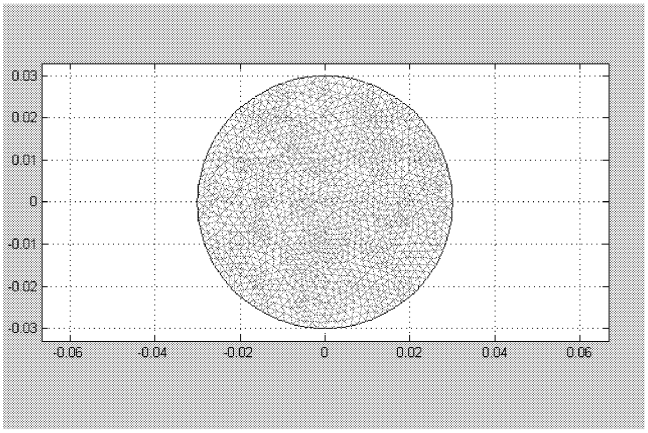

Figure 1: Mesh

EXPERIMENTS

Experiments were used to estimate the mass convection coefficient, and to validate the model.
They consisted in the manufacture of French bread stick, with determination of water loss and measure of center temperature during chilling.
The dough composition for 100 g of flour is given in Table 2, and the manufacturing process in Table 3.

Table 2: Dough Composition

Flour	100 g
Water	60 g
Compressed yeast	3 g
Salt	2.2 g
Baking aid	0.7 g

Table 3: Manufacturing Process

Mixing	3 min at 40 rpm, then 9 min at 60 rpm
Division	round pieces of 140 g
1st proving	ambient T, 15 min
Molding	28 cm long cylinders, 6 cm diameter
Proving	27 °C, 100% RH, 1h30
Scarification	2 cuts
Partial baking	150 °C, 12 min
Chilling	20 °C, 1 h

The water temperature was adjusted so that the base temperature ($T_{room} + T_{flour} + T_{water}$) was 42 °C. In a first stage at 40 rpm, the flour, the water and the baking aid was mixed in a spiral mixer (VMI, Montaigu, France). The yeast was

introduced after 3 min, and the salt 5 min before the end. The division in round pieces was achieved manually. After a 15 min rest period at ambient temperature, molding was carried out using mechanical molding equipment (Puma, Mitry-le-Neuf, France). Proving was realized in proving cabinet (Panimatic, Souppes-sur-le Loing, France). Scarification was achieved with razor blades wet beforehand. Partial baking taken place in a baking oven (Sofinor, Bois-Grenier, France) pre-heated with 150 °C. The oven chimney was closed the first 7 minutes and was opened the five last. The chilling was achieved in a proving cabinet, in order to control the ambient temperature and humidity. At the end of the process, the sticks had a length of approximately 28 cm, and a diameter close to 6 cm. The sticks were weighed just after partial baking, and after chilling in order to calculate the loss water. During the chilling, the center temperature was logged using a data acquisition system (Datalog 20, AOIP, Evry, France), the product being placed in a cell saturated in humidity and at temperature of 20 °C.

MODEL VALIDATION

Computed and experimental temperatures at the product center are close (Figure 2). The differences observed can be explained by the use of constant thermophysical parameters. Certain parameters vary significantly with the temperature and the local water content, in particular the thermal conductivity, and consequently might modify the temperature variation.

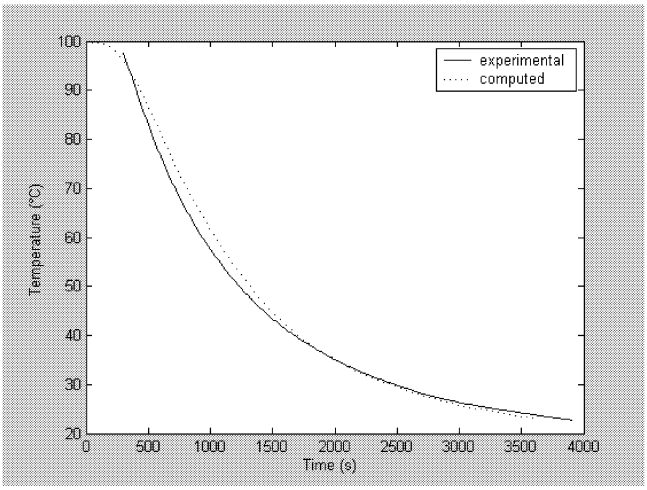

Figure 2: Comparison of Experimental and Computed Temperatures at Center

RESULTS AND DISCUSSIONS

Chilling without Spraying

The simulator allowed firstly to evaluate the importance of selected phenomena on the chilling rate without spraying. For instance, the respective importance of radiation and evaporation on the chilling can be compared. Figure 3 presents the temperature variation at the center with the three terms of heat transfer at the surface, without radiation and without evaporation.

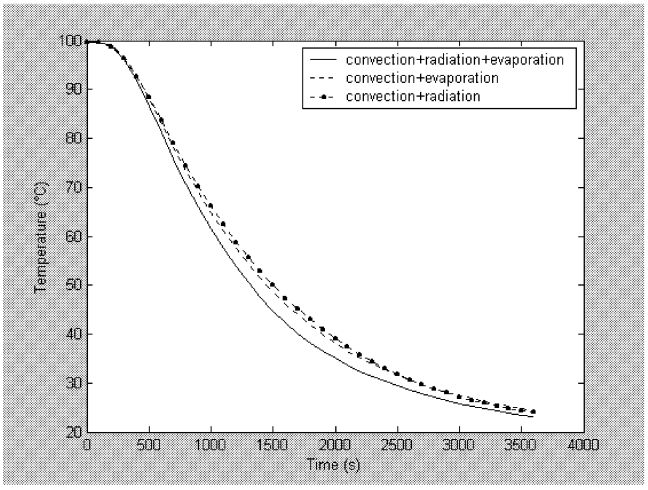

Figure 3: Computed Temperature Variation at the Center with Convection, Radiation and Evaporation, without Radiation, and without Evaporation

Convection is the main transfer mechanism at surface; nevertheless, radiation and evaporation play a significant role in the chilling. The figure shows that suppression of evaporation delays the chilling more than suppression of radiation. At 1700 s, the temperature difference between the line for the three modes and the line without radiation is 3.8 °C, whereas it is 5.2 °C with the line without evaporation.

Table 4 presents water losses for the three cases.

Table 4: Water Losses with Convection, Radiation and Evaporation, without Radiation, and without Evaporation

Convection+radiation+evaporation	3,7 g
Convection +evaporation	4,9 g
Convection+radiation	0 g

Water loss when there are only convection and evaporation is 4.9 g, and is not any more that 3.7 g when the radiation is present. The radiation, by accelerating chilling, limits the water loss of almost 25%.

Figure 4 presents the time variation of the local water content profile. Bread surface undergoes in the first seconds a significant water loss because of importance of evaporation when the bread is taken off the oven, then a rehydration by water diffusion from the bread inside.

Chilling with Spraying

Spraying of bread surface has no significant influence on the chilling rate (Figure 5).
On the other hand, water loss is stopped and water diffusion from the surface into the center can be observed (Figure 6), which can be a problem for the industrial.

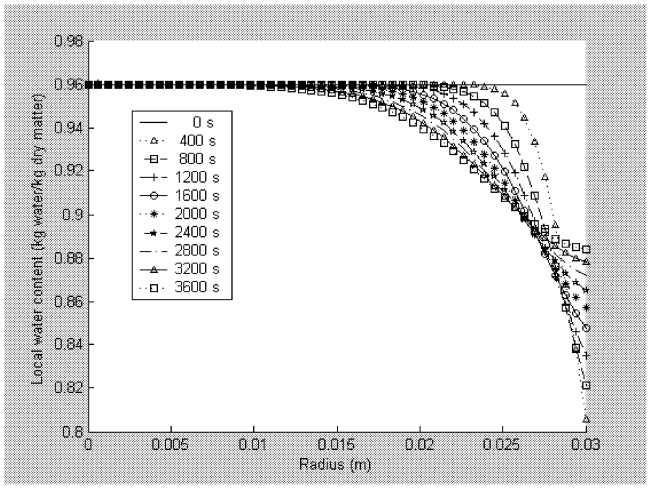

Figure 4: Time Variation of the Computed Local Water Content Profile without Spraying

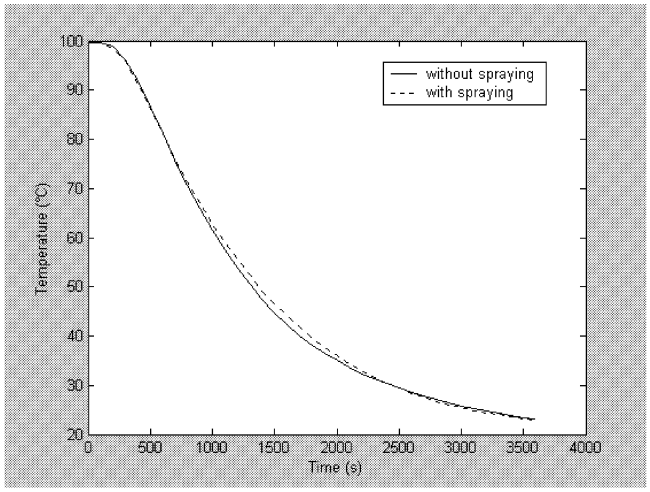

Figure 5: Computed Temperature Variation at the Center with and without Spraying

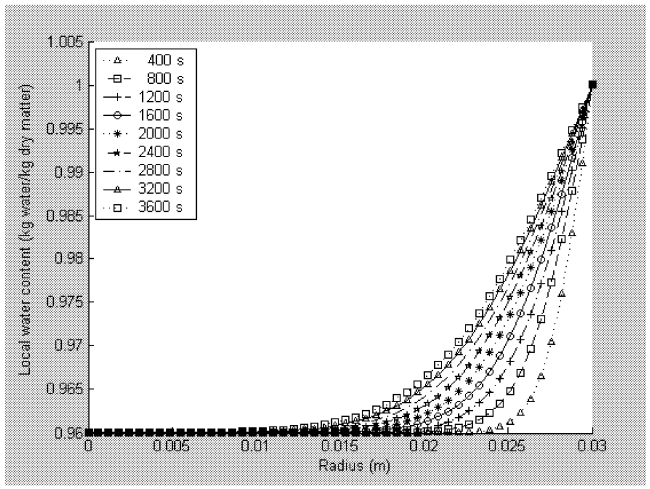

Figure 6: Time Variation of the Computed Local Water Content Profile with Spraying

CONCLUSION

Spraying thus seems to be an interesting technique. It does not significantly improve the chilling rate, but can help to limit water losses. The model, though simple, allowed to have an idea about the interest of the spraying technique. It would be interesting to confirm these results by using a more accurate model, with for example temperature and moisture dependent thermophysical parameters. In addition, the experiments showed that the temperature of sprayed water had a significant influence on chilling, which indicates that heat transfer by conduction exists between the bread and the sprayed water. It would be necessary to supplement the model by taking into account this transfer. The model could then be used to optimize the method, for example by adopting a time dependant spraying rather than a continuous one. This method could be interesting to apply to the baking industry with the sprayed water equal or close to the water loss in order to compensate the evaporation; indeed, the evaporation is the major cooling phenomenon at the beginning of the chilling. Experiments showed that water should be sprayed at the beginning of post baking chilling.

NOMENCLATURE

a_w	water activity
Cp	specific heat, J kg^{-1} K^{-1}
D	mass diffusivity, m^2 s^{-1}
h	heat transfer convection coefficient, W m^2 K^{-1}
L	latent heat of water vaporization, J kg^{-1}
k	mass transfer convection coefficient, s m^{-1}
n	normal to the surface, towards outside
p	water loss, kg
P_v	vapor pressure, Pa
R	radius of the French bread stick, m
RH	relative humidity
t	time, s
T	temperature, K
W	local water content, kg water/ kg dm

Greek Symbols

ε	emissivity
λ	thermal conductivity, W m^{-1} K^{-1}
ρ	density, kg m^{-3}
σ	Stéfan-Boltzmann constant, 5.67051 10^{-8} W m^{-2}K^{-4}

Subscripts

a	ambient
dm	dry mater
s	surface
sat	saturated

REFERENCES

Hamdami, N.; J.-Y. Monteau and A. Le Bail. 2003. "Moisture diffusivity and water activity of frozen part baked bread." *International Journal of Food Science and Technology*. Submitted for publication

Hamdami, N.; J.-Y. Monteau and A. Le Bail. 2004. "Thermophysical properties evolution of French partly baked bread during freezing." *Food Research International*. Submitted for publication

Kuitche, A.; J. D. Daudin and G. Letang. 1996. "Modelling of temperature and weight loss kinetics during meat chilling for time-variable conditions using an analytical-based method - I. The model and its sensitivity to certain parameters." *Journal of Food Engineering*, 28, 55-84.

Taine, J. and J.-P. Petit. 2003. *Transferts thermiques : introduction aux sciences des transferts*. Dunod, Paris.

COMBINED THERMODYNAMIC AND POPULATION BALANCE MODEL OF THE BATCH TOP-SPRAY FLUIDISED BED COATING PROCESS OF INERT SPHERES

Frederik Ronsse[a,b], Jan G. Pieters[a] and Koen Dewettinck[b]
[a] Biosystems Engineering
[b] Food Technology and Engineering
Ghent University
Coupure Links 653
B-9000 Ghent, Belgium
E-mail: Frederik.Ronsse@UGent.be

KEYWORDS
Fluidisation, Mass transfer, Heat transfer, Simulation, Coating mass distribution, Monte Carlo

ABSTRACT

A combined population balance and thermodynamic model was developed using a multi-compartment representation of a top-spray fluidised bed coater. This model enables the prediction of the coating mass distribution changing over time in the fluidised bed during batch operation. In addition, this model predicts the one-dimensional thermodynamic behaviour of the fluidised bed. An event-driven Monte Carlo technique was chosen for the simulation of the particle exchange. The simulation results were validated using the results from tests on a Glatt GPCG-1 fluidised bed unit in the top-spray configuration. This model helps in understanding the impact of process variables and could prove useful in the design of model predictive controllers in fluidised bed coating.

INTRODUCTION

Coating operations have been widely employed in various industries, including the food industry, as an effective technique for altering the surface properties of solid particles and consequently, to tune the effect of functional constituents (Abe et al., 1998). Fluidised bed coating is among the most widespread methods. In top-spray fluidised bed coating, the solid particles are fluidised in a stream of hot air. The coating, mostly in the form of an aqueous solution, is applied onto the particles by spraying the coating solution onto the fluidised bed. The supplied hot air also delivers the energy to evaporate the coating solution deposited on the surface of the suspended particles. The coating solution is usually supplied to the fluidised bed by means of a pneumatic nozzle, in which the use of compressed air results in very strong shear forces at the gas-liquid interface, producing droplets with a size ranging from 10 to 40 μm (Guignon et al., 2002).
As the fluidised bed coating process requires a complex equilibrium of mass and heat transfer between the particle bed and the fluidising air, a number of side effects are likely to occur, resulting in the formation of out-of-specification waste products. If the drying capacity of the supplied air is too high, the droplets containing the solution evaporate completely before impinging on the particle surface. The spray-dried coating material is partially collected by the filter system, partially entrapped within the coating film, resulting in coating imperfections. In contrast, if the drying capacity of the supplied air is too low, the wetted particles tend to form liquid bridges between them. These bridges could persist beyond the point of drying and consequently, dry agglomerates are formed (Guignon et al., 2002).
In order to control the coating process, there is a need to balance the drying rate and the rate at which the coating solution is sprayed onto the particles. Attempts have been made to construct thermodynamic models, considering the reactor as a uniform black box (Dewettinck et al., 1999). However, Maronga and Wnukowski (1998) demonstrated that broad temperature and humidity profiles exist inside the fluidised bed and consequently, the modelling of the fluidised bed into different compartments or zones is more appropriate.
The objective of this study was the development of a dynamic heat and mass transfer model for the description of coating mass, temperature and humidity fields along the vertical axis of a fluidised bed coater. This model will serve, in future studies, as a basis for process control to further increase process efficiency and to reduce out-of-specification waste product formation.

MODEL

The top-spray fluidised bed coating reactor usually has the geometry of an inverted truncated cone. Particles are retained by means of a perforated plate, the air distributor. A downward aiming spraying nozzle is positioned above the fluidised bed; the distance between the nozzle and the bed should be kept to a minimum to avoid premature droplet evaporation.
To develop the model, the fluidised bed was horizontally divided into n cylindrically shaped control volumes, each having a constant volume V_b/n and a constant number of particles N_{tot}/n (Figure 1). Furthermore, particles and

process air were assumed to be perfectly mixed in each control volume. In order to simulate the mixing behaviour of the fluidised bed, particles are continuously being exchanged between two adjacent control volumes and this is expressed by the particle exchange rate r_i, as the fraction of the entire particle population exchanged between the volumes S_i and S_{i+1} per unit of time. It was further assumed that, for a given interface between two control volumes, particle exchange rate was equal for any given particle, irrespective of its properties such as particle diameter and particle shape.

Distinction was made between two types of control volumes: coating volumes, where droplet deposition takes place, and non-coating volumes, where both drying and heating take place. Furthermore, it was assumed that all spraying liquid is deposited on the particles and the amount of liquid deposited on each particle is directly proportional to the particle surface.

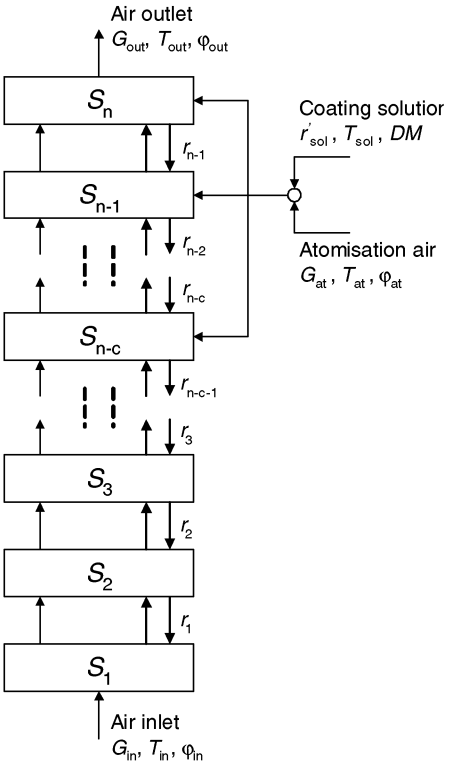

Figure 1a: Schematic overview of the model

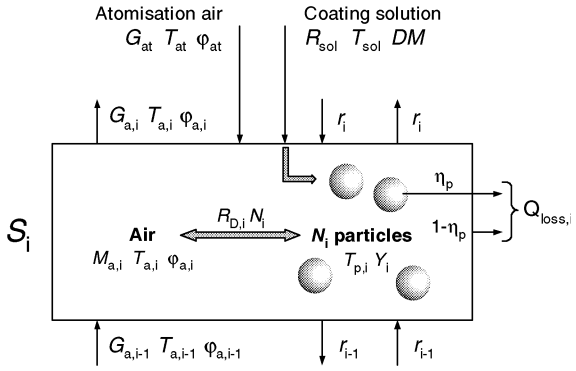

Figure 1b: Control volume in detail

In each control volume the population balance for the particles along with the dynamic heat and mass balances for air, moisture, particles and coating material were established. The particle/fluidum heat and mass transfer coefficients were approximated by means of the dimensionless Nusselt and Sherwood numbers. Heat losses were calculated according to the fluidised bed theory as described by Kunii and Levenspiel (1991).

To solve the model, a discrete approach consisting of an event-driven Monte Carlo technique for the simulation of the particle exchange was chosen. Particle exchange rates were estimated using a kinetic submodel (Ronsse et al., 2003). To generate the random numbers for the Monte Carlo simulation, the Mersenne Twister pseudo-random number generator with a period of 2^{19937}-1 was selected (Matsumoto and Nishimura, 1998).

RESULTS AND DISCUSSION

Figs. 2, 3 and 4 present the results of an example simulation with a number of simulated particles N_{sim} = 1000 and with a time step, Δt_{sim} = 1 ms. The model consisted of 8 control volumes of which the top control volume was a coating control volume. The simulated process consisted of two distinct phases. In the heating phase, no coating solution was sprayed. Next, after 500 seconds of simulated time, a step input was given for the spraying rate. The simulated air temperature and air relative humidity evolutions during a coating process are graphically presented in Figure 2 and their profiles along the reactor z-axis are shown in Figure 3.

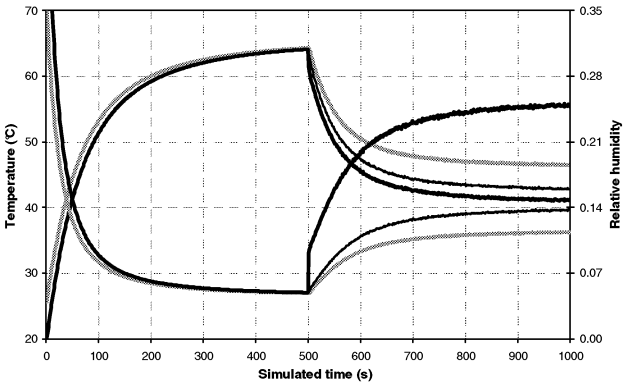

Figure 2: Simulated air temperature and air relative humidity profiles of the coating control volume, S_8 (—); the highest positioned non-coating control volume, S_7 (—); and the lowest positioned non-coating control volume, S_1 (—). Spraying was initiated after 500 s.

In Figure 3, the loss of moisture from the coating solution on the particle surface was very fast in such a way that significant higher air moisture contents are predicted only in the coating control volume (S_8). Due to evaporative cooling in the coating region and due to the release of compressed air at ambient temperature, air temperature was significantly lower than the rest of the reactor. Also, a significant air temperature decrease occurred between the control volume situated closest to the air distributor (S_1) and its adjacent

control volume (S_2). This temperature difference could be explained by the fact that the fluidised particles mainly exchange heat in the region closest to the air distributor: the particle-fluidum heat transfer in S_1 accounted for 64 % of all heat transferred between the particles and the fluidising air.

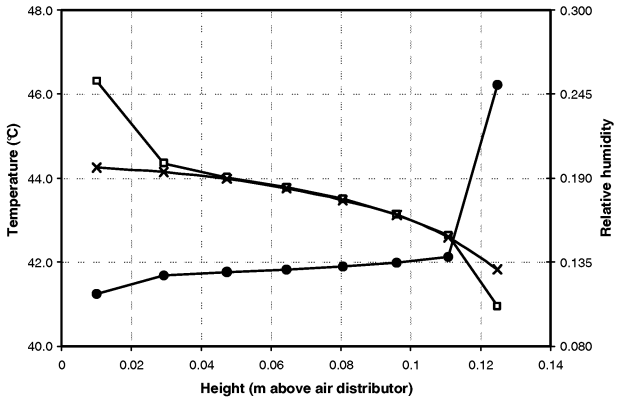

Figure 3: Simulated air temperature (-□-), air relative humidity (-●-) and average particle temperature (-✕-) profiles along the vertical reactor axis during steady-state coating.

Figure 4 presents the coating mass distributions, expressed as mass of coating material per mass of core, taken at different times during the simulation. Due to the constant spraying rate and assuming that no spray drying occurs, the relationship between average coating mass and process time is linear. The simulated coating weight distribution started as a hyperbolic distribution and evolved to a normal distribution when the process would have been run infinitely long. A linear relationship (R^2 = 0.9995) was found between the predicted coating mass standard deviation and the square root of the process time.

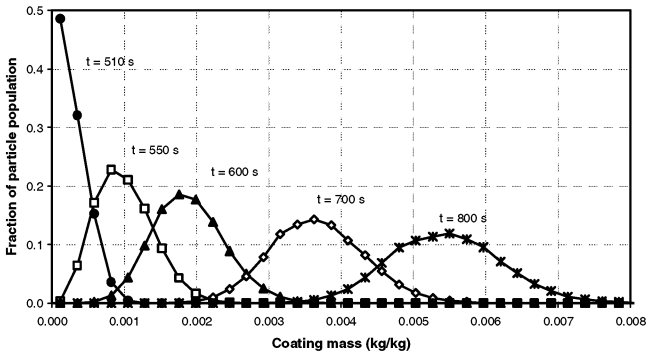

Figure 4: Predicted coating mass distributions, expressed as kg coating per kg core material, simulated at different times during the spraying stage. Spraying was initiated after 500 s.

The effect of variation of several parameters on the outlet air temperature and outlet air humidity was studied. To describe the sensitivity of the model, each parameter (ζ) was varied individually and the translation factor ($1/K_\zeta$) was calculated using the equation, with Ω being the output variable:

$$\frac{1}{K_\zeta} = \frac{\partial \Omega}{\Omega} \cdot \frac{\zeta}{\partial \zeta}$$

Based on the data presented in Figures 5 and 6, both inlet air temperature and atomisation air temperature were found to have the most significant effect on the combined outlet air temperature and outlet air humidity. Next, in decreasing order of importance, came the inlet air volumetric flow rate, the spraying rate, the inlet air relative humidity, the atomisation air pressure and the dry matter content. The solution temperature and the external temperature have virtually no effect upon the model-predicted outlet air temperature and humidity.

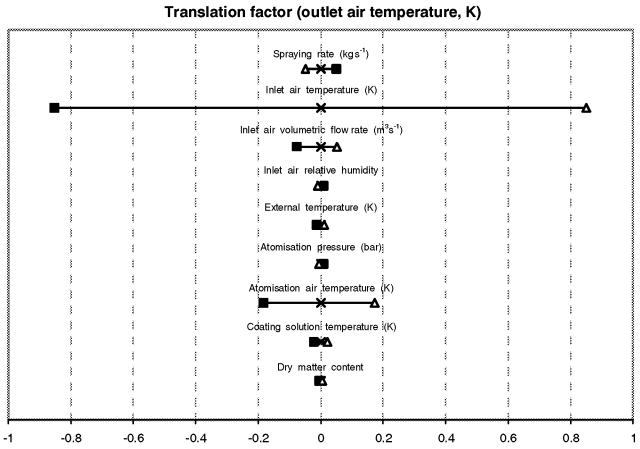

Figure 5: Translation factors describing the predicted effects of variation (negative parameter variation, ■ ; positive parameter variation, Δ) of several model parameters on the outlet air temperature (K).

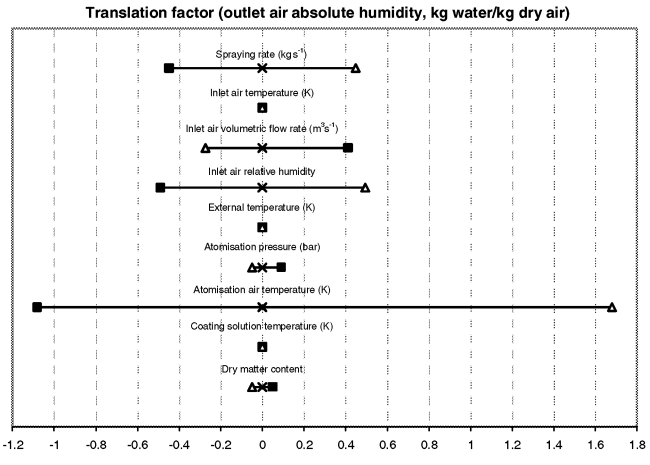

Figure 6: Translation factors describing the predicted effects of variation (negative parameter variation, ■; positive parameter variation, Δ) of several model parameters on the outlet air absolute humidity (kg water kg dry air^{-1}).

Experimental steady-state coating data, generated by Dewettinck et al. (1999) have been used to validate the model in this study. In these experiments, 750 g of glass beads with a volume weighted average diameter of 365 μm (Sovitec Micropearl®, B) were fluidised in a Glatt GPCG-1 with top-spray insert (Glatt GmbH, D). The spraying liquid used was distilled water at ambient temperature. Reactor outlet air temperature was measured at the top of the reactor by means of a T-type thermocouple during steady state coating regime.

The process variables, having the largest impact on the thermodynamic operation point were varied and the experimentally measured steady-state outlet temperature was compared with the model predicted outlet temperature. The correlation between the experimentally measured values and the model predicted values is presented in Fig. 7. Regression analysis performed on the model predicted and experimental data yielded $T_{exp} = 0.996 \times T_{sim} + 0.433$ (R^2=0.96).
Hereby it can be concluded that model proposed in this paper approached closely the experiments for the tested range of process variables.

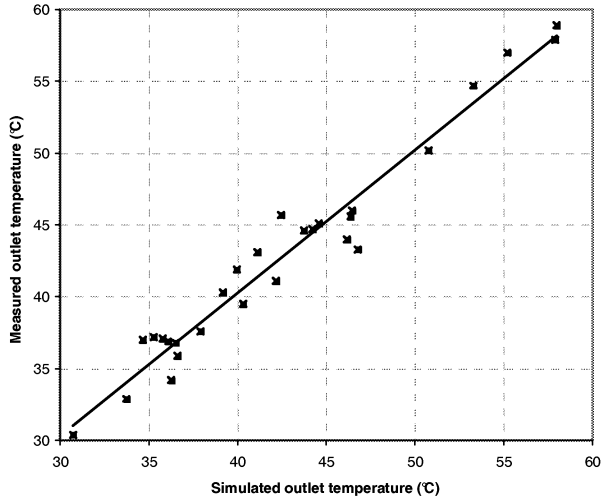

Figure 7: Correlation between simulated and experimental outlet air temperature.

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REFERENCES

Abe, E., Yamada, N., Hirosue, H. & Nakamura, H. 1998. "Coating mass distributions of seed particles in a tumbling fluidized bed coater." *Powder Technology*, 97, 85-92.

Dewettinck, K., De Visscher, A., Deroo, L. and Huyghebaert, A. 1999. "Modeling the steady-state thermodynamic operation point of top-spray fluidized bed processing." *Journal of Food Engineering*, 39, 131-143.

Guignon, B., Duquenoy, A. and Dumoulin, E.D. 2002. "Fluid bed encapsulation of particles: principles and practice." *Drying Technology*, 20(2), 419-447.

Kunii, D. and Levenspiel, O. 1991. "Fluidisation Engineering, 2nd edn." Butterworth-Heinemann, Stoneham.

Maronga, S.J. and Wnukowski, P. 1998. "The use of humidity and temperature profiles in optimizing the size of fluidized bed in a coating process." *Chemical Engineering and Processing*, 37, 423-432.

Matsumoto, M. and Nishimura, T. 1998. "Mersenne Twister: A 623-dimensionally equidistributed uniform pseudorandom number generator." *ACM Transactions on Modeling and Computer Simulation*, 8(1), 3-30.

Ronsse, F., Depypere, F., Dewettinck, K. and Pieters, J.G. 2003. "Monte Carlo simulation for top spray fluidised bed coating processes." *Proceedings of the Sixth National Congress on Theoretical and Applied Mechanics*, 26-27 May 2003, Gent, Belgium. 5p.

BIOGRAPHY

FREDERIK RONSSE was born in Kortrijk, Belgium on November 3rd, 1978. He graduated from the Ghent University in 2001 with a M.Sc. in Biological Engineering Sciences. Currently he is a doctoral researcher at the Ghent University in the Faculty of Agricultural and Applied Biological Sciences. His current research deals with the process optimisation and control of fluidised bed systems in the food industry, a joint project between the Biosystems Engineering Research Group (Department of Agricultural Engineering) and the Food Technology and Engineering Research Group (Department of Food Technology and Nutrition). Among his prime research interests are food microencapsulation, population balance modelling in powder technology, industrial automation and machine prototyping.

FDA/FSIS *LISTERIA MONOCYTOGENES* RISK ASSESSMENT: ANALYSIS OF THE ANALYSIS

Marcel Zwietering
Laboratory of Food Microbiology
Wageningen University
Bomenweg 2
6703 HD Wageningen, The Netherlands
E-mail: Marcel.Zwietering@wur.nl

KEYWORDS
Listeria monocytogenes, risk assessment, product groups, exposure distribution, scenario analysis

ABSTRACT

An extensive risk-assessment (540 pages) of *Listeria monocytogenes* in ready-to-eat foods has been carried out (FDA/FSIS 2003). This very extensive risk assessment contains a wealth of information. However, in order to use the information, this needs first to be analysed. Very useful information can be extracted, both for managing the problem of listeriosis, as well as for the assessment of other microbiological risks, microbiological research, sampling of food products, etc. Deli-meats results as the most relevant product group, followed by pasteurised milk. Very exceptional, very high levels are of large importance for the public health burden. Furthermore, the quantitative effect of various interventions can be estimated by scenario analysis.

ANALYSIS OF THE ANALYSIS

The risk assessment of *Listeria monocytogenes* in ready-to-eat foods (FDA/FSIS 2003) consists of a hazard identification, exposure assessment, hazard characterisation and a risk characterisation. In the hazard identification it is argued to focus on *Listeria monocytogenes* in ready to eat foods, and endpoints are defined as death and serious illness for the intermediate-age subpopulation and two readily identifiable vulnerable subpopulations: perinates (fetuses and newborns) and the elderly (60 years of age and older). In the exposure assessment an identification of ready-to-eat foods associated with *L. monocytogenes* is carried out from outbreaks, sporadic cases, recalls, etc. Furthermore the amounts consumed per serving for each of these food category and estimates of the annual number of servings in the U.S. are estimated. Also distributions of contamination levels at retail and growth, decline, or inactivation between retail and consumption are determined. Specifically for frankfurters also the effect of reheating is included. Combination of all these data results in frequencies and levels of contamination, i.e. the distributions of food consumption frequency, amount, contamination frequency and levels
In the hazard characterisation epidemiological data (incidence) are used to anchor dose data and incidence to estimate the dose response parameter for the three populations. Finally in the risk characterisation the exposure assessment and the hazard characterisation are coupled to determine illness from the 23 categories. Moreover, a Monte Carlo analysis is carried out to evaluate the effect of variability and uncertainty and scenario analyses are carried out to quantify the effect of various interventions and factors.
From this risk-assessment it follows (Table 1) that deli-meats is the most important source of listeriosis. Unexpectedly pasteurised milk is on the second place, if the risks are considered on a yearly basis. The distribution of levels of contamination is very large and it seems that the very occasional very high levels are of the largest importance of the total exposure (Table 2). These very high levels were found due to surveys with huge amount of samples. The global results of these investigations seem consistent if data in a limited time period in only the US are compared to literature data over a larger time-frame and from all parts of the world (Table 3). It should be noted that data presented in this table are data at retail, and before consumption growth is still possible.

Table 1: Median Number of Cases of listeriosis per Year in the US (FDA/FSIS 2003)

deli meats	1599	(89%)
pasteurised milk	91	(5%)
high fat and other dairy products	56	(3%)
frankfurters not reheated	31	(2%)
soft unripened cheese	8	
pate and meat spreads	4	
unpasteurised fluid milk	3	
crustaceans	3	
smoked seafood	1	
rest	2	

Table 2: *L. monocytogenes* in RTE Foods (Gombas et al. 2003) 31705 samples investigated 577 positive (1.8%)

C (cfu/g)	number	P(%)	exposure	
<0.1	402	1.3	40.2	
<1	82	0.26	82	
<10	52	0.16	520	
<100	20	0.063	2E3	
<1E3	16	0.050	1.6E4	
<1E4	3	0.0095	3E4	99.1%
<1E5	0	0	0	97.6%
<1E6	2	0.0063	2E6	97.6%

total exposure is 2.0E6

Table 3: *L. monocytogenes* in RTE Foods (FDA/FSIS 2003) 336228 samples investigated 6459 positive (1.9%)

C (cfu/g)	number	P(%)	exposure	
<0.1	5219	1.6	522	
<1	100	0.030	100	
<10	300	0.089	3000	
<100	533	0.16	5.3E4	
<1E3	173	0.051	1.7E5	
<1E4	92	0.027	9.2E5	98.9%
<1E5	25	0.0074	2.5E6	94.4%
<1E6	17	0.0051	1.7E7	82.3%

total exposure is 2.1E7

The effect of various interventions can be estimated on a quantitative manner by simulating various scenarios. Also following logic, the number of consumed products, the concentration distribution, the storage temperature and storage time are of relevance. But the great advantage of this analysis is that one gets insight in the quantitative effects of different interventions. This is therefore a strong tool in the managing of the risk of *L. monocytogenes*. But in order to use the tool one has first to digest the information and be very critical in using it. This should, however, not impede from using this highly valuable source of information.

REFERENCES

FDA FSIS (2003) Quantitative Assessment of Relative Risk to Public Health from Foodborne *Listeria monocytogenes* Among Selected Categories of Ready-to-Eat Foods. FDA/Center for Food Safety and Applied Nutrition, USDA/Food Safety and Inspection Service, Centers for Disease Control and Prevention, September 2003 http://www.foodsafety.gov/~dms/lmr2-toc.html.

Gombas D.E., Chen Y., Clavero R.S. and Scott V.N. 2003. Survey of *Listeria monocytogenes* in Ready-to-Eat Foods. *Journal of Food Protection* 66, 559-569.

PREDICTING TASTE DEVELOPMENT OF CHEESE USING HYBRID MODELING

M. Verschueren
P. de Jong
J. Straatsma
M. Vissers
G. van den Berg
Department of Processing, Quality and Safety
NIZO food research
P.O. Box 20
6710BA, Ede
The Netherlands
E-mail: maykel.verschueren@nizo.nl

KEYWORDS
cheese, ripening, taste, flavour, hybrid modeling, .

INTRODUCTION

The quality of products in the food industry is often strongly connected to variations in both raw material and processing conditions. In case of cheese production both variations in the milk composition and variations in the processing and storage conditions have an effect on cheese quality parameters such as taste and texture (see figure 1) In the modern food industry there is, therefore, a growing interest in models that can predict variations in complex product properties such as taste as a result of variations in processing and storage conditions (see figure **2**). Such models can be used to optimise processes, to improve product quality or to reduce operating costs or for product development.

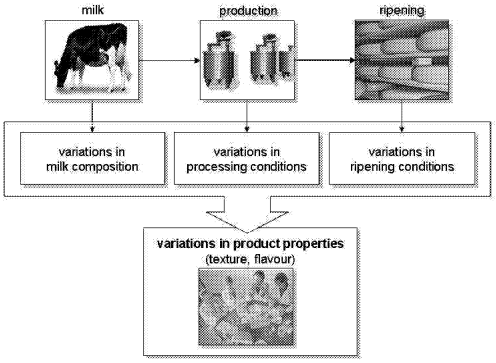

Figure 1

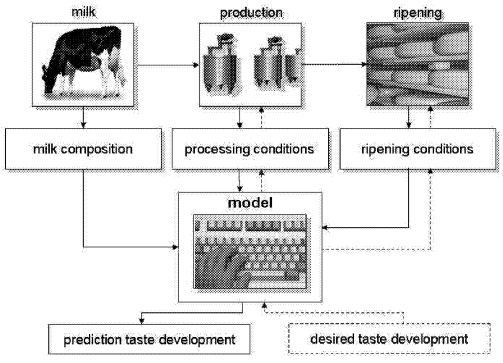

Figure 2

HYBRID MODELING

Very often processing steps can be described by so-called white-box models, which are based on physico-chemical relations. White-box models have some advantages over black-box models: a small amount of data is required to develop a white-box model and it can be extrapolated outside the range for which data is available. Taste parameters, however, are often quantified by means of sensory evaluations. White-box approaches can not be used to include the sensory data in the model. In this case black-box approaches can be used (e.g. neural networks, fuzzy logic). The effect of changes in processing conditions on taste parameters can therefore be described most accurately by a hybrid model, consisting of both white- and black-box elements (figure 3). Within the framework of a project funded by the J. Mesdag Foundation, NIZO food research has developed a hybrid model, CHERMO[1], which is able to predict several taste parameters of cheese as a function of variations in the processing and storage conditions.

