Modelling for Control of Industrial Fermentation

Jan Kamyno Rasmussen, Henrik Madsen and Sten Bay Jørgensen

CAPEC, Department of Chemical engineering, Technical University of Denmark,
Building 229, DK-2800 Lyngby, Denmark
Informatics and Mathematical Modelling, Technical University of Denmark,
Building 321, DK-2800 Lyngby, Denmark

Abstract
This paper presents application of a grey-box stochastic modelling framework for developing continuous time models for dynamic systems based on experimental data. The framework will be used to develop predictive models suited for control purposes. The main idea behind the framework is to combine stochastic modelling with data to obtain information on parameter values and model (in-)validity. In case the initial model is falsified the method can point out specific deficiencies which facilitates further model development. The industrial fermentation case is production of an amylase enzyme by a filamentous fungus.

Keywords: Parameter estimation, industrial fermentation, modelling for control, grey-box modelling

1. Introduction
Fed-batch processes play a very important role in chemical and biochemical industry. Fermentations are widely used in biochemical industry and are most often carried out as fed-batch processes. Present control schemes do not utilise the full potential of the production facilities and may often fail to achieve uniform product quality and optimal productivity. Application of advanced multivariable control schemes can help solve this problem. The introduction of model based control strategies is considered difficult because suitable models are not readily available and require a significant investment in experimental work for their development.

First principles engineering models can be used in the controller assuming that they possess satisfactory predictive capabilities. Parameter estimation in a first principles engineering model can be very time consuming and can cause problems when scaling up from laboratory to industrial fermentors. Especially parameters for mass and heat transfer models may change when the volume of the fermentor is changed. These phenomena can not be investigated in laboratory scale equipment which therefore makes large scale experiments necessary.

The approach taken in this paper is to combine first principle engineering models with operational data to produce predictive models suited for control purposes. The method described in this paper is grey-box stochastic modelling which consists of a set of stochastic differential equations describing the dynamics of the system in continuous
time and a set of discrete time measurements. An important advantage using this approach compared to using ordinary differential equations type model is that they can account for random variations in data.

A framework for this kind of model development has already been developed (Kristensen et al., 2004) and is described in figure 1.

![Figure 1. Grey-box modelling cycle.](image)

One of the key ideas behind the grey-box stochastic modelling framework is to use all prior information for formulation of an initial first principles engineering model. Unknown parameters of the initial model are then estimated from experimental data and a residual analysis is carried out to evaluate the quality of the resulting model. The next step in the modelling cycle is the model falsification or unfalsification which determines if the model is sufficiently accurate to serve its intended purpose. If the model is unfalsified the model development is completed. In case of falsification the modelling cycle must repeated by reformulating the initial model. In this case statistical tests can be used to provide indications of which parts of the model that are deficient. Nonparametric modelling can be applied to estimate which functional relationships are needed to improve the model.

### 2. Process description

The process studied is fermentation of the filamentous fungi *Aspergillus oryzae* for production of the enzyme amylase. The fermentation is initiated by transferring the contents of a seed tank to the main fermentation tank when a certain transfer criterion has been satisfied. The main fermentation tank contains an initial amount of substrate and the main fermentation process starts immediately after inoculation. The main fermentation is carried out in a batch and fedbatch phase. When the initial substrate has been consumed by the microorganisms the fedbatch phase is initiated. Feed dosing is started at a low level and increased to its final value within a certain time span. The fedbatch phase continues for the rest of the fermentation process. The fermentors are equipped with sensors for online measurements of different variables but some values are only available as offline measurements which makes closed loop control more difficult and requires a more accurate model for predicting the variable values.
The first principles engineering model to be studied here is proposed by Agger et al. (Agger et al., 1998). The model is based on the assumption that the total filamentous biomass can be divided into three distinct regions:

- **Active region** ($x_a$): Responsible for uptake of substrate and growth of the hyphal element. $\alpha$-Amylase synthesis occurs in this region.
- **Extension zone** ($x_e$): Building of new cell wall.
- **Hyphal region** ($x_h$): Contains the degenerated part of the hyphal elements and can be considered as an inactive region.

The original model contains 5 states, (The 3 morphological states, substrate concentration ($s$) and product concentration ($p$)). During the development of the model Agger et al. has assumed that no oxygen limitation is present. In order to be able to model the behaviour at low dissolved oxygen values and relate the morphological states to off-gas measurements the model has been extended (see Zangirolami, 1998). The oxygen concentration has been introduced as an extra state and as the volume is changing this constitutes an additional state of the system.

