Dynamic simulation of benzene vapor treatment by a two-phase partitioning bioscrubber
Part II: Model calibration, validation, and predictions

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Abstract

The two-phase partitioning bioscrubber (TPPB) model presented in Part I has been validated using experimental data under constant and transient operating conditions for the treatment of benzene waste gases by *Achromobacter xylosoxidans* Y234 with *n*-hexadecane as an immiscible, organic phase. Model calibration was performed to account for observed enhancements of benzene biodegradation rates in biphasic media, postulating that direct benzene uptake from dispersed organic droplets increased substrate bioavailability. This led to the use of an 'effective dissolved substrate concentration' to model cell specific growth rates. Model predictions were greatly improved using this empirical modification. The characteristics of the organic phase, both in terms of the volume fraction selected and its high equilibrium solubility, are found to be of critical importance for minimizing effluent gas VOC concentrations and stabilizing performance during transient operation. The biokinetic parameters μ_{max} and K_S are also particularly important, greatly influencing the response of the TPPB both during and while recovering from transient periods. K_S was found to be important for influencing steady-state benzene removal efficiencies under even invariant operation, leading to the conclusion that microorganisms which can maintain high rates of biological activity under very dilute substrate concentrations will make ideal biocatalysts in the TPPB.
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1. Introduction

Two-phase partitioning bioscrubbers (TPPBs) continue to emerge as a viable alternative to physicochemical waste gas treatment processes, as well as to the more traditional vapor phase bioreactors, such as biofilters. Demonstrated high elimination rates and efficiencies and increasing ease of operation have arisen from their continual development [1–4]. In Part I of this paper, a mechanistic, dynamic model for the TPPB process was formulated to describe its primary constituent phenomena. Here, the model is validated using experimental data, beginning with a calibration step to adapt the generic model equations to our particular experimental conditions and address discrepancies arising from non-ideal behavior in the TPPB experiments. Much of the model validation is conducted using dynamic conditions, because they represent more practical operating conditions, and

because they allow for a more rigorous assessment of the predictive capability of the model for adequately capturing the characteristic time constants of the TPPB. The validated model is then used to make predictions regarding the performance potential of the TPPB, as well as to identify key design variables that may be adjusted to further improve operation.

2. Materials and methods

2.1. Validation experiments

Validation of the proposed model was performed in multiple stages, each representing various limiting cases or practical operating scenarios. Firstly, mass transfer in the TPPB was studied using a cell-free system in order to validate both the model structure and parameter estimates used to describe the associated absorption/desorption and partitioning processes. A feed stream containing 5.6 ± 0.3 mg/L benzene was applied at 1 L/min to a TPPB containing 2 L of sterile aqueous medium and 1 L of sterile *n*-hexadecane. After the liquid phases reached saturation, a step

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Nomenclature

$a_{A/O}$	aqueous–organic interfacial area (m^2)
A_s	amplitude of transient spike
c_1, c_2, c_3	empirical parameters of the expression of Chatzi et al. [15]
C_A	dissolved oxygen concentration in the aqueous phase (mg O$_2$/L)
C_A^*	aqueous dissolved oxygen concentration equilibrated with gas phase (mg O$_2$/L)
$C_{A,SS}$	steady-state dissolved oxygen concentration in the aqueous phase (mg O$_2$/L)
C_O	dissolved oxygen concentration in the organic phase (mg O$_2$/L)
d_{SM}	Sauter mean diameter of dispersed organic droplets (m)
D_i	impeller diameter (m)
D_C	dissolved oxygen aqueous–organic partitioning coefficient
H_{SO}	Henry's Law coefficient of substrate in the organic phase
K_C	half-saturation constant of oxygen (mg O$_2$/L)
$k_L a_{CA}$	lumped, volumetric mass transfer coefficient of oxygen absorption in the aqueous phase (h^{-1})
$k_L a_{CO}$	lumped, volumetric mass transfer coefficient of oxygen absorption in the organic phase (h^{-1})
k_v	inverse time constant of flowmeter valve actuation (h^{-1})
K_S	half-saturation constant of substrate (mg substrate/L)
m_C	specific maintenance rate on oxygen (mg O$_2$ mg cells^{-1} h^{-1})
n	empirical parameter of the Luong kinetic model
N	bioreactor agitation rate (rpm)
S_A	dissolved substrate concentration in the aqueous phase (mg substrate/L)
S_A^e	effective dissolved substrate concentration in biphasic media (mg substrate/L)
S_G^{in}	influent gas substrate concentration (mg substrate/L)
$S_{G,0}^{in}$	nominal influent gas substrate concentration (mg substrate/L)
S_m	inhibitory substrate concentration of the Luong kinetic model (mg substrate/L)
S_O	dissolved substrate concentration in the organic phase (mg substrate/L)
t	time (h)
t_s	duration of transient spikes (h)
V_T	total liquid volume of the TPPB (L)
W_e	Weber number
X_{SS}	steady-state biomass concentration (mg cells/L)
$Y_{X/C}$	biomass-to-oxygen yield coefficient (mg cells mg O$_2$$^{-1}$)

Greek letters

α	interfacial affinity constant
ϕ_{ORG}	organic phase volume fraction
μ	specific growth rate (h^{-1})
μ_{max}	maximum specific growth rate (h^{-1})
ρ_d	density of the biphasic dispersion (g m^{-3})
σ	aqueous–organic the interfacial tension (dyn m^{-1})

change in the influent benzene concentration to 9.3 ± 0.7 mg/L was performed and maintained until a new steady-state was achieved. The TPPB was then stripped of dissolved benzene by aeration at 2 L/min until no benzene was detected in the liquid phases or effluent gas.

The performance of the cell-containing TPPB under constant conditions from start-up through establishment of steady-state operation was validated in bioscrubbers with 3 L total liquid volumes and organic phase volume fractions of 0, 0.10, and 0.33. Experimental data were obtained from experiments which were conducted by following the procedures reported by Nielsen et al. [5]. A constant influent gas benzene concentration of 3.1 ± 0.3 mg/L was used in each of these experiments at an aeration rate of 60 L/h.

Finally, predicted TPPB performance in response to transient operating conditions was validated for both long-term (4 h) and short-term (5 min) fluctuations in the influent gas benzene concentration. Transient data were obtained from Nielsen et al. [4] for an organic phase volume fraction of 0.33. Data were also obtained by analogous experimentation at organic phase volume fractions of both 0 and 0.10.

2.2. Numerical methods

Solution of the proposed model was performed using Matlab® and the intrinsic ordinary differential equation solver, *ode15s*. Transient responses of the TPPB were investigated experimentally beginning from the process steady-state, corresponding to the 'maintenance state' of the culture. Accordingly, initial conditions for all of the state variables were determined by simultaneous solution of the steady-state form of the model presented in Part I via Newton's method using the routine presented by Constantinides and Mostoufi [6].

