Lyapunov stability of a microbioreactor under the influence of external noise

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***Abstract*—**Microreactors are being increasingly preferred over traditional macroreactors for sophisticated microbial processes because the accurate controls possible in the former are desirable for sensitive biological reactions. Sensitivity to disturbances is an important consideration here because cellular processes are constantly under the influence of noise from different sources. While intra-cellular noise has been analyzed in some detail, the effects of noise from the environment are less well understood. Since external noise is a ubiquitous feature of many microbial processes, the present communication analyzes its effect on microbioreactor stability. This is done by using the Lyapunov exponent as an index of stability. For glucose fermentation by immobilized *Saccharomyces cerevisiae* as a model system, simulation results show that the microbioreactor loses stability beyond a threshold variance of the noise. This threshold increases with the concentration of glucose and it is larger for an optimal distribution of cells than for a conventional uniform distribution. However, owing to the Crabtree effect, the glucose concentration has to be optimized between robustness to noise and inhibition at large concentrations. Previous results for macrobioreactors suggest that a similar optimization may be beneficial for filtering of the noise inflow in order to promote stochastic resonance.

Keywords— External noise, Lyapunov exponent, Microbioreactor, Optimization, Reactor stability.

I. Introduction

MICROREACTORS are being increasingly preferred for many biological and biochemical applications that were being carried out in larger macroreactors. Possibly the most widely used application is for DNA detection and polymerase chain reaction (PCR) analysis [1,2]. The success of microbioreactor-based PCR has spawned variations of this theme. These include mixing of DNA and a restriction enzyme, followed by separation of the fragments [3] and integration of DNA analysis with other steps such as electrophoresis [4].

Integration of microbioreactors with other analytical devices has been useful in a variety of enzyme assays, such as the reaction kinetics of β-galacotsidase [5] and on-line reaction activities of HAB-mutase and soyabean peroxidase [6] and aspartate transaminase [7]. Like the combination of a microbioreactor with capillary electrophoresis, enzymatic reactions also have benefited from such integration, Spross and Sinz's [8] recent combination of a microreactor, an ESI device and a MALDI mass spectrometer illustrates the capabilities of expanded integration.

Similar to enzyme activity monitoring, immunoassays too have benefited from microreaction devices. Recently Yang et al. [9] employed a streptavidin-functionalised capillary microreactor to perform highly efficient immunoassays of α-fetoprotein analyte. Utsumi and coworkers [10] reported the use of a bundle of microbioreactors for the analysis of mouse immunoglobulin and nonylphenol using UV absorption spectroscopy. Both studies reported much higher sensitivities, lower costs and easier fabrication and automation by microreactors than by conventional assay methods.

Other areas of microbioreactor applications encompass flow cytometry [11], cellular biosensors [12] and fementations using free or immobilized cells or enzymes. The last group is another major area of applications, as DNA analysis and PCR were mentioned earlier, and it is the subject of the present analysis. Among recent applications for fermentation studies is Szita et al.'s [13] multiplexed microbioreactor for *Escherichia coli* cultivations. Zhang et al. [14] and Lee et al. [15] also studied *E. coli* fementations, as did Buchenauer and associates [16] more recently. A common focus of all these authors was on the monitoring and control of optical density, dissolved oxygen and pH in real time. Their microbioreactors showed excellent performance for all three variables and displayed good reproducibility. The scope of these reactors was extended by Samorski et al. [17] and Funke et al. [18], who moved from a few microreactors in parallel to an array of microtiter plates. This extension was driven by the lack of a miniature reactor that realizes simple process control in high throughput, mainly to obtain accurate and sufficient data for scale-up.

The initial studies have been based on *E. coli*, the work-horse of biologists and biotechnologists. However, the yeast *Saccharomyces cerevisiae* has also attracted significant research attention. Studies by Zhang et al. [19], Reis et al. [20], Au et al. [21], Rahman et al. [22] and Schapper et al. [23] indicate that similar benefits can be obtained with *S. cervisiae*. Of these, Schapper et al.'s [23] work is a useful departure from those of others in that it deals with the spatial distribution of *S. cerevisiae* cells to maximize product formation. Since the present analysis is based on their work, it will be discussed later in detail.

