Integrated Nonlinear Optimization of Bioprocesses via Linear Programming

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The problem of integrated design and control of bioprocess plants is considered. A previously presented optimization approach for biochemical systems based on linear programming and modeling using the power law formalism (the Indirect Optimization Method, IOM) is extended. This method is enhanced in order to take into account both static and dynamic measures, and its use for the optimization of the integrated design of a bioprocess is illustrated. The chosen case study is a wastewater treatment plant, a bioprocess which typically presents controllability problems in real practice due to bad design methodologies. After defining an objective function reflecting both investment costs and "paracosts" (such as stability, flexibility, and controllability), a set of constraints determined by the system components and technical and economical factors is defined. A comparison of the results obtained with this new method and with a global optimization method reveals that, in both cases, significant improvements in both controllability and cost reduction are achieved, although the global method yields somewhat better improvements. The advantages and limitations of both methods are evaluated, concluding that the IOM, through its incorporation to a dynamic process simulator, can be successfully used to obtain, in a quick, inexpensive and interactive way, near-optimal integrated designs for bioprocess plants.

Introduction

The proper design and operation of any microbial-based biotechnological process requires the quantitative description of the variables relevant for the system's kinetics. Once this information is available, it will be possible to derive an optimal process design and to attain its optimal operation. However, the nonlinear nature of these processes has impaired such optimizations (Vagners, 1983; Heinrich and Schuster, 1996). In order to circumvent these difficulties chemical and biochemical engineers have developed strategies to minimize or even avoid nonlinearities. Focusing on specific properties of chemical networks and by using prudent approximations, different techniques and approaches have been developed within the field of process engineering, which permits the process improvement through the right choice of operating parameters (Stephanopoulos, 1998; Stephanopoulos et al., 1998).

One approach that fully acknowledges the nonlinearities, yet ultimately leads to linear optimization tasks, is based on a modeling framework called Biochemical Systems Theory (Savageau 1969a,b; Voit, 2000). The hallmark of this theory is the approximation of rate laws and other processes with products of power-law functions. Mathematically, this type of representation is very convenient and solidly grounded in Taylor's theory of approximating functions with polynomials. The Biochemical System Theory framework offers some choices for the formulation of biochemical systems, among which the most relevant for our present purposes is the S-system (see Voit (2000) for a recent review). Within this formalism, our group has developed, presented, and applied to different systems of biotechnological relevance an integrated approach for modeling, analyzing, and optimizing biochemical systems, the so-called Indirect Optimization Method, (IOM) (see Torres et al. (1997) for a presentation of the method, and Torres et al. (1998), Marín-Sanguino and Torres

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(2000, 2002) and Álvarez-Vasquez et al. (2000) for different applications).

The above mentioned IOM approach is based on the fact that, although S-system models are nonlinear, the steady-state equations are linear when the variables are expressed in logarithmic coordinates. The key argument now is that the logarithmic transformation does not change the locations of maxima and minima. In other words, the two functions $f(x)$ and $\ln f(x)$, for positive x-values, have their maxima and minima for the same values of x, and this is also true for functions of many variables.

So far, the IOM approach has been used to optimize biochemical systems operating at static, steady-state conditions. On the contrary, this article is focused on demonstrating how IOM can be applied to integrated design problems of bioprocess plants, that is, problems where both static and dynamic measures of bioprocesses are taken into account. In this context the selection of a wastewater treatment plant as a case study is not arbitrary: these plants do present many controllability problems in real practice due to bad designs (designs which were obtained with only static measures in mind). Thus, the optimization approach must consider the optimal steady state with respect to some economic measure. However, also important from the economic point of view is the process dynamics arising from the given design. In this vein, a suitable objective function should reflect some monetary costs, shadow prices, or investment costs and also some "paracosts" such as stability, flexibility, or controllability (Górak et al., 1987). The constraints are dictated by the system components and by technical and economical factors. Moreover, we will compare the optimum profiles and the optimized responses with the ones obtained for the same problem by using selected global optimization methods, such as the GLOBAL method, a hybrid global optimization clustering technique (Csendes, 1988) based on a modification of the algorithm presented by Boender et al. (1982).

Case Study: Wastewater Treatment Plant Model

In order to test the applicability and reliability of the proposed optimization approach to process design and control we have used a wastewater treatment plant model originally presented by Gutiérrez and Vega (2000) and slightly modified by Moles et al. (2001). In Appendix A the full formulation used by the latter authors is presented. In Figure 1 the plant scheme is presented. The plant is composed by two aeration tanks working in series acting as bioreactors and two settlers. A flocculating microbial population (biomass) that transforms the biodegradable pollutants (substrate) is kept inside each bioreactor. Oxygen is provided by aeration turbines. Effluents coming from the aeration tanks are separated in their associated settlers into a clean water stream and an activated sludge, which is recycled to the corresponding aeration tank. Due to the excess of activated sludge production, the amount that cannot be transferred to the recycle tanks is eliminated via a purge stream. The objective of the control system is to keep the substrate concentration at the output (q_{sal}) under a given admissible value. The main disturbances come from large variations in both the flow rate and substrate concentration (q_i and s_i) of the input stream. Although there are several possibilities for the manipulated

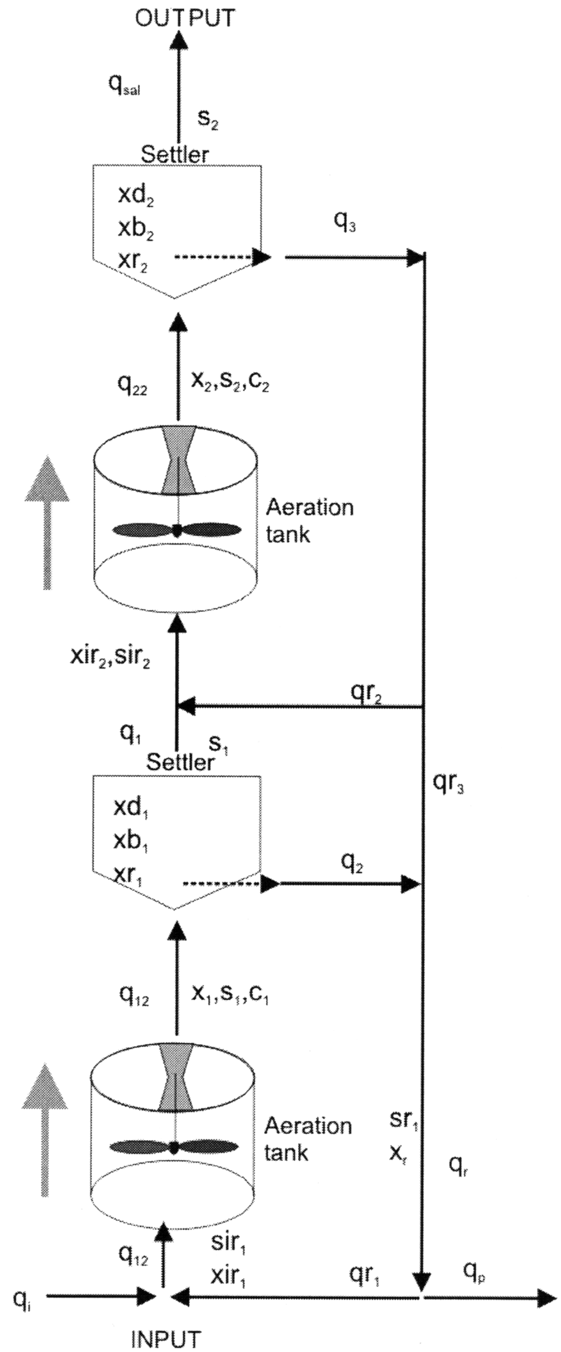

Figure 1. Plant scheme of the activated sludge process.

variable, we have considered here the flow rate of the sludge recycle to the first aeration tank, as considered by Gutierrez and Vega (2000). It should be noted that although this control configuration might appear as relatively simple, the corresponding integrated design problem has proved to be very challenging, as reported by Moles et al. (2001). The mathematical model (Gutiérrez and Vega, 2000) consists of 14 ordinary differential equations and 19 algebraic equations, with 14 dependent and 11 independent variables. In Table 1 the classification, definitions, basal values, and equivalencies among the original and S-system nomenclature are shown.

Based on the original model equations (see Appendix), the following S-form representation can be obtained

$$\frac{dX_1}{dt} = 0.2131 X_1^{0.0556} X_3^{0.0495} X_{11}^{0.908} X_{12}^{0.0143} X_{13}^{0.9892} X_{14}^{-0.9443}$$

$$\times X_{22}^{-0.1585} X_{24}^{-0.0651} - 0.2131 X_1^{1.016} X_{13}^{0.2935} X_3^{-0.016} X_{14}^{-0.9833}$$

$$\frac{dX_2}{dt} = 0.2037 X_2^{0.0339} X_4^{0.0317} X_7^{0.1717} X_{12}^{0.7942} X_{22}^{0.1717} X_{23}^{0.7942} X_{15}^{-0.966}$$

$$-0.2037 X_2^{1.003} X_{22}^{0.8573} X_{23}^{0.1389} X_4^{-0.003} X_{15}^{0.9963}$$

$$\frac{dX_3}{dt} = 1.5724 X_1^{0.0605} X_3^{0.0066} X_4^{0.0019} X_{13}^{0.0396} X_{14}^{-0.9691} X_{22}^{-0.0034}$$

$$\times X_{24}^{-0.0014} - 1.5724 X_1^{0.8667} X_3^{0.9052} X_{13}^{0.0397} X_{14}^{0.1332}$$

$$\frac{dX_4}{dt} = 0.3454 X_2^{0.0096} X_3^{0.9127} X_4^{0.0775} X_{22}^{0.9127} X_{23}^{0.0819} X_{15}^{-0.9947}$$

