Lyapunov Comparison of Noise-Filtering Methods for Oscillating Yeast Cultures

Pratap R. Patnaik
Institute of Microbial Technology, Sector 39-A, Chandigarh 160 036, India

DOI 10.1002/aic.10156

Keywords: continuous yeast cultures, oscillations, noise-induced chaos, noise filter

Under certain conditions, continuous cultures of the budding yeast *Saccharomyces cerevisiae* exhibit steady oscillations with time in some key concentrations such as those of the cell mass, carbon substrate (glucose), product (ethanol), storage carbohydrate, and dissolved oxygen. These oscillations have been reported in small, laboratory-scale reactors (Bellgardt, 1994; Beuse et al., 1998; Murray et al., 2001; Satroutdinov et al., 1992), which are largely unaffected by environmental disturbances. The nature of the oscillations, including the absence of oscillatory behavior itself, depends on the operating conditions, notably the dilution rate and the mass transfer rate of oxygen to the fermentation broth (Beuse et al., 1998; Jones and Kompala, 1999).

Both metabolic processes and transport between the cells and the extracellular fluid contribute to the occurrence and control of oscillations, and their interactions are complex and not yet fully understood (Patnaik, 2003). This limitation makes it difficult to propose quantitative descriptions of oscillatory fermentations that are adequate without being too complicated. Most modeling efforts have thus focused either in detail on the cellular metabolism with little attention to extracellular transport or primarily on the macroscopic variables of interest through mass balances coupled to heavily lumped kinetics. Both kinds of models have been reviewed recently (Duboc et al., 1996; Patnaik, 2003) and are therefore not discussed here.

These models were based on observations with small, laboratory-scale reactors, where nonideal features such as process noise and spatial gradients can usually be ignored. However, these effects become significant in the realistic conditions of production-scale bioreactors, thus making it difficult to translate laboratory-scale models directly to larger bioreactors (Gillard and Tragardh, 1999; Rohner and Meyer, 1995). To facilitate this scale-up and be able to replicate the high performances achievable in the laboratory, the control of disturbances is one important aspect of bioreactor optimization.

Process noise complicates measurements and control, and can seriously affect bioreactor performance. Published industrial data (Glassey et al., 1994; Montague and Morris, 1994; Rohner and Meyer, 1995) illustrate the extent of fluctuations possible. Analyses of ethanol production by *S. cerevisiae* (Sweere et al., 1988) and *Zymomonas mobilis* (Patnaik, 1994) under nonoscillatory conditions show that noise carried by a feed stream can undermine productivity and also drive a fermentation to chaotic behavior. Because fed-batch and continuous fermentations are practiced in many applications, inflow noise is of serious concern on an industrial scale.

Despite the recognized importance of noise in bioreactor operation, there is limited information on transitions from monotonic to chaotic behavior and on restoration of the original noise-free performance. Such information is even more scanty for oscillating fermentations. However, in view of the prevalence and importance of oscillating microbial cultures, and the difficulties of formulating adequate mathematical models for realistic conditions (Duboc et al., 1996; Murray et al., 2001; Patnaik, 2003), it is purposeful to analyze the effect of noise on deterministic oscillations. Because of the availability of biochemical and physiological studies, its industrial importance and many observations of oscillatory behavior, *S. cerevisiae* is a good organism for such analyses.

As described later, a continuous fermentation model established through laboratory experiments was subjected to Gaussian noise in the substrate feed stream and solved under different conditions for which noise-free oscillations have been reported. The parameters of the noise were varied until chaotic behavior was generated. These data were then passed through commonly used noise filters and their performances were compared by means of Lyapunov exponents.

Correspondence concerning this article should be addressed to P. R. Patnaik at pratap@imtech.res.in.

Fermentation Description and Data Generation

Practical difficulties and commercial restrictions limit the availability and public use of industrial data. Moreover, industrial data are sometimes not sufficiently detailed or sampled in a manner suitable for modeling; interventions to sample more data or more variables may be costly or may upset production schedules. Therefore many authors (Chen and Rollins, 2000; Schubert et al., 1994; Simutis and Lubbert, 1997; Tian et al., 2002) have found it expedient to generate data mimicking industrial conditions by adding noise to a model established through laboratory experiments and solving the model under the conditions of interest. This method also allows exploration of wide ranges of parameters and operating conditions, so that a few promising choices thus obtained may be applied in plant operation.

