

Fuzzy Estimation of a Yeast Fermentation Parameters

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Abstract: *The dynamics of fermentation processes are very complex and not completely known. Some state variables are non-measurable, and the process parameters are strongly time dependent. Recently, there are some control methods like fuzzy learning and neural networks, which are promising in dealing with non-linearity, complexity, and uncertainty of these processes. These methods are suitable for the modelling of these systems, which are difficult to describe mathematically. The fuzzy learning methods are useful for the modelling, they are less demanding on the mathematical model and a priori knowledge about the processes. Different techniques for estimating the state variables (that are non-measurable) in the fermentation process have been investigated. A non-linear auto-regressive with exogenous input (NARX) model was developed using process data from a pilot bioreactor. The fermentation process is splitted into three phases, where each phase was treated separately. Generally, fuzzy models have a capability of dividing an input space into several subspaces (fuzzy clustering), where each subspace is supposed to give a local linear model. In our work, we used global learning where the local models are less interpretable, but the global model accuracy is satisfying, and the fuzzy partition matrix is obtained by applying the Gustafson-Kessel algorithm. The fermentation parameters are estimated for a batch and a fed-batch culture. The number of inputs to our fuzzy model are three for a first simulation. We used four inputs for a second simulation, in order to detect some correlations among inputs. The results show that estimated parameters are close to the measured (or calculated) ones. The parameters used in the computation are identified using batch experiments.*

Keywords: *Fermentation, batch, fed-batch, Takagi-Sugeno model, process.*

Received November 18, 2003; accepted July 8, 2004

1. Introduction

Fuzzy identification is a useful tool for the estimation of complex and uncertain non-linear systems parameters, based on experimental data. Dynamic Takagi-Sugeno (TS) fuzzy model has been extensively used in the fuzzy modelling. This model consists of if-then rules with fuzzy antecedents and the consequent is a mathematical function of the antecedents. The input space is partitionned into a number of fuzzy regions, while the consequent functions describe the process's behavior in these regions [1, 7, 8].

The construction of a TS fuzzy model requires two steps. In a first step, the membership functions in the rule antecedents are determined. This can be done using data-driven techniques (application of the Gustafson-Kessel (GK) algorithm). In a second step, the parameters of the consequent functions are estimated. The consequent functions are usually linear, in their parameters; standard linear least-squares methods are used. One difficulty of the construction procedure is the identification of the antecedent membership functions, which is a non-linear optimisation problem. For the solution, some techniques are used [2, 3, 5, 14].

Fuzzy clustering in the Cartesian product-space of the inputs and outputs has also been used recently. In this paper, we use Gustafson-Kessel algorithm [3].

2. Takagi-Sugeno Fuzzy Model

The particularity of this model is that the consequent parts of linguistic rules are expressed as functions of linguistic variables. Let the dynamic model be of the form

$$y = f(u) \quad (1)$$

Based on some available input-output data: $u_k = [u_{1k}, \dots, u_{nk}]^T$ and y_k , respectively, k denoting data samples and varying from 1 to N .

It is often difficult to find a model to describe the unknown system globally, then it becomes necessary to construct local linear models around selected operating points. In this case, the model is generally given by [4, 8, 12].

$$\hat{y} = \sum_{i=1}^c \Phi_i(u) (a_i^T u + b_i) \quad (2)$$

where $\Phi_i(u)$ is the validity function for the i th operating regime; $\theta_i = [a_i^T \ b_i]^T$ is the parameter vector

of the corresponding local linear model; and c is the number of rules. There are n individual components of u , and a rule R_i can be stated as follows [4]:

R_i : If u_1 is $A_{i,1}(u_1)$ and ... and u_n is $A_{i,n}(u_n)$ then

$$\hat{y}_i = a_i^T u + b_i \quad (3)$$

The degree of fulfillment $\beta_i(u)$ of the rule R_i is calculated as the product of the individual membership degrees and the rule's weight w_i .