$$-0.3454 X_2^{0.4123} X_4^{0.9737} X_{22}^{0.5056} X_{23}^{0.0819} X_{15}^{-0.5876}$$

$$\frac{dX_5}{dt} = 0.7579 X_{18} - 0.7579 X_1^{0.0011} X_3^{0.001} X_5^{0.9988} X_{13}^{0.0825}$$

$$\times X_{18}^{0.7223} X_{14}^{-0.2764}$$

$$\frac{dX_6}{dt} = 0.7375 X_5^{0.1909} X_{19}^{0.809} X_{22}^{0.1909} X_{15}^{-0.1909}$$

$$-0.7375 X_2^{0.00005} X_4^{0.00005} X_6^{0.9999} X_{19}^{0.7247} X_{22}^{0.2368} X_{23}^{0.0383} X_{15}^{-0.2752}$$

$$\frac{dX_7}{dt} = 1.6487 X_9 X_{22} X_{16}^{-1} - 1.6487 X_7^{0.9644} X_{16}^{-0.0977} X_{22}^{0.0977}$$

$$\frac{dX_8}{dt} = 1.7567 X_{10} X_{22}^{1.0403} X_{17}^{-1} X_{24}^{-0.0403} - 1.7567 X_8 0.9974 X_{22}^{0.1084}$$

$$\times X_{17}^{-0.1042} \ X_{24}^{-0.0042}$$

$$\frac{dX_9}{dt} = 0.7353 X_1^{0.8847} X_7^{0.1107} X_{13}^{0.2641} X_{16}^{-0.8847}$$

$$-0.7353 X_9^{0.6697} X_{13}^{0.0538} X_{16}^{-0.1802}$$

$$\frac{dX_{10}}{dt} = 1.004 X_2^{0.9066} X_8^{0.093} X_{22}^{0.7802} X_{23}^{0.1264} X_{17}^{-0.9066}$$

$$-1.004 X_{10}^{0.9761} X_{22}^{0.1084} X_{23}^{0.0175} X_{17}^{-0.126}$$

$$\frac{dX_{11}}{dt} = 0.2702 X_9^{0.6213} X_{13}^{0.0617} X_{16}^{-0.06} X_{22}^{-0.1464}$$

$$-0.2702 X_{11} X_{13}^{1.025} X_{16}^{-1} X_{22}^{-2.4333}$$

$$\frac{dX_{12}}{dt} = 0.153 X_{10}^{0.9733} X_{23}^{0.0196} X_{24}^{0.0046} X_{17}^{-0.0243}$$

$$-0.153 X_{12} X_{23}^{0.8068} \ X_{24}^{0.1931} X_{17}^{-1}$$

$$\frac{dX_{13}}{dt} = 0.0453 X_2^{0.1159} X_4^{0.9926} X_{21}^{0.7189} X_{22}^{0.1421} X_{23}^{0.023} X_{15}^{-0.1651}$$

$$X_{20}^{-0.7189} - 0.0453 X_2^{0.0027} X_3^{0.2565} X_4^{0.0218} X_{21}^{0.7189} X_{22}^{0.2565} X_{23}^{0.023}$$

$$X_{15}^{-0.279} X_{20}^{-0.7189}$$

Aggregation of fluxes in total input and output fluxes is an essential characteristic of *S*-system formalism. However, this property of *S*-system models has important effects in the model's structure, and it can vary significantly the relative value of kinetic orders in the model (Voit, 2000).

Quality Assessment of the Model Representation

One significant advantage of representing a complex system with an *S*-system rather than some other nonlinear model is the fact that its steady state can be expressed explicitly in the form of a system of linear equations (Savageau, 1969b; Voit, 2000). One important consequence is that the steady state itself, as well as derived features like sensitivities, are easily computed (such as with the freeware PLAS, Ferreira, 2000).

Stability of the steady state

The numerical values for the system variables at steady state coincide with those previously defined (Gutiérrez and Vega, 2000). The local stability is confirmed by examining the eigenvalues for the local representation of the system showing that the real parts of all the eigenvalues are negative (results not shown). Moreover, there are imaginary parts, indicating that dumping or oscillatory behavior is to be expected, which coincides with the observed experimental behavior. The quality of the representation was subsequently analyzed in order to check whether the model represented a realistic picture of the system and was, thus, worth optimizing.

Sensitivity analysis

The system sensitivity analysis indicates whether the model is able to tolerate structural changes. There are two types of parameters for analyzing system sensitivity in an *S*-system: the rate constants and kinetic orders. Accordingly, there are two types of sensitivity coefficients: the rate constant sensitivities and the kinetic order sensitivities.

System Sensitivities. A rate constant or kinetic order sensitivity coefficient is defined as the ratio of a relative change in a dependent variable Z_i to a relative change in a rate constant α_j or β_j or kinetic order g_{jk} or h_{jk}. They can be determined by differentiation of the explicit steady-state solution

$$S(X_i, p_j) = \left(\frac{\partial Z_i}{\partial p_j} \frac{P_j}{Z_i} \right)_0 = \frac{\partial(\log Z_i)}{\partial(\log p_j)}$$

where p_j stands for the rate constants α_j or β_j, or for the kinetic order g_{jk} or h_{jk}. The subscript 0 refers to the steady state. Sensitivities values are related with the tolerance of dependent variables and fluxes with respect to the rate constants or kinetic orders values. High sensitivities (that is, absolute values upper than 50) indicate an extreme sensitivity of the model to slight changes (inaccuracies) in the corresponding parameter, which is an inadequate behavior in metabolic system's models. However, this interpretation must be translated with the best attention to other kind of models (like bioprocess system models).

Table 1. Set Points (Fixed), Dependent Variables, S-System Equivalence and Basal Values for the Model Optimization of the Wastewater Treatment Plant

Dependent Variables	Definition	S-System Equivalence	Basal Value	Unit
x_1	Biomass concentration bioreactor 1	X_1	2516.7	mg/L
x_2	Biomass concentration bioreactor 2	X_2	250.21	mg/L
s_1	Organic matter concentration bioreactor 1	X_3	36.826	mg/L
s_2	Organic matter concentration bioreactor 2	X_4	20.4016	mg/L
c_1	Oxygen concentration bioreactor 1	X_5	5.7791	mg/L
c_2	Oxygen concentration bioreactor 2	X_6	7.1666	mg/L
xd_1	Suspended organic matter concentration settler 1	X_7	50.134	mg/L
xd_2	Suspended organic matter concentration settler 2	X_8	3.6248	mg/L
xb_1	Settling organic matter concentration settler 1	X_9	512.79	mg/L
xb_2	Settling organic matter concentration settler 2	X_{10}	34.774	mg/L
xr_1	Sedimented organic matter concentration settler 1	X_{11}	8518.6	mg/L
xr_2	Sedimented organic matter concentration settler 2	X_{12}	1430.4	mg/L
qr_1	Activated sludge net flux into bioreactor 1	X_{13}	553.3	m^3/h
Independent Variables				
Optimization parameters				
υ_1	Bioreactor 1 volume	X_{14}	8843.95	m^3
υ_2	Bioreactor 2 volume	X_{15}	7520.32	m^3
ad_1	Settler 1 surface	X_{16}	3994.72	m^2
ad_2	Settler 2 surface	X_{17}	3447.27	m^2
fk_1	Bioreactor 1 aeration factor	X_{18}	0.7822	dimensionless
fk_2	Bioreactor 2 aeration factor	X_{19}	0.7636	dimensionless
τi	Integral time of the controller	X_{20}	10.76	h
k_p	Gain of the controller	X_{21}	-9.51	$m^3 \cdot L/mg \cdot h$
Constant parameters				
q_1	Flux from settler 1			
bioreactor 2	X_{22}	1313.5	m^3/h	
qr_2	Flux from settler 2			
bioreactor 2	X_{23}	212.85	m^3/h	
qr_3	Flux from settler 2			
bioreactor 1	X_{24}	50.942	m^3/h	

In the present case, only the rate constant sensitivities showed values different from zero, with all the kinetic order sensitivities having null values (see Figures 2a and 2b). In regard to the $S(V_i, \alpha_j)$ sensitivities (Figure 2a), from a total number of 169 values, 93 are less than 1 and the remaining are never bigger than 5. The values for the $S(X_i, \alpha_j)$ sensitivities (Figure 2b) are of the same magnitude except for some of those related with X_{11} and X_{13} that reach values of about 25. In the case of X_{13}, high sensitivities are a consequence of the special dynamic of this variable (the control variable); thus, high sensitivities are being allowed. In the case of X_{11} the sensitivities around 15 may indicate a specific structural characteristic of this system.