3. Results

3.1. Model calibration

Preliminary analysis of the experimental data sets quickly indicated that benzene biodegradation rates measured during transient operation were higher in the TPPB than in a single-phase bioscrubber under otherwise identical conditions. Comparison of these data to preliminary simulations confirmed that observed biodegradation rates were also faster in the two-phase system than would be predicted using the Luong kinetic

model and its previously measured parameter estimates obtained from single-phase batch kinetic studies in Part I. As a result, unacceptable offsets arose between the experimental data and model predictions, particularly with respect to S_G^{out}, the key performance indicator of this process. The discrepancies were most apparent during transient operation during which significant amounts of benzene would rapidly accumulate in both liquid phases in response to an increase in S_G^{in}, leading to subsequently higher rates of biological activity. These unanticipated high rates of biological activity suggest that benzene was actually more bioavailable in the two-phase system than previously predicted, even when assuming that a spontaneous partitioning equilibrium exists between the liquid phases. Efroymson and Alexander [7] reported similar findings for the biodegradation of phenanthrene by a *Pseudomonas* sp. using various organic phases.

There are many plausible biological and physical explanations for this observed inconsistency. For instance, as has been reported for naphthalene [8], substrate partitioning equilibrium between the organic and the aqueous phases may be inadequately represented by a linear isotherm for the range of concentrations experienced. Alternatively, microorganisms may actively promote greater substrate bioavailability by increasing the surface area of dispersed organic droplets or reducing interfacial tension between the phases through the secretion of surfactants or solubilizing agents [9]. Finally, it is also possible that substrate uptake occurs directly from the organic phase by cells in contact with its surface, a mechanism that is frequently reported among partitioning bioreactors [10–12]. This mechanism involves hydrophobic bacteria that attach to dispersed organic droplets, thereby increasing the bioavailability of the partitioned substrate through its direct uptake. Accordingly, the biomass is segregated to include a subpopulation of cells that exists at the surfaces of the dispersed organic droplets with $\mu = f(S_O)$, while the remainder is subjected to lower concentrations in the aqueous phase with $\mu = f(S_A)$. The overall biodegradation rate is therefore, a function of both S_A and S_O, however their individual contributions are not easily identified experimentally [12]. Modelling these effects individually can also be challenging due to difficulties in predicting the relative distribution of the segregated portions of the biomass.

Emulsification of a portion of the organic phase (up to 25 vol.%) was routinely observed, further indicating that there was some degree of cellular interaction with the organic phase. Microscopic examination of biphasic liquid samples removed from the TPPB routinely showed ample interaction by *Achromobacter xylosoxidans* Y234 with *n*-hexadecane droplet surfaces in the TPPB, however, cellular accumulation at the organic interface was not excessive and the majority of the cells resided in the aqueous phase. This indicates that direct uptake of benzene from *n*-hexadecane droplets was possibly a contributing, but not the sole mechanism of biodegradation. To capture the effect of enhanced bioavailability in this biphasic medium the use of an effective substrate concentration, S_A^e, is now proposed to empirically account for organic phase interactions as a function of its volume fraction, ϕ_{ORG} using an 'interfacial affinity constant', α, as:

$$S_A^e = S_A + \alpha \phi_{ORG} S_O \tag{1}$$

Note that Eq. (1) predicts S_A^e will reduce to S_A in the case of the single-phase system ($\phi_{ORG} = 0$). By its definition, α is a lumped parameter which intends to combine the pertinent phenomena influencing cellular behavior at the organic surface and the associated effect on the observed biodegradation rate. Using this approach, segregation of the biomass population is not required and all cells are assumed to have equal opportunity to intermittently access the organic interface in the well-mixed system. With this change, the kinetic growth expression used in the originally derived model is now replaced here by Eqs. (1) and (2):

$$\mu(S_A, S_O, C_A) = \mu_{max} \frac{S_A^e}{S_A^e + K_S} \left[1 - \frac{S_A^e}{S_m} \right]^n \frac{C_A}{C_A + K_C} \tag{2}$$

Estimates of α were obtained via non-linear regression with the objective of minimizing deviations between predicted and observed values of S_G^{out} with the ultimate goal of eliminating the offset between experimental data and model predictions. This calibration step was performed using data obtained during transient operation in the form of 4-h step changes in S_G^{in} of various amplitudes with TPPBs containing different values of ϕ_{ORG} with S_G^{out} selected as the operating variable for which to perform the calibration. Fig. 1 demonstrates how modifying the original model with Eq. (2) has dramatically improved the predictions with the initial sum-of-squared-errors (SSE$_0$) being reduced through this calibration step (see SSE$_{fit}$) by a minimum of 94.1% for each set of dynamic data. The best-fit estimates of α obtained are also provided in Fig. 1, and average to 0.055 ± 0.011, thus indicating its statistical significance and hence, independence of step magnitude or organic fraction studied. Since the introduction of the interfacial affinity constant, α,
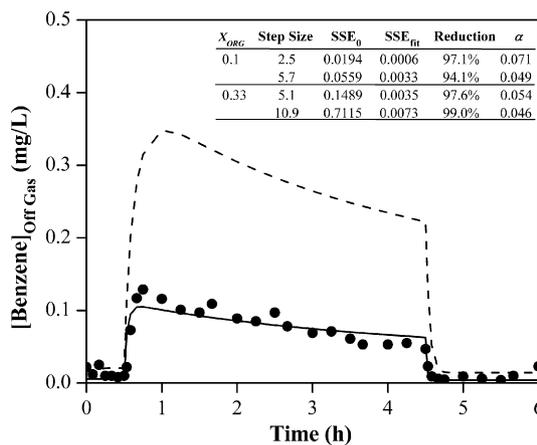

X_{ORG}	Step Size	SSE$_0$	SSE$_{fit}$	Reduction	α
0.1	2.5	0.0194	0.0006	97.1%	0.071
	5.7	0.0559	0.0033	94.1%	0.049
0.33	5.1	0.1489	0.0035	97.6%	0.054
	10.9	0.7115	0.0073	99.0%	0.046

Fig. 1. An example of model calibration by introduction and estimation of the 'interfacial affinity parameter', α, to improve predicted off-gas benzene concentrations during a 4 h, 11-fold increase in the influent gas benzene concentration with an organic phase volume fraction of 0.33. Experimental data (closed circles) are compared with both the original and calibrated model predictions (dashed and solid lines, respectively). Results of similar dynamic calibrations under various dynamic conditions are also listed in the inset table.
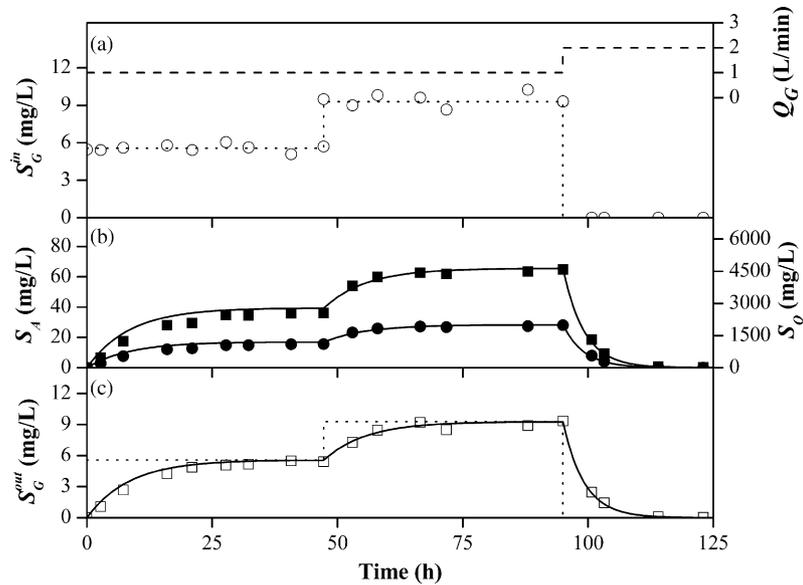
Fig. 2. Experimental (points) and model predicted (solid lines) benzene concentration profiles during an absorption experiment using a cell-free TPPB in: (a) the feed gas (open circles), along with the average feed gas concentration (dotted line) and gaseous volumetric flow rate (dashed line), (b) the aqueous (solid circles) and organic (solid squares) phases, and (c) the off-gas (open squares), as also compared with the average feed gas concentration (dotted line).