$$\beta_i(u) = w_i A_i(u) = w_i \prod_{j=1}^n A_{i,j}(u_j) \quad (4)$$

By using the fuzzy-mean formula, the output \hat{y} is given by [4]

$$\hat{y} = \left(\sum_{i=1}^c \beta_i(u) (a_i^T u + b_i) \right) / \sum_{i=1}^c \beta_i(u) \quad (5)$$

from (2) and (5), the Takagi-Sugeno fuzzy model is equivalent to the model when $\Phi_i(u)$ is the normalized degree of fulfillment.

$$\Phi_i(u) = \beta_i(u) / \sum_{i=1}^c \beta_i(u) \quad (6)$$

The only assumption we make on the rules, is that it's complete in the sense that $\beta_i(u) > 0$, for some i for all u , such that (6) is well defined.

3. Fuzzy Model Identification Based on Gustafon-Kessel Clustering

In our application, the data are the sampled measurements of S , E , r_q , and q_s which are respectively, the glucose concentration (g/l), the ethanol concentration (g/l), respiratory coefficient and specific glucose uptake rate (h^{-1}) [6].

Generally, n measured variables (including input and output variables) are grouped into an n -dimensional column vector $z_k = [z_{1k}, \dots, z_{nk}]^T$. A set of N observations is denoted by $Z = \{z_k | k=1, \dots, N\}$, and is represented as an $n \times N$ matrix

$$Z = \begin{bmatrix} Z_{11} & Z_{12} & \dots & \dots & Z_{1N} \\ Z_{21} & Z_{22} & \dots & \dots & Z_{2N} \\ \vdots & \vdots & \dots & \dots & \vdots \\ Z_{n1} & Z_{n2} & \dots & \dots & Z_{nN} \end{bmatrix} \quad (7)$$

In the analysis of fermentation measurements, each column of Z is generally the observations of one measured variable; the last column contains the output samples. The data set Z is partitioned into c clusters.

Fuzzy partition of Z is a family of fuzzy subsets $\{A_i | 1 \leq i \leq c\}$. The subsets are characterised by their membership functions represented in the partition matrix $U = [\mu_{ik}]_{c \times N}$. Each row of this matrix contains values of the membership function of one fuzzy subset

of Z . The partition matrix satisfies the following conditions:

$$\mu_{ik} \in [0,1], 1 \leq i \leq c, 1 \leq k \leq N \quad (8a)$$

$$\sum_{i=1}^c \mu_{ik} = 1, 1 \leq k \leq N \quad (8b)$$

$$0 \leq \sum_{i=1}^N \mu_{ik} \leq N, 1 \leq i \leq c \quad (8c)$$

Equation (8a) states that the membership degrees are real numbers with values in the interval $[0, 1]$. Equation (8b) constrains the sum of each column of U to 1, and thus the total membership of each Z_k in all the clusters equals one. Equation (8c) means that none of the fuzzy subsets is empty, nor it contains all the data.

The GK algorithm is based on the minimization of the well-known fuzzy c-means functional

$$J(Z, U, V, \{A_i\}) = \sum_{i=1}^c \sum_{k=1}^N (\mu_{ik})^m D_{ik}^2 A_i \quad (9)$$

where Z is the set of observations, U is the fuzzy partition matrix of Z , $V = [V_1, V_2, \dots, V_c]$, $V_i \in \mathbb{R}^n$ is a vector of cluster centers, and

$$D_{ik}^2 = (z_k^T - v_i)^T A_i^{-1} (z_k^T - v_i) \quad (10)$$

is the squared inner-product distance norm. Matrices A_i are computed in the optimisation algorithm using the local covariance of the data around each cluster center. Each cluster has its own matrix; allowing it to adapt the distance norm to the local distribution of data.

4. Process Description

Fermentations can be operated in batch, fed-batch or continuous reactors. In batch reactors, all components, except gaseous substrates such as oxygen, pH-controlling substances, are placed in the reactor in the beginning of the fermentation; and during process there is no input nor output flows. In fed-batch process, nothing is removed from the reactor during the process, but one substrate component is added in order to control the reaction rate by its concentration. In a continuous reactor, there are both input and output flows, but the reaction volume is kept constant.