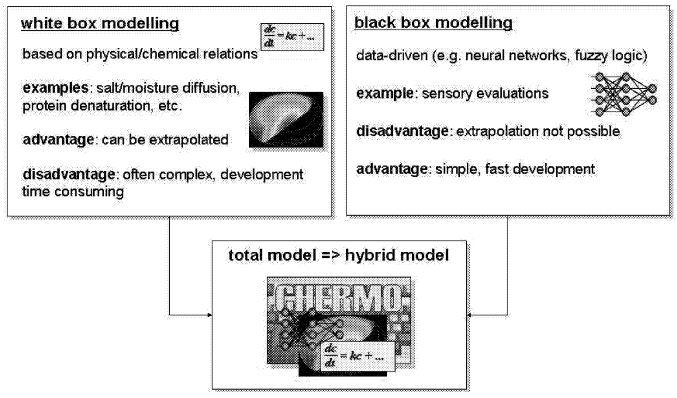

Figure 3

[1] CHEese Ripening MOdel

NIZO *PREMIA*

Over the past 20 years, NIZO food research has developed a wide range of models for cheese production processes, based on the results of research projects and available knowledge and know-how. These models correspond to different operating units and processes taking place during cheese manufacturing (e.g. pasteurisation, renneting, acidification, pressing, brining and storage). All these models, initially only available as stand alone applications, were integrated in one software platform called NIZO Premia. In NIZO Premia all these models can be combined in a user-friendly way to model complete cheese production processes from milk storage tanks to storage of cheese in warehouses and at retailers (see figure 4). The integrated modelling concept in NIZO Premia allows the user to predict the effect of variations in processing and ripening conditions on various process and product properties such as cheese yield, renneting time, the degree of denaturation of whey proteins, inactivation of enzymes, moisture and salt content, pH of the cheese.

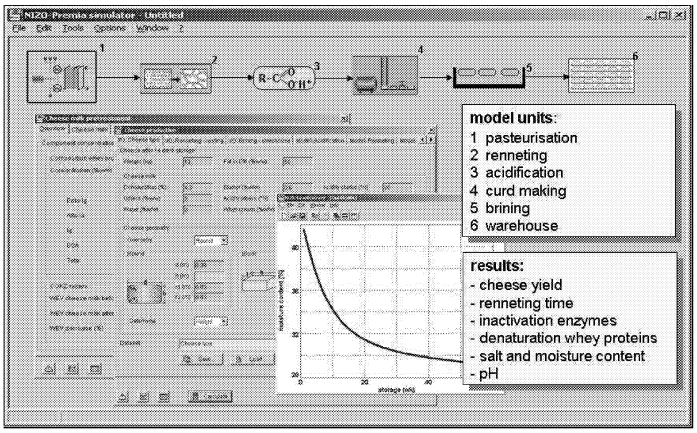

Figure 4

CHERMO

The models mentioned above all have a white-box character. However, as stated above, variations in processing and ripening conditions often also have an effect on cheese quality parameters such as taste and flavour development. To be able to predict the taste development of cheese during ripening as a function of processing and storage conditions, a hybrid model (CHERMO) was developed using the white-box elements of NIZO-*Premia* as a starting point. First, the key parameters for taste development and the relevant processing and storage conditions were identified and summarized in knowledge diagrams. Based on the results of this investigation the database on which the already available NIZO-*Premia* modules were based was extended with the relevant experimental results (e.g. protein breakdown, enzyme activities, sensory panel evaluations etc.). This resulted in a large database based on the evaluations of about 400 cheeses (Gouda). The database was used for model development and validation. According to the knowledge diagrams, the already available models were extended and additional models were developed, e.g. for protein breakdown, for several taste parameters (using neural networks) and for moisture and salt diffusion (CFD[2] models). The extended and added models were also integrated in the NIZO Premia environment (see figure 5)

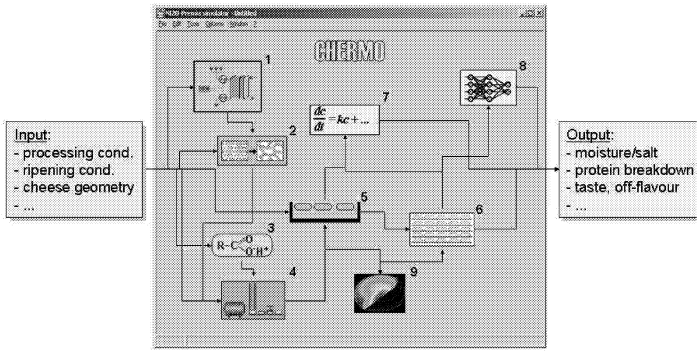

Figure 5

CHERMO firstly relates the relevant processing conditions to the relevant parameters (e.g. moisture, salt, pH, enzyme activities) in the cheese at the end of the manufacturing process. After that the fermentation process during ripening is modelled by considering protein breakdown as a function of the storage conditions (e.g. temperature, relative humidity) and the relevant, time-dependent parameters (e.g. salt, moisture). Finally, the sensory evaluations are related to the relevant parameters in the cheese (e.g. SN, AN, pH).

Neural networks were used to model the sensory evaluations as a function of the relevant ripening parameters. Figure 6 shows two examples for estimated age (left) and bitter scores (right): the neural network predictions vs. the sensory evaluations are shown. The graph on the left hand side also shows the results for a model based on multiple linear regression techniques (MLR). The predictions by the neural network are clearly better: the standard deviation for the MLR model is 2.3 weeks and for the neural network 1.8 weeks. Figure 7 shows model predictions for the estimated age as a function of storage temperature varying from -15 to +18°C (left) and the salt score as a function of the brining time varying from 80 to 110 hr. The neural network for the age estimation correctly predicts the increase in the estimated age of the cheese with increasing storage temperature. The neural network for the salt score predicts a clear switch from 'not salt' to 'salt' at a brining time of 96 hours.

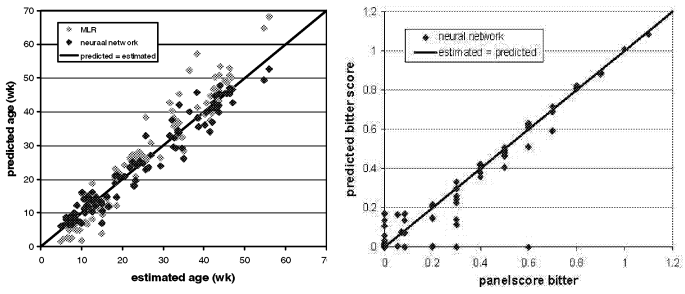

Figure 6

[2] Computational Fluid Dynamics

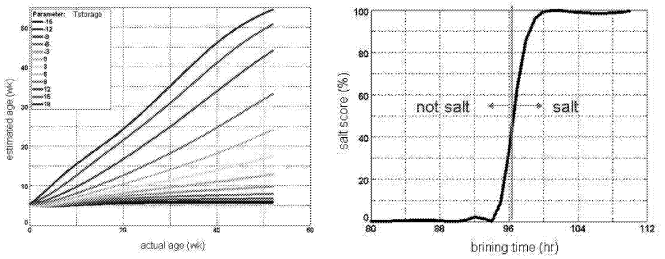

Figure 7

CONCLUSIONS

A hybrid model was developed which is able to predict the effect of variations in the processing and storage conditions on the relevant parameters in the cheese during ripening and the sensory evaluations. By using white-box elements in the hybrid global model the predictive capacities of the model increase. Moreover, hybrid modelling allows for an optimal use of the available data and knowledge since (white-box) models that were developed in the past can be integrated.

CHERMO was designed for practical applications. The integrated modelling concept of NIZO Premia and its user-friendliness, ensures user accessibility of the models. Furthermore, the architecture of NIZO Premia allows the user to (re)calibrate the models using in-company data. For the black-box models this calibration procedure is essential, since sensory evaluations can be unique for different companies. By using own data, companies can fine-tune CHERMO to their own production sites.

REFERENCES

Verschueren, M., Berg, G. van den, Jong, P. de. 2002. "Predicting taste development of cheese using hybrid modeling." Australian Journal of Dairy Technology 57 (2), 165

Verschueren, M. "Hybrid modelling of cheese manufacturing and ripening." Proceedings 7th cheese symposium, October 2003, Cork, Ireland.

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MAYKEL VERSCHUEREN was born in the Netherlands in 1971 and obtained his MSc in Applied Physics at the Technical University of Eindhoven in 1995. After a research project on modeling structure development in flow he obtained his PhD in 1999 at the department of Mechanical Engineering at the Technical University of Eindhoven. Since then he has worked for NIZO food research. Currently he is group leader of the predictive modeling group at NIZO food research.

A QUANTITATIVE RISK ASSESSMENT OF BUTYRIC ACID BACTERIA SPORES IN SILAGE AND RAW MILK

Marc Vissers
Frank Driehuis
Meike te Giffel
Peter de Jong
NIZO Food Research
P.O. Box 20
6710 BA Ede, The Netherlands
E-mail: Marc.Vissers@nizo.nl

Jos Lankveld
_Wageningen University
Chair of Dairy Science
P.O. Box 8129
6700 EV Wageningen, The Netherlands

KEYWORDS
Risk Analysis, Sensitivity analysis, Raw Milk, Butyric acid Bacteria, Farm management.

ABSTRACT

Spores of Butyric acid bacteria (BAB) present in cheese milk after pasteurization can cause the late-blowing defect in e.g. Gouda cheese. BAB spores originate from the farm environment and farmers have to take efficient measures to assure low concentrations in their raw milk. A quantitative risk analysis has been performed to identify the relative influence of various managerial and environmental factors on the contamination of raw milk with BAB. Concentrations of BAB in silage was found to be the most important. High concentrations in silage cannot be corrected through good farm hygiene or milking practices. Silage heterogeneity seems to be the main factor determining the average concentration of BAB in silage at the point of feeding.

INTRODUCTION

Raw milk generally contains low concentrations of spores of the anaerobic, sporeforming, bacterium *Clostridium.* The dairy- associated species are often called butyric acid bacteria (BAB). *C. tyrobutyricum* is capable of converting lactic acid into butyric acid and causes off-flavours and excessive gas formation, so-called late-blowing, in semi-hard cheeses such a Gouda (Figure 1). Spores of clostridia survive pasteurisation of cheese milk. *C. tyrobutyricum* spore concentrations in cheese milk as low as 1 colony forming unit (CFU) per ml can cause the late-blowing defect (Stadhouders-J 1990).

C. tyrobutyricum spores in milk originate from the dairy farm environment. In The Netherlands milk supplies of farmers are analyzed monthly for the presence of spores of BAB by the van Beynum&Pette MPN-method. The farmer receives a penalty on the milk price when two out of two tubes with 0,1 ml raw milk test positive on BAB(MCS, 2001).

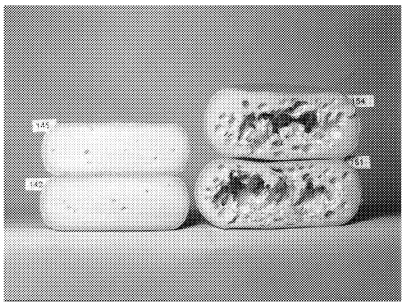

Figure 1: Late-blowing of Gouda cheese

Since the introduction of this system in The Netherlands 22 years ago a significant reduction of the average contamination level of BAB spores in raw milk is achieved. This reduction has levelled off in the 1990's from approximately 20% to 4% BAB-positive samples. Historical data show that the majority of the positive samples originate from a small group of farmers, which repeatedly test double positive on BAB ('recidivists') (MCS 2001).

Because of the impact of BAB spores on cheese quality, reducing the contamination of raw milk is of major interest to the dairy industry. The present study aims to determine the influence of farm management and farm environment conditions on BAB contamination by quantitative risk analysis and to identify effective control points. A number of quantitative microbial risk assessment studies on foodborn pathogens are published, but the application of the same methods on a non-pathongenic micro-organism and farm processes is new(Bemrah, Sanaa et al. 1998; Cassin, Lammerding et al. 1998; Lindqvist and Westoo 2000). Since silage is considered to be the most important source of BAB spores in the farm environment (Stadhouders, Hup et al. 1983), the first step in the analysis was to quantify the importance of silage in relation to a number of control measures a farmer can apply. The second step is to identify risk factors related to silage. In this paper the first step is described.

Silage

Silage from forage crops, such as maize and grass, forms a major part of the feed ration of dairy cows (up to 90%).

Silage making is the most popular method to preserve these crops. It involves a natural fermentation process dominated by lactic acid bacteria. The main preservation principles of silage are a rapid decrease of pH, a relative low water activity (a_w) and the maintenance of anaerobic conditions. The storage period of silages on the farm varies from two weeks to more than two years.

In successful silage making growth of BAB and other harmful micro-organisms should be prohibited. In practice, however, growth of BAB in silage frequently occurs, in particular in grass silages. The concentration of BAB spores in silage varies between $<10^2$ and 10^7 per g silage (Pahlow et al. 2003). The initial concentration (after harvesting, prior to ensiling) varies between $<10^2$ and 10^4 BAB spores per g, and originates mainly from soil. Factors that influence BAB growth in silage include pH, rate of pH decline, dry matter concentration (relates to a_w) and nitrate concentration (Pahlow et al. 2003). Another factor of importance is the heterogeneity of silages. As a result, there is significant spatial variation in conditions (pH, a_w), which may lead to local spots with conditions suitable for growth of BAB (Pauly-TM 1999).

APPROACH

The contamination route of BAB spores in milk is shown in Figure 2. Monte Carlo simulations were performed in order to assess the importance of the different steps in the route and to reveal the importance of silage quality with respect to other factors quantitatively. In this calculation all cows were assumed to be equal with respect to factors such as dirt on udder pre-milking, cleaning efficiency and mixed feed ration. Initial concentrations and transmission rates were derived from published data (Stadhouders-J and Jorgensen 1990) (Table 1). Growth of BAB was assumed only to occur in silage and mixed feed ration. Since BAB spores survive the gastro-intestinal tract and 90% of the feed is digested the concentration of spores in the faeces was assumed to be 10 times the concentration in the mixed ration (Stadhouders-J and Jorgensen 1990).

Growth of BAB was modelled using an exponential microbial growth model with lag time. Growth rate was described as a function of pH, a_w and temperature, using the Gamma concept (Zwietering-MH, Wit et al. 1996). The probability of growth in the different phases of silage (fermentation, storage and after opening of the silage) was analysed. Profiles for temperature, pH and a_w were derived from literature. Further assumptions were the absence of oxygen and the presence of sufficient lactic acid for growth. The effect of heterogeneity was also investigated.

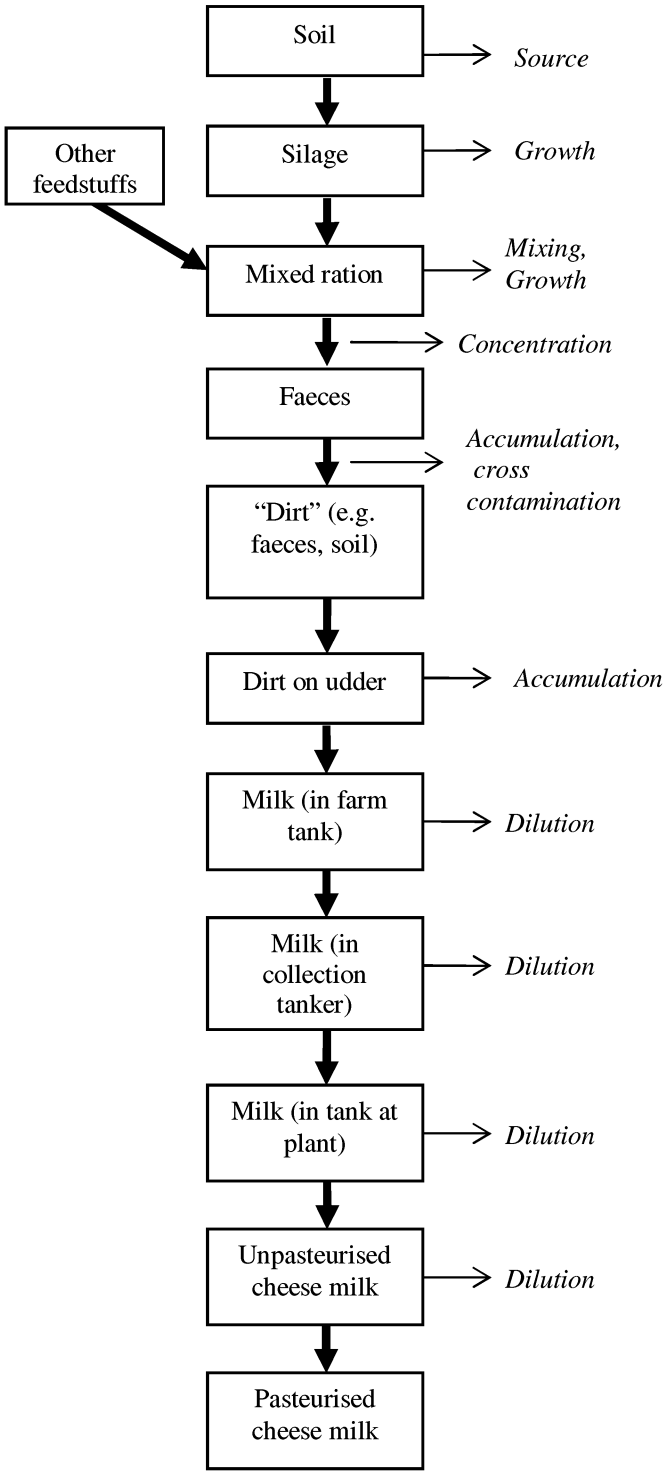

Figure 2: Schematic representation of microbial pathway of spores of BAB from soil to pasteurised cheese milk with possible *processes.*

Table 1: Assumptions for the Main Variables in the BAB Model (Minimum, average and maximum)

Variable	Min.	Average	Max.	Ref.
Concentration in silage (CFU/g)	10^1	10^4	10^6	1
Concentration in other feedstuffs (CFU/g)	0	10^2	10^4	1
Fraction of silage in ration (%)	0	40	80	2
Temperature in cattle house (°C)	13	19	25	2
Feeding interval (hour)	6	9	12	2
Faeces on udder (g)	0,1	5	10	3
Reduction by udder cleaning (%)	0	50	90	3
Transmission from udder to milk (%)	0	0,75	1,5	3
1 Measurements 2 Personal communication 3 (Stadhouders, 1990 #100)				

RESULTS

The computer simulation analysis confirmed the importance of the concentration of spores in silage for the contamination of raw milk (Figure 3). The concentration of BAB spores in silage is significantly more important than other factors, including milking hygiene. Obviously, the importance of these other factors increase when silage with a low spore concentration is fed.

Calculation of growth in silage showed that when conditions after ensiling are suitable for growth the long storage time will result in high concentrations (10^7 – 10^9 CFU/g) at the point of feeding. Measurements of actual silage concentrations showed lower levels of contamination (see Table 1). This is probably due to the heterogeneity of the silage, resulting in a dilution of the spots with high concentrations in the remainder of the silage with low concentrations (no growth). More research is needed to confirm the hypothesis and to derive control measures for farmers in order to reduce the contamination levels in silage.

CONCLUSIONS AND FURTHER RESEARCH

Model simulations have shown the significant importance of BAB concentration in silage with respect to the contamination levels of raw milk. Heterogeneity of the silage, resulting in local spots with conditions suitable for growth of BAB seems to be extremely important for the average concentration in the silage at the point of feeding. Further quantitative analyses will be performed to establish the importance of silage heterogeneity and to evaluate possible control measures to reduce the average concentration of the fed silage.

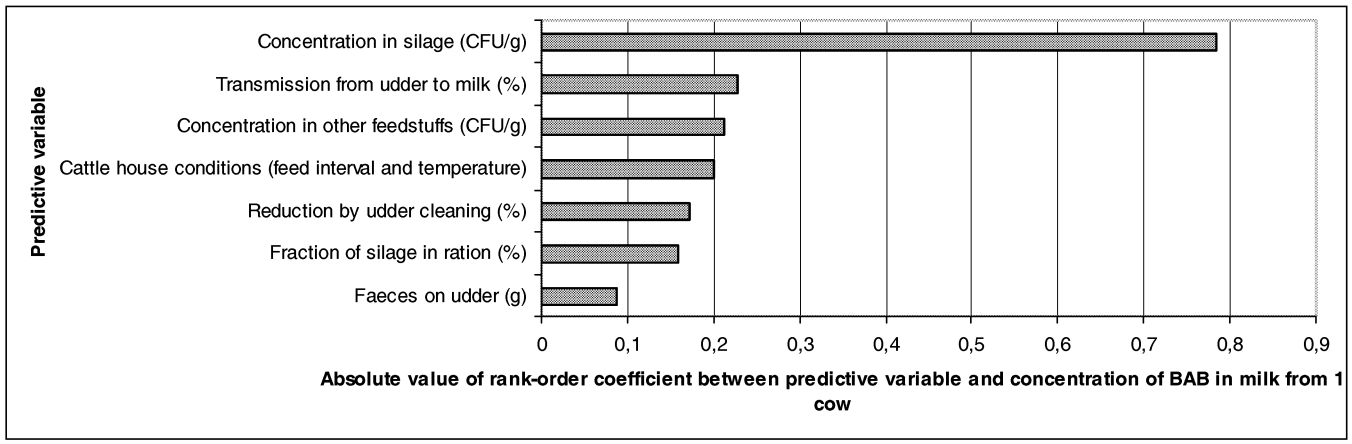

Figure 3: Variables with the most significant impact on the contamination of raw milk with spores of butyric acid bacteria (for assumptions for variable values see Table 1).

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REFERENCES

Bemrah, N., M. Sanaa, et al. (1998). "Quantitative risk assessment of human listeriosis from consumption of soft cheese made from raw milk." Preventive Veterinary Medicine **37**: 139-145.

Cassin, M., A. Lammerding, et al. (1998). "Quantitative risk assessment for *Escherichia coli O157:H7* in ground beef hamburgers." International Journal of Food Microbiology **41**(1): 21-44.

Lindqvist, R. and A. Westoo (2000). "Quantitative risk assessment for *Listeria monocytogenes* in smoked or graad salmon and rainbow trout in Sweden." International Journal of Food Microbiology **58**(3): 181-196.

MCS (2001). "Annual report 2001 - Netherlands Milk Control Station."

Pauly-TM (1999). "Heterogeneity and hygienic quality of grass silage." Acta-Universitatis-Agriculturae-Sueciae - Agraria **157**.

Stadhouders, J., G. Hup, et al. (1983). "Silage and cheese quality." NIZO-Medelingen **M19**: 1-39.

Stadhouders-J (1990). "Prevention of butyric acid fermentation by the use of nitrate." Bulletin-of-the-International-Dairy-Federation **251**: 40-46.

Stadhouders-J and K. Jorgensen (1990). "Prevention of the contamination of raw milk by a hygienic milk production." Bulletin-of-the-International-Dairy-Federation **251**: 32-36.

Zwietering-MH, J. C. d. Wit, et al. (1996). "Application of predictive microbiology to estimate the number of *Bacillus cereus* in pasteurised milk at the point of consumption." International Journal of Food Microbiology **30**: 55-70.

MARC VISSERS was born in Veghel, The Netherlands and obtained his MSc in Food Processing Technology at Wageningen University in 1999. In 1999 he started working at NIZO food research where he is involved in the application and development of predictive models for process optimisation. In 2003 he started a PhD entitled “Modelling microbial farm ecology”. In this project he tries to describe microbial behaviour at dairy farms in order to determine effective control measures for the reduction of animal diseases and microbial contamination of raw milk.

ENTHALPY OF FROZEN MEAT FROM COMPOSITION DATA

R.G.M. van der Sman, Agrotechnological Research Institute
p.o. box 17, 6700 Wageningen, the Netherlands
Email: ruud.vandersman@wur.nl

May 28, 2004

INTRODUCTION

For the design of freezing operations on foods it is important to have knowledge about their freezing behaviour. There is an ongoing effort to predict freezing behaviour from composition data. As such, in a previous paper we have presented a model predicting the water activity (and equivalently the initial freezing point) of meat products, given their composition [van der Sman & Boer,2004]. Amongst other aspects this model involves 1) the estimation of bound water, and 2) the estimation of the non-ideal contribution of sodium-chloride (NaCl) to the water activity using an approximation of the Pitzer equation. We extend this model to the prediction of enthalpy during freezing.

THEORY

The enthalpy $H(T)$ of frozen food at temperature T can be computed as follows [Mannapperuma & Singh,1988]:

$$H(T) = \sum_s c_{p,s} y_s + c_{p,w} y_w(T) + (c_{p,ice} + \lambda) y_{ice}(T) \quad (1)$$

Here $c_{p,s}$, $c_{p,w}$, $c_{p,ice}$ are respectively the specific heats of solids (enumerated with index s), water and ice, y_s, y_w, y_{ice} are the mass fractions of solids, water and ice, and λ is the latent heat of fusion.

It must be noted that in broad temperature range (between initial freezing point and -40^oC) ice and water coexists. Due to ice formation the unfrozen aqueous phase will be freeze concentrated, inducing freezing point depression. The ice fraction y_{ice} as a function of temperature T. Ice formation can be computed by integrating Clausius-Clapeyron equation:

$$\ln a_w(y_{ice}) - \ln a_{w0} = \int_{T_f}^{T} \frac{\lambda}{RT^2} dT \quad (2)$$

Here a_w is the water activity of the unfrozen aqueous phase, which depends on the ice fraction, a_{w0} is the water activity at the initial freezing point of the food T_f, and R is the gas constant. The water activity and the initial freezing point (where $y_{ice} = 0$) can be determined using the model in our previous paper [van der Sman & Boer,2004], which is briefly described below.

The initial freezing point T_f is related to the water activity a_{w0}, also via Clausius-Clapeyron (using T_{f0} the freezing point of pure water):

$$\ln a_{w0} = \left(\frac{1}{T_f} - \frac{1}{T_{f0}}\right) \frac{\lambda}{R} \quad (3)$$

The water activity of the frozen food can be computed from the composition of the unfrozen aqueous phase, following the model [van der Sman & Boer,2004]. Composition is given in mass fractions of water (y_w), proteins (y_p), fat (y_f), carbohydrates (y_c), ash (y_{ash}), sodium chloride (y_{NaCl}), where y_{ash} is exclusive y_{NaCl}.

Below initial freezing point T_f water in food can be in one of three states:
1) in solid state as ice crystals, 2) in liquid state in the unfrozen aqueous phase, and 3) in an amorphous state as water of hydration bounded to biopolymers. This partitioning can be expressed as: $y_{w0} = y_{ice} + y_w + y_b$, where y_{w0} is the total mass fraction of water.

Due to its amorphous state, and bound water is unavailable to dissolution of solutes. The mass fraction of bound water y_b is assumed to be temperature independent, and is a linear

function of the mass fractions of soluble solids [van der Sman & Boer,2004]

$$y_b = 0.29y_p + 0.10y_c \tag{4}$$

The water activity of the unfrozen aqueous phase is mainly determined by its salt content. It is assumed that the contributions of different salts in the chemical potential ($\mu = RT \ln a_w$) are additive, and therefore the Ross equation holds:

$$a_w = a_{w,ash} a_{w,NaCl} \tag{5}$$

with $a_{w,ash}$ given by Raoults law:

$$a_{w,ash} = \frac{y_w}{y_w + n_{ash} M_w y_{ash}/M_{ash}} \tag{6}$$

and a_{NaCl} follows the approximation of the Pitzer equation [Chen,1990, van der Sman & Boer,2004]:

$$\frac{1}{a_{w,NaCl}} = 1 + M_w(\beta_e + Bm^n)m \tag{7}$$

The molar mass of water is $M_w = 18$ g/mol, the molar mass of sodium chloride is $M_{NaCl} = 58.15$ g/mol, and the effective molar mass of ash is $M_{ash} = 72$ g/mol [van der Sman & Boer,2004]. The dissociation numbers of the salts are $n_{ash} = 2$ and $n_{NaCl} = 2$. The molality of sodium chloride is m, and is given by $m = M_{NaCl} y_{NaCl}/y_w$ The parameter values of the approximation of the Pitzer equation are: β_e=1.868, B=0.0582, and n=1.618.

Due to freeze-concentration at a certain temperature the molatity of NaCl can rise above its saturation point (6m). In this case NaCl will crystallize out of the unfrozen solution and consequently $a_{w,NaCl}$ is maintained at the value of 0.75, the water activity of a saturated NaCl solution.

In order to compute the enthalpy one has to take into account that the specific heats of water and ice, and the latent heat of fusion are strongly temperature dependent. The specific heat of water shows a thermodynamic anomaly, and can be described as a combination of a linear function and a term with a Boltzmann factor accounting for the anomalous behaviour [Tanaka,2000]:

$$\begin{aligned} c_{p,w} &= c_{p,w0} + c_{p,w1}(T - T_{f0}) + \\ &\quad c_{p,wa} \exp(\Delta E_a/T) \end{aligned} \tag{8}$$

The parameter values are: $c_{p,w0} = 4.02$ kJ/kg.K, $c_{p,w1} = 1.6\ 10^{-3}$ kJ/kg.K^2, $c_{p,wa} = 0.26\ 10^{-3}$ kJ/kg.K, and $\Delta E_a = 1800$ K. The specific heat of ice follows a normal linear function [Sanz et al.,1999]:

$$c_{p,ice}(T) = c_{p,ice0} + c_{p,ice1}(T - T_{f0}) \tag{9}$$

with $c_{p,ice0} = 2.12$ kJ/kg.K, and $c_{p,ice1} = 7.8$ J/kg.K^2. The latent heat of fusion follows a polynomial function [Sanz et al.,1999]:

$$\begin{aligned} \lambda(T) &= \lambda_0 + \lambda_1(T - T_{f0}) + \\ &\quad \lambda_2(T - T_{f0})^2 \end{aligned} \tag{10}$$

with λ_0=6004 J/mol, λ_1=95.23 J/mol.K, and λ_2=0.14 J/mol.K^2.

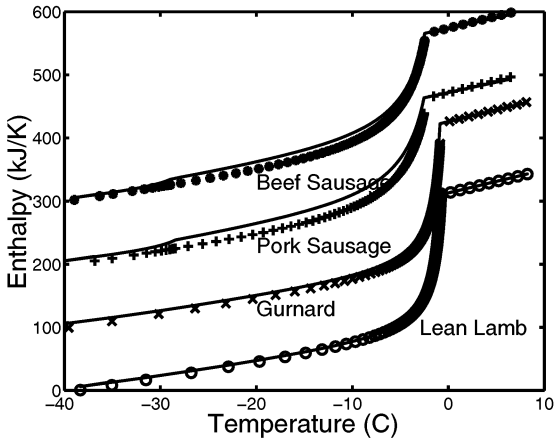

Figure 1: Experimental (symbols) and predicted (solid lines) values of the enthalpy for 4 types of meat. For clarity the curves are shifted with respect to each other to prevent overlap.

RESULTS

The results of the above model are compared with experimental data [Lindsay & Lovatt,1994]. These results are presented in figure 1. Lindsay and Lovatt have fitted the following equations to the enthalpy data:

$$\begin{aligned} H(T) &= H_0 + c_u(T - T_{f0}) \text{ if } T > T_f \\ H(T) &= A + + c_f(T - T_{f0}) \\ & \quad B/(T - T_{f0}) \text{ if } T \leq T_f \end{aligned} \tag{11}$$

Here A is an integration constant rendering $H(-40) = 0$. c_u is the heat capacity of the unfrozen food at T_{f0}, and c_f is the heat capacity of the food at approximately -40^o C, where they assumed nearly all water (except bound water) is frozen. In Table I we have listed the values c_u, c_f and T_f according 1) to the experiments of Lindsay and Lovatt, and 2) to our model (indicated with superscripts *). Also we have determined the L_2-norm between experimental and the predicted $H(T)$ curves, defined as:

$$L_2 = \sum_n \frac{(H(T_n) - H^*(T_n))^2}{NH_0^2} \tag{12}$$

In figure we have plotted the predicted and experimental values. Without any adjustments of parameters good agreement is obtained between experiment and model prediction. For pork and beef sausages the model predicts a abrupt change in the enthalpy if NaCl gets above 6m, and crystallizes out. Due to unavailability of the original experimental data, it is not known whether the experimental data also showed this abrupt change in enthalpy.

Also for other type of meat products good agreement is found, as shown in Table I. These results shown there are good opportunities to develop freezing models for meat products, purely based on composition data.

References

[Chen,1990] C.S. Chen. Predicting water activity in solutions of mixed solutes. J. Food Sci. 55(2): 494-497 (1990).

[Lindsay & Lovatt,1994] D.T. Lindsay, and S.J. Lovatt. Further enhalpy values of foods measured by an adiabatic calorimeter. *J. Food Eng.* **23** (4): 609-620 (1994).

[Mannapperuma & Singh,1988] J.D. Mannapperuma, and R.P. Singh, Prediction of freezing and thawing times of foods using a numerical-method based on enthalpy formulation *J. Food Sci.* **53** (2): 626-630 (1988)

[Sanz et al.,1999] P.D. Sanz, M. Ramos, and J. Aguirre-Puente. One-stage model of foods freezing. *J. Food Eng.* **40**:233-239(1999).

[Schwartzberg,1976] H.G. Schwartzberg. Effective heat capacities for the freezing and thawing of food. *J. Food Sci.* **41**:153 (1976).

[van der Sman & Boer,2004] R.G.M. van der Sman, and E. Boer, Predicting initial freezing point and water activity of meat products from composition data. *J. Food. Eng.*, in press (2004).

[Tanaka,2000] H. Tanaka. Simple physicla model of liquid water. *J. Chem. Phys.* **112**(2):799-809 (2000).

Table I: Comparison of model predictions and experimental values of Lindsay and Lovatt [Lindsay & Lovatt,1994].

Name	c_u kJ/kg.K	c_u^* kJ/kg.K	c_f kJ/kg.K	c_f^* kJ/kg.K	T_f ^{o}C	T_f^* ^{o}C	L_2 %
Lamb	3.62	3.66	2.19	2.02	-0.78	-0.77	0.01
Beef	3.64	3.67	2.26	2.00	-0.70	-0.69	0.01
Chicken	3.71	3.72	2.07	2.02	-0.80	-0.75	0.06
Pork	3.62	3.68	2.06	2.02	-0.79	-0.83	0.09
Venison	3.69	3.64	2.12	2.03	-0.87	-0.86	0.11
Mutton	3.66	3.53	2.09	2.05	-0.95	-1.09	0.03
Gurnard	3.77	3.73	2.14	2.00	-0.76	-0.87	0.28
Tarakihi	3.69	3.70	2.13	2.02	-0.79	-0.82	0.06
P. Sausage	3.81	3.30	1.93	1.94	-2.23	-2.54	0.37
B. Sausage	3.79	3.33	1.95	1.95	-2.34	-2.47	0.13

A MICROMECHANICAL APPROACH FOR SIMULATING PLANT TISSUE

Jimmy Loodts, Engelbert Tijskens, Herman Ramon
Laboratory for Agricultural Machinery and Processing
K.U.Leuven, Kasteelpark Arenberg 30, B 3001, Leuven, Belgium
E-mail: Jimmy.Loodts@agr.kuleuven.ac.be
Internet: www.agromechatronics.be

KEYWORDS

Micromechanical Model, Numerical Simulation, Discrete Element Method, Onion Epidermis.

ABSTRACT

In this paper a generic micromechanical modelling approach is introduced that allows for dynamical simulations of cellular biological tissue. It is derived from the discrete element approach in the sense that the tissue is discretised such that microscopic features and histological aspects like cell geometry and the cellular arrangement within the tissue can be fully incorporated into the model. This makes dynamical simulations of arbitrarily shaped cellular tissues feasible in an elegant and robust way, while providing room for future extensions to incorporate intercellular fluid transport and tissue failure. The validity of this simulation technique is demonstrated by a case study on the unicellular epidermis layer of the Spanish onion (*Allium cepa*). The parameters of a two dimensional model are determined using the stress-strain relation in a tension test for longitudinal strips. The model is then validated quantitatively against the data for transversal strips.

INTRODUCTION

In order to come up with thorough innovations in agro-technology, it is becoming increasingly important to deepen our knowledge of the soft tissue mechanics in agricultural products. A fruitful approach towards this goal is the use of numerical simulation techniques for conducting virtual experiments and for the indirect determination of material properties. On the one hand such an understanding can help to obtain the desired firmness of fruits and vegetables and thus increase product quality. On the other hand it is an essential tool for damage minimization during handling, storage and transportation. Mechanical damage can be caused by impacts, vibrations, static load and friction.

Since plant tissues are complex conglomerates of cells whose integrity depends on the mechanical properties of the cell wall, the cellular turgor pressure, the presence of an adhesive middle lamella between individual cells and the presence or absence of intercellular spaces, it is clear that the cellular structure is a predominant factor in determining the mechanical behaviour of such tissues (Pitt 1982; Kerstens et al. 2001).

A *micromechanical approach* is ideally suited to account for these cellular and histological attributes on the overall mechanical behaviour of fruits and vegetables.

In literature both qualitative and quantitative micromechanical models of uniformly stressed cellular tissues are described with symmetry and uniformity assumptions on cell shape and cell wall deformations (Nilsson et al. 1958; Gao and Pitt 1991). The symmetry and uniformity assumptions made in the above mentioned work cannot always describe the geometry of the tissue under study, indicating the need for methodologies that can handle random shaped cells, like finite elements (Cooke et al. 1976; Wu and Pitts 1999; Pitt and Davis 1984). Another approach is to tackle the problem using techniques from mechanics of large elastic deformations (Zhu and Melrose 2003). Some phenomena typically encountered in the study of tissue mechanics like intercellular fluid transport or the fast changing boundaries accompanying crack propagation cannot be dealt with in an elegant way by the finite element method. This is why Schembri and Harris (1996) developed a two dimensional discrete element model to describe failure of sugar cane tissue. They represented the parenchyma cells and fibrovascular bundles as different kinds of rigid polygonal objects, called discrete elements, connected by springs mimicking adhesion and volume exclusion forces. Then the dynamics of the tissue is simulated by tracking the motion of every individual element through integration of the acceleration resulting from the forces between the elements.

The goal of this paper is to develop a model incorporating microscopic information in order to understand macroscopic mechanical behaviour in terms of these microscopic features. Ultimately, the microscopic information will form the basis for the development of models at any larger scale. In order to achieve this goal the approach in this paper is based on the discrete element method because of its computational efficiency and, more important, because it allows for an elegant incorporation of histological features, intercellular fluid transport and tissue failure. More precisely, a two dimensional mechanical model of an epidermis layer of Spanish onion (*Allium cepa*) is constructed using this new approach. The unicellular epidermis layer was chosen as a test case because its cellular arrangement can be studied using conventional optical techniques and accurate measurements for validation were readily available.

RESULTS

Theoretical Background

The two major biological factors that determine the mechanical response of plant tissue to an applied load are the cell wall and the turgor pressure of the individual cells.

The cell wall consists of cellulose microfibrils and a matrix of pectin and hemicellulose together with water (Cosgrove 1997). As long as the linear elastic range is not exceeded, the matrix and the microfibrils will both deform elastically and thus can be treated as one single material (Köhler and Spatz 2002) of which the mechanical properties related to tension will be predominantly determined by those of the cellulose microfibrils since they are by far the least extensible component in the cell wall (Ha et al. 1997). This is why the cell wall has successfully been modelled as a membrane made from a linear elastic material for small strains (Hiller et al. 1996). Microfibrils are long and thin, consequently they can only resist tension load while they will offer practically no resistance to bending. This means that the cell wall can be modelled as a flexible structure that exhibits a linear elastic response to tension within the linear elastic range.

The *turgor pressure* is the force exerted by the cell fluid in the protoplast on its encapsulating structure. Since this cell fluid is incompressible it can be replaced by a volume conservation constraint per cell for modelling purposes when limited timescales are considered. However, over longer periods of time fluid transport may occur between cells through plasmodesmata and other transport mechanisms.

Mathematical Model

When constructing a model, the above physical principles have to be translated into a simplified model that can be verified through measurement and that can be computed. In order to focus thoughts, a two dimensional model for deformable polygonal cells, with turgor pressure will be elaborated upon. This model was designed in such a way that it can deal with irregular geometries and non-uniform loads in an elegant way, while allowing for extensions such as tissue failure and intercellular fluid transport.

Every cell in the tissue is described as a polygon whose corner points will be termed as *nodes* (see fig. 1).

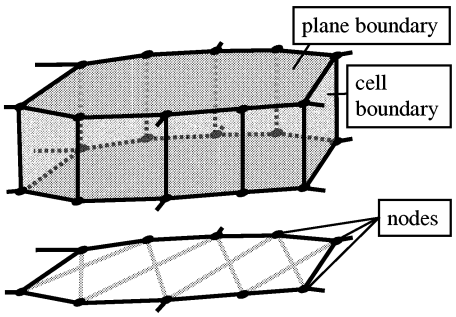

Figure 1: 2D Model as a Projection of the Actual 3D Tissue

These nodes are the only objects in our model that exhibit inertia. External loads can be applied on every node in the tissue.