has altered the structure of the equations it is prudent to revisit the sensitivity analysis in order to investigate the influence of α on the predictions and to determine what effect this modification may have had on the roles of the other model parameters. Such was performed for the calibrated model under the same conditions used in Part I, and the results have been included in Appendix A.

3.2. Validation of mass transfer effects: abiotic TPPB simulation

In order to confirm the validity of the key assumptions, model structure, and parameter estimates used to simulate the biphasic absorption of benzene, an absorption experiment was conducted using a cell-free system. The experimental results are compared
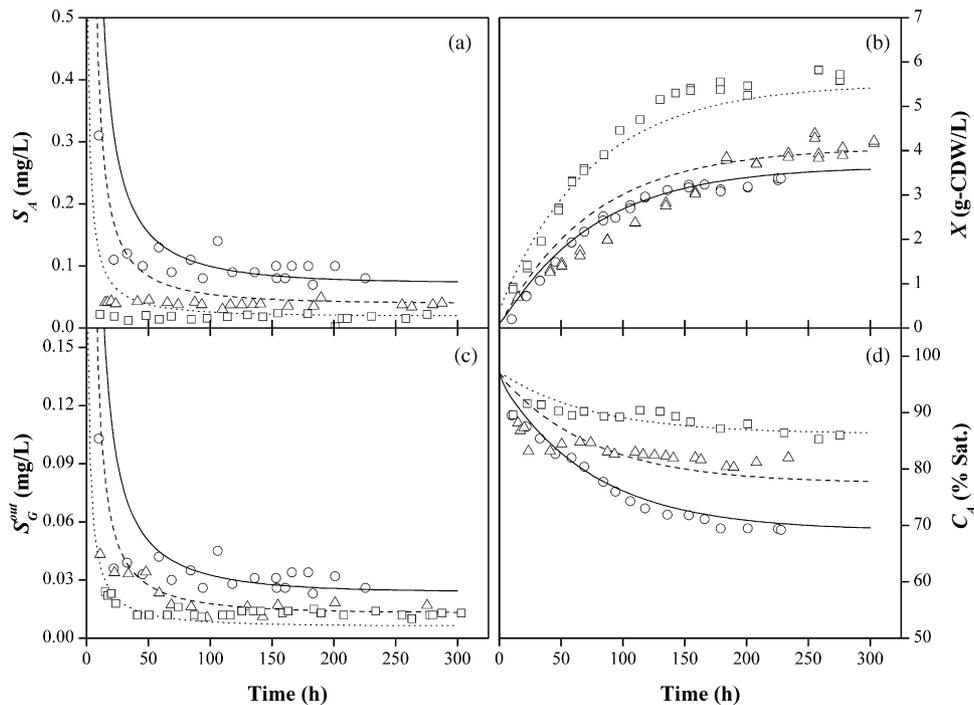

Fig. 3. Validation of TPPB start-up and steady-state operation under constant conditions in terms of (a) aqueous phase benzene concentration, (b) biomass concentration, (c) effluent gas benzene concentration, and (d) aqueous dissolved oxygen concentration using experimental data and predictions at organic phase volume fractions of: 0 (solid line, open circles), 0.10 (dashed line, open triangles), and 0.33 (dotted line, open squares). Each TPPB was operated using a feed gas stream containing 3.1 ± 0.3 mg/L benzene fed at 60 L/h, while maintained at 30 °C, pH 6.6, and agitated at 800 rpm.

with the model predictions in Fig. 2a–c. The model captures the experimental trends associated with the mass transfer processes under all conditions examined, even when subjected to perturbations of the influent benzene concentration and gas flow rate, and is able to predict the characteristic time constants of absorption and desorption at different aeration rates, as well as the steady-states.

3.3. Validation of constant operation: start-up through steady-state

Constant operation implies that the operating variables of the TPPB are maintained at their nominal values at all times. Since the proposed model incorporates an unstructured cell model to predict growth and biodegradation rates, simulation of the lag phase is not possible and can only begin after the lag, by which time a significant amount of benzene has accumulated in the liquid phases and the off-gas stream. This state is used as the initial condition for generating model solutions for constant operating conditions. The TPPB always responded quickly after this lag period, consuming dissolved substrate and rapidly reducing all benzene concentrations to their steady-state levels. The required response time depends upon the total mass of benzene dissolved as well as the inoculum size, both of which varied between experiments. As can be seen from Fig. 3a and c, the response of the model from the initial start-up period is more sluggish than observed experimentally for all organic fractions studied. This

discrepancy is most apparent in the aqueous phase benzene concentration, as effluent gas concentrations more closely follow predictions for these start-up trends. The predicted biomass and dissolved oxygen concentrations, shown in Fig. 3b and d, respectively, remain essentially insensitive to this preliminary transient period because only relatively small quantities of substrate are being consumed at this time.

