Analysis of non-linear process of biodegradation and kinetics for the removal of toluene, biomass growth and carbon dioxide production

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Abstract

We present for the first time a mathematical model for the removal of toluene, biomass growth, and carbon dioxide production by Rhodococcus erythropolis. A model system of three coupled nonlinear equations under steady-state conditions were reported for the first time. The analytical model was successfully applied to solve biochemical reactions which occurred in the bioreactor. Within the catalyst, those biochemical reactions were defined by the substrate, biomass, and product concentrations. Simple analytical expressions for the concentration of substrate, biomass, and product have been derived for all values of reaction parameters using the new homotopy perturbation method. Numerical simulation of concentration profile for non-steady state condition is carried out and compared with our analytical results. A good agreement is noted. A novel graphical procedure for estimating the yield of biomass, saturation constant, inhibition constant, maximum specific growth rate, growth, and non-growth-associated constant is also suggested. Differential sensitivity analysis of controllable parameters such as yield coefficient, saturation, and inhibition constant are reported. This modeling simulation study provides a better understanding of biomass growth on toluene mineralization. Also, it has been predicted that the yield coefficient has more impact on the production of carbon dioxide, biomass growth and toluene degradation.

Keywords
Toluene biodegradation; Rhodococcus erythropolis; Mathematical modeling; Non-linear rate equations; New Homotopy perturbation method.

Highlights
• A theoretical model of reaction in aerobic biodegradation of toluene into carbon dioxide and biomass
• Nonlinear system of reaction equations are solved to describe the concentrations.
• The concentrations of substrate and products have been obtained using the NHPM.
• The analytical results were compared with numerical values and were in good agreement.

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**Introduction**

Volatile organic compounds have been reported to be hazardous for the environment and humans [1]. There are several methods reported in the literature for the waste air treatment development, however more recently, biological waste air treatment processes have attracted a lot of interest due to its cost-effectiveness and environment technologies [1]. Instauration or eradication coupled with physical, chemical, and biological treatments, are the main methods to remove VOCs [2]. The rate of polluted gases in the atmosphere comprises of some dissolved organic compounds which affect the environment. One such compound which is highly toxic to human beings and additionally present in the gaseous state is toluene [3]. Toluene has attracted great interest since it has been widely used as the sole carbon and energy source. Over the last years, the aerobic biodegradation of toluene into carbon dioxide, water and biomass employing bacteria have been widely utilized [4]. Biodegradation techniques have attracted a lot of interest due to the effective and economical intervention compared to more traditional techniques. Quantification techniques have been extensively utilized to the elucidation of toluene, which contributes to an assortment of various techniques employed at different levels of investigation [4]. Modeling of growth kinetics for Pseudomonas spp. During benzene degradation on Haldane-Andrews kinetics model is discussed [5]. Recently the analytical expressions for VOC concentrations and oxygen by solving the system of non-linear equations related to Monod, Haldane-Andrews kinetics, etc., have been obtained using the Adomian decomposition method [6].

A mathematical model on hydrogen production in batch cultures of the photosynthetic bacterium Rhodococcus capsulatus was represented by a classical Monod or Michaelis-Menten kinetics [7]. Recently a mathematical model for kinetic characterization of VOC-degrading microorganism was described. This model was formulated as a set of non-linear differential equations describing the growth of biomass, toluene degradation and carbon dioxide production. This model was solved using Runge-Kutta method. It is observed that, even though the results are compared well with experimental data, they could only be obtained at discrete points using Runge-Kutta method. This creates a shortcoming since toluene degradation is a dynamical model [8].

To the best of our knowledge, no rigorous analytical expression for the growth of biomass, utilization of substrate, and production of CO2 has been previously reported. This manuscript aims to derive the approximate analytical expression for the concentrations of substrate, biomass, and CO2 in liquid and gas phase. Moreover, these expressions help us to analyze the behavior of the model parameters to degrade the substrate (toluene). In general, analytical results are more stimulating and beneficial than the results of numerical simulation as they are amenable to various kinds of data manipulation, optimization of parameters, and data analysis.

