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Application of the Lyapunov Exponent to Evaluate Noise Filtering Methods for a Fed-batch Bioreactor for PHB Production

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***Abstract**: Large-scale fed-batch fermentations are often subject to noise carried by the feed streams. This noise corrupts the process data and may destabilize the fermentation. So it is important to retrieve clear signals from noisy data. This is done by noise filters. The performances of some commonly used filters have been studied for poly-β-hydroxybutyrate production by Ralstonia eutropha. In simulated experiments, Gaussian noise was added to the flow rates of the carbon and nitrogen substrates. The filters were compared by means of the Lyapunov exponents of the outputs and their closeness to the noise-free performance. Negative exponents indicate a stable fermentation. An auto-associative neural filter performed the best, followed by a combination of a cusum filter and an extended Kalman filter. Butterworth filters were inferior and inadequate.*

***Keywords**: Poly-β-hydroxybutyrate, Fed-batch fermentation, Inflow noise, Filter performance, Lyapunov exponents.*

Introduction

Microbial fermentations operated under production conditions are invariably subject to noise from the environment. Since optimal operation and control depend on the timely availability of clear and reliable measurements, it is important to filter out the noise from corrupted data and generate clearly identifiable signals. The presence and the effects of noise on bioreactor monitoring and performance have been documented by many authors [15, 19, 29]. These and other studies show that, in addition to complicating measurements, process noise may alter the nature of the fermentation itself, such as its stability [14] and monotonicity [26].

Continuous and fed-batch fermentations are more likely to be affected by noise than are batch fermentations because of fluid flow from the environment into the bioreactor. Noise is predominantly carried by feed streams of liquid substrates and gases (typically oxygen in aerobic cultures). Because kinetic and metabolic considerations, together with economic viability on a large scale, favor fed-batch and continuous fermentations [12], noise is of serious concern on an industrial scale.

To generate clearly identifiable values of process variables from noisy measurements, the raw data are processed (preferably on-line) through noise filters. These are software devices that act on noisy inputs and generate reasonably noise-free outputs. The epithet ‘reasonably’ is significant because different types of filters generate outputs with different amounts of residual noise. It will be seen from the descriptions that follow that noise filters differ both structurally and algorithmically. For a particular type of filter, the extent of removal of noise depends also on the noise-affected variable and the initial intensity of the noise.

Previous studies [17, 20, 23] have identified some filter configurations that are effective for applications to bioreactors. These filters and two others were applied here to a fed-batch fermentation by *Ralstonia eutropha* to produce poly-β-hydroxybutyrate (PHB). The filters used and the kinetics of PHB formation are described below. PHB was chosen in view of its emergence as a biopolymer with good biodegradability, biocompatibility and properties that make it potentially competitive with synthetic polymers for many applications.

Noise filters for bioreactors

Controllers for bioreactors require clear data that represent the performance faithfully at all times. This involves removal of noise that clouds many practical measurements, especially in large bioreactors that interact with the environment. While complete removal of noise may be idealistic, most filters attenuate the noise to allow identification of the process signals. The filters are essentially electronic realizations of software methods that act on raw data and prune aberrations, outliers, fluctuations and other extraneous features. Both standard and customized filters are available, and the choice is guided by the application and the resources available.

Previous applications [7, 17, 20, 23, 28] have identified five types of filters suitable for bioreactors. Since their detailed descriptions are available in the literature, the filters are briefly introduced here, with references for further information.

(a) Low pass Butterworth filter (LPBF) [18]

Given a noise-affected measurement vector $\overline{x}_k$ at the k-th (current) sampling point, the LPBF generates filtered values $\widetilde{x}_k$ according to the performance equation:

$$\widetilde{x}_k = \left(\frac{T_f}{T_f + T_s}\right)\widetilde{x}_{k-1} + \left(\frac{T_f}{T_f + T_s}\right)\overline{x}_k \qquad (1)$$

T_f is the filter time constant, T_s is the sampling interval, and $\widetilde{x}_{k-1}$ are the estimated values at the previous point in time.

Eq. (1) represents a first-order LPBF. Filters of second or higher orders are created by placing two or more first order filters in series.