When one wants to grasp the essential features of a three dimensional tissue in a two dimensional description, it is most convenient to conceptually divide the cell wall into two categories. The first category, the *cell boundaries*, comprises the cell walls which are perpendicular to the plane of the tissue strip (the vertical walls in fig. 1). The other category, the *plane boundaries*, consists of the cell walls that cover the top and bottom of the cells and thus are parallel to the plane of the tissue strip (the horizontal walls in fig. 1). The linear elastic behaviour of the cell walls for small strains is modelled by means of massless linear springs between the nodes (see fig. 2). Actually the two cell walls of neighbouring cells, together with the corresponding middle lamella are modelled as one single cell boundary spring. The mechanical properties of the cell wall sheets that make up the plane boundaries are modelled as an isotropic spring network. The isotropy follows from the specific geometry of the model and will be elaborated upon in the section 'Initial conditions'. The fact that the resistance to bending of the cell wall is negligible is incorporated into the model by representing the nodes as frictionless hinges, which allow for free rotation of the springs representing the cell wall.

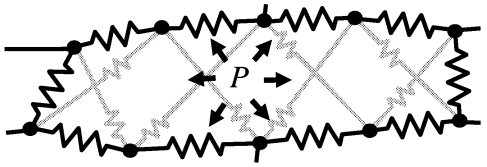

Figure 2: Schematic Model of a Cell; Black Springs: Cell Boundaries, Grey Springs: Plane Boundaries

In summary, the following assumptions are made: 1) The cell wall is linear elastic and offers no resistance to bending. 2) The cell wall sheets are isotropic. 3) No intercellular spaces are present. 4) The cell fluid is incompressible. 5) There is no intercellular fluid transport on the timescale simulated.

When a simulation is started all parameters are set to the appropriate values and initial conditions are applied, this gives one enough information to calculate all forces on each node. These forces are used to determine the new positions of the nodes for the next time step through numerical integration. All the information that is needed to analyse the system afterwards is saved. The forces are evaluated with the new positions and the above cycle is repeated until a stop condition is met.

Governing Equations

The resultant force, $\mathbf{F}_{R,i}$,on each node i can be written as the sum of the spring force, $\mathbf{F}_{S,i}$, the pressure force, $\mathbf{F}_{P,i}$, and the external force, $\mathbf{F}_{E,i}$, acting on that node. In fact the dynamics of a node is determined by the forces that act on

the cell walls within its 'zone of influence' (the grey zone in fig. 3), which reaches halfway the connected cell wall pieces. All forces acting on the cell walls in this zone will be lumped together in the central node.

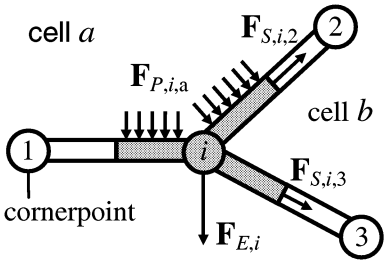

Figure 3: The 'Zone of Influence' of Node *i*

The *spring forces*, which are due to the tension in the cell wall, are calculated for each piece of cell wall that is connected to the node *i*. Linear elastic massless springs are used with spring constants $k_{SCB,i}$ and $k_{SPB,i}$ for the cell and the plane boundaries respectively. In order to implement the incompressibility of the cell fluid the cell volume is kept constant, which comes down to maintaining a constant cell surface for this two dimensional model. This is achieved by means of a *pressure force* that has a proportional and a differential action to the difference between the current cell surface and the desired one. The proportionality factor can be chosen in such a way that the regulation of the volume is achieved on a smaller timescale than the dynamics of the system, which assures volume conservation. The third factor is the sum of the *external forces* acting on the i^{th} node, so the resultant force on this node can be written as

$\mathbf{F}_{R,i} = \mathbf{F}_{S,i} + \mathbf{F}_{P,i} + \mathbf{F}_{E,i}$.

From the above considerations it is clear that $\mathbf{F}_{R,i}$ depends on the position and velocities of all the nodes belonging to one of the cells to which the node *i* belongs. This locality results in a linear dependence of the computational cost on the system size. Conform the second law of Newton this resultant force will cause the node *i* with mass m_i to accelerate according to $\mathbf{F}_{R,i} = m_i\mathbf{a}_i$, where $\mathbf{a}_i$ is the acceleration of node *i*. The forward Euler integration scheme is used to solve these differential equations numerically such that the only additional information needed to perform simulations is a set of initial conditions and the appropriate values for the model parameters.

Initial Conditions

The initial conditions consist of an initial position for every node, from which the rest length for every spring and the surface of every cell in rest can be derived. Since our model can handle any polygonal cell shape, it is possible to work with realistic tissue samples. These can be digitalized as shown in fig. 4 and stored as an input file that contains the positions of the nodes together with the information of which points belong to which cell and which points are connected through springs.

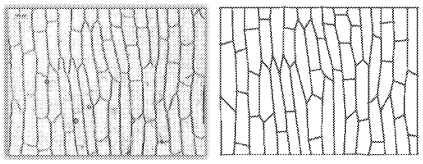

Figure 4: A Typical Piece of Onion Tissue (on the left) and its Digital Version (on the right)

For tissues with a limited number of cells (such as the one in fig. 4) it is feasible to create the appropriate input files manually. In the experiments performed by Wei et al., (2001), rectangular strips of 18 mm by 3 mm were cut such that the long axis of the cut was either parallel or perpendicular to the long axes of the cells, resulting in *longitudinal* and *transverse strips* respectively. Because of these larger strips, containing circa a thousand cells, an automated procedure had to be developed which creates a tissue with an alternating brick structure as can be seen in the upper part of fig. 5 and 6. The cells in the brick structure have an aspect ratio of 4 which corresponds to the average aspect ratio of onion epidermis cells. The springs of the plane boundaries are initially placed under an angle of 45 degrees with the long axes of the cells to assure that they will cause no difference in the stress measured while stretching in the longitudinal versus the transversal direction and thus represent an isotropic network with respect to these two directions. The entire strips used in the experiments measured 18 mm by 3 mm and where 120 µm thick while a single epidermis cell measures 480 µm by 120 µm on average.

Experimental Determination of Model Parameters

The model parameters are determined from experimental measurements performed by Wei et al. (2001). The Cauchy strain is determined as the current length of the strip divided by the original length and expressed as a percentage. For the stress the forces in all the springs connecting the left and the right half of the tissue were computed. The sum of these forces is then divided by the initial transectional area of the tissue to obtain the nominal stress.

The true density of onion tissue is 1040 ± 90 kg/m³ (Abhayawick, 2002). From this we can calculate the mass of a single strip ($6.74\ 10^{-6}$ kg) which has to be divided over the 3625 nodes to obtain the mass of a single node; $1.86\ 10^{-9}$ kg. The relation between the spring constants of the plane and cell boundaries was calculated analytically and verified through simulations to be $k_{SCB} = 1.0265\ k_{SPB}$ for stretches up to 5.3 % (the average of 3.2 % and 7.4 %). Since the spring constants for the cell boundaries and the plane boundaries are related to one another, they can both be determined from one parameter optimisation in relation to the measurements on the longitudinal strips (stretches up to 3.2 %) performed by Wei et al., 2001 (see fig. 5).

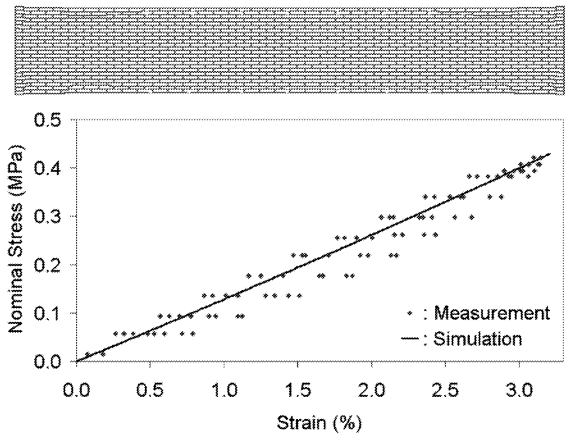

Figure 5: The Stretched Longitudinal Strip (initially 3 mm by 18 mm) with the Corresponding Stress-Strain Curve

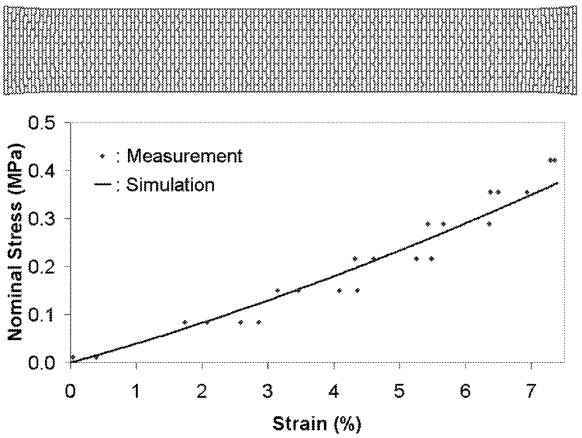

Figure 6: Validation of the Model with Parameters obtained from the Longitudinal Strip Experiment

The measurements on the transversal strips (stretches up to 7.4 %) were used to validate the model predictions quantitatively.
The proportionality factor to regulate the volume for each cell was chosen high enough to ensure volume preservation per cell. The maximal variation of the individual cell volume during the entire virtual experiment was 0.05 % of its initial volume. This variation can be made arbitrarily small by increasing the value of this proportionality factor, resulting in smaller timesteps and a higher computational cost. The timestep is chosen small engough to ensure numerical stability.

Validation

Since the model parameters were determined by taking into account only the information of the longitudinal strip, a qualitative agreement with the data for the transversal strip is a powerful validation. The result of the virtual stretching experiment for the transversal strip is shown in fig. 6. It is clear that a very good quantitative agreement is obtained between the simulation results and the measured data.
This implies that the cellular arrangement (the alternating brick structure) and the elongated form of individual cells (an aspect ratio of 4) can effectively account for the difference in mechanical properties between longitudinal and transversal strips.

Parameterspace Exploration

In order to study the influence of the different aspects in our model such as the relative importance of the cell and plane boundaries and the internal pressure, some virtual experiments were performed with different values for the corresponding parameters. The virtual tissue used was identical to the tissue used in the previously described stretching experiments except for the dimensions which were 3 mm by 3 mm. To show the non-linear behaviour of the overall tissue more clearly, the tissue is stretched up to 10 % strain.
When no action is taken to keep the volume constant, one can observe in fig. 7 that the tissue becomes less stiff in comparison with a simulation in which the volume is kept constant. This is in agreement with previous studies of the influence of turgor pressure on tissue stiffness (Jackman, 1992; Pitt and Chen, 1983), where a stiffening of the tissue with increasing turgor pressure is reported.
Removing the plane boundary springs results in a maximal difference in stiffness between the longitudinal and the transversal direction (see fig. 7).
It is obvious that there should be no difference in the stiffness between the longitudinal and the transversal direction when all histological information is excluded by removing the springs that represent the cell boundaries. This is shown clearly in fig. 7 and follows from the fact that the plane boundaries are modelled as an isotropic spring network.

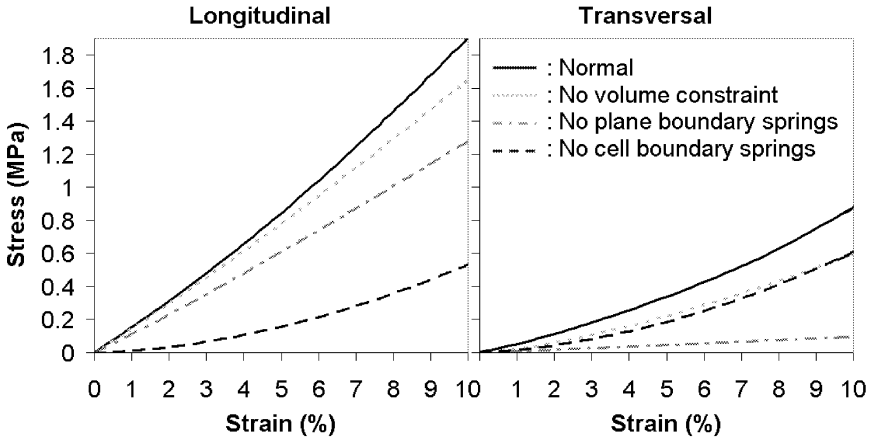

Figure 7: Exploration of the Parameterspace of the Model for the Longitudinal Strip (left) and the Transversal Strip (right)

CONCLUSION

A generic modelling approach is introduced for the dynamical simulation of cellular biological tissue. It is based on the discrete element approach and results in a mass-spring network with linear springs representing both the cell and plane boundaries. The presence of cellular fluid is incorporated by setting a volume conservation constraint for each individual cell. This approach is flexible enough to incorporate histological aspects such as the individual cell geometry and the cellular arrangement in the tissue. The validity of this simulation technique is demonstrated through a case study on the unicellular epidermis layer of the Spanish onion (*Allium cepa*). With the model parameters optimised from experiments on a longitudinal strip, the model is able to generate good quantitative predictions for measurements on transversal strips. The positive correlation between turgor pressure and tissue stiffness was also illustrated.

The flexibility of this modelling approach enables future extensions for the incorporation of intercellular fluid transport, large strain deformations, tissue failure, etc. such that progress towards well-founded simulations of the mechanical behaviour and texture of fruits and vegetables as well as the mechanical aspects of food processing can be expected.

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REFERENCES

Abhayawick L., Laguerre J.C., Tauzin V., Duquenoy A. 2002. "Physical properties of three onion varieties as affected by the moisture content." *J Food Eng* 55: 253-262.

Cooke R.J., De Baerdemaeker J.G., Rand R.H., Mang H.A. 1976. "A finite element shell analysis of guard cell deformations." *Trans ASAE* 19: 1107-1121.

Cosgrove D.J. 1997. "Assembly and enlargement of the primary cell wall in plants." *Annu Rev Cell Dev Biol* 13: 171-201.

Gao Q., Pitt R.E., Ruina A. 1990. "A mechanics model of the compression of cells with finite initial contact area." *Biorheology* 27: 225-240.

Ha M.-A., Apperley D.C., Jarvis M.C. 1997. "Molecular rigidity in dry and hydrated onion cell walls." *Plant Physiol* 115: 593-598.

Hiller S., Bruce D.M., Jeronimidis G. 1996. "A micro-penetration technique for mechanical testing of plant cell walls." *J Texture Stud* 27: 559-587.

Jackman R.L., Marangoni A.G., Stanley D.W. 1992. "The effects of turgor pressure on puncture and viscoelastic properties of tomato tissue." *J Texture Stud* 23: 491-505.

Kerstens S., Decraemer W.F., Verbelen J.-P. 2001. "Cell walls at the plant surface behave mechanically like fiber-reinforced composite materials." *Plant Physiol* 127: 381-385.

Köhler L., Spatz H.-C. 2002. "Micromechanics of plant tissues beyond the linear elastic range." *Planta* 215: 33-40.

Nilsson B.S., Hertz H.C., Falk S. 1958. "On the relation between turgor pressure and tissue rigidity II. Theoretical calculations on model systems." *Physiol Plantarum* 11: 818-837.

Pitt, R.E. 1982. "Models for the rheology and statistical strength of uniformly stressed vegetative tissue." *Trans ASAE* 25: 1776-1784.

Pitt R.E., Chen H.L. 1983. "Time-dependent aspects of the strength and rheology of vegetative tissue." *Trans ASAE* 26: 1275-1280.

Pitt R.E., Davis D.C. 1984. "Finite element analysis of fluid-filled cell response to external loading." *Trans ASAE* 27: 1976-1983.

Schembri M.G., Harris H.D. 1996. "Modelling impact on a biological material (sugar cane) using the discrete element method." *AgEng'96*, Madrid.

Wei C., Lintilhac P.M., Tanguay J.J. 2001. "An Insight into cell elasticity and load-bearing ability. Measurement and Theory." *Plant Physiol* 126: 1129-1138.

Wu N., Pitts M.J. 1999. "Development and validation of a finite element model of an apple fruit cell." *Postharvest Biol Tec* 12: 1-8.

Zhu H.X., Melrose J.R. 2003. "A mechanics model for the compression of plant and vegetative tissues." *J Theor Biol* 221: 89-101.

Application of Multivariate Statistical Analysis and Human Expert Knowledge in Fuzzy Logic Modelling of the Multi-Stage High-Pressure Inactivation of *Lactococcus lactis* ssp. *cremoris* MG 1363

Klaus V. Kilimann
Technische Universität München
Weihenstephaner Steig 23
85354 Freising
Germany
Tel: +498161713247
Fax:+498161714510
kilimann@wzw.tum.de

Christoph Hartmann
Technische Universität München
Weihenstephaner Steig 23
85354 Freising
Germany
Tel: +498161713276
Fax:+498161714510
Christoph.hartmann@wzw.tum.de

Michael G. Gänzle
Technische Universität München
Weihenstephaner Steig 16
85354 Freising
Germany
Tel: +498161713204
Fax:+498161713327
Michael.Gaenzle@wzw.tum.de

KEYWORDS
High Pressure Inactivation; Fuzzy Modelling; Principal Component Analysis

ABSTRACT

Kinetic data on the multi-step inactivation of *Lactococcus lactis* ssp. *cremoris* MG 1363 are obtained by use of ex-situ staining methods and cell counts. The data reflects inactivation as a function of pressure, temperature, various additives to the substrate and pressure holding time. They are analysed with a principal component analysis. Results imply that metabolic activity correlates with the viablility of cells under high pressure, while the counts of pressurized but undamaged cells correlates with the activity of a membrane bound transport enzyme LmrP. These correlations have been used for the formulation of a fuzzy logic model that relates four input variables to two intermediate (output) variables and three dependent output variables. With current model it is possible to predict five states of high pressure induced inactivation in dependence of above mentioned parameters with an accuracy in the range of the experimental error.

INTRODUCTION

High pressure treatment (HP) with pressures from 200 to 800 MPa is a novel process in food technology employed to selectively affect the activity of food enzymes, to improve food texture, to stabilise colour, and to inactivate microorganisms. Compared to heat treatment, HP-technology also allows the elimination of the spoilage and of pathogenic microorganisms as well as the inactivation of enzymes while retaining "fresh" attributes of food properties. Therefore, pressure processed foods generally are of higher quality compared to thermally treated products (Harte et al., 2002; Krebbers et al., 2002). The use of pressure processes in industrial practice requires suitable mathematical models to predict the bactericidal effect of pressure on relevant target microorganisms. Described here are the effects of various pressure treatments on *Lactococcus lactis*, and the development of a multi-step fuzzy-logic model to predict pressure effects on microbial survival.
Previous investigations with *L. lactis* in milk buffer or milk buffer with bactericidal or baroprotective additives have shown that pressure treatment results in various degrees of lethal and sublethal injury of the bacteria. Hence, successful inactivation of the bacteria strongly depends on environmental conditions. Five relevant cell properties are available to describe the lethal or sublethal effects of HP/temperature-treatment on *L. lactis*., i. e. the viable cells (CFU), the number of undamaged cells (CFUsub), the membrane integrity (MI), the metabolic activity (MA) and the activity of a membrane bound multiple-drug-resistance (MDR) transport system LmrP (LmrP).
A multi-step fuzzy model is established which accounts for the multiple effects of HP/temperature-treatment on the properties of *L. lactis*. The fuzzy logic modelling approach is motivated by different aspects: Fuzzy logic allows the simple integration of expert knowledge. It compensates a large bandwidth in the experimental data arising from variable experimental conditions. The elimination of obvious errors in the experimental data can be carried out without changing the overall-structure of the model. It can easily be adapted to the requirement of a multi-level approach. Finally, many high pressure induced effects are still unexplained and therefore, a deterministic approach based on clear microbiological and physical concepts seems inappropriate at present.
The model is based on experimental data covering a temperature range from 5°C to 50°C, a pressure range from 0.1 MPa to 600 MPa, pressure application times from 0 to 120 minutes and 21 additives. A Principal Component Analysis (PCA) is carried out on all data in order to detect most powerful measurands. Afterwards, the model is established using different data based tools. To generate knowledge out of data, fuzzy clustering is done using fuzzy C-means algorithm. Results represent barycentres of the data, which are then analysed with respect to their dependency on the environmental conditions, using transfer functions or analysis of sensitivity. Afterwards, data are transferred to non-sharp logic, by the description of linguistic variables. The inactivation is then described through fuzzy rules, which have been automatically generated by WinROSA, an evolutionary search algorithm (Krone and Kiendl 1996). These rules are checked and completed by a human expert on validity.

MICROBIOLOGICAL METHODS AND ANALYSIS

The quantity of the data is divided using nearly 50% for model establishment and 50 % for model validation. Therefore, a data pool of nearly 180 kinetics exists.

Viable Cells and Undamaged Cell Counts

After pressure treatment, cell suspensions of each vial are diluted and plated on M17 agar (Merck, Darmstadt, Germany) supplemented with 1% of glucose or M17 agar containing 3% NaCl for determination of viable (CFU) and undamaged cell counts (CFUsub), respectively. The plates are incubated for 24 h at 30°C under aerobic conditions, and for 48 h to assess undamaged cells under same conditions as described above.

Membrane Integrity

The determination of membrane integrity of pressure treated *L. lactis* is carried out with the LIVE/DEAD® BacLight™ kit (Molecular probes, Eugene, U.S.A.) with propidium iodide as membrane-impermeant probe essentially according to the instructions of the manufacturer. 1 ml pressurised cell suspension is harvested by centrifugation. The supernatant is removed and the pellet resuspended in 1 ml phosphate buffer (PBO [g l^{-1}], H_2KPO_4, 6.8; $MgSO_4 \cdot 7H2O$, 0.1; $MnSO_4 \cdot 1H_2O$, 0.05). A stock solution of LIVE/DEAD® BacLight™ is prepared, the final concentration of each dye was 33,4 µM Syto® 9 and 200 µM propidium iodide (PI). 100 µl of each of the bacterial cell suspensions are mixed in 100 µl of the stock solution, mixed thoroughly and incubated for 5 minutes in the dark at 30°C. The fluorescence intensities of Syto® 9 and PI were measured with excitation and emission wavelengths of 485 nm and 520 nm, and 485 nm and 635 nm, respectively, using a spectrafluor microtiter plate reader (TECAN, Grödig, Austria). The ratio of Syto® 9 to PI fluorescence intensity is used as measure for membrane integrity (MI).

Metabolic Activity

The determination of metabolic activity (MA) of pressure treated *L. lactis* is carried out according to Ulmer et al. (2000). Cells from 1 ml pressure treated cultures are harvested by centrifugation. The supernatant is removed and the pellet resuspended in 1 ml PBO. A stock solution of tetrazolium is prepared mixing 4-iodonitrotetrazolium violet (INT, 2-(4-iodophenyl)-3-(-4-nitrophenyl)-5-phenyltetrazolium chloride) and glucose in PBO. The final concentration of each is 4 mM and 20 mM respectively. 100 µl of each of the bacterial cell suspensions are mixed with 100 µl of the stock solution. The absorbance is measured at 590 nm with a spectrafluor microtiter plate reader (TECAN, Grödig, Austria) and the results are reported as % MA.

LmrP-Actvity

Ethidium bromide (EB) is a substrate for the membrane bound enzyme LmrP and other multi drug resistance mdr-transport enzymes. EB stock solutions are prepared by dissolving 40 µmol of EB l^{-1} in PBO. Cells are washed, harvested by centrifugation and resuspended in a stock solution of PBO and EB, the final concentration is 20 µmol l^{-1}. After this treatment, the cell suspension is stained for 2 hours at 30°C in the dark without an energy source. Then glucose is added to a final concentration of 20 g l^{-1}. Cells reenergised with glucose export EB, resulting in a lower fluorescence of the EB-DNA complex. Immediately after glucose addition, the fluorescence of the EB-DNA complex is measured over 30 min in a spectrafluor microtiter plate reader (TECAN, Grödig, Austria) using excitation and emission wavelengths of 485 nm and 595 nm. The initial rate of EB efflux as measured by the decrease of EB fluorescence intensity upon glucose addition was calculated as described (Ulmer et al., 2002) and reported as LmrP activity (LmrP).

Data Preprocessings

For each parameter combination, measurements were carried out at least in duplicate and, in many cases, in triplicate. An averaging on the duplicate and triplicate data sets has been done in order to reduce the amount of raw data. Averages reflect the characteristics of the original data. Subsequent to averaging data are normalised in order to shift them from their original range to a range between zero and one. Further logarithmic transformation is done to access data of the states viable cells and undamaged cells for fuzzy logic modelling.

Principal Component Analysis

A principal component analysis (PCA) is done to detect correlations between measurands and thus to determine those physiological properties that are most useful to describe sublethal and lethal injury in *L. lactis*. Principal components (PCs) are optimal combinations of the original measurement quantities, wich represent new, artificial variables. In other words, PCA performs a rotation of the axes of a multivariate space of the original variables along orthogonal directions of maximal variance.
Therefore, pretreated data are combined in a matrix X. Eigenvectors and eigenvalues are then calculated out of the correlation matrix $X \cdot X^T$. The transformation relation between data matrix X and the matrix of eigenvectors can be written as

$$\underset{n\times m}{X} = \underset{n\times m}{S}\ \underset{n\times n}{V^T} \qquad (1)$$

where S indicates the matrix of un-normed scores. Subsequently, eigenvectors are referred to as loadings as commonly done in the PCA related literature. In order to reduce the number of significant variables, the number of loadings is reduced to g<n, which causes a reduction of the score matrix to a dimension g<n and which introduces an error E, if data X will be calculated with the reduced number of loadings

$$\underset{n\times m}{X} = \underset{g\times m}{S}\ \underset{g\times n}{V^T} + \underset{n\times m}{E} \qquad (2).$$

The number g of significant eigenvalues is calculated using the residual variance V of the r^*-th eigenvector (Vandeginste et al., 1998).

Fuzzy Logic

The principal structure of the established model is shown in figure 1.
Relationship between input variables and output variables are described in the form of fuzzy rules. Two of the output variables (CFU and LmrP) are described in dependence of the input variables (autonomous output variable), the remaining three output variables (MI, MA, CFUsub, termed dependent output variables) are described predominantly in dependence of the autonomous output variables.

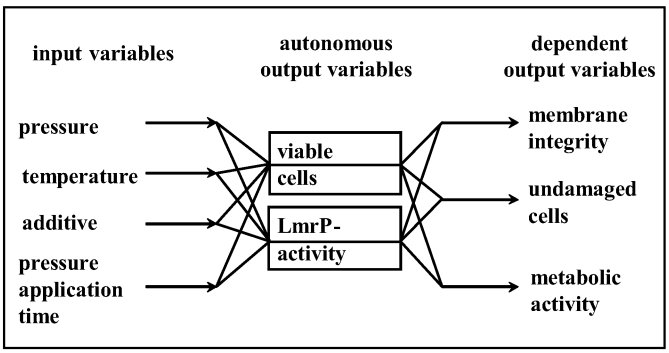

Figure 1: Principal model structure

Fuzzy rules are built up by an "if"-part, which describes a premise. The fuzzy "then"-part describes the conclusion. The rules can be defined from the knowledge of an expert (e. g. the person who carried out the experiments), or automatically by use of the following procedure. The fuzzy-C-means algorithm determines clusters (studied with sensitivity analysis and transfer functions) in the preprocessed experimental data and suggests a fuzzyfication, i. e. a transformation of sharp data to linguistic variables. The fuzzy sets and the desired input and output quantities are then analysed by evolutionary search algorithms which yield the above mentioned rules. Since the conclusion is a linguistic variable a defuzzyfication on the basis of the centre-of-gravity-method is employed to obtain a sharp model output value (Kiendl 1996, Kilimann et al. 2004)

RESULTS

Pressure effects on *L. lactis* are studied using a pressure range between 0.1 to 600 MPa, a temperature range from 5 to 50°C, pressure application between 0 and 120 min and 21 different food relevant additives (different pH, different concentrations of sucrose, sodium chloride and glycerine/betaine as well as mannitol). The data set consists of nearly 180 kinetics to describe all 5 states in detail. Using bactericidal additives (pH ranging from pH=4.0 to pH=6.0), a synergistic effect with HP/temperature-treatment is observed. In contrast, *L. lactis* is protected against lethal effects of pressure in the presence of 1.5M sucrose. Sublethal injury of the entire population occured after treatments at 600 MPa. Addition of 4M NaCl protects the viability of *L. lactis* to a pressure of up to 400 MPa. Using 600 MPa, a complete inactivation is reached after short pressure application.
To study the effect of combined HP/temperature-treatments, a pressure of 200 MPa and a temperature range between 5 and 50°C is chosen. Treatments at all combinations of pressure and temperature were also carried out in milk buffer and the presence of 1.5M sucrose or 4M NaCl. A subset of results is given in figure 2. Using temperatures smaller than 40°C and 1.5M sucrose, nearly no inactivation was observed. Using 4M NaCl, a total protection is achieved at temperatures greater than 20°C.

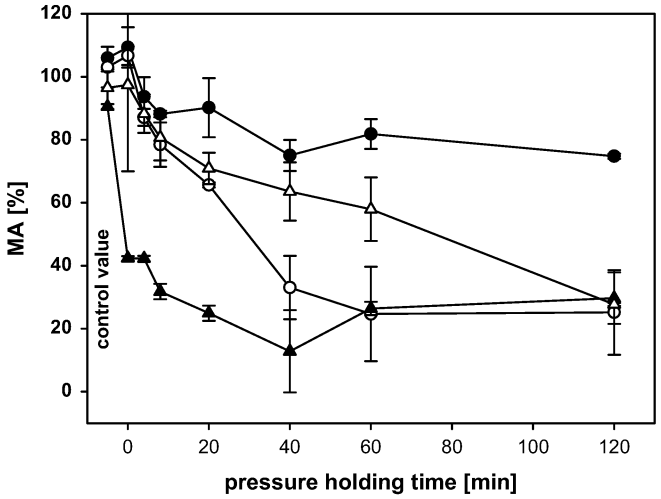

Figure 2: MA after combined HP/temperature treatment. Data are measured using 200 MPa.Cycles represent data with 1.5M sucrose/ ●10°C and ○ 50°C, triangles represent data using 4M NaCl/▲ 10°C and Δ/50°C.

Results are integrated in the multi-step fuzzy logic model, which is able to predict data in the range of used environmental conditions. A validation of the data is done using pressures at 250 MPa, 350 MPa, 400 MPa and 500 MPa, as well as temperatures of 10°C, 15°C and 45°C and for the most bactericidal and baroprotective additives as well as for milk buffer. Model prediction quality is shown for LmrP in figure 3.
Figure 3 A represents a prediction of LmrP through the model is in an early state of development. At that time, MA and CFU were used as autonomous output variables. Because of the total failure of prediction quality, the PCA was performed to identify the most powerful measurands as autonomous output variables and to detect correlations between the measured states.
For the actual model, a good correlation exists between MA and CFU as well as between LmrP and CFUsub using data measured in milk buffer (data not shown). Using 1.5M sucrose and 4M NaCl, other correlations are observed. Using 1.5M sucrose, MI is correlated with CFU and LmrP, MA and CFUsub could be detected as a cluster (data not shown). Using 4M NaCl, no correlations have been detected. The results suggest, that different kinds of inactivation mechanisms exist for *L. Lactis*. Autonomous output variables have been denominated as is shown in figure 1. With these settings, it was possible to predict all 5 states in a comparable quality or better than shown in figure 3B.

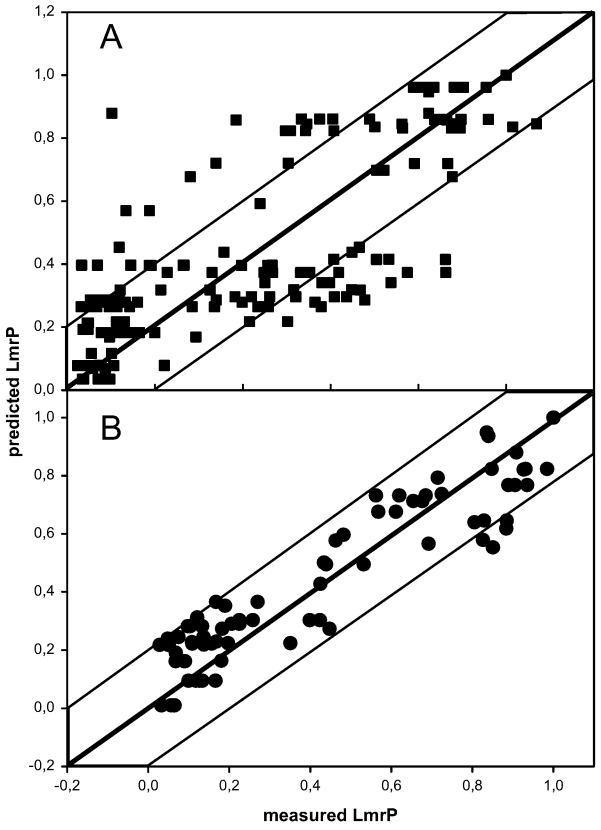

Figure 3: Comparison of predicted and measured LmrP-activity: A) before PCA, B) after PCA

CONCLUSION

In this work, the effect of combined HP/temperature treatment is studied by means of PCA and fuzzy logic on the basis of a data set consisting of 180 inactivation kinetics of *L. lactis*. A multi-step fuzzy logic model is proposed to describe the multiple effects of HP/temperature-treatment on *L. lactis*. Similarities between CFU and MA as well as between LmrP and CFUsub are detected and therefore CFU and LmrP can be used as autonomous output variables. These two variables contain all relevant information on sublethal and lethal injury of *L. lactis* after pressure treatments. From input data for the pressure level ranging from 0.1 to 600 MPa, the application time up to two hours, temperatures between 5 to 50°C and for different additives to the fluid medium, a prediction of five sates of the bacteria is possible: the membrane integrity, the metabolic activity, concentrations of surviving damaged and undamaged cells and the activity of a membrane transport system. The predicted data are in very good agreement with independently measured data. The deviation of calculated to measured values remains below 15 % for all quantities. The range covered by the model covers the range of practical interest. Extrapolations are presently not supported.

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REFERENCES

Harte, F., Amonte, M., Luedecke, L., Swanson, B.G., Barbosa-Canovas, G.V., 2002. Yield stress and microstructure of set yogurt made from high hydrostatic pressure-treated full fat milk. Journal of Food Science 67, 2245-2250

Kiendl, H., 1997. Fuzzy Control methodenorientiert. Oldenbourg Verlag. München

Kilimann, K.V., Hartmann, C., Delgado, A., Vogel, R.V., Gaenzle, M.G., 2003. A fuzzy logic based model for the multi-stage high pressure inactivation of *Lactococcus lactis* ssp. *cremoris* MG 1363. accepted in International Journal of Food Microbiology

Krebbers, B., Matser, A. M., Koets, M., van den Berg, R.W., 2002. Quality and storage-stability of high-pressure preserved green beans. Journal of Food Engineering 54, 27-33

Krone A. and Kiendl H., 1996.Rule based decision analysis with Fuzzy-ROSA method. Proceedings of European Workshop on Fuzzy Decision Analysis for Management, Dortmund. 109-114

Ulmer, H. M., M. G. Ganzle, and R. F. Vogel. 2000**.** Effects of high pressure on survival and metabolic activity of Lactobacillus plantarum TMW1.460. Applied and Environmental Microbiology 2000. 66: 3966-73.

Ulmer, H. M., Herberhold, H., Fahsel, S., Gänzle, M. G., Winter, R., and Vogel, R. F., 2002. Effects of pressure-induced membrane phase transitions on inactivation of HorA, an ATP dependent multidrug resistance transporter, in *Lactobacillus plantarum*. Applied and Environmental Microbiology 2002. 68, 1088-1095.

Vandeginste, B.G.M., Massart, D.L., Buydens, L.M.C., De Yong, S., Lewi, P.J., Smeyers-Verbeke, J., 1998. Data handling in science and technology. Handbook of chemometrics and qualimetrics Part A/Part B. Elsevier Science B.V.

AUTHOR BIOGRAPHY

KLAUS VALENTIN KILIMANN was born in Augsburg, Germany and went to the Technische Universität München (TUM), where he studied brewing sciences and he obtained his degree in 2002. He works now since 2002 at the Lehrstuhl für Fluidmechanik und Prozessautomation/Lehrstuhl für Technische Mikrobiologie (both TUM) as a PHD-student and is educating students in mathematics and high pressure in biotechnology. Further on, he is studying business at Universität Hagen/Germany where he obtained his pre-degree in March 2004.

CHRISTOPH HARTMANN studied Mechanical Engineering at the Technische Hochschule Darmstadt, Germany. After a one year stay in the Ecole Centrale de Lyon, France, he obtained his diploma in 1992. He continued his studies preparing a PhD in Computational Fluid Dynamics within the Mechanics Department of the Technische Hochschule Darmstadt. He defended his PhD in 1996. After several month of post-doctoral activities he joined the Engineering Systems International (ESI) Company in 1997 as a research and development engineer

for Finite-Element-Software (PAM-CRASH) for the simulation of occupants' safety in the automotive industry. In 1998, he joined the department of Fluid Dynamics and Process Control of the Technische Hochschule München as the head of the fluid dynamics research group. In January 2004, he defended his Habilitation thesis. His global field of interest are interaction processes between thermofluid-dynamics and food- and biotechnology preferably solved by simulation techniques.

MICHAEL G. GÄNZLE studied Food Science and Technology at the Universität Hohenheim, Stuttgart, Germany, and Oregon State University, USA. After obtaining this diploma in Food Technology in 1994, he prepared his doctoral thesis in the laboratory of food microbiology, Universität Hohenheim, and obtained a doctoral degree in 1998. Since 1998, he is working as a researcher and assistant professor (since 2003) at the TU München, Lehrstuhl Technische Mikrobiologie. His major research are the characterisation of functional metabolic traits of lactic acid bacteria for use in food fermentations, and the use of high pressure processes to control microbial activity in foods.

COMBINING EXPERIMENTAL DATA AND *IN SILICO* ANALYSIS TO MODEL THE METABOLIC NETWORK OF *LACTOBACILLUS PLANTARUM*

Bas Teusink[1,2,3]
Frank JH van Enckevort[2,3]
Arno Wegkamp[1,2]
Jos Boekhorst[3]
Douwe Molenaar[1,2]
Jeroen Hugenholtz[1,2]
Eddy J Smid[1,2]
Roland J Siezen[1,2,3]

[1]Wageningen Centre for Food Sciences
PO Box 557
6700 AN Wageningen
The Netherlands

[2]NIZO food research BV
PO Box 20
6710 BA Ede
The Netherlands

[3]Centre for Molecular and Biomolecular Informatics
University of Nijmegen
PO Box 9010
6500 GL Nijmegen
The Netherlands

KEYWORDS
Genome-scale modeling, metabolic reconstruction, phenotype prediction, functional genomics, systems biology

ABSTRACT

The complete genome of *Lactobacillus plantarum WCFS1* has recently been sequenced within the Wageningen Centre for Food Sciences. *Lactobacillus plantarum* is a versatile lactic acid bacterium that is important in many food and feed fermentation processes. Putative biological functions could be assigned to 2,120 (70%) of the 3,052 predicted protein-encoding genes. After prediction of gene function, the focus is now on the development and improvement of methods and tools to go from genome sequence to gene annotation, to pathway reconstruction and to prediction of phenotype through metabolic models. Important aspects are how and where to incorporate and use experimental (genomics) data, and how and to what extent parts of the process can be automated.

We have set up different bioinformatics tools, including web-interfaced databases and simulation software. This paper described some of these tools, and how they are used and combined with experimental data to come to a model of the metabolic network of *Lactobacillus plantarum*. The use and type of questions that can be addressed with these type of models will be discussed.

INTRODUCTION

Food production must be consistent and safe to provide nutrition and health for an increasing population demanding products of ever-higher quality. Fermentation with starter cultures containing lactic acid bacteria (LAB), such as lactobacilli and lactococci, plays an essential role for many products made from milk, meat, vegetables, and cereals. During their fermentative growth these bacteria produce mainly lactic acid, which has a preserving effect. Various important flavor and texturing compounds are also produced through the activity of LAB enzymes, both during fermentation and product maturation.