This approach was applied to generate data from a model proposed by Jones and Kompala (1999) for continuous cultures of *S. cerevisiae*. It differs from previous models in a fundamental way. Whereas other models have followed a mechanistic approach (Patnaik, 2003), Jones and Kompala adopted a cybernetic approach. Apart from having greater physiologically fidelity to the behavior of the cells, their model is simpler than that of many others and captures most of the observed features of oscillating cultures.

The cybernetic approach formalizes the evolutionary concept that microorganisms try to follow those metabolic pathways that are most favorable to their survival under the prevailing conditions. This concept has been able to explain excursions between different growth patterns and consumption patterns in multisubstrate environments and the consequences of genetic manipulations where conventional mechanistic models have struggled to elucidate.

In open fermentation systems (that is, continuous and fed-batch operations), the substrate feed stream is a common carrier of noise, mainly in the flow rate. Previous studies (Montague and Morris, 1994; Patnaik, 1999a; Simutis and Lubbert, 1997) have shown that this noise may be characterized by a set of Gaussian distributions with mean values equal to the instantaneous values of the flow rate and time-dependent variances. Details of the noise generation procedure are described elsewhere (Patnaik, 1994, 1999a). To generate data simulating industrial operation, Gaussian noise was added to the flow rate (or, equivalently, the dilution rate) and the Jones and Kompala (1999) model was solved with the parameter values specified by them. Although the deterministic flow rate profiles provided the starting values for the means of the Gaussian distributions, the variances were increased progressively until the oscillations became chaotic. The filtering devices described next were then applied to the noisy data to regain the original smooth oscillations.

Noise Filters for Bioreactors

Noise filters are essentially electronic realizations of software methods that act on measured data, prune aberrations, outliers, noisy signals, and other extraneous features, and generate outputs that hopefully reflect the true performance of a system. In view of the variations among biological processes and in the conditions in which they may function, the choice of a filtering method depends on the application and the objective. For bioreactors, two kinds of filters have been commonly used and these have therefore been selected for the present study. Because details about them are available elsewhere (Dochain and Perrier, 1997; Nelles, 2000), they are only briefly described here.

One common filter is the low-pass Butterworth filter; it may be of any order, the first order being the basic configuration. It has the following performance equation

$$\tilde{x}_k = \left(\frac{T_f}{T_f + T_s}\right)\tilde{x}_{k-1} + \left(\frac{T_f}{T_f + T_s}\right)x_k \tag{1}$$

Here x_k is the vector of noisy measurements at the kth sampling instant, $\tilde{x}_k$ are the filtered values, T_f is the filter time constant, and T_s is the sampling interval.

Filters of second or higher orders are created by placing two or more first-order filters in series.

The other common noise filter is the extended Kalman filter, which performs according to the following model

$$\tilde{x}_k = \hat{x}_k + A(\tilde{x}_{k-1} - x_{k-1}) + Ww_{k-1} \tag{2}$$

where $\hat{x}_k$ is the set of values from a process model, x_{k-1} are the a posteriori estimates at one time interval before the current time, and w_{k-1} is the vector of the process noise.

The bioreactor model is usually expressed in the form

$$\hat{x}_k = f(\hat{x}_{k-1}, u_k) \tag{3}$$

where u_k is the vector of forcing functions. Knowing this model, the two Jacobian matrices **A** and **W** can be calculated as

$$\mathbf{A} = \left[\frac{\partial f}{\partial \hat{x}_{k-1}}\right] \tag{4}$$

and

$$\mathbf{W} = \left[\frac{\partial f}{\partial w_{k-1}}\right] \tag{5}$$

Both kinds of filters require a model for the bioprocess. Under the realistic conditions of large-scale operations, where ideal conditions are "corrupted" by noise and incomplete dispersion in a reactor, it is often difficult to propose a model that is sufficiently simple and accurate for on-line use in a dynamic situation (Rohner and Meyer, 1995). Then nonalgorithmic filters based on fuzzy logic and neural networks offer a viable alternative. They require none or a minimum process model, they are robust to disturbances in the data, they are flexible, and they learn and improve with use.