All of the predicted variables approach steady-states that are numerically consistent with their respective experimental data (Fig. 3). For example, it is both predicted and observed that an increased presence of the organic phase drives the steady-state aqueous benzene concentration lower through partitioning of benzene into the second liquid phase. Likewise, apart from the case of $\phi_{ORG} = 0.10$ where the biomass appears to have accumulated in a linear manner, predicted characteristic time constants of biomass growth and accumulation in the TPPB are consistent with the remaining experimental observations. Similarly, the consistent prediction of the total cellular mass present at steady-state in each TPPB system agrees well with previous work [5]. The enhancing effect of the organic phase on oxygen absorption rates is confirmed in Fig. 3d. As was previously predicted [13], when ϕ_{ORG} is increased the correspondingly higher rates of oxygen transfer lead to greater total dissolved oxygen concentrations for identical total rates of benzene degradation and associated dissolved oxygen consumption. Steady-state values of S_G^{out} decreased as ϕ_{ORG} was increased due to the beneficial effect that the favorable organic phase gas–liquid affinity has on
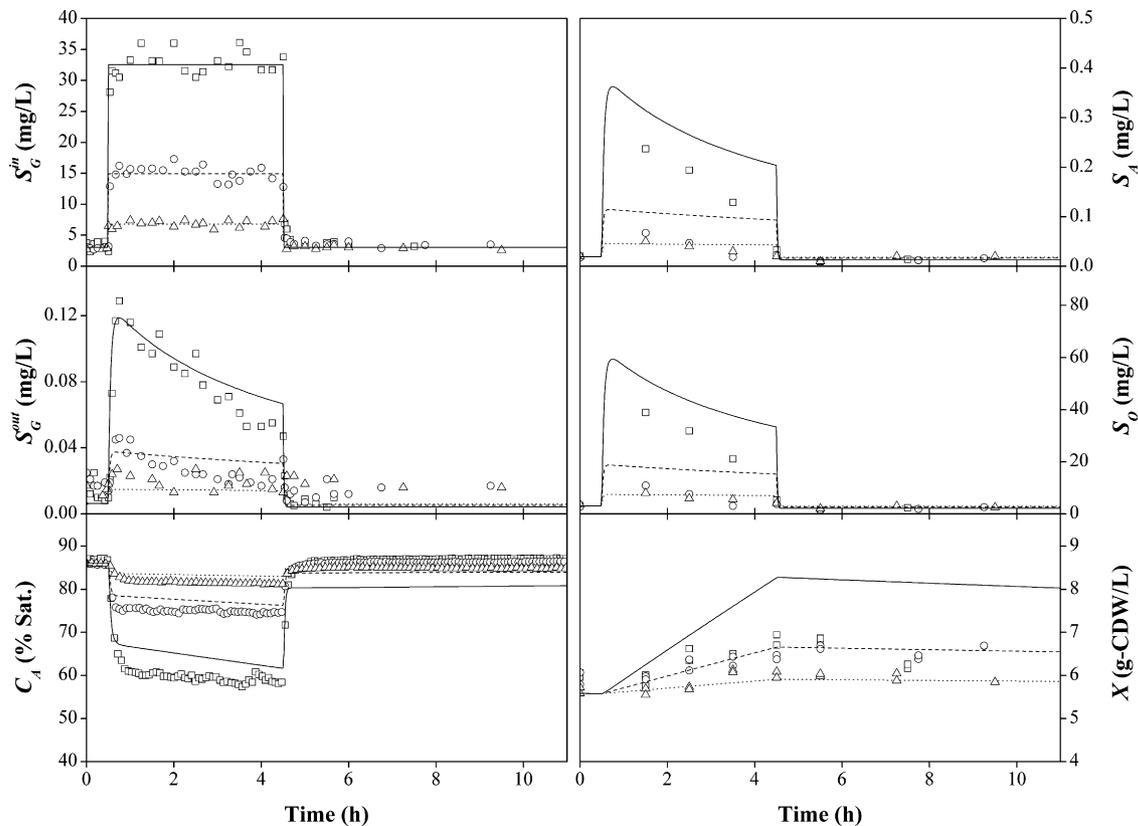

Fig. 4. Experimental and model predicted response of a steady-state TPPB with an organic volume fraction of 0.33 to 4 h step changes in the influent gas benzene concentration by magnitudes of 2.3-fold (open triangles, dotted line), 5-fold (open circles, dashed line), and 11-fold (open squares, solid line) its nominal value (3.1 mg/L).
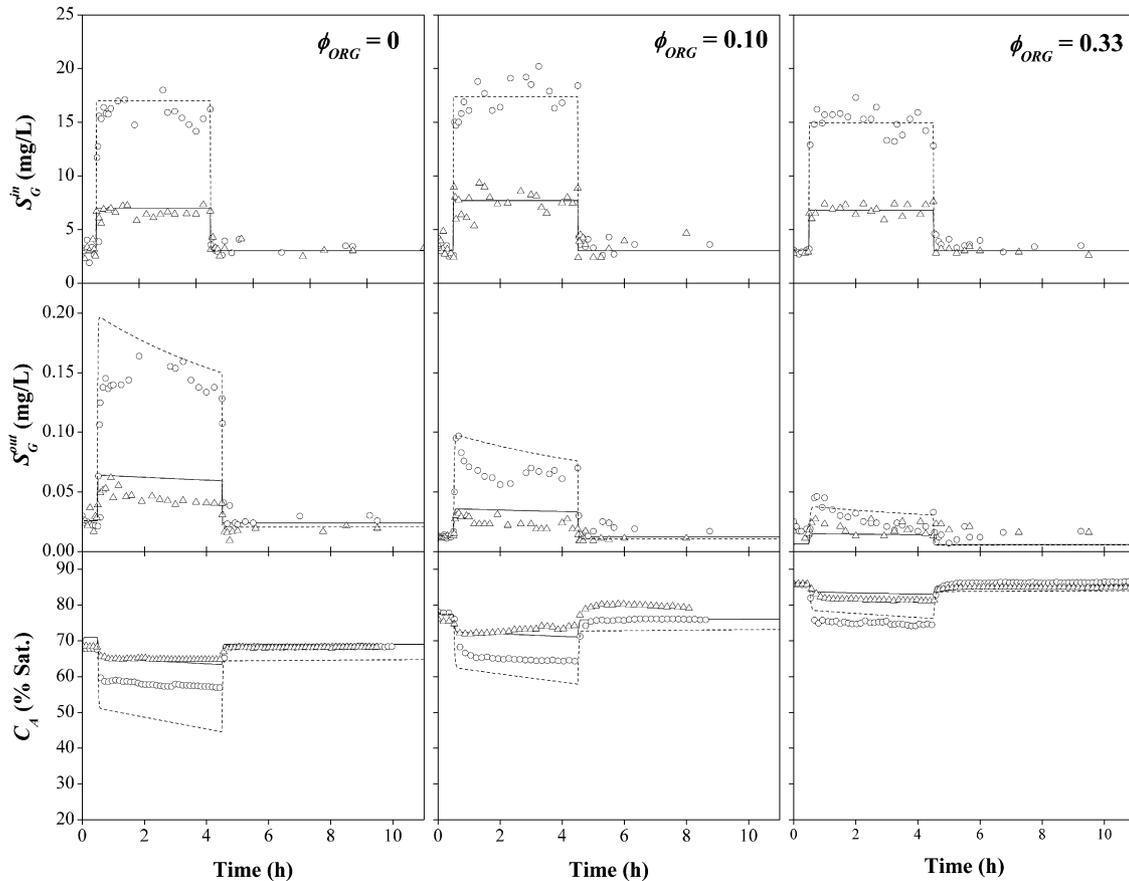
Fig. 5. Effect of the organic phase volume fraction on the predicted and experimental transient TPPB performance in response to 4 h step changes in the influent gas benzene concentration by magnitudes of approximately 2-fold (open triangles, solid line) and 5-fold (open circles, dashed line) its nominal value (3.1 mg/L).

the overall absorption rate, however, both the experimental data and model predictions demonstrate that the resultant effect on TPPB performance may be only subtle under constant conditions and particularly at steady-state. This is a consequence of following an operating policy that encourages high levels of biomass in the TPPB, thereby providing higher potential rates of biodegradation. Operating in this manner ensures that dissolved benzene concentrations in both liquid phases will be maintained at a minimum, allowing for high rates of benzene absorption at steady-state, regardless of the organic phase volume fraction selected.