**Mathematical modeling**

Several microbial growths and biodegradation kinetic models have been developed, proposed and used in bioremediation schemes. Some of these models include Monod’s, Haldane-Andrews, Bungay’s weighted model and general substrate inhibition models. It was found
that the Haldane-Andrews model is the best fit for toluene biodegradation. The specific growth rate of the substrate (toluene) in Haldane-Andrews model is given as follows [8]:

\[
\mu(S) = \frac{\mu_{\text{max}} S}{K_S + S + \frac{S^2}{K_I}}
\]  

(1)

where \(\mu_{\text{max}}\) is the maximum specific growth rate, \(S\) is a substrate concentration, \(K_S\) and \(K_I\) are the toluene half-concentration and toluene inhibition constants respectively. The relation between concentration of \(CO_2\) in liquid phase (\(CO_{2L}(t)\)) and biomass \(X(t)\) is given by:

\[
\frac{dCO_{2L}(t)}{dt} = \alpha \frac{dX(t)}{dt} + \beta X(t)
\]

(2)

where \(\alpha\) and \(\beta\) are constants which are related to the biomass growth and the biomass concentration respectively. The concentration of \(CO_2\) in gas phase (\(CO_{2G}(t)\)) and liquid phase (\(CO_{2L}(t)\)) have the following relation.

\[
\frac{dCO_{2G}(t)}{dt} = H_{CO_2} \frac{dCO_{2L}(t)}{dt}
\]

(3)

where dimensionless Henry’s law constant is given by \(H_{CO_2} = 1.17\). The substrate (toluene) utilization rate and biomass growth rate can be written as a following system of non-linear differential equations.

\[
\frac{dS(t)}{dt} = -\frac{\mu(S)}{Y_{X/S}} X(t)
\]

(4)

\[
\frac{dX(t)}{dt} = \mu(S) X(t)
\]

(5)

The model is established by solving the system of mass transport equations to determine the substrate and product concentration. For the development of this model the following assumptions were made [8]. (i) The liquid phase is assumed perfectly mixed and thus no mass transfer limitation occurred for the kinetics. (ii) The Henry’s law constant remains same throughout the experimental system. The initial conditions for Eqs. (2) – (5) are described as follows:

\(CO_{2L}(t = 0) = CO_{2L_0}, CO_{2G}(t = 0) = CO_{2G_0}, S(t = 0) = S_0, X(t = 0) = X_0\)

(6)

The names and symbols for the SI base and supplementary units is also given in nomenclature.

### Dimensionless form of the problem

To compare the analytical results with simulation results, we make the above non-linear differential equations (2-5) in dimensionless form by defining the following dimensionless parameters:

\[
\begin{align*}
    s &= \frac{S}{X_0}, & x &= \frac{X}{X_0}, & \gamma &= \frac{K_S}{X_0}, & \delta &= \frac{X_0}{K_I}, & c_i &= \frac{CO_{2L}}{X_0}, & c_s(t) &= \frac{CO_{2G}}{X_0}, & \tau &= \mu_{\text{max}} t
\end{align*}
\]

(7)

Now the Eqs. (4) and (5) becomes in dimensionless form as follows:
\[
\frac{dx(\tau)}{d\tau} = \frac{s(\tau)x(\tau)}{\gamma + s(\tau) + \delta s(\tau)^2}
\]  
\(8\)

\[
\frac{ds(\tau)}{d\tau} = -\frac{s(\tau)x(\tau)}{Y_{x/s} [\gamma + s(\tau) + \delta s(\tau)^2]}
\]  
\(9\)

The Eqs. (2) and (3) are expressed as follows:

\[
\frac{dc_l(\tau)}{d\tau} = \alpha \frac{dx(\tau)}{d\tau} + \beta x(\tau)
\]  
\(10\)

\[
\frac{dc_g(\tau)}{d\tau} = \frac{dc_l(\tau)}{d\tau} - H_{CO_2}
\]  
\(11\)

To solve the Eqs. (8)-(10), the following dimensionless initial conditions are defined.

\[x(\tau = 0) = 1, \quad s(\tau = 0) = \frac{S_0}{X_0} = s_0, \quad c_{l}(\tau = 0) = \frac{CO_{2L}}{X_0} = c_{u}, \quad c_{g}(\tau = 0) = \frac{CO_{2G}}{X_0} = c_{s_0}\]  
\(12\)