(b) Extended Kalman filter (EKF) [5]

The EKF determines the current (noise-free) estimates of a set of variables by linearizing through the partial derivatives of the process and measurement functions at the (known) previous instant of time. The linearized performance equation has the form:

$$\widetilde{x}_k = \hat{x}_k + A(\widetilde{x}_{k-1} - \overline{x}_{k-1}) + W\overline{w}_{k-1}, \qquad (2)$$

where $\hat{x}_k$ are the outputs of a process (bioreactor) model, usually expressed as:

$$\hat{x}_k = \overline{f}(\hat{x}_{k-1}, \overline{u}_k) \qquad (3)$$

In Eqs. (2) and (3), $\overline{u}_k$ are the forcing functions at the k-th (current) point in time, $\overline{w}_{k-1}$ is the vector of process noise, A is the Jacobian matrix of $\overline{f}$ with respect to $\hat{x}_{k-1}$, and W is the Jacobian with respect to $\overline{w}_{k-1}$.

(c) Cusum filter (CF) [28]

In this filter, the mean of a set of noisy data is considered to change significantly due to changes in the process and not the noise when the cumulative sum (cusum) of the differences between the current measurements and the previous ones exceeds three standard deviations.

$$\text{Let cusum} = \sum_{k=1}^{N} \frac{\bar{x}_k - \bar{x}_{k-1}}{\sigma_x}, \tag{4}$$

where $\bar{x}_k$ is the vector of measurements at the k-th (current) time, σ_x is the set of standard deviations, and N is the number of samples.

Then, the filter acts if |cusum| > $\alpha\sqrt{N}$. The value for α may be chosen according to the desired confidence level for the decision. Typically, α = 2 represents 95% confidence and α = 3 corresponds to 99.7% confidence.

(d) Combined CF-EKF [7]

The working model of this filter is of the form:

$$\tilde{x}_k = \tilde{x}_{k-1} + K_k(\bar{x}_k - \tilde{x}_{k-1}) \tag{5}$$

Note the similarity to Eq. (2). As before, $\tilde{x}_{k-1}$ and $\tilde{x}_k$ are filtered data and $\bar{x}_k$ the noisy data. The Kalman gain K_k may be related to the Jacobian A, mentioned above with reference to Eq. (3), as:

$$K_k = \frac{A_{k-1}}{A_{k-1} + R_e}, \tag{6}$$

where R_e is the variance of the measurement noise.

This essentially Kalman filtering algorithm is implemented through the cusum condition of Eq. (4).

(e) Auto-associative neural filter (ANF) [21, 24]

All the filters described above require a model of the bioprocess. However, under noise-affected industrial conditions it is often difficult to formulate mathematical models that are sufficiently simple, accurate and flexible. Then it is useful to have a filter that does not depend on a model. Artificial neural networks offer this possibility.

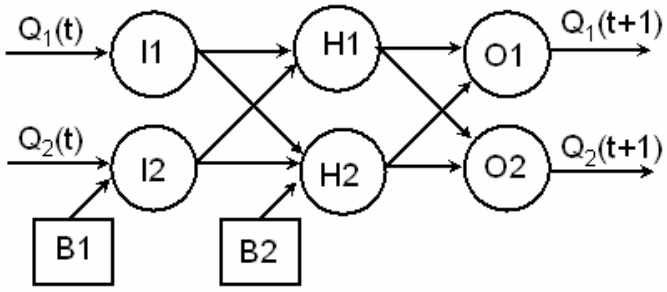

Fig. 1 Architecture of the auto-associative neural filter.
I1, I2 – input neurons; H1, H2 – hidden neurons;
O1, O2 – output neurons; B1, B2 – bias neurons.

Previous studies [18, 21, 22, 23] have shown that an auto-associative network performs effectively for different kinds of fermentations. This is understandable because, in a way, such

a neural network is germane to the filtering process, i.e. the outputs and inputs are the same, except for the reduction of noise. The ANF used here had the architecture shown in Fig. 1. There are two input and two output neurons, one each for the flow rates of the two substrates. The number of neurons in the hidden layer was varied to obtain the best performance; this number also turned out to be two.

Description and modeling of the fermentation

PHB is an energy storage polymer that is synthesized by certain microorganisms under conditions adverse to their growth. Such conditions are created in bioreactors by depriving the cells of essential nutrients such as nitrogen and phosphorus, with nitrogen starvation being preferred.

While a shortage of nitrogen favors PHB synthesis, it is detrimental to cell growth. Sufficient amounts of carbon during the fermentation are also required for growth. Like nitrogen, excess of carbon also suppresses growth [9]. Thus, the C:N ratio is critical in determining both cell growth and polymer formation. The optimum ratio seems to depend on the substrates, the organism and the fermentation conditions, thus making it difficult to generalize. Nevertheless, we know that the optimum C:N ratio for *Ralstonia eutropha* is between 10 and 20 [8, 9]. However, the optimum ratio also varies with time. These features favor fed-batch fermentation for PHB production.