3.4. Validation of transient operation: step change fluctuations

The response of the steady-state TPPB to 4 h step changes in the influent gas benzene concentration is shown in Fig. 4. The particular case of $\phi_{ORG} = 0.33$ was used to examine the ability of the model to properly capture the pertinent transient phenomena. Step change amplitudes of approximately 2-, 5-, and 11-times the nominal influent gas benzene concentration of 3.1 ± 0.3 mg/L are considered. Although some offset between the experimental data and certain predicted variables was observed under some sets of transients, there was notable consistency between the observed trends in the experimental data and the profiles of their respective predictions. In particular, the consistency of the responses suggests that the order of

the dynamic model equations is correct. On this basis, the model structure is deemed to be adequate for predicting TPPB behavior. However, improved parameter estimates could still possibly produce better agreement with the experimental data by reducing the observed offset.

Good agreement during transient operation is also attained when $\phi_{ORG} = 0$ and 0.10, as seen in Fig. 5. Although the predicted effluent gas benzene concentrations may not capture all of the intricate details which were displayed experimentally throughout the dynamic period, the predictions are able to capture the relative magnitude of the effluent concentration at all times. The data and predictions both consistently demonstrate that the presence of the organic phase helps to lower benzene release from the bioscrubber during step changes. Furthermore, the model provides good predictions of the characteristic response times of both the effluent gas benzene concentrations and dissolved oxygen concentrations, as the step increase is both introduced, and returned to the nominal condition. A substantial offset does exist, however, between the experimental and predicted dissolved oxygen concentration which becomes more pronounced both as the amplitude of the step input is increased, and ϕ_{ORG} is decreased. This discrepancy is likely a result of a low estimate of the oxygen mass transfer coefficient of the aqueous phase, $k_L a_{CA}$. Meanwhile, although oxygen transfer in the two-phase system is similarly a function of $k_L a_{CA}$, it also depends upon estimates of $k_L a_{CO}$, allowing the combined effect
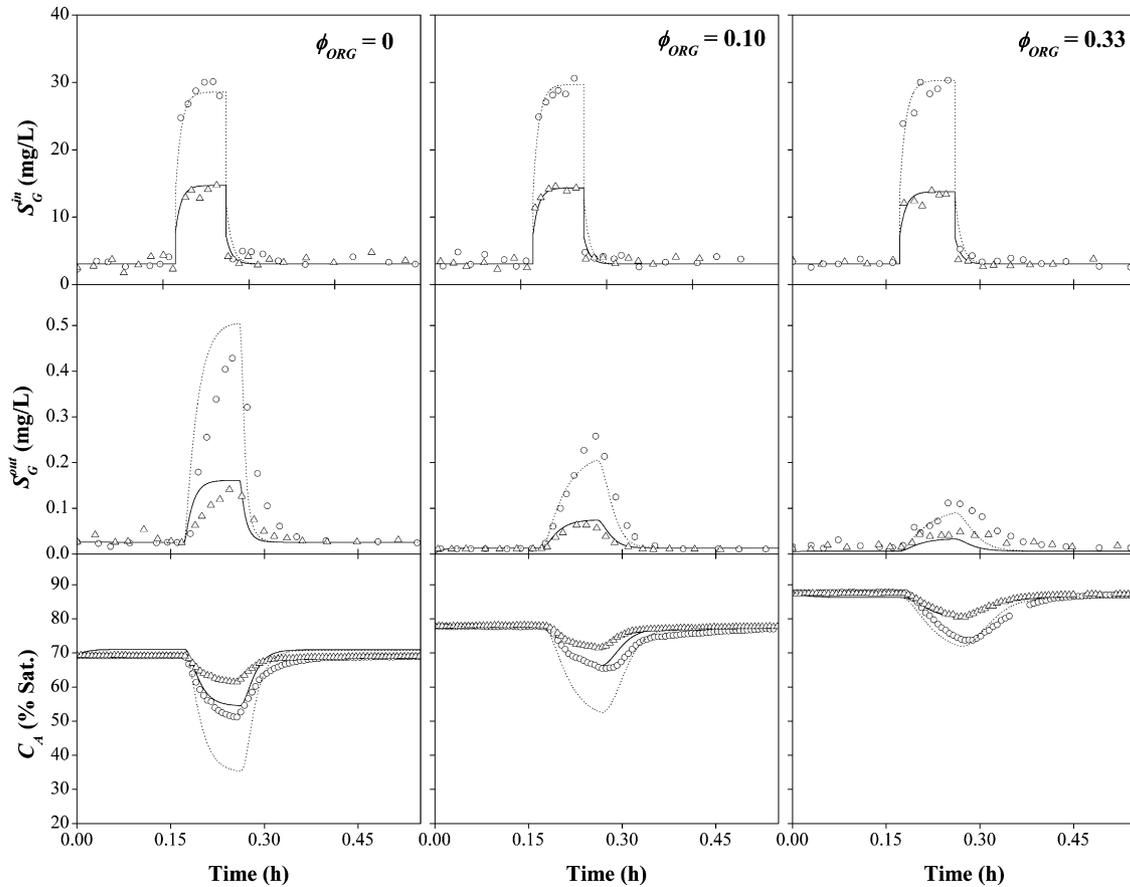
Fig. 6. Effect of the organic phase volume fraction on the predicted and experimental transient TPPB performance in response to 5 min spikes in the influent gas benzene concentration by magnitudes of approximately 5-fold (open triangles, solid line) and 10-fold (open circles, dotted line) its nominal value (3.1 mg/L).

to mitigate the impact of a potentially poor estimate of k_La_{CA} on the overall prediction.

3.5. Validation of transient operation: spike fluctuations

The spikes introduced were of particularly short duration (5 min) and produced by manually adjusting a rotameter. It was found that a simple step-up, step-down function of S_G^{in} in the model poorly represented the data because the time required to adjust the rotameter was significant relative to the transient period itself. In order to more accurately represent this mathematically, the following expressions (which result solely from fitting the data to an empirical model) were used for the dynamic input variable.

$$S_G^{in}(t) = S_{G,0}^{in} \quad \text{for } t < t_0 \tag{3a}$$

$$S_G^{in}(t) = S_{G,0}^{in}[A_s + (1 - A_s) e^{-k_v(t-t_0)}] \quad \text{for } t_0 < t < t_0 + t_s \tag{3b}$$

$$S_G^{in}(t) = S_{G,0}^{in}[1 + (A_s - 1) e^{-k_v(t-t_0-t_s)}] \quad \text{for } t > t_0 + t_s \tag{3c}$$

where $S_{G,0}^{in}$ is the nominal influent gas benzene concentration at steady-state, A_s the amplitude of the spike input, t_0 the time at which the spike is initiated, and t_s is the duration of the spike.