4.1. Batch Process

4.1.1. Reactor Model

X , S , E , O_F , and C_F are respectively the concentrations of the biomass, the glucose, the ethanol, the dissolved oxygen and the carbon dioxide.

In the continuously operating stirred fermentor, which is shown in Figure 1. The yeast cells, represented by the cell mass concentration X are growing in the

liquid phase; they are consuming the glucose, substrate, dissolved oxygen and they are producing carbon dioxide; ethanol can be a product or substrate depending on the concentrations of glucose and dissolved oxygen. In batch culture (Dilution rate $D = 0$), some model parameters are identified. The first phase in the growth, where the growth rate stays almost constant, is the lag phase. The lag phase is due to the fact that the cells have to adapt to the medium, before they can use it for growth. The next phase is the declining growth phase, in which a substrate begins to limit the growth rate. The stationary phase is the phase where the growth rate is zero. The last phase is called the death phase.

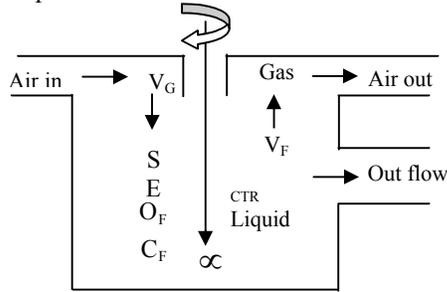


Figure 1. Schematic diagram of the fermentor.

The specific growth rate μ depends on S , r_{AC} , q_{O_2} and r_E , which are respectively glucose concentration (g/l), specific acetyl-CoA-consumption rate (h^{-1}), specific oxygen uptake rate (h^{-1}), ethanol reaction rate (h^{-1}) where g/l denotes grams/liter and h denotes hour. Maximisation of μ with the constraints that are the rate limiting steps [15].

$$\text{Ethanol limitation } r_{E2} < 1.1E \quad (11)$$

$$\text{Oxygen limitation } q_{O_2} < 12800O_F \quad (12)$$

$$\text{Crabtree effect } r_{AC} < r_{ACmax} \quad (13)$$

$$\text{Regulation of gluconeogenesis } r_S > r_{Smax} \quad (14)$$

$$\text{Regulation of respiration } q_{O_2} < q_{O_2max} \quad (15)$$

$$\text{Glucose limitation } q_S = q_{Smax} \frac{S}{(0.45+S)} \quad (16)$$

where r_S , q_S , and q_{Smax} are, respectively, the specific rate of glycolysis, specific glucose uptake rate, and maximum specific glucose uptake rate; where all these rates are expressed in h^{-1} which form a multiple Monod-Blackman kinetic. The maximisation of μ , under given constraints, can be replaced by partitioning the process into three phases, depending on the specific glucose uptake rate [15, 16]:

- *Phase 1*: $q_S > q_{Slim}$ fermentative growth.
- *Phase 2*: $q_{Smin} < q_S < q_{Slim}$ oxidative growth.
- *Phase 3*: $q_S < q_{Smin}$ gluconeogenesis.

where:

$$q_{Slim}[k] := \left(\frac{((9.561416 * p[k] + 49.212672) * our[k] / X[k]) - 1.05783498168}{276.4874} \right)$$

$$q_{Smin}[k] := 0.795318 * ((1 + 6 * p[k]) * our[k] / X[k] + 1.98414) / (144.615044)$$

4.1.2. Computation of the Variable RQ and the Parameters

The respiratory quotient RQ is defined as:

$$RQ = \frac{CPR}{OUR} = \frac{\text{Carbon production rate}}{\text{Oxygen uptake rate}}$$

The parameters μ , r_{AC} and r_E which are respectively the specific growth rate, the acetyl-CoA consumption rate and the ethanol reaction rate, are computed for each phase [15, 16]:

Phase 1:

$$\mu[k] = 0.0376 * q_{Smax} * S[k] / (0.45 + S[k] + (0.0078 + 0.042 * P / O[k]) * OUR[k] / X[k] - 0.01388898)$$

$$r_{AC}[k] = 0.1062 * q_{Smax} * S[k] / (0.45 + S[k] + (0.3140.0036 * P / O[k]) * OUR[k] / X[k] + 0.001190484)$$

$$r_E[k] = 1.554 * q_{Smax} * S[k] / (0.45 + S[k] - (0.277 + 0.0538 * P / O[k]) * OUR[k] / X[k] + 0.01779112)$$

Phase 2:

$$\mu[k] = 0.042 * q_{Smax} * S[k] / (0.45 + S[k] + (0.007 + 0.042 * P / O[k]) * OUR[k] / X[k] - 0.01388898)$$

$$r_{AC}[k] = 0.0002 * q_{Smax} * S[k] / (0.45 + S[k] + (0.333 + 0.0002 * P / O[k]) * OUR[k] / X[k] + 0.00006613)$$

$$r_E[k] = 1.9332 * q_{Smax} * S[k] / (0.45 + S[k] - (0.344 + 0.0668 * P / O[k]) * OUR[k] / X[k] + 0.02209009)$$

Phase 3:

$$\mu[k] = 0.0812 * q_{Smax} * S[k] / (0.45 + S[k] + (0.0068 + 0.0406 * P / O[k]) * OUR[k] / X[k] - 0.013426014)$$

$$r_{AC}[k] = 0.0004 * q_{Smax} * S[k] / (0.45 + S[k] + (0.3334 + 0.0002 * P / O[k]) * OUR[k] / X[k] - 0.000066138)$$

$$r_E[k] = 1.9808 * q_{Smax} * S[k] / (0.45 + S[k] - (0.3442 + 0.0646 * P / O[k]) * OUR[k] / X[k] + 0.021362574)$$

4.1.3. Simulations

The sampling period is 20 mn for a cultivation duration of 20 hours, giving a total of 60 data samples. The dilution rate D is zero. The choice of the input variables was difficult. Different variables affect the output variables in the three phases of the process. We used in a first part of simulation, the input variables S ,

E, and RQ and the inputs are X, μ , r_{AC} , r_E , P/O, and q_{O_2} where X: Biomass concentration [9, 10].

- μ , r_{AC} , and r_E are defined above.
- P/O: Phosphor Oxygen effectiveness.
- q_{O_2} : Oxygen uptake rate.

The outputs are illustrated on Figures (2-7) respectively.

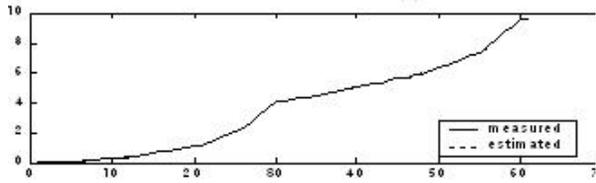


Figure 2. Cell mass concentration (x).

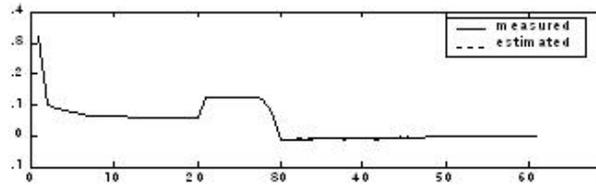


Figure 3. Specific growth rate (μ).

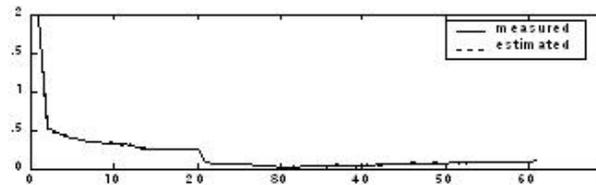


Figure 4. Specific acetyl-CoA-consumption rate (r_{AC}).