For filtering of disturbances, an autoassociative neural network has been shown to be an appropriate and efficient device (Nelles, 2000): it has the same inputs and outputs, and the vital processing of noise-infected data is done by the hidden neurons, whose number is adjustable. The superiority of a neural filter over the other two kinds described above has been demonstrated for fermentations without oscillations (Patnaik, 1999a,b). The present work is thus intended to extend the applications to an oscillating fermentation under variations in

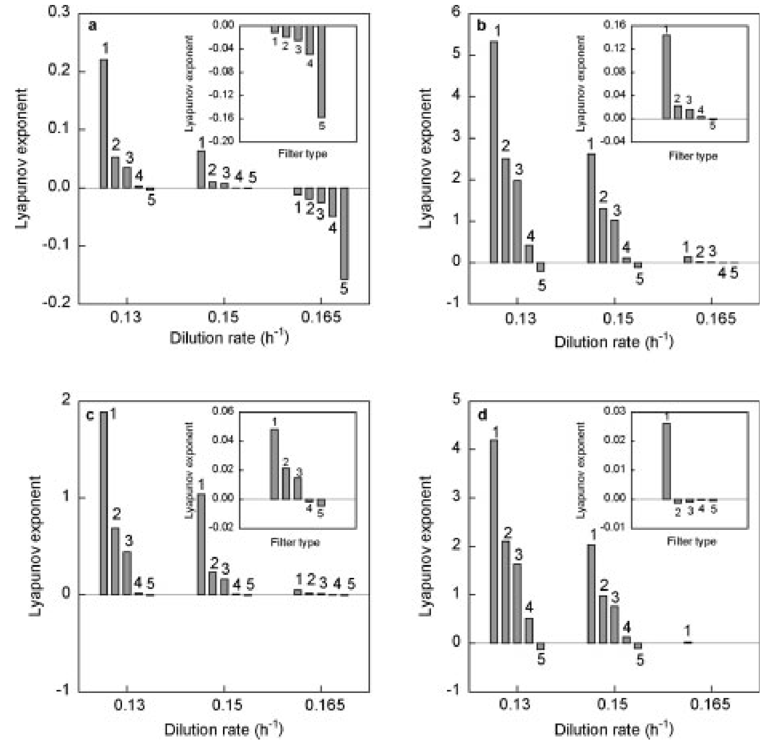

Figure 1. Lyapunov exponents for (a) glucose, (b) cell mass, (c) dissolved oxygen, and (d) ethanol at different values of the dilution rate.

Indices on the bars denote: (1) no filter; (2) first-order low-pass Butterworth filter; (3) second-order low-pass Butterworth filter; (4) extended Kalman filter; (5) neural filter. The insets contain magnified images of the exponents for a dilution rate of 0.165 h^{-1}.

the dilution rate and the dissolved oxygen mass-transfer coefficient.

The Lyapunov Exponent

A prime objective of using a noise filter is to regain the (oscillating) profiles that existed before the intrusion of noise. Using these deterministic profiles as reference values, the overall departure of a disturbed profile from a reference profile may be conveniently characterized by the Lyapunov exponent. This exponent provides a single number indicating the continuing presence or removal of noise, and its rapid evaluation facilitates its use in automatic control systems. Because the theory of Lyapunov exponents is available in the standard literature (Dewdney, 1991; Elert, 2000), only a short overview is provided below.

Consider two trajectories in two-dimensional space. In this study, these trajectories are profiles of concentrations vs. duration of fermentation before and after the onset of some disturbance or noise. Let x_0 be the value of a concentration just before the start ($t = 0$) of a disturbance, and let this value be displaced by $\Delta x(x_0, t)$ as time progresses. The initial displacement is obviously $\Delta x(x_0, 0)$. The mean exponential rate of divergence of the two trajectories may then be calculated as

$$\lambda = \underset{t\to\infty, |\Delta x_0 \to 0|}{\text{limit}} \frac{1}{t} \frac{|\Delta x(x_0, t)|}{|\Delta x_0|} \tag{6}$$

The number λ is called the Lyapunov exponent, and it applies to both continuous and discrete systems.