The formation rate of the three regions is given in equation (1)-(3).

$$\frac{dx_e}{dt} = q_1 - Dx_e$$  \hspace{1cm} (1)

$$\frac{dx_a}{dt} = q_3 - q_1 - q_2 - Dx_a$$  \hspace{1cm} (2)

$$\frac{dx_h}{dt} = q_2 - Dx_h$$  \hspace{1cm} (3)

Substrate, product and oxygen concentration are described by (4)-(6)

$$\frac{ds}{dt} = -\left(\frac{1}{\alpha} q_3 + r_{ps} \frac{1}{Y_{sp}} x_a + m_s (x_e + x_a + x_h)\right) + D(s_f - s)$$  \hspace{1cm} (4)

$$\frac{dp}{dt} = r_{ps} x_a - Dp$$  \hspace{1cm} (5)

$$\frac{dC_{O_2}}{dt} = -r_{O_2} (x_e + x_a + x_h) + k_{1a}(C_{O_2}^* - C_{O_2}) - DC_{O_2}$$  \hspace{1cm} (6)

$$D = \frac{F}{V}$$  \hspace{1cm} (7)

The three kinetic expressions are given in equation (8) and (9). It is assumed that the concentration of hyphal elements is above the critical value at all times, meaning that only the second inequality in (8) needs to be considered.
In order to account for the decrease of growth rate for the active region under oxygen limiting conditions the last Monod term in equation (9) has been introduced. The specific growth rate and rate of product formation and carbon dioxide and rate of uptake of oxygen is given in equations (9)-(11).

\[
\mu = \frac{q_3}{x_e + x_a + x_b}
\]

\[
r_{ps} = \frac{k_{ps} s}{(s + K_{s3})(1 + \exp(k_{p2}(s - s_{rop}))} + k_c
\]

\[
r_{CO_2} = Y_{xc} \frac{q_3}{x_e + x_a + x_b} + m_c ; \quad r_{O_2} = Y_{xo} \frac{q_3}{x_e + x_a + x_b} + m_o
\]

\[
k_1 = Y_{xc} \frac{k_{bran} \cdot 10^4}{\frac{\pi}{4}(d \cdot 10^{-4})^2(1-w)f \rho}
\]

\[
k_3 = k_{tp,max} \cdot 10^{-7} \frac{\pi}{4}(d \cdot 10^{-4})^2(1-w)f \rho
\]

\[
d = 11.25 \cdot \mu + 1.1 ; \quad a = \left(\frac{1}{2} \left(\frac{1}{2} \cdot d \cdot 10^{-4}\right)^3 \frac{4\pi}{3}(1-w)\rho\right)^{-1}
\]

The relations to the off-gas measurements are given in (16).

\[
OUR = r_{O_2} \left(x_e + x_a + x_h\right) ; \quad CER = r_{CO_2} \left(x_e + x_a + x_h\right) ; \quad DOT = \frac{C_{O_2}}{C_{O_2}}
\]

For more information on the details of the model please refer to Agger et al., 1998.
3. Simulation

