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Biodegradation of Mixed Wastes in Continuously Operated Cyclic Reactors

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ABSTRACT

The problem of simultaneous biodegradation of two dissimilar substrates in a continuously operated cyclic reactor was studied both at the theoretical and experimental levels using a simple model system. The system involved media containing mixtures of glucose and phenol as carbon sources. A pure culture of *Pseudomonas putida* (ATCC 17514) was employed. Independent kinetic experiments have revealed that glucose and phenol are involved in a crossinhibitory uncompetitive kinetic interaction. The dynamics of a cyclically operated reactor were analyzed using the principles of bifurcation theory for forced systems. Experimental results have confirmed the theoretical predictions. Implications of the results for the design of waste-treating facilities are discussed.

Index Entries: Biodegradation; cyclic bioreactors; bioreactor dynamics; mixed wastes; glucose/phenol removal.

INTRODUCTION

Biological treatment of domestic and industrial liquid wastes is a well-established technology. However, new and stricter environmental standards will require large capital expenditures for building larger facilities unless existing units can be optimized so that their efficiencies increase. Optimization has been already performed based on empirical or semiempirical approaches. There is, though, a lot of room for further optimization of waste-treating facilities through a fundamental understanding of the kinetics of biodegradation, the population interactions, and the dynamics of the reactors employed in the process. The work presented here focuses on understanding the basic kinetics of biodegradation of mixtures of dissimilar substrates (pollutants) and the dynamics of a cyclically operated reactor.

Semicontinuous reactor operation, known as sequencing batch reactor (SBR) technology, has been studied and applied extensively in treatment of liquid wastes. An overview of SBRs and their advantages over continuously operated (steady-state) activated sludge systems can be found in the literature *(1,2)*. Continuously operated cyclic bioreactors have been investigated in detail for various systems involving removal of pollutants. Dikshitulu et al. *(3)* investigated phenol removal by two competing cultures and showed the effects of biomass composition changes

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on the process. Wang et al. *(4)* investigated biological reduction of nitrate and nitrite in a cyclic bioreactor, and showed that process analysis can lead to correct selection of operating conditions in order to avoid process instabilities and ensure avoidance of nitrite accumulation in the reactor. Such a process analysis based on fundamental kinetics and dynamics has been also used in the design of an actual facility built for denitrification of munition wastes *(5)*. Using advanced computational algorithms, Lenas et al. *(6)* performed design optimization studies for phenol biodegradation by a pure culture in a cyclic reactor.

The question of kinetic interactions between dissimilar substrates has been addressed at various levels by a number of investigators. For example, it has been reported that, depending on the acclimation procedure of a mixed culture, glucose may or may not inhibit the biodegradation of phenol *(7)*. The same study concluded that phenol had no impact on the glucose utilization rate. Another more detailed, kinetic study has concluded that glucose and 2,4-dichlorophenoxyacetate are involved in a crossinhibitory interaction when they are simultaneously removed by a mixed culture of *Pseudomonas* species *(8)*. Glucose has been reported to enhance the mineralization rate of *p*-nitrophenol *(9)*, although this enhancement has been attributed to higher biomass presence owing to growth on glucose. Thus, the effect of glucose on the specific rate of *p*-nitrophenol removal is unclear. The same study has concluded that phenol inhibits the mineralization rate of *p*-nitrophenol. Studies on the effect of glucose on the degradation of 2,4-dinitrophenol in SBRs have concluded that the degradation rate of the pollutant is enhanced, provided that the presence of glucose does not exceed a certain limit *(10,11)*.

The intent of the present study was to investigate the dynamics of a continuously operated cyclic bioreactor employed in treatment of liquid wastes containing mixtures of dissimilar compounds (pollutants). A simple system containing glucose and phenol was selected to simulate wastes of domestic (urban) and industrial origin, respectively. A pure culture of *Pseudomonas putida* (ATCC 17514) capable of growing both on glucose and phenol as sole carbon and energy sources was used in the experiments. The biodegradation kinetics were revealed in independent batch kinetic studies. The expressions for the removal rates were then used in deriving general equations describing operation and performance of a continuously operated cyclic reactor. The model was subjected to a detailed analysis of its dynamics. Subsequently, experiments were performed with a 7-L unit operating in a periodic mode, and the results were compared to the theoretical predictions.