If $\lambda < 0$, the disturbed trajectory is attracted eventually to a stable periodic orbit, just as the noise-free oscillations are for *S. cerevisiae* cultures. This represents an asymptotically stable, dissipative system; in the limit $\lambda \to -\infty$, the system is superstable. By contrast, $\lambda > 0$ denotes an unstable and chaotic trajectory. In the present study, the variances of the components of the noise were increased until positive values of λ were obtained for all the concentrations in Figure 1.

Sandwiching these two situations is $\lambda = 0$, which signifies a neutrally stable orbit. Physically this means the disturbed oscillations and the original deterministic oscillations stay apart by a constant distance. Such a system is said to be Lyapunov stable.

Application and Discussion

Jones and Kompala (1999) applied their deterministic model to study the effects of changes in dilution rate and the oxygen mass transfer coefficient on oscillatory behavior. These two variables can change significantly the occurrence and the nature of oscillations (Beuse et al., 1998; Duboc et al., 1996; Sweere et al., 1988) and are commonly used as manipulated variables for reactor control, with the dilution rate being preferred (Dochain and Perrier, 1997; Henson and Seborg, 1992).

Two sets of results were used from the work of Jones and Kompala (1999). In one set, the dilution rate was maintained at 0.13 h^{-1} for 100 h, then changed to 0.15 h^{-1} until 200 h, and

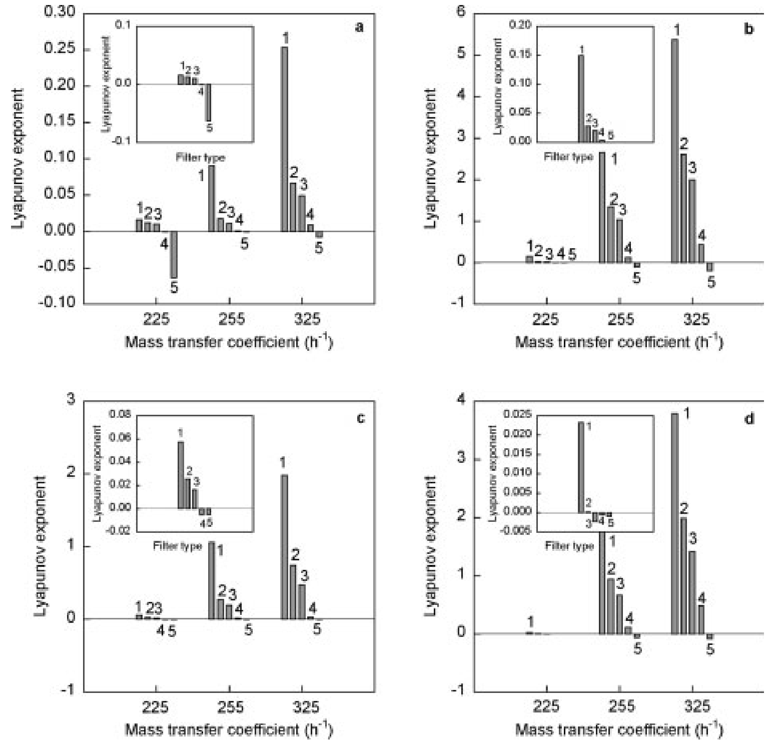

Figure 2. Lyapunov exponents for (a) glucose, (b) cell mass, (c) dissolved oxygen, and (d) ethanol at different values of the oxygen mass transfer coefficient.

Indices on the bars denote: (1) no filter; (2) first-order low-pass Butterworth filter; (3) second-order low-pass Butterworth filter; (4) extended Kalman filter; (5) neural filter. The insets contain magnified images of the exponents for a mass transfer coefficient of 225 h^{-1}.

then changed again to 0.165 h^{-1} until the end of the fermentation at 300 h. With each increase, oscillations in all the variables decayed in both amplitude and frequency. In the second set, the mass transfer coefficient for oxygen was similarly increased stepwise: 225 h^{-1} for 0–100 h, 255 h^{-1} for 100–200 h, and 325 h^{-1} for 300–400 h. The oscillations now amplified with the mass transfer coefficient. These two sets of results thus provide a wide spectrum of behavior to study the effect of noise. Significantly, there was no deterministic chaos. This observation is important because it ensures that the chaotic oscillations, arising subsequently when noise is added, are triggered by the noise and are not intrinsic to the system. A useful implication of this inference is that the Lyapunov exponent used to assess the effectiveness of a filter in restoring the noise-free performance provides reliable comparisons of filtering devices.