LAB not only play a role in fermentation. A large diversity of species has been found in different environmental niches, including the gastro-intestinal tract, with several having probiotic properties. Probiotics are live microbial food and feed supplements which are reported to improve the microbial balance of the intestine. Mediation presumably occurs through stimulation of the commensal flora and competitive exclusion of pathogens.

To fully exploit their potential, LAB have been the subject of considerable research and commercial development. Focus has however primarily been on empirical strain selection and the study of individual enzymes or simple metabolic pathways. With the genomics revolutions in biology, and hence the genome sequencing of numerous LAB initiated in the past few years (see Fig 1 for one of these), we now have a unique opportunity to radically change that. Knowledge of the complete genetic potential has paved the way for the integration of high-throughput functional genomics data into comprehensive models of cell-factories.

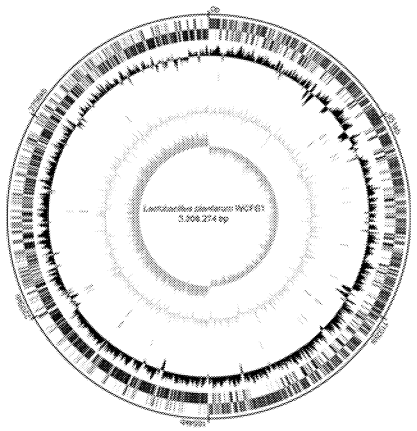


Fig. 1 Circular map of the genome of *Lb. plantarum*. Bars in the outer ring indicate the genes, other gene-related information can be projected in additional rings. Picture generated with the in-house developed tool Microbial Genome Viewer (Kerkhoven et al. 2004. *Bioinformatics*, in press)

Methods for data integration, data storage and data analysis at the size of hundreds to thousands of genes, compounds and reactions, are still in development, as the amount and magnitude of data is new to biology. Important aspects are standardization of vocabulary, functional interactions (ontologies), biological concepts for data integration and visualization. New software needs to be developed, and existing tools that traditionally dealt with only a few genes or reactions, need to be scaled up. These developments go extremely fast, and some of the required concepts and tools are emerging.

We have recently sequenced the genome of *Lactobacillus plantarum* WCFS1 (Kleerebezem et al. 2003). *Lb. plantarum* is a versatile lactic acid bacterium that is important in many food and feed fermentation processes. Putative biological functions could be assigned to 2,120 (70%) of the 3,052 predicted protein-encoding genes. This paper describes the development of a model of the complete metabolic network, based on the genome annotation. Emphasis will be on the tools used, the information required for the model development and the type of questions that can be addressed.

METABOLIC NETWORK RECONSTRUCTION

For a reconstruction of the metabolic network, information is required about the (putative) functions of genes, and databases with information of metabolic pathways. Annotation data describing the function of genes, comments of curators and further additional information are stored in an in-house developed, web-interfaced, mySQL database. The database stores information on different microorganisms and different versions (updates) of the annotated genomes. Queries can be performed, and comments can be added via the web interface.

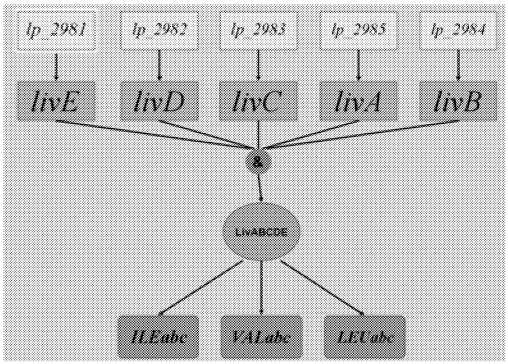


Fig 2. example of many-to-many relationship between genes (*livA* to *livE*), proteins (the LivABCDE transporter complex) and the reactions (active transport of the three branched-chain amino acids). Picture is from Simpheny™.

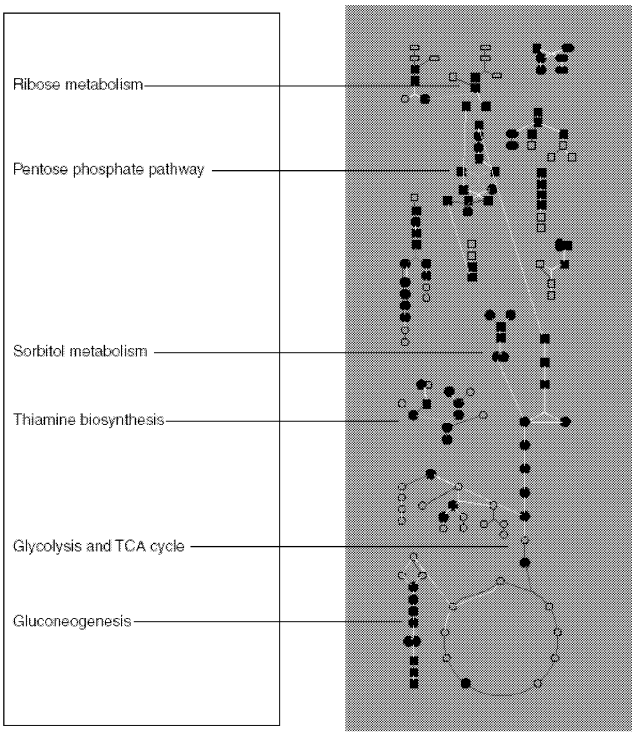
For information on metabolic pathways, we used primary literature and a number of databases. These include Kegg (http://www.genome.ad.jp/kegg/kegg2.html), ERGO Bioinformatics Suite (Integrated Genomics), Brenda (http://www.brenda.uni-koeln.de/), MetaCyc (Karp et al. 2002, www.metacyc.org) and Simpheny™ (Genomatica Inc.). All these databases contain similar information, but there are many errors, and therefore the different databases are supplementary but also contradictory. Moreover, not only information about reactions is required, but also on the enzymes that carry out these reactions. There exist many-to-many relationships between genes, proteins and the reactions they carry out. For example, alcohol dehydrogenase is encoded by one gene, but the enzyme can oxidize many alcohols. ATP synthase, however, carries out only one (very important) reaction, the synthesis of ATP, and it consists of many subunits, encoded by many different genes. The branched chain amino acid transporter LivABCDE even combines these two features (Fig. 2). These complicated relations between genes and reactions, and the inevitable mistakes in the databases, make the construction of the first model very labor intensive, but once a first high-quality model is made, it can form the basis of new models of other strains.

Experimental data that is required to reconstruct the metabolic network are the potential inputs and outputs. These comprise the substrates that the organism can consume, the products that it can make, and the composition of the biomass. The latter is very important to resolve issues in membrane and cell wall biochemistry, leading to significant sinks of, *e.g.*, carbon and phosphate. Half of the 20 amino acids, and 7 out of 10 known cofactors and vitamins, needed to be supplied in a minimal growth medium (A. Wegkamp, unpublished results), reflecting the relatively rich environments *Lb. plantarum* grows in. Yet, all but three amino acid biosynthesis routes in *Lb. plantarum* appear to be complete. There are many possible explanations, including kinetic constraints (regulation), mutations that render genes inactive, errors in the prediction of gene functions, and others. We are in the process of resolving some of these issues, which has led to

many new hypotheses about the function of particular genes and metabolic pathways.

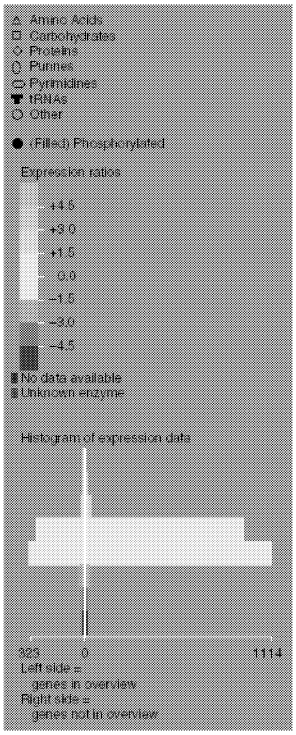
MODELING

A specific roadmap of the metabolism of the organism of interest is in itself extremely useful. Exploring the network allows one to predict viability and product formation of knockout strains, optimal yields, and perhaps even regulatory sites (Stelling et al. 2002). These are all qualitative aspects of the network. Other questions that can be addressed and that have a more quantitative nature, are: given a certain fixed growth rate, what is the maximal by-product formation, and which fluxes within the network go up to achieve that? Given a certain rate of glucose uptake, what is the maximal growth rate and what is the optimal flux distribution to achieve that (see for an introductory paper, Covert et al. 2001)? We use the tool Simpheny™ (Genomatica Inc.) for answering these more quantitiative questions. Moreover, as Simpheny contains a large database with biochemical reactions and lots of QA control features, it facilitates in the reconstruction and quality analysis of large-scale metabolic networks. Our current network comprises 655 genes (21% of the genome), 540 reactions and 600 metabolites, but is still under construction. When finished, the null space of the network's stoichiometry matrix will be analyzed through Simpheny's built-in linear programming tool, to obtain optimal flux distributions given constraints and objective functions.



INTEGRATION AND VISUALISATION

Within the genomics revolution, new high-throughput methods have been developed that allow the measurement of activities of all the genes and or proteins in the system. Obviously there are many relevant statistical analyses that can be performed on these data sets. However, visualization of the data sets in a biological context is extremely important to help interpreting these data from a biological viewpoint. Once the connections between genes and reactions in a metabolic map have been defined, high-throughput transcriptome or proteome data can be projected on metabolic maps (Fig. 3). This can be done with MetaCyc related software and within Simpheny™. Moreover, these data sets can be projected on circular maps (see Fig. 1) and on linear genome-maps through the Microbial Genome Viewer (Kerkhoven et al. 2004. *Bioinformatics*, in press).



Having a large scale metabolic model allows one to relate the changes in activity of genes to changes in the flux distribution. Understanding the changes on a metabolic level will then help understanding the regulatory mechanisms that made these changes in fluxes possible. The metabolic model can thus form a solid biochemical basis on which to build and interpret other functions of the cell, such as signaling and regulatory networks.

CONCLUSIONS

In the next years, detailed analysis and comparison of the complete genome content (presence/absence of genes for certain metabolic routes, regulatory networks, etc.) of many (LAB) species and strains will provide key insights towards understanding the natural diversity of their capabilities, roles, and interactions. This knowledge will greatly assist efforts to select for specific traits, and to maintain and design stable genomic arrangements in existing strains and in new derivatives. With the

elucidation of entire genomes sequences, the future approach to metabolic analysis will be the reconstruction of metabolic potential using bioinformatics tools and databases, followed by targeted experimental verification and exploration of the metabolic network properties. For this, models and simulations will become essential. We anticipate that in the future, networks for particular applications will be designed in the computer, not unlike what is commonplace for most of today's high-tech products.

REFERENCES

Kleerebezem, M., et al. 2003. "Complete genome sequence of *Lactobacillus plantarum* WCFS1." *Proc Natl Acad Sci USA* 100, 1990-1995.

Covert, M.W.; Schilling, C.H.; Famili, I.; Edwards, J.S.; Goryanin, I.I.; Selkov, E.; and Palsson, B.O. 2001. "Metabolic modeling of microbial strains in silico." *Trends Biochem Sci* 26, 179-186.

Stelling, J.; Klamt, S.; Bettenbrock, K.; Schuster, S.; and Gilles, E.D. 2002. "Metabolic network structure determines key aspects of functionality and regulation." *Nature* 420, 190-193.

Karp, P.D.; Riley, M.; Paley, S.M.; and Pellegrini-Toole, A. 2002. "The MetaCyc Database." *Nucleic Acids Res* 30, 59-61.

AUTHOR BIOGRAPHY

BAS TEUSINK studied Chemistry at the University of Amsterdam, The Netherlands. After graduation in 1993, he started his PhD work in the group of Mathematical Biochemistry at the University of Amsterdam, under supervision of Prof. Hans V Westerhoff. In 1996 he obtained a Marie Curie fellowship from the EU to work for one year in Manchester, UK, in the lab of Prof. Stephen G Oliver, at that time coordinator of the Yeast functional analysis program EUROFAN. Back in Amsterdam he continued his PhD work and in 1999 he defended his thesis entitled: "exposing a complex metabolic system: glycolysis in Saccharomyces cerevisiae". At the end of 1998 he joined the group of Prof. Louis M Havekes at TNO-Prevention and Health, Leiden, The Netherlands, to study the interactions between glucose, fatty acid and lipoprotein metabolism in several transgenic and knockout mouse models. In 2002 he started at NIZO food research, Ede, The Netherlands, where he is involved in Systems Biology of lactic acid bacteria. He combines bioinformatics and modeling to turn the high throughput data of modern genomics into biological knowledge suitable for rational metabolic engineering.

Bas.Teusink@nizo.nl

TOOLS TRAINING AND SIMULATION

THE USE OF OPTIMAL EXPERIMENTAL DESIGN CONCEPTS IN BIOPROCESS MODELLING

Kristel Bernaerts, Ilse Smets, Kristel Gysemans, Astrid Cappuyns and Jan F. Van Impe
BioTeC-Bioprocess Technology and Control
Department of Chemical Engineering
Katholieke Universiteit Leuven
W. de Croylaan 46
B-3001 Leuven, Belgium
E-mail: jan.vanimpe@cit.kuleuven.ac.be

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System identification, bioprocess modeling, optimal experiment design, sensitivity function analysis, parameter estimation

ABSTRACT

Bioprocess modelling presents a challenging subject which however requires a meticulous modelling strategy. During the modelling process, experimental data form a key ingredient during structure characterisation and parameter estimation. Accurate system identification can only be guaranteed if the experimental data contain sufficient information on the process dynamics. In this respect, sufficient effort should be spent on *optimal* experiment design in order to maximise the information that can be extracted from data; especially because experimental data generation for bioprocesses usually presents a time-consuming, labor-intensive and costly job.
This contribution summarises the bioprocess modelling cycle emphasising the need for careful experimental data collection. Concepts of optimal experiment design for parameter estimation are revised using examples on (*i*) microbial growth kinetics as a function of temperature, and (*ii*) microbial growth kinetics as a function of limiting substrate. A two-parameter and multi-parameter estimation problem are tackled by means of either static or dynamic process input design. Complementary, the use of sensitivity function analysis for model reduction is illustrated.

INTRODUCTION

This contribution summarises the bioprocess modelling cycle emphasising the need for careful experimental data collection. Concepts of optimal experiment design for parameter estimation are revised using examples on (*i*) microbial growth kinetics as a function of temperature, and (*ii*) microbial growth kinetics as a function of limiting substrate. A two-parameter and multi-parameter estimation problem are tackled by means of either static or dynamic process input design. Complementary, the use of sensitivity function analysis for model reduction is illustrated.

The paper is organised as follows. First, the bioprocess modelling cylce is outlined. Subsequently, the general concepts of optimal experiment design for parameter estimation are addressed and two examples are given. Finally, the application of model output sensitivity function analysis in the context of structure characterisation is discussed.

THE MODELLING CYCLE

Mathematical modelling basically consists of the following tasks, to be performed within an *iterative cycle*: collection of available mechanistic process knowledge, experimental data collection, data processing, system identification, and model validation (e.g., Ljung, 1999). System identification in itself basically consists of three main phases, for all of which prior (mechanistic) knowledge, experimental data and modelling objectives are to be considered as inputs. *Relationship detection* is the qualitative detection of the causal relationships between state variables. The quantitative description for such relationships is obtained through the *structure characterisation* (SC) and the *parameter estimation* (PE) steps. A mutual feedback between the two latter identification steps is indispensable, as the *theoretical identifiability* of the model parameters (also called *structural identifiability* of the model) is determined by the model structure itself. On the other hand, *practical identifiability* of the model parameters depends on the amount of information that can be extracted out of experimental data.

Within a biological context mathematical modelling and system identification are not straightforward. As an example, unique and accurate estimation of kinetic parameters out of an arbitrary experimental data set is most often not possible (e.g., Holmberg and Ranta 1982, Bernaerts et al. 2000). Frequently occurring reasons for this problem are, e.g., (*i*) a too small amplitude of process output sensitivities with respect to the model parameters, (*ii*) correlation of model parameters, (*iii*) measurements with limited accuracy and/or small measurement frequency, and (*iv*) a lack of measurements for certain (biologically important) state variables (e.g., Ljung 1999, Vanrolleghem and Dochain, 1998).

In order to overcome these problems, experiments for bioprocess identification need to be carefully designed. Experimental data need to be sufficiently *rich* (in other words, they should contain a lot of *information* concerning the process dynamics) in order to enable correct model structure characterisation, and accurate and unique parameter estimation. The information content can be optimised by using concepts and techniques of *optimal experimental design* (*OED*): the (time-varying) process inputs are designed such that the resulting process outputs

have maximum information content, and this within the validity region of the proposed model.

Mathematical models for biological systems are mostly (highly) non-linear models which are empirically derived from experimental data. As there is a lack of generally applicable *structure characterisation* techniques for non-linear systems, the structure characterisation problem is usually formulated as a *model discrimination* problem: the most suitable model has to be selected out of a pre-specified (finite) set of candidate models. The available literature on experimental design for structure discrimination is relatively limited. Often the discrimination criterion is parameter dependent, implying that a parameter estimation step is involved during optimisation of the discrimination criterion.

The methodology for optimal experiment design for parameter estimation, however, is well-established. The overall objective is to define the experimental protocol such that the model parameters can be estimated uniquely and with the greatest statistical confidence, i.e., the estimated values approach the true parameter values with a minimum error. Hereto, a *persistently exciting inputs* yielding data that contain sufficient *information* needs to be found. Optimisation of the experimental protocol includes specifications on initial conditions, experimental inputs (treatment combinations or dynamic inputs), sample spacing, sensor location, and others. In the domain of system and control engineering, the methodology of *optimal experiment design* (based on the so-called Fisher information matrix) was already introduced by Mehra in 1974. However, it is only during the last decade that these concepts for the design of input signals for identification experiments are applied in the domain of bioprocess modeling.

OPTIMAL EXPERIMENTAL DESIGN FOR PARAMETER ESTIMATION

General concepts

Once the model structure has been characterised (i.e., the model structure is taken to be correct), optimal experiment design for parameter estimation (OED/PE) can be addressed to establish accurate and unique parameter estimation. The corner stone of OED/PE is the *Fisher information matrix* (**F**), the application of which is based on the following interesting properties (e.g., Walter and Pronzato, 1997).

- The inverse of the Fisher information matrix corresponds to the Cramér-Rao lower bound on the parameter covariance matrix for any unbiased estimator.
- Based on the linearisation of the model output in the vinicity of true process parameters $\mathbf{p}^*$, it can be demonstrated that the expectation value of the identification cost $E(J(\mathbf{p}))$ for a parameter set $\mathbf{p}$ slightly different from the optimal parameter set $\mathbf{p}^*$ can be approximated by (Munack, 1989):

$$E\left(J(\mathbf{p}+\delta\mathbf{p}^*)\right) \approx \left(\mathbf{p}-\mathbf{p}^*\right)^{\mathrm{T}} \cdot \mathbf{F}(\mathbf{p}) \cdot \left(\mathbf{p}-\mathbf{p}^*\right) \qquad (1)$$

The Fisher information matrix thus quantifies the shape of the cost hypersurface near $\mathbf{p}^*$, and levels of equal identification cost form hyperellipsoids defined by expression (1). In addition, the Fisher information matrix determines the statistical *joint confidence region* of parameter estimates (e.g., Bates and Watts, 1988).

The Fisher information matrix thus provides an approximate quantification of the attainable parameter estimation quality (in the neighbourhood of the true parameter vector $\mathbf{p}^*$) for a particular experiment. Depending on the model system (inputs and outputs), and the experimental conditions to be optimised, the Fisher information matrix is defined as follows.

- For a multiple output system and discrete-time measurements, the Fisher information matrix reads as follows:

$$\mathbf{F}(\mathbf{p}) = \sum_{i=1}^{n_t} \left[\frac{\partial \mathbf{y}}{\partial \mathbf{p}}(t_i,\mathbf{p})\right]_{\mathbf{p}=\mathbf{p}^*} \cdot \mathbf{Q} \cdot \left[\frac{\partial \mathbf{y}}{\partial \mathbf{p}}(t_i,\mathbf{p})\right]_{\mathbf{p}=\mathbf{p}^*}^{\mathrm{T}} \qquad (2)$$

  where $(\partial\mathbf{y}/\partial\mathbf{p})$ represents the *sensitivity matrix* of the model outputs to the parameters, $\mathbf{Q}$ is a weighting matrix usually chosen equal to the inverse of the measurement error covariance matrix, n_t is the number of observations for each model output, $\mathbf{p}^*$ is the vector of true model parameters. The elements of $\mathbf{F}$ thus consist of products of the sensitivities summed over all data points. In case that the measurement errors are mutually independent and Gaussian distributed $N(0,\sigma^2)$, the measurement error covariance matrix is a square matrix with the error variances σ^2 on the main-diagonal and zeros on off-diagonal positions. The measurements errors may, however, depend on the independent variable, e.g., time ($\sigma^2(t_i)$).
- For continuous-time design, the Fisher information matrix for an experiment of duration t_f can be written as follows.

$$\mathbf{F}(\mathbf{p}) = \int_{t_o}^{t_f} \left[\frac{\partial \mathbf{y}}{\partial \mathbf{p}}(t_i,\mathbf{p})\right]_{\mathbf{p}=\mathbf{p}^*} \cdot \mathbf{Q} \cdot \left[\frac{\partial \mathbf{y}}{\partial \mathbf{p}}(t_i,\mathbf{p})\right]_{\mathbf{p}=\mathbf{p}^*}^{\mathrm{T}} dt \qquad (3)$$

  The summation over the data points in (2) is replaced by an integral over the interval $[t_o,t_f]$. Strictly speaking, this transition from a discrete function to a continuous function implies solving a slightly different problem; however, the errors observed as a consequence of such approximation are much smaller than errors, for example, introduced by linearisations in the solution of the experimental design problem (Espie and Machietto, 1989, and cited references).

Optimal design criteria are specific real-valued functionals of $\mathbf{F}$ expressing the *efficacy* of an experiment with respect to the parameter identifiability and the estimation accuracy expected from the collected experimental data. The criterion selection shall depend on the requirements imposed by the application. In general, experiment designs of which the determinant of the corresponding Fisher information matrix is equal to zero, are termed *non-informative* and models parameters are practically not identifiable (Goodwin and Payne, 1977). Equally, a zero eigenvalue of $\mathbf{F}$ indicates that the experimental data cannot yield unique parameter values. Some prevalent design criteria are listed in Table 1. Mehra (1974) and others emphasise that the resulting optimal

design depends on the chosen information criterion, meaning that a careful criterion selection is needed.

Table 1: Examples of optimal design criteria for parameter estimation (see, e.g., Munack 1991, Steinberg and Hunter 1984, Vanrolleghem et al. 1998, Walter and Pronzato, 1997).

Criterion	Interpretation
D-criterion: $\min[\det(\mathbf{F})]$	Minimise geometric mean of parameter estimation error
A-criterion: $\min[\text{trace}(\mathbf{F}^{-1})]$	Minimise arithmetic mean of parameter estimation error
E-criterion: $\max[\lambda_{min}(\mathbf{F})]$	Minimise largest parameter estimation error
Modified E-criterion: $\min[\Lambda(\mathbf{F})]$	Equalise parameter estimation errors

The optimal design problem can be formulated as an optimal control problem which can be solved using techniques described in Barton et al. (1998): (*i*) indirect approaches based on the Pontryagin minimum principle, or (*ii*) direct approaches where the problem is transformed into a non-linear programming problem by control input parameterisation. Application of the Pontryagin minimum principle requires specific mathematical optimisation techniques as well as a large computational capacity as numerical calculations become computationally involved. As a results, this is not always practical for complex nonlinear biological models. On the contrary, the control input may be parameterised yielding a finite dimensional optimisation problem. In many publications, the control input is parameterised by using piecewise constant or piecewise linear functions ([see, e.g., Espie and Macchietto, 1989, Körkel et al., 1999, Sydall et al., 1998, Asprey and Macchietto, 2000). Within the biological context, practical feasibility as well as model validity must be taken into account. The *trade-off* between (*i*) *excitation* of the biological system for a higher information release, and (*ii*) guaranteed balanced growth which is the basic assumption when using unstructured growth models, can be taken into account as an additional cost (penalty function) in the design criterion or by constraining the domain of admissible inputs (see, e.g., Baltes et al., 1994 and Bernaerts et al., 2002, respectively).

As the true parameters $\mathbf{p}^*$ are *a priori* unknown during experiment design, they are replaced by some initial guess, i.e., the so-called *nominal parameters* $\mathbf{p}^o$. These nominal parameter values can be extracted from literature or estimated from preliminary experiments. For linear models, optimal design results will not depend on the parameter values. On the contrary, the optimal experiment designs for non-linear models are function of the selected nominal parameters, which has implications for OED/PE. The optimality of the designed experiment will be determined by the agreement between the nominal and true model parameters. As suggested by, e.g., Steinberg and Hunter, 1984, Walter and Pronzato 1997, a *sequential design strategy*, i.e., the iterative application of the optimal experiment design may be needed to obtain convergence from $\mathbf{p}^o$ to $\mathbf{p}^*$. The parameter estimates are updated after each trial and the next design is performed with the aid of the improved estimates. The design cycle is repeated until the parameter estimates are *sufficiently close* to $\mathbf{p}^*$. Exact values for $\mathbf{p}^*$ cannot be expected due to (*i*) inevitable measurement errors, and (*ii*) model non-linearity. For non-linear models, it is advisable to include all previous experiments into the parameter estimation procedure, hereby minimising the possibility for biased parameter estimation (Walter and Pronzato, 1997).

Example 1: optimal dynamic experimental design

Unique estimation of the parameters of the Monod growth kinetics by means of optimal experiment design for parameter estimation, for example, has been extensively explored (e.g., Ejiofor et al., 1994, Merkel et al., 1996, Munack 1989, 1992, Munack and Posten, 1989). Optimal feed rate profiles (determining the substrate concentration) within a fed-batch process are designed to allow simultaneous parameter estimation from (a single set of) biomass data. The following example considers the growth of and the production of indol-3-acetic acid (IAA) by *Azospirillum brasilense* Sp245 (see, Smets et al., 2004).

Azospirillum brasilense Sp245 produces indol-3-acetic acid (IAA) when grown on malate in the presence of tryptophane. Malate is used as a carbon source and the biomass growth obeys the Monod kinetics. In a first research step, batch experiments were performed during which the biomass growth, the consumption of substrate, the IAA production and the consumption of tryptophane (i.e., an essential precursor) were measured. Based on the available data, two prototype models, either considering IAA production as growth-related or not, have been proposed. Dealing with batch data, accurate estimation of the Monod parameters is impossible as can be easily decuded from a contour plot of the identification functional or the condition number of the Fisher information matrix. Multiple parameter combinations yield the same descriptive quality. To achieve accurate Monod parameter estimation, a *fed-batch feeding strategy* should be applied. *Fed-batch experiment*s inspired by the optimal feeding strategy for product optimisation are here designed according to the OED/PE methodology explained above (see, e.g., Versyck et al., 1999, Van Impe et al., 1995). The optimal feeding profile consists out of a batch phase followed by a (singular) feeding phase which keeps the substrate concentration in the bioreactor at a constant level. The process input is optimised with respect to the modified E-criterion which has the advantage of knowing the optimum solution beforehand. The sole degrees of freedom during optimisation are the initial substrate concentration and the substrate concentration during the singular feeding phase. It can be shown that multiple optimal solutions (i.e., combination of set points yielding a condition number of **F** equal to one) can be found. Further selection of the process input can thus be driven from a practical point of view.

Example 2: optimal static experimental design

When the model is an explicit function of the independent variable, the OED/PE reduces to the optimal selection of (static) treatment combinations of the independent variable. As an example, the estimation of the cardinal values describing microbial growth kinetics as function of temperature is given. Microbial growth within the entire growth temperature domain can be described by the *Cardinal Temperature model with Inflection point* which embeds four model parameters (Rosso, 1995). Finding the optimal *dynamic* temperature input for such four-parameter estimation problem is not straightforward. As a possible onset to solving this complex parameter estimation problem *static* optimal experimental designs are computed (see, Bernaerts et al., 2003, Gysemans et al., 2004, Bernaerts et al., 2004).

During static optimal experimental design, the maximum specific growth rate is considered as the *measured* model output and an optimal combination of temperature treatment levels needs to be found. Besides common design criteria focusing on parameter estimation accuracy, G-optimal design maximising the model prediction accuracy is considered (Steinberg and Hunter, 1984). For nonlinear models as often in the field of bioprocess modelling or in this case predictive microbiology, this prediction accuracy is not always linearly related with the parameter estimation accuracy.

A model output sensitivities analysis yields a first indication of relevant temperature inputs. Although lowering the information content of a set of experiments, boundary values on the design region need to be imposed during optimisation to exclude unworkable experiments and partly account for the *uncertainty* on the nominal parameter values. Optimal design results show that, opposed to the frequently applied equidistant or arbitrary treatment placement within a factorial design, typically four informative temperature are selected and replicate experiments are to be performed at certain levels. Informative experiments are typically placed at points with an extreme model output sensitivity. Constrained G-optimal designs show great similarity with D-optimal designs (minimising the overall parameter estimation error variance).

SENSITIVITY FUNCTION ANALYSIS AS A TOOL FOR MODEL REDUCTION

Knowledge-based modelling of advanced biological processes typically escalates into mathematical relations including (*i*) a large number of state variables, and (*ii*) complicated kinetic expressions containing a large number of model parameters. Inspired by the use of sensitivity functions in the context of optimal experiment design for parameter estimation, a sensitivity function analysis is introduced as a powerful tool to reduce the complexity of knowledge-based models (thus contributing to structure charactisation) (Smets et al., 2002).

Example

Mathematical models for microbial conversion processes essentially describe two kinds of phenomena (Bastin and Dochain, 1990). Firstly, the (micro-)biological, chemical, and biochemical reactions that transform some reactants into some products. Secondly, mass transfer processes due to exchanges with the environment. The number of reactions and species that are involved in the process may be very large. An accurate description of such complex systems can therefore result in very involved models containing a large number of state variables and model parameters. These should all be calibrated against experimental data. Since full state measurements are in general not available and parameters of complex model structures are hardly identifiable a *trade-off* should be searched for between model parsimony and goodness-of-fit and this with respect to the goal of the model.

In this context, a generic methodology based on sensitivity function analysis is developed to reduce the model complexity at the level of the kinetics, while maintaining the high predictive power. As a case study, the influence of the dissolved oxygen concentration on the *cytN* gene expression in *Azospirillum brasilense* Sp7 is modelled. As a first modelling approach, available mechanistic knowledge has been incorporated into a mass balance equation model with 3 states and 14 parameters which has been identified on batch experiments with a time-varying dissolved oxygen concentration. The large differences in order of magnitude of the model parameters identified on the available experimental data indicated (*i*) possible structural problems in the kinetic model, and, associated with this, (*ii*) a possibly too high number of model parameters.

For the given experimental inputs, the 3x14 sensitivity functions of the model outputs with respect to the model parameters are computed. To allow proper comparison between all sensitivities, each sensitivity is rescaled by multiplying with the corresponding parameter value (yielding so-called semirelative sensitivity functions). *Essential* model parameters are selected based on the comparison of the order of magnitude of its sensivity function to the average order of magnitude of the sensitivity function related with considered state variable. Only model parameter with substantially larger sensitivities ought to be retained. Doing so, the original model could be reduced to a mass balance equation model containing 7 model parameters.

REFERENCES

Asprey, S.P., and S. Macchietto. 2000. “Statistical tools for optimal dynamic model building”. *Computers and Chemical Engineering,* 24, 1261-1267.

Baltes, M., R. Schneider, C. Sturm and M. Reuss. 1994. “Optimal experimental design for parameter estimation in unstructured growth models”. *Biotechnological Progess*, 10, 480-488.

Baranyi, J. and T.A. Roberts. 1994. “A dynamic approach to predicting bacterial growth in food”. *International Journal of Food Microbiology,* 23, 277-294.

Barton, P.I., R.J. Allgor, W.F. Feehery and S. Galán. 1998. “Dynamic optimisation of a discontinuous world”. *Industrial and Engineering Chemistry Research*, 37(3), 966-981.

Bastin, G. and D. Dochain. 1990. “On-line estimation and adaptive control of bioreactors”. Elsevier, Amsterdam.

Bates, D.M. and D.G. Watts. 1988. “Nonlinear regression analysis and its applications”. John Wiley and Sons, Inc. New York.

Bernaerts, K., R.D. Servaes, S. Kooyman, K.J. Versyck and J.F. Van Impe. 2002. Optimal temperature input design for estimation of the Square Root model parameters: parameter accuracy and model validity restrictions. *International Journal of Food Microbiology, Special issue*, 73(2-3), 147-159.

Bernaerts, K., T. Nhan Minh and J.F. Van Impe. 2003. “Critical evaluation of a nonlinear model from predictive microbiology using sensitivity analysis and optimal experimental design”. In: *Proceedings 4th MathMod Vienna,* I. Troch and F. Breitenecker (Eds.), ARGESIM Report No. 24, Volume 2, Technical University Vienna, 1274-1280.

Bernaerts, K., K. Gysemans, T. Nhan Minh and J.F. Van Impe. 2003. “Optimal experiment design for cardinal values estimation: instructions for data collection”. In: *Predictive Modelling in Foods - Conference Proceedings*, J.F.M. Van Impe, A.H. Geeraerd, I. Leguérinel and P. Mafart (Eds.), KULeuven/BioTeC, Belgium, 111-113.

Bernaerts, K., K.P.M. Gysemans, T. Nhan Minh, J.F. Van Impe. 2004. “Optimal experiment design for cardinal values estimation: instructions for data collection”. *International Journal of Food Microbiology, Special issue (accepted with minor revision).*

Ejiofor, A.O., C.H. Posten, B.O. Solomon and W.-D. Deckwer, 1994. A robust fed-batch feeding strategy for optimal parameter estimation for baker's yeast production. *Bioprocess Engineering,* 11, 135-144.

Espie, D. and S. Machietto. 1989. “The optimal design of dynamic experiments”. *AIChE Journal,* 35(2), 223-229.

Goodwin, G.C. and R.L. Payne. 1977. “Dynamic system identification: experiment design and data analysis”. Academic Press. New York.

Gysemans, K.P.M, K. Bernaerts and J.F. Van Impe. 2004. Constrained input optimization for optimal parameter estimation of a predictive biokinetic model. In: *Proceedings of the 9th International Symposium on Computer Applications in Biotechnology (in press)*.

Holmberg, A. and J. Ranta. 1982. Procedures for parameter and state estimation of microbial growth process models. *Automatica* 18(2), 181-193.

Körkel, S., I. Bauer, H.G. Bock and Schölder J.P., 1999. “A sequential approach for nonlinear optimum experimental design in DAE systems. In: *Proceedings of the international workshop on scientific computing in chemical engineering* (F. Keil, Ed.). Vol. 2. Springer. Berlin. pp. 338-345.

Ljung, L. 1999. *System identification: theory for the user* (*Second edition*). Prentice Hall, Inc.. Upper Saddle River, NJ.

Mehra, R.K. 1974. Optimal input signals for parameter estimation in dynamic systems - Survey and new results. *IEEE Transactions on Automatic Control* 19(6), 753-768.

Merkel, W., A. Schwarz, S. Fritz, M. Reuss and K. Krauth. 1996. “New strategies for estimating kinetic parameters in anaerobic wastewater treatment plants”. *Water Science and Technology* 34(5-6), 393-401.

Munack, A. 1989. “Optimal feeding strategy for identification of Monod-type models by fed-batch experiments”. In: *Computer applications in fermentation technology, modelling and control of biotechnological processes* (N.M. Fish, R.I. Fox and N.F. Thornhill, Eds.). pp. 195-204. Elsevier. Amsterdam.

Munack, A. and C. Posten. 1989. “Design of optimal dynamical experiments for parameter estimation”. In: *Proceedings of the American Control Conference*. Pittsburgh. pp.2010-2016.

Munack, A. 1991. Optimization of sampling. In: *Biotechnology, a multi-volume comprehensive treatise*, Vol. 4 Measuring, modelling and control} (H.-J. Rehm and G. Reed, Eds.). Vol.4. pp.252-264. VCH. Weinheim.

Munack, A. 1992. Some improvements in the identification of bioprocesses. In: *Modeling and Control of Biotechnical Processes*. Pergamon Press, Oxford. pp. 89-94.

Rosso, L. 1995. Modèlisation et microbiologie prévisionnelle: Elaboration d'un nouvel outil pour l'agro-alimentaire. PhD thesis, Université Claude Bernard-Lyon I. Villeurbannne Cedex (France).

Steinberg, D.M. and W.G. Hunter. 1984. “Experimental design: review and comment”. *Technometrics* 26(2), 71-97.

Smets, I., K. Bernaerts, J. Sun, K. Marchal, J. Vanderleyden and J. Van Impe. 2002. “Sensitivity function based model reduction: a bacterial gene expression case study”. *Biotechnology and Bioengineering*, 80(2), 195-200

Smets, I., K. Bernaerts, A. Cappuyns, O. Ona, J. Vanderleyden, E. Prinsen and J.F. Van Impe 2004. A prototype model for indole-3-acetic acid (IAA) production by *Azospirillum brasilense* Sp 245. In: *Proceeding of the 7th International Symposium on DYnamics and COntrol of Process Systems*, Cambridge (Massachusetts, USA), July 5-7, 2004 *(accepted)*

Sydall, M.T., G.C. Paul and C.A. Kent. 1998. “Improving the estimation of parameters of penicillin fermentation models”. In: *Preprints of the 7th International Conference on Computer Applications in Biotechnology* (T.~Yoshida and S.~Shioya, Eds.). Osaka (Japan). pp.23-28.

Van Impe, J.F. and G. Bastin. 1995. “Optimal adaptive control of fed-batch fermentation processes”. *Control Engineering Practice*, 3 (7), 939-954

Vanrolleghem, P.A. and D. Dochain 1998. “Bioprocess model identification”. In: *Advanced instrumentation, data interpretation and control of biotechnological processes* (J.F. Van Impe, P.A. Vanrolleghem and D.M. Iserentant, Eds.). pp. 250-318. Kluwer Academic Publishers. Dordrecht.

Vanrolleghem, P.A., M. Van Daele and D. Dochain 1995. Practical identifiability of a biokinetic model of activated sludge respiration. *Water Research* 29(11), 2561-2570.

Versyck, K.J., and J.F. Van Impe. 1999. “Feed rate optimization for fed-batch bioreactors: from optimal process performance to optimal parameter estimation”. *Chemical Engineering Communications*, 172, 107-124

Walter, E. and L. Pronzato. 1997. *Identification of parametric models from experimental data*. Springer. Masson.

BIOGRAPHY

KRISTEL BERNAERTS was born in Bonheiden, Belgium, in 1974. She received her Master's degree Engineering in Chemistry and Biochemistry with specialisation in industrial microbiology from the Katholieke Universiteit Leuven in 1997. From 1997 till 2002, she was employed as a research assistant at BioTeC - Bioprocess Technology and Control, K.U.Leuven. She received a doctorate in applied biological sciences from the Katholieke Universiteit Leuven in 2002. Currently, she is a Postdoctoral Fellow of the Fund for Scientific Research Flanders (Belgium), at the same research group. Her main research interests are in the field of bioprocess modelling and predictive microbiology and, more particularly, the design of optimal experiments aiming at accurate model structure characterisation and model parameter estimation.
E-mail: Kristel.Bernaerts@cit.kuleuven.ac.be

JAN F. VAN IMPE was born in Varsenare, Belgium, in 1965. He received his master's degree in electrical and mechanical engineering from the University of Ghent in 1988, and a doctorate in applied sciences from the Katholieke Universiteit Leuven in 1993 (ESAT Laboratory).

Thereafter, he joined the Department of Food and Microbial Technology of the same university (since 1995 as an associate professor). Since 2001, he is professor at the Department of Chemical Engineering. During that period he started the BioTeC research group, which has nowadays about 20 members. He teaches courses in system analysis, bioreactor engineering, and process engineering and control. His research interests are mainly in nonlinear systems and identification, nonlinear and adaptive control, and its applications in model-based optimization and control of (bio-)chemical conversion processes. He has (co-)authored about 300 papers in these areas. In 1995 he received the Barco Award of the Fund for Scientific Research, in 1996 he was laureate of the Belgian Royal Academy, and in 1997 he was awarded the KIWANIS prize of the KULeuven Research Council. In 2002 he became a member of the board of the EUCA, the European Union Control Association. Furthermore, he is member of the Editorial Board of International Journal of Food Microbiology.

