ADAPTIVE ALGORITHMS FOR ESTIMATION OF MULTIPLE BIOMASS GROWTH RATES AND BIOMASS CONCENTRATION IN A CLASS OF BIOPROCESSES

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Abstract. An approach for multiple biomass growth rates and biomass concentration estimation is proposed for a class of bioprocesses characterizing by on-line measurements of dissolved oxygen concentration and off-line measurements of biomass concentration. The approach is based on adaptive observer theory and includes two steps. In the first one, an adaptive estimator of two biomass growth rates is designed using on-line measurements of dissolved oxygen concentration. In the second step, the third biomass growth rate and the biomass concentration are estimated on the basis of other estimator using additionally off-line measurements of biomass concentrations. The simulation results demonstrated the good performance of the estimators under fed-batch conditions.

Keywords: adaptive systems, biotechnology, estimators

1. INTRODUCTION

The kinetic rates are ones of the most important parameters of the bioprocesses. Their exact estimation allows to be realized on-line by adaptive control algorithms. Moreover, the information about their changes in the time is important and useful with regard to the investigations and study of every bioprocess.

In many practical cases, it is impossible to measure the kinetic rates directly. Their estimation becomes a necessary step. Some approaches, proposed in the literature, are based on extended Kalman filter (Dochain and Pauss, 1988; Lubenova, 1993), adaptive system theory (Lubenova, 1993; Bastin and Dochain, 1989; Cazzador and Lubenova, 1995; Ferreira, 1995; Oliveira *et al.*, 1996; Pomerleau and Perrier, 1990), high gain approach (Farza *et al.*, 1997), hybrid neural network based approach (Chen *et al.*, 1995) etc.

A considerable part of the authors direct their efforts to estimation of the specific growth rate in simple culture. Multiple rates estimation is realized in small number of works (Ferreira, 1995; Oliveira *et al.*, 1996; Pomerleau and Perrier, 1990). Pomerleau and Perrier (1990) used the observer-based estimator (Bastina and Dochain, 1989) and a pole placement based tuning in the estimation of three specific growth rates of baker's yeast fed-batch fermentation. Ferreira (1995) and Oliveira *et al.* (1996) proposed a second order dynamic based tuning for the design parameters of the same general estimator to estimate these rates in the same fermentation process. In both cases, two partial models are used to describe the process. The estimation is carried out using a switch between two partial observer based estimators using on-line measurements of the dissolved oxygen and carbon dioxide concentrations.

This paper is dedicated to the estimation of the multiple biomass growth rates and the biomass concentration of a class of bioprocesses, which is characterized by the following reaction network:

$$S_1 + O \xrightarrow{\mu_1} X + C \qquad (1)$$

$$S_1 \xrightarrow{\mu_2} X + C + S_2\,(P) \qquad (2)$$

$$S_2\,(P) + O \xrightarrow{\mu_3} X + C \qquad (3)$$

where S_1 is the first substrate; O – dissolved oxygen; X – biomass; S_2 (P) – second substrate or product depending on the metabolic pathway; C – carbon dioxide; μ_1, μ_2, and μ_3 are the specific growth rates for the three metabolic pathways.

In the sequel O, X, S_1, S_2, C will represent the concentrations of these variables.

Pathways (1), (2) and (3) refer respectively to the respiratory growth on substrate S_1, fermentative growth on substrate S_1 and the respiratory growth on S_2. It is considered the case when the processes, belong to the class above, are described using only one model. Two adaptive algorithms are proposed. The first one estimates two of the biomass growth rates R_1 and R_3 using on-line measurements of dissolved oxygen concentration. The second algorithm estimates the biomass growth rate R_2 and the biomass concentration using additionally off-line measurements of biomass concentration. The second estimator need the estimates R_1 and R_3, obtained by the first algorithm. The performance of proposed estimators is investigated by simulation.

2. PROBLEM STATEMENT

A general dynamical model of a stirred tank reactor (Bastina and Dochain, 1989) has the form:

$$\frac{d\boldsymbol{\xi}}{dt} = \mathbf{K}\,\mathbf{r}(\xi) - D\boldsymbol{\xi} + \mathbf{F} - \mathbf{Q} \qquad (4)$$

where $\boldsymbol{\xi}$ is the n vector of state variables; **K** - the n×m yield coefficient matrix; D - the dilution rate; **F** - n feed rate vector, **Q** – n gaseous outflow rate vector, **r** ($\boldsymbol{\xi}$)- a m×r reaction rate vector.