There are two main feed streams. The carbon stream is glucose or fructose, and the nitrogen stream is ammonium chloride or ammonium sulfate. Recent studies [9, 30] have shown that maximization of PHB production requires nonlinear variations in both flow rates and in their ratio. These variations may become more pronounced for large nonideal bioreactors, such as the one analyzed here.

Recognizing these difficulties early on, Yoo and Kim [33] proposed a cybernetic model for PHB synthesis. Their model is based on *R. eutropha*, which is the most widely employed organism for PHB. The preference for *R. eutropha* over other PHB-producing bacteria is based on its easy cultivation, its well-understood physiology and its ability to synthesize large amounts of PHB inside the cells. Yoo and Kim used the NCIB 11599 strain in batch fermentations with ammonium sulfate and glucose.

A central premise in Yoo and Kim's [33] model was that "the carbon source is optimally allocated to the key enzyme synthesis system so that the cells have a high degree of flexibility under nitrogen starvation". Like other workers [9, 11, 16], they divided each cell into a PHB component and the residual biomass. The rate of growth of residual biomass was expressed as:

$$r_X^R = \frac{\mu_1 E_1 S_1 X_R}{K_1 + S_1} \tag{7}$$

S_1 the concentration of the nitrogen source and E_1 is the key enzyme (or bottle-neck enzyme) that catalyses the growth. A similar equation was proposed for PHB synthesis.

$$r_P = \frac{\mu_2 E_2 S_2 X_R}{K_2 + S_2} \tag{8}$$

In terms of the metabolic network [1], E_1 may be RNA-polymerase or glutamate dehydrogenase, whereas E_2 may be identified with β-ketothiolase or PHB polymerase.

A basic principle of cybernetic modeling is that the cells respond to their environment in a manner that is most favorable to their own survival [2]. Yoo and Kim [33] expressed this objective as the maximization of the cell mass at each instant of time. This involves dynamic allocations of carbon and nitrogen according to

$$\nu_i = \frac{\exp(R_i)}{\exp(\sum_j R_j)}; i, j = 1 \text{ or } 2, \tag{9}$$

where ν_i is the fraction of the total substrate that is allocated to the i-th component.

As explained above, the utilization of each substrate is triggered by a key enzyme E_i, whose activity varies as:

$$\gamma_i = \frac{\exp(R_i)}{\max_j[\exp(R_j)]}; i, j = 1 \text{ or } 2 \tag{10}$$

The γ_i are the cybernetic variables.

The rate of change of enzyme concentration is the net result of induced synthesis, degradation and dilution due to cell growth.

$$r_i^e = \frac{\alpha_i S_i \nu_i}{K_i + S_i} - E_i \nu_j \left(\beta_i + \frac{d\ln x}{dt}\right); i = 1 \text{ or } 2 \tag{11}$$

Given the basis of Eqs. (9) and (10), the cybernetic equations for the rates of consumption of the key substrates may be written as:

$$-r_1^S = \frac{\mu_1 r_X^R}{Y_1}\gamma_1 \tag{12}$$

$$-r_2^S = \frac{\mu_1 r_X^R}{Y_1'}\gamma_1 + \frac{\mu_2 r_P}{Y_2} + mX_R \tag{13}$$

Since PHB is accumulated intra-cellularly, the rate of growth of the total biomass is:

$$r_X = r_X^R + r_P \tag{14}$$

Following Kompala et al. [10], Yoo and Kim [33] expressed the specific growth rate on the i-th substrate as:

$$\mu_i = \frac{\mu_{mi}(\mu_{mi} + \beta_i)}{\alpha_i}; i = 1 \text{ or } 2 \tag{15}$$

The batch kinetics may be inserted into the fed-batch model presented below.