Logarithmic Gains. The logarithmic gains are specific types of sensitivity coefficients that are defined as

$$L(Z_i, p_k) = \left(\frac{\partial Z_i}{\partial p_k} \frac{p_k}{Z_i} \right)_0 = \frac{\partial(\log Z_i)}{\partial(\log p_k)}$$

where Z_i stands for concentrations or fluxes and p_k for an independent variable. The subscript 0 refers to the steady state. As in the previous sensitivity analysis, in metabolic systems log gains should have low values (less than 10 in absolute value). A high value in a log gain indicates that the dependent variable or flux is very sensitive to changes in the

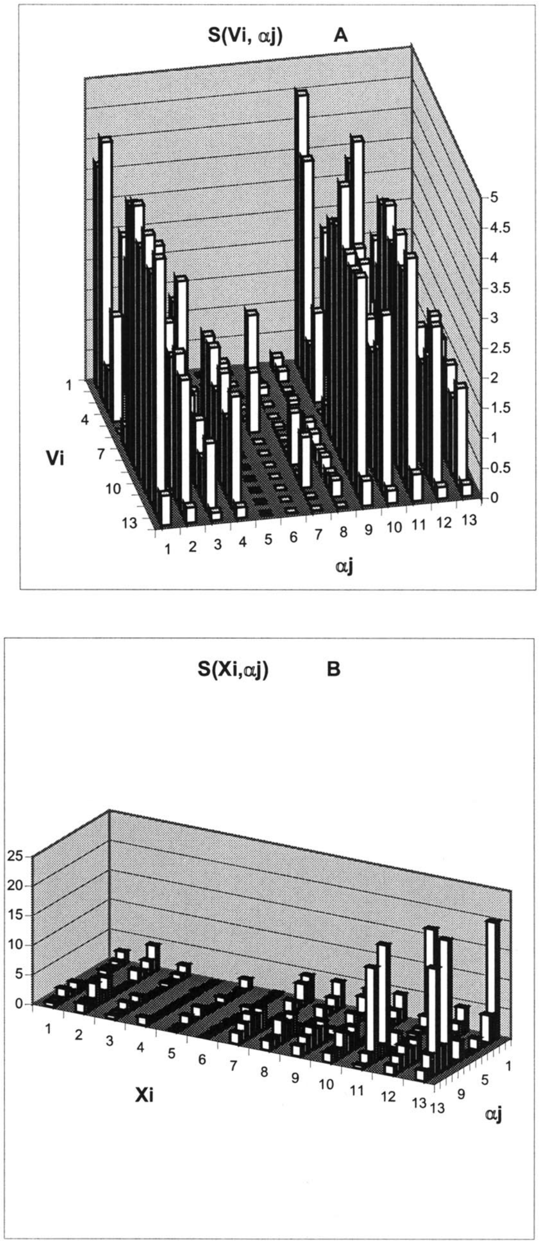

Figure 2. Influence of rate constant parameters on fluxes (a) through metabolite pools and metabolite concentrations as determined by the magnitudes of the parameter sensitivities.

The 3-D plot (panel a) displays the magnitudes of sensitivities as a function of the fluxes V_i and the rate constant parameters α_j. Panel *B* displays the magnitudes of sensitivities as a function of the metabolite concentrations X_i and the rate constant parameters α_j. The sensitivities with respect to the β parameters, can be obtained by simply negating the value for the corresponding α parameter, except for the sensitivities on the diagonal, from which we first subtract 1 and then negate the value. The key to the dependent variables is given in Figure 1 and the Model section.

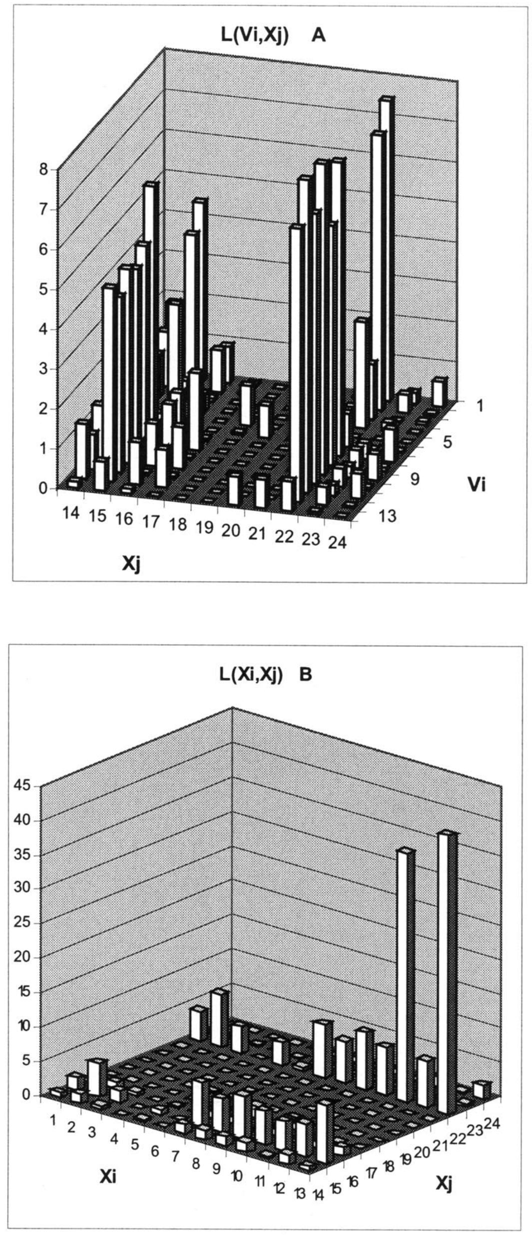

Figure 3. Influence of independent variables on fluxes (a) through metabolite pools and on metabolite concentrations (b) as determined by the magnitudes of the logarithmic gains.

The 3-D plot (a) displays the magnitude of the logarithmic gains as a function of the fluxes V_i and the independent variables X_j. Panel b displays the magnitude of the logarithmic gains as a function of the dependent metabolite concentrations X_i and the independent variables X_j.

corresponding independent variable; thus, a small change will translate the system to a completely different steady state.

The influence of the independent variables on the concentrations and fluxes through the pools has the distribution shown in Figures 3a (and 3b). Most of the dependent variable logarithmic gains (107; 75%) are below 1 (Figure 3a), with the bigger ones (values from 2 to 8) being related to the X_{15} (the volume of the second aeration tank) and X_{22} (the main flux). The same pattern is observed in the concentration logarithmic gains (Figure 3b).

The value of log gains $L(X_{11},X_{22})$, $L(X_{13},X_{22})$ are high, indicating that changes in X_{22} (the flux from settler 1 to bioreactor 2, the main system's flux) strongly affect X_{11} and X_{13}. Initial explorations allowing changes in the variable X_{22} returned unfeasible and unstable solutions. All these features lead us to keep X_{22} constant in all our explorations.

Dynamics

Finally, we can consider the dynamic features that characterize the transient responses to temporary perturbations or permanent alterations. Such analyses often identify problems of consistency and reliability of the mathematical representation (Shiraishi and Savageau, 1992; Ni and Savageau, 1996). As part of the quality assessment procedure of the proposed model, we simulated the system response after a 3% change in the input flux. This perturbation value was taken from the original formulation, inspired in a real plant. Figure 4 shows that the system returns to the steady state previous to the predisturbance with a maximal variation in the other dependent variables of less than 3%, showing damped or oscillatory behavior as it corresponds to the non-zero imaginary parts of the eigenvalues. As a whole, such a response can be considered acceptable in a biotechnological setting.

Application of the Indirect Optimization Method to the Simultaneous Optimization of the Design and Control of the Wastewater Treatment Plant Model

Once we are provided with an S-system model version and the steady-state analysis and quality assessment have been carried out, we are in a condition to perform the logarithmic transformation and, subsequently, to define the linear programming problem (Torres et al., 1998). The implementation of this step is as follows.

Objective function

The optimization problem consists of evaluating the physical and control parameters of the plant simultaneously, while the investment and the operation costs are minimized. In addition, the dynamics of the system should be within desired ranges. These desired dynamic characteristics of the system are good for rejection of the disturbances at the output variable S_2 and to ensure the stability of the resulting system. Accordingly, an objective function C was originally defined as a weighted sum of controllability (ISE) and economic (ϕ_{econ}) cost terms as

$$\text{Minimize} \qquad C = \omega_1 \cdot \text{ISE} + \phi_{\text{econ}}$$

The economic term ϕ_{econ} has the following expression

$$\phi_{\text{econ}} = \left(\omega_2 \cdot v_1^2\right) + \left(\omega_3 \cdot v_2^2\right) + \left(\omega_4 \cdot ad_1^2\right) + \left(\omega_5 \cdot ad_2^2\right) + \left(\omega_6 \cdot fk_1^2\right) + \left(\omega_7 \cdot fk_2^2\right)$$

where v_1, v_2, ad_1, and ad_2 are related to the bioreactor volumes and the surface of the settler tanks, respectively, and fk_1 and fk_2 to the aeration devices. In all cases ω represents the vector of weighting factors $\omega = [10^3;\ 2 \cdot 10^{-5},\ 2 \cdot 10^{-5},\ 10^{-5},\ 10^{-5},\ 12,\ 12]$. If we translate the objective function to Power-Law formalism, the function takes the form

$$\phi_{\text{econ}}^{S-S} = \left[\alpha_{\text{econ}} \cdot \prod_{i=14}^{19} X_i^{g\text{econ},i}\right]$$

where

$$g_{\text{econ},i} = \left.\frac{2 \cdot \omega_{i-12} \cdot X_i^2}{\phi_{\text{econ}}}\right|_{X^o}, i = 14, 15, \ldots 19$$

$$\alpha_{\text{econ}} = \left.\frac{\phi_{\text{econ}}}{\prod_{1=14}^{19} X_i^{g\text{econ},i}}\right|_{X^o}$$

In contrast with the economic objective function, the control design objective ISE poses particular difficulties in its translation to the power law formalism. The objective of the control system is to keep the substrate concentration of the output (S_2) close to a predefined set point value. ISE is the integral square error of the output (S_2) evaluated considering a step disturbance to the input substrate concentrations Si_i (see Eqs. A14 and A33 in Appendix A).