Lyapunov exponents for four of the five concentrations are compared in Figures 1 and 2, the first for the dilution rate and the second for the mass transfer coefficient. The bars for internal storage carbohydrate are not shown because they were similar to those for ethanol. In all the plots, the exponents for a fermentation without any filtering of noise are the reference values. Comparisons across the four sets of plots and within each set enable a number of inferences. First, all Lyapunov exponents decrease progressively with increasing dilution rate and increase with the mass transfer coefficient. Considering that oscillatory behavior also follows the same trends, these two figures suggest that monotonic profiles and low intensity oscillations are less likely to be driven to chaotic behavior and, even when this does happen, the fermentation can be brought back to its original performance. Because smaller exponents indicate movement toward stability (or stable oscillations), a reason for this trend may be that at large dilution rates the disturbance-affected stream spends less time in the bioreactor, thus diminishing its scope of disturbing the fermentation process.

A second inference follows from the quantitative values of the exponents for different concentrations. Glucose and dissolved oxygen have smaller exponents than those of ethanol and the cell mass. The model proposed by Jones and Kompala (1999) predicts that the former two variables have much smaller concentrations than the other pair. Thus, like low-amplitude oscillations, variables expressed in low concentrations in the broth appear less likely to be destabilized by noise in the feed stream.

There is also a possible mechanistic basis for this trend. The cybernetic model of Jones and Kompala (1999) was derived from the mechanism shown below (Kompala et al., 1984). The growth of cell mass X on the ith substrate S_i (in a multisubstrate broth) may be expressed schematically as

$$X + S_i \xrightarrow{E_i} (1 + Y_i)X + \cdots \tag{7}$$

where E_i is the key enzyme required for the use of S_i.

Although Kompala et al. did not address product formation, later studies based on this mechanism expressed product formation either in terms of the substrate and cell mass concentrations multiplied by suitable yield factors or in a Luedeking–

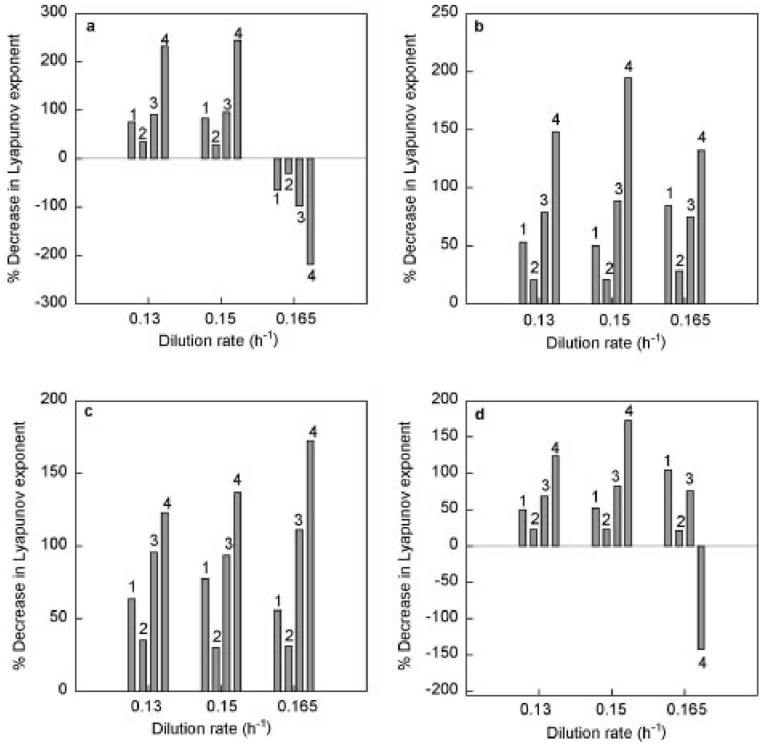

Figure 3. Percentage reductions in the Lyapunov exponents shown in Figure 1 for (a) glucose, (b) cell mass, (c) dissolved oxygen, and (d) ethanol.