The theme of this communication is the effect of external noise on microbioreactor performance. Many earlier studies

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[24-29] have shown that noise from the environment can have injurious effects on bioreactor performance, and it can change the behavior of a bioprocess drastically. Excessive noise many destabilize the steady operation of a bioreactor, and even drive the process toward stochastic chaos [27,28]. These studies highlight the importance of understanding the effects of external noise on bioreactor performance. However, while these effects have been analyzed for macro-scale reactors, the influence of noise on a microbioreactor has not yet been analyzed. Since many chemical and biological processes are moving from macroreactors to microreactors, it is relevant to perform a similar analysis for a microreactor. This is the motivation for the present work.

II. Problem Statement

Although most of the studies of noise-affected bioreactors have focused on *E. coli* fermentations, the yeast *S. cervisiae* is no less important. Like *E .coli*, *S. cervisiae* is wide by preferred as a model organism to understand the behavior of yeasts. Moreover, *S. cerevisiae* may display a variety of outputs under different conditions, ranging from normal monotonic outputs to different kinds of oscillations and even chaotic behavior [30-32]. Noise from the environment, carried typically by a feed stream, can propel a culture exhibiting smooth monotonic outputs into one of the other types. Of these, chaotic performance is understandably the most undesirable, and therefore it is useful to analyze when such transitions are likely and how to avoid them. This short communication addresses the issue of occurrence of noise-induced unstable behavior by determining the variations of the Lyapunov exponent of a key variable such as biomass concentration as the intensity of noise inflow, measured in terms of its variance, increases. The Lyapunov exponent and its use for stability analysis are introduced in the next section.

Here we provide a brief description of the microbial system to which it has been applied. Schapper et al. [23] studied *S. cervisiae* cultivation in a microbioreactor with the objective of determining the best spatial distribution of cells so as to maximize the production of a recombinant protein. The strain was *S. cervisiae* C468 (ATCC 20690) containing the plasmid pGAC9, which expresses the *Aspergillus awamori* glucoamylase gene and secretes glucoamylase into the extracellular medium. While most cultivations are carried out with a homogeneous distribution of cells, Schapper et al. [23] used topology optimization [33] to determine the best (non-uniform) distribution so as to maximize glucoamylase production. Details are available in their publication.

Briefly, the growth of *S. cervisiae* comprises there metabolic processes: (a) glucose fermentation, (b) glucose oxidation and (c) ethanol oxidation. Glucose is the main carbon source. The synthesis of recombinant glucoamylase is growth-associated and occurs via oxidative metabolism. According to Schapper et al. [23], biomass growth along the three pathways may be described by Monod equations as presented below.

$$\mu_1 = \mu_{1,\max}\left(\frac{G}{K_{1'}+G}\right)\left(\frac{1+k_{a'}G}{k_{b'}+k_{a'}G}\right) \quad (1)$$

$$\mu_2 = \mu_{2,\max}\left(\frac{G}{K_{2'}+G}\right)\left(\frac{1+k_{c'}G}{1+k_{c'}k_{d'}G}\right) \quad (2)$$

$$\mu_3 = \mu_{3,\max}\left(\frac{E}{K_3+E}\right)(1+\tanh(G)) \quad (3)$$

The microbioreactor was loaded with cells immobilized in porpous beads. From a practical perspective, the model accounted for the detachment of some cells from the supports; these cells add to the cells which did not got attached initially. Thus, there are always some free *S. cerevisiae* cells in the culture medium. Following the observation of Branyik et al. [34], only a constant fraction of the immobilized biomass, $X_{im,}^{act}$ was considered to be actively growing, and the rate of detachment of cells was described by

$$k_{det=}^{*} k_{det}^{sst}\left(\frac{G}{K_s+G}\right)+C_3\left(\frac{E}{K_s+E}\right) \quad (4)$$

where C_3 reflects a switch to growth on ethanol (when glucose is depleted).