The dynamics of the class of bioprocesses, which is characterized by the scheme (1), (2) and (3) is given in the matrix form (4), (5) by the following vectors and matrix:

$$\xi = \begin{bmatrix} X \\ S_1 \\ S_2(P) \\ O \\ C \end{bmatrix}; \ \mathbf{K} = \begin{bmatrix} 1 & 1 & 1 \\ -k_1 & -k_2 & 0 \\ 0 & k_3 & -k_4 \\ -k_5 & 0 & -k_6 \\ k_7 & k_8 & k_9 \end{bmatrix};$$

$$\mathbf{F} = \begin{bmatrix} 0 \\ DS_{in1} \\ 0 \\ OTR \\ 0 \end{bmatrix}; \mathbf{Q} = \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ CTR \end{bmatrix} \ \mathbf{r}(\xi) = \begin{bmatrix} \mu_1 X \\ \mu_2 X \\ \mu_3 X \end{bmatrix} = \begin{bmatrix} R_1 \\ R_2 \\ R_3 \end{bmatrix} \quad (5)$$

where $k_1 \div k_9$ are yield coefficients, OTR is the oxygen transfer rate, CTR is the carbon dioxide transfer rate, and S_{in1} is the first substrate concentration in the feed.

For the system (4), (5), it is assumed that:

A1. Only dissolved oxygen concentration O, the oxygen transfer rate OTR, and the carbon dioxide transfer rate CTR, and carbon dioxide concentration C are measured on-line.

A2. The elements of the yield coefficient matrix **K** are known and constants.

A3. The off-line measurements of biomass concentration X are available.

For the class of bioprocesses, given by the system (4), (5) and under the assumptions A1÷A3, the following problem is considered: Estimation of the biomass growth rates R_1, R_2 and R_3, as well as the concentration of biomass on the basis of adaptive algorithms, derived by the theory of adaptive estimation using on-line measurements of the dissolved oxygen concentration and the off-line X measurements.

3. ADAPTIVE ALGORITHMS DESIGN

3.1 *Estimator of R_1 and R_3 (Estimator I)*

According to Eq. 5, the dynamics of the dissolved oxygen concentration is presented by:

$$\frac{dO}{dt} = -k_5 R_1 - k_6 R_3 - DO + OTR \qquad (6)$$

From Eqs. (6), it follows that the oxygen uptake rate (OUR) is given by:

$$OUR = k_5 R_1 + k_6 R_3 \qquad (7)$$

Then the time derivative of the reaction rate R_1 can be presented in the following way:

$$\frac{dR_1}{dt} = \frac{1}{k_5}\frac{dOUR}{dt} - \frac{k_6}{k_5}\frac{dR_3}{dt} \qquad (8)$$

Define the auxiliary parameter:

$$\rho_1 = \left(\frac{1}{k_5}\frac{dOUR}{dt} - \frac{k_6}{k_5}\frac{dR_3}{dt} + C^I R_1\right)\frac{1}{C^{II}} \qquad (9)$$

with C^I and C^{II} - positive constants or time-varying parameters.

Combining Eqs. (6), (8), and (9), it is possible to derive the following adaptive estimator of R_1 and R_3:

$$\frac{d\hat{O}}{dt} = -k_5\hat{R}_1 - k_6\hat{R}_3 - DO + OTR + C_1\left(O - \hat{O}\right) \quad (10a)$$

$$\frac{d\hat{R}_1}{dt} = C^{II}\hat{\rho}_1 - C^I\hat{R}_1 + C_2\left(O - \hat{O}\right) - C_4 V_1\left(O - \hat{O}\right) \quad (10b)$$

$$\frac{d\hat{R}_3}{dt} = -C_3\left(O - \hat{O}\right) \quad (10c)$$

$$\frac{d\hat{\rho}_1}{dt} = -C_4\left(O - \hat{O}\right) \quad (10d)$$

$$\frac{V_1}{dt} = -C^I V_1 + C^{II} \quad (10e)$$

where $\hat{O}, \hat{R}_1, \hat{R}_3$ and $\hat{\rho}_1$ are estimated values for O, R_1, R_3 and ρ_1, while C^I, C^{II}, C_1, C_2, C_3 and C_4 are estimator parameters, which must be chosen according to stability conditions, V_1 is an auxiliary variable.