As described by the Luedeking-Piret [9] approach, the product formation is composed by two parts: a non-growth-associated and a growth. The change of product concentration \(dc_g(\tau)/d\tau\) is calculated by means of the growth–associated product constant \(\alpha\), the change of biomass concentration \(dx(\tau)/d\tau\), the non-growth-associated product constant \(\beta\) and the biomass concentration \(x(\tau)\). Dividing the Eq.(9), by Eq.(10) and using the initial condition (Eq.(12)), we can obtain the following relation between the concentration of substrate and biomass:

\[s(\tau) = \frac{1}{Y_{x/s}} \left[1 + Y_{x/s} S_0 - x(\tau)\right]\]  
\(13\)

**Analytical expression of concentrations using a new approach to the homotopy perturbation method**

Non linear differential equations play a crucial role in many branches of physical sciences. Solving systems of non-linear differential equations have gained importance and popularity in recent years, mainly due to the necessity of analytical solutions in diverse fields of science and engineering. Many authors have paid attention to study the solutions of non-linear differential equations by using various advanced analytical methods like Homotopy perturbation method [10], Homotopy analysis method [11], variational iteration method [12], Laplace Adomian decomposition method [13] and a new approach to Homotopy perturbation method [14] among others. Among these, a new approach to the Homotopy perturbation method is employed to solve the non-linear ordinary differential equations (2) – (5). The advantage of this method is that it has been results in a simple approximate solution in the zeroth iteration [14]. Using this method the concentration of biomass \(x(\tau)\) is obtained as follows (Appendix A):

\[x(\tau) = \frac{x_{ss}}{1 + Y_{x/s} S_0 e^{-\sigma x_{ss} \tau}}\]  
\(14\)

where the steady state substrate concentration \(x_{ss}\) and the parameter \(\sigma\) are given by the following equation:

\[x_{ss} = 1 + Y_{x/s} S_0\]  
\(15\)
\[
\sigma = \frac{1}{Y_{X/S}[\gamma - Y_{X/S}s_0 + \delta(Y_{X/S}s_0)^2]} 
\]

Substituting Eq. (14) and (15) in the Eq. (13), the concentration of substrate \( s(\tau) \) can be obtained as follows:

\[
s(\tau) = \frac{x_{ss}}{Y_{X/S}} \left[ 1 - \frac{1}{1 + Y_{X/S}s_0 e^{-\sigma x_{ss} \tau}} \right] 
\]

The concentration of carbon dioxide in the liquid phase \( c_i(\tau) \) can be obtained using Eqs. (8) and (10) as given below:

\[
c_i(\tau) = c_{i0} + \frac{(x_{ss})^2}{6} \left\{ \frac{1}{(Y_{X/S}e^{-\alpha x_{ss} \tau} + 1)^2} \left( 3s_0 Y_{X/S} \frac{\beta e^{-\alpha x_{ss} \tau}}{\mu_{max}} + \frac{2x_{ss} Y_{X/S}s_0 \alpha}{Y_{X/S}(\gamma + s_0 + \delta s_0^2) - \mu_{max}} e^{-\alpha x_{ss} \tau} + \frac{3\beta}{\mu_{max}} \right) \right\} 
\]

By using the above equation, the analytical expression for the carbon dioxide in gas phase \( c_i(\tau) \) can be obtained as follows:

\[
c_i(\tau) = H_{CO_2} (c_i(\tau) - c_{i0}) + c_{i0} 
\]

When \( \alpha = 0 \), the product (carbon dioxide) formation is completely non-growth-associated. The product formation is completely growth-associated when \( \beta = 0 \). In these cases the concentration of carbon dioxide in liquid phase becomes as follows:

\[
c_i(\tau) = c_{i0} + \frac{(x_{ss})^2}{6} \left\{ \frac{1}{(Y_{X/S}e^{-\alpha x_{ss} \tau} + 1)^2} \left( 3s_0 Y_{X/S} \frac{\beta e^{-\alpha x_{ss} \tau}}{\mu_{max}} + \frac{2x_{ss} Y_{X/S}s_0 \alpha}{Y_{X/S}(\gamma + s_0 + \delta s_0^2) - \mu_{max}} e^{-\alpha x_{ss} \tau} + \frac{3\beta}{\mu_{max}} \right) \right\} - \frac{1}{(Y_{X/S} + 1)^2} \left( 3s_0 Y_{X/S} \frac{\beta e^{-\alpha x_{ss} \tau}}{\mu_{max}} + \frac{3\beta}{\mu_{max}} \right) 
\]

\[
c_i(\tau) = c_{i0} + \frac{(x_{ss})^2}{6} \left\{ \frac{1}{(Y_{X/S}e^{-\alpha x_{ss} \tau} + 1)^2} \left( 2x_{ss} Y_{X/S}s_0 \frac{\alpha}{Y_{X/S}(\gamma + s_0 + \delta s_0^2)} e^{-\alpha x_{ss} \tau} \right) + \frac{1}{(Y_{X/S} + 1)^2} \left( \frac{2x_{ss} Y_{X/S}s_0 \alpha}{Y_{X/S}(\gamma + s_0 + \delta s_0^2)} \right) \right\} 
\]