CONSTRAINED OPTIMIZATION OF PIECEWISE-CONSTANT INPUTS FOR OPTIMAL SQUARE ROOT MODEL PARAMETERS ESTIMATION

Kristel Gysemans

Kristel Bernaerts

Ifor Verhoeven

Jan F. Van Impe
BioTeC-Bioprocess Technology and Control
Katholieke Universiteit Leuven
W. de Croylaan 46, B-3001 Leuven, Belgium
E-mail corresponding author: jan.vanimpe@cit.kuleuven.ac.be
Tel. +32-16-32.14.66 Fax. +32-16-32.29.91

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Optimal experiment design, parameter estimation, control input parameterization, piecewise-constant input, Square Root model, predictive microbiology.

ABSTRACT

In this paper, the methodology of optimal experiment design is applied to design an optimal temperature profile for the estimation of the two kinetic model parameters from the Square Root model. This model describes the temperature dependence of the specific growth rate of microorganisms within the suboptimal growth temperature range. The optimization problem is solved by parameterization of the control input, i.e., the temperature, and by subsequent optimal selection of the degrees of freedom of the parameterized input with a Sequential Quadratic Programming method. In previous work, first and second order polynomials were optimized. Imposing necessary constraints, the latter resulted in an optimal step temperature profile. In this study, piecewise-constant functions allowing for multiple step temperature changes are optimized. Results show that the dynamic optimization problem becomes difficult to solve as the problem becomes ill-conditioned when the number of degrees of freedom increases; but, overall, a single step spanning the full range of valid temperatures seems to be the most optimal.

INTRODUCTION

To render reliable predictions, models must be accurate. This means that models must contain, besides a proper model structure, parameter estimates of high quality. Given an accurate model structure, high quality parameter estimates can only be obtained on the basis of informative data. The information content of data can be maximized by optimal selection of the experimental input. This can be achieved by the technique of optimal experiment design for parameter estimation.

Optimal experiment design for parameter estimation has already been applied in several research domains, including the domain of predictive microbiology. The models developed in this domain predict the evolution of microorganisms in foods. In (Bernaerts et al. 2000; Bernaerts et al. 2002) the technique was applied in order to acquire unique estimates for the two parameters of the predictive Square Root model (Ratkowsky et al. 1982). This model describes the maximum microbial growth rate (μ_{max}) as function of temperature (T) in the suboptimal growth range. Dynamic optimal experiment design consists, in this case, of the selection of the most optimal temperature profile. To solve this optimization problem, the authors firstly parameterized the temperature input as a first and second order polynomial and optimized the degrees of freedom of these polynomials within the region of model validity. A quadratic temperature profile equivalent to a step spanning the suboptimal growth temperature domain was proven to yield the most unique parameter estimates. On the basis of this result, a step profile was also considered for optimal estimation of the Square Root parameters. Optimization of its degrees of freedom resulted in a profile equal to the optimal quadratic profile.

Since the number of input parameterizations considered in (Bernaerts et al. 2000; Bernaerts et al. 2002) is limited, it is possible that even more optimal profiles exist. In this paper it will be examined if this is the case. Hereto, piecewise-constant temperature profiles are optimized. The interval lengths of these profiles are gradually shortened. The shorter the length of the intervals is, the more the temperature profiles approximate continuous functions. Unfortunately, this also often makes the optimization problems more difficult to solve since the number of intervals to be optimized usually increases. In this paper, it is investigated to which extent convergence to global solutions is hampered when more intervals are involved.

In summary, the goals of this study are: (*i*) optimize piecewise-constant temperature profiles in order to examine

if the optimized step profile presented in (Bernaerts et al. 2000; Bernaerts et al. 2002) is the most optimal profile for estimation of the Square Root parameters, (*ii*) study to which extent convergence to global solutions is hampered when the number of degrees of freedom of the piecewise-constant functions is increased.

The paper is organized as follows. In Section 2, the model under study is presented. In the third section, the technique of optimal experiment design for parameter estimation is briefly explained, followed by a description of how this method is applied on the case study. In Section 3, the optimal step profile discussed in (Bernaerts 2002), is displayed. In Section 4, the optimized piecewise-constant temperature profiles are depicted. These profiles are discussed in Section 5. In the sixth section, the optimized piecewise-constant profiles are compared with the optimal step profile. Some general conclusions are finally formulated in Section 7.

CASE STUDY: THE SQUARE ROOT MODEL PARAMETERS

The Square Root model (Ratkowsky et al. 1982) describes the temperature (T) [°C] dependence of the microbial maximum specific growth rate (μ_{max}) [h^{-1}] in the suboptimal temperature range:

$$\sqrt{\mu_{\max}(T(t))} = b \cdot (T - T_{\min}) \qquad (1)$$

The model parameters are b, a regression coefficient [$°C^{-1}.h^{-1/2}$], and T_{min} the (theoretical) minimum temperature for growth [°C].

The combination of the growth model of Baranyi and Roberts (1994) (Eq. 2) with the Square Root model of Ratkowsky et al. (1982) (Eq. 1) describes the microbial growth under suboptimal time-varying temperature conditions $T(t)$, under the assumption that microorganisms adapt immediately to changing temperatures.

$$\frac{dn}{dt} = \frac{Q(t)}{1+Q(t)} \cdot \mu_{\max}(T(t)) \cdot [1 - \exp(n(t) - n_{\max})] \qquad (2)$$

$$\frac{dQ}{dt} = \mu_{\max}(T(t)) \cdot Q(t)$$

$n(t)$ denotes the natural logarithm of the cell density [ln(CFU.ml^{-1})], n_{max} is the natural logarithm of the maximum cell density [ln(CFU.ml^{-1})], $Q(t)$ is a measure for the physiological state of the cells.

DESIGN OF OPTIMAL EXPERIMENTS FOR UNIQUE ESTIMATION OF THE SQUARE ROOT MODEL PARAMETERS

For a proper comparison between the information content of the step profile discovered by (Bernaerts 2002) and the information content of the piecewise-constant temperature profiles optimized in this study, the approach followed to design the optimal experiments should be identical for the vast majority. The two approaches may only differ in the parameterizations used to solve the optimization problem.

This section comprises of three subsections. In the first subsection, the common part of the two approaches is discussed. Afterwards, the difference between the two is highlighted. Finally, it is explained which computer language is chosen and which routines are used to perform the optimizations.

Common part

Optimal experiments are designed on the basis of the Fisher information matrix **F** (Walter and Pronzato 1997). Selection of the most informative experiments is namely achieved by minimization/maximization of a scalar function of **F**. The type of scalar function determines which aspect of the parameter estimation quality is optimized. Different design criteria are described in literature (e.g., Steinberg and Hunter 1984). In this case, the Modified Ecriterion is applied. This criterion minimizes the condition number of the Fisher information matrix, i.e., (Λ(**F**)), hereby guaranteeing unique parameter estimation.

Optimal *dynamic* experiments are designed instead of optimal *static* experiments. The latter approach consists of the selection of the optimal *set* of temperatures at which *static* experiments should be performed. In the former approach, however, the *evolution* of the temperature during *one* experiment is optimized. Performance of this dynamic experiment will yield cell density data $n(t)$ from which the Square Root model parameters, i.e., $\mathbf{p} = [b\ T_{min}]^T$, can be estimated. The former approach is more advantageous since it provides information on the microbial dynamics under dynamic conditions and since it can be time-saving.

Mathematically, the above discussed optimal design problem can be formulated as follows:

$$\min_{admissible\ T(t)} \left[\Lambda(\mathbf{F}(\mathbf{p}^*))\right] \qquad (3)$$

with

$$\mathbf{F}(\mathbf{p}^*) = \int_0^{t_f} \left[\left(\frac{\mathbf{p}}{n(t,\mathbf{p})}\right)\left(\frac{\partial n}{\partial \mathbf{p}}(t,\mathbf{p})\right)\right]_{\mathbf{p}=\mathbf{p}^*} \cdot \left[\left(\frac{\mathbf{p}}{n(t,\mathbf{p})}\right)\left(\frac{\partial n}{\partial \mathbf{p}}(t,\mathbf{p})\right)\right]_{\mathbf{p}=\mathbf{p}^*}^T dt$$

$(\partial n/\partial \mathbf{p})$ represents the sensitivity function vector. For reasons explained elsewhere (Bernaerts et al. 2000; Bernaerts et al. 2002), fully relative sensitivity functions $[(p_i/n)(\partial n/\partial p_i)]_{|\mathbf{p}=\mathbf{p}^*}$ are used. The vector $\mathbf{p}^* = [b^*\ T_{min}^*]^T$ contains the true parameters. Since these true values are *a priori* unknown, they have to be replaced by nominal model parameters $\mathbf{p}^0$. Specific values applied in this work are: $\mathbf{p}^0$ = [5.261 x 10^{-2} $°C^{-1} \cdot h^{-1/2}$ 8.689°C] (*Escherichia coli* K12 MG1655), $n(0)$=ln(10^3), $Q(0)$=200 (representing absence of initial lag) and n_{max} = ln(3 x 10^9) (Bernaerts et al. 2000; Bernaerts et al. 2002; Bernaerts 2002).

During optimization, constraints have to be imposed on the temperature ($T \in [T_{low}\ T_{high}]$=[10°C 37°C]) to ensure that the temperature profiles are restricted to the suboptimal temperature range, i.e., the validity region of the Square Root model.

In general, dynamic optimization problems can be solved using (*i*) direct approaches where the problem is transformed into a nonlinear programming problem by control input parameterization, or (*ii*) indirect approaches based on the Pontryagin minimum principle (see, e.g., Banga et al. 2002). In this case, the direct approach is selected. Application of the Pontryagin minimum principle requires specific mathematical optimization techniques as well as a large computational capacity as numerical calculations become computationally involved, and is thus not used in this case.

The direct approach consists of the parameterization of the control input and the subsequent optimal selection of the degrees of freedom of the parameterized input with respect to the cost criterion. In this case, the control input is the temperature *T(t)* and the cost criterion is Eq. (3).

Parameterization approaches

Approach followed in (Bernaerts et al. 2000; Bernaerts et al. 2002).

In previous research, linear, quadratic and step profiles were considered as parameterizations of the control input, *T(t)*. The duration of these experiments was determined by the following stop criteria: the experiment was stopped when (*a*) the maximum cell density was approximately attained, or when (*b*) $\Lambda(\mathbf{F})$ reached a minimum. A survey of this research is given in the next section.

Approach followed in this paper.

In this study, the control input is parameterized as piecewise-constant profiles. This approach is illustrated in Figure 1. Intervals of equal length Δt are considered. In each interval, a constant temperature is assumed, i.e., T_1, T_2, ... These temperatures are the degrees of freedom that have to be optimized according to criterion (3). The number of degrees of freedom is determined by the length of the intervals and by the final time t_f. In this paper, different interval lengths are considered, namely, Δt = 25h, 20h, 10h and 5h. The experiment duration, t_f, is determined by the two stop criteria described above. In this approach, however, it is also useful to *a priori* fix the experiment duration (the reason for this is given below). The results obtained with this approach are presented in Section 5.

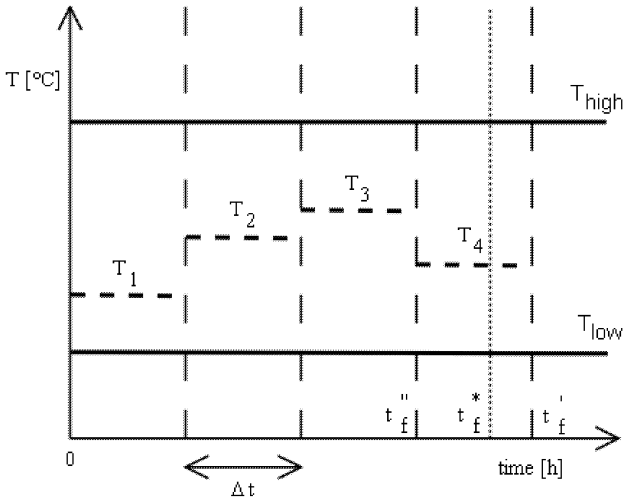

Figure 1: Illustration of Control Input Parameterizations Based on Piecewise-constant Functions.

Programming language

Programs are written in Fortran using NAG-routines (Numerical Algorithms Group) for (*i*) optimization (E04UCF) and (*ii*) random initialization (G05FAF). E04UCF uses a Sequential Quadratic Programming (SQP) method. Pseudo-random initializations of all degrees of freedom are generated by the G05FAF routine. For all optimization problems, 200 initializations are initially applied. If this number does not suffice to find a global minimum, 2000 pseudo-random initializations are generated. When even more initializations are required, 4000 pseudo-random numbers are used. Furthermore, in that case, an additional NAG-routine is used, namely the G05CBF-routine. This routine is used to set different internal seeds used by the pseudo-random number generator mechanism (G05FAF). In this way, different sequences of pseudo-random numbers can be generated which is not possible when solely the G05FAF-routine is employed.

REFERENCE OPTIMAL TEMPERATURE INPUT

As discussed above, linear, quadratic and step profiles were considered in (Bernaerts et al. 2000; Bernaerts et al. 2002). Firstly, linear and quadratic profiles were optimized. This resulted in a quadratic temperature profile equivalent to a step spanning the admissible temperature range. Based on this result, the optimization of a step profile was considered. The degrees of freedom of a step profile are: the initial temperature T_1 [°C], the time of the temperature shift t_{shift} [h], and the final temperature T_2 [°C]. Optimization yielded a step profile equivalent to the optimal quadratic temperature profile (see Table 1 and Figure 2). The optimal experiment duration of this profile is equal to 73.203h because the maximum cell density is approximately attained at that time (see Figure 3). At that time, the condition number of the Fisher information matrix, i.e., $\Lambda(\mathbf{F})$, is also almost minimal. A prolongation of the experiment would only lead to an insignificant decrease of the condition number.

Table 1: Optimized Step Temperature Profile

T_1 [°C]	T_2 [°C]	t_{shift} [h]	t_f^* [h]	$\Lambda(\mathbf{F})$
10.00	37.00	61.429	73.203	4.094

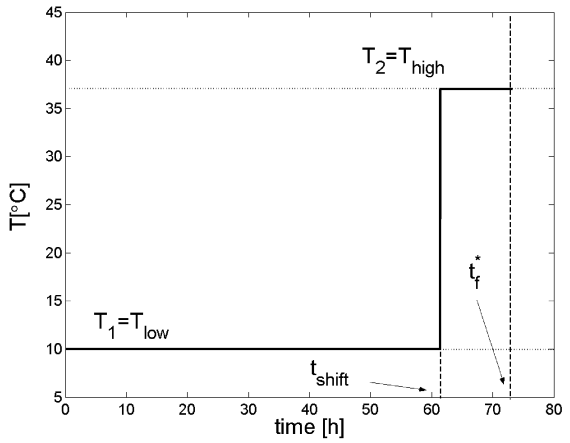

Figure 2: Optimized Step Temperature Profile.

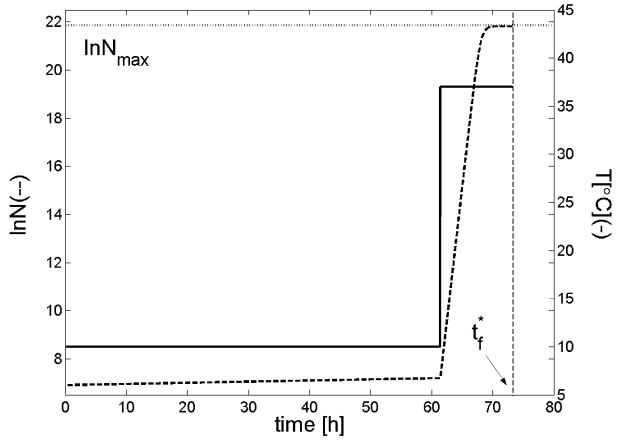

Figure 3: Optimized Step Temperature Profile with Accompanying Growth Curve.

RESULTS FOR THE PIECEWISE-CONSTANT TEMPERATURE PROFILES

In this section, the optimized piecewise-constant temperature functions of which the duration is determined by the above-specified stop criteria are shown, followed by the experiments with fixed duration.

Experiment duration determined by stop criteria

In Table 2, the results obtained for the interval lengths 25h, 20h, 10h and 5h are summarized.

Fixed experiment duration

The experiment duration is first fixed at $t_f^* = 73.203$h, i.e., the experiment duration of the optimized step temperature profile. Secondly, the influence of an elongation or shortening of the experiment duration is investigated. Hereto, the experiment duration is varied between (*i*) $t_f = t_f'$, i.e., end of interval after t_f^* and (*ii*) $t_f = t_f''$, i.e., end of interval before t_f^* (see Figure 1).

Experiment duration fixed at $t_f = t_f^* = 73.203h$.
In Figure 4, the optimization results obtained with interval lengths of (*i*) 25h, (*ii*) 20h, (*iii*) 10h and (*iv*) 5h are depicted.

Influence of an elongation or shortening of the experiment duration.
For Δt = 20h the optimization results for experiment durations varied between $t_f = t_f'$ and $t_f = t_f''$ are summarized in Table 3.

Table 2: Most Optimal Temperature Profiles Resulting from 200 Initializations (*), 2000 Initializations (°) or 4000 Initializations ($^+$). t_{shift} Indicates the Time at which the Largest Temperature Shift Occurs.

Δt [h]	Degrees of freedom T_i [°C] (with i = 1,...,10)	t_f [h]	t_{shift} [h]	$\Lambda(\mathbf{F})$
*25	10.00-10.00-37.00	54.719	50h	4.237292
*20	10.00-10.00-10.00-37.00	71.786	60h	4.101809
°10	10.00-10.00-10.00-10.00-10.00-10.00-37.00-36.47	71.855	60h	4.101809
°10	10.00-10.00-10.00-10.00-10.00-10.00-37.00-21.93	78.161	60h	4.101809
$^+$5	10.00-10.00-10.00-10.00-10.00-10.00-10.00-10.00-10.00-37.00	49.033	45h	4.304204

Table 3: Most Optimal Temperature Profiles Resulting from 200 Initializations. t_{shift} Indicates the Time at which the Largest Temperature Shift Occurs.

t_f [h]	Degrees of freedom T_i [°C] (with i = 1,…,4)	t_{shift} [h]	$\Lambda(\mathbf{F})$
60.000	10.86-10.02-37.00	40h	5.391754
61.000	10.86-10.02-37.00-26.5	40h	5.391754
62.000	10.86-10.00-37.00-17.10	40h	5.391754
63.000	10.86-10.00-37.00-19.70	40h	5.391754
64.000	10.00-10.00-10.00-37.00	60h	5.309592
65.000	10.00-10.00-10.00-37.00	60h	4.393988
66.000	10.00-10.00-10.00-37.00	60h	4.134375
67.000	10.00-10.00-10.00-37.00	60h	4.103630
68.000	10.00-10.00-10.00-37.00	60h	4.101870
69.000	10.00-10.00-10.00-37.00	60h	4.101810
70.000	10.00-10.00-10.00-37.00	60h	4.101809
73.203	10.00-10.00-10.00-37.00	60h	4.101809
80.000*	10.00-10.00-10.00-37.00	60h	4.101809

* The same results are obtained with t_f = 71.000h, 72.000h, 74.000h, 75.000h, 76.000h, 77.000h, 78.000h and 79.000h.

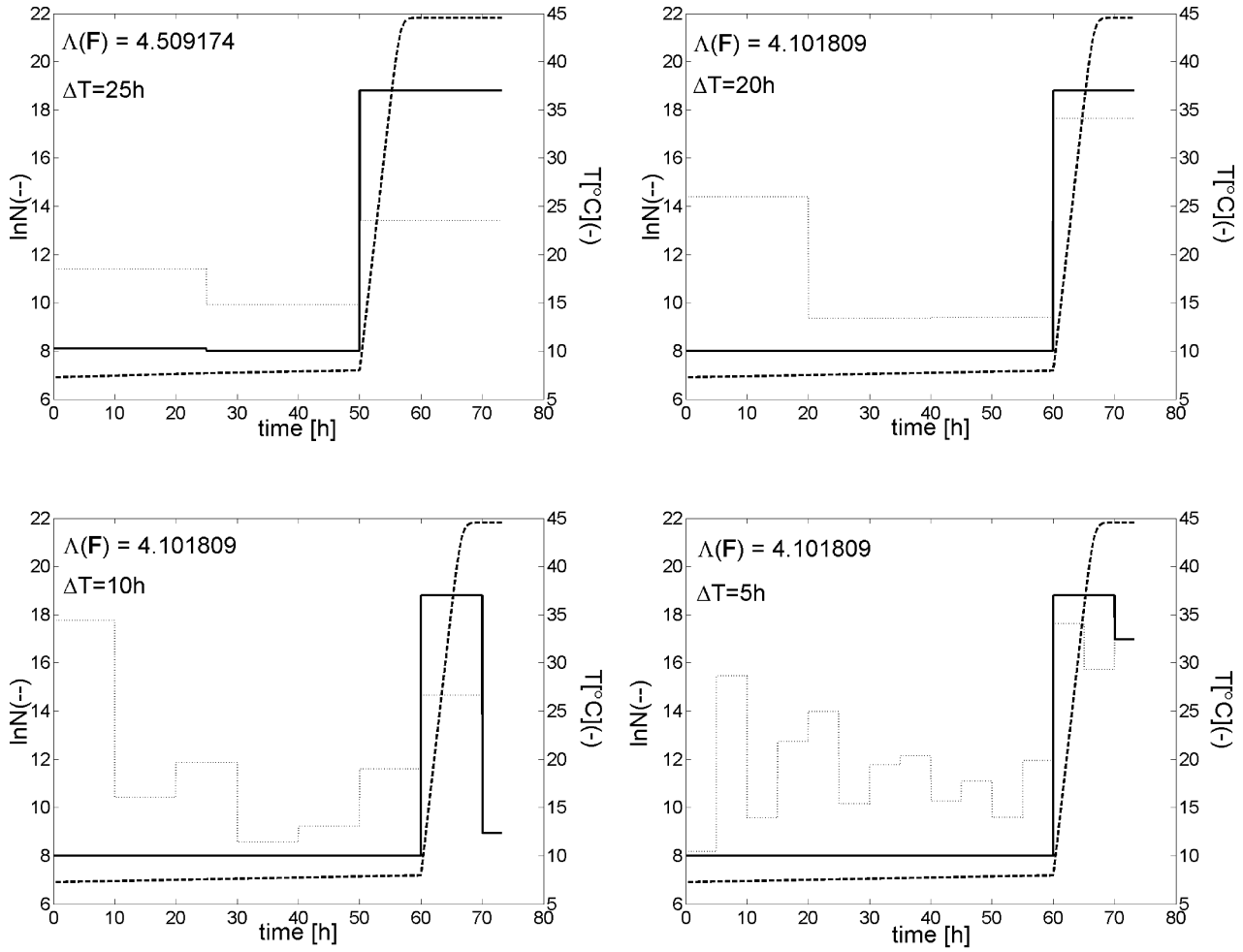

Figure 4: Most Optimal Temperature Profiles (-) with Accompanying Growth Curves (--) Resulting from 200 Initializations (The Upper Plots and the Left Lower Plot) or 4000 Initializations (Right Lower Plot). The Initializations of the Degrees of Freedom are Depicted by the Dotted Grey Lines.

DISCUSSION OF RESULTS

The following general conclusions can be made with respect to conditioning of the optimization problem, number of intervals, type of temperature input, interval length and experiment duration.

- *Conditioning of the optimization problem.* As can be seen in the tables and figures displayed above, several temperature profiles have a similar information content, i.e., their corresponding condition numbers only differ slightly. This points out that the optimization problem is rather ill-conditioned (i.e., there is only a weak minimum), which makes it difficult to obtain global solutions.

- *Number of intervals*. The higher the number of intervals is, the more difficult it becomes to find a global solution. The number of initializations necessary to find the global minimum namely increases with increasing number of intervals. This can be due to the fact that the problem becomes more ill-conditioned. The influence of the number of intervals on the solvability of the optimization problem can be illustrated by Figure 4. This figure shows that, when t_f is fixed to t_f^*, a limited number of initializations, namely 200, is sufficient to find the presumable global minimum when only three, four or eight intervals need to be optimized (Δt = 25h, Δt = 20h and Δt = 10h). Increasing the number of intervals to 15, leads to an increment in the number of initializations necessary (i.e., 4000).

- *Type of temperature input.* Table 2 and Figure 4 show that optimization of piecewise-constant profiles either yields step profiles from T_{low} to T_{high} (Δt = 25h and Δt = 20h) or two-step profiles consisting of the same positive step across the whole suboptimal temperature range followed by a negative step (Δt = 10h and Δt = 5h). For the latter case, it must however be remarked that the temperature after the second temperature jump is systematically equal to the initialization value. Moreover it can be replaced by any other temperature value without changing the condition number of the Fisher information matrix accompanying this profile (simulation results not shown). This indicates that the

temperature in the last interval is not important; it has no influence on the information content of the profile. Consequently, this temperature can be replaced by T_{high}, which converts the two-step profile into a simple single-step profile.

In summary, this study points out that the most optimal temperature structure for estimation of the Square Root parameters is a step profile spanning the admissible temperature range. This can however not be known with certainty, since the number of intervals tested in this study is limited.

- *Interval length*. The points in time where temperature shifts can take place are, in this case, discrete and not continuous as is the case for, e.g., the optimization of a step profile. With other words, t_{shift} cannot adopt each value. The interval length determines which values it can take. For example when Δt = 20h, t_{shift} can take the following values: 20h, 40h, 60h,...

  The value of t_{shift}, has an important influence on the information content of the temperature input. Table 2 exemplifies this. From this table it can be derived that the optimization of a piecewise-constant temperature profile with intervals of 25 hours leads to a less informative profile ($\Lambda(\mathbf{F})$ = 4.237) than is obtained with a profile with interval lengths of 20 hours ($\Lambda(\mathbf{F})$ = 4.102). The difference in information between the two profiles is due to the difference in t_{shift} (t_{shift} = 50h versus t_{shift} = 60h).

- *Experiment duration*.

  - *t_f determined by stop criteria versus t_f a priori fixed.* In this study two different approaches are applied to determine the optimal experiment duration. The first approach makes use of stop criteria. With these stop criteria a precise value for the optimal t_f can be obtained. From Table 2, it can however be derived that with this approach a large number of initializations is necessary to find the most optimal profiles. For the case Δt = 5h, the global minimum is even not found with 4000 initializations. Therefore, a second approach is also considered. In this approach, the optimal experiment duration is determined by testing several *a priori* fixed experiment durations. As a starting point, t_f is firstly fixed at t_f^* = *73.203h*, i.e., the experiment duration of the optimized step profile (see Table 1). Afterwards, it is tested if longer or shorter experiments give better results. A comparison of Figure 4 with Table 2, shows that with the latter approach less initializations are necessary to find the solution compared to the first approach. For example, when Δt = 10h, 200 initializations are necessary when the experiment duration is fixed versus 2000 initializations when the duration is determined by stop criteria. The disadvantage of this method is, however, that numerous *a priori* fixed experiment durations should be tested in order to find the precise value of the optimal t_f.

  - *Optimal experiment duration when t_f is determined by stop criteria.* Experiments are stopped when the maximum cell density is approximately attained (data not shown). The experiments can already be terminated earlier since the change of $\Lambda(\mathbf{F})$ is limited after the stationary phase is reached.

  - *Optimal experiment duration when t_f is fixed.* The experiments optimized in this paper should be ended after the microorganisms have entered the stationary phase and t_f should not interfere with the most optimal value that t_{shift} can take at a certain interval length. This can be deduced from the following reasoning. Table 3 shows that when the interval length is equal to 20h, (*i*) a prolongation of the experiment duration from t_f^* has no effect, (*ii*) a shortening of the duration (*a*) has limited effect on the optimality of the profile if t_f is greater than or equal to 68h, (*b*) lowers the optimality of the profile if t_f is less than 68h and greater than or equal to 64h, and (*c*), in all other cases, induces a change in the time at which the temperature step takes places, hereby decreasing the information content of the profile. In this context, it must be remarked that the microorganisms enter the stationary phase at approximately 68h, when a step from T_{low} to T_{high} at 60h is applied (see Figure 4).

    In these results the following general pattern can be recognized. A prolongation of the experiment duration from t_f^* has no effect on structure and information content of the optimal profile (case (*i*)). Similarly, a shortening from t_f^* has limited effect as long as the decreased t_f is part of the stationary phase (case (*ii*)(*a*)). A shortening does, however, influence the optimality of the profile when the experiment is ended before the stationary phase has started (case (*ii*)(*b*)). If decreased t_f interferes with the most optimal value that t_{shift} can take at a certain interval length, then a less optimal value is adopted by t_{shift}, hereby lowering the information content of the profile (case (*ii*)(c)).

COMPARISON BETWEEN THE REFERENCE OPTIMAL TEMPERATURE INPUT AND THE OPTIMIZED PIECEWISE-CONSTANT PROFILES

Optimization of piecewise-constant temperature profiles yields temperature steps from the lower bound T_{low} to the upper bound T_{high}. Thus, the parameterization method used in this study gives rise to the same temperature structure as the parameterization method used in (Bernaerts et al. 2000; Bernaerts et al. 2002). This strengthens the suspicion that a step profile is the most optimal profile for estimation of the Square Root parameters.

The parameter estimates that can be obtained with the reference optimal temperature input are sligthly more unique ($\Lambda(\mathbf{F}) = 4.094$) compared with the estimates that can be obtained with the most optimal piecewise-constant profile presented in this paper ($\Lambda(\mathbf{F}) = 4.102$). This is caused by the different values of t_{shift}. In the former case, t_{shift} is optimal ($t_{shift} = 61.429$h), while in the latter case, t_{shift} is suboptimal ($t_{shift} = 60$h). The optimal time of the shift can only be found in case of the step profile, since in that case t_{shift} can adopt each value and is not dependent on the size of the intervals. In theory, the most optimal value for t_{shift} can also be found with piecewise-constant functions if the interval length is infinitesimally short. But with the method proposed in this paper this will be almost impossible, given the fact that the dynamic optimization problem is difficult to solve when a high number of intervals is involved.

CONCLUSIONS

Piecewise-constant temperature profiles have been optimized for optimal estimation of the Square Root model parameters. The results indicate that the most optimal temperature input for estimation of the Square Root parameters is probably the step profile presented in (Bernaerts 2002). This is a temperature step spanning the complete admissible temperature range and encompassing an optimized t_{shift}. To be fully certain, optimization of piecewise-constant temperature profiles with *high* numbers of intervals also need to be performed. This will be a next step in the investigation. This can however be a difficult task since the number of initialization necessary to solve the optimization problem increases when the number of intervals increases. Consequently, application of another optimization method than the Sequential Quadratic Programming method used in this paper can be useful.

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REFERENCES

Banga, J.R.; K.J. Versyck; and J.F. Van Impe. 2002. "Computation of optimal identification experiments for nonlinear dynamic process models: a stochastic global optimization approach". *Industrial and Engineering Chemical Research* 41, 2425-2430.

Baranyi, J. and T.A. Roberts. 1994. "A dynamic approach to predicting bacterial growth in food". *International Journal of Food Microbiology* 23, 277-294.

Bernaerts, K.; K.J. Versyck; and J.F. Van Impe. 2000. "On the design of optimal dynamic experiments for parameter estimation of a Ratkowsky-type growth kinetics at suboptimal temperatures". *International Journal of Food Microbiology* 54, No. 1-2, 27-38.

Bernaerts, K.; R.D. Servaes; S. Kooyman; K.J. Versyck; and J.F. Van Impe. 2002. "Optimal temperature input design for estimation of the Square Root model parameters: parameter accuracy and model validity restrictions". *International Journal of Food Microbiology* 73, No.2-3, 147-159.

Bernaerts, K. 2002. *Dynamic experiment design and validation in predictive microbiology: optimal estimation of two-parameter microbial growth kinetics as function of temperature.* Ph.D. thesis 519. Department of Food and Microbial Technology, Faculty of Agricultural and Applied Biological Sciences. Katholieke Universiteit Leuven.

Ratkowsky, D.A.; J. Olley; T.A. McMeekin; and A. Ball. 1982. "Relationship between temperature and growth rate of bacterial dultures". *Journal of Bacteriology* 149, No.1, 1-5.

Steinberg, D.M. and W.G. Hunter. 1984. "Experimental design: review and comment". *Technometrics* 26, No.2, 71-97.

Walter, E. and L. Pronzato. 1997. *Identification of Parametric Models from Experimental Data.* Springer, Masson.

BIOGRAPHY

KRISTEL GYSEMANS was born in Leuven, Belgium, in 1979. She received her master's degree in engineering in chemistry and biochemistry with specializations in food science and technology and industrial microbiology from the Katholieke Universiteit Leuven in 2002. Since 2002, she is working as a research assistant at BioTeC – Bioprocess Technology and Control, KULeuven. Her main research interests are in the field of predictive microbiology, more particularly, the design of optimal experiments aiming at accurate model building.

JAN F. VAN IMPE was born in Varsenare, Belgium, in 1965. He received his master's degree in electrical and mechanical engineering from the University of Ghent in 1988, and a doctorate in applied sciences from the Katholieke Universiteit Leuven in 1993 (ESAT Laboratory). Thereafter, he joined the Department of Food and Microbial Technology of the same university (since 1995 as an associate professor). Since 2001, he is professor at the Department of Chemical Engineering. During that period he started the BioTeC research group, which has nowadays about 20 members. He teaches courses in system analysis, bioreactor engineering, and process engineering and control. His research interests are mainly in nonlinear systems and identification, nonlinear and adaptive control, and its applications in model-based optimization and control of (bio-)chemical conversion processes. He has (co-) authored about 300 papers in these areas. In 1995 he received the Barco Award of the Fund for Scientific Research, in 1996 he was laureate of the Belgian Royal Academy, and in 1997 he was awarded the KIWANIS prize of the KULeuven Research Council. In 2002 he became a member of the board of the EUCA, the European Union Control Association. Furthermore, he is member of the Editorial Board of International Journal of Food Microbiology.

TOWARDS THE ACCURATE ASSESSMENT OF NON-LOGLINEAR MICROBIAL SURVIVOR KINETICS: DEVELOPMENT OF A FREEWARE USER-FRIENDLY TOOL IN THE AREA OF PREDICTIVE MICROBIOLOGY

Annemie H. Geeraerd
Vasilis P. Valdramidis
Jan F. Van Impe
Department of Chemical Engineering
Katholieke Universiteit Leuven
B-3001 Leuven
Belgium
E-mail: annemie.geeraerd@cit.kuleuven.ac.be or jan.vanimpe@cit.kuleuven.ac.be

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Predictive microbiology, microbial inactivation kinetics, freeware tool, Excel Add-In.

ABSTRACT

This contribution focuses on the presentation of GInaFiT (Geeraerd et al. Inactivation Model Fitting Tool), a freeware Add-inn for Microsoft® Excel aiming at bridging the gap between people developing predictive modeling approaches and end-users in the food industry not disposing over advanced non-linear regression analysis tools. More precisely, the tool is useful for testing eight different types of microbial survival models on user-specific experimental data relating the evolution of the microbial population with time. The eight model types are: (i) classical log-linear curves, (ii) curves displaying a so-called shoulder before a log-linear decrease is apparent, (iii) curves displaying a so-called tail after a log-linear decrease, (iv) survival curves displaying both shoulder and tailing behavior, (v) concave curves, (vi) convex curves, (vii) convex/concave curves followed by tailing, and (viii) biphasic inactivation kinetics. Next to the obtained parameter values, the following statistical measures are automatically reported: standard errors of the parameter values, the Sum of Squared Errors, the Mean Sum of Squared Errors, the R^2 and the *adjusted* R^2. The tool is downloadable via the KULeuven/BioTeC-homepage http://www.kuleuven.ac.be/cit/biotec/index.htm at the topic "Downloads" (Version 1.3, Release date July 2004).

INTRODUCTION

Microbial inactivation, whether due to a thermal or non-thermal food processing technique like high hydrostatic pressure, pulsed electric field, ohmic heating, …, can exhibit one of the eight shapes illustrated in Figure 1 (see, for example, Xiong et al. 1999, Mafart et al. 2002 or Devlieghere et al. 2004). It is clear that the classical concept of loglinear inactivation modeling fails to assess accurately the majority of these survival curves.

In this research, the focus is on the development of a user-friendly interface enabling easy identification of (one of) these curvatures on an experimental data set provided by the end-user. After testing several of the possible models included in GInaFiT, the models can be compared based on some reported statistical measures.

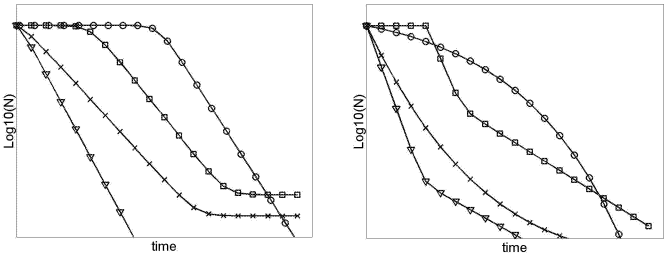

Figure 1: Commonly Observed Types of Inactivation Curves. Left plot: Linear (∇, shape I), Linear with Tailing (x, shape II), Sigmoidal-like (□,shape III), Linear with a preceding Shoulder (O, shape IV). Right plot: Biphasic (∇,shape V), Concave (x, shape VI), Biphasic with a Shoulder (□, shape VII), and Convex (O, shape VIII)

MODEL STRUCTURES INCLUDED IN GINAFIT

In total, eight different model types are implemented in the current GInaFiT version (Version 1.3), covering all but one (namely, shape VII) of the eight shapes of survival curves. The eight different model structures are grouped in four Menu Items, as will be explained in the next paragraphs. For each Menu Item, the shapes of survivor curves covered are indicated in brackets.

Menu Item 1 : Log-Linear model (shape I)

The first menu item provides the end-user with the possibility to use the traditional log-linear approach for describing microbial inactivation curves (see, for example, Anonymous, 2000). In static conditions, this can be written as

$$\log 10(N) = \log 10(N(0)) - \frac{t}{D} = \log 10(N(0)) - \frac{k_{\max}\, t}{\log(10)} \quad (1)$$

Herein, N represents the microbial cell density [cfu/mL], $N(0)$ the initial microbial cell density [cfu/mL], k_{max} [1/time

unit] the first order inactivation constant and D [time unit] the decimal reduction time. In this model it is assumed that all cells in a population have equal heat sensitivity and that the death of an individual is dependent upon the random chance that a key molecule or "target" within it receives sufficient heat (Cole et al. 1993). Despite the world-wide use of this model, especially in the canning industry for the so-called "12D process" of the proteolytic strains (Group I) of *Clostridium botulinum* spores (ICMSF 1996), a lot of deviations have been observed (particularly at lower temperatures and for vegetative cells) indicating that inactivation kinetics are not always following first order loglinear relationships (Anonymous, 2000) as was also illustrated in Figure 1. This observation was at the basis of the development of a number of non-loglinear modeling equations as covered by the remaining GInaFiT Menu Items.

Menu Item 2 : Biphasic model (shapes I, II and V)

Cerf (1977) proposed a two-fraction model, which can be formulated as follows

$$\log10(N) = \log10(N(0)) + \log10(f \cdot \exp(-k_{\max 1} \cdot t) + \cdots \quad (1-f) \cdot \exp(-k_{\max 2} \cdot t))$$

Herein, f is the fraction of the initial population in a major population, (1-f) is the fraction of the initial population in a subpopulation (which is more heat resistant than the previous one), k_{max1} and k_{max2} [1/unit time] are the specific inactivation rates of the two populations, respectively. The model describes biphasic curves, which were generally considered to represent a mix of two species or strains having different heat resistances. Biphasic inactivation curves have been observed in the framework of thermal inactivation (see, e.g., Cerf 1977, Humpherson et al. 1998), non-thermal inactivation due to lethal water activity (Shadbolt et al. 1999) or lethal pH levels (Shadbolt et al. 2001). Zhang et al. 1994, amongst others, support biphasic Pulsed Electric Field inactivation curves. Given this experimental evidence, it is explicitly included in GInaFiT that k_{max1} is always larger then k_{max2}, implying the faster inactivation of the most sensitive population.