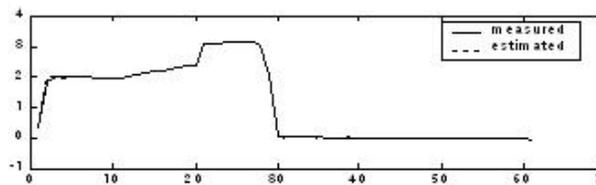


Figure 5. Ethanol reaction rate (r_E).

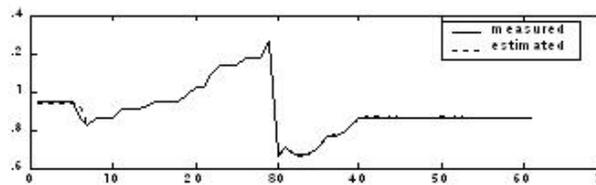


Figure 6. Effectiveness of Oxidative Phosphory (P/O).

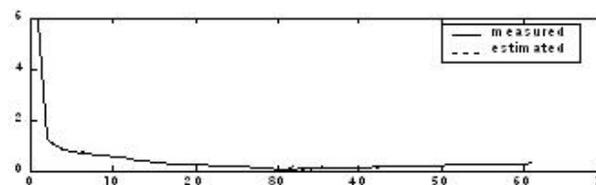


Figure 7. Specific oxygen uptake rate (q_{O_2}).

We used in a second part of simulation the input variables S, E, RQ, and q_S and we showed the same outputs which are illustrated on Figures (8-13) respectively.

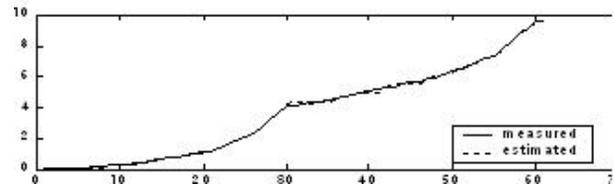


Figure 8. Cell mass concentration (x).

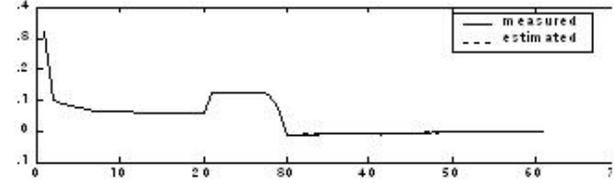


Figure 9. Specific growth rate (μ).

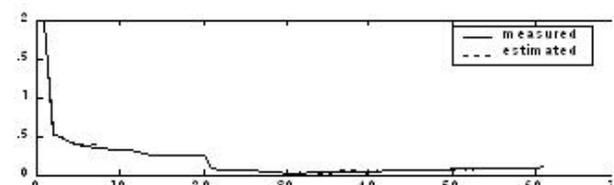


Figure 10. Specific acetyl-CoA-consumption rate (r_{AC}).

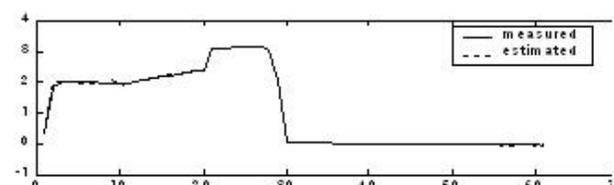


Figure 11. Ethanol reaction rate (r_E).

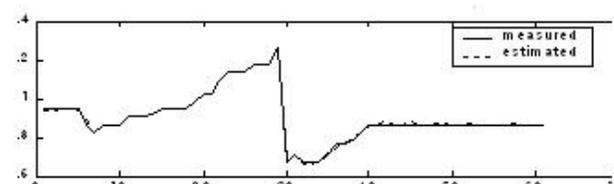


Figure 12. Effectiveness of Oxidative Phosphory (P/O).

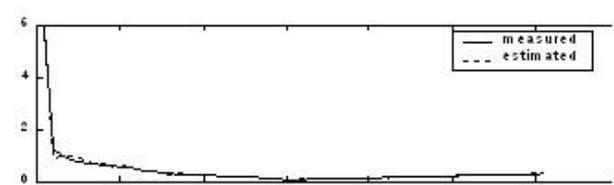


Figure 13. Specific oxygen uptake rate (q_{O_2}).