The difficulties arising for the integral character of the ISE function can, however, be overcome by substituting the origi-

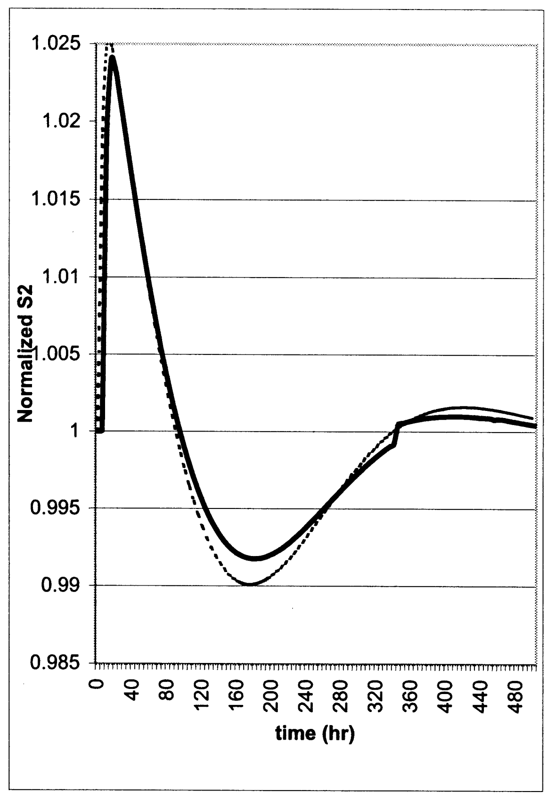

Figure 4. Comparison of the s_2 evolution after a 3% perturbation in the input flux as predicted by the S-system and the original Monod kinetic model.

The continuous line represents the original model solution, while the dotted line corresponds to the S-system model.

nal formulation by a similar, equivalent one. After some exploration, we finally chose to represent the original objective control function ISE as the following.

$$\mathrm{Max}\left[\frac{d\dot{X}_{\mathrm{cont}}}{dP_{\mathrm{pert}}}\right]$$

In this equation X_{cont} represents the control variable and P_{pert} represents the perturbation parameter, that represents the step disturbance. So, expressing the maximization of $dX_{\mathrm{cont}}/dP_{\mathrm{pert}}$ is equivalent to maximizing the sensitivity of the flux through the control variable with regard to the perturbation parameter. This is so because changes in the control variable are determined by changes in the flux. Also, due to the fact that the flux changes are controlled by changes in the perturbation parameter, maximizing the system's controllability is just maximizing the sensitivity of the flux with respect to the perturbation parameter.

Using the power-law formalism, it is easy to transform the above expression into the following, which will be used in our optimization setup

$$\mathrm{Max}\left[\frac{J^{+}_{\mathrm{cont}}}{P_{\mathrm{pert}}}K(X_{\mathrm{cont}},P_{\mathrm{pert}})\right]$$

In this expression J^{+}_{cont} represents the input flux to the control variable X_{cont}, and $K(X_{\mathrm{cont}}, P_{\mathrm{pert}})$ is a constant, defined as

$$K(X_i,P_j) = \sum_k (g_{ik} - h_{ik})L(X_k,P_j) = cte$$

where $g_{i,k}$, $h_{i,k}$ are the J^{+}_{cont} and J^{-}_{cont} kinetic orders respectively; $L(X_k, P_j)$ the logarithmic gains and P_{pert} are the perturbation parameter value. In our system setting $X_{\mathrm{cont}} = X_{13}$, thus, we have

$$\mathrm{Max}\left[\frac{d\dot{X}_{\mathrm{cont}}}{dP_{\mathrm{pert}}}\right] = \max\left[\frac{J^{+}_{13}}{P_{\mathrm{pert}}}K(X_{13},P_{\mathrm{pert}})\right]$$

Combining both objective functions, taking logarithms, and simplifying, we finally obtain the following combined economic and control objective function

$$\mathrm{Max}\left[\sum_{k=14}^{19}(g_{13,k} - g_{\mathrm{econ},k})\cdot Y_k + \sum_{\substack{k=1\\ k\notin[14,19]}}^{24} g_{13,k}\cdot Y_k\right]$$

A detailed derivation of the final, numerical expression of the objective function, used for optimization purposes, is shown in the Appendix. After substitution of the numerical values at the basal, reference steady state, we finally obtain as the objective function

$$\mathrm{Max}[0.116X_2 + 0.993X_4 - X_{14} - 0.965X_{15} - 0.1X_{16} - 0.08X_{17} - 0.005X_{18} - 0.005X_{19} - 0.719X_{20} + 0.719X_{21} + 0.142X_{22} + 0.023X_{23}]$$

Constraints

In addition to the steady-state constraints there are four sets of constraints to be imposed in the formulation of the optimization problem.

Flux Constraints. These constraints were already imposed in the original presentation of the wastewater treatment system (Gutiérrez and Vega, 2000). They impose upper and lower limits to some dependent fluxes, which are related to the tank's dimensions. The constraints can be straightforwardly translated into a power law expression

$$Q_i = f(X_1,X_2,\ldots) \quad Q_i \approx \alpha\cdot\textstyle\prod_j X_j^{gj} \quad L_i \le \alpha\cdot\prod_j X_j^{gj} \le U_i$$

Taking the logarithm and using logarithm properties

$$\mathrm{Log}(L_i) \le \mathrm{Log}\left(\alpha\cdot\textstyle\prod_j X_j^{gj}\right) \le \mathrm{Log}(U_i)$$

$$\mathrm{Log}(L_i) \le \mathrm{Log}(\alpha) + \pi_j X_j^{gj}\mathrm{Log}(X_j) \le \mathrm{Log}(U_i)$$

Substracting $\mathrm{Log}\,\alpha$ and expressing the result in terms of y-variables ($y_i = \mathrm{Log}\ X_i$), we transform this constraint into the linear form

$$\mathrm{Log}\left(\frac{L_i}{\alpha}\right) \le \sum_j g_j\cdot y_j \le \mathrm{Log}\left(\frac{U_i}{\alpha}\right)$$

Fluxes	*Linear constraints*
Q2	$-0.4312 < 1.0250y_{13} - 2.4335y_{22} < 0.7449$
Q3	$-0.1202 < .8061y_{23} + 0.1931y_{24} < 1.0559$
Q12	$-1.5690 < 0.2985y_{13} < 0.2761$
Q22	$-1.4847 < 0.8605y_{22} + 0.1394y_{23} < 0.3604$
Qsal	$-1.1013 < 1.0403y_{22} - 0.0403y_{24} < 0.3759$
Qr	$-1.0724 < 0.9366y_{13} - 2.2235y_{22} + 0.0862\ y_{24} < 0.5296$

where y_i stands for $\ln(X_i)$.

Mixing Coefficient Constraints. As in the previous case, these constraints were defined in the original model (Gutiérrez and Vega, 2000). They serve to limit the biomass and other organic matter present in the fluxes coming from the mixing flux nodes. These constraints serve to guarantee the growth and development of the microbial cultures. These constraints are obtained by following the very same procedure used in the previous case

Variable	*Linear constraint*
*Xir*1	$-0.7811 < 0.9615\ y_{11} + 0.0152y_{12} + 0.7489y_{13} - 0.1679y_{22} - 0.0690y_{24} < 0.0147$
*Sir*1	$-0.7288 < 0.0375y_3 + 0.0020y_4 - 0.2576y_{13} - 0.0035\ y_{22} - 0.0014y_{24} < 0.2718$
*Xir*2	$-0.0839 < 0.1778y_7 + 0.8222y_{12} - 0.6827y_{22}\ 0.6827y_{23} < 0.9161$
*Sir*2	$-0.0611 < 0.9176y_3 + 0.0824y_4 + 0.0571y_{22} - 0.0571y_{23} < 1.1607$
Xr	$-0.5970 < 0.9844y_{11} + 0.0156y_{12} + 0.0724y_{13} - 0.1719y_{22} - 0.0706y_{24} < 0.0440$

where y_i stands for ln (X_i).

Dependent Variable Constraints. The dependent variables (see Figure 1 and Table 1) were allowed to vary between different limits ranging from 0.75 to 10 times the basal values. As an exception, an additional constraint was imposed in order to assure that, at any optimized solution found, the value of X_4 was the predefined "set point" $(S_2)_0$. The corresponding expression of these constraints after taking the logarithm and expressing the result in terms of y-variables is the following

$$
\begin{array}{ccccc}
-1.3862 & \leq & y_1 & \leq & 0.2231 \\
-0.2876 & \leq & y_2 & \leq & 2.3025 \\
-0.2876 & \leq & y_3 & \leq & 1.6094 \\
-1.3862 & \leq & y_5 & \leq & 0.2231 \\
-1.3862 & \leq & y_6 & \leq & 0.2231 \\
-0.6931 & \leq & y_7 & \leq & 1.6094 \\
-0.2876 & \leq & y_8 & \leq & 2.3025 \\
-1.3862 & \leq & y_9 & \leq & 0.9162 \\
-0.2876 & \leq & y_{10} & \leq & 2.3025 \\
-0.6931 & \leq & y_{11} & \leq & 0.2231 \\
-0.2876 & \leq & y_{12} & \leq & 1.6094 \\
-1.3862 & \leq & y_{13} & \leq & 0.9162
\end{array}
$$

where y_i stands for ln (X_i).

Independent Variables. Following the same procedure, we imposed limits to the independent variables ranging from 0.25 and 10 times the base line steady-state values, except for X_{22}, X_{23}, and X_{24} that were kept at the base line steady-state values

$$
\begin{array}{ccccc}
-1.3862 & \leq & y_{14} & \leq & 0 \\
-1.3862 & \leq & y_{15} & \leq & 0 \\
-1.3862 & \leq & y_{16} & \leq & 0 \\
-1.3862 & \leq & y_{17} & \leq & 0 \\
-2.3025 & \leq & y_{18} & \leq & 0 \\
-2.3025 & \leq & y_{19} & \leq & 0 \\
-2.3025 & \leq & y_{20} & \leq & 2.3025 \\
-2.3025 & \leq & y_{21} & \leq & 2.3025
\end{array}
$$

where y_i stands for ln (X_i).