**Discussion**

Eqs. (14) and (17) represent the new simple analytical expression for the concentration of biomass and substrate respectively in terms of the kinetic parameters \( Y_{X/S} \), \( \gamma \) and \( \delta \). Recently substrate utilization, biomass formation and \( CO_2 \) production are reported based on the experimental and simulation results [8].

Fig 1. represents the concentration of growth of biomass, utilization of substrate and product formation versus time for some fixed values of other kinetic parameters. From Figure 1, it is observed that the substrate concentration is a decreasing function whereas biomass and carbon dioxide are an increasing function. The concentration-time curves shown in Fig. 1 can be divided into two phases acceleration/deceleration and stationary phase. The acceleration phase in biomass concentration and deceleration phase in substrate concentration starts at the
time $\tau = 0$. When dimensionless time $0 < \tau < 40$, the concentration of biomass and substrate are in the exponential phase. Also when $\tau \approx 40$, both curves attain the stationary phase.

![Graph](image)

**Fig. 1:** Plot of dimensionless concentration of biomass $x(\tau)$, substrate (toluene) $s(\tau)$ carbon dioxide in liquid phase $c_l(\tau)$, carbon dioxide in gas phase $c_g(\tau)$, versus dimensionless time $\tau$ using Eq. (14) and (17-19) for various values of parameters

Comparison of our analytical results with numerical results for the concentration of biomass with various values of kinetic parameters $\gamma$ and $\delta$ is given in Fig. 2(a-b) and Table 1. The maximum average relative error between our analytical results and numerical results is 0.003%. It is well known that higher the $K_S$ value worse the utilization ability of the substrate. Hence the concentration of biomass decreases when saturation constant $\gamma$ or $K_S$ increase (Fig 2(a)). Also, it is observed that, the concentration of biomass rises rapidly and reaches a steady state value when $\tau = 80$. From Fig 2(b), it is noted that the concentration of the biomass does not vary significantly for the experimental values of parameter $\delta$ (inhibition constant). From Fig. 2(c), it is noted that the yield coefficient is increasing with the increase in biomass concentrations, due to the consumption of the substrate.
Fig. 2: Plot of dimensionless concentration of biomass $x(\tau)$ versus dimensionless time $\tau$ using Eq. (14) for various values of parameters $\gamma, \delta, Y_{X/S}$. The solid line represents Eq[14] and dotted line represent numerical result.

The change in substrate concentration with respect to time $\tau$ for various values of parameters is compared with numerical results in Fig. 3(a) – (b) and Table 2. Fig. 3(a) illustrates that as $\gamma$ increases, the substrate concentration decline quickly and reaches the steady state value and growth stops. This can be attributed to the fact that the (toluene) substrate is consumed by the *Rhodococcus erythropolis*. Fig 3(b) shows that $\delta$ is relatively independent on the shape of the concentration-time profile. From Fig. 3(c), it is inferred that the concentration of substrate increases when the biomass-to-toluene yield increases.
Fig. 3: Plot of dimensionless concentration of substrate (toluene) $s(\tau)$ versus dimensionless time $\tau$ using Eq.(17) for various values of parameters $\gamma$, $\delta$, $Y_{X/S}$. The solid line represents Eq(17) and dotted line represent numerical result.

Eqs. (18) and (19) represent the concentration of CO$_2$ in liquid and gas phases, respectively in terms of the kinetic parameters. Significant information can be extracted from Figs. 4(a-c) concerning the CO$_2$ production in the liquid phase. From Figs. 4(a) and 5(a), it is noted that the concentration of CO$_2$ in liquid and gas phases increases when non-growth-associated constant $\alpha$ increases. From Figs. 4(b) and 5(b) it is inferred that the parameter growth-associated constant $\beta$ and CO$_2$ production is positively correlated. When $\beta/\mu_{max}$ increases, the corresponding CO$_2$ production also increases.
Fig. 4: Plot of dimensionless concentration of carbon dioxide in liquid phase \( c_l(\tau) \) versus dimensionless time \( \tau \) using Eq. (18) for values of parameters \( \alpha, \beta/\mu_{max}, S_{X/S} \).