The total immobilized biomass, X_{im}, is:

$$X_{im} = (1-\gamma)X_{im}^{max} \quad (5)$$

where $(1-\gamma)$ is the fraction of carrier used by the immobilized biomass. The plasmid- bearing content of X_{im} is:

$$X_{im}^{+} = (1-p)X_{im} \quad (6)$$

The transport of suspended (free) biomass, glucose and ethanol occurs by fluid convection and diffusion. So the combined rates may be expressed in Fickian form as:

$$\bar{u}\bar{\nabla}X_f = \mu\left[\left(\frac{X_{im}^{act}}{X_{im}^{act}+X_{im}}\right)\left(\frac{X_{im}P_c}{V_r}\right)X_f\right] \quad (7)$$

$$\bar{u}\bar{\nabla}X_f^{+} = \mu\left[\left(\frac{X_{im}^{act}}{X_{im}^{act}+X_{im}^{+}}\right)\left(\frac{X_{im}^{+}P_c}{V_r}\right)+X_f^{+}\right]+D_{x_f}+X_f^{+} \quad (8)$$

$$\bar{u}\bar{\nabla}G = \left[\frac{\mu_1}{Y_{X/G}^{F}}+\frac{\mu_2}{Y_{X/G}^{0}}\right]\left[\left(\frac{X_{im}^{act}}{X_{im}^{act}+X_{im}}\right)\left(\frac{X_{im}P_c}{V_r}\right)+X_f\right]+D_G\nabla^2 G \quad (9)$$

$$\bar{\mu}\bar{\nabla}E = \left[Y_{E/X}\mu_1-\frac{\mu_3}{Y_{X/E}}\right]\left[\left(\frac{X_{im}^{act}}{X_{im}^{act}+X_{im}}\right)\left(\frac{X_{im}P_c}{V_r}\right)+X_f\right]+D_E\nabla^2 E \quad (10)$$

Schapper et al. [23] argued that since the flow of fluid through the microtube was not axially uniform, neither was the availability of substrates along the length at any time and with the passage of time at any position. Therefore a uniform distribution of the biocatalyst would not necessarily generate the highest possible amount of the product glucoamylase. Hence Schapper et al. determined the optimal longitudinal distribution of the immobilized cells in the microbioreactor by maximizing the total glucoamylase production rate. The objective function thus becomes:

$$\max.\,\phi(r) = \max.\int_{\Omega} R_P\,(t)dv \qquad (11)$$

$$\text{where } R_p = (\alpha_2\mu_2 - \alpha_3\mu_3)\left[\frac{X_{im}^{+}P_c}{V_r} + X_f^{+}\right] \qquad (12)$$

As stated earlier, the problem was solved by topology optimization [33]. For a homogeneous distribution of cells, Eqs.(1)–(10) are solved without any topological optimization; in this case, γ stays constant at its initial value.

III. DESCRIPTION OF NOISE INFLOW

Microbial cultures are constantly under the influence of noise from within the cells and from outside. Intra-cellular noise is associated with the metabolic processes and is manifested in the expression of proteins by specific genes. There are two kinds of intra-cellular noise – intrinsic or extrinsic. Intrinsic noise may be detected through differences between the expressions of two reporter genes inside a single cell. Extrinsic noise affects both, or all, genes equally but generates differences between cells in a population. Both experimental methods [35] and mathematical models [36] for intra-cellular noise have been proposed.

While there are a sufficient number of studies of intra-cellular noise to generate a number of recent reviews [37-39], the understanding of external noise and the combined effects of both sources of noise are still evolving. Nevertheless, there is sufficient evidence to underscore the significance of extra-cellular noise in guiding the performance of a microbial culture. Both experimental [40-42] and simulation [43,44] studies provide ample information of the possible deleterious or beneficial effects of noise inflow to cellular systems. These studies have also indicated that external noise may be modeled by a set Gaussian distributions with time-dependent mean values and different variances.

External noise permeates the cells and interacts with intra-cellular noise. The nature of the interactions determines the subsequent course of a microbial process. Although there are few quantitative analyses of these phenomena, qualitative considerations (reviewed by Patnaik [45]) allow at least two important inferences. One is that noise may have either a harmful effect or a helpful effect on a biological system. These are discussed later. The second inference is that external noise is more likely to resonate with or nullify extrinsic noise rather than intrinsic noise. The reason is that the former two have comparable auto-correlation times of several minutes whereas that of intrinsic noise is much smaller [37,46].