Menu Item 3: Log-Linear model with shoulder and/or tailing (shapes I, II, III and IV)

The dynamic model, consisting of two coupled differential equations, reads as follows (Geeraerd et al. 2000).

$$\frac{dN}{dt} = -k_{\max} \cdot N \cdot \left(\frac{1}{1+C_c}\right) \cdot \left(1 - \frac{N_{res}}{N}\right)$$

$$\frac{dC_c}{dt} = -k_{\max} \cdot C_c \qquad (2)$$

Herein, C_c is related to the physiological state of the cells [-], k_{max} is the specific inactivation rate [1/time unit], and N_{res} is the residual population density [cfu/ml]. The model has four degrees of freedom: two states $N(0)$ and $C_c(0)$, and two parameter values k_{max} and N_{res}. The first factor at the right-hand side of Equation (2) models the loglinear part of the inactivation curve and is equivalent to the classical first-order inactivation kinetics (as in Equation (1) when written in dynamic format). The second factor describes the shoulder effect and is based on the hypothesis of the presence of a pool of protective or critical components C_c around or in each cell (Mossel et al. 1995). Gradually, this pool is destroyed. In case of a shoulder, $1/(1+C_c(0))$ takes on a small (positive) value. Towards the end of the shoulder region $1/(1+C_c(t))$ becomes (approximately) equal to one, due to the component C_c undergoing heat inactivation following a first-order model. Finally, the last factor of Equation (2) implies the existence of a more resistant subpopulation N_{res}, which can be framed within the established mechanistic or vitalistic concepts (Cerf 1977). For more details about the derivation of this model as based on literature arguments (Mossel et al. 1995, Cerf 1977), reference is made to (Geeraerd et al. 2000).

This model can exhibit a loglinear behavior with and without shoulder and/or tailing revealing a smooth transition between each phase. It is important to remark that, for this model, tailing is considered for a population remaining *constant* in time or, otherwise stated, not undergoing any significant subsequent inactivation. This is in contrast with the Whiting model (Whiting, 1993) able to describe shape VII curves (see Figure 1, right) or the afore-mentioned biphasic model.

The model structure has been successfully applied to survival data of different micro-organisms and different treatments, such as, *Listeria monocytogenes* and *Lactobacillus sakei* during a mild thermal inactivation (Geeraerd et al. 2000), *Monilinia fructigena* and *Botrytis cinerea* during a pulsed white light treatment (Marquenie et al. 2003), the Acid Tolerance Response (ATR) of *Salmonella enterica* and *L. monocytogenes* (Greenacre et al. 2003) and the inactivation of *L. monocytogenes* in a pH-modified chicken salad during cold storage (Guentert et al. 2003).

The explicit solution of the original dynamic model reads as follows, after substituting $C_c(0)$ by $e^{k_{\max} S_l} - 1$ with S_l [time units] a parameter representing the shoulder as derived in (Geeraerd et al. 2000).

$$N(t) = (N(0) - N_{res}) \cdot e^{-k_{\max} t} \cdot \left(\frac{e^{k_{\max} S_l}}{1 + (e^{k_{\max} S_l} - 1) \cdot e^{-k_{\max} t}}\right) + N_{res}$$

Observe that in this formulation, all parameters have a clear biological/graphical meaning, which is interesting with respect to the parameter estimation procedure for this nonlinear model (initial values are easy to obtain) and because they can be interpreted afterwards.

Menu Item 4: Weibull type models (shapes I, VI and VIII)

The Weibull model, when applied to describe microbial inactivation (see, for example, Peleg and Cole, 1998, Van Boekel 2002), is the cumulative form of the distribution of the individual heat resistance of microbial cells. In GInaFiT, it is the version as proposed by Mafart et al. (2002) which is included based on the possibility to reduce in a natural way to the classical log-linear model (depending on data behavior).

$$\log10(N) = \log10(N(0)) - \left(\frac{t}{\delta}\right)^p$$

Herein, δ [min] is a scale parameter and can be denoted as the *time for the first decimal reduction*, and p [-] is a shape parameter. For $p > 1$, convex curves are obtained, while for $p<1$, concave curves are described.

Van Boekel (2002) and Mafart et al. (2002) observed a strong correlation between parameters δ and p. The dependency of the parameters is due to the model structure (i.e., an error in δ will be balanced by an error in p). This drawback can be circumvented by fixing the value of p (see also, e.g., Peleg and Penchina 2000). Fixing the p-parameter is one of the options under Menu Item 4 of GInaFiT. In this case, a pop-up window appears asking the user which value of p he wants to select. In practice, this option is suitable only when describing a range of different inactivation curves individually (for example, collected at increasing constant temperature values) by using the full Weibull model. Afterwards, if an average p-value seems appropriate, this mean value can be used for all curves (as illustrated in Mafart et al 2002) by using this additional option under Menu Item 4.

The last option under this Menu Item, is the model recently proposed by Albert and Mafart (2003), and which is able to describe concave, convex or linear curves followed by a tailing effect. The model can be written as follows.

$$\log10(N) = \log10[(N(0) - N_{res}) \cdot 10^{\left(-\left(\frac{t}{\delta}\right)^p\right)} + N_{res}]$$

Parameters have the same meaning as in the Weibull model (for δ and p) and as in the Geeraerd et al. model (for N_{res}). The obtained curvature is more smooth then the Geeraerd et al. model presented above.

STATISTICAL MEASURES INCLUDED IN GINAFIT

The following statistical measures are generated for each of the eight models included in GInaFiT:

1. The Sum of Squared Errors (SSE), obtained by summing the squared differences between the experimental data and the model prediction, both in log10-scale.
2. The Mean Sum of Squared Errors (MSE), which can be derived by dividing SSE by the number of degrees of freedom, i.e. the number of data points minus the number of parameters/initial values used (two, three or four). MSE, being the variance of the residuals, is assumed to be a measure for the true variance on the experimental data.
3. The two, three or four parameter values identified, together with their Asymptotic Standard Error. This Standard Error is obtained by taking the square root of the diagonal elements of the asymptotic variance-covariance matrix AVC, with $AVC = MSE \cdot \left(J^T \cdot J\right)^{-1}$. Herein, J corresponds to the Jacobian matrix containing the partial derivatives of the model output with respect to the model parameters evaluated at each experimental time instant. Generally, an *underestimation* of the actual confidence range of the parameter estimates by using Standard Errors for a non-linear model is to be expected as covariance terms are neglected. More appropriate measures for non-linear models include the construction of joint confidence regions (see, for example, Beale 1960) or a Monte Carlo analysis based on knowledge of the experimental error. These measures are not included in the present version of GInaFiT.
4. R^2, the coefficient of determination which equals 1 - SSE/SSTO, with SSTO the sum of the squared differences between the measured values and the mean of these measured values.
5. R^2_{adj}, the adjusted coefficient of determination (see, for example, Wonacott and Wonacott 1990), which equals

   $$R_{adj}{}^2 = \frac{(n-1)\cdot R^2 - k + 1}{n-k}$$

   with n the number of data points and k the number of parameter values (two, three or four). In comparison with the original R^2, the R^2_{adj} attempts to penalize the inclusion of an irrelevant variable, in casu, a redundant parameter.

PROGRAMMING LANGUAGE

For the development of this tool Microsoft® Excel was chosen based on three criteria: (i) Microsoft Office is commonly available on most personal computers, (ii) MS-Visual Basic offers a large flexibility for automating the process and developing the user interface, and (iii) the Solver Add-In for the non-linear parameter estimation of the selected model structures is available. In order to make the installation as user-friendly as possible the tool is written as an Add-In for MS-Excel available to the user at any time as an additional Menu Item.

In order to guarantee the convergence of the non-linear optimization, GInaFiT has some built-in constraints on the parameter values to be estimated, for example, $k_{max} > 0$ and $N_{res} < N(0)$ for the Geeraerd et al. model.

AN ILLUSTRATIVE CASE-STUDY ON THE THERMAL INACTIVATION OF *ESCHERICHIA COLI* K12

Data generation

Survival data of early stationary phase cultures of *E. coli* K12 MG1655, a surrogate for the food-borne pathogen *E. coli* O157:H7 are collected. The inactivation experiment takes place in BHI broth in capillaries immersed in a constant temperature circulating water bath at 56.6°C (GR150-S12, Grant). Cell density is determined by plate counting on BHI.

Importing data into GInaFiT and model application

The user selects in an open MS-Excel sheet the experimental data consisting of time (first column) and the (decimal logarithm) of the population observed (second column). Next, the user selects in the menu item GInaFiT the model of his choice, as shown in Figure 2.

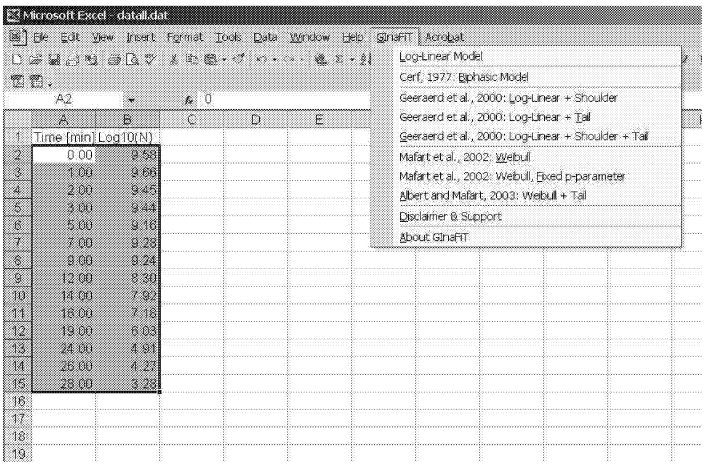

Figure 2: Screendump of the Additional Menu-Item GInaFiT and the Eight Model Types Available

Based on the model selected by the user the tool will perform the parameter estimation/calibration minimising the Sum of Squared Errors criterion. For end-users having already a qualitative idea of the general shape of their survival curves, the choice for one or some of the available model types is obvious. On the other hand, if the end-user does not have a clear idea yet, the different types can be tested and compared based on their Adjusted R^2.

GInaFiT will deliver error messages when parameters which are fairly unlikely or unreliable are obtained, for example, a negative shoulder length or an estimated value of the residual population N_{res} lower then the smallest measured population value.

GInaFiT model outputs

Experimental data are fitted with seven out of the eight models of the tool and their fitting capacity is compared based on the available statistical measures (Table 1). The Weibull model with fixed p (model 7) is not applied as it is only suitable when describing a range of experimental data sets.

Table 1. Statistical Measures Obtained when Applying the Models (1: Log-Linear, 2: Biphasic, 3: Log-Linear + Shoulder, 4: Log-Linear + Tail, 5: Log-Linear + Shoulder + Tail, 6: Weibull, 8: Weibull + Tail) Available in GInaFiT Version 1.3 on the Experimental Data Set at Hand.

Model types	SSE	MSE	R^2	R^2_{adj}
1	3.95	0.3291	0.9374	0.9322
2	3.95	0.3949	0.9374	0.9186
3	0.33	0.0304	0.9947	0.9937
4	3.95	0.3590	0.9374	0.9260
5	0.33	0.0335	0.9947	0.9931
6	0.55	0.0502	0.9912	0.9897
8	0.55	0.0502	0.9912	0.9886

Even more important than these statistical measures, are some observations following the parameter estimation. Firstly, the biphasic model fit (model 2) is actually equal to the log-linear model fit (model 1). The unnessary f parameter is estimated as being zero. Secondly, for the models with tailing (4, 5, and 8), the program warns that this tailing is not substantiated by the data and that a model without tailing should be selected. Nevertheless, convergence for the residual population value N_{res} is still obtained (at a very low level with an unrealistic high Standard Error, which also indicates the redundance), while the other parameter values converge to the ones obtained by model 3 or 6, respectively. The GInaFiT output for model 3 is shown in Figure 3. The minimum variance of parameters obtained in the case of model 3 if compared with model 6 (not shown) indicates that the former model is the most suitable for this experimental case study.

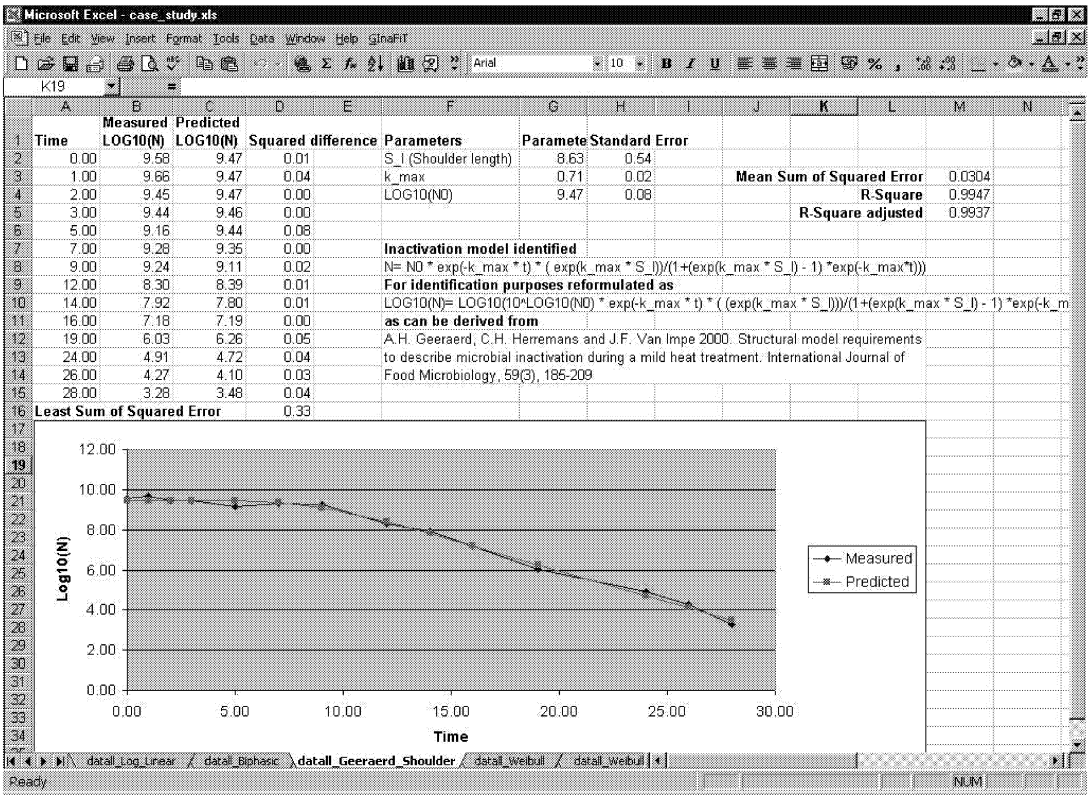

Figure 3. GInaFiT output Results when Applying Model 3 (Log-linear + Shoulder) on the Data Set at Hand

CONCLUDING REMARKS

GInaFiT Version 1.3 is useful to quickly test in a quantitative and user-friendly way a range of microbial survival models on user-specific data, and to derive the associated parameter estimates, standard errors, and some goodness-of-fit indicators. The software, which is currently available for Office97 (English and French), Office2000 (English) and OfficeXP/2002 (English), will be distributed under a freeware license agreement. If necessary, the GInaFiT code will be adapted in order to be suitable for other languages.
In the future, it is envisaged to incorporate the last one of the remaining survivor curve types (biphasic with a preceding shoulder). More importantly, an extension towards the influence of several (possibly interacting) environmental and processing factors like temperature, pH, water activity, recovery conditions, food structure and composition, … on the shape and extent of the microbial survival curves is necessary. This step would enable food producers to have an overall view on the influence of their processing conditions on microbial survival, and hence, to assess in an accurate way their processes performances in the framework of, for example, tackling Food Safety Objectives.

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REFERENCES

Albert I. and P. Mafart 2003. "A modified Weibull model for bacterial inactivation." In: J.F.M. Van Impe, A.H. Geeraerd, I. Leguérinel and P. Mafart (Eds.), *Predictive Modelling in Foods - Conference Proceedings*, Katholieke Universiteit Leuven/BioTeC, Belgium, pp. 143-145 (ISBN 90-5682-400-7) (Selected for the Special Issue of *International Journal of Food Microbiology*)

Anonymous 2000. "Kinetics of microbial inactivation for alternative food processing technologies – IFT´s response to task order 1, US Food and Drug Administration: How to quantify the destruction kinetics of alternative processing technologies." *Journal of Food Science*, suppl, 4-108.

Beale, E.M.L. 1960. "Confidence regions in non-linear estimation." *Journal of the Royal Statistical Society* B, 22, 41-88.

Cerf, O. 1977. "A review. Tailing of survival curves of bacterial spores." *Journal of Applied Microbiology*, 42, 1-19.

Cole, M.B., K.W. Davies, G. Munro, C.D. Holyoak and D.C. Kilsby 1993. "A vitalistic model to describe the thermal inactivation of *Listeria monocytogenes*." *Journal of Industrial Microbiology*, 12, 232-239.

Devlieghere, F. K. Francois, K.M. Vereecken, A.H. Geeraerd, J.F. Van Impe and J. Debevere 2004. "Effect of chemicals on the microbial evolution in foods." *Journal of Food Protection (in press)*

Geeraerd, A.H., C.H. Herremans and J.F. Van Impe 2000. "Structural model requirements to describe microbial inactivation during a mild heat treatment." *International Journal of Food Microbiology*, 59, 185-209.

Greenacre, E.J., T.F. Brocklehurst, C.R. Waspe, D.R. Wilson and P.D.G. Wilson 2003. "*Salmonella enterica* Serovar Typhimurium and *Listeria monocytogenes* Acid Tolerance Response Induced by Organic Acids at 20°C: Optimization and Modeling." *Applied and Environmental Microbiology*, 69(7), 3945-3951.

Guentert, A.M., R.H. Linton, R.H. Mohtar, M.L. Tamplin, J.B. Luchansky and M.A. Cousin 2003. "Growth and inactivation of *Listeria monocytogenes* in pH-modified chicken salad during cold storage." In: J.F.M. Van Impe, A.H. Geeraerd, I. Leguérinel and P. Mafart. *Predictive Modelling in Foods - Conference Proceedings* (pp. 167-169). KULeuven/BioTeC, Belgium (ISBN 90-5682-400-7)

Humpherson, L., M.R. Adams, W.A. Anderson and M.B. Cole 1998. "Biphasic Inactivation kinetics in *Salmonella enteriditis* PT4." *Applied and Environmental Microbiology*, 64, 459-464.

ICMSF 1996. *Microorganisms in foods 5. Characteristics of microbial pathogens*. Blackie Scientific Publications, London.

Mafart, P., O. Couvert, S. Gaillard and I. Leguerinel 2002. "On calculating sterility in thermal preservation methods: application of the Weibull frequency distribution model." *International Journal of Food Microbiology*, 72, 107-113.

Marquenie, D., A.H. Geeraerd, J. Lammertyn, C. Soontjens, J.F. Van Impe, C.W. Michiels and B.M. Nicolaï 2003. "Combinations of pulsed white light and UV-C or mild heat treatment to inactivate conidia of *Botrytis cinerea* and *Monilinia fructigena*." *International Journal of Food Microbiology*, 85, 185-196.

Mossel, D.A.A.; J.E.L. Corry, C.B. Struijk and R.M. Baird 1995. *Essentials of the microbiology of foods*. John Wiley & Son, Inc., West Sussex.

Peleg, M. and M.B. Cole 1998. "Reinterpretation of microbial survival curves." *Critical Reviews in Food Science and Nutrition*, 38, 353-380.

Peleg, M. and C.M. Penchina 2000. "Modeling microbial survival during exposure to a lethal agent with varying intensity." *Critical Reviews in Food Science and Nutrition*, 40, 159-172.

Shadbolt, C., T. Ross T.A. and McMeekin 1999. "Nonthermal death of *Escherichia coli*." *International Journal of Food Microbiology*, 49, 129-138.

Shadbolt, C., T. Ross and T.A. McMeekin 2001. "Differentiation of the effects of lethal pH and water activity: food safety implications." *Letters in Applied Microbiology*, 32, 99-102.

Van Boekel, M.A.J.S. 2002. "On the use of Weibull model to describe thermal inactivation of microbial vegetative cells. *International Journal of Food Microbiology*, 74, 139-159.

Whiting, R.C. 1993. "Modeling bacterial survival in unfavorable environments." *Journal of Industrial Microbiology*, 12, 240-246.

Wonacott, T.H.; and R.J. Wonacott. 1990. *Introductory statistics*. John Wiley & Sons, New York.

Xiong, R., G. Xie, A.E. Edmondson and M.A. Sheard 1999. "A mathematical model for bacterial inactivation." *International Journal of Food Microbiology*, 46, 45-55.

Zhang, Q., A. Monsalve-González, G.V. Barbosa-Cánovas and B.G. Swanson 1994. "Inactivation of *E. coli* and *S. cerevisiae* by pulsed electric fields under controlled temperature conditions." *Transactions of the ASAE*, 37, 581-587.

IMPROVEMENT OF THE COOPERATION BETWEEN THE OPERATOR AND THE PROCESS – AN EXPERIMENTAL APPROACH OF THE IMPERFECTION IMPACT ON A CHEESE RIPENING FUZZY MODEL

Irina Ioannou, Nathalie Perrot, Gilles Trystram
UMR Génie Industriel Alimentaire – Equipe Automatique et Qualité Alimentaire (Cemagref), 24, Avenue des Landais, BP 50085, 63172 Aubière cedex, tel: (33) 473-44-06-69 – fax: (33) 473-44-06-97 email : nathalie.perrot@cemagref.fr

Gilles Mauris
LAMII Université de Savoie 74000 Annecy
gilles.Mauris@esia.univ-savoie.fr

ABSTRACT

In the food processes, it is relevant to build tools taking human measurements into account for the control of the sensory quality of the food products. Despite the methodology used to formalize these measurements, they are subject to more imperfections (imprecision, unreliability,…). Our aim is to take these imperfections into account in our approach of modelling by processing them in the built models. When it is possible, we try to extract high level informations and some guidelines towards an optimized cooperation between the operator, the sensors and the process.

INTRODUCTION

Finished food products must meet specific sensory, sanitary and technological requirements. Among these requirements, sensory properties are essential because they condition the choice and the preferences of consumers [1]. As a consequence, it is relevant to develop methods which allow firstly to measure those properties on the manufacturing line and secondly to integrate them in a feedback control of food processing [2]. In this context, the use of the fuzzy symbolic approach for representing human assessments and reasoning in a decision support system is interesting to help people in charge of the process control (operator) [3]. These systems have as inputs sensory measurements made by the operator at line during the manufacturing and/or instrumental measurements. It allows a cooperation between the human operator and the automation system. However, the operator sensory measurements are subjected to higher imperfections than the conventional sensory measurements. The causes are, for example, the place of the measurement (operator influenced by his environment: temperature, humidity, light) or the time of the measurement (pressure due to the need to control, several tasks to do at the same time). From a model developed on an application dealing with the control of a cheese ripening process, we are looking for determining the impact of the imperfections, coming from the expert evaluations, on the fuzzy model output. In this paper, we are concerned with two aspects of this kind of imperfections: the lack of a sensory measurement and the imprecision of the sensory measurements.

The paper is organized as follows. The first section explains the model developed on the application of cheese ripening. The methodology of the experimental analysis and results are presented in the second section.

THE FUZZY SYMBOLIC APPROACH APPLIED TO CHEESE RIPENING

Having a mathematical support to represent human assessments and reasoning expressed by linguistic expressions is one of the originate motivation of fuzzy subset theory [4,5]. A fuzzy symbolic description of phenomena consists in affecting degrees (between 0 and 1) to considered terms, e.g. 0.8/High;0.2/Medium;0./Small. The latter can be directly obtained from human assessments or indirectly from the numeric measurements by the concept of fuzzy symbolic measurement, the meaning of terms being encoded in their associated membership functions [6,7]. The interest of the fuzzy symbolic approach is thus the possibility to take both numeric and linguistic assessments into account and also to process such information further, e.g making information fusion.

This approach was applied to build a decision support system in cheese ripening [8], that is a ageing process lasting about one month. This system was established to inform an operator on the potential drift of the sensory trajectory of the cheese each day in order to help him to control the process. To control the process, the operator uses a variable called ripening degree which represents an indication of the ripening state at a time t of ripening. This variable is assessed on an ordinate scale (from 4 (state A) for a cheese no ripened cheese to 0 (state E) for a ripened cheese) with graduations of 0.25. Despite these graduations, the operators say that their sensitivity on the ripening degree is 0.5. That is why the two sensitivity levels 0.5 and 0.25 are tested for the validation of the model (see fig. 2). The explanatory model of cheese ripening is shown on figure 1. This model takes five variables as input data : the ripening time and four sensory variables : the cheese coat (MS1), its color (MS2), its humidity (MS3) and its consistency (MS4). The output of the model is the ripening degree.

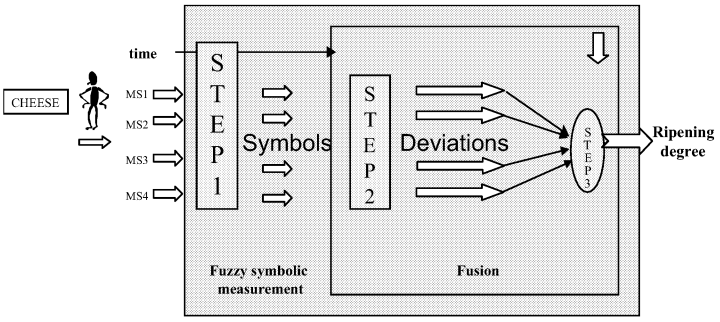

Fig. 1. Principle of the ripening model

The proposed model consists of three steps. The first step allows to transfer the sensory measurements made on an ordinate scale (from 1 to 6) in a symbolic space (different symbols are defined for each sensory measurement). In the second step, the deviations are calculated for each sensory measurement in comparison with the objective trajectories of the sensory measurement. The third step allows to combine the deviations on each sensory measurement by rules treated in a fuzzy symbolic mathematical frame [7]. The used rules depend on the ripening time. Table 1 presents the rule base for the period from day 8 to day 12. It corresponds to a standard ripening degree C.

Table 1. Rules base for the standard ripening degree C (day 8 to day 12)

Deviations on cheese coat / Deviations on cheese consistency	(Very few covered)	(Few covered)	(Medium covered)	(Very covered)
(No proteolysis)	*late (-1)*	*late(-1)*	*late (-0.5)*	
(Few proteolysis)		*late (-0.25)*	Standard state	*early (+0.5)*
(Medium proteolysis)			***EARLY. (+1)***	***EARLY (+1)***
(Total proteolysis)			***EARLY (+2)***	***EARLY (+2)***

For example: at day 10 of the ripening time, the cheese coat is measured to 3 and the cheese consistency to 2. The corresponding fuzzy linguistic descriptions are: for cheese coat *medium covered* with a membership degree of one and for cheese consistency: *few proteolysis* with a degree of 1. The standard trajectory of these measurements give the following symbols like standards at day 10: *medium covered* and *few proteolysis*. So, deviations of both these measurements are equal to 0. Table 1 combines these deviations to obtain the ripening degree: the cheese is in standard state C at day 10.

EXPERIMENTAL IMPACT ANALYSIS OF SENSORY MEASUREMENT IMPERFECTIONS

In this paper, we propose a methodology in two parts to analyze the impact of sensory measurement imperfections. The first one is an analysis of the degradation of the structure of the model led by a lack of a sensory measurement. The second one is an analysis of sensitivity of the input variables, it consists in propagating imprecision of input measurements on the model output.

1 Impact of lack of a sensory measurement

To reproduce the lack of a sensory measurement, an input sensory measurement of the model is deleted. This deletion corresponds to a degradation of the ripening model. It brings information on the importance of each sensory measurement in the structure of the model. The identification of measurements that are more important than others can induce a more attentive assessment by the expert for this measurement : increased vigilance , repetition of the measurement or addition of an information to confirm the sensory measurement.

Method. Six degradations are made. We obtain six damaged models (table 2). Each degradation corresponds to the deletion of one sensory measurement for the three first damaged models and two sensory measurements for the three last damaged models. For example, for the first damaged model (MD1), the sensory measurement deleted is the humidity of the cheese (MS3), so the inputs of the model staying are the cheese coat (MS1) and the cheese consistency (MS4). The input data, color of the cheese (MS2), is considered as having little impact on the model to find the ripening degree, but it is useful at the end of the ripening to determine a default of color. Therefore, this measurement is deleted for each degradation of the ripening degree model.

Table 2. Composition of the different damaged models

Name of the damaged model	Variables of deleted input	Variables of input staying	Name of he damaged model	Variables of deleted input	Variables of input staying
MD 1	MS3, MS2	MS1,MS4	MD 4	MS3, MS2, MS1	MS4
MD 2	MS1,MS2	MS3, MS4	MD 5	MS3, MS2, MS4	MS1
MD 3	MS4, MS2	MS1, MS3	MD 6	MS1, MS2, MS4	MS3

The deletion of one or several sensory measurements leads to a cancellation of the rule tables of the ripening model, giving the ripening degree. This task is realized with the help of the expert by a reformulation of the original rules. For example, from table 1 shown in part 2, we obtain after the deletion of the measurement cheese consistency table 3.

Table 3. Rules base for the standard ripening degree C with deletion of the cheese consistency

DEVIATIONS ON CHEESE COAT	Very few covered	Few covered	Medium covered	Very covered
RIPENING DEGREE	***DEFECT***	***LATE (-0.5)***	*STANDARD*	***EARLY (+0.5)***

In this way, for the original model, the standard state was defined by a cheese coat *medium covered* and a cheese consistency *few proteolysis*. Now for the damaged model MD1 (cheese consistency deleted), the standard state is defined only by a cheese coat *medium covered.*
The answer of the damaged model (ripening degree) is considered like compatible if it does not differ from the expert answer more than the tolerance thresholds (fixed in function of the precision degree of the human measurements: 0.25 and 0.5 on a scale 0-4). The performance of the model is defined as the number of answers compatible in comparison with the number of cheeses to assess. To compare the performances of the different damaged models with the performance of the original model, they are plotted on a histogram (see fig.2).

Results. On the figure 2, are presented the performances of the different damaged models in comparison with the original model.

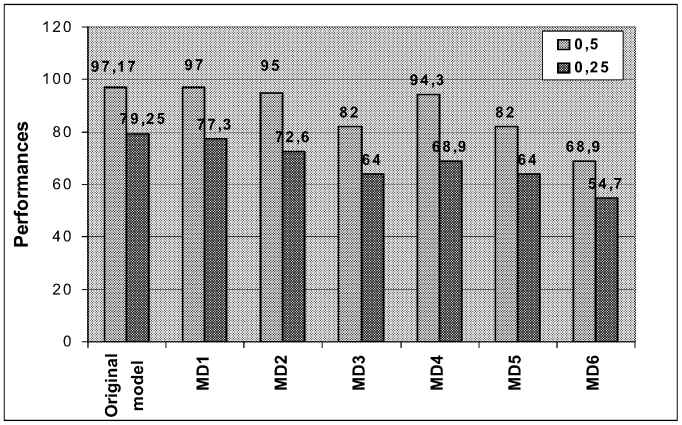

Fig. 2. Performance of the original and the damaged models at two thresholds

A degradation of the model structure linked to the cheese humidity (MD1) does not act upon the performances of the model whatever the different thresholds tested. But, when the degradation of the structure of the model is linked to the cheese consistency (MD3), it leads to a loss of 15 % for the two thresholds.
A degradation of the structure of the model linked to the cheese coat (MD2) leads to a decrease of the performances unequalled for the two thresholds. For a threshold of 0.5, 2 % of compatible answers are lost and 8 % to a threshold of 0.25. If the measurement cheese humidity is also deleted (MD4), we notice a loss of 3% of compatible answers to a threshold of 0.5 and 9 % to a threshold of 0.25.
This analysis enables to conclude that the sensory measurement with the highest impact is the cheese consistency. It is responsible for 97 % of compatible answers to a threshold of 0.5. Moreover, we can conclude to a strong impact of the cheese coat to give a more refined answer.

2 Impact of sensory measurement imprecision

Method. The principle of this analysis is to propagate in the model an imprecision applied on an input variable, the others input variables being fixed. The study is achieved on ripening kinetics, obtained on a pilot able to represent the different ripening dynamics. An example of kinetic is shown on figure 3.

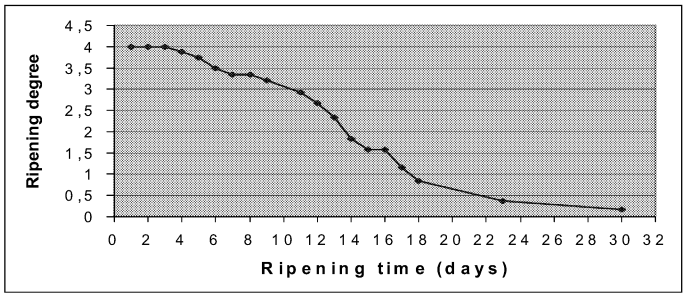

Fig. 3. Evolution of the ripening degree versus the ripening time

This kind of analysis enables to answer the following questions : What is the impact on the model output (the ripening degree expressed on a 4 to 0 scale) if we increase the imprecision on the input measurements? How does the model propagate the imprecision (amplification, attenuation)? These questions are particularly important when, for example, a crusting default appears on the cheese and increases the imprecision in the cheese consistency measurement (MD4). Another more fundamental question is : "what kind of high level informations about the aid operator system built can be deduced from such an approach?". To achieve this study, we associate a fuzzy number to each input variable as to represent this imprecision. The fuzzy number associated to the cheese consistency input variable (MD4) characterizing the operator measurement is represented as example figure 4. Thus, for a measurement MD4 = 3 achieved by an operator, its imprecision is represented by a fuzzy number centered on 3 with a half support (Sinput/2)of 0.5. It means that the measurement is actually included between 2.5 and 3.5 with a possibility to be 2.8 for example, three times higher than the possibility to be 2.6. Experimental simulations are achieved processing different fuzzy numbers as input for each given variable from Sinput = 0.5 to Sinput = 3. The properties of monotonicity of the model of cheese ripening allows us to propagate the imprecision of the inputs in the model, using the extension principle defined by [9]. Simulations are computed input by input. Others inputs are fixed to a standard value. For example, for computations about the measured cheese consistency, cheese colour is fixed to yellow, cheese humidity to few humid and cheese coat to medium covered from day 8 to day 16 and to very covered from day 16 until the ripening end. The result is an output fuzzy number describing the ripening degree. The half support (Soutput/2) of this fuzzy number is compared to the imprecision brought by the expert measurement about the ripening degree, which is between 0.5 and 0.25 upon the period of the kinetic.Thus if Soutput/2 > 0.5, we consider the model "very sensitive" to the imprecision propagate on a

given input variable; if 0.25<Soutput/2 <0.5, we consider the model "sensitive" to imprecision and finally if Soutput/2 < 0.25, we consider the model robust to the imprecision propagate on a given input variable and as a consequence "no sensitive" to imprecision. The experimental simulations are achieved on a data basis representative of three different characteristic dynamics of ripening acquired during experiments led on a pilot with around 20 points each one.

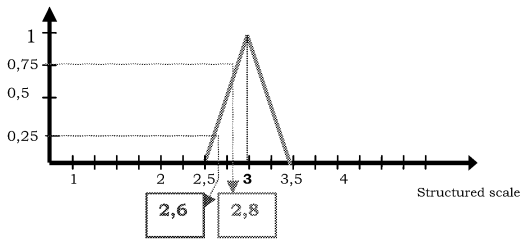

Fig. 4. Evolution of the ripening degree in function of the ripening time

Results. Figure 5 is an example of results acquired for simulations concerning the sensitivity of the model to the measurement cheese consistency (MS4) for a given representive kinetic of ripening. We notice that the impact of the imprecision on this measurement is crucial between the 8th and 24th days with a maximum of sensitivity at around 16-18 days. It means that in this period a strategy of measurement or state estimation, in cooperation with the operator, should be established in order to avoid imprecision as much as possible. For example, the strategy could be to duplicate the sensory measurement in this period or to check the sensory measurement using an indirect measurement. An hypothesis could also be deduced from this result : the maximum of sensitivity is indirectly linked to a key microbiological instant in the cheese consistency elaboration. To complete the analysis of all the results, figure 6 presents an aggregation of the results acquired during those simulations on one kinetic. If the operator precision on his sensory measurements is not really high between the 4th and 24th days (+/- 4 days), consequence on his evaluation is critical only after the 8th day (model "sensitive") and the key measurement on this period is the cheese consistency variable. Such a result offers a sort of guideline to improve the estimation of the degree of ripening achieved by the operator . It leads to focus on the periods where (i) a strategy of indirect measurement could be really interesting to develop and (ii) a better understanding or estimation of the state variables is enhanced.

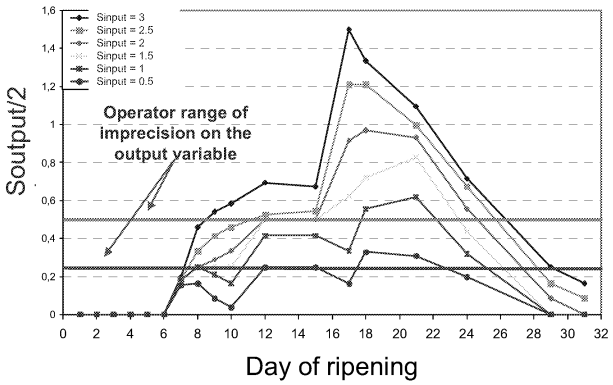

Fig. 5. Results of the propagation of different fuzzy numbers (from support 0.5 to support 3) describing the input variable cheese consistency on the half support (Soutput/2) of the output of the model : the degree of ripening- comparison to the operator imprecision on this value-kinetic 3.

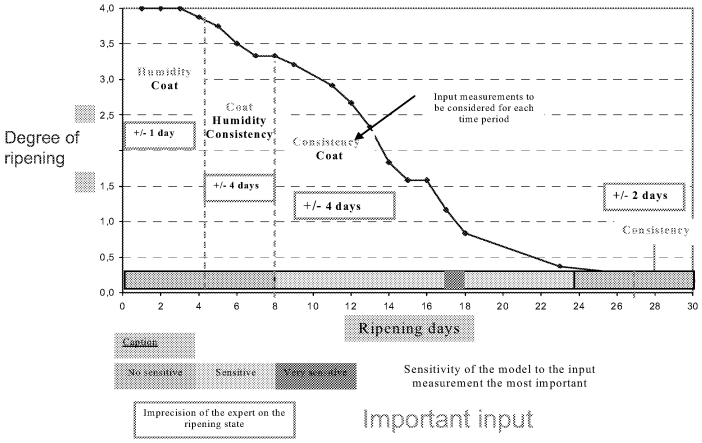

Fig. 6. Synthetic representation of the results obtained about the impact of the imprecisioons on the input measurement on the model estimation.

CONCLUSIONS

The contribution of this paper is to bring information on the impact of imperfections coming from the expert evaluations, on a fuzzy symbolic model describing cheese ripening. Especially, the experimental analysis made allows to indicate the sensory variables that must be measured in priority and also variables for which precautions must be taken to achieve a « precise measurement ». In this sense, the cheese consistency is the most important sensory variable. Moreover, we observed that the sensitivity of the model to the measurements increased during the second half of the ripening period. A better understanding or estimation of the state variables could be value added in this period. This study opens an interesting avenue with regards to the issue of food process control. Experimentations are in progress to analyse other kinds of imperfections identified in the application like the structure of the model.