4.1.4. Comments on Simulation Results

- X is increasing monotonically with a slope changed at 10 hours ($k = 30$).
- μ is constant at the beginning (up to $k = 20$), it is the lag phase; then the growth phase begins, followed by a stationary phase ($\mu = 0,14h^{-1}$) then finally the death phase ($\mu \approx 0$).
- r_{AC} is decreasing with slope changed at $k = 3$ and $k = 20$; to decay at around zero.
- r_E is constant at the beginning (up to $k = 20$) at around $2h^{-1}$, then increases to $3h^{-1}$ to stay constant

until $k = 30$; where E begins to decay to be used in the growth; r_E then decreases to zero.

- P/O is increasing up to $k = 30$, to decrease at around 0,65 to increase again up to $k = 40$ to reach 0,85 where it stays until the end of the simulation.
- q_{O_2} is decreasing, to be constant from $k = 20$ up to the end of the simulation.

In increasing the number of input variables from three to four, the error between the measured variables and the estimated ones becomes smaller as it appears in the figures.

4.2. Fed-Batch Process

4.2.1. Reactor Model

For the fed-batch process, a tank is added to the reactor which contains the components (X^T, S^T, E^T, O_F^T , and C_F^T). The yeast cells are growing in the liquid phase: They are consuming the glucose Substrate S and dissolved Oxygen O_F and producing Carbon dioxide C_F . Ethanol E can be a product or a substrate.

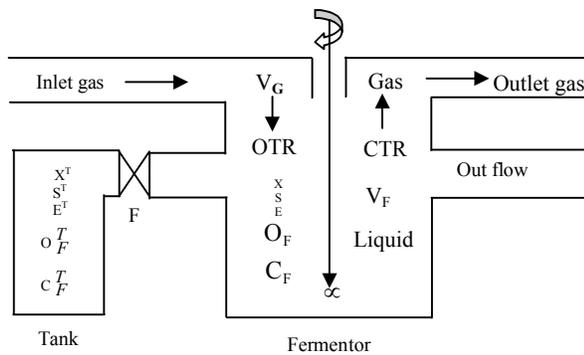


Figure 14. Bioreactor.

The bioreactor is composed of a tank and a fermentor. In the fed-batch fermentation process, the inlet substrate feed should be as concentrated as possible to minimize dilution and to avoid process limitation caused by the reactor size.

The fermentation is started with a small amount of biomass and substrate in the fermentor. The substrate feed is started when most of the initially added substrate has been consumed. The growth rate can be controlled by the substrate concentration to avoid sugar-overflow metabolism or glucose effect which occurs when glucose concentration exceeds a critical value. The growth rate μ depends on the variables (S, q_{O_2}) and on the parameters r_{AC} and r_E [11, 13, 17].

4.2.2. Computation of the Variable RQ and the Parameters

The variable RQ and the parameters μ, r_{AC} , and r_E are as defined before; The parameters μ, r_{AC} , and r_E are computed for each phase (the phases are in the number of three, depending on q_S as seen before).

4.2.3. Simulations

The sampling period is 18 mn for a fed-batch cultivation duration of 30 hours, resulting in a total of 98 data samples. The process starts in fed-batch mode, and for a duration of 10 hours, the dilution rate D is $0,2h^{-1}$, and for the next 20 hours the dilution rate D is $0,34 h^{-1}$.

The process is operating continuously. The glucose is metabolised and its concentration must be kept low. Here, the parameter q_{Smax} is not observable, and the simulation doesn't change this parameter value. This non-observability causes no problems, because the parameter has no effect on growth when glucose concentration is low. We used in a first part of simulation, the input variables S, E , and RQ and the ourputs are $X, \mu, r_{AC}, r_E, P/O$, and q_{O_2} ; these quantities are defined before. The outputs are illustrated on the Figures (15-20) respectively.