MATERIALS AND METHODS

Inocula of *P. putida* (ATCC 17514) were originally grown in a nutrient broth of BBL product 11479 (Dickinson Microbiology Systems, Cockeysville, MD) and then acclimated in a synthetic medium that contained phenol and/or glucose as carbon sources. The acclimation procedure was the same as that of Dikshitulu et al. *(3)*. The synthetic medium used in the batch kinetic experiments and the experiments with the cyclically operated bioreactor was prepared by adding phenol and/or glucose, as required, from stock solutions carrying each substrate at 2 kg/m^3 to a 50-m*M* phosphate buffer (K_2HPO_4 and KH_2PO_4) solution of pH 7.2. The following compounds were also present per liter of buffer: 0.5 g $(NH_4)_2SO_4$, 0.1 g $MgSO_4$, 0.01 g $MnSO_4$, 0.0005 g $FeCl_3$, and 100 mL of tap water. Before utilization, the synthetic waste (medium) was sterilized in an autoclave at 121°C for 20 min.

During experiments, samples were taken from the reactor for assaying the biomass, phenol, and glucose concentrations. The biomass concentration was determined through the optical density of samples measured with a Varian DMS-200 spectrophotometer (Varian Instrument Group, Palo Alto, CA) at 540 nm. Optical densities up to 0.6 were converted to biomass concentrations (in g/m^3) when multiplied by a factor of 273.88. Samples with optical densities higher than 0.6 were diluted with deionized water and their optical densities were measured again.

Phenol concentrations were measured via high-pressure liquid chromatography (HPLC) with a unit SP8800 (Spectra Physics Analytical, Piscataway, NJ). The samples were filtered through a 0.2-μm filter paper (Gelman Sciences, Ann Arbor, MI) before injection to the HPLC. The flow rate of the mobile phase, which consisted of 55:45 (v:v) methanol and deionized water, was 1 mL/min. Both methanol and water also contained acetic acid at 1% by volume. The column and detector of the unit have been described elsewhere *(3)*. The retention time for phenol was 2.4 min.

Glucose concentrations were determined through the use of glucose diagnostic kits obtained from Sigma (St. Louis, MO). Kit Sigma 510 was used in cases where experiments were performed with glucose as sole carbon and energy source, whereas kit Sigma 115 was used in cases where experiments were performed with mixtures of glucose and phenol. Phenol has been found to interfere with glucose detection when kit Sigma 510 is used. Both methods are based on the use of enzymes that yield colored final products. The details for using these diagnostic kits are given by the manufacturer and were followed step by step as described in the manuals.

The experimental unit for the cyclic reactor operation is given schematically in Fig. 1 and is almost identical with the one used in earlier studies *(3,6)*. The main unit (reactor vessel) had a volumetric capacity of 7 L. During each cycle, the volume of the reactor contents was varied from 2 to 4 L. The unit was fully automated through the use of a programmable time sequence controller (Model SCY-PO, Omron, Peabody, MA). The reactor was fed and discharged by using two flow pumps (Masterflex L/S Unified drives, Cole-Parmer Instr., Niles, IL). The same unit, but without the time sequence controller, was used in batch experiments for determining the kinetics of glucose and phenol/glucose mixtures utilization by the culture. The working volume of the reactor during the batch runs was 0.5 L. All experiments were performed at a constant temperature of 28°C.

The pH of samples was monitored and found to be essentially constant at values between 7.0 and 7.2. The level of dissolved oxygen in the reactor was continuously monitored with a probe (Series 900, New Brunswick Scientific Co., New Brunswick, NJ) and found to be at levels no less than 85% of saturation, which ensured that all experiments were under conditions that were not only aerobic, but also nonoxygen-limiting.

MATHEMATICAL DESCRIPTION OF THE PROCESS

Consider a vessel that contains at time t = 0 an amount of culture suspension of volume V_o. The concentration of the biomass in the suspension is b_o. The medium contains two substitutable substrates (carbon sources) S_1 and S_2 at growth-limiting concentrations $s_{1,0}$ and $s_{2,0}$, respectively. At t = 0, the vessel starts being fed with a medium carrying substrates S_1 and S_2 at concentrations s_{1f} and s_{2f}, respectively. The externally fed stream carries no biomass, whereas all required nutrients except S_1 and S_2 are present in it in excess quantities. The medium is fed at a constant flow rate