The inverse of time constant for valve actuation, k_v, was estimated as $100 \, h^{-1}$. These expressions of the input function have rounded the corners on the steps up and down that form the spike, and simply allow for better representation of the data, as seen in Fig. 6. Note that these transitions could have been included when modelling the longer (4 h) step changes, however, the time required to adjust the rotameter was insignificant relative to the duration of the step, thus allowing a simple step-up, step-down function to suffice. Although there are modest inaccuracies in the predicted rates of response in the effluent gas for the single-phase system, the predicted magnitudes of benzene concentrations in the effluent gas are consistent with the experimental data. As with the experimental data, the model is again able to predict that the presence of the organic phase helps to reduce benzene release from the bioscrubber. Similar to the predicted response of the TPPB to step changes in S_G^{in}, considerable offset exists in dissolved oxygen concentrations. As previously argued, these discrepancies are likely a result of inaccuracies in the estimates of k_La_{CA}. In general, the model predicts a faster return of S_G^{out} to its previous, steady-state concentration after the spike has ended, particularly in the single-phase system. This offset is possibly due to an experimental delay which is not modelled but occurs as the effluent gas travels between the liquid surface and the sampling port. With a headspace volume of approximately 3.6 L, the residence time of the effluent gas through this volume would be approximately 0.06 h.
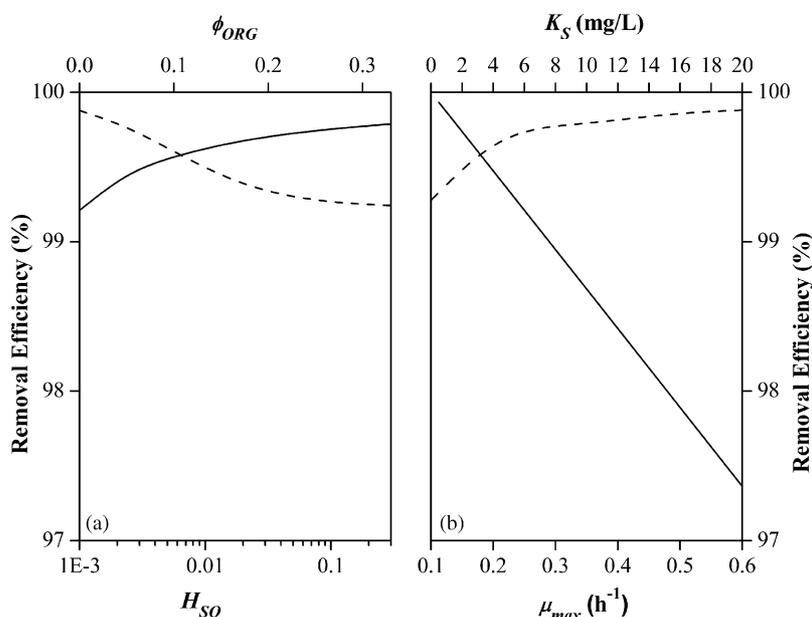
Fig. 7. Effect of the key design parameters on the TPPB steady-state removal efficiency as predicted by the independent variation (from the nominal values reported in Part I) of (a) ϕ_{ORG} (solid line), H_{SO} (dashed line), (b) K_S (solid line), and μ_{max} (dashed line) between their respective, practical parameter spaces. The influent gas benzene concentration is constant at 3.1 mg/L.

3.6. Performance predictions

Now validated, the proposed model is used to simulate the TPPB under a variety of practical operating scenarios and to further explore the influence of those potential design parameters which were identified via sensitivity analysis (in Part I) as having the greatest potential influence on performance. To reiterate, of the parameters to which S_G^{out} was most sensitive, it was argued that H_{SO}, μ_{max}, and K_S will be those whose manipulation will be most practical via selection of an alternate organic phase compound or biocatalyst, respectively. In addition, ϕ_{ORG} was identified as having an influence on transient performance, even though it may only slightly benefit removal rates under constant loading conditions. The effect of these parameters is studied by independently perturbing their values from the nominal values reported in Part I. The design parameters are perturbed over a range of values representative of those found throughout the literature in order to maintain physically realistic conditions.

From Fig. 7, under constant loading conditions, only variation of K_S has a significant effect on the steady-state removal efficiency. Although the steady-state predictions of S_G^{out} are sensitive to H_{SO} and μ_{max} in Part I, the effect was ultimately found to be insignificant over the parameter regions considered in Fig. 7. As previously predicted, the steady-state performance remained quite insensitive to ϕ_{ORG}, consistent with the results of Fig. 3.

The influence of the selected design parameters on transient TPPB performance was analyzed by predicting their individual effect on S_G^{out} predictions while S_G^{in} was subjected to an intermediate dynamic step change duration of 1 h and amplitude of five-times the nominal when compared to those studied in Figs. 4–6. These predictions are compared in Fig. 8. A decrease in biological activity (lower μ_{max} or higher K_S) greatly affects the rate of response of the cells and their influence on

removal rates not allowing TPPB performance to stabilize during shorter transitory periods. For example, when $\mu_{max} = 0.1$ h^{-1} or $K_S = 20$ mg/L, benzene is predicted to accumulate in the liquid phases and the effluent gas throughout the entire 1 h period. Furthermore, a less active culture will delay recovery to pre-transitory performance after step changes are completed. When $K_S = 20$ mg/L, this recovery took in excess of 1 h, as seen in Fig. 8. Increasing the total equilibrium solubility of the liquid phases (by increasing ϕ_{ORG} or decreasing H_{SO}) promotes higher absorption affinities, and greater VOC capture. As noted, an organic phase with high equilibrium VOC solubility will decrease the activity of the dissolved compounds, resulting in greater retention and lower losses in the effluent gas stream [14]. While this effect was obvious for the present benzene treatment system, it would become even more apparent for more highly volatile target compounds whose Henry's Law constants in water are even higher, further increasing the role of the organic phase.

While each of the identified design parameters are available for manipulation, ϕ_{ORG} is most easily changed by simply adding more or less solvent. Furthermore, its modification will not compromise the material or biological compatibility of the pre-designed TPPB system, making it the preferred design parameter to be first considered. Although the organic phase has only a modest impact on steady-state performance, Fig. 8 illustrates the positive result it can yield in reducing effluent VOC concentrations during transients. Furthermore (Fig. 9), the beneficial effect during transient operation becomes more pronounced as the magnitude of the fluctuation increases. At greater ϕ_{ORG}, the TPPB is capable of maintaining lower levels of S_G^{out}. If the maximum magnitude of a fluctuation can be anticipated, then the TPPB can be designed to include the lowest required level of ϕ_{ORG} while still maintaining a target maximum S_G^{out}. Such an optimization would then ensure desired performance even
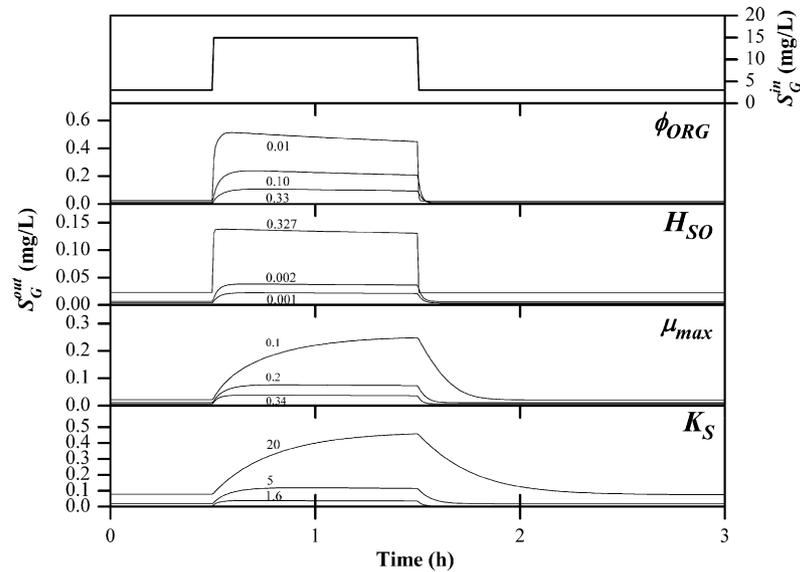
Fig. 8. Effect of the key design parameters on the dynamic response of the TPPB to a 1 h step change in the influent gas benzene concentration of five-times its nominal value (3.1 mg/L). Design parameters are independently varied (from the nominal values reported in Part I) within their respective, practical parameter regions.
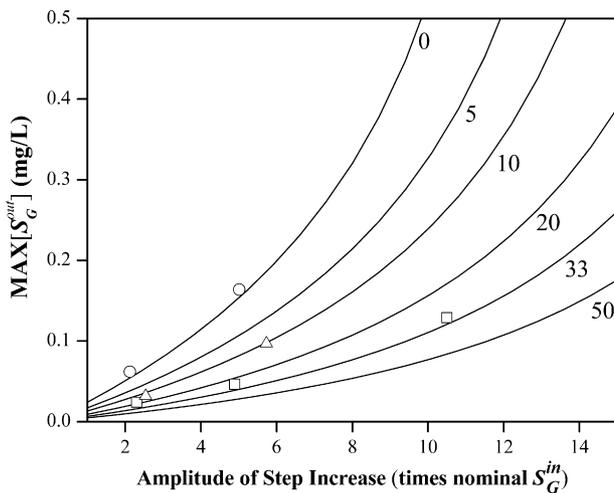