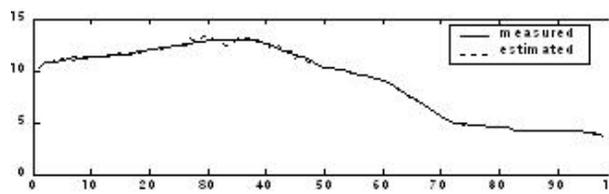


Figure 15. Cell mass concentration (x).

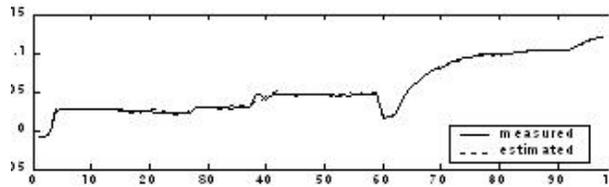


Figure 16. Specific growth rate (μ).

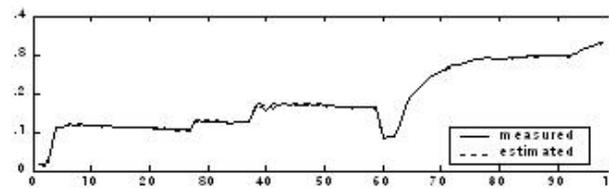


Figure 17. Specific acetyle-CoA-consumption rate (r_{AC}).

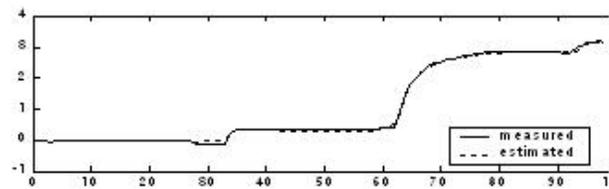


Figure 18. Ethanol reaction rate (r_E).

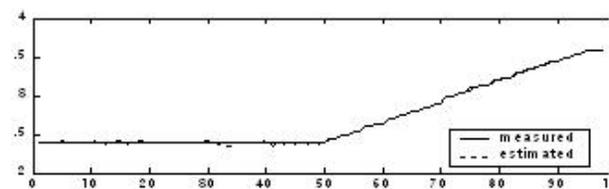
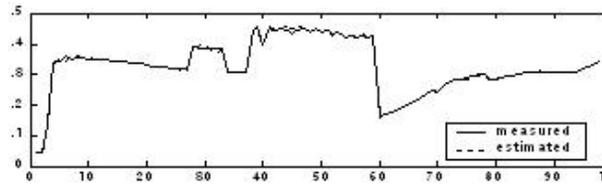


Figure 19. Effectiveness of Oxidative Phosphory (P/O).

Figure 20. Specific Oxygen uptake rate (q_{O_2}).

We used in a second part of simulation, the input variables S, E, RQ, and q_s , and we showed the same outputs which are illustrated on Figures (21-26) respectively.