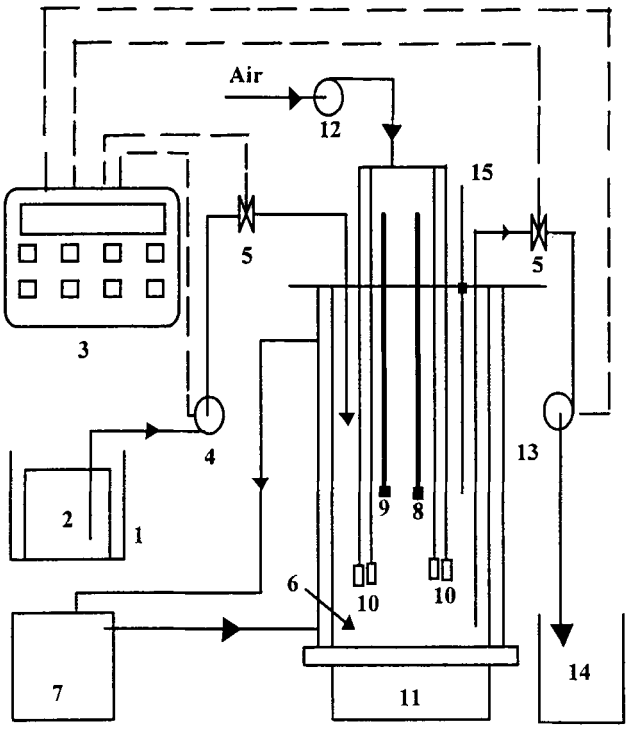

Fig. 1. Schematic of the experimental reactor unit: (1) constant temperature water bath for feed tank, (2) tank containing the medium (untreated waste), (3) programmable time sequence controller for automating the cyclic operation of the reactor, (4) peristaltic feed pump, (5) solenoid valve, (6) jacketed reactor; (7) circulating water bath, (8) thermometer, (9) dissolved oxygen probe, (10) air diffusion tube, (11) magnetic stirrer, (12) air pump, (13) peristaltic discharge pump, (14) discharge tank, (15) sampling port.

Q_f^* for a period up to time $t = t_1$. This is the first period of the cycle, and during it, there is no output from the vessel. Consequently, the volume of the suspension increases linearly with time, and at time $t = t_1$, it reaches a maximum value of V_m. During this first phase of the cycle, the contents of the reactor are aerated and, thus, reaction occurs. At time $t = t_1$, feeding of the vessel stops while there is still no outlet for the reactor contents. This lasts up to time $t = t_2$. Hence, for a period of time, the extent of which is given by $t_2 - t_1$, the vessel operates as a batch reactor with a constant volumetric hold-up equal to V_m. The period of time from $t = t_1$ to $t = t_2$ constitutes the second phase of the cycle. At time $t = t_2$ a pump is activated and reactor contents start being drawn at a constant flow rate Q, while there is still no external feeding of the vessel. During this third phase of the cycle, which lasts up until time $t = t_3$, the volume of the reactor contents decreases linearly with time and at time $t = t_3$, it becomes equal to V_o, which was the value of the suspension at the beginning of the cycle (i.e., $t = 0$). At that instant of time, the discharge pump is deactivated while the feed pump is activated, and a second cycle starts for the reactor. This cyclic pattern is repeated continuously.

Assuming that the liquid (suspension) density is constant at all times, and that the two substitutable substrates, which are fed to the reactor at growth-limiting concentrations, can be simultaneously used by the culture, the process can be

described by four equations. These equations represent the total mass balance, mass balance on substrates S_1 and S_2, and balance on the biomass. Their form is, correspondingly, as follows if one also assumes that biomass maintenance requirements are negligible.

$$dV/dt = Q_f - Q \tag{1}$$

$$ds_j/dt = (Q_f/V)\,(s_{jf} - s_j) - b\mu_j\,(s_1,s_2)/Y_j,\ j = 1,2 \tag{2}$$

$$db/dt = [-Q_f/V + \mu_1\,(s_1,s_2) + \mu_2\,(s_1,s_2)]b \tag{3}$$

The specific form of the equations above depends on what phase of the cycle the system is at. For example, $Q_f = Q_f^*$ during the first phase, whereas $Q_f = 0$ during the following two phases. Symbols that appear in Eqs. (1)–(3) and have not yet been defined are: V, volume of reactor contents; s_j, concentration of substrate j (j = 1,2) in the reactor; b, biomass concentration in the reactor; Y_j, yield coefficient of the culture on substrate j; $\mu_j(s_1,s_2)$, specific growth rate of the culture on substrate j. Observe that $\mu_j(s_1,s_2)$ is indicated to be a function of the concentrations of both substrates, which is true when substrate interactions are present.

Equations (1)–(3) are generally true for any system involving two substitutable rate-limiting substrates that can be utilized simultaneously by a culture. Their form changes depending on the kinetics of the specific system. For the case of interest here, if S_1 represents phenol and S_2 stands for glucose, the following expressions are valid, as discussed later.

$$\mu_1(s_1,s_2) = \mu_1^* s_1/[K_1 + s_1 + s_1^2/K_I + K_3 s_1 s_2]$$
$$\mu_2(s_1,s_2) = \mu_{m2} s_2/(K_2 + s_2 + K_4 s_2 s_1) \tag{4}$$