$$\frac{dX}{dt} = r_X - \frac{Q}{V}X \tag{16}$$

$$\frac{dS_1}{dt} = r_1^S + \frac{Q_1}{V}(S_{1f} - S_1) \tag{17}$$

$$\frac{dS_2}{dt} = r_2^S + \frac{Q_2}{V}(S_{2f} - S_2) \tag{18}$$

$$\frac{dP}{dt} = r_P - \frac{Q}{V}P \tag{19}$$

$$\frac{dE_1}{dt} = r_1^e - \frac{Q}{V}E_1 \quad (20)$$

$$\frac{dE_2}{dt} = r_2^e - \frac{Q}{V}E_2 \quad (21)$$

$$\frac{dV}{dt} = Q = Q_1 + Q_2 \quad (22)$$

In a recent communication [27], the feed rates Q_1 and Q_2 were determined in the absence of noise so as to maximize PHB production. The values of the parameters and initial conditions are shown in Table 1. To study the effect of noise and its filtering, previous studies of large bioreactors [4, 13, 20, 21, 26, 29] data from large bioreactors were used as the basis to each of the flow rates noise that follows a Gaussian distribution with a mean equal to the current value of the flow rate and different variances. Then Eqs. (16)-(22) were solved first without a noise filter and then with each of the filters described in Section 2. For each set of concentration profiles thus obtained, the Lyapunov exponents were calculated as described next.

Table 1. Values of the parameters and initial conditions [27, 33]

Parameter	Units	Value	Variable	Units	Initial Value
$\mathbf{K_2}$	$g\ l^{-1}$	0.254	$\mathbf{X_R}$	$g\ l^{-1}$	0.29
m	h^{-1}	0.010	$\mathbf{S_1}$	$g\ l^{-1}$	1.26
$\mathbf{Y_1}$	$g\ g^{-1}$	1.653	$\mathbf{S_2}$	$g\ l^{-1}$	20.0
Y_1'	$g\ g^{-1}$	0.460	**P**	$g\ l^{-1}$	0.12
$\mathbf{Y_2}$	$g\ g^{-1}$	0.439	$\mathbf{E_1}$	$g\ l^{-1}$	$8.0.10^{-4}$
α_i	h^{-1}	0.001	$\mathbf{E_2}$	$g\ l^{-1}$	$1.7.10^{-3}$
β_i	h^{-1}	0.050	$\mathbf{S_{1f}}$	$g\ l^{-1}$	1.50
μ_{m1}	h^{-1}	0.176	$\mathbf{S_{2f}}$	$g\ l^{-1}$	20.0
μ_{m2}	h^{-1}	0.098	**V**	l	30.0

The Lyapunov exponents

The Lyapunov exponent provides a convenient quantitative measure of system stability in terms of its time-dependent displacement after a disturbance. In other words, it measures the rate of divergence of two trajectories with increasing time. In the present context, one trajectory may be a noise-free concentration profile and the other a noise-affected profile. Both filtered and unfiltered profiles are considered (in the latter case) to assess the effectiveness of different filtering methods. Since the theory underlying Lyapunov exponents has been described adequately by others [3, 32], only a short introduction relevant to the present application is provided here.

Let x_0 be the value of a concentration just prior to the start (initial time t = 0) of a disturbance or noise signal, and let this value be displaced by $\Delta x(x_0, t)$ as time progresses. The initial separation of the disturbance-free and disturbed trajectories is obviously $\Delta x(x_0, 0)$. A dynamic system, such as the present one, is stable if the separation of the two trajectories does not increase with time. Mathematically this means:

$$\sup_{\Delta x_0} |\Delta x(x_0, t)| \le Ce^{(\lambda t|\Delta x_0|)}, C \in R \quad (23)$$

The number λ is called the Lyapunov exponent, and it applies to both continuous and discrete processes. A system with many variables may have more than one Lyapunov exponent; then the largest exponent, λ_{max}, is sufficient to characterize stability [3, 32]:

$$\lambda_{max} = \lim_{t\to\infty, |\Delta x_0|\to 0} \frac{1}{t} \frac{|\Delta x(x_0, t)|}{|\Delta x_0|} \tag{24}$$

If $\lambda_{max} < 0$, the noise-affected trajectory is attracted eventually to a stable orbit. For PHB fermentation, this means the concentration profiles with noise eventually merge with the deterministic profiles. [How fast (or slowly) the disturbed system regains its original state depends on the value of λ_{max}]. In the limit $\lambda_{max} \to -\infty$, the system is said to be superstable, i.e. no disturbance of any magnitude can permanently shift the fermentation to a different state. By contrast, $\lambda_{max} > 0$ denotes an unstable trajectory; following a disturbance, such a trajectory will diverge increasingly from its original path and eventually either annihilate the microbial processes or generate chaotic behavior.