Just as for deterministic studies, *E. coli* and *S. cerevisiae* have been the main vehicles to understand the effects of both intra-cellular and extra-cellular noise. Analyses of continuous cultures of *S. cerevisiae* at the genetic level [47,48] have shown how variations in the noise experienced by the cells can induce them to undergo both metabolic and stability transitions that do not arise spontaneously in the noise-free system. Since noise is a ubiquitous phenomenon experienced by living cells, it becomes useful to analyze its effects.

The analyses cited above were all for homogeneous populations, i.e. they did not consider differences among the cells and the effects of different environmental conditions experienced by an optimally distributed population. In view of the benefits demonstrated by Schapper et al. [23] for a topologically optimum distribution of *S. cerevisiae* cells in a noise-free microbioreactor, it is useful to understand the effects of external noise on such a system. This has been done in the present study and the performance has been compared with that of a similar noise-affected homogeneous distribution. Owing to the importance and possibility of stability transitions, the focus here is on how noise may generate loss of stability. Previous studies by this author [27-29,43] have shown that the Lyapunov exponent is a convenient and reliable index of the stability of a physical, chemical and biological system. Before it is applied to the present system, the method is introduced in the next section.

IV. THE LYAPUNOV EXPONENT

The Lyapunov exponent, λ, provides a convenient quantitative measure of the stability of a system in response to a disturbance. This is done by measuring the rate of divergence of a disturbed trajectory of the system from its path prior to the disturbance. The faster and more expansive the divergence, the greater is the likelihood of the disturbed system being propelled toward instability.

Let x_0 be the value of a concentration just before the start of a disturbance or noise signal. This is the starting time t=0. Let $\Delta x(x_0,t)$ denote the distance between the two concentration trajectories at any time t. Then the initial displacement is obviously $\Delta x(x_0,0)$. A dynamic system is stable if the separation of the disturbed path from the initial stable path does not increase with time; this condition is expressed mathematically as:

$$\sup|\Delta x(x_0,t)| \le C\exp(\lambda t|\Delta x_0|, C \in R \qquad (13)$$

where R is a finite real number. The smaller is the value of R, the closer are the two paths, indicating greater stability.

The number λ is called the Lyapunov exponent, and it applies to both continuous and discrete processes. A multi-variable system may have more than one Lyapunov exponent; then the largest exponent, λ_{max}, is sufficient to characterize stability. This is calculated as:

$$\lambda_{max} = \lim_{t\to\infty,|\Delta x_0|} \frac{1}{t}\frac{|\Delta x(x_0,t)|}{|\Delta x_0|} \quad (14)$$

If $\lambda_{max} < 0$, the noise-affected trajectory will eventually return to its initial stable orbit. In the present context this means the disturbed concentration profile will gravitate back to its profile before the onset of noise. However, during the interim period the system may digress unacceptably far away from a stable situation, and the magnitude of λ_{max} provides an indication of this. From the foregoing discussion it may be inferred that a system is stable if $\lambda_{max} < 0$ and unstable if $\lambda_{max} > 0$. 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 equilibrium to another state.

The exact value $\lambda_{max} = 0$ denotes neutral stability, where the disturbed path eventually remains at a constant distance from the initial path. In most practical situations, owing to the interactions of noise with different characteristics from different sources, strict neutral stability is rarely observed and there is usually a gray area within which a system eventually settles after a disturbance; this is called marginal stability. The presence of marginal stability implies that the transition from stable to unstable behavior is not as clear and rigid as the Lyapunov exponent may indicate; instead, there is a transition window from one to the other phase. A more detailed discussed on the Lyapunov exponent is not warranted here; detailed discussions on this are available elsewhere [49-51].

V. APPLICATION AND DISCUSSION

As stated above, one of the main routes for the inflow of external noise to a bioreactor is through fluctuations in the flow rate(s) of the feed stream(s). These fluctuations may be modeled by a set of Gaussian distributions with different variances [40,42,43,52].

Schapper et al. [23] compared the production rates of recombinant glucoamylase in a microbioreactor with a uniform distribution of immobilized cells with one containing a topologically optimized distribution of cells. To be consistent with their work, four representative values of the inlet concentration of glucose were chosen from the range of concentrations studied by them; the values of the parameters were also maintained the same as in their study (see Table 1). The values of the largest Lyapunov exponent for the biomass were computed at each inlet concentration for mean variances of the feed stream noise ranging from 0% (no noise) to 10%. The mean variance of a mixture of Gaussian distributions may be calculated as explained by Trailovic and Pao [53].