Equation (1) is linear and, thus, it can be easily integrated analytically and incorporated into Eqs. (2) and (3). When this is done, and after performing an analysis the details of which have been presented elsewhere *(3,4,6)*, one can show that Eqs. (1)–(3) can be written as follows.

$$du/d\theta = (u_f - u)M/(\sigma_1\delta + \theta - \beta) \times f(u,z) \tag{5}$$

$$dz/d\theta = (z_f - z)M/(\sigma_1\delta + \theta) - \eta\gamma x\beta g(u,z) \tag{6}$$

$$dx/d\theta = -xM/(\sigma_1\delta + \theta) + [f(u,z) + \eta g(u,z)]\beta x \tag{7}$$

where

$$f(u,z) = u/(1 + u + \omega u^2 + \lambda_1 uz) \qquad g(u,z) = z/(1 + z + \lambda_2 uz) \tag{8}$$

and

$$M = 1, \text{ for } 0 \le \theta \le (1 - \delta)\sigma_1;\ M = 0, \text{ for } (1 - \delta)\sigma_1 \le \theta \le 1 - \delta \tag{9}$$

Equations (5)–(7) are dimensionless versions of Eqs. (2) and (3), whereas expressions (8) are dimensionless versions of the specific growth rates given by expressions (4).

The dimensionless quantities appearing in the model Eqs. (5)–(9) have been defined as follows.

$$\theta = tQ_f^*\sigma_1/V_m,\ u = s_1/K_1,\ u_f = s_{1f}/K_1,\ z_f = s_{2f}/K_2,\ z = s_2/K_2,$$
$$x = b/(Y_1K_1),\ \sigma_1 = t_1/t_3,$$
$$\gamma = Y_1K_1/(Y_2K_2),\ \eta = \mu_{m2}/\mu_1^*,\ \omega = K_1/K_I,\ \lambda_1 = K_2K_3,\ \lambda_2 = K_1K_4,$$
$$\delta = V_o/V_m,\ \beta = \mu_1^* V_m/(Q_f^*\sigma_1)$$

The physical meaning of the dimensionless quantities above is as follows; u,z: concentrations of substrates S_1 and S_2 in the reactor, respectively; u_f,z_f: concentrations of substrates S_1 and S_2 in the medium fed to the reactor; x: biomass concentration in the reactor; γ, η, ω, λ_1, λ_2: kinetic parameters related to those appearing in expressions (4) and to the yield coefficients; θ: time; δ: ratio of minimum to maximum volume of reactor contents or, alternatively, $1 - \delta$: fraction of reactor contents exiting from the reactor per cycle and being substituted for by fresh medium; σ_1: fraction of cycle time devoted to filling the reactor with medium; β: measure of the inverse dilution rate.

In general, rendering model equations dimensionless reduces the amount of computational—or even experimental—work needed for studying a process. This is also true for the process considered here. For example, for a specific system, the seven kinetic constants appearing in expressions (4) and the two yield coefficients are specified. Thus, dealing either with nine dimensional constants or their five dimensionless equivalents is immaterial. However, if one wants to investigate the behavior of other similar systems, the dimensionless model allows capturing the entire range of possibilities by varying five rather than nine parameters. In case of a specified system, there are seven dimensional operating parameters that affect the behavior of the system, namely, V_o, V_m, Q_f^*, t_1, t_3, s_{1f}, and s_{2f}. With the dimensionless model, one needs to study the effect of five rather than seven parameters, namely, δ, σ_1, β, u_f, and z_f. In addition, two of the dimensionless parameters (δ, σ_1) are fractions and need to be varied between 0 and 1, whereas none of the dimensional parameters is really confined in any particular range. It is also worth mentioning that, as reflected in Eq. (9), the analysis shows that the extent of each cycle—in dimensionless form—is given by $1 - \delta$.

RESULTS AND DISCUSSION

Detailed batch experiments with media containing glucose as the sole carbon and energy source at various initial concentrations have been performed, and the analysis of the data has shown that $\mu_{m2} = 0.84/h$, $K_2 = 29.84\ g/m^3$, and $Y_2 = 0\ 44$ g biomass/g glucose. The data have also confirmed that maintenance requirements are negligible.

The culture used in the present study had been also used in an earlier study *(3)*. Kinetic experiments with phenol as the sole carbon and energy source had revealed in that study, that $\mu_1^* = 0.897/h$, $K_1 = 12.20\ g/m^3$, $K_1 = 203.68\ g/m^3$, and $Y_1 = 0.768$ g biomass/g phenol. During the present study, only few batch experiments were performed with media containing phenol, but not glucose. The results could be perfectly described when the above-mentioned kinetic constants were used. In the absence of glucose, i.e., $s_2 = 0$, expression (4) implies that $\mu_1(s_1)$ is the Andrews *(12)* inhibitory model.