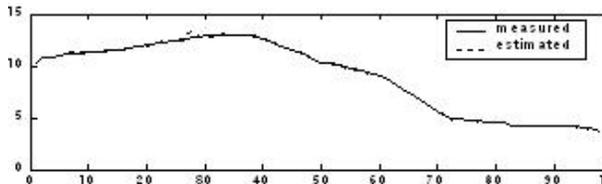
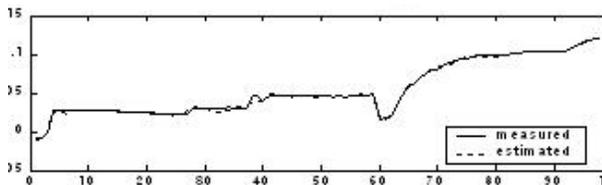
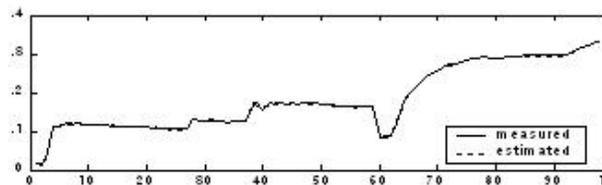
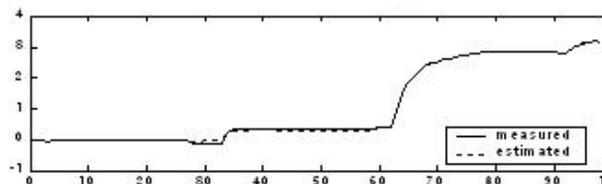
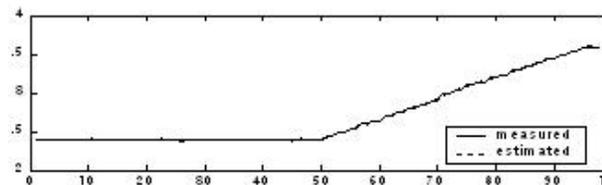
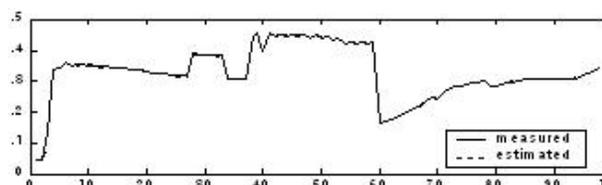
Figure 21. Cell mass concentration (x).Figure 22. Specific growth rate (μ).Figure 23. Specific acetyly-CoA-consumption rate (r_{AC}).Figure 24. Ethanol reaction rate (r_E).

Figure 25. Effectiveness of Oxidative Phosphory (P/O).

Figure 26. Specific Oxygen uptake rate (q_{O_2}).

4.2.4. Comments on Simulation Results

- X is increasing uniformly up to $k = 26$ ($t = 8$ h) where it reaches a stationary operation; at $k = 33$ ($t = 10$ h) X decreases; ethanol is produced with a high r_E ; at $k = 60$ ($t = 18$ h) the oxygen supply is reduced by changing the inlet gas components. X decreases with a changed slope; at $k = 70$ ($t = 21$ h) growth is strongly oxygen limited.
- μ begins at $0,02h^{-1}$ to reach $0,05h^{-1}$ at $k = 60$ ($t = 18$ h) where it falls at zero and stays there for a relatively short time, to increase again to reach $0,12h^{-1}$ at the end of the simulation.
- r_{AC} begins at a value of $0,02h^{-1}$ and does not change until $k = 60$ ($t = 18$ h), to fall at $0,08h^{-1}$ where it remains shortly to increase again from there to reach finally $0,35h^{-1}$.
- r_E begins at 0, to increase $0,5h^{-1}$ at $k = 33$ ($t = 10$ h); and it stays at that value until $k = 60$ ($t = 18$ h) to increase to reach at $k = 80$ ($t = 24$ h) $2,8h^{-1}$ and remains at that value until the end.
- P/O begins at 2,4 and stays at the value until $k = 60$ ($t = 18$ h) to increase linearly to reach a final value of 3,6.
- q_{O_2} begins at $0,36h^{-1}$; and during the period from the beginning to $k = 60$ ($t = 18$ h), q_{O_2} does not change a lot (except some small variations for $28 \leq k \leq 60$); at $k = 60$, q_{O_2} decreases sharply to $0,15h^{-1}$ to increase again to reach finally $0,35h^{-1}$.

5. Conclusion

The fermentation process is complex, non-linear, uncertain and not very well known; the estimation of its parameters for control or prediction purposes, is a difficult task. In this application, the methodology used is of type Takagi-Sugeno fuzzy modelling, which proved to be useful in many non-linear estimation problems. The simulations show that satisfactory results are obtained, in comparing estimation error and measurement error. One of the important motivations of using the TS model is to gain insights into the model, it is important to achieve a good trade-off between global approximation and local interpretation. Developing alternative computationally efficient procedures for including global learning and local learning for large data sets modelling problems, and availability of more accurate sensors will certainly improve the research results in this area.

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