Briefly, a k-component Gaussian mixture has a probability distribution function (pdf)

$$f_k(x) = \sum_{j=1}^{k} w_j \Phi(X; m_j, \sigma_j) \quad (15)$$

where $\Phi(X; m_j, \sigma_j)$ is a Gaussian pdf with mean m_j, standard deviation σ_j and weights w_j satisfying

$$\sum_{j=1}^{k} w_j = 1;\ w_j \geq 0 \quad (16)$$

Given the mixture parameters
$\{w_j, m_j\sigma_j\}, j = 1,2 \ldots \ldots, k$
the mean $\bar{m}$ and variance $\bar{\sigma}^2$ of the distribution are:

$$\bar{m} = \sum_{j=1}^{k} w_j m_j \quad (17)$$

$$\bar{\sigma}^2 = \sum_{j=1}^{k} w_j(\sigma_j^2 + m_j^2) - \bar{m}^2 \quad (18)$$

Table 1. Values of the parameters used in computing the Lyapunov exponents (from [23]).

Parameter	Value	Units
Model parameters		
X_{im}^{act}	0.62	$g_{X_{im}} g_c^{-1}$
P_c/V_r	13.6	gL^{-1}
P	0.05	--
$Y_{X/G}^{F}$	0.12	$g_X g_G^{-1}$
$Y_{X/G}^{0}$	0.48	$g_X g_G^{-1}$
$Y_{E/X}$	3.35	$g_X g_G^{-1}$
$Y_{X/E}$	0.65	$g_X g_E^{-1}$
α_2	32.97	Ug_X^{-1}
α_3	33.80	Ug_X^{-1}
$\mu_{1,max}$	0.38	h^{-1}
$\mu_{2,max}$	0.25	h^{-1}
$\mu_{3,max}$	0.10	h^{-1}
K_1,	0.18	$g\ L^{-1}$
K_2,	0.01	$g\ L^{-1}$
k_a,	-0.004	--
k_b,	2.3	--
k_c,	20	--
k_d,	2.9	--
Simulation parameters		
d_p	0.1	Pa
D_{Xf}	$1*10^{-10}$	$m^2\ s^{-1}$
D_G	$1*10^{-9}$	$m^2\ s^{-1}$
D_E	$1*10^{-9}$	$m^2\ s^{-1}$
D_P	$1*10^{-9}$	$m^2\ s^{-1}$
l	0.012	m
w	0.012	m
h	0.001	m
η	0.001	Pa s
G_{inlet}	5, 30, 100, 500	$mg\ L^{-1}$

Figures 1 and 2 trace the variation in the largest Lyapunov exponent with the mean variance of feed stream noise for a uniform distribution and an optimal distribution *S. cervisiae* cells. In both cases the bioreactor loses stability as the variance crosses a threshold value (indicated by $\lambda_{max} = 0$). This threshold decreased with increasing values of the glucose concentration; a possible explanation for this trend is that the inflow of noise has a smaller effect for low concentrations since here the glucose has a weaker role in the metabolic reactions. However, this does not *ipso facto* suggest maintaining a high concentration in the reactor because glucose becomes inhibitory at high concentrations (Crabtree effect). Hence the optimum concentration is a balance between cell growth inhibition and robustness to noise.

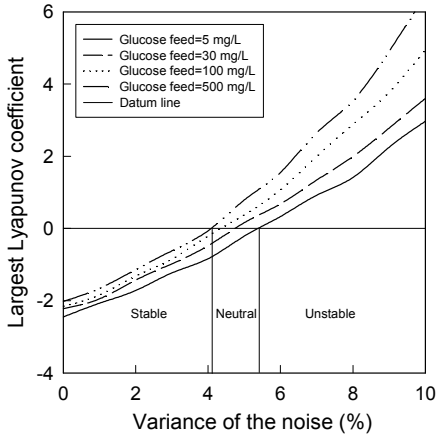

Fig. 1. Variation of the largest Lyapunov exponent with the variance of the external noise for a uniform distribution of immobilized cells.