Fig. 9. Effect of the organic phase volume fraction on the dynamic performance of the TPPB in response to 4 h step change fluctuations in the influent gas benzene concentration of different amplitudes (from its nominal value 3.1 mg/L) as measured by the maximum effluent gas benzene concentration emitted during the transient period. Experimental data obtained with TPPBs containing 0 (open circles), 10 (open triangles), and 33% (vol.) (open squares) organic phase are provided for comparison.

under this worst case scenario, while minimizing material costs and wastes.

4. Discussion

The overall effect that direct substrate uptake from the organic phase will elicit on the total biodegradation rate will depend on the degree of cellular interaction at the organic interface. Interfacial interaction will be a function of the population density and the specific properties of the cells [12], as well as the aqueous–organic interfacial surface area, $a_{A/O}$ [10], all of which may be time-varying. The following expression can be derived

to relate $a_{A/O}$ to the organic phase volume fraction, ϕ_{ORG}:

$$a_{A/O} = \frac{6\phi_{ORG}}{d_{SM}} V_T \qquad (4)$$

where V_T is the total liquid volume and d_{SM} represents the Sauter mean diameter of the dispersed organic droplets which can be determined experimentally by drop size distributions [10], or estimated using an empirical relationship, such as that of Chatzi et al. [15]:

$$d_{SM} = c_1(1 + c_2\phi_{ORG})W_e^{-c_3} D_i \qquad (5)$$

where $c1$, $c2$ and $c3$ represent empirical parameters that are characteristic of the fluids and the Weber number, $W_e = \rho_d N^2 D_i^3 / \sigma$. The impeller speed and diameter are represented by N and D_i, respectively, ρ_d is the dispersion density and σ is the interfacial tension. Although the combination of Eqs. (4) and (5) suggests a non-linear dependence of $a_{A/O}$ on ϕ_{ORG}, note that as per the structure of the equations, although $a_{A/O}$ is a strong function of ϕ_{ORG} at low solvent fractions, its value will eventually reach a maximal value where it will be relatively insensitive to changes in ϕ_{ORG}. Although it is clear that both non-linear and linear regions will exist in the dependency of $a_{A/O}$ on ϕ_{ORG}, without specific data regarding droplet formation patterns in the particular application it is difficult to predict where this transition will occur. These data can be difficult to obtain as accurate estimation of $a_{A/O}$, is of noted difficulty to perform [16]. As such, it cannot be expected that our proposed approach may be sufficient for accurately capturing the interfacial effect over all ranges of ϕ_{ORG}, however it will likely provide sufficient results when using relatively large organic fractions, as performed throughout this study. Furthermore, our approach may also be insufficient for predicting the influence of different operating conditions on specific growth and biodegradation rates without re-estimating α.

Other researchers have attempted to capture the positive potential effect that enhanced bioavailability in biphasic media can have on specific growth and biodegradation rates by use of μ_{max} and K_S estimates which vary as a function of ϕ_{ORG} [17]. For the sake of comparison, we have re-estimated μ_{max} and K_S using non-linear least-squares regression by the Gauss–Newton method with the same transient step change data presented in Fig. 1. It was found that the best-fit estimates of μ_{max} and K_S were slightly increased and decreased, respectively, from their original estimates obtained from batch studies. The magnitude of each variation from their original estimates was observed to be a function of ϕ_{ORG}. For example, although the original set of parameter estimates provided for sufficient prediction of the single-phase bioscrubber, by reducing K_S from 1.6 mg/L to an optimal value of just 0.5 mg/L a TPPB containing $\phi_{ORG} = 0.33$ could also have been as well simulated as was demonstrated in Fig. 1. Likewise, an optimal K_S value of 1.0 mg/L would provide more accurate model predictions when $\phi_{ORG} = 0.10$. These data suggest that a non-linear dependence of K_S on ϕ_{ORG} would have also been likely. In this way, the model predictions could have alternatively been improved without the introduction of α and the use of an effective substrate concentration. However, while our approach brings with it the introduction and estimation of another empirical parameter, in our opinion it is a preferred alternative to manipulation of the kinetic parameters because it preserves the integrity of the traditional notion of the biological constants. Currently, the ability of *A. xylosoxidans* Y234 to uptake benzene directly from the surfaces of *n*-hexadecane droplets is conjecture, as there is insufficient experimental evidence to fully support this claim. Therefore, additional work should be directed towards obtaining further insight into this phenomenon. Such will allow the further development of an interfacial uptake model (by use of an interfacial affinity constant, or even better predicting K_S as a function of ϕ_{ORG}) and allow for accurate prediction of specific growth rates in biphasic cultures. Furthermore, alternative approaches beyond simple batch techniques which are also suitable for use with volatile substrates should be pursued to re-estimate the kinetic parameters with greater accuracy and precision and improve their significance.

Although it was predicted from Fig. 9 that the transient performance of the TPPB will be improved only through the use of higher organic phase volume fractions, there are other issues to consider before selecting ϕ_{ORG} when designing a TPPB system. For instance, although increasing ϕ_{ORG} will directly enhance the equilibrium VOC solubility, and thereby the buffering capacity of the TPPB, using an excessively large ϕ_{ORG} could result in an over-engineered process with excessive material costs. As noted, anticipation of the maximum magnitude of fluctuation to be experienced can help to select an ideal organic phase volume fraction. Other practical features must be considered when selecting an appropriate ϕ_{ORG}, including the effect it may have on the hydrodynamic conditions in the bioscrubber, as well as the potential effects it may have on foaming and cell entrainment.