Fig. 4(c) and 5(c) show that, the concentration increases with the increase in biomass-to-toluene yield. It was found that a sharp increase in the \( CO_2 \) when \( \tau \leq 10 \) is due to the uptake of toluene (substrate) by the \( Rhodococcus \) erythropolis. From this figure, it is inferred that as the initial substrate concentration increases, the \( CO_2 \) production is also increasing.
Fig. 5: Plot of dimensionless concentration of carbon dioxide in liquid phase $c_g(\tau)$ versus dimensionless time $\tau$ using Eq. (19) for values of parameters $\alpha$, $\beta/\mu_{max}$ and $Y_{X/S}$.

The relation between the specific biomass growth rate and substrate concentration is plotted in Fig. 7. Figure 7 shows an increase of biomass growth when the substrate concentration increases. After this increment, there is a dramatic decrease in the biomass growth, this is due to the Haldane model contribution. Also, the specific growth rate reaches the maximum value when the following equation gives the substrate concentration:

$$S = \sqrt{K_s K_I}$$

(22)

for all values of the maximum specific growth rate $\mu_{max}$. The maximum specific growth rate is directly proportional to the square root of Haldane inhibition ($K_I$) and saturation constant ($K_s$). Also from the figure, it is observed that the specific growth rate is directly proportional to the maximum specific growth rate. The maximum and minimum values of the biomass formation, toluene utilization, and $CO_2$ production in terms of kinetic parameters are summarized in Table 4.
Specific reaction rate versus substrate concentration for the Haldane-Andrews model.

Here $\mu_{\text{max}} = 0.21, 0.55, 0.92$ and fixed $K_s = 0.39, K_i = 54$

**Determination of kinetic parameters**

It is very interesting to estimate the kinetic constant, such as maximum specific growth rate $\mu_{\text{max}}$, saturation constants $K_s, K_i$, yield coefficients $Y_{x/s}$ theoretically. The Eqs.(8, 9) can be rewritten as follows:

$$\frac{s(\tau) - s_0}{x(\tau)} = \frac{1}{Y_{x/s}} \frac{s(\tau)}{x(\tau)} - \frac{1}{Y_{x/s}}$$

(23)

The plot of $(s(\tau) - s_0)/x(\tau)$ versus $1/x(\tau)$ (Fig. 6(a)) gives the slope $1/Y_{x/s}$ and intercept $-1/Y_{x/s}$. From the slope, we can obtain the biomass-to-toluene yield $Y_{x/s}$. Also, this can be determined from the Eq (22):

$$\frac{dx}{d\tau} = -Y_{x/s} \frac{ds}{d\tau}$$

(24)

The plot of $dx/d\tau$ versus $ds/d\tau$ (Fig 6(b)) gives the slope $-Y_{x/s}$. The change in biomass concentration $dx/d\tau$ is depicted as the function of biomass concentration $x(\tau)$ (Eq. [5]). From this linear correlation, the specific growth rate $\mu$ should be estimated as the slope of the curve based on the equation $\frac{dx}{d\tau} = \mu x(\tau)$. Also the rate equation (1) can be written as:

$$\mu_{\text{max}} \frac{S}{\mu(S)} = K_s + S + \frac{S^2}{K_i}$$

(25)

$\mu_{\text{max}} S / \mu(S)$ is a quadratic polynomial in $S$. Now, here a data set of $S / \mu(S)$ and $S$ are available. The coefficients $\mu_{\text{max}}$, $K_s$ and $1 / K_i$ are calculated by solving the following equations simultaneously (Least-Square method).

$$\mu_{\text{max}} \sum \frac{S_i}{\mu(S_i)} = K_s n + \sum S_i + \frac{1}{K_i} \sum S_i^2$$

(26)

$$\mu_{\text{max}} \sum \frac{1}{\mu(S_i)} = K_s \sum \frac{1}{S_i} + n + \frac{1}{K_i} \sum S_i$$

(27)
\[ \mu_{\max} \sum \frac{1}{\mu(S_j) S_j} = K_s \sum \frac{1}{S_j^2} + \sum \frac{1}{S_j} + \frac{n}{K_I