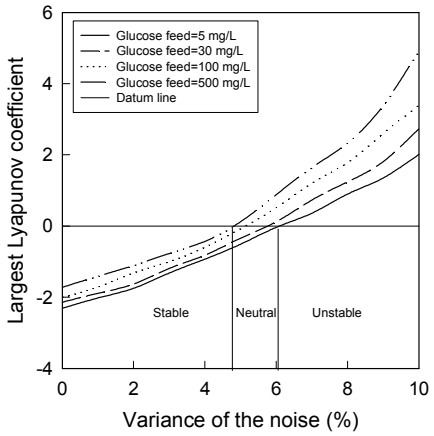

Fig. 2. Variation of the largest Lyapunov exponent with the variance of the external noise for a topologically optimum distribution of immobilized cells.

One interesting difference between the two sets of plots is that for a given inlet glucose concentration, the stability threshold is crossed at a smaller mean variance for a uniform distribution of cells. The larger variance threshold for an optimal distribution has a practically useful consequence. Stabilization of a noise-distorted microbioreactor requires filtering of the noise such that the mean variance of the noise in the filtered inflow stream is below the threshold value. Hence a large threshold requires less rigorous filtering, which is easier to implement.

Figure 3 displays the fractional change in λ_{max} between the two types of reactors at different values of the mean variance. Here again it is worth observing that the largest fractional changes occur approximately in the so-called neural stability interval, i.e. the span of variances within which the largest Lyapunov coefficient for different inlet glucose concentrations crosses the stability threshold. This suggests the biggest gains on moving from a uniform distribution to an optimal distribution of cells occurs in the vicinity of the stability threshold. This is plausible because at low variances it is relatively easy to filter the noise such that both microbioreactors are as noise-free as desired, whereas at high mean variances it is much more difficult to remove the noise, and hence both reactors remain substantially noise-affected.

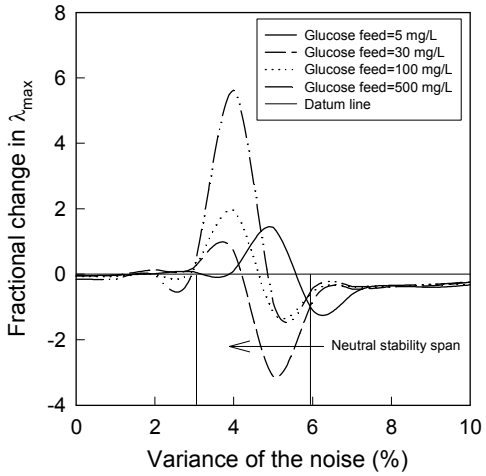

Fig. 3. Fractional change in the largest Lyapunov exponent with the variance of the external noise on moving from a uniform distribution to a topologically optimum distribution of immobilized cells.

In the preceding paragraph it was stated that at low mean variances it is comparatively easy to filter out the noise inflow. While this may be true, previous studies [27-29,42] indicate that the best performance is obtained not by complete removal of noise inflow but by an optimum level of filtering. In other words, controlled noise is more favorable than no noise. These studies and others [54,55] have attributed this apparently paradoxical observation to resonance between the noise entering from outside and the noise present inside cells. Stochastic resonance has in fact been invoked to explain many biological phenomena, thereby enhancing its credibility.

Resonance between two or more sources of noise by means of controlled filtering is now recognized as a major factor in the processing of information by nerve cells in the brain [56], in the evolution of certain phenotypes that are resistant to treatment by drugs [37,57], the movements of populations of cells through microtubes in response to chemical stimuli [58] and, on a more macroscopic scale, the performances of bioreactors with complex microbial reactions [59]. It is of

interest to nose that the first application, from the neurophysiology area, is the conceptual basis for noise filters based on artificial intelligence that have proven to be very effective for bioreactors such as those studied in the last application [59].

Although the applications of noise filters to microreactors is at a nascent stage, the few investigations reported suggest considerable benefits. While Lee et al. [60] reported transport facilitation through coherence resonance in a single nanotube, Enomoto and co-workers [61] analyzed an array of microtubes for improvement of internal signal transmissions through resonant noise filtering. The latter study blends nicely with the earlier studies by Moss et al. [56] for sensory information processing and Patnaik [58] for chemotactic signal transduction, thus establishing persuasive arguments in favor of controlled noise as a beneficial factor for microbioreactor operations. On the basis of the present results of the raw effects of external noise, the next communication will report on filter designs and their effects.