Steady-State Constraints. This set of constraints, derived from the S-system model formulation, assures that the optimized system is in a steady state

$$\frac{dX_i}{dt} = 0 \quad \alpha_i \Pi_j X_j^{gij} - \beta_i \Pi_j X_j^{hij} = 0$$

Reconfiguring equations and taking logarithms

$$\mathrm{Log}\left(\alpha_i \Pi_j X_j^{gij} \right) = \mathrm{Log}\left(\beta_i \Pi_j X_j^{hij} \right)$$

Using logarithms' properties and y-variables

$$\mathrm{Log}(\alpha_i) + \sum_j g_{ij} y_j = \mathrm{Log}(\beta_i) + \sum_j h_{ij} y_j$$

In the present case $\alpha_i = \beta_i$ for all differential equations. Reconfiguring and simplifying, these constraints take the following form

$$\sum_j (g_{ij} - h_{ij}) y_j = 0$$

where g_{ij} and y_{hij} are kinetics orders in the S-system representation of dynamic system. In the present case these constraints take the following form

$$-0.9604\ y_1 + 0.0656 y_3 + 0.9080 y_{11} + 0.0144 y_{12} + 0.6957 y_{13} + 0.039\ y_{14} - 0.1586 y_{22} - 0.0652 y_{24} = 0$$

$$-0.9691 y_2 + 0.0348 y_4 + 0.1718 y_7 + 0.7943 y_{12} + 0.0303 y_{15} - 0.6856 y_{22} + 0.6554 y_{23} = 0$$

$$-0.8062 y_1 - 0.8986 y_3 + 0.0019 y_4 - 0.0001 y_{13} - 0.8359 y_{14} - 0.0034 y_{22} - 0.0014 y_{24} = 0$$

$$-0.4027\ y_2 + 0.9128 y_3 - 0.8962 y_4 - 0.4071 y_{15} + 0.4071 y_{22} = 0$$

$$-0.0012 y_1 - 0.0010\ y_3 - 0.9989\ y_5 - 0.0825 y_{13} + 0.2765 y_{14} + 0.2776 y_{18} = 0$$

$$-0.00005 y_2 - 0.00005 y_4 + 0.1910 y_5 - 0.9999 y_6 + 0.0842 y_{15} + 0.0843 y_{19} - 0.0459 y_{22} - 0.0384 y_{23} = 0$$

$$-0.9645 y_7 + y_9 - 0.9022 y_{16} + 0.9022 y_{22} = 0$$

$$-0.9975\ y_8 + y_{10} - 0.8958 y_{17} + 0.9319 y_{22} - 0.0361 y_{24} = 0$$

$$0.8847 y_1 + 0.1107 y_7 - 0.6698\ y_9 + 0.2103\ y_{13} - 0.7045 y_{16} = 0$$

$$0.9066 y_2 + 0.0931 y_8 - 0.9761 y_{10} - 0.7806 y_{17} + 0.6718 y_{22} + 0.1089 y_{23} = 0$$

$$0.6214 y_9 - y_{11} - 0.9633 y_{13} + 0.9398 y_{16} + 2.2869 y_{22} = 0$$

$$0.9733 y_{10} - y_{12} + 0.9757 y_{17} - 0.7873\ y_{23} - 0.1884 y_{24} = 0$$

$$0.1132 y_2 - 0.2566 y_3 + 0.9708 y_4 + 0.1144 y_{15} - 0.1144 y_{22} = 0$$

Other Constraints. This is a set of constraints of a different nature that forces the plant to operate within the suitable operative range. These constraints limit some flux ratios (i and ii); the residence times (iii), the settler rates (iv and v), and the ratio of organic matter input flux over the biomass (vi, vii, viii and ix).

Table 2. Optimum Parameter Profile Obtained through the Application of the IOM to the Wastewater Treatment Plant*

		Optimum solutions	
		(X_i)opt/(Xi)basal	
Independent	Basal	IOM Solution	GLOBAL
X_{14} (v_1)	8843.95	1.0	0.621
X_{15} (v_2)	7520.32	0.282	0.53
X_{16} (ad_1)	3994.72	0.495	0.575
X_{17} (ad_2)	3447.27	1.0	1.16
X_{18} (fk_1)	0.7822	0.1	0.132
X_{19} (fk_2)	0.7636	0.1	0.081
X_{20} (τi)	10.76	0.1	0.072
X_{21} (k_p)	−9.51	10.52	10.52
ISE	10.75	0.43	0.3986
ϕ_{econ}	2988.2	1812.5	1133.2
C	13738.2	2242.5	1538.205
Improvement	—	83.7	88.8

*Improvement in the objective function C is defined as $100 \cdot (1 - C/C_{\text{basal}})$, where C_{basal} stands for the objective function value at the basal, reference steady state. The IOM solution corresponds to the translation of the optimized parameter profile obtained from the original kinetic model. The GLOBAL solution corresponds to the optimization of the same system as obtained by Moles et al. (2001) by using the GLOBAL clustering method.

Linear constraint

(i) $-0.2808 < -0.2985y_{13} + y_{14} < 0.2244$

(ii) $-1.3368 < -X_1 + 0.0385y_3 + 0.002y_4 + 0.0421y_{13} - y_{14} - 0.0036y_{22} - 0.0015y_{24} < 0.9643$

(iii) $-1.4474 < -y_2 + 0.9176y_3 + 0.0824y_4 - y_{15} + 0.9176y_{22} + 0.0824y_{23} < 0.3307$

(iv) $0.2985\, y_{13} - y_{16} < 0.5096$

(v) $-X_{17} + 0.8606y_{22} + 0.1395\, y_{23} < 0.5299$

(vi) $-0.2331 < 0.5668y_1 - 0.5668y_{11} + 0.5668y_{14} + 0.4333y_{16} + 35.0887y_{22} - 1.3608y_{24} < 0.2897$

(vii) $-0.0522 < 0.4329y_2 - 0.4329y_{12} + 0.4329y_{15} + 0.5671y_{16} + 0.3509y_{22} - 1.3608y_{24} < 0.4707$

(viii) $-0.0921 < 0.6885y_{13} - 1.6345y_{22} + 0.2649y_{23} + 0.0634y_{24} < 0.1632$

(ix) $-0.1911 < -0.689y_{13} - 33.4541y_{22} - 0.2645y_{23} + 1.2975y_{24} < 0.1769$

where y_i stands for ln (x_i).

Results and Discussion

As the result of the optimization procedure presented above, we obtained the optimum solution vector profile shown in Table 2.

The optimized steady state is stable and does not violate any of the imposed constraints. This solution has a robust behavior (results not shown) and better perturbation dynamics than the original setup, as shown in Figure 5. In this figure it can be seen that the time course of the substrate concentration X_4 (S_2) after a disturbance in S_i (approx. +3%) shows less and smaller oscillations than the basal steady state.

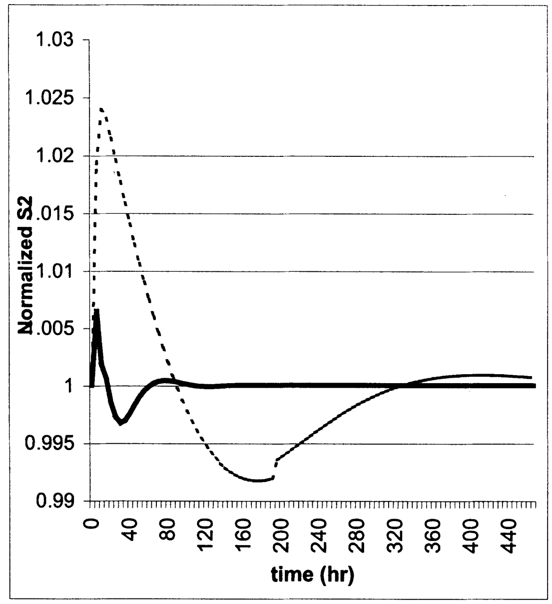

Figure 5. Time course of the substrate concentration normalized with respect to the operation point value, at the output of the wastewater treatment plant X_4 (s_2) after a perturbation in S_i.

The continuous line represents the optimum solution, while the dotted line corresponds to the original model.

A second observation refers to the level of improvement both in cost and controllability obtained by using this method. In fact, examination of Table 2 shows that a significant improvement in both controllability and cost can be attained through the use of the IOM approach applied here. The ISE value changes from 10.75 in the original setup down to 0.575, that is, just a 5.34% of its initial value. Moreover, the cost function ϕ_{econ} changes from 2988.2 to 1729.79, 57.9% of the original value. Finally, the C function changes 13738 to 2304.79, 83.22% better than the original value. Table 2 also provides us with a comparison of these results with those obtained by other approaches. One type of approach used so far to deal with this type of complex nonlinear optimization problem addressed is the use of global optimization methods. This problem has been in particular addressed in the past through the use of several adaptive stochastic methods (including ICRS, a sequential, adaptative random search method that handles inequality constraints via penalty functions; see Banga and Casares (1987)) and other clustering and determining global optimization methods. Using the GLOBAL clustering method, these authors found out the optimum parameter profile shown in Table 2. This solution was slightly further refined using the ICRS and DE stochastic methods, and a final local search using SQP. As can be seen for the three responses considered, ISE, ϕ_{econ} and C, the solution obtained with the GLOBAL method is better than the IOM, with improvements in about 30% in three cases.

However, when the GLOBAL method was used, the computation time required was about 250 s (Fortran implementation, PC Pentium III). The other adaptative stochastic methods, implemented in the Matlab, required computational efforts which were four times larger, although they could be reduced by using parallel versions running on a local network of 5 Pentium III platforms. When using the IOM (once the original model had been translated to the S-system form), the computation time was at its minimum, since the optimization engine used the simplex LP algorithm. The IOM approach can solve this type of problem in almost real time, since solving a LP can be done very fast and the symbolic manipulation needed by the IOM approach can be fully automated by means of suitable software (such as Maple). This means that we envision that the IOM procedure can be incorporated into a modern process simulator (such as gPROMS, Abacuss). By doing this, users will be able to define a bioprocess model and a tentative control structure and, almost immediately, obtain via the IOM the optimal integrated design ensuring low capital and operating costs plus maximum controllability. The use of global NLP methods will be two or three orders of magnitude slower, thus precluding suitable interactivity. Furthermore, the IOM scales up very well, since huge LPs can be solved very rapidly with low cost hardware. In contrast, methods like GLOBAL do not scale well with problem size, with computational cost often increasing exponentially.

The evaluation of the IOM reliability as a general optimization method was taken further ahead through a series of optimization runs within a wide range of parameters values. In Figure 5 the ISE is evaluated at the original and at the S-system models. What can be seen is that the ISE derived from the S-system representation deviates from the predicted by the original model, although these observed discrepancies are within acceptable ranges. It has been obseved by ourselves (see Figure 6) that the original model shows sustained oscillating behavior (limit cycles) for certain sets of parameter values (especially in the unfeasible search space), which lead to very high ISE values. In our explorations we have found out that the S-system shows such periodic solutions. These solutions occur at high values of the parameter ratio *Kp/ti*, an indicator of the sensitivity of the waste-water control system.

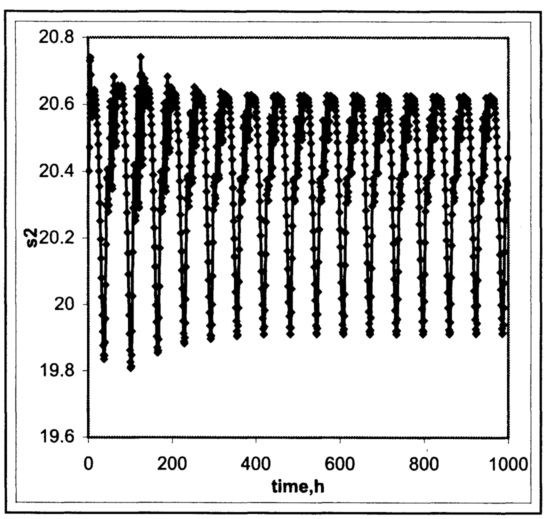

Figure 6. Sustained oscillating behavior for the variable $s2$ in the original model with a certain set of decision variables in the unfeasible search space.

$\upsilon_1 = 5942.82$, $\upsilon_2 = 6011.19$, $ad_1 = 2916.98$, $ad_2 = 1191.6$, $fk_1 = 0.07822$, $fk_2 = 0.07636$, $kp = -100$, $ti = 0.65$, $qr2 = 212.85$, $qr3 = 50.942$, $qp = 37.434$.

The canonical form of the S-system representation poses other additional advantages in an optimization study as the one carried out here. It is of foremost importance in a model and optimization program, either in the engineering or bioengineering fields, that the model structure should be independent of the number of constituents. In this regard, the most significant advantage of a canonical form, such as the S-system representation used in this work, is the fact that its homogeneous structure allows the standardization of a typical analysis, which includes model design, steady-state analysis, dynamic analysis and simulation, which in many instances are difficult within other modeling formalisms.

Another advantage of the IOM refers to the optimization issue itself. Linear programming allows us to tackle multiobjective optimization problems that are not readily accessible by using direct, global optimization methods. In the present case we have approached the multiobjective optimization by transforming a multiobjective optimization problem into a mono-objective problem through the definition of a combined objective function that measures the plant's economics and its controllability. This approach has, however, some drawbacks since it constitutes a simplification that may mask important aspects of the problem. Relevant for our purposes here is the fact that, from all the efficient solutions provided by the Pareto Optimum definition, we only obtain those corresponding to the weights chosen for each objective subfunctions (economic and controllability).

There are, however, some ways to mitigate this problem. In those cases where a great deal of information on the system is available we can assign the most adequate weights to the objective subfunctions. When the knowledge about the systems is not complete, some techniques can be applied that give us an approximation to the multiobjective problem without high computational requirements (sequential variation of subobjective weights or goal programming). The multiobjective resolution usually requires high computational capabilities, and needs more computational time.

The Multiobjective Indirect Optimization Method (MIOM; Vera et al., 2003) is an alternative approach to this problem using *S*-system formalism. In this algorithm, we profit from the properties of *S*-system models to solve a multiobjective nonlinear problem using multiobjective linear programming with low computational requirements.

Acknowledgments

This work was supported by a research grant from the Ministerio de Ciencia y Tecnología, ref. no. BIO2002-04157-C02-02 Julio Vera was the recipient of a research grant from the Ministerio Ciencia y Tecnología, ref. PN99-4320298.

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Table A1. Parameters of Model

Parameter	Value (units)
μ	0.1824 (h^{-1})
yy	0.5948
kd	$5 \cdot 10^{-5}$ (h^{-1})
kc	$1.3333 \cdot 10^{-4}$ (h^{-1})
ks	300.0 (h^{-1})
fkd	0.2
nnr	3.1563
aar	−0.00078567
kla	0.7 (h^{-1})
k_{01}	0.0001 (h^{-1})
cs	8.0 (h^{-1})
lr_1	0.5 (m)
lr_2	0.5 (m)
ld_1	2.0 (m)
ld_2	2.0 (m)
lb_1	3.5 (m)
lb_2	3.5 (m)
x_i	80.0 (mg/L)
q_i	1300.0 (m^3/h)
$s_{i,s}$	366.7 (mg/L)

Appendix A: Detailed Formulation of the Nonlinear Dynamic Model of the Wastewater Treatment Plant

This model was originally presented by Gutiérrez and Vega (2000) and subsequently slightly modified by Moles et al. (2001). The model was derived using a first principles approach, and it consists of a set of differential-algebraic equations. Regarding differential equations, Eqs 1–6 are the mass balances (mg/L·h) of the aeration tanks (subscripts 1 and 2). They represent the rate of change of oxygen (c_1,c_2), biomass (x_1,x_2) and organic substrate (s_1,s_2). In the mass balances for biomass, (Eqs. 1–2), the first term on the righthand side corresponds to a Monod-type biomass growth rate, the second is a first-order death rate for biomass, the third term reflects the dead biomass which is eaten by live biomass (especially in situations of high biomass and low substrate concentrations), and the last term is the biomass efflux due to flow. The same phenomena are taken into account in the mass balances for organic substrate and oxygen (Eqs. 4–6).

Equations 7–12 are the mass balances for the two settlers (subscripts 1 and 2) where three layers of different and increasing biomass concentration (xd_1, xb_1, xr_1, and xd_2, xb_2, xr_2) are considered. These are simple balances where the settling velocities follow a standard exponential decay. Finally, Eqs. 13 and 14 correspond to the integral term of the proportional integral (PI) controller and to the integral square error (ISE) measure respectively.

$$\frac{dx_1}{dt} = \frac{yy \cdot \mu \cdot s_1 \cdot x_1}{ks + s_1} - kc \cdot x_1 - \frac{kd \cdot x_1^2}{s_1} + \frac{q_{12} \cdot (xir_1 - x_1)}{v_1} \quad \text{(A1)}$$

$$\frac{dx_2}{dt} = \frac{yy \cdot \mu \cdot s_2 \cdot x_2}{ks + s_2} - kc \cdot x_2 - \frac{kd \cdot x_2^2}{s_2} + \frac{q_{22} \cdot (xir_2 - x_2)}{v_2} \quad \text{(A2)}$$

$$\frac{ds_1}{dt} = -\frac{\mu \cdot s_1 \cdot x_1}{ks + s_1} + fkd \cdot \left(\frac{kd \cdot x_1^2}{s_1} + kc \cdot x_1 \right) + \frac{q_{12} \cdot (sir_1 - s_1)}{\upsilon_1} \tag{A3}$$

$$\frac{ds_2}{dt} = -\frac{\mu \cdot s_2 \cdot x_2}{ks + s_2} + fkd \cdot \left(\frac{kd \cdot x_2^2}{s_2} + kc \cdot x_2 \right) + \frac{q_{22} \cdot (sir_2 - s_2)}{\upsilon_2} \tag{A4}$$

$$\frac{dc_1}{dt} = kla \cdot fk_1 \cdot (c_s - c_1) - \frac{k_{01} \cdot \mu \cdot x_1 \cdot s_1}{ks + s_1} - \frac{q_{12} \cdot c_1}{\upsilon_1} \tag{A5}$$

$$\frac{dc_2}{dt} = kla \cdot fk_2 \cdot (c_s - c_2) - \frac{k_{01} \cdot \mu \cdot x_2 \cdot s_2}{ks + s_2} + \frac{q_1 \cdot c_1}{\upsilon_2} - \frac{q_{22} \cdot c_2}{\upsilon_2} \tag{A6}$$

$$\frac{dxd_1}{dt} = \frac{(q_{12} - q_2) \cdot xb_1 - q_1 \cdot xd_1}{ad_1 \cdot ld_1} - \frac{\upsilon sd_1}{ld_1} \tag{A7}$$

$$\frac{dxb_1}{dt} = \frac{q_{12} \cdot x_1 - q_1 \cdot xb_1 - q_2 \cdot xb_1}{ad_1 \cdot lb_1} + \frac{\upsilon sd_1 - \upsilon sb_1}{lb_1} \tag{A8}$$

$$\frac{dxr_1}{dt} = \frac{q_2 \cdot (xb_1 - xr_1)}{ad_1 \cdot lr_1} + \frac{\upsilon sb_1}{lr_1} \tag{A9}$$

$$\frac{dxd_2}{dt} = \frac{(q_{22} - q_3) \cdot xb_2 - qsal \cdot xd_2}{ad_2 \cdot ld_2} - \frac{\upsilon sd_2}{ld_2} \tag{A10}$$

$$\frac{dxb_2}{dt} = \frac{q_{22} \cdot x_2 - q_{sal} \cdot xb_2 - q_3 \cdot xb_2}{ad_2 \cdot lb_2} + \frac{\upsilon sd_2 - \upsilon sb_2}{lb_2} \tag{A11}$$

$$\frac{dxr_2}{dt} = \frac{q_3 \cdot (xb_2 - xr_2)}{ad_2 \cdot lr_2} + \frac{\upsilon sb_2}{lr_2} \tag{A12}$$

$$\frac{dI}{dt} = \frac{k_p}{\tau_i} \cdot (s_{2s} - s_2) \tag{A13}$$

$$\frac{dISE}{dt} = (s_{2s} - s_2) \cdot (s_{2s} - s_2) \tag{A14}$$

It should be noted that Eqs. 1 and 2, which give the dynamic of biomass in tanks 1 and 2, do not take into account oxygen concentration, that is, the Monod-type growth kinetics only depend on substrate and biomass concentration. However, Eqs. 5 and 6 (dynamic of oxygen in the tanks) do reflect the consumption of oxygen by the biomass. The reason is that the model is assuming a high enough oxygen concentration so this is not a limiting factor for the growth. To ensure model validity during the integrated design process, the lower limit to the oxygen concentration is set to 1 mg/L.

Regarding algebraic equations, Eq. A15 describes the control law (qr_{1s} corresponds to the value in the steady state), while Eqs. A18–A21 state the rate of settling of sludge, and Eqs. A22–A28 correspond to the different balances among the flow rates (m^3/h). Equation A33 describes the disturbance in the input (substrate) considered for the computation of the ISE. In the above equation the disturbance is introduced at $t = 25$ h, but this time can be changed to any other suitable value

$$qr_1 = qr_{1s} + k_p \cdot (s_{2s} - s_2) + I \tag{A15}$$

$$sr_1 = \frac{s_1 \cdot q_2 + qr_3 \cdot s_2}{q_r} \tag{A16}$$

$$x_r = \frac{xr_1 \cdot q_2 + xr_2 \cdot qr_3}{q_r} \tag{A17}$$

$$\upsilon sd_1 = nnr \cdot xd_1 \cdot e^{aar \cdot xd_1} \tag{A18}$$

$$\upsilon sb_1 = nnr \cdot xb_1 \cdot e^{aar \cdot xb_1} \tag{A19}$$

$$\upsilon sd_2 = nnr \cdot xd_2 \cdot e^{aar \cdot xd_2} \tag{A20}$$

$$\upsilon sb_2 = nnr \cdot xb_2 \cdot e^{aar \cdot xb_2} \tag{A21}$$

$$q_2 = qr_1 + q_p - qr_3 \tag{A22}$$

$$q_3 = qr_3 + qr_2 \tag{A23}$$

$$q_{12} = q_i + qr_1 \tag{A24}$$

$$q_{22} = q_1 + qr_2 \tag{A25}$$

$$q_{sal} = q_i - q_p \tag{A26}$$

$$q_1 = q_{12} - q_2 \tag{A27}$$

$$q_r = q_2 + qr_3 \tag{A28}$$

$$xir_1 = \frac{q_i \cdot x_i + qr_1 \cdot x_r}{q_{12}} \tag{A29}$$

$$sir_1 = \frac{q_i \cdot s_i + qr_1 \cdot sr_1}{q_{12}} \tag{A30}$$

$$xir_2 = \frac{q_1 \cdot xd_1 + xr_2 \cdot qr_2}{q_{22}} \tag{A31}$$

$$sir_2 = \frac{q_1 \cdot s_1 + s_2 \cdot qr_2}{q_{22}} \tag{A32}$$

$$s_i = \left\{ \begin{matrix} s_{i,s} \\ s_{i,s} \end{matrix} + (10 - 10 \cdot e^{-2.5 \cdot t}) \begin{matrix} t < 25h \\ t \geq 25h \end{matrix} \right\} \tag{A33}$$

The optimization problem (NLP-DAEs) discussed by Moles et al. (2001) has eight design variables: υ_1, υ_2 volume of the aeration tanks, m^3), ad_1, ad_2 (areas of the settlers, m^2), fk_1, fk_2 (aeration factors), and k_p, τ_i (parameters of the PI controller). Apart from the DAEs given above, which act as equality constraints, the minimization is also subjected to the following nonlinear inequality constraints

$$2.5 \leq \frac{\upsilon_1}{q_{12}} \leq 8.0 \tag{A34}$$

$$0.001 \leq \frac{q_i \cdot s_i + qr_1 \cdot sr_1}{\upsilon_1 \cdot x_1} \leq 0.6 \tag{A35}$$

$$0.001 \leq \frac{(q_i + qr_3 - q_p) \cdot s_1 + qr_2 \cdot s_2}{\upsilon_2 \cdot x_2} \leq 0.06 \tag{A36}$$

$$\frac{q_{12}}{ad_1} \leq 1.5 \tag{A37}$$

$$\frac{q_{22}}{ad_2} \leq 1.5 \tag{A38}$$

$$3.0 \leq \frac{\upsilon_1 \cdot x_1 + ad_1 \cdot lr_1 \cdot xr_1}{q_p \cdot xr_1 \cdot 24} \leq 10.0 \tag{A39}$$

$$3.0 \leq \frac{\upsilon_2 \cdot x_2 + ad_2 \cdot lr_2 \cdot xr_2}{q_p \cdot xr_2 \cdot 24} \leq 10.0 \tag{A40}$$

$$0.5 \leq \frac{q_2 + q_3}{q_i} \leq 0.9 \tag{A41}$$

$$0.03 \leq \frac{q_p}{q_2 + q_3} \leq 0.07 \tag{A42}$$

The values of the model parameters are given in the Table A1, where μ (h^{-1}) is the microbial specific growth rate, *yy* is the metabolized substrate fraction converted in biomass, *kd* (h^{-1}), *kc* (h^{-1}) and *ks* (h^{-1}) are rate constants for any operating conditions, *fkd* is the fraction of death biomass converted into the substrate, *nnr* is the mass rate constant in the settlers, while *kla* (h^{-1}), k_{01} (h^{-1}), *cs* (h^{-1}) are the kinetic parameters in the oxygen equations. Besides, lr_1, lr_2, ld_1, ld_2, lb_1 and lb_2 (m) are the height of each layer in the settlers x_i (mg/l) and q_i (m^3/h) correspond to the input concentration and flow rate respectively, and $s_{i,s}$ (mg/L) establishes the steady-state before the disturbance considered.

Appendix B: Symbolic S-System Equations Derived from the Original Model

The corresponding S-system equations derived from the original model are as follows

$$\frac{dX_1}{dt} = \alpha_1 X_1^{g1,1} X_3^{g1,3} X_{11}^{g1,11} X_{12}^{g1,12} X_{13}^{g1,13} X_{14} \times - X^{g1,14} X_{22}^{g1,22} X_{24}^{g1,24} - \beta_1 X_1^{h1,1} X_3^{h1,3} X_{13}^{h1,13} X_{14}^{h1,14}$$

$$\frac{dX_2}{dt} = \alpha_2 X_2^{g2,2} X_4^{g2,4} X_7^{g2,7} X_{12}^{g2,12} X_{15}^{g2,15} X_{22}^{g2,22} X_{23}^{g2,23} - \beta_2 X_2^{h2,2} X_4^{h2,4} X_{15}^{h2,15} X_{22}^{h2,22} X_{23}^{h2,23}$$

$$\frac{dX_3}{dt} = \alpha_3 X_1^{g3,1} X_3^{g3,3} X_4^{g3,4} X_{13}^{g3,4} X_{14}^{g3,14} X_{22}^{g3,22} X_{24}^{g3,24} - \beta_3 X_1^{h3,1} X_3^{h3,3} X_{13}^{h3,13} X_{14}^{h3,14}$$

$$\frac{dX_4}{dt} = \alpha_4 X_2^{g4,2} X_3^{g4,3} X_4^{g4,4} X_{15}^{g4,15} X_{22}^{g4,22} X_{23}^{g4,23} - \beta_4 X_2^{h4,2} X_4^{h4,4} X_{15}^{h4,15} X_{22}^{h4,22} X_{23}^{h4,23}$$

$$\frac{dX_5}{dt} = \alpha_5 X_{18}^{g5,18} - \beta_5 X_1^{h5,1} X_3^{h5,3} X_5^{h5,5} X_{13}^{h5,13} X_{14}^{h5,14} X_{18}^{h5,18}$$

$$\frac{dX_6}{dt} = \alpha_6 X_5^{g6,5} X_{15}^{g6,15} X_{19}^{g6,19} X_{22}^{g6,22} - \beta_6 X_2^{h6,2} X_4^{h6,4} X_6^{h6,6} X_{15}^{h6,15} X_{19}^{h6,19} X_{22}^{h6,22} X_{23}^{h6,23}$$

$$\frac{dX_7}{dt} = \alpha_7 X_9^{g7,9} X_{16}^{g7,16} X_{22}^{g7,22} - \beta_7 X_7^{h7,7} X_{16}^{h7,16} X_{22}^{h7,22}$$

$$\frac{dX_8}{dt} = \alpha_8 X_{10}^{g8,10} X_{17}^{g8,17} X_{22}^{g8,22} X_{24}^{g8,24} - \beta_8 X_8^{h8,8} X_{17}^{h8,17} X_{22}^{h8,22} X_{24}^{h8,24}$$

$$\frac{dX_9}{dt} = \alpha_9 X_1^{g9,1} X_7^{g9,7} X_{13}^{g9,13} X_{16}^{g9,16} - \beta_9 X_9^{h9,9} X_{13}^{h9,13} X_{16}^{h9,16}$$

$$\frac{dX_{10}}{dt} = \alpha_{10} X_2^{g10,2} X_8^{g10,8} X_{17}^{g10,17} X_{22}^{g10,22} X_{23}^{g10,23} - \beta_{10} X_{10}^{h10,10} X_{17}^{h10,17} X_{22}^{h10,22} X_{23}^{h10,23}$$

$$\frac{dX_{11}}{dt} = \alpha_1 X_9^{g11,9} X_{13}^{g11,13} X_{16}^{g11,16} X_{22}^{g11,22} \times \beta_{11} X_{11}^{h11,11} X_{13}^{h11,13} X_{16}^{h11,16} X_{22}^{h11,22}$$

$$\frac{dX_{12}}{dt} = \alpha_{12} X_{10}^{g12,10} X_{17}^{g12,17} X_{23}^{g12,23} X_{24}^{g12,24} - \beta_{12} X_{12}^{h12,12} X_{17}^{h12,17} X_{23}^{h12,23} X_{24}^{h12,24}$$

$$\frac{dX_{13}}{dt} = \alpha_{13} X_2^{g13,2} X_4^{g13,4} X_{15}^{g13,15} X_{20}^{g13,20} X_{21}^{g13,21} X_{22}^{g13,22} X_{23}^{g13,23} - \beta_{13} X_2^{h13,2} X_3^{h13,3} X_4^{h13,4} X_{15}^{h13,15} X_{20}^{h13,20} \times X_{21}^{h13,21} X_{22}^{h13,22} X_{23}^{h13,23}$$

The kinetic orders corresponding to the *S*-system representation were determined from the original data and presentation. In the translation process we realized that there were rather wide ranges for the values of the variables, from 10^{-1} to 10^4. This feature reduces the model's tractability and makes the system prone to calculation errors. In order to avoid such difficulties, previous to any numerical evaluation, we normalized all the variables with respect to the references steady state, changing the differential equations accordingly. These changes leave the numerical values of the rate constant and kinetic orders unaffected, with the system showing the very same dynamics. The value of each rate constant is subsequently determined with any convenient set of values for the rate, variables, and kinetic orders that have just been determined.

Appendix C: Derivation of the Objective Function

The chosen expression to represent the original objective control function, ISE, was the following

$$\mathrm{Max}\left[\frac{d\dot{X}_{\mathrm{cont}}}{dP_{\mathrm{pert}}}\right] \tag{C1}$$

The control design objective function was obtained by differentiation, translation to the power law formalism, and the subsequent use of the definition of the logarithm's gain and the properties of the S-system representation followed by the substitution of the original basal values. By derivation of Eq. C1 we obtain

$$\frac{d\dot{X}_{\mathrm{cont}}}{dP_{\mathrm{pert}}}=\frac{\partial\dot{X}_{cont}}{\partial P_{\mathrm{pert}}}+\sum_k\frac{\partial\dot{X}_{\mathrm{cont}}}{\partial X_k}\frac{\partial X_k}{\partial P_{\mathrm{pert}}}$$

Using the power-law formalism, it is easy to transform the above expression into the following

$$\frac{d\dot{X}_{\mathrm{cont}}}{dP_{\mathrm{pert}}}=\frac{\partial\dot{X}_{\mathrm{cont}}}{\partial P_{\mathrm{pert}}}+\sum_k\left(g_{\mathrm{cont},k}\frac{J^+_{\mathrm{cont}}}{X_k}-h_{\mathrm{cont},k}\frac{J^-_{\mathrm{cont}}}{X_k}\right)\frac{\partial X_k}{\partial P_{\mathrm{pert}}}$$

where $g_{\mathrm{cont},k}$, $h_{\mathrm{cont},k}$ are the J^+_{cont} and J^-_{cont} kinetic orders, respectively, and P_{pert} is the perturbation parameter value. For every steady state, J^+_{cont} and J^-_{cont} have identical values. Then

$$\dot{X}_{\mathrm{cont}}=0\Rightarrow J^+_{\mathrm{cont}}=J^-_{\mathrm{cont}}$$

$$\frac{d\dot{X}_{\mathrm{cont}}}{dP_{\mathrm{pert}}}=\frac{\partial\dot{X}_{\mathrm{cont}}}{\partial P_{\mathrm{pert}}}+\sum_k(g_{\mathrm{cont},k}-h_{\mathrm{cont},k})\cdot\frac{J^+_{\mathrm{cont}}}{X_k}\frac{\partial X_k}{\partial P_{\mathrm{pert}}}$$

Using the definition of logarithmic gains, we have

$$L(X_k,P_{\mathrm{pert}})=\frac{\partial X_k}{\partial P_{\mathrm{pert}}}\frac{P_{\mathrm{pert}}}{X_k}\qquad \frac{\partial X_k}{\partial P_{\mathrm{pert}}}=L(X_k,P_{\mathrm{pert}})\frac{X_k}{P_{\mathrm{pert}}}$$

Due to the fact that in this case $\left(\frac{\partial\dot{X}_{\mathrm{cont}}}{\partial P_{\mathrm{pert}}}=0\right)$ we obtain

$$\frac{d\dot{X}_{\mathrm{cont}}}{dP_{pert}}=\frac{J^+_{\mathrm{cont}}}{P_{\mathrm{pert}}}\sum_k(g_{\mathrm{cont}_{,k}}-h_{\mathrm{cont}_{,k}})L(X_k,P_{\mathrm{pert}})$$

In the above expression.

$$K(X_{\mathrm{cont}},P_{\mathrm{pert}})=\sum_k(g_{\mathrm{cont},k}-h_{\mathrm{cont},k})L(X_k,P_{\mathrm{pert}})=cte$$

Thus, we obtain

$$\mathrm{Max}\left[\frac{d\dot{X}_{\mathrm{cont}}}{dP_{\mathrm{pert}}}\right]=\mathrm{Max}\left[\frac{J^+_{13}}{P_{\mathrm{pert}}}K(X_{13},P_{\mathrm{pert}})\right]$$

where $X_{\mathrm{cont}}=X_{13}$ and $K(X_{\mathrm{cont}},P_{\mathrm{pert}})$ are P_{pert} constants in all the optimization solutions.

Given that the logarithmic transformation does not change the locations of maxima and minima we can write

$$\mathrm{Min}[\phi_{\mathrm{econ}}]\Rightarrow\mathrm{Min}[\mathrm{Log}\,\phi_{\mathrm{econ}}]$$

$$\mathrm{Max}\left[\frac{J^+_{13}}{P_{\mathrm{pert}}}K(X_{13},P_{\mathrm{pert}})\right]\Rightarrow\mathrm{Max}\left[\mathrm{Log}\left(\frac{J^+_{13}}{P_{\mathrm{pert}}}K(X_{13},P_{\mathrm{pert}})\right)\right]$$

Accordingly, the total maximization objective function can be expresssed as

$$\mathrm{Max}\left[\gamma_{\mathrm{cont}}\cdot\mathrm{Log}\left(\frac{J^+_{13}}{P_{\mathrm{pert}}}K(X_{13},P_{\mathrm{pert}})\right)-\gamma_{\mathrm{econ}}\cdot\mathrm{Log}\,\phi_{\mathrm{econ}}\right]$$

where γ_{cont} and γ_{econ} are the weighting factors for both global objective's parts. By substituting J^+_{13} and ϕ_{econ} by its corresponding power law expressions, we obtain

$$\mathrm{Max}\left[\gamma_{\mathrm{cont}}\cdot\log\left(\frac{\alpha_{13}}{P_{\mathrm{pert}}}K(X_{13},P_{\mathrm{pert}})\cdot\left(\prod_{k=1}^{24}X_k^{g13k}\right)\right)-\gamma_{\mathrm{econ}}\cdot\log\left(\alpha_{\mathrm{econ}}\cdot\prod_{k=14}^{19}X_k^{\mathrm{gecon}k}\right)\right]$$

By separating all the terms in the objective, we obtain

$$\mathrm{Max}\left[\gamma_{\mathrm{cont}}\cdot\log\left(\frac{\alpha_{13}}{P_{\mathrm{pert}}}K(X_{13},P_{\mathrm{pert}})\right)-\gamma_{\mathrm{econ}}\cdot\log\alpha_{\mathrm{econ}}+\gamma_{\mathrm{cont}}\cdot\log\left(\prod_{k=1}^{24}X_k^{g13k}\right)-\gamma_{\mathrm{econ}}\cdot\log\left(\prod_{k=14}^{19}X_k^{\mathrm{gecon}k}\right)\right]$$

The two first terms are constants, and we can ignore them

$$\mathrm{Max}\left[\gamma_{\mathrm{cont}}\cdot\log\left(\prod_{k=1}^{24}X_k^{g13k}\right)-\gamma_{\mathrm{econ}}\cdot\log\left(\prod_{k=14}^{19}X_k^{\mathrm{gecon}k}\right)\right]$$

In the normalized basal state the two terms equals 1, thus, in order to give them the same importance, we assign both a weighting factor of 1

$$\mathrm{Max}\left[\log\left(\prod_{k=1}^{24}X_k^{g13k}\right)-\log\left(\prod_{k=14}^{19}X_k^{\mathrm{geconk}}\right)\right]$$

By rearranging and using logarithmic properties, we obtain

$$\mathrm{Max}\left[\prod_{k=14}^{19}(g_{13,k}-g_{\mathrm{econ},k})\cdot Y_k+\prod_{\substack{k=1\\k\notin[14,19]}}^{24}g_{13,k}\cdot Y_k\right]$$

where the variables are replaced by its logarithmic counterparts. Finally, expanding ϕ_{econ} around the basal steady state and substituting the numerical values we obtain

$$\mathrm{Max}\,[\,0.116X_2+0.993X_4-X_{14}-0.965X_{15}-0.1X_{16}$$
$$-0.08X_{17}-0.005X_{18}-0.005X_{19}0.719X_{20}+0.719X_{21}$$
$$+0.142X_{22}+0.023X_{23}]$$

Manuscript received Sept. 18, 2002, and revision received May 6, 2003.