REFERENCES

1. I. Ioannou, N. Perrot and al. (2002). The fuzzy set theory: a helpful tool for the estimation of sensory properties of crusting sausage appearance by a single expert. *Food quality and preference*. 13(7-8): 589-595.
2. N. Perrot, G. Trystram and al. (2000). Feed-back quality control in the baking industry using fuzzy sets. *Journal of food process engineering*. 23: 249-279.
3. V. Davidson, R. B. Brown and al. (1999). Fuzzy control system for peanut roasting. *Journal of food engineering*. 41 : 141-146.
4. L. Zadeh (1965). Fuzzy sets. Information and control, 8: 338-353.
5. L. Zadeh (1999). From computing with numbers to computing with words-From Manipulation of measurements to manipulation of perceptions. *IEEE Transactions on circuits and systems* 45(1): 105-19.
6. L. Zadeh (1971). Quantitative fuzzy semantics. *Inf. Sci*. 3: 159-176.
7. G. Mauris, E. Benoit and al (1996). The aggregation of complementary information via fuzzy sensors. *Measuremen*t, 17(4): 235-249.
8. D. Dubois, L. Foulloy, S. Galichet, H. Prade, Performing approximate reasoning with words, in, Computing with words in information/intelligent systems 1, 1999. 24-29.
9. N. Perrot, L. Agioux, I. Ioannou, V. Chevallereau, G. Trystram, G. Mauris, G. Corrieu, . A fuzzy symbolic approach to estimate the sensory state of a food product : application to cheese ripening, Foodsim'02 - International conference on simulation in food and bioindustry, (2002), 99-102.

PROCESS AND PLANT CONTROL

MODELLING AND CONTROL OF EVAPORATORS AND SPRAY DRYERS

Peter Cunningham
Barry O'Connor
AMT Ireland,
Process Engineering Department,
University College Cork,
Cork,
Ireland.
Email: p.j.cunningham@student.ucc.ie
b.oconnor@ucc.ie

Donal J. O'Callaghan
Dairy Products Research Centre,
Teagasc.
Moorepark,
Fermoy,
Co. Cork,
Irish Republic
E-mail: djocallaghan@moorepark.teagasc.ie

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Evaporation, Spray Drying, Advanced Process Control

ABSTACT

This paper presents a review of modelling and Advanced Process Control (APC) when applied to both evaporators and spray dryers. APC techniques such as neural networks and model based predictive controllers are already an accepted technology. A number of case studies are presented. However, some of these techniques are only applied to a process when it is operating in steady-state or in some cases they are only applied when specific products are being manufactured. There is an increased demand for value added products, which will result in a move away from long production runs of a single product type. Therefore with an increased frequency of product changes and shorter run times, start-up and shut-down will become more frequent. As a result, start-up and shut-down are taking up a greater percentage of production time and more consideration needs to be given to possibilities for energy savings and other efficiencies such as product recovery and water treatment during these times.

INTRODUCTION

Preserving foodstuffs by drying and thereby depriving micro-organisms of the water necessary for growth has been known for centuries. Skim-milk powder has a maximum shelf life of about 3 years while whole milk powder has a maximum shelf life of about 6 months (Alpha Laval 1995). The first recorded reference to the manufacture of milk powder was by Marco Polo who observed the Mongol soldiers producing milk powder by drying milk in the sun in the 13th Century (Kelly et al. 2003). Today milk powder is produced on a large scale in modern plants most commonly consisting of multiple effect falling-film evaporators and spray dryers (Masters 1985; Písecký 1997). A flow diagram of the general process of milk powder production is given in Figure 1 (Luo 1998).

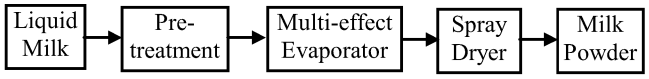

Figure 1: Milk Powder Production Process

Drying is one of the most important and most energy intensive industrial operations. It is estimated (Mujumdar and Passos 2000) that thermal dehydration processes account for approximately 9 to 25% of national industrial energy consumption in developed countries. It is noted that the specific energy consumption of the spray drying process is about 10-20 times higher than the energy consumption of a multi-effect evaporator. Therefore it is desirable to remove as much moisture as possible in the evaporation stage. There is however a limit to the amount of moisture that can be removed in the evaporation stage. If the total solid content of the concentrate is too high then the product becomes too viscous which will hinder the subsequent drying process. Less variation in the total solid content of the concentrate leaving the evaporator allows this limit to be closely approached without violating it and therefore improves the efficiency of the overall process (Verdurmen and De Jong 2003).

The remainder of the paper is organised as follows, firstly a discussion about the current methods used in modelling of evaporators and spray dryers. Then methods of control that are commonly used are presented. Then a number a number of case studies of the application of APC to industrial evaporators and spray dryers are presented. Finally, the paper concludes with suggestions for possible improvements to current methods and possibilities for an increase in the use of APC.

MODELLING STRATEGIES

In the dairy industry both the evaporator and spray dryer are controlled separately. The overall goal of each operation is to control the final moisture content of the product. In general, two distinct kinds of models exist (van Wijck et al. 1994). These are black-box modelling and physical or first principles modelling (also known as white-box modelling). Both approaches offer certain advantages and disadvantages.

Traditionally, food science focused on white box modelling (Perez-Correa and Farías 1995; Zbiciński 1995). These models give an insight into the physical phenomenon that occurs during the evaporation and drying. They are also accurate over a wide operating range. However, they require an extensive knowledge of physical properties such as heat and mass transfer coefficients, viscosities

dimensions of machinery, etc. A major problem is that some of the required properties maybe unknown and therefore certain assumptions may have to be incorporated into the model. These assumptions affect the accuracy of the model and can limit the range of validity of the model. Development of a white-box model can be a time consuming task, in comparison to the black-box techniques, and may require significant calculation time on a computer. However, mathematical models that can predict the properties of the final product would be of great benefit to the food industry, in particular if these models are used to optimise the process control in relation to desired product quality instead of selecting process controls to achieve pre-determined values of temperatures, pressures, flow rates etc. from past process knowledge (De Jong et al. 2002).

Black-box models are developed using data extracted from the process in conjunction with an identification technique. The most commonly used methods are neural network or fuzzy logic models. A black-box model does not contain the physical details of a process and is very specific to the process and product on which they are trained. An alternative to using a single black-box model to represent the entire process it is to build an overall model that comprises of a number of sub-networks each modelling a specific element of the overall system (Russell et al 2000). This sub-network approach offers benefits in terms of model flexibility and interpretability.

The main advantage of the black-box approach is that minimal knowledge of the process being modelled is required. Once there is sufficient data then it is possible to estimate a model. Also, black-box models can be very accurate around the specific operating region for which they are trained. A major drawback of black-box modelling is that changes in product properties or process conditions cannot be predicted. Therefore if any changes do occur then the entire identification procedure must be repeated for the new conditions.

CONTROL STRATEGIES

In general, there are two distinct types of control strategies. The first technique aims to maintain constant values of process conditions, e.g. temperature, flow, pressure etc., at values that have been selected using experience gained over years of plant operations. The methodology of this is that constant process conditions will result in a constant product quality. Therefore it is possible to build a database of process conditions that ensure the desired product quality (Kelly and Kelly 2000). However, a problem occurs due to the fact that a small standard deviation in process parameters may result in a large deviation in product properties. In the second strategy, APC, process set-points are determined by mathematical models, which determine the interaction between process and product properties. Currently the models used in this instance are black-box models with little or no physical or chemical background.

Evaporators

The control of evaporators mainly focuses on producing concentrate with a constant total solid content at a constant flow rate. (De Jong and Verdurmen 2001). A variation in the total solid content of the concentrate leaving the evaporator is one of the major sources of disturbances in the drying process. It is also important to match the flow rate of the concentrate leaving the evaporator to the rate that the dryer is using the concentrate. Failure to achieve closely matched flow rates increases the need for large buffers in the process. The main variables manipulated to control the evaporator are the flow-rate of the product entering the process and the steam used for evaporation.

A control strategy commonly used is the implementation of a number of Single-Input Single-Output (SISO) PID controllers each used to ensure a specific parameter of the process is maintained at the desired set-point. This strategy is used in conjunction with the methodology described earlier where constant product properties are achieved by ensuring constant process conditions. However a multiple effect falling film evaporator consists of a number of complex interacting systems and therefore the use of SISO PID loops will perform poorly in comparison to multi-variable controllers. If the multi-variable option is used then the use of an accurate model, either first principles or black-box, is essential to the overall success of the scheme.

Spray Dryers

The control of spray dryers focuses mainly on the final moisture content of the powder. In modern dryers infrared, resistance or capacitance are often used to determine the moisture content of a product. While moisture content is one of the most important properties of a dried product it is also important to note that other properties, such as bulk density, insolubility index, instant properties are important to the overall quality of powder produced. Often the temperature of the outlet air is used to determine the amount of drying that has taken place in the dryer. The variable manipulated to achieve the desired moisture content is usually either the feed rate of the concentrate entering the dryer or the temperature of the air entering the dryer. The product flow rate is usually the parameter that is selected as the variable to be manipulated, as it is generally the cheaper option. This method can require the use of large buffers between the evaporator and dryer as it becomes more difficult to match the feed rate of the product leaving the evaporator with the feed-rate used by the dryer.

As was the case in the evaporator it is quite common to use a number of SISO PID loops to maintain process parameters at set-points which have been established through prior experience as giving a dried product of a certain quality. This approach also suffers from the same drawbacks reported for the evaporator case. Therefore there are incentives to using model based multi-variable controllers. Again the model can be either a first principles model or a black-box model. Very few first principles approaches have been reported (Straatsma et al 1999a;

Straatsma et al 1999b) and since the use of neural networks is a specialised field specialised software packages can be required. Some of the commonly used software packages for APC are described in the case studies presented in the following section. A number of examples of these packages being applied to evaporators and spray dryers with the resultant benefits are also presented.

CASE STUDIES

A number of successful applications of model based multi-variable controllers have been reported. A selection of the tools used is Connoisseur, Process Perfector, Predictive Controls, MonitorMV, QuickStudy and Q-MAST. A number of different benefits have been reported resulting from the application of these tools. The following subsections detail some of the results achieved on industrial examples.

Ingredia Dairy Ingredients

Invensys' Connoisseur® was successfully applied at Ingredia Dairy Ingredients, France, to an evaporator and dryer (APV 2004). Invensys is the market leader in the application of commercial APC for evaporator and dryer processes. Connoisseur Model Predictive Control is a comprehensive APC software tool that uses plant data to identify and model the significant cause and effect relationships in a process.

Ingredia Dairy Ingredients produces a wide range of liquid, concentrate and powdered food for the dairy and confectionary market. The aims of the company when applying APC was to increase product yield, improve energy efficiency and produce a more consistent product quality. The results reported due to the application of Connoisseur at Ingredia Dairy Ingredients were improved product quality through a reduction in the variation of key variables by 40-50%, a reduction in heat energy used per ton of powder by 2-3%, an increase in plant throughput and an extra 0.2% powder produced per litre of liquid feed. Due to these improvements it is estimated that a return on investment payback period of less than twelve months will be achieved.

Abbott Laboratories

Abbott Laboratories, Ireland, also installed Connoisseur to control their evaporator and dryer plant (APV 2003). Abbott Laboratories is a global, broad-based health care company devoted to the discovery, development, manufacture and marketing of pharmaceutical, nutritional and medical products. In the plant in Ireland infant nutritional products, such as infant formula and follow-on formula, are manufactured using evaporators and spray dryers. The solution employed in Abbott Laboratories was to use a number of model predictive controllers, linked together to provide co-ordinated control of the overall process. The evaporator controller ensured a concentrate with consistent total solid content was passed to the dryer. The dryer controller consisted of two multivariable controllers connected in cascade. The main dryer outlet temperature controller takes its set-point from the powder moisture controller. Finally an optimiser was used to oversee the whole process and set targets for parameters such as throughput rates to ensure maximum production is achieved while matching the evaporator feed rate to the drying rate. This eliminated worries about level control issues in the relatively small dryer balance tank used by Abbott Laboratories. The recorded process improvements were an increase in the powder production rate of up to 10% and an energy efficiency improvement of 5%. A reduction in the variation of the total solid level of concentrate leaving the evaporator was also achieved allowing this value to be held closer to its optimum value. As stated earlier, this has the advantage of improving efficiency (due to more water being removed in the evaporator which is more energy efficient). Again the return on investment period was estimated to be less than twelve months.

Fonterra

Pavilion's Process Perfecter® was applied at Fonterra's Kauri K2 milk powder plant in New Zealand to the two evaporators and one spray dryer (Pavillion 2003). Pavilion's Process Perfector software solution is a dynamic, non-linear, multi-variable model predictive controller and is widely applied in a number of multi-variable manufacturing environments. Process Perfecter combines steady-state optimisation with model predictive control to deliver an advanced process control solution capable of managing process set-points and transitions. The following paragraph describes the application of Process Perfecter to a Fonterra milk powder production plant.

Fonterra is a leading multinational dairy company. Fonterra manufactures and markets over 1.8 million tonnes of product annually. The two falling film evaporators feed the spray drying process and processes 6.5-9 tonnes per hour. The main objectives of this project were to reduce the variations in total solid content leaving the evaporator by 50% and to reduce the variation of the moisture content of the dried powder by 50%. When Process Perfector was applied to the evaporators the variation in total solid content was reduced by 68-73% allowing the total solid content targets to be increased without violating the viscosity limits. A 0.5% increase in total solids from the evaporator can lead to a 2% increase in dryer throughput. In the case of the spray dryer the variation in moisture content was reduced by 52% and also the variation in the chamber outlet temperature was reduced by 43%. Overall, this allowed the target moisture to be moved closer to the maximum specification limit thereby producing additional powdered product for the same raw milk feed.

FMC FoodTech Citrus Systems

FMC FoodTech Citrus Systems, Florida, used QuickStudy® and Q-MAST® in the control of an evaporator used to concentrate orange juice (Morris 2001). QuickStudy and Q-MAST are an adaptive, model predictive controller software and process modelling software respectively and

are designed by Adaptive resources. The overall goal of the APC was to more tightly regulate the final Brix of the concentrated orange juice to minimise post-blending, post-processing and waste. Typically only the product feed rate is the control variable used. In this case the feed concentration and the steam pressure are measured and using these it is possible to predict the effect of changes on the final product and what correction action to take to account for variations in these parameters. To develop a model of the process several hundred data points were taken and used in Q-MAST to create a model of the process using statistical modelling. QuickStudy then uses this model in the control of the system. The model is also evaluated online and updated with new data as the process is running. When QuickStudy was applied to the process it was possible to reduce the variation of the Brix from its typical value of ±1 degree Brix to a variation of ±0.1 degree Brix. Although it was not measured, it was believed that this reduction in variation will lead to an increase in throughput and reduced fouling that will result in longer production runs between CIP cycles.

British Sugar

British sugar operates nine beet sugar refining processes in the UK producing sugar and molassed sugar beet animal feed. Mixing beet fibre together with molasses produces the animal feed. This is then dried and usually palletised. British Sugar introduced a model based predictive controller, implemented by Predictive Control Ltd., with the intention of controlling the moisture level of the product to eliminate under-drying and over-drying, better control of product temperatures to eliminate the need for pellet cooling to minimise energy costs (Caddet 2000). The result of implementing the model based predictive control strategy was an energy savings totalling GBP 32,900 per year. Product yield was increased by 0.86%, which is worth a total of GBP 61,600 per year. Product quality was improved resulting in a reduction in reworking off-specification product from 11% to 4%. Based on these figures it is estimated that the return on investment period will be 1.4 years.

CONCLUSIONS

From the case studies listed above the advantages of applying APC to evaporators and dryers is obvious. There is a reduction in total solid content of the concentrate and moisture content of the powder in all cases, a reduction in the amount of energy used, an increase in the throughput of the plants and extra product being produced per litre of liquid feed. All these factors result in a more efficient process with a more consistent quality of product being produced. In these case studies the modelling was performed using black-box techniques and will suffer from the drawbacks earlier.

It is not uncommon for such APC being used only when the process has reached steady-state. Therefore, both start-up and shut-down procedures are often performed manually be the plant operators. As the variation of products being used in evaporators and spray dryers increases it is becoming more common to produce a large number of products and therefore the length of time that a single product is being produced is decreased. Therefore, start-up and shut-down becomes more frequent. The possibilities for savings, similar to those achieved using APC during steady-state, is also possible during start-up and shut-down. Another problem with the introduction of new products is that the models obtained previously may not be valid for this product. Therefore the entire process may have to be remodelled for this new product. Often this results in APC only being applied to certain products.

However, it is clear that the industry has actually accepted advanced control systems as an effective weapon to maximise product quality and output. If a first principle model of the process were used in the APC strategy then this model would also be valid during start-up and shut-down. It also offers the potential for overcoming the difficulty of adding new products. Once the properties of a new product are known it would be possible to uses these properties in the equation governing the operation of the evaporator and spray dryer and thereby easily construct new models of the process.

REFERENCES

Alpha Laval. 1995. “Milk Powder” in *Dairy Processing Handbook*. Tetra Pak, 361 – 373.

APV. 2004. “Powdered Milk Production Benefits from APV and Foxboro Solutions”. *APV News*, 2 January. http://www.apv.com/news/default.htm

APV. 2003. “Evaporator and Dryer Optimisation Delivers Significant Benefits for Abbott Laboratories in Ireland”. *APV Food and Drink*, March. http://www.apv.invensys.com/news/FoodAndDrink/Default.htm

Caddet. 2000. “Model-Based Predictive Control System Saves Energy”. *Caddet Energy Efficiency*. http://www.portalenergy.com/caddet/ee/R371.pdf

De Jong, P. and Verdurmen, R.E.M. 2001. “Concentrated and dried Dairy Products”. In *Mechanisation and Automation in Dairy Technology*, A.Y. Tamime and B.A. Law (Eds.). Sheffield Academic Press, 95 – 118.

De Jong, P., te Giffel, M.C., Straatsma, H., and Vissers, M.M.M. 2002. “Reduction in fouling and contamination by predictive kinetic models”. *International Dairy Journal.* No. 12, 285-292.

Luo, G. 1998. “Modelling of Evaporators in the Production of Milk Powder”. Research Report EE/JVR/98/1, School of Electronic Engineering, Dublin City University, Dublin, Ireland.

Kelly, A.L., O’Connell, J.E. and Fox, P.F. 2003. “Manufacture and Properties of Milk Powders”. In *Advanced Dairy Chemistry Part B Volume* 1: *Proteins*, P.F. Fox and P.L.H. McSweeney (Eds.). Kluwer Academic/Plenum Publishers, 1027 – 1061.

Kelly, J. and Kelly, P. 1999. “Ingredient Development Using a Pilot Scale Tall-Form Drier – A database of process operating conditions and physico-chemical

properties of resulting powder". Teagasc Project Report http://www.teagasc.ie/research/reports/
Masters, K. 1985. "Spray Drying Handbook". *George Godwin*, London.
Morris, C.E. 2001. "EngineeringR&D: Predictive Control Tightens Evaporation Results". *Food Engineering*, January. http://www.foodengineeringmag.com
Mujumdar, A.S. and Passos, M.L. 2000. "Innovation in Drying Technologies". In *Drying Technology in Agriculture and Food Science*, A.S. Mujumdar (Ed.). Science Publishers, 291-310.
Pavillion. 2003. "Fonterra Case Study – Achieving Real-time Performance Goals". *Pavillion Technologies* http://www.pavtech.com/pavilion/value/customer/nzmp.html
Perez-Correa, J.R. and Farías, F. 1995. "Modelling and control of a spray dryer: a simulation study". *Food Control*. Vol. 6, 219-227.
Písecký, J. 1997. "Handbook of Milk Powder Manufacture". Niro, A/S, Denmark.
Russell, N.T., Bakker, H.H.C. and Chaplin, R.I. 2000. "Modular neural network modelling for long-range prediction of an evaporator". *Control Engineering Practice*. No. 8 (Jan), 49-59.
Straatsma, J., Van Houwelingen, G., Steenbergen, A.E., and De Jong, P. 1999a. "Spray drying of food products: 1. Simulation model". *Journal of Food Engineering*. No. 42 67-72.
Straatsma, J., Van Houwelingen, G., Steenbergen, A.E., and De Jong, P. 1999b. "Spray drying of food products: 2. Prediction of insolubility index". *Journal of Food Engineering*. No. 42 73-77.
Van Wijck, M.P.C.M., Quaak, P. and van Haren, J.J. 1994. "Multivariable Supervisory Control of a Four-Effect Falling Film Evaporator". *Food Control*, Vol. 5, Issue 2, 83 - 89
Verdurmen, R.E.M. and De Jong, P. 2003. "Optimising Product Quality and Process Control for Powdered Dairy Products". In *Dairy Processing, Improving Quality*, G. Smit (Ed.). Woodhead Publishing, 333 – 365.
Zbiciński, I. 1995. "Development and experimental verification of momentum, heat and mass transfer model in spray drying". *The Chemical Engineering Journal*. No. 58, 123-133.

BIOGRAPHY

PETER CUNNINGHAM was born in Cork, Ireland. He attended Cork Institute of Technology where he studied Electronic Engineering and received his BEng and MEng in 2001 and 2003 respectively. In December 2003 he began work on a Ph.D. under the supervision of Dr. Barry O'Connor in the area of advanced control of evaporators and spray dryers at AMT Ireland in University College Cork.

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NIZO Premia and Premic
Off-line and in-line product and process control tools for the food industry

F. Smit
J. Straatsma
M.M.M. Vissers
M. Verschueren
P. de Jong
NIZO food research BV
PO Box 20,
710 BS Ede, The Netherlands
E-mail: Erik.Smit@nizo.nl

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Predictive modeling, premia, premic, model based process control, in-line, optimisation.

NIZO PREMIA

A substantial amount of the knowledge and experience of research institutes and universities never reach the food industry. The main bottleneck is the interpretation of the scientific research results. Thus the objective is to find a way to benefit the food industry by application of these results in practice. Experience with industrial projects shows that predictive kinetic models are an effective tool to apply state-of-the-art knowledge in the factory. NIZO food research has been working on predictive (kinetic) models for the food industry for the past 20 years. A lot of models have been developed covering a wide range of products and processes. Three types of predictive models can be distinguished:

1. Process models
   Describe process units in terms of model reactors (e.g. temperature vs. time profiles). Examples are: models for holding tubes, (plate) heat exchangers, falling film evaporators, spray drying, etc.
2. Product models
   (mostly) Kinetic predictive models that describe the transformation of food components and contaminants.
   For example: Models to predict the concentration of micro-organisms in final products as a result of growth, adherence, release and inactivation in process equipment, kinetic models that predict the transformation of food components related to the food properties recognised by the consumer (e.g. viscosity, colour, shelf-life) etc.
3. Cost models
   Predictive models that estimate the effect of process operation on production costs.

NIZO food research has developed a user friendly, dynamic and flexible software framework (see figure 1), called NIZO Premia, that can be used behind an office desk (off-line) to calculate product properties and optimise process condition.

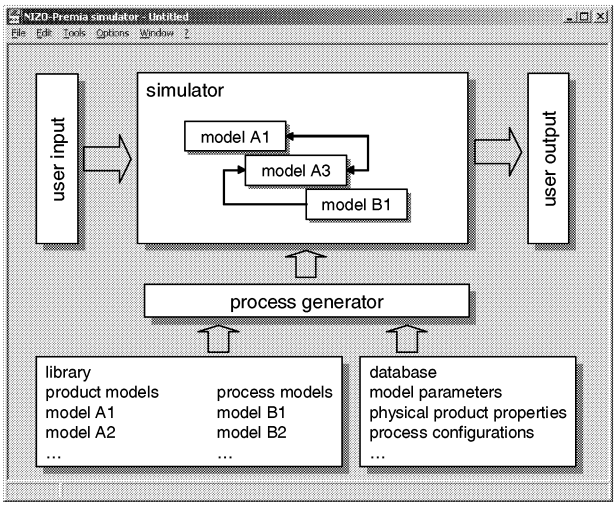

Figure 1. Schematic representation of the NIZO Premia framework

NIZO Premia (PRedictive models ready for industrial application) makes it possible to use all predictive models and combined them with each other to generate a production chain (see figure 2).

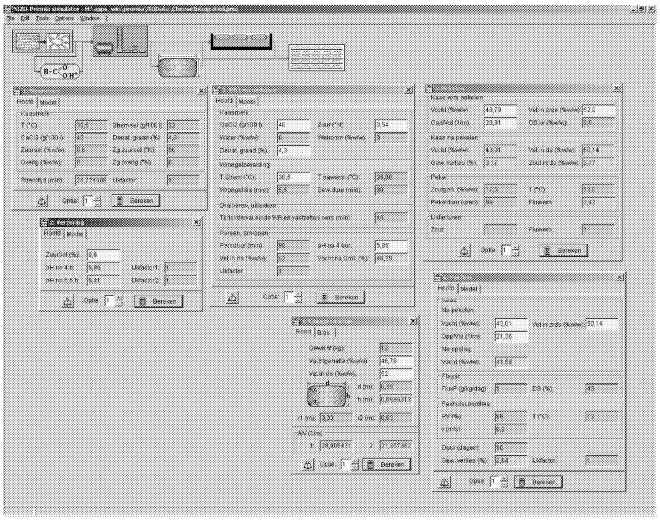

Figure 2. Production process of cheese predicted with NIZO Premia

Processes can be defined via the process generator (see figure 3) and kinetic parameters can be defined via the product editor (see figure 4).

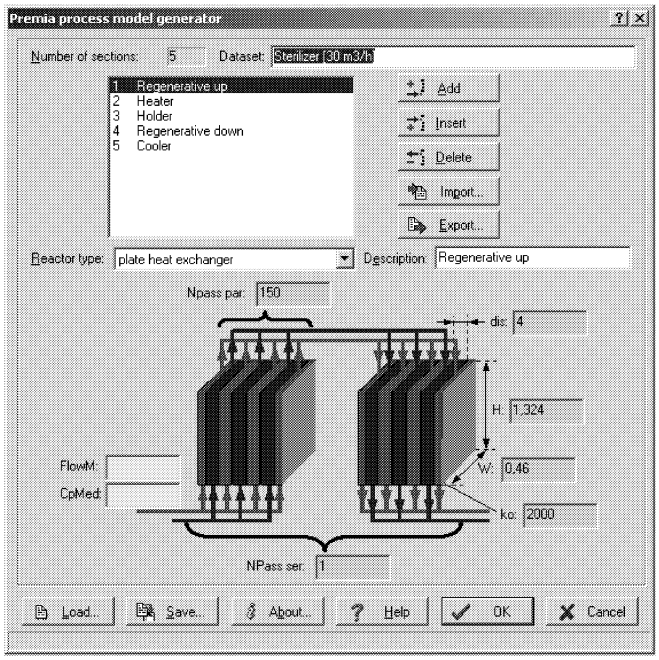

Figure 3. Process generator within NIZO Premia

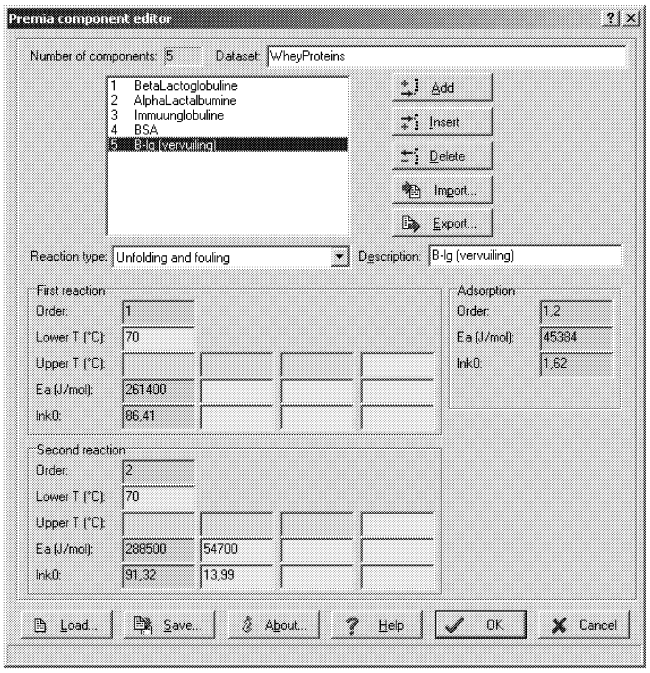

Figure 4. Component editor within NIZO Premia

Simulation results can be displayed in graphs and tables (see figure 5).

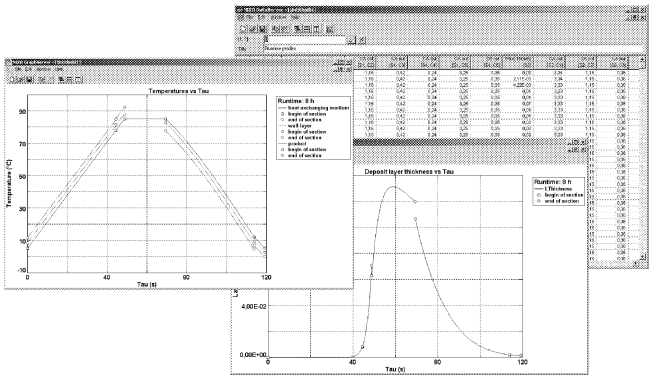

Figure 5. Graphs and tables generated with NIZO Premia

PREMIC

Predictive models as described turn out to be an effective tool to translate scientific knowledge to practical applications in the food factories. The most effective way to ensure the benefits of using predictive models would be to integrate them into process control systems.
NIZO food research and Honeywell have developed a model based process control (MBC) system that is focused on optimal product quality and minimal operating costs, called Premic (PREdictive Models ready for Industrial Control). Based on actual process data and the composition of the raw materials the models can predict the state of the process (e.g. amount of fouling, bio-film thickness, energy usage) and the state of product (degree of contamination, stability, texture). This means that the process can be controlled on product specifications instead of process conditions. In figure 6 the Premic system is shown in general terms. Based on process design (e.g. dimensions, apparatus configuration, in-line measured process conditions) the temperature-time history of the product is calculated with the process model. Together with the given composition this information is used to predict the product properties using the kinetic product models. The predicted product properties are compared with the given desired product properties. In the optimization module the production process set points are optimized to meet the desired product properties as closely as possible with minimum operating costs.

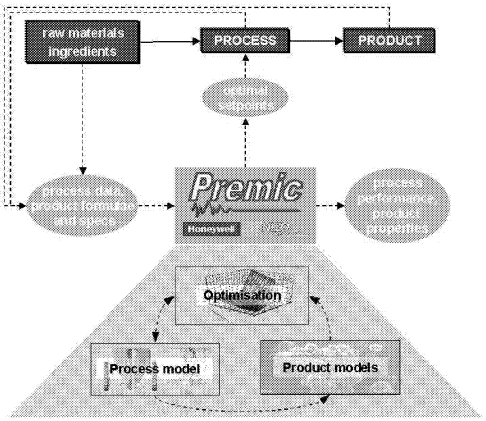

Figure 6. Schematic representation of predictive-model based process control in food processing

A screenshota of the system is shown in figure 7.

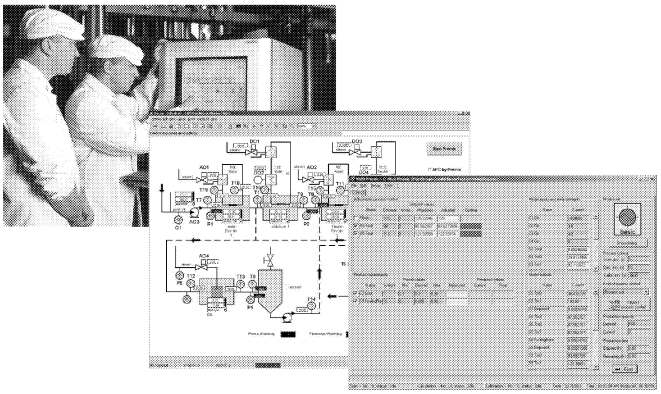

Figure 7. Screenshots of the Honeywell-NIZO food research product Premic

In the ideal situation the process is controlled based on the desired product specifications and minimum operating costs. The system corrects itself automatically when:

- Fouling changes the temperature-time history of the product
- The product specifications change
- The composition of the raw materials changes
- Disturbances occur (e.g. temperature changes, flow instabilities)

The estimated savings amount up to 10-50 % in production costs.

TOWARDS AN ON-LINE MEASUREMENT OF THE PURITY CANE SUGAR FACTORY JUICES : A NEURAL NETWORK APPROACH

Teddy Libelle, Michel Benne , Brigitte Grondin-Perez, Jean-Pierre Chabriat
Industrial engineering laboratory
University of La Réunion
15, av. R. Cassin, B.P. 7151, 97715 Saint-Denis,
Messageries Cedex
France
E-mail: teddy.libelle@univ-reunion.fr

KEYWORDS
conductivity, purity, sugar juice solution, on-line measurement, neural model, multiple polynomial regressions, sugar factory.

ABSTRACT

For most semi-batch crystallization processes, it is still difficult to obtain on-line relevant information about the purity of sugar solutions. Consequently, very few studies dealing with the estimation of the purity, can be found in the literature.
Therefore, the aim of this article is to present a new method of on-line measurement of the purity of cane sugar factory juices used during the crystallization.
The first results reinforce the reliability of neural networks and recommend their uses to estimate the purity which is a significant parameter when dealing with crystallization supervision.

INTRODUCTION

As part of developments we have undertaken in collaboration with Bois Rouge sugar mill (BRSM, groupe USDA, Saint-André, La Réunion), we are aiming at optimising two manufacturing processes: crystallization (Lauret et al. 2000) and evaporation (Benne et al. 2000). This aim is based partially on the improvement of the supervision of the physicochemical variables representative of the processes evolution, among which the purity of the cane sugar factory juices constitutes an essential parameter. Traditionally, this information is measured in laboratory from manually taken samples. Two major constraints result from this:

- the duration of the analysis and the number of products limit the multiplication of measurements;
- the out-line measurements are available only after an analysis time incompatible with an automated management.

Based on the works of (Dubourg 1938), (Ponant and Nebout 1962), (Parker 1958; Parker 1959) and (Ponant and Windal 1976), a neuronal estimator of the purity was developed. While breaking sampling constraints, this soft sensor allows to estimate the purity from on-line measurements: brix, conductivity and temperature.

This paper describes the implementation of the neural model and presents the results of a soft sensor tested in simulation from experimental data.

PROPOSITION OF A SOFT SENSOR FOR THE MEASUREMENT OF THE PURITY OF CANE SUGAR FACTORY JUICES

The limits of the conductimetric method for on-line measurement

Ponant and Windal's works show that the conductivity y of a sugar solution at a given temperature T is expressed according to the brix Bx and the purity P :

$$y = A\left(100 - P\right) x\, e^{-c\, x^{n}} \qquad (1)$$

$$x = \frac{B_x}{100 - B_x}$$

$$P = 100 - k\, y^{T}_{max}$$

Several conductimetric studies undertaken in laboratory from BRSM solutions show that n depends on the temperature and the purity, just as y^{T}_{max}, A, c (Ponant and Windal 1976) and k (Pidoux 1961). Under these conditions, the correlation (1) does not allow to deduce P from the measurements of y, Bx and T.
The introduction of complementary relations (Ponant and Windal 1976) allows to directly express y according to Bx, T, P and n (correlation (2), figure 1):

$$y = \frac{y^{T}_{max}}{x_{y^{T}_{max}}} x \exp\left(\frac{1}{n}\left(1 - \left(\frac{x}{x_{y^{T}_{max}}}\right)^{n}\right)\right) \qquad (2)$$

$$x_{y^{T}_{max}} = \frac{Bx_{y^{T}_{max}}}{100 - Bx_{y^{T}_{max}}}$$

$$B_{x y^{T}_{max}} = 29.100 + 0.096\ T - 0.027\ P$$

$B_{x y^{T}_{max}}$ is the value of the brix for a temperature T and a purity P with maximum conductivity (Ponant and Windal 1976).

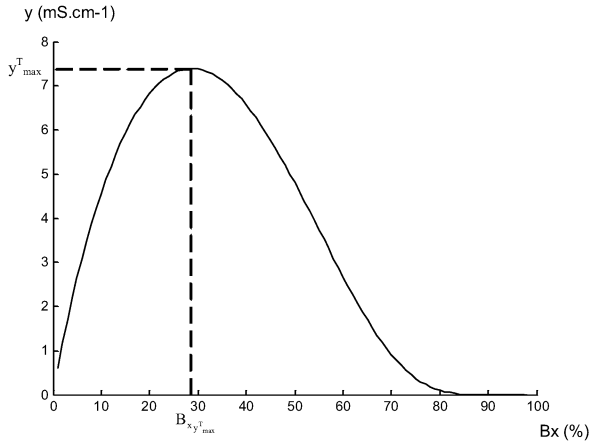

Figure 1: Example of representation of the evolution of conductivity according to the Bx.

The correlation (2) being not reversible, we propose to identify a function P(Bx, T, y) from a systemic approach. Established on line, the identified model constitutes a soft sensor of purity (Cheruy 1998).

Soft sensor structure

It is a neural network (NN) of perceptron type with 3 inputs, a hidden layer allowing a number of sigmoid neurons to define and 1 output. Figure 2 represents the neuronal model with brix, temperature and conductivity measured on-line as inputs, and purity as output.

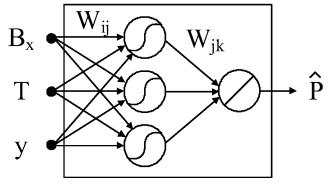

Figure 2: Neural model with three inputs and one ouput

IMPLEMENTATION OF THE SOFT SENSOR

The identification of a neural model is based on the estimation of the weighting coefficients Wij and Wjk (Figure 1) from example databases {inputs, outputs}, it is the learning phase. To have a sufficient number of available informations, we generated a learning database from experimental and simulated data.

Identification of y^{T}_{max} and n as polynomial function

This step is based on experimental values of Bx, T, y^{T}_{max} and P measured from about 50 factory juices. Thus, the identification of y^{T}_{max} and n as functions of T and P is realised by multiple polynomial regression at order 4, according to the nonlinear canonical analysis (Makarenkov and Legendre 1999).

Generation of database

A 3000 samples database of the triplet [Bx, T, P] has been generated from the Monté Carlo method. For that, we have realised a draw at random, for each one of these variables, according to a normal law. Table 1 gives the $(\overline{x})$ average and the (σ) standard deviation of these distributions for the brix, the temperature and the purity.

Table 1: The $(\overline{x})$ average and the (σ) standard deviations of brix, temperature and purity.

	$\overline{x}$	σ
Bx (%)	70,3	11,6
T (°C)	69,9	5,8
P (%)	72,6	13,0

This choice of $(\overline{x})$ and (σ) allows to have values of Bx, T and P representative of the experimental conditions observed in sugar factory. Thus, we calculate y^{T}_{max} and n corresponding to 3000 examples of couple [T, P] from multiple polynomial regression at order 4 (Makarenkov and Legendre 1999).
The relation (2) gives the value of y for these 3000 samples (Figure 3).

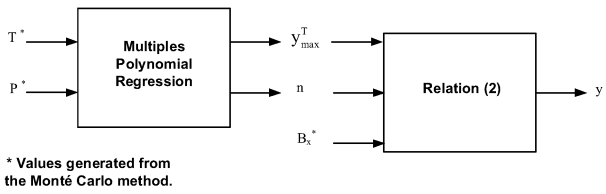

Figure 3: Summary diagram to obtain the conductivity

Identification of the Neural Network (NN) model

The training (learning and validation) phase of the NN model is based on 2000 samples of the simulated database. The selected architecture presents 12 hidden neurons with a MQE (Mean Quadratic Error) of about 0.002.

TEST OF THE SOFT SENSOR IN LABORATORY

Tested in simulation on the 1000 other samples of the generated database, and on experimental values measured in laboratory, the NN model presents good capacities of generalization.
For example, applied to a cane sugar factory juices of purity 53.3 %, brix 68.5 %, conductivity 11.12 $mS.cm^{-1}$ and temperature 65°C, the prediction model gives a value of purity equal to 52.7 %, which represents a relative error of 1.21 %.
Table 2 and figure 4 show the comparison between experimental measurements and model estimations for a set of factory solutions (constant purity: 53.3 %).

Table 2: Comparison between experimental (P_exp) and model (P_model) values.

Bx (%)	P_exp (%)	P_model (%)
68.5	53.3	52.0
65	53.3	51.9
63	53.3	51.9
60	53.3	53.4
58	53.3	53.6
55	53.3	52.6
53	53.3	52.2
50	53.3	52.2
48	53.3	52.1
45	53.3	53.8

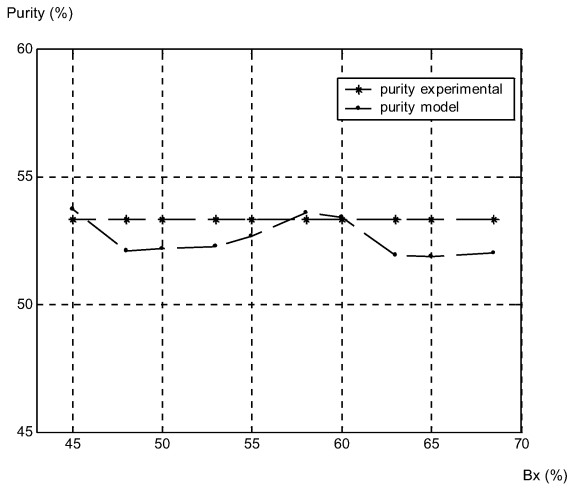

figure 4: Comparison between experimental and model purities.