For multi-variable complex processes, as many biological systems are, there is rarely a clear transition from $\lambda_{max} < 0$ to $\lambda_{max} > 0$. A disturbed (or noise-affected) system usually passes through a gray area around $\lambda_{max} = 0$, within which the system is marginally stable. In a strict Lyapunov sense, $\lambda_{max} = 0$ signifies neutral stability, where a disturbed path eventually stays at a constant distance from the original path until perturbed again. However, in real systems a strictly constant separation can rarely be maintained. Since noise is prevalent more in real applications than in ideal cases, marginal stability is a more useful concept than neutral stability. Further, in microbial processes a certain amount of fuzziness is unavoidable [31], due to noise as well as measurement uncertainties, thereby creating to a window of marginal or transitional stability between the unstable and stable regions.

In view of its success in earlier applications [23, 25, 26], the Lyapunov exponent was employed here to study the effect of inflow of noise on fed-batch fermentation for PHB.

Application and discussion

In fed-batch and continuous fermentations the feed stream is the main source of noise. Observations have shown [15, 20, 26, 29] that the inflow of noise can seriously impair the performance, especially when more than one species of microorganism and/or substrate are involved. In PHB synthesis there are two main substrates, glucose and ammonium sulfate, and their ratio has a singular influence on the fermentation [8, 9, 11].

The noise carried by the feed stream(s) typically has a Gaussian distribution with a mean equal to the instantaneous deterministic value of the noisy variable [13, 20, 29]. In fed-batch fermentations this mean is obviously a function of time. So time-dependent Gaussian noise was added to the flow rates of glucose and ammonium sulfate determined earlier [27] for a noise-free fermentation, and the cybernetic model was solved for different values of the variance of the noise. A range of variances was explored because of observations from other fermentations [14, 21, 23] that large variances may destabilize the fermentation and cause chaotic behavior.

Fig. 2 shows the variation in the Lyapunov exponent over a range of variances for four key concentrations characterizing the fermentation. Until a variance of about 8%, all four plots are negative and increase slowly. Beyond 8%, the exponents become positive and there is a pronounced increase in their rates of change. Owing to the presence of both process noise and

measurement noise, the transition from a negative to a positive Lyapunov exponent is neither abrupt nor exactly the same for all variables. Therefore, as seen in Fig. 2, a band of variances separates the stable region (negative exponents) from the unstable region. Within this band all the exponents cross the axis of neutral stability.

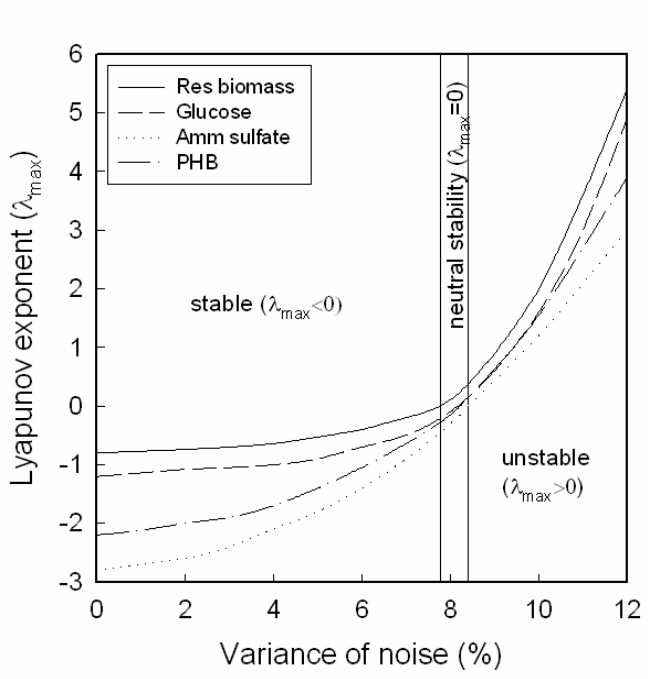

Fig. 2 Variations of the largest Lyapunov exponents with the variance of the noise

Fig. 2 also shows that the residual biomass has the largest Lyapunov exponent for all variances and ammonium sulfate the smallest. This suggests that the residual biomass is the most susceptible to noise-induced destabilization and ammonium sulfate the most robust. Time-domain plots of these variables for an optimally dispersed broth [9] show that the biomass concentration is more than an order of magnitude larger than that of ammonium. This implies that small concentrations are more stable than large concentrations. This has also been observed for an oscillating continuous fermentation by *Saccharomyces cerevisiae* [23], implying that the inference could be valid for different types of organisms and fermentations.