3.2 *Stability analysis*

Defining the estimation errors:

$\tilde{O} = O - \hat{O}$, $\quad \tilde{R}_1 = R_1 - \hat{R}_1$,

$\tilde{R}_3 = R_3 - \hat{R}_3$, $\quad \tilde{\rho}_1 = \rho_1 - \hat{\rho}_1$

consider the following error system:

$$\frac{d\mathbf{x_1}}{dt} = \mathbf{A_1 x_1} + \mathbf{u_1} \quad (11)$$

where $\mathbf{x_1} = \begin{bmatrix} \tilde{O} \\ \tilde{R}_1 \\ \tilde{R}_3 \\ \tilde{\rho}_1 \end{bmatrix}$; $\mathbf{u_1} = \begin{bmatrix} 0 & 0 \\ 0 & 0 \\ 1 & 0 \\ 0 & 1 \end{bmatrix} . \begin{bmatrix} \frac{dR_3}{dt} \\ \frac{d\rho_1}{dt} \end{bmatrix}$;

$$\mathbf{A_1} = \begin{bmatrix} -C_1 & -k_5 & -k_6 & 0 \\ -C_2 + C_4 V_1 & -C^I & 0 & C^{II} \\ C_3 & 0 & 0 & 0 \\ C_4 & 0 & 0 & 0 \end{bmatrix}$$

It is assumed that:

A5. $C_1 > 0$; $C_1 = h_1 + h_2 - C^I$;

A6. $C_2 > 0$; $C_2 = [(h_2 - h_1)^2 - (C_1 - C^I)^2]/4.k_5$

A7. $c_1 > 0$; $c_1 = - C_2/k_5$

A8. $C_3 = c_1 k_6$; $C_4 = c_1 k_5 V_1$

where $h_1 = -h_1'$, $h_2 = -h_2'$ with h_1, h_2 positive constants, h_1', h_2' - eigenvalues of the matrix:

$$\mathbf{B_{11}} = \begin{bmatrix} -C_1 & -k_5 \\ -C_2 & -C^I \end{bmatrix}$$

A9. The vector $[k_6, k_5 V_1]^T$ is a persistently exciting signal.

A10. $\left|\frac{dR_3}{dt}\right| \leq M_1$, and A11. $\left|\frac{d\rho_1}{dt}\right| \leq M_2$

where M_1 and M_2 are upper bounds.

Statement 1: Under assumptions A1 to A11, there exist positive finite constants l_{01}, l_{11} and l_{21} such that the error $\mathbf{x_1}$ is bounded as follows, for all t:

$\|\mathbf{x_1}(t)\| \leq l_{01} \|\mathbf{x_1}(0)\| + l_{11} M_1 + l_{21}$

Proof:

1. Defining $\tilde{R}_1^* = \tilde{R}_1 - V_1(\tilde{\rho}_1)$

the following system is equivalent to the error system (11):

$$\frac{d}{dt}\begin{bmatrix} \tilde{O} \\ \tilde{R}_1^* \\ \tilde{R}_3 \\ \tilde{\rho}_1 \end{bmatrix} = \begin{bmatrix} -C_1 & -k_5 & -k_6 & -V_1 k_5 \\ -C_2 & -C^I & 0 & 0 \\ C_3 & 0 & 0 & 0 \\ C_4 & 0 & 0 & 0 \end{bmatrix} \begin{bmatrix} \tilde{O} \\ \tilde{R}_1^* \\ \tilde{R}_3 \\ \tilde{\rho}_1 \end{bmatrix} + \begin{bmatrix} 0 & 0 \\ 0 & 0 \\ 1 & 0 \\ 0 & 1 \end{bmatrix} . \begin{bmatrix} \frac{dR_3}{dt} \\ \frac{d\rho_1}{dt} \end{bmatrix} \quad (12)$$