A detailed series of batch kinetic runs with medium containing both glucose and phenol at various initial concentrations was performed. The data could not be described unless the Andrews expression for phenol and the Monod expression for glucose were modified as indicated by expressions (4). These modifications concern the introduction of the extra terms in the denominators that involve products of the concentrations of the two substrates. Keeping the analogy with enzyme kinetics, these extra terms imply that phenol and glucose are involved in a crossinhibitory uncompetitive interaction. All constants except K_3 and K_4 in the kinetic expressions are those obtained from experiments with media containing either phenol or glu-

cose. The values of K_3 and K_4 were determined as 0.04 and 0.02 m^3/g, respectively, by fitting a large number of kinetic data from experiments with media containing glucose and phenol mixtures to expressions (4). The so-determined K_3 and K_4 values were then used unaltered in describing data sets that had not been used in the fitting approach. The agreement was very good and, thus, these values can be used with confidence. The magnitudes of K_3 and K_4 imply that glucose exerts a degree of inhibition on the removal of phenol higher than that which phenol exerts on the glucose utilization rate.

Having the values of all kinetic constants from the batch experiments, Eqs. (5)–(9) involve the following parameters: σ_1, δ, β, u_f, and z_f. The concentrations of phenol and glucose in the feed stream (u_f and z_f, respectively) may vary, whereas the remaining three parameters (β, σ_1, δ) can be selected at the experimenter's/designer's will. For the results reported here, δ and σ_1 were set at a value of 0.5. The equations were then analyzed in the three-dimensional space ($\beta - u_f - z_f$). Since the system is periodically forced, it cannot attain a steady state (equilibrium point). Instead, and after transients decay, it reaches a so-called limit cycle. This means that the phenol, glucose, and biomass concentrations vary periodically in the reactor, and the same patterns are repeated in each cycle. These periodic patterns (orbits) can be found numerically based on computer codes, which have been discussed in the literature *(13,14)*. The stability (or instability) of periodic orbits is determined by the magnitude of the Floquet multipliers of the Poincaré map for the system. These methodologies have been discussed earlier *(3,4,6)* in conjunction with different physical systems that were studied with the reactor operation protocol which was also followed in the present study. The analysis of the system examined here has shown that the operating parameter space ($\beta - u_f - z_f$) is divided into three regions. In region I, the culture washes out of the reactor. Because of the fact that some biomass is lost during the third phase of the cycle, culture washout is a possible outcome. In region II, the analysis predicts that the system can, under the same operating conditions, reach either a state of culture survival (and, thus, treatment of phenol/glucose) or a state of culture washout. Hence, region II is one in which multistability occurs. Which one of the two possible outcomes is actually reached depends on the way the process is started up. Furthermore, even if the startup is properly done and a culture survival state is reached, temporary operational upsets may lead the system to the washout state. Finally, there is a third region in the $\beta - u_f - z_f$ space (region III) where it is predicted that there is a single possible outcome for the system, and it is a state of culture survival. In this case, temporary operational upsets are not detrimental to the process, since the system returns to its original pattern once conditions are restored. Hence, region III is the safest one to operate in. These results are shown graphically in Fig. 2 in the form of two-dimensional diagrams in the $\beta - u_f$ plane for three different z_f values. The boundaries of regions I, II, and III are obtained through a bifurcation analysis of the periodic states. The boundary of regions I and II is the locus of points where a limit point bifurcation occurs. The physical significance of this boundary is that it indicates the maximum allowable value for the flow rate during feeding (first phase of the cycle) in order for the culture to have a chance to establish itself in the reactor. The boundary of regions II and III is the locus of points where a transcritical bifurcation occurs. Physically, this boundary represents the maximum allowable value for the feed flow rate (for given u_f and z_f values) in order to ensure that the culture will definitely establish itself in the reactor.