Having identified the stable and unstable regions, one variance from each region was selected to study the effects of different filters. The corresponding Lyapunov exponents are plotted in Figs. 3-6. From left to right the order of the bars in each set is: 1 – no filter, 2 – 1-st order LPBF, 3 – 2-nd order LPBF, 4 – EKF, 5 – CF, 6 – CF-EKF and 7 – ANF. For zero variance, i.e. no noise, all the exponents are negative, indicating a stable fermentation. The exponents are also equal for each variable, which is expected since the filters become redundant in the absence of noise. However, the exponents are progressively more negative from the residual biomass through glucose, PHB and ammonium sulfate, implying increasing stability.

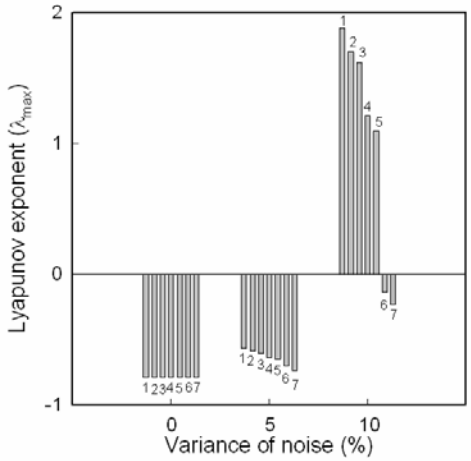

Fig. 3 Values of the largest Lyapunov exponents of the residual biomass without a filter and with different filters for noise variances of 0% (no noise), 5% (stable) and 10% (unstable). Bars: 1 – no noise, 2 – LPBF(1), 3 – LPBF(2), 4 – EKF, 5 – CF, 6 – CF-EKF, 7 – ANF.

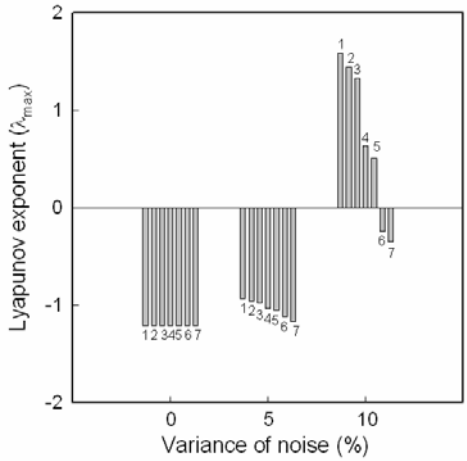

Fig. 4 Values of the largest Lyapunov exponents of glucose without a filter and with different filters for noise variances of 0% (no noise), 5% (stable) and 10% (unstable). Bars: 1 – no noise, 2 – LPBF(1), 3 – LPBF(2), 4 – EKF, 5 – CF, 6 – CF-EKF, 7 – ANF.

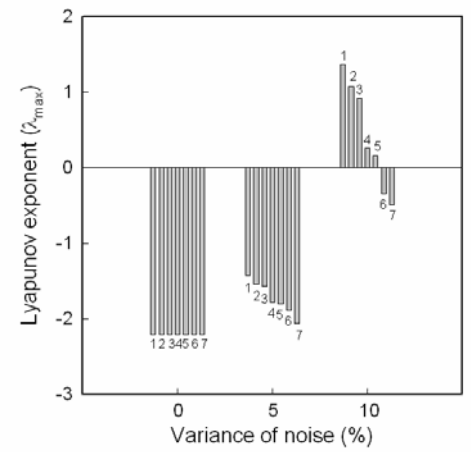

Fig. 5 Values of the largest Lyapunov exponents of PHB without a filter and with different filters for noise variances of 0% (no noise), 5% (stable) and 10% (unstable). Bars: 1 – no noise, 2 – LPBF(1), 3 – LPBF(2), 4 – EKF, 5 – CF, 6 – CF-EKF, 7 – ANF.

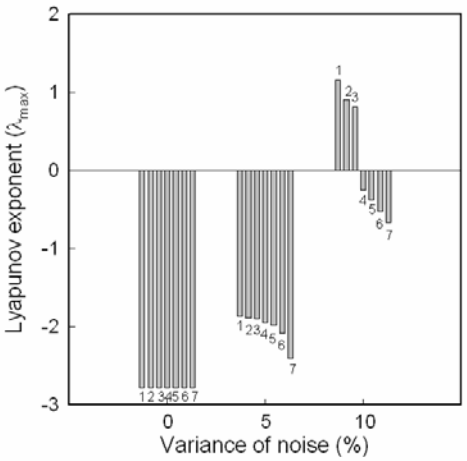

Fig. 6 Values of the largest Lyapunov exponents of ammonium sulfate without a filter and with different filters for noise variances of 0% (no noise), 5% (stable) and 10% (unstable). Bars: 1 – no noise, 2 – LPBF(1), 3 – LPBF(2), 4 – EKF, 5 – CF, 6 – CF-EKF, 7 – ANF.