We can note that the estimated values are very close to the experimental measurements.
More generally, the study of the error distribution shows that around 75% of estimations present less than 2 points of purity (figure 5). Furthermore, the best results concern the value from 50% to 75% (figure 6, table 3), which corresponds to our interval of interest.

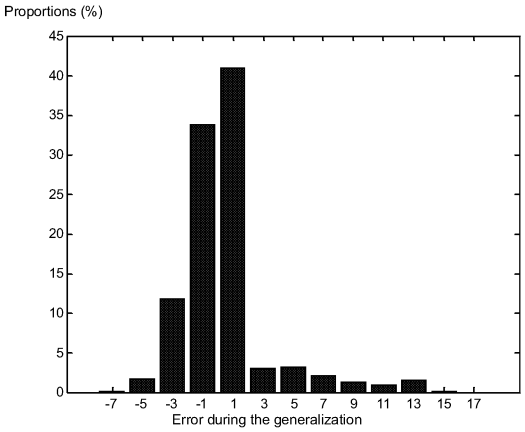

Figure 5: Error values during the generalization step.

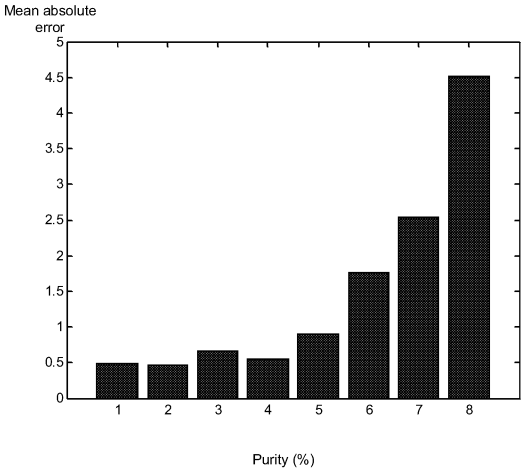

Figure 6: Evolution of the mean absolute error associated with intervals of purity

Table 3: Correspondence between the abscissa values and the purities values.

abscissa values	Purity (%)
1	[50;55[
2	[55;60[
3	[60;65[
4	[65;70[
5	[70;75[
6	[75;80[
7	[80;85[
8	[85;90[

CONCLUSION

In sugar factory, the lack of experimental information, among which the purity of feeding solutions (syrup, standard liquors, molasses, etc), hinders the development of computerised supervision and control applications. The proposed soft sensor giving good estimations from the generated database, we plan on-line validations *in situ* (July 2004). Its introduction at Bois Rouge should eventually allow to solve mass balances taking into account this essential parameter, all along the production line.

REFERENCES

Benne, M.; B. Grondin-Perez; J.-P. Chabriat; and P. Hervé. 2000. "ANNs for modelling and predictive control of an industrial evaporation process". Journal of Food Engineering, 2000, vol. 46, 227-234.

Cheruy A. 1998. "L'apport de l'automatique dans la maîtrise des procédés agro-alimentaires illustré par une étude de cas industriel en brasserie". Industries alimentaires et agricoles, 17-20.

Dubourg J. 1938. "Considérations théoriques sur la relation entre la conductibilité électrique et la teneur en cendres d'une solution sucrée ". Journal des fabricants de sucre, 201-202.

Lauret, P.; H. Boyer; and J.-C. Gatina. 2000, "Hybrid modelling of a sugar boiling process". Control Engineering Practice, 8, 299-310.

Makarenkov, V. and P. Legendre. 1999. "Une méthode d'analyse canonique non-linéaire et son application à des données biologiques". Mathématiques, informatique et sciences humaines, 37e année, No. 147, 135-147.

Parker Wm. H. 1958. "The determination of the apparent purity of beet sugar factory juices and syrups, Part I". British Sugar Corporation Ltd, 355-357.

Parker Wm. H. 1959. "The determination of the apparent purity of beet sugar factory juices and syrups, Part II". British Sugar Corporation Ltd, 9-13.

Pidoux G. 1961. "Mesures conductimétriques sur les sirops de sucre à haute pureté". Zucker, 10-14.

Ponant, J., Nebout, J.P. 1962. "Considérations sur la détermination de la pureté des solutions sucrées par méthode conductimétrique". Sucrerie Française, 429-434.

Ponant, J., Windal, G. 1976. "Mesure en ligne de la pureté en sucrerie". Industries alimentaires et agricoles, 869-877.

BIOGRAPHY

TEDDY LIBELLE was born in La Réunion, France, and continued his scientific studies at the University of La Réunion. He obtained his master's degree in 2000. Since 2002, he's studying food sciences for his PhD in the industrial engineering laboratory (L.G.I : Laboratoire de Génie Industriel), with the A.C.T.E.S team (Analyse et Contrôle des Transferts Energétiques et des Systèmes).

Tel. 0262 938679 (en France)
(+262) 262 938679 (from abroad)
fax. 0262 938673 (en France)
(+262) 262 938673 (from abroad)

MODELING AND CONTROL OF A PH NEUTRALIZATION PROCESS : FUZZY CLUSTERING WITH A GUSTAFSON-KESSEL'S ALGORITHM AND TAKAGI-SUGENO TYPE OF FUZZY MODEL.

S. Beyou, B. Grondin-Perez, M. Benne, and R. Lorion
Industrial Engineering Laboratory, Faculty of Sciences and Technologies
University of La Reunion
15, Av. R. Cassin, BP 7151, 97715 Saint-Denis,
France
E-mail address : sebastien.beyou@univ-reunion.fr

KEYWORDS
Fuzzy logic, Fuzzy clustering, Gustafson-Kessel's algorithm, pH-neutralization.

ABSTRACT

Inherent time-varying nonlinearity and complexity usually exist in chemical processes. The main objective of this study is to propose a full approach for the modeling and control of a pH neutralization process thanks to fuzzy logic. The Gustafson and Kessel's fuzzy clustering algorithm is an interesting way to modelize a non linear process such as the pH neutralization process. Presented results will show the efficiency of this approach.

INTRODUCTION

Since the beginning of our collaboration with local industries in Reunion Island, our aim and main objective is to perform the processes using new technologies. Industrial sugar processes are complex and need high performance control and modelisation strategies. Fuzzy logic is one of the tools that we propose to use.Since its introduction in 1965, fuzzy set theory has found applications in a wide variety of disciplines. Modeling and control of dynamic systems belong to the fields in which fuzzy set techniques have received main interest, not only from the scientific community but also from industry. (Babuška 1998; Grisales 2002). The purpose of our work is to evaluate this modelling. We have chosen to apply fuzzy techniques to an academic problem : pH neutralization process which presents strong non linearity. This paper is organized into three parts : In a first part, we present the fuzzy modeling concepts through the main models we use. In a second part, we introduce the fuzzy clustering principle with *c-mean* and Gustafson-Kessel's algorithms. Finally, we present an application and its results on pH neutralization process.

CONCEPTS OF FUZZY MODELING SYSTEM

L.A. Zadeh, the father of the fuzzy logic, was the first to suggest the employment of fuzzy sets to describe complex systems. This description, like human reasoning, is based on IF-THEN type of rules (If *Antecedent* Then *Consequent*). The considered information accepts, due to a special treatment, uncertainty and/or imprecision, and imitate mechanisms of apprenticeship. Fuzzy modeling of systems is typically divided into two categories which differ from their capacity to represent different type of information. The first one is the Mamdani's type of model, based on experiment, the second one, is the Takagi-Sugeno's type of model, which is suitable to a data based approach (Babuška and Verbruggen 1996). Equations (1) and (2) give the rules respectively used by the different models.

Rules of a Mandani's type of model : (1)

$$R^i : If \quad \{(X_1 \ is \ A_1^i) \ and \ (X_2 \ is \ A_2^i) \ and \ ... \ and \ (X_m \ is \ A_m^i)\}$$
$$Then \quad (Y \ is \ B_n^i)$$

Rules of a Tagaki-Sugeno' type of model : (2)

$$R^i : If \quad \{(X_1 \ is \ A_1^i) \ and \ (X_2 \ is \ A_2^i) \ and \ ... \ and \ (X_m \ is \ A_m^i)\}$$
$$Then \quad (Y^i = f^i(X_1,...,X_m))$$

FUZZY CLUSTERING AND IDENTIFICATION

Cluster analysis is the classification of objects according to their similarities and the arranging of data in groups. Clustering techniques, suitable for quantitative and/or qualitative data, determine the belonging of an object in a group simultaneously and attribute level of adherence. The similarity of these objects is defined as the mathematical concept expressed with a norm of the distance between : the data row vectors, or a data row vector and a prototype object of the group. (Grisales 2002).

Fuzzy clustering c-means : FCM

Most of the fuzzy clustering algorithms are based on this type of clustering. It uses reciprocal distance (induced norm) to compute fuzzy weights(Bezdek 1981). The idea of FCM consists in using the weights that minimize a total weighted mean-square error.(Zhang 2001).

Clustering with fuzzy covariance matrix

To detect different geometrical forms of the clusters in the space of data, Gustafson and Kessel have extended the FCM algorithm with an adaptative norm of the distance. Thus, each group has its own induced norm matrix which are used as optimization variables in the *c-means*

functionals to enforce each group to adapt their distance norm to the local topological structure of the data.

IDENTIFICATION OF A PROCESS, APPLICATION TO A PH NEUTRALIZATION REACTOR

We use a knowledge based model (mathematical model) of the pH neutralization process to simulate the output of the process at each sample time. Then we identify the Takagi-Sugeno's type of fuzzy model using these data. The simulation and calculation platform is the software Matlab® v6.5 and the Fuzzy Model IDentification Toolbox (FMID Toolbox).(Babuška 1998).The knowledge model has been developed and validated by Saint Donat. (Saint-Donat et al 1991).

Description of the process

The process that we have simulated (*Figure 1*) is a mix of acid (acetic acid) and base (sodium hydroxide) in a reactor perfectly agitated in a constant volume. The knowledge model depends on, firstly, the water ionic product, secondly, the acidity constant and thirdly on the different material assessments of acetate and sodium (Grondin-Perez 1994). Thus, pH neutralization process would be assumed as an academic problem of non-linearity.

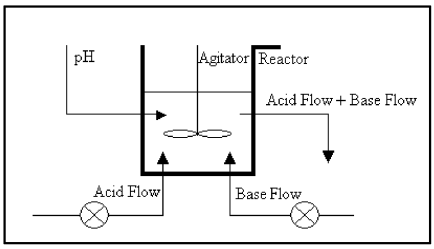

Figure 1 : Continuous Stired Tank Reactor.

Applying identification

The process is a SISO system, with sodium hydroxide flow as an input (acid flow is constant) and pH as an output. We choose following values (*Table 1*) (Grondin-Perez 1994) :

Acetic acid dissociation constant	K_a = 1.8e-05
Water dissociation constant	K_w = 1e-14
Volume of the Reactor in liters	V = 1000
Flow rate of acid in liter/min	D_a = 81
Acid/Base concentration in mol/l	C_a = 0.3178, C_b = 0.05
Sample time	T_e = 40 seconds

Table 1 : Parameters of the pH neutralization reactor.

The learning data base is composed with a set of normalized commands between 0.1 and 0.9 with a precision of 2e-4 and of the corresponding outputs. This represents a data base of 5000 inputs and outputs (Top of the *figure 2*).

Takagi-Sugeno's type of linear modeling

Using the input-output data base (flow of sodium hydroxide– pH) and FMID Toolbox, we have identified the model with the Gustafson and Kessel's algorithm. Parameters of the fuzzy model are as follows (*Table 2*) :

Fuzzy parameter	$m = 2$
Number of clusters (rules)	$c = 9$
Antecedent : type of calculation	*ante* = 2 (projection)
Stop criterion of the algorithm	*tol* = 0.01
Number of input/output/pure delay	$n_u = 1$, $n_y = 0$, $n_d = 0$

Table 2 : Parameters of the fuzzy model.

Local models of the consequent and membership functions of the antecedent are represented *Figure 2*, generated from the data base, and the Takagi-Sugeno's type of rules which describe the dynamic of the system. There, we can see that the fuzzy model is a piecewise linear representation of the input-output database (Top), with their membership levels (Bottom).

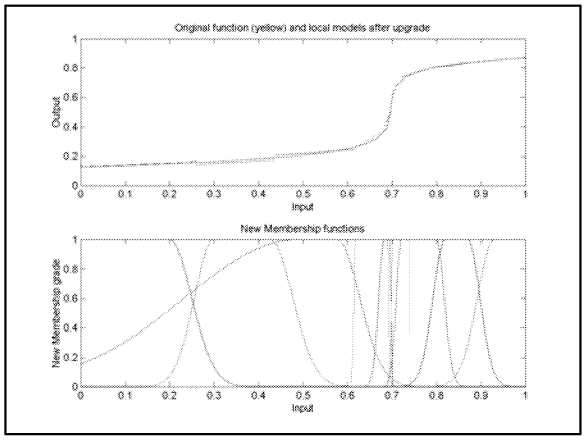

Figure 2 : Local models of the consequent and membership functions of the antecedent.

If u is A_1 **then**
$y(k) = 1.16*10^{-1}u+1.27*10^{-1}$

If u is A_2 **then**
$y(k) = 1.88*10^{-1}u+9.22*10^{-2}$

If u is A_3 **then**
$y(k) = 2.24*10^{-1}u+1.09*10^{-1}$

.
.
.

If u is A_9 **then**
$y(k) = 2.67*10^{-1}u+6.05*10^{-1}$

Table 3 : Example of Rules

We choose linear functions of the consequent, f^i :

$$R^i : If \quad (X \quad is \quad A^i) \qquad (3)$$
$$Then \quad Y^i = a_i^T X + b_i$$

With, $a_i \in \Re^m$ a row vector of parameters and b_i, the offset. This is the Takagi-Sugeno's linear type of model. Here, the main interest is to decompose a non linear behaviour into local linear sub models.

VALIDATION OF THE MODEL

Validation of the fuzzy model is based on the evaluation of the error between the output of the knowledge model, considered as a reference of the model, and the output of the fuzzy model in response to the same input. This response is a 5000 points long PRBS (pseudo-random binary sequence). A variance based criterion (4), where Y is the ideal output and Y_m the prediction of the fuzzy model, gives the following result : 96.5 %. This is near of the ideal result : 100%.

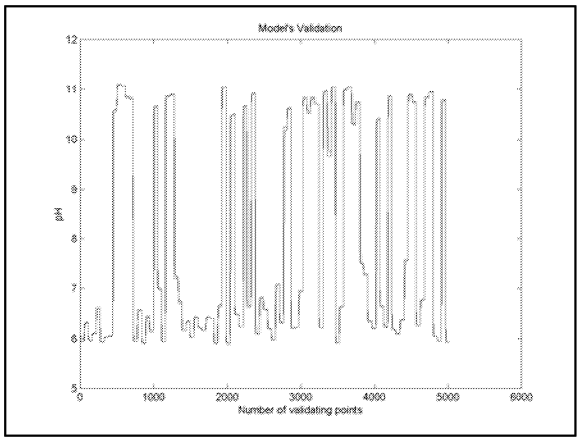

Figure 3 : Comparison between the output of the process and the output of the fuzzy model.

This figure (*Figure 3*) illustrates the comparison between the output of the process (mathematical model) and the output of the Takagi-Sugeno's type of fuzzy model. Variance based criterion : (Babuška 1998).

$$VAF = 100\% \frac{[1-(\mathrm{var}(Y-Y_m))]}{\mathrm{var}(Y)} \qquad (4)$$

SYSTEM CONTROL

To control this system, we have used a forward fuzzy model (*Figure 4*) :

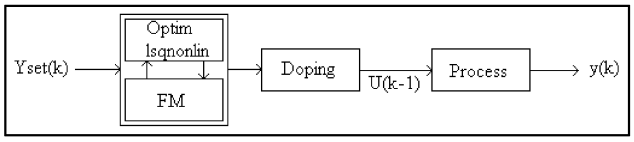

Figure 4 : Scheme of the control system.

We have used of *lsqnonlin* which does belong to the Optimization Toolbox of Matlab as the optimization algorithm. This algorithm, based on the Levenberg-Marquardt method, minimizes a least square non linear criterion. The doping module is a simple algorithm which increases (dopes) variations of the command. Results are presented in *Figure 5*.

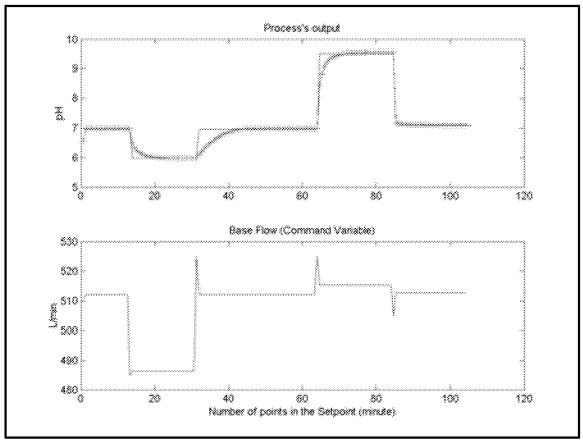

Figure 5 : (Up) Setpoint consign and output of the process, (Down) Corresponding command.

On this figure (*Figure 5*) we can see that the setpoint consign, continuous line, is well followed by the output of the process (++). To validate the control of the system, we use the variance based criterion, the result is 97%, and this is an excellent result.

CONCLUSION AND PERSPECTIVES

The use of the fuzzy clustering technique with Gustafson-Kessel's algorithm has allowed to group a set of data generated by a non linear system into a fuzzy set of data which locally describe linear models. Takagi-Sugeno's type of models is built by a concatenation of its local sub models. The results show that this modeling is very efficient, and does present the benefit of allowing an easy way to control the system. The application of these techniques to a pH neutralization process has shown its efficiency. The main objective of this work was to test these methods on an academic problem presenting interesting nonlinear properties in order to be able to apply them on food industry processes. Perspectives of future works could be oriented through different kind of process control structure, particularly those based on inverse fuzzy models, but also through the use of other optimization methods, like genetic algorithms.

BIBLIOGRAPHY

Babuška, R. (1998). "Fuzzy Modeling and Identification Toolbox for use with Matlab."

Babuška, R. and H. B. Verbruggen (1996). "An Overview Of Fuzzy Modeling For Control."

Bezdek, J. C. (1981). "Pattern Recognition with Fuzzy Objective Function Algorithms." (Plenum Press, New York).

Grisales, V. H. (2002). "Identification et modélisation floue de type Takagi-Sugeno pour un bioréacteur anaerobie."

Grondin-Perez, B. (1994). Les réseaux de neurones pour la modélisation et la conduite des réacteurs chimiques : simulations et expérimentations. Bordeaux, Université de Bordeaux I.

Saint-Donat and al (1991). "Neural net based model predictive control." Int. J. Control **54**(6): 1453-1468.

Zhang, L. (2001). "Comparison of Fuzzy c-means Algorithm and new Fuzzy Clustering and Fuzzy Merging Algorithm."

CHALLENGES IN MODEL APPLICATIONS

Management of innovation in the life science industries

By
Felix Janszen
Professor in Management of technology and Innovation
at the EUR and WU
Managing director of Inpaqt b.v.

General introduction

While according to Kumpe and Bolwijn (1990) the emphasis in the sixties was on efficiency, in the seventies on quality, and in the eighties on flexibility, since the nineties the emphasis has been on innovation. So without exaggerating we can say that we are living in the age of innovation.

Successful innovations rarely take place in a single instant. They evolve while they are being developed in the laboratory, drawing room or work floor, or in contact with customers during the market development stage. Using the innovation provides an understanding of its strengths and weaknesses. The weaknesses will lead to further improvements and a constant flow of innovations. Innovations are the result, then, of the actions of a number of players and can be seen as a process rather than a single event.

An innovation can be defined, according to the Austrian economist Schumpeter, as a new combination that may consist of the following elements:

1. The application of a new technology in an existing product, service or process
2. The introduction of a new application in the form of a product, service or process using a new combination of technologies
3. The opening up of a new market to an existing or new product or service
4. The introduction of a new organizational form, work method or market approach

All these 4 elements, new technologies, new applications, new markets and adaptations of the organization, will contribute to the innovation during its trajectory from start to maturity.
To innovate successfully therefore knowledge about all 4 elements have to be used and integrated. Nobody masters all these fields. You need a team of people with different expertise that complements each other. Innovation is therefore very much a team play.

The innovation process

In most organizations there is no lack of ideas. Despite this, very few ideas eventually make it to the market. In most organizations the level of innovativeness is disappointingly low. An important question is how to ensure that more ideas are successful.

The innovation process can be seen as a knock-out race, with only 1 in 10 ideas at best reaching the market. Within the general innovation process (the funnel) we can place a mortality curve. A mortality curve is given its shape by positioning the number of ideas against the development timeline. Most ideas are quickly abandoned while they are being developed into a product concept.

This is presented in the figure on the following page.

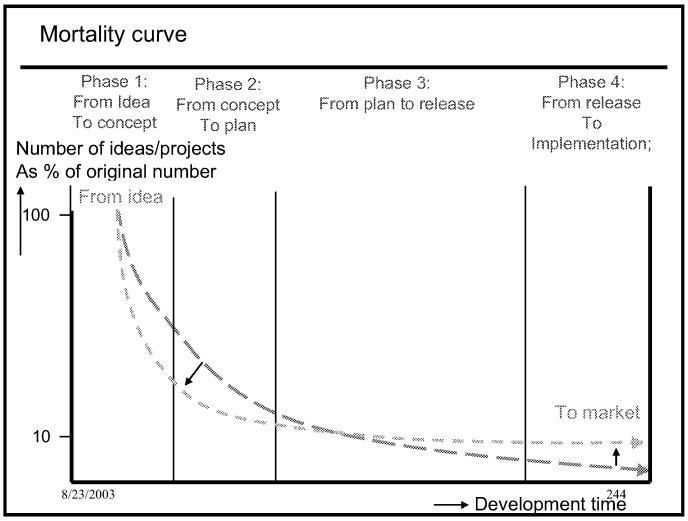

Figure 1. Mortality curve

I

n this figure we have divided the development of the idea into a product or service into 4 phases: 1. from idea to concept, 2. from concept to plan, 3. from plan to the release of the product or service, and 4. from release to market introduction. Most companies adhere to a similar phased approach to the development process, with the first 2 phases being considered pre-project phases that precede the actual development.

Research indicates that some 10% of all ideas are eventually realized. A mere fraction of the ideas that make it to the implementation stage succeeds in achieving its commercial objectives. This knock-out race is indicated in red in the mortality curve presented above.

If we could decide at an earlier stage which ideas are viable, this would enable us to divert more means to their development. This would also increase the likelihood of an idea being commercially successful. This is indicated in green in the mortality curve presented above.

The possibility of improving the mortality curve depends on the speed with which we are able to collect information regarding the idea and use that information to formulate a go/no-go decision.

If we manage to speed up and broaden the collection of information at the start, this may lead to a swifter and more informed go/no-go decision, and consequently result in better plans with regard to the remaining projects. This means we are looking for a way to shift the red curve towards the green one.

We can do this by improving the information gathering or learning process in the various phases. It is important to note that the kind of learning process taking place in the *first* two phases is different from the one taking place in the *last* two phases. In the first two phases we have to get an idea of the customer. Which customers are interested in the product idea (market definition and market segmentation)? What will determine whether or not he or she will buy the product (customer value proposition)? What product attributes and functionalities will create customer value (product definition)? Who are the competitors and what are they offering (competitor analysis)?

When general answers to these questions are provided, the question arises at which price the product or service can be offered (revenue model). What is the size of the market? What are the production costs? What investments have to be made in terms of development and production facilities? What are the costs involved in distribution and market development (cost-benefit analysis and defining the business case)? It is only when these questions are answered that a company can decide

whether or not the idea is financially interesting.

To answer these questions it is not enough just to gather data. We need a model to interpret the data and transform them into useful information. First we have to make a model of the customer, the market and the development and production process, before we are in a position to come up with the questions that will enable us to collect information regarding the customer, market and competition.

At the beginning a model of the product and customer will be drawn up, which if needed can be adjusted in discussions with others (articulation and modelling). After developing the model we will have to start by validating this model that we have concerning the customer, market, production process, etc.

This takes place through qualitative customer and market research. After that research is conducted we collect the data through qualitative research and use them as input for our model. We can then see if we can offer the customer better value than our competitors, whether the margin is large enough and the expected market share sufficiently big to turn the project into a commercial success.

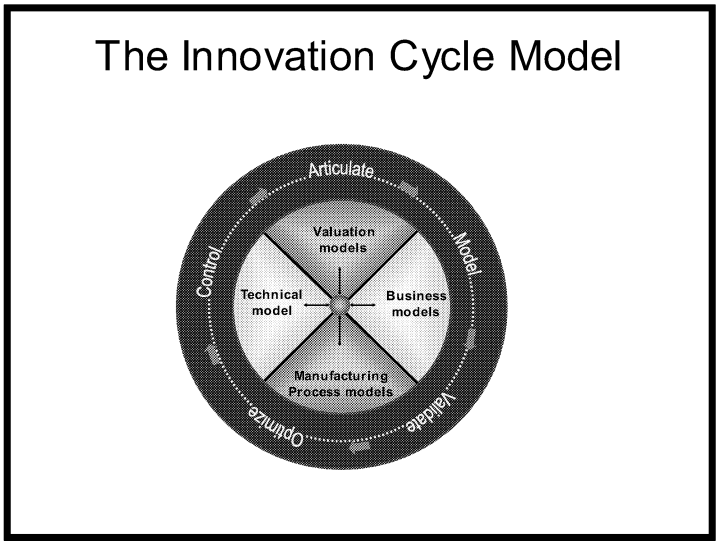

Figure 2. The innovation cycle model

The drafting, validation, and adjustment of the product, customer, market and production process models are called “double loop learning”. When we have developed the right models it is merely a matter of optimization. This means that the product characteristics are defined in such a way as to achieve maximum customer value, in a market segment that is sufficiently attractive, at an attractive price and at minimal production costs. Optimization ideally takes place during the development phase. This learning process is called “single loop learning”.

Technicians are trained in the methods and techniques to set up and test technical models perfectly[1]. Marketers are trained to do the same for customer and market models, as do production staff for the production and business models, while controllers use their own specific financial models to determine whether or not a project is still commercially attractive. To draw up a valid financial model, however, information from the production and commercialization models is needed, and vice versa. In addition, during the optimization of the product features and process there will be a continuous need for feedback from the market as well as financial calculations. In other words, the models have to match. This is presented in the figure above.

[1] They often start with rough outlines, which become increasingly specific. Later on the graphic models are transformed into physical models, often via computer models that are used to conduct various simulation tests.

"Drafting, validating and filling these models has to take place in a multifunctional team"

These models must provide us with answers about the questions such as about market segmentation, customer value proposition, market positioning cost benefit analysis etc can be answered.
In the figure below we have defined these questions and mapped these to the various types of models such as technical-, business-, organizational- and valuation c.q. decision models.

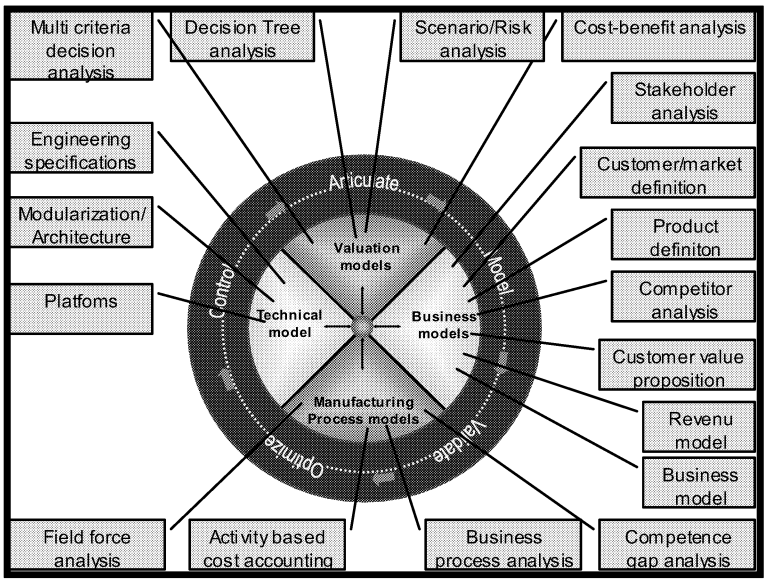

Figure 3. Relations between business issues and the innovation cycle model

In this course we will discuss most of the models as pictured in the figure above. Therefore at this moment we will only mention these topics but not define them.

In addition, criteria have to be defined to monitor the project's progress and the decision-making process concerning the termination, continuation or adjustment of the innovation (valuation c.q. decision models). In general, these criteria will be deduced from a long-term vision and strategic plan. On the other hand any knowledge gained in the course of the project has to lead to adjustments of the strategy and even the long-term vision. Innovation teams have to be able to respond quickly to opportunities. A large degree of self-management is needed to give entrepreneurship and innovation a chance.

Whereas in the pre-project phase the development and validation of concepts through the drafting of interrelated business-technical and financial and economic models is essential, in the development phase it is of vital importance to use these models to optimize product and market positioning. In the implementation and commercialization phases it is necessary to check continuously whether or not the assumptions on which the models were based are still valid. This makes it possible to respond quickly to any changes in customer wishes, competition, economic bottlenecks etc. Such changes may lead to a new phase in the innovation process.

We are slowly approaching the area of Innovation Management. We have discussed the cyclical nature of innovations as well as the central role played by the various models. We will now introduce and briefly discuss the Innovation Management Cycle model. The Innovation Management Cycle Model® provides a general framework within which the above-mentioned processes and issues regarding Innovation Management can be placed.

Innovation Value Chain and Innovation management

The Innovation Value Chain

Main activities of Innovation Management
Within Innovation there are three main activities: 'innovation', 'development' and 'commercialization'. 'Innovation' refers to all activities aimed at combining various observations into ideas that can then be transformed into concepts or models, and eventually be developed into business plans. 'Development' has to do with the development of plans into products/services and process within the established frameworks concerning quality, time, costs and scope. 'Commercialization' is all about the activities that are needed to maximize the added market value of the products/services during the various stages of their life cycles.

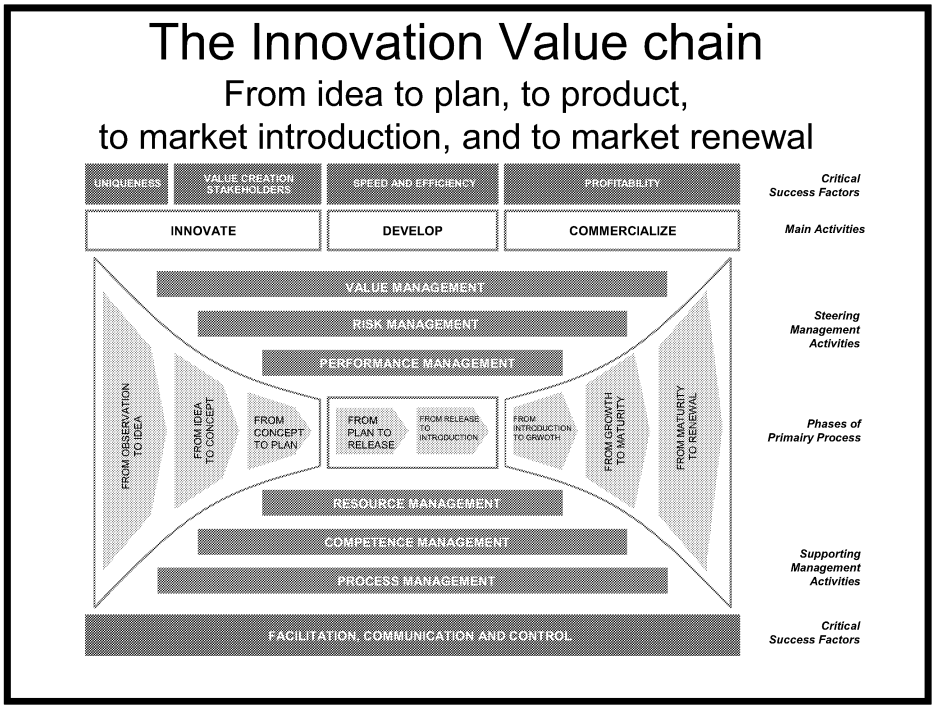

Figure 4. The Innovation Value Chain

With this activity the Innovation cycle begins all over again. Market trends, bottlenecks in existing processes, emerging technologies, etc. are observed and must be transformed in an adequate way into new and unique business ideas.

Critical success factors
Within Innovation there are five critical success factors. In 'innovation', the focus is on generating *unique* business ideas that will allow an organization to distinguish itself. These ideas are then developed into business concepts and plans with the emphasis on a maximum *value creation* for the various stakeholders.

As far as 'development' is concerned, *speed* and *efficiency* are essential. It is important to realize a short time-to-market and an optimal allocation of resources. During 'commercialization' the innovation has to be optimally exploited, which means that *profitability* is the critical factor. The fifth and final critical success factor refers to the process as a whole. To ensure a continuous creation of value it is important to treat Innovation as an integrated process. That means that the right balance has to be struck between *facilitating*, *communicating* and *monitoring*.

Controlling/supporting management activities
Within Innovation Management there are two clusters of management activities: *controlling* and *supporting* management activities.

Innovations have to be controlled with regards to *value creation*, *risk* and *performance*. Supporting management activities refer to *process*, *-competence* and *resource* management. We will discuss these steering and supporting

management activities in more length during the subsequent sessions of this course.

Management of the Innovation

Process:

Innovation is a never ending, cyclic process and management must address this cyclic character. We have depicted that in the figure below.

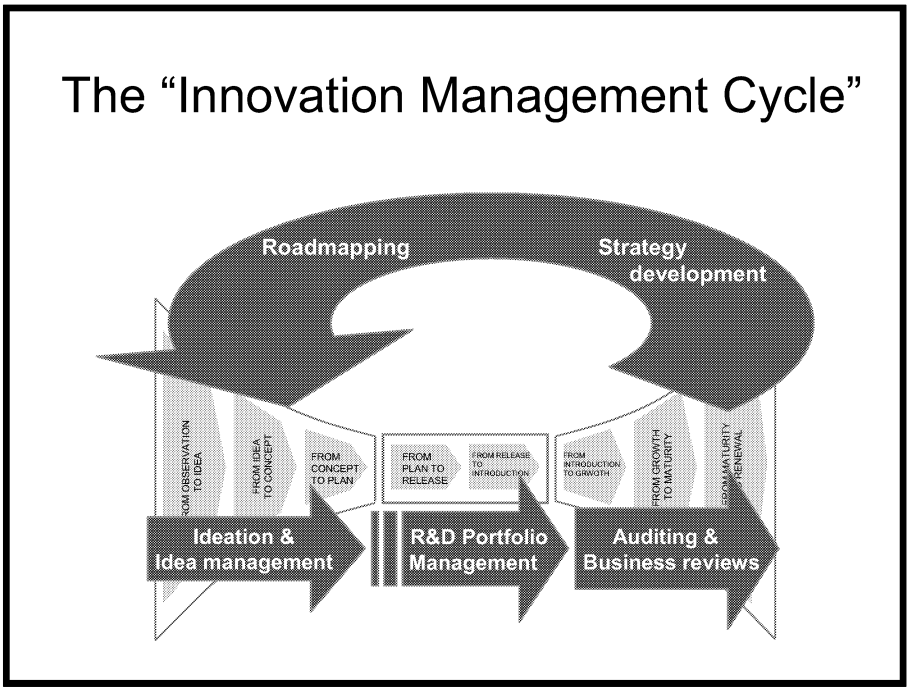

Figure 5. The Innovation Management Cycle Model

The innovation process in the pre-project phase from business idea to business plan is sometimes named ideation and its management idea management. This is one of the most critical phases of the innovation process. In that phase the real innovation take place. Although without the other parts of the process the innovation will never materialize and innovation stays a hollow phrase.

During the development phases data are collected. Through a cycle of drafting, testing and redrafting the optimal product is developed. Here the process is predominantly aimed at realizing the plans and optimizing the product-market combination. When we relate the test results to the decision-making criteria that have been developed, a decision can be reached as to how to proceed.
During this development phase many projects may run in parallel. Resources are allocated to the projects. However the future always enrolls differently than anticipated. There is a competition for resources among these projects and constantly decisions have to be taken about reallocations of these resources to these projects. This is the field of portfolio management.

The commercialization phase above all has to do with the commercial exploitation of the developed product. It is important that continuous monitoring take place to determine whether or not the assumptions regarding customers, market and competitors are still valid. Changes in the market or the emergence of new technologies may lead to new technological, product and market developments.
When the innovation is implemented we have to monitor constantly the environment and the activities inside the company to control whether we are still on course. When we monitor changes in customer preferences, or the introduction of some competitive products or emerging disturbing technologies we have to react and adjust our strategy.

After adjustment of the innovation strategies these strategies have to be planned and translated into a roadmap. In this way the whole process of ideation and idea management start all over again.

Below we have listed a number of problems we came across in the various organizations we have supported the last years. We have sorted these problems in 4 areas according to the subsequent phases of the innovation process.

From idea to project or business plan:

- Not enough good business ideas. Too much inward looking!
- Many good ideas, but too few are introduced successfully into the market!
- Development of the business case and business plan takes too long and is chaotic. There doesn't exist a real process!

From project plan to exploitation

- Too many projects; none of the projects are stopped in time, most are just fading away!
- Too low productivity; Developers are involved in too many projects and most of their time is wasted in meetings discussing why they missed milestones!
- Projects are not in line with strategy!

From exploitation to renewal; Auditing and business reviewing

- Too many red traffic lights, but how to improve the situation?
- How to recognize disruptive technologies before it is too late?

Strategy Development and planning

- Opportunities are missed and entrepreneurial coworkers are leaving the company or are becoming frustrated. How combining long term strategic planning with emerging entrepreneurial strategies?
- Tactics replaces strategy. How to combine short term plans with long term vision?
- How to translate strategic objectives into project selection criteria?

"Well-planned is half-finished" and "look before you leap" are statements that certainly apply to innovations. On the other hand we must beware of the danger of "Paralysis by Analysis". The figure presented below shows that any additional work required at the outset will offer substantial rewards further down the line, where it is possible to save time and resources because less work has to be done.

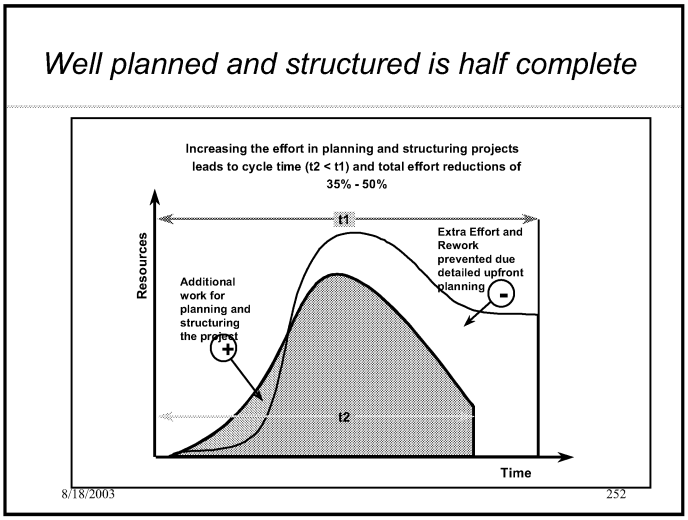

Figure 6. Relation between allocation of resources during the innovation process phases and time to market and costs

In addition to saving costs the yields are higher because the product or service can be marketed at an earlier date, thus ensuring a longer product life cycle and a larger market share. A good preparation in the pre-project (innovation) phase has to do with the development and validation of the models of the customer, market and business models, and financial and economic models (business case) in

relation to (a) technical model(s) of the product or service. These models indicate what data have to be collected, and they are essential to the translation of these data into information).

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