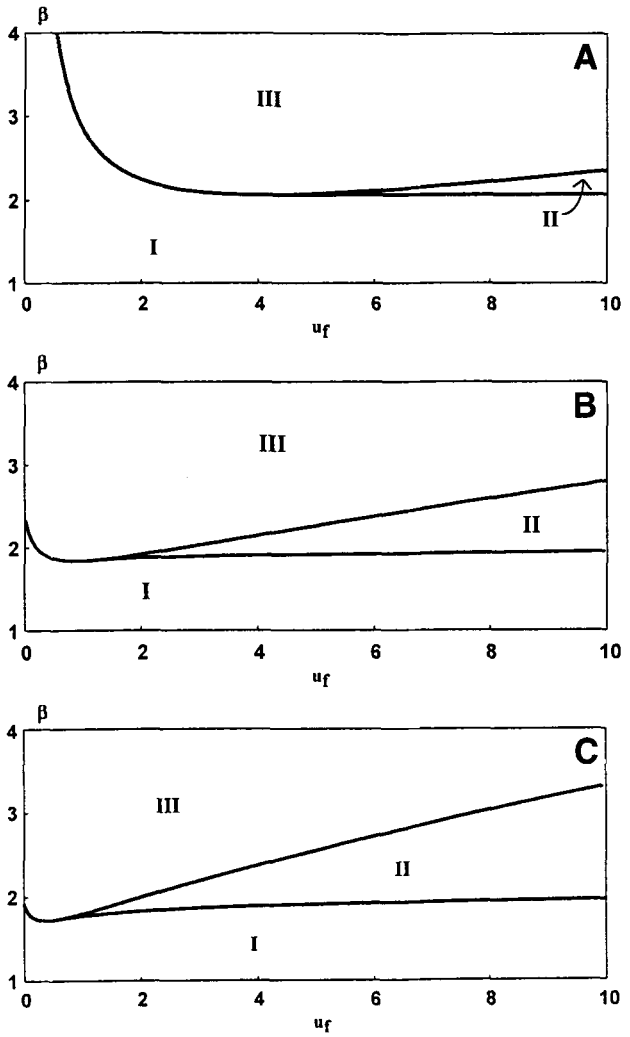

Fig. 2. Operating diagrams for the system in the β–u_f plane. In all cases $\sigma_1 = \delta = 0.5$, whereas s_{2f} is 0, 50, and 100 g/m^3 for cases **(A)**, **(B)**, and **(C)**, respectively.

Figure 2A is for a case where the medium contains phenol as the sole carbon and energy source. In this case, the existence of region II where multistability occurs is the consequence of the inhibitory kinetics during the biodegradation of phenol. This has been discussed for the case of an ideal chemostat having time invariant inputs by Andrews *(12)*. The presence of glucose introduces an extra inhibitory term in the expression f(u,z) as shown by expressions (8). The higher the glucose presence is, the higher the degree of crossinhibition is in f(u,z) and, thus, the larger the extent of region II is as reflected in Figs. 2B and 2C.

For the case of an ideal chemostat having time invariant inputs, one can show the following. When z_f is specified, as is the case in Fig. 2, the $f(u_f,z_f)$ curve goes through a maximum for a value of u_f, which will be called u_f^*. In such cases, region III arises when $1/\beta < f(u_f,z_f) + g(u_f,z_f)$. Region II arises when $u_f \geq u_f^*$ and if $f(u_f,z_f) + g(u_f,z_f) < 1/\beta < f(u_f^*,z_f) + g(u_f^*,z_f)$, whereas region I arises in all other cases. For the case of the chemostat, $1/\beta$ stands for the dilution rate. These results can be used in

Table 1
Operating and Start-Up Conditions for the Experiments

Parameter	Experiment 1	Experiment 2	Experiment 3
t_1 (h)	0.613	0.613	0.7245
$t_2 - t_1$ (h)	0.446	0.446	0.5578
t_3 (h)	1.226	1.226	1.449
Q_f^* (L/h)	3.262	3.262	2.76
s_{1f} (g/m^3)	102.3	100.2	51.7
s_{2f} (g/m^3)	50	54	97
$s_{1,0}$ (g/m^3)	0	80	0
$s_{2,0}$ (g/m^3)	0	40	0
b_o(g/m^3)	94.782	4.921	81.145
V_o (L)	2	2	2
V_m (L)	4	4	4
u_f	8.382	8.21	4.236
z_f	1.675	1.809	3.25
σ_1	0.5	0.5	0.5
δ	0.5	0.5	0.5
β	2.2	2.2	2.6
Region in Fig. 2	II(B)	II(B)	III(C)

understanding the diagrams of Fig. 2, although things are not exactly analogous. In the case considered in the present study, there is no single value of the dilution rate, and β is only a measure of it. The results could be possibly better compared if for the case of cyclic reactors, one defined β as a time average of the dilution rate over a cycle.

The experiments reported here were performed in order to examine whether the key predictions of the theory can in fact be seen in a real system. The various operating and start-up conditions for the experiments are given in Table 1. Experimental results and model predictions regarding concentration profiles are given in Figs. 3–5. It needs to be mentioned that since sampling was done manually and samples needed immediate processing, data were collected for some of the cycles, only. The unit kept operating, and after a few cycles data collection was repeated. Experimentally determined concentrations are shown as symbols on the graphs, whereas the solid curves represent predicted concentration profiles. Predicted profiles were generated through numerical integration of Eqs. (5)–(9), and no curve-fitting was involved.