2. The homogeneous part of (12) can be written:

$$\frac{d}{dt}\begin{bmatrix} \tilde{O} \\ \tilde{R}_1^* \\ \tilde{R}_3 \\ \tilde{\rho}_1 \end{bmatrix} = \begin{bmatrix} \mathbf{B_{11}} & -\mathbf{B_{21}}^T \\ \mathbf{B_{21} P_1} & 0 \end{bmatrix} \begin{bmatrix} \tilde{O} \\ \tilde{R}_1^* \\ \tilde{R}_3 \\ \tilde{\rho}_1 \end{bmatrix} \quad (13)$$

with $\mathbf{B_{11}} = \begin{bmatrix} -C_1 & -k_5 \\ -C_2 & -C^I \end{bmatrix}$; $\mathbf{B_{21}} = \begin{bmatrix} k_6 & 0 \\ k_5 V_1 & 0 \end{bmatrix}$;

$\mathbf{P_1} = \begin{bmatrix} c_1 & 0 \\ 0 & 1 \end{bmatrix}$

The matrix

$$\mathbf{P_1 B_{11}} + \mathbf{B_{11}}^T \mathbf{P_1} = \begin{bmatrix} -2C_1 c_1 & -k_5 c_1 - C_2 \\ -k_5 c_1 - C_2 & -2C^I \end{bmatrix}$$

is negative definite by the assumptions A5÷A8.

The matrix $\mathbf{B_{11}}$ is stable by the same assumptions. Then the exponential stability of (13) follows from Theorem 3.2 (Bastin and Dochain, 1989).

3. The forcing term of (12) is bounded by assumptions A10 and A11.

4. Then it is a result of adaptive system theory that the state of (11) is bounded (Theorem A2.6, Bastin and Dochain, 1989).

3.3 *Estimator of R_2 and X (Estimator II)*

Define the auxiliary parameters:

$$\rho_2 = OUR + X \quad (14)$$

$$\rho_3 = \left(-R_2 - C^{III} X\right)/C^{IV} \quad (15)$$

where C^{III} and C^{IV} are parameters, which can be positive constants or time-varying parameters.
Write the dynamical model of dissolved oxygen concentration, given by Eq. (6) and using Eq. (14) as:

$$\frac{dO}{dt} = -\rho_2 + X - DO + OTR \qquad (16)$$

According to Eq. (15), the dynamical model of X can be written as:

$$\frac{dX}{dt} = R_1 + R_3 - C^{IV}\rho_3 - C^{III}X - DX \qquad (17)$$

Using Eqs. (16), (17) and on-line measurements of the dissolved oxygen concentration and the off-line measurements of the biomass concentration, the following adaptive estimator can be derived:

$$\frac{d\hat{O}}{dt} = -\hat{\rho}_2 + \hat{X} - DO + OTR + C_5(O - \hat{O}) \qquad (18a)$$

$$\frac{d\hat{X}}{dt} = \hat{R}_{1I} + \hat{R}_{3I} - C^{IV}\hat{\rho}_3 - C^{III}\hat{X} - D\hat{X} + C_6(O - \hat{O}) +$$
$$+ C_8 V_2 (O - \hat{O}) + C_{x1}(X_L - \hat{X}_L) \qquad (18b)$$

$$\frac{d\hat{\rho}_2}{dt} = -C_7(O - \hat{O}) \qquad (18c)$$

$$\frac{d\hat{\rho}_3}{dt} = -C_8(O - \hat{O}) - C_{x2}(X_2 - \hat{X}_2) \qquad (18d)$$

$$\frac{dV_2}{dt} = -(C^{III} + D)V_2 + C^{IV} \qquad (18e)$$

where $\hat{O}, \hat{X}, \hat{\rho}_2$ and $\hat{\rho}_3$ are estimated values for O, X, ρ_2 and ρ_3, while C^{III}, C^{IV}, C_5, C_6, C_7, C_8, C_{x1} and C_{x2} are estimator parameters, V_2 - an auxiliary variable, X_L and $\hat{X}_L$ - the off-line measurements and the estimates of X, $\hat{R}_{1I}$, $\hat{R}_{3I}$ - the estimated values of the R_{1I}, R_{3I} obtained by the first algorithm.

An estimation of R_2 is obtained using ρ_3, X estimates and the relationship (15) as follows:

$$\hat{R}_2 = -\hat{\rho}_3 C^{IV} - C^{III}\hat{X} \qquad (18f)$$

The stability of the estimator (18) can be proved like estimator I under the following assumptions:

a) in the case where the off-line X measurements are not available:

A12. $C_5>0$; $C_5 = 2h - (C^{III} + D)$, h – positive constant.