The order of stability is preserved even when noise flows in with the feed streams. At 5% variance all the filters are able to maintain stable fermentations even though the extents of stability differ. Again this may be expected since 5% variance of the noise inflow is unable to destabilize the process, as revealed by the negative Lyapunov exponents without a filter in all four figures. Nevertheless, within the increasing order of effectiveness, there are only marginal differences between the first and second order LPBF and between an EKF and a CF. The ANF is seen to be substantially better than other filters.

The differences between the noise filters become really significant and informative under conditions where noise destabilizes the fermentation. This happens above 8% variance (Fig. 2) and illustrative results for 10% variance are shown in Figs. 3-6. All Lyapunov exponents without a filter are strongly positive; while the use of filters reduces the exponents in the same order as for 5% variance, some filters are now unsuccessful in restoring stability. For the relatively less stable variables (Figs. 3-5), only the CF-EKF and the ANF are able to filter out the noise sufficiently, whereas for ammonium sulfate (Fig. 6), which is less affected, the EKF and CF are also effective.

The superiority of an ANF over other kinds of filters arises from its flexibility, versatility, robustness, and the ability to learn from previous experience and make 'intelligent' decisions [6]. Static filters such as the LPBF and EKF are restricted in these features and therefore in their ability to act effectively when there are large deviations from stable performance. However, the EKF is better than the LPBF in its design methodology, connection with a plant model and its ability to restore stable performance even in the presence of limited chaos [5, 23, 26]. Figs. 3-6 also show that while the EKF and the CF are about equally good, their combination (the CF-EKF) improves filtering efficiency remarkably. While more studies are required to generalize and elucidate this observation, Jacobsson et al.'s [7] work suggests an explanation. For complex information networks, they surmised that efficient attenuation of noise had to be linked to reduction of bottlenecks in the network since noise tended to accumulate at such points. This requirement implies combining a filtering algorithm with another for change detection. An EKF provides the former requirement and a CF the latter. Therefore, as in Jacobsson et al.'s study, a CF-EKF filter has been efficient in the present application also since the metabolic network for PHB synthesis [1] also has complex information flow and key enzymes corresponding to the bottleneck points.

The differences in the filtering efficiencies for different designs are quantitatively compared in Table 2. Each value depicts the extent of noise reduction, expressed as:

$$\text{Reduction}(\%) = 100\left(\frac{\lambda_f - \lambda_0}{\lambda_0}\right), \tag{25}$$

where λ_0 is the (largest) Lyapunov exponent without a filter and λ_f the corresponding exponent with a filter. Therefore, in the absence of noise all the values in Table 2 are zero. For 5% variance, the reductions are positive since both λ_0 and λ_f are negative in all cases (Figs. 3-6), whereas for 10% variance they are negative because $\lambda_0 > 0$ and $\lambda_f < \lambda_0$ for all variables.

Table 2 indicates that the six filters may be paired into three groups according to the closeness of their performances. Group 1 has the two LPBFs, group 2 contains the EKF and the CF, and group 3 comprises the CF-EKF combination and the ANF. The differences between the members in each group are much smaller than between the groups. There is a 3- to 4- fold improvement from group 1 to group 2, and group 3 filters are twice as good as those in group 2. It is also seen that the extent of stabilization increases down the table, in the same order as the plots in Fig. 2. The theoretical limit of $-\infty$ in Table 2 denotes infinite stability, a notion that is consistent with the superstability signified by a Lyapunov exponent $\lambda \to -\infty$ [3, 32].

Table 2. Comparison of the noise reduction performances of different filters

Concentration	Variance of noise(%)	Percentage reduction in Lyapunov exponent					
		LPBF(1)	LPBF(2)	EKF	CF	CF-EKF	ANF
Biomass	0	0.0	0.0	0.0	0.0	0.0	0.0
	5	3.51	7.02	12.28	14.03	22.81	29.82
	10	-9.57	-13.83	-35.64	-42.02	-107.45	-112.23
Glucose	0	0.0	0.0	0.0	0.0	0.0	0.0
	5	3.23	5.38	10.75	12.90	20.43	25.81
	10	-8.86	-16.46	-60.13	-67.72	-115.19	-122.15
PHB	0	0.0	0.0	0.0	0.0	0.0	0.0
	5	7.69	9.79	24.48	25.87	32.17	44.06
	10	-21.32	-33.09	-80.88	-88.24	-125.74	-136.03
Ammonium	0	0.0	0.0	0.0	0.0	0.0	0.0
	5	1.61	2.15	4.84	6.45	11.83	29.57
	10	-22.42	-30.17	-121.55	-132.76	-144.83	-157.76