Simulations have been performed to compare the predictions of the model with actual data from an industrial fermentation. The industrial data has been supplied by Novozymes A/S. The parameters for the simulation are provided in Table 1. The only input used in the simulation is the feed profile (figure 2) which has been applied to one of the batches from the industrial data set. The figures in the following show characteristic results obtained in the simulation and experiments.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Unit</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{bran}$</td>
<td>$1.7 \times 10^{-3}$</td>
<td>$\text{tip} \cdot \mu\text{m}^{-1} \cdot \text{h}^{-1}$</td>
<td>Agger et al.</td>
</tr>
<tr>
<td>$K_{tip,\text{max}}$</td>
<td>49</td>
<td>$\mu\text{m}^{-1} \cdot \text{h}^{-1}$</td>
<td>Agger et al.</td>
</tr>
<tr>
<td>$k_2$</td>
<td>0.08</td>
<td>$\text{h}^{-1}$</td>
<td>Agger et al.</td>
</tr>
<tr>
<td>$K_{s1}$</td>
<td>$3 \times 10^{-1}$</td>
<td>$\text{g} \cdot \text{L}^{-1}$</td>
<td>Agger et al.</td>
</tr>
<tr>
<td>$K_{s3}$</td>
<td>$6 \times 10^{-1}$</td>
<td>$\text{g} \cdot \text{L}^{-1}$</td>
<td>Agger et al.</td>
</tr>
<tr>
<td>$m_s$</td>
<td>0.01</td>
<td>$\text{g glucose} \cdot \text{g DW}^{-1} \cdot \text{h}^{-1}$</td>
<td>Agger et al.</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>0.57</td>
<td>$\text{g active DW} \cdot \text{g glucose}^{-1}$</td>
<td>Agger et al.</td>
</tr>
<tr>
<td>$\rho$</td>
<td>1</td>
<td>$\text{kg/L}$</td>
<td>Agger et al.</td>
</tr>
<tr>
<td>$w$</td>
<td>0.67</td>
<td>$\text{g/g DW}$</td>
<td>Agger et al.</td>
</tr>
<tr>
<td>$f$</td>
<td>0.8</td>
<td></td>
<td>Agger et al.</td>
</tr>
<tr>
<td>$Y_{sp}$</td>
<td>5316</td>
<td>$\text{FAU} \cdot \text{g glucose}^{-1}$</td>
<td>Agger et al.</td>
</tr>
<tr>
<td>$k_c$</td>
<td>8</td>
<td>$\text{FAU} \cdot \text{g active DW} \cdot \text{h}^{-1}$</td>
<td>Agger et al.</td>
</tr>
<tr>
<td>$k_{p1}$</td>
<td>32</td>
<td>$\text{FAU} \cdot \text{g active DW} \cdot \text{h}^{-1}$</td>
<td>Agger et al.</td>
</tr>
<tr>
<td>$k_{p2}$</td>
<td>5000</td>
<td>$\text{L} \cdot \text{g}^{-1}$</td>
<td>Agger et al.</td>
</tr>
<tr>
<td>$s_{rep}$</td>
<td>$9.5 \times 10^{-3}$</td>
<td>$\text{g} \cdot \text{L}^{-1}$</td>
<td>Agger et al.</td>
</tr>
<tr>
<td>$K_{s4}$</td>
<td>$6 \times 10^{-4}$</td>
<td>$\text{g} \cdot \text{L}^{-1}$</td>
<td>Agger et al.</td>
</tr>
<tr>
<td>$Y_{xc}$</td>
<td>0.01786</td>
<td>$\text{mol CO}_2 \cdot \text{g DW}^{-1}$</td>
<td>Carlsen et al.</td>
</tr>
<tr>
<td>$Y_{xo}$</td>
<td>0.01563</td>
<td>$\text{mol O}_2 \cdot \text{g DW}^{-1}$</td>
<td>Carlsen et al.</td>
</tr>
<tr>
<td>$m_g$</td>
<td>$6.3 \times 10^{-5}$</td>
<td>$\text{mol CO}_2 \cdot \text{g DW}^{-1} \cdot \text{h}^{-1}$</td>
<td>Carlsen et al.</td>
</tr>
<tr>
<td>$m_a$</td>
<td>$6.6 \times 10^{-4}$</td>
<td>$\text{mol O}_2 \cdot \text{g DW}^{-1} \cdot \text{h}^{-1}$</td>
<td>Carlsen et al.</td>
</tr>
<tr>
<td>$k_{l,a}$</td>
<td>79</td>
<td>$\text{h}^{-1}$</td>
<td>Zangirolami</td>
</tr>
<tr>
<td>$k_{O2}$</td>
<td>$2.25 \times 10^{-5}$</td>
<td>$\text{mol} \cdot \text{L}^{-1}$</td>
<td>Fitted</td>
</tr>
</tbody>
</table>

Figure 3 shows that to some extent the model is able to predict the behaviour of the OUR. During the batch phase the OUR predicted by the model is somewhat lower than actually measured. As the feed dosing is initiated at t=25h a large drop in OUR occurs which is captured by the model. The prediction for the fed-batch phase is somewhat higher than the experimentally observed.

Figure 4 shows the evolution of the biomass concentrations with time. It is seen that the concentration of active region and extension zone decreases during the fermentation and the concentration of the hyphal region increases. This behaviour can be explained by the decrease in substrate and oxygen availability occurring after the batch phase. The low concentrations decrease $q_3$ (eq. 9) which reduces the rate of formation of active cells.
4. Discussion

The morphologically structured model presented is able to predict a large part of the behaviour of the industrial fermentation. The model has been developed in laboratory scale equipment where no oxygen limitation has occurred in the experiments. In order to be able to simulate an industrial scale fermentor some of the model parameters need to be reestimated and new functional relationships have to be introduced. It has been shown that the morphology changes drastically under oxygen limitation (Zangirolami, 1998). This runaway phenomenon will also be modelled. During low dissolved oxygen concentration the filamentous fungus changes its filaments which increases viscosity and impairs oxygen transfer. Hence the oxygen concentration becomes even lower.

Parameter (re-)estimation and investigation of new functional relationships in the model based on experimental data are carried out in the software program CTSM (Continuous Time Stochastic Modelling) (Kristensen et al., 2004). CTSM provides a graphical user interface which allows the user to specify how the model parameter should be estimated. After specifying which experimental data sets to use, the program determines the parameter estimates and evaluates statistical tests.

References