Indices on the bars denote: (1) first-order low-pass Butterworth filter; (2) second-order low-pass Butterworth filter; (3) extended Kalman filter; (4) neural filter. Each percentage change is with respect to the preceding value in Figure 1.

Piret form (Gadgil and Venkatesh, 1997). In either case, product is synthesized from the substrate by the cell mass. Because of the intervention of the cell mass, noise in the substrate inflow directly affects the cell mass and becomes attenuated by the product-formation stage. This kind of buffering effect by a large intermediate resource has also been able to explain differences in the effects of feed interruptions on different variables during fed-batch fermentation for β-galactosidase from recombinant *Escherichia coli* (Patnaik, 1998), and thus may have wider validity beyond oscillations in continuous yeast cultures.

A final important question that Figures 1 and 2 answer is how different methods for the filtering of noise perform in restoring the stable oscillations of Figure 1. We observe first that all the Lyapunov exponents in the absence of a filter (bars labeled 1) are positive, signifying chaotic trajectories. Upon applying a noise filter, there is a progressive improvement toward regeneration of stable oscillations in the case of all variables, and the order of improvement is: first-order low-pass Butterworth filter (LPBF) (bar 2), second-order LPBF (bar 3), extended Kalman filter (bar 4), and neural filter (bar 5). Within this order, there are significant decreases from a second-order LPBF to an extended Kalman filter and again to a neural filter. The superiority of a Kalman filter over other algorithmic filters is attested by many applications. Nevertheless, both the LPBF and the Kalman filter are model-based static devices with limited ability to adapt to uncertainties and dynamic variations. Because of their greater flexibility and versatility, and their ability to learn and improve continually, neural networks can adapt more readily to complex and changing situations. Previous studies of nonoscillating fermentations (Patnaik, 1999a,b; Zorzetto and Wilson, 1996) have shown that their greater effectiveness than static filters is linked to their ability to make "intelligent" decisions on allowing optimally filtered noise to accompany the feed stream. This superiority is now seen to be sustained for an oscillating fermentation also. A corollary observation from Figures 1 and 2 is that the smallest improvements occur on moving from a first-order LPBF to a second-order LPBF; higher orders provide only marginally better performances.

Figures 3 and 4 provide a more direct comparison of the relative effectivenesses of different noise-filtering methods. Each bar represents the percentage reduction in the Lyapunov exponent relative to the preceding bar in either Figure 1 or Figure 2. It is significant that the reductions are often as large as two- to threefold. It may also be noted that some ordinates are positive, whereas others are negative. These differences arise because of the definition of the ordinates; the reduction in the value of a Lyapunov exponent is defined as

$$p_j = \left(\frac{\lambda_{j-1} - \lambda_j}{\lambda_{j-1}}\right) \times 100 \tag{8}$$

where p_j is the percentage reduction for the jth type of filter, λ_j is the value of the exponent for that filter, and λ_{j-1} is the corresponding value for the preceding type of filter. Thus, the sign of p_j depends on the signs of λ_{j-1} and λ_j. Because $\lambda_{j-1} > \lambda_j$ in both Figure 1 and Figure 2, three combinations are possible, as follows:

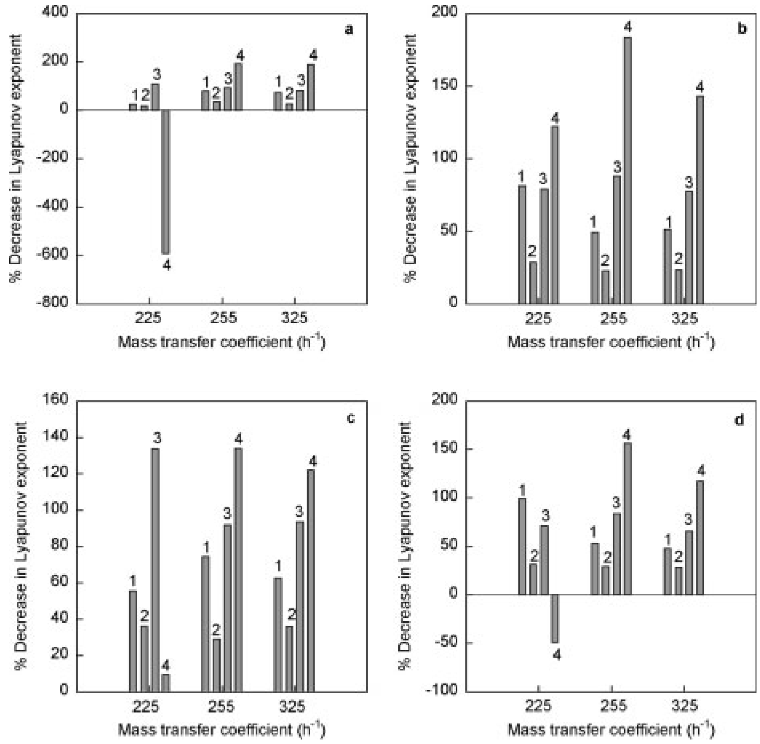

Figure 4. Percentage reductions in the Lyapunov exponents shown in Figure 2 for (a) glucose, (b) cell mass, (c) dissolved oxygen, and (d) ethanol.

Indices on the bars denote: (1) first-order low-pass Butterworth filter; (2) second-order low-pass Butterworth filter; (3) extended Kalman filter; (4) neural filter. Each percentage change is with respect to the preceding value in Figure 2.

	Sign of	
λ_{j-1}	λ_j	p_j
+	+	+
+	−	+
−	−	−

The fourth combination of $\lambda_{j-1} < 0$ and $\lambda_j > 0$ does not satisfy $\lambda_{j-1} > \lambda_j$. This table shows that a negative ordinate in Figure 2 indicates a further reduction in the Lyapunov exponent, suggesting more stable oscillations. By analogy with the "approximate entropy," proposed by Pincus (1991) as a measure of disorder, this "relative stability" of two stable processes may be interpreted as their relative propensity to be displaced from stable trajectories. This interpretation also satisfies the limiting situation of $p_j \rightarrow -\infty$, which now denotes a transition from finite to infinite stability, similar to the superstability indicated by a Lyapunov exponent $\lambda_j \rightarrow -\infty$ (Elert, 2000).

Acknowledgment

This article is IMTECH communication No. 013/2003.

Literature Cited

Bellgardt, K.-H., "Analysis of Synchronous Growth of Baker's Yeast. Parts I and II," *J. Biotechnol.*, **35**, 19 (1994).

Beuse, M., R. Bartling, A. Kopman, H. Diekman, and M. Thoma, "Effect of Dilution Rate on the Mode of Oscillation in Continuous Cultures of *Saccharomyces cerevisiae*," *J. Bacteriol.*, **61**, 15 (1998).

Chen, V. C. P., and D. K. Rollins, "Issues Regarding Artificial Neural Network Modeling for Reactors and Fermenters," *Bioproc. Biosyst. Eng.*, **22**, 85 (2000).

Dewdney, A. K., "Mathematical Recreations: Leaping into Lyapunov Space," *Sci. Am.*, **Sep.**, 178 (1991).

Dochain, D., and M. Perrier, "Dynamic Modeling, Analysis, Monitoring and Control Design for Nonlinear Bioprocesses," *Adv. Biochem. Eng. Biotechnol.*, **56**, 147 (1997).

Duboc, Ph., I. Marison, and U. von Stockar, "Physiology of *Saccharomyces cerevisiae* during Cell Cycle Oscillations," *J. Biotechnol.*, **51**, 57 (1996).

Elert, G., *The Chaos Hypertextbook*, available on the web at http://hypertextbook.com/chaos/ (2000).

Gadgil, C. J., and K. V. Venkatesh, "Structured Model for Batch Culture of *Lactobacillus bulgaricus*," *J. Chem. Technol. Biotechnol.*, **68**, 89 (1997).

Gillard, F., and C. Tragardh, "Modeling the Performance of Industrial Bioreactors with a Dynamic Microenvironmental Approach: A Critical Review," *Chem. Eng. Technol.*, **22**, 187 (1999).