A13. $C_6>0$; $C_6 = [C_5 - (C^{III} + D)]^2/4$

A14. $c_2>0$; $c_2 = C_6$

A15. $C_7 = c_2$, $C_8 = c_2 V_2$

A16 The vector $[1, V_2]^T$ is a persistently exciting signal

A17. $\left|\frac{d\rho_2}{dt}\right| \le M_3$;

A18. $\left|\frac{d\rho_3}{dt}\right| \le M_4$

where M_3 and M_4 are upper bounds.

b) in the case where the off-line X measurements are available:

A19. $C_5>0$; $C_5 = 2h - (C^{III} + C_{x1} + D)$, with h – positive constant.

A20. $C_6>0$; $C_6 = [C_5 - (C^{III} + C_{x1} + D)]^2/4$

A21. $c_2>0$; $c_2 = C_6$, A22. $C_7 = \gamma_1$

A23. $C_8=0$

A24. $C_{x2} = \gamma_2 C^{IV}$ with γ_1, γ_2 positive constants.

4. SIMULATION INVESTIGATIONS

The behaviour of proposed adaptive estimators is investigated by simulations of a process model, which belongs to the class defined by Eqs. (4), (5) where specific growth rates are given by Monod models with constant parameters μ_{max1}, μ_{max2}, μ_{max3} maximum specific growth rates, K_{s1}, K_{s2}, K_{s3} saturation constants. The values of the model parameters are given in Table 1.

It is considered fed-batch process. The initial values of variables and parameters X, S_1, R_1, R_2, R_3 are given in Table 2. In Fig. 1, the simulation results from estimator I under different values of design parameter C^I are shown. The simulations results of estimator II are plotted in Fig. 2.

On the basis of the obtained results, the following conclusions can be drawn:

1. Although the proposed estimators have not small number parameters, the proposed tuning procedures reduce their number to: three for estimator I: C^I, h^I and $V_I(0)$; four for estimator II: C^{III}, h^{II}, C_{x1} and C_{x2}

2. The estimates under different values of the sampling period T_x coincide.

3. More accurate estimates are obtained for R_1, R_3 and X in comparison with R_2.

4. In the considered simulation investigations, the values of the tuning parameters are chosen using a trial-and-error approach. It is possible to be proposed and applied other tuning methods under the

experimental validation of the proposed software sensors. One possibility to be searched is a reasonable trade-off between noise sensitivity and convergence using criteria proposed by Claes and Van Impe (1997) on the basis of the experimental data.

5. CONCLUSION

An approach for estimation of multiple biomass growth rates and biomass concentration for a class of aerobic bioprocesses is proposed. It is based on adaptive observer theory and requires on-line measurements of dissolved oxygen concentration and off-line measurements of biomass concentration.

The practical applicability of the proposed approach is a direct consequence of several important factors. The estimators (i) are not depend on any particular models of the biomass growth rates, which are assumed to be unknown time-varying parameters; (ii) require only on-line measurements of dissolved oxygen concentration, which can be performed easily using cheap and reliable sensors.

The results by simulation demonstrated the good performance of the proposed estimators under fed-batch conditions. The values of tuning parameters were chosen using a trial and error approach. The experimental validation of the proposed strategy gives the possibilities to be applied other tuning procedures, connecting with the use of experimental data.

Table 1: Model parameters

μ_{max1}=0.5 h^{-1}	μ_{max2}=0.8 h^{-1}	μ_{max3}=0.1 h^{-1}	
K_{s1}=1 g l^{-1}	K_{s2}=2 g l^{-1}	K_{s3}=1 g l^{-1}	
k_1=0.5	k_2=0.1	k_5=0.1	k_6=0.08
k_7=0.1	k_8=0.05	k_9=0.01	
S_{in1}=10 g l^{-1}			
OTR=0.3 g l^{-1} min^{-1}			
CTR=0.35 g l^{-1} min^{-1}			

Table 2: Initial conditions

$X(0)$=34.56 g l^{-1}
$S_1(0)$=0.1086 g l^{-1}
$R_1(0)$=1.6934 g l^{-1} min^{-1}
$R_3(0)$=0.3386 g l^{-1} min^{-1}
$R_2(0)$=1.4245 g l^{-1} min^{-1}
D=0.1 h^{-1}