A number of conclusions can be reached from Figs. 3–5. The first is that phenol and glucose can be simultaneously utilized by the culture in the continuously operated cyclic reactor. The second is that (Figs. 3 and 5) the concentrations of the substrates can in fact become zero at the end of each cycle (complete waste treatment). The agreement between experimental data and model predictions is almost perfect. Furthermore, it is shown that the system can reach culture survival states (Figs. 3 and 5) or a culture washout state (Fig. 4). It is also very interesting to observe from Table 1 that experiments 1 and 2 were performed under identical operating, but different start-up conditions. The operating conditions were such that they defined a point in region II of graph (B) in Fig. 2. This is a region where

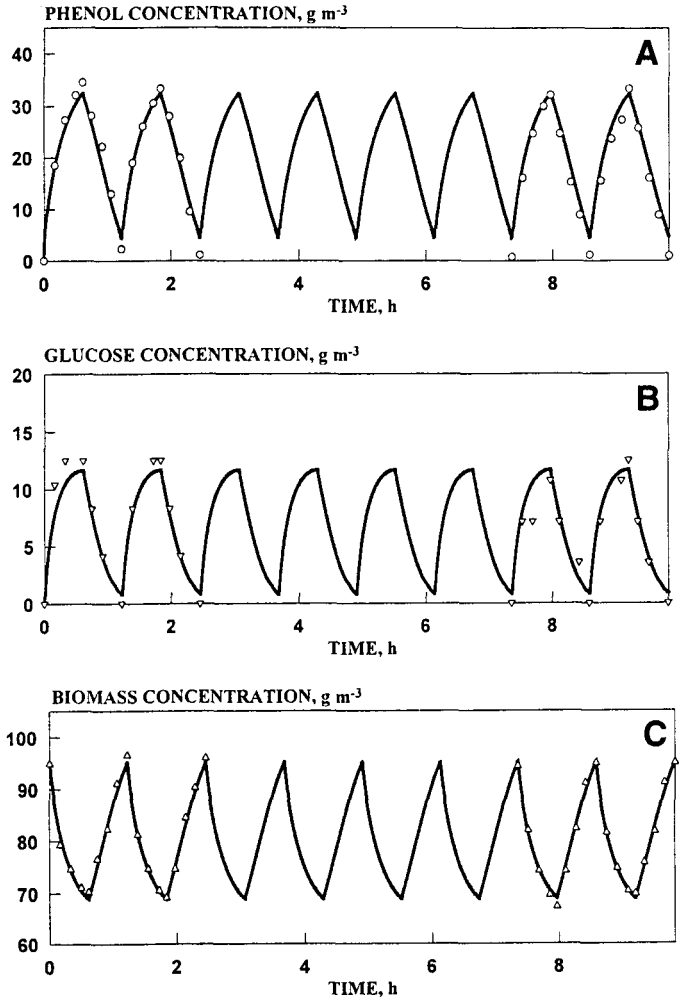

Fig. 3. Experimental and model-predicted concentration profiles for phenol **(A)**, glucose **(B)**, and biomass **(C)** for experiment 1. Conditions for this experiment are given in Table 1.

theory predicts multistability, and the results of experiments 1 and 2 confirm that this is in fact true. From the practical point of view, if it is decided to design a system so that it operates in a region of multistability, an appropriate control strategy has to be used in order to avoid the unwanted state (washout), even if there are temporal operational upsets. The conditions for experiment 3 were such that they define a point in region III of graph (C) in Fig. 2. This is a region where the system has only a survival state, and culture washout cannot occur. It is also interesting to observe that the results shown in Figs. 3 and 5 indicate that the system enters its steady (limit) cycle almost immediately after startup. This was by design and was achieved by appropriate selection of startup conditions so that they would fall close to the eventual cyclic pattern. This approach reduced lengthy transients and allowed the experiment to run for a relatively long time under the desired conditions of limit cycle.