Glassey, J., G. A. Montague, A. C. Ward, and B. V. Kara, "Artificial Neural Network Based Experimental Design Procedures for Enhancing Fermentation Development," *Biotechnol. Bioeng.*, **44**, 397 (1994).

Henson, M. A., and D. E. Seborg, "Nonlinear Control Strategies for Continuous Fermenters," *Chem. Eng. Sci.*, **47**, 821 (1992).

Jones, K. D., and D. S. Kompala, "Cybernetic Model of the Growth Dynamics of *Saccharomyces cerevisiae* in Batch and Continuous Cultures," *J. Biotechnol.*, **71**, 105 (1999).

Kompala, D. S., D. Ramkrishna, and G. T. Tsao, "Cybernetic Modeling of Microbial Growth on Multiple Substrates," *Biotechnol. Bioeng.*, **26**, 1272 (1984).

Montague, G. A., and A. J. Morris, "Neural Network Contributions in Biotechnology," *Trends Biotechnol.*, **12**, 312 (1994).

Murray, D. B., S. Roller, H. Kuriyama, and D. Lloyd, "Clock Control of Ultradian Respiratory Oscillation Found during Yeast Continuous Culture," *J. Bacteriol.*, **183**, 7253 (2001).

Nelles, O., *Nonlinear System Identification: From Classical Approaches to Neural Networks and Fuzzy Models*, Springer-Verlag, New York (2000).

Patnaik, P. R., "Fractal Characterization of the Effect of Noise on Biological Oscillations: The Biosynthesis of Ethanol," *Biotechnol. Technol.*, **8**, 419 (1994).

Patnaik, P. R., "Analysis of the Effect of Interruptions in Substrate Inflow on a Fed-Batch Fermentation with Recombinant Bacteria," *Biochem. Eng. J.*, **1**, 121 (1998).

Patnaik, P. R., "Improvement of the Microbial Production of Streptokinase by Controlled Filtering of Process Noise," *Process. Biochem.*, **35**, 309 (1999a).

Patnaik, P. R.,"Coupling of a Neural Filter and a Neural Controller for Improvement of Fermentation Performance," *Biotechnol Technol.*, **13**, 735 (1999b).

Patnaik, P. R., "Oscillatory Metabolism of *Saccharomyces cerevisiae*: An Overview of Mechanisms and Models," *Biotechnol. Adv.*, **21**, 183 (2003).

Pincus, S. M., "Approximate Entropy as Measure of System Complexity," *Proc. Natl. Acad. Sci. U.S.A.*, **88**, 2297 (1991).

Rohner M., and H.-P. Meyer, "Application of Modeling for Bioprocess Design and Control in Industrial Production," *Bioprocess Eng.*, **13**, 69 (1995).

Satroutdinov, A. D., H. Kuriyama, and H. Kobayashi, "Oscillatory Metabolism of *Saccharomyces cerevisiae* in Continuous Culture," *FEMS Microbiol. Lett.*, **98**, 261 (1992).

Schubert, R., R. Simutis, M. Dors, I. Havlik, and A. Lubbert, "Bioprocess Optimization and Control: Application of Hybrid Modeling," *J. Biotechnol.*, **35**, 51 (1994).

Simutis, R., and A. Lubbert, "Exploratory Analysis of Bioprocesses Using Artificial Neural Network Based Methods," *Biotechnol. Prog.*, **13**, 479 (1997).

Sweere, A. P. J., J. R. Mesters, L. Janse, K. Ch. A. M. Luyben, and N. W. F. Kossen, "Experimental Simulation of Oxygen Profiles and Their Influence on Baker's Yeast Production—I: One-Fermenter System," *Biotechnol. Bioeng.*, **31**, 567 (1988).

Tian, Y., J. Zhang, and A. J. Morris, "Optimal Control of a Fed-Batch Bioreactor Based upon an Augmented Recurrent Neural Network Model," *Neurocomputing*, **48**, 919 (2002).

Zorzetto, L. F. M., and J. A. Wilson, "Monitoring Bioprocesses Using Hybrid Models and an Extended Kalman Filter," *Comput. Chem. Eng.*, **20**, S689 (1996).

Manuscript received Apr. 23, 2003, and revision received Oct. 29, 2003.