VI. CONCLUSIONS

Microreactors sustaining microbial reactors are subject to noise from within the cells and from the environment. While intra-cellular noise has been understood in some detail, the effects of noise from the external environment are not well explored. A preliminary analysis of the magnitude and nature of their effects is presented in this study.

The focus was on stability of a microbioreactor, for which the Lyapunov exponent has been presented as a convenient and reliable index. For a multi-variable system, negative values of the largest Lyapunov exponent, λ_{max}, indicate stability and positive values signify instability. The variation of λ_{max} with the (mean) variance of the external noise was compared for two microbioreactors, one with a uniform distribution of immobilized cells of recombinant *S. cerevisiae*, and the other with a topologically optimized distribution [23]. The main substrate was glucose, and ethanol and glucoamylase were the principal products.

For both distributions, λ_{max} increased from negative to positive values with increasing variance of the external noise. For a given inlet concentration of glucose, the cross-over point from a negative to a positive domain was larger for an optimum distribution of cells. Since the external noise often comprises a mixture of components with different variances, a large cross-over threshold implies less stringent filtering of the noise; this advantage in a noise-affected situation adds to the advantages already shown [23] for a noise-free microbioreactor. These results motivate the exploration of the effects of noise filtering, which will be part of the continuing studies.

VII. NOMENCLATURE

D_G diffusion coefficient for glucose, $m^2\ s^{-1}$
D_E diffusion coefficient for ethanol, $m^2\ s^{-1}$
D_{X_f} diffusion coefficient for total suspended biomass, $m^2\ s^{-1}$
$D_{X_f^+}$ diffusion coefficient for plasmid-containing suspended biomass, $m^2\ s^{-1}$
E concentration of ethanol in microbioreactor, $g\ l^{-1}$
G concentration of glucose in microbioreactor, $g\ l^{-1}$
K_1, saturation constant for μ_1, $g\ l^{-1}$
K_2, saturation constant for μ_2, $g\ l^{-1}$
K_3 saturation constant for μ_3, $g\ l^{-1}$
K_S saturation constant for k^*_{det}, $g\ l^{-1}$
k_a, enzyme pool regulation constant, --
k_b, enzyme pool regulation constant, --
k_c, enzyme pool regulation constant, --
k_d, enzyme pool regulation constant, --
k^*_{det} rate of detachment of immobilized cells, $g\ l^{-1}$
k^{sst}_{det} steady state value of k^*_{det}, $g\ l^{-1}$
p probability of plasmid loss, --
P_C total mass of carrier, g
X_{im} total concentration of immobilized cells, $g\ l^{-1}$
X^{max}_{im} maximum value of X_{im}, $g\ l^{-1}$
X^{act}_{im} active component of X_{im}, $g\ l^{-1}$
X^+_{im} concentration of plasmid-bearing immobilized cells, $g\ l^{-1}$
X_f concentration of suspended (free) biomas, $g\ l^{-1}$
X^+_f concentration of plasmid-bearing suspended (free) biomas, $g\ l^{-1}$
V_r total volume of microbioreactor, l
$Y^0_{X/G}$ yield coefficient of biomass on glucose for glucose oxidation, $g\ g^{-1}$
$Y^F_{X/G}$ yield coefficient of biomass on glucose for glucose fermentation, $g\ g^{-1}$
$Y_{E/X}$ yield coefficient of ethanol on glucose for glucose fermentation, $g\ g^{-1}$
$Y_{X/E}$ yield coefficient of biomass on ethanol for ethanol oxidation, $g\ g^{-1}$

Greek letters

α_2 protein yield coefficient for glucose oxidation, U/g
α_3 protein yield coefficient for ethanol oxidation, U/g
γ free (unused) fraction of the carrier, --
λ Lyapunov exponent, --
μ_1 specific growth of biomass via glucose fermentation, h^{-1}
μ_2 specific growth of biomass via glucose oxidation, h^{-1}
μ_3 specific growth of biomass via ethanol oxidation, h^{-1}

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