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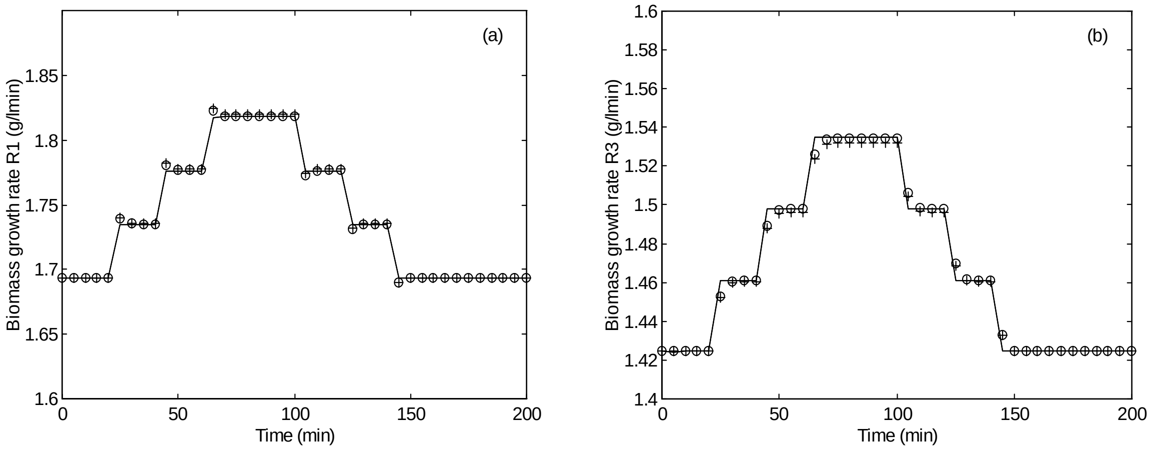

Figure 1. Estimates of R_1, R_3, and ρ_1 by estimator I and the true value of the same parameters (lines) under different values of the design parameter C^I (C^{II}=1):
C^I =1.05 (o points), C^I =1.03 (+ points), $h_{1,2}^I$= -5.

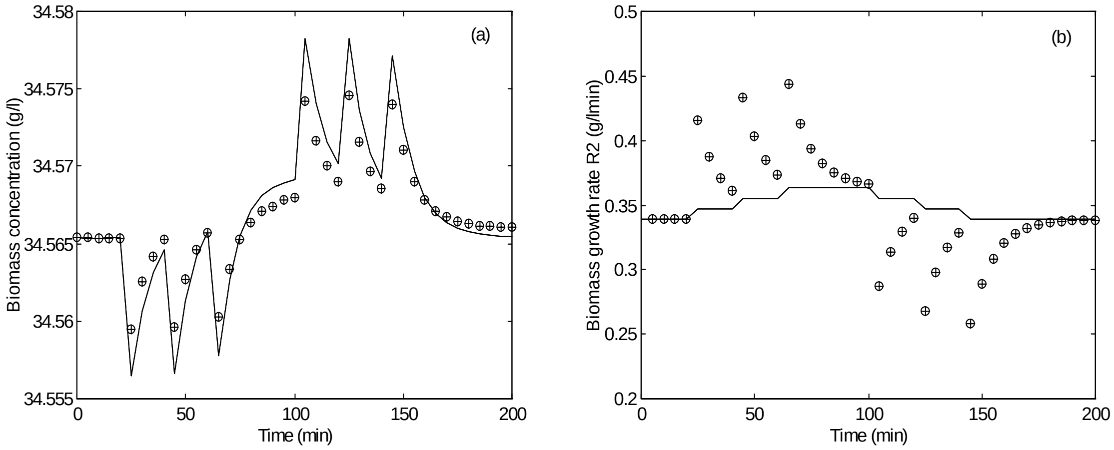

Figure 2. Estimates of X, R_2, ρ_2, and ρ_3 by estimator II and the true value of the same parameters (lines) under different sampling times T_x of biomass measurements.
Values of design parameters: estimator I: C^I =1.05, C^{II} =1, $h_{1,2}^I$ = -5, estimator II: C^{III} = $-D$+0.1, C^{IV}=1, $h_{1,2}^{II}$=-10 and C_{x1}=10, C_{x2}= C_7=50 (o points for T_x =25 min and with + points for T_x = 50 min).