As previously hypothesized [18], rates of oxygen absorption in biphasic media can be enhanced using solvents with a high equilibrium solubility of dissolved oxygen. Furthermore, Nielsen et al. [13] have analytically shown for the present system that this enhancing effect depends on the volume fraction employed. This important prediction has now been demonstrated during active aerobic biodegradation. Since nearly equivalent rates of benzene biodegradation were achieved in each constant loading experiment, and because equal total masses of cells were obtained at each steady-state, the total rates of steady-state dissolved oxygen consumption are roughly equivalent between experiments and independent of ϕ_{ORG}. The enhanced rates of oxygen absorption in the two-phase system led to steady-state dissolved oxygen concentrations which were elevated as a function of ϕ_{ORG} (Fig. 3). Under transient conditions (Figs. 5 and 6), this same phenomenon allowed the TPPB to treat fluctuations of greater magnitude with less concern about approaching dissolved oxygen limiting conditions.

One specific area for improving the model predictions is the better representation of the biosynthetic response of the steady-state culture to the additional substrate that is rapidly made available during step change fluctuations (Fig. 4). It is possible that the steady-state culture, after experiencing prolonged exposure to nearly starved conditions, could become physiologically distinct from its actively growing counterpart. When the culture in such a 'maintenance state' is then perturbed by the re-introduction of excess amounts of substrate it is possible that a period of 're-acclimation' may be required before cellular metabolism can be redirected towards biosynthesis. These conditions could have induced a lag phase in the cells, leading to a time-delay in observed growth and lower associated biomass yields than those predicted. As with start-up operation, the unstructured kinetic model will not allow for adequate prediction during such a state. This possible cellular response may be accentuated by the magnitude of the change in substrate concentrations, which could account for the poor predictions during the 4 h, 10-fold increase to S_G^{in}, while satisfactory agreement was obtained for 2- and 5-fold changes. Finally, the inconsistent biodegradation rates that were observed may have simply been a result of subtle physiological differences in the culture used in the various experiments, for example, cell viability. The difficulty of predicting any of these plausible mechanisms of behavior stems from the fact that they cannot be represented without the use of a structured cell models or segregation of the cell population.

This general mechanistic model has been validated for the particular system of interest: degrading high concentration, low flow rate benzene waste gases by *A. xylosoxidans* Y234 using *n*-hexadecane as the organic phase. Further experimentation and simulation of an alternate TPPB system will provide useful structural validation of the proposed equations. However, since this model has been derived from fundamental material balances incorporating well-understood phenomena, it is anticipated that it will readily translate for use with other TPPB systems of interest.

Finally, extrapolation of the predictions made here much beyond the operating regions which have already been examined experimentally should be done with caution because certain phenomena may have been oversimplified in developing this model. This may become particularly evident with respect to the behavior of the biocatalyst.

5. Conclusion

The predictions of the fundamental model presented in Part I of this work provide adequate representation of the experimental data during constant and transient operation. Calibration of the model was required in order to generate adequate predictions, and this was accomplished by introducing an "interfacial affinity constant", α, which is largely an empirical parameter, and the use of an effective, biphasic dissolved substrate concentration to predict cell specific growth rates. The proposed modification suggests that increased bioavailability is the most plausible explanation for the macroscopic observation of enhanced biodegradation rates in this biphasic system.

The modest performance improvements that were predicted and observed during constant operation may seem to provide inadequate justification for employing an organic phase in a bioscrubber process. However, by helping to stabilize and maintain minimal effluent gas benzene concentrations when subjected to transient feed fluctuations, it is clear that the dynamic performance of a bioscrubber can be significantly improved as a direct result of the inclusion of a second, immiscible organic phase. The previously predicted ability of the organic phase to enhance oxygen absorption rates has also now been demonstrated experimentally.

Although the potential for improved performance is proportional to the organic phase volume fraction selected, ϕ_{ORG} should be carefully chosen, and even optimized, to ensure that the TPPB is not over-engineered for a desired practical application. The Henry's Law coefficient of the organic phase was also found to be a relatively important parameter, particularly for helping to maintaining low effluent gas concentrations during transient operation. For this reason, it is recommended that it be treated as a critical design parameter for consideration in the preliminary design of any TPPB process.

The particular importance of the biological parameters μ_{max} and K_S suggest that selection of an effective biocatalyst is key for maintaining high VOC removal rates under stable conditions and, particularly, during transient operation. Microorganisms capable of maintaining high rates of biological activity under even the most dilute conditions will make ideal candidates for use in TPPBs.

Appendix A

As can be seen from Fig. A1, the model is most sensitive to α during start-up when the TPPB is not mass transfer limited and significant dissolved substrate concentrations exist. Both S_A and S_G^{out} were found to be sensitive to α at all times following start-up, including at the steady-state. The reason for this result is as was analogously reported for K_S because α also greatly influences the biodegradation rate in the same manner, particularly under substrate limiting conditions. This will in turn affect the rate of substrate accumulation in the liquid phases of the TPPB, and consequently the removal efficiency of VOC from the waste gas itself. In general, calibrating the model by structural modification did not alter any of the qualitative results of the original sensitivity analysis. However, doing so did result in a heightened sensitivity of certain predicted variables to particular parameters. Most notably, a greater sensitivity of S_A (and S_O) and S_G^{out} to VOC mass transfer parameters ($k_L a_{SA}$, $k_L a_{SO}$, H_{SA}, H_{SO}) was observed. These sensitivities were found to be between 2- and 4-fold larger than the results from the uncalibrated model. Since the simulated biodegradation rates are enhanced as a result of interfacial uptake of substrate, the modelled culture becomes further limited by mass transfer, making those parameters all the more important to the predicted performance. An increased sensitivity to ϕ_{ORG} was also observed, which is intuitive as this parameter controls the amount of material from which interfacial uptake may occur. Meanwhile, incorporation of α had no effect on the characteristic sensitivities of either X or C_A (or C_O).
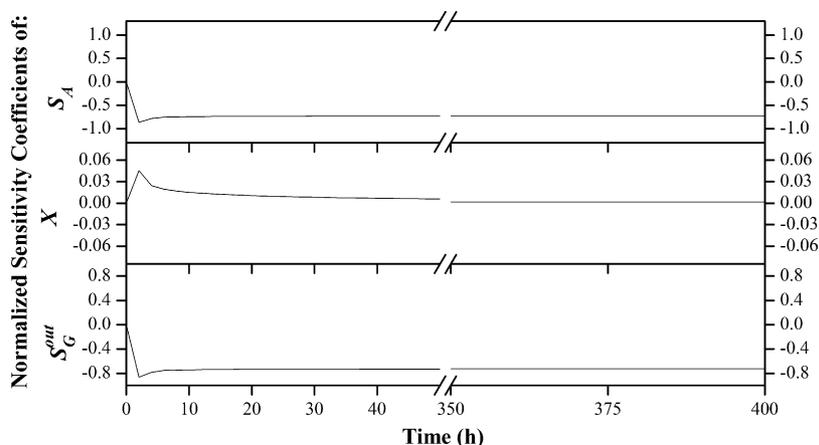

Fig. A1. Normalized sensitivity coefficient profiles predicted for the proposed model with respect to α, corresponding to the simulated operation of the TPPB from start-up through steady-state, as performed in Part I.

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