Concluding observations

Noise is a ubiquitous feature of large-scale fermentations. Inflow streams are the main carriers of noise, thus making continuous and fed-batch fermentations more likely to be affected than batch operations. Previous studies have shown that noise in the flow rates of feed streams may be modeled by a Gaussian distribution.

For PHB production in fed-batch fermentation by *R. eutropha*, an experimentally validated cybernetic model was used to study the effect of noise in the flow rates of the main carbon source (glucose) and the main nitrogen source (ammonium sulfate). The focus was on stability of the fermentation, quantitatively described by the Lyapunov exponent. Up to about 8% in the variance of the noise, the Lyapunov exponents were negative, indicating stability. Larger variances caused instability. So, variances of 5% and 10% were selected to study the effect of filtering the noise.

At 5% variance, the fermentation was inherently stable, so filtering the noise was of marginal benefit. Nevertheless, filtering did reduce the already negative exponents, thus increasing the stability. The effectiveness of noise filters is, however, properly tested for a noisy unstable fermentation. A variance of 10% allowed this evaluation. Lyapunov exponents of the noise-affected unfiltered fermentation had revealed that residual biomass was most severely affected and ammonium the least, while glucose and PHB were intermediate. While low pass Butterworth filters could not restore stability, an EKF and a CF could stabilize only the ammonium sulfate concentration. On the contrary, the CF-EKF combination and ANF were effective for all concentrations.

Despite their superior performances, the CF-EKF and the ANF need not always be the most suitable choice. At least two considerations moderate the selection. One is the requirement of the application. Efficient filters also tend to be complex and slow, so an application that requires fast but not very accurate data acquisition and control may served better by an EKF or a CF. Even for these filters, a realistic application often involves a balance between speed and accuracy [26, 28]. Secondly, more than one criterion may be applied to evaluate the performance of a filter, and the choice depends on the relative importance assigned to different criteria [23, 24].

Nomenclature

E_1	concentration of key enzyme for nitrogen assimilation (g l^{-1})
E_2	concentration of key enzyme for glucose assimilation (g l^{-1})
K_1	Monod constant for biomass growth (g l^{-1})
K_2	Monod constant for PHB synthesis (g l^{-1})
m	maintenance coefficient (g g^{-1} h^{-1})
P	concentration of PHB (g l^{-1})
Q	total feed rate (l h^{-1})
Q_1	feed rate of ammonium sulfate (l h^{-1})
Q_2	feed rate of glucose (l h^{-1})
r_i^e	rate of synthesis of E_i (g l^{-1} h^{-1})
r_1^S	rate of consumption of ammonium sulfate (g l^{-1} h^{-1})
r_2^S	rate of consumption of glucose (g l^{-1} h^{-1})
r_P	rate of PHB synthesis (g l^{-1} h^{-1})
R_i	r_X^R for i = 1 and r_P for i = 2 (g l^{-1} h^{-1})
r_X^R	rate of growth of residual biomass (g l^{-1} h^{-1})
S_1	concentration of ammonium sulfate (g l^{-1})
S_{1f}	feed concentration of ammonium sulfate (g l^{-1})
S_2	concentration of glucose (g l^{-1})
S_{2f}	feed concentration of glucose (g l^{-1})
t	time (h)
V	volume of broth in the reactor (l)
X	total biomass concentration (g l^{-1})
X_R	concentration of residual biomass (g l^{-1})
Y_1	residual biomass yield from ammonium sulfate (g g^{-1})
Y_1'	residual biomass yield from glucose (g g^{-1})
Y_2	PHB yield from glucose (g g^{-1})
α_i	synthesis rate constant for E_i (h^{-1})
β_i	decay constant for E_i (h^{-1})
γ_i	cybernetic variable controlling the activity of E_i
μ_1	specific rate of growth of biomass on ammonium sulfate (h^{-1})
μ_2	specific rate of growth of biomass on glucose (h^{-1})
μ_{mi}	maximum value of μ_i (h^{-1})
ν_i	fractional allocation of i-th resource

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