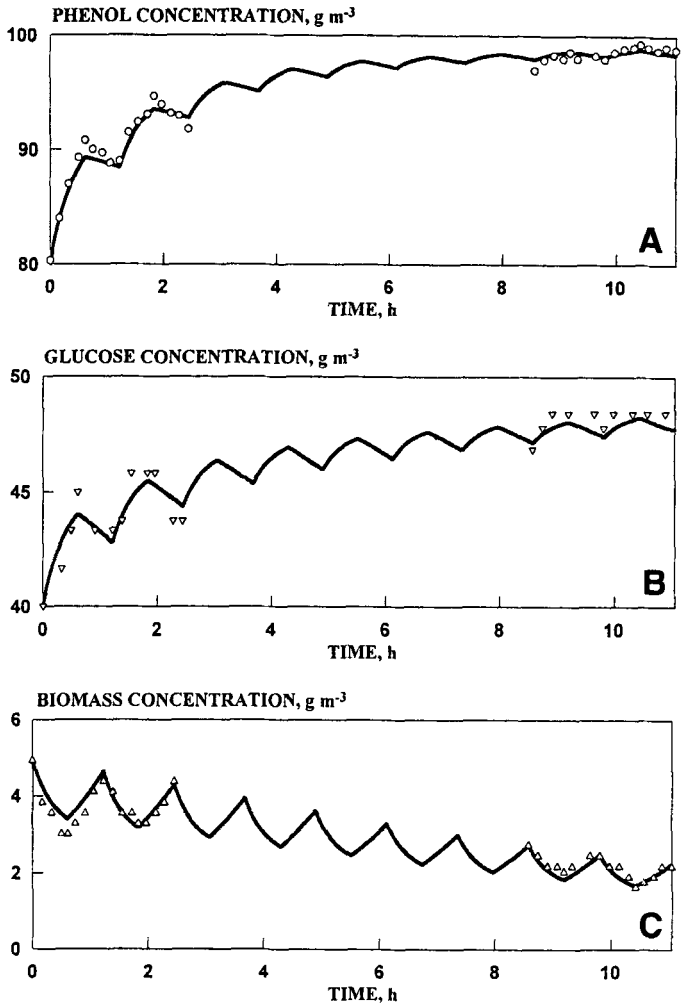

Fig. 4. Experimental and model-predicted concentration profiles for phenol **(A)**, glucose **(B)**, and biomass **(C)** for experiment 2. Conditions for this experiment are given in Table 1.

It is believed that a model has been derived and experimentally validated for the simultaneous biodegradation of two dissimilar carbon sources in a cyclic reactor. This model can be now used with confidence in computational studies in order to complete the analysis of the system dynamics by studying the effect of parameters δ and σ_1, and then determine the optimal σ_1, δ, and β values for given glucose/phenol concentration values in the inlet stream. The intent of optimization studies should be to determine parameter values for achieving a desired degree of substrate conversion with the minimum reactor volume and/or processing time. Such studies are currently under way. Finally, it is recognized that the system considered here is too simple to represent real waste treatment. However, the methodology could be applied to systems of any complexity provided that there is enough information on the biodegradation kinetics of all pollutants in the waste stream.

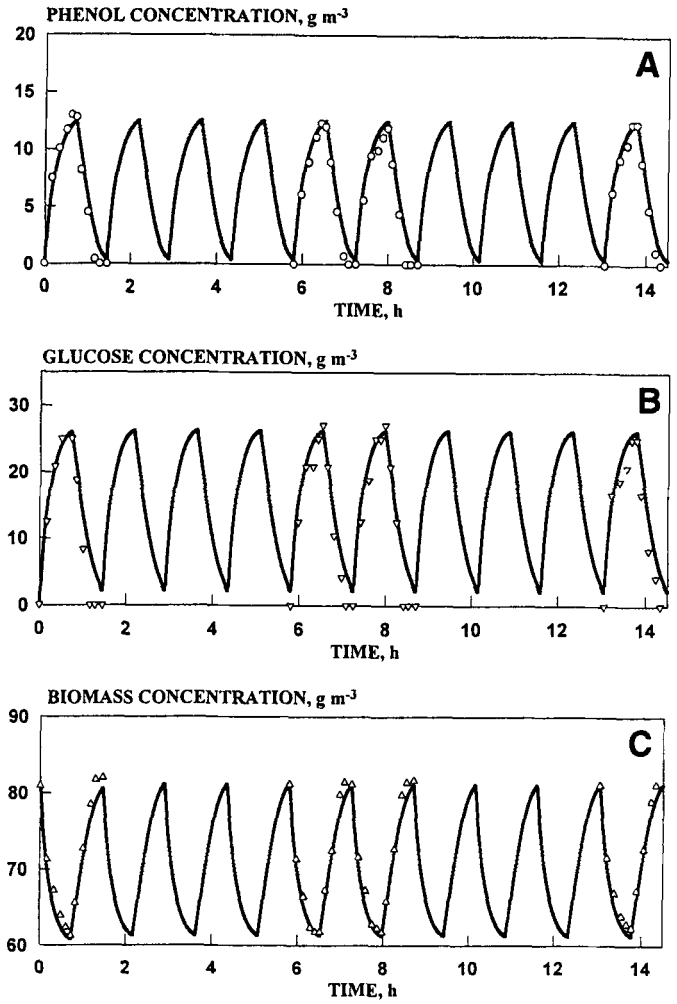

Fig. 5. Experimental and model-predicted concentration profiles for phenol **(A)**, glucose **(B)**, and biomass **(C)** for experiment 3. Conditions for this experiment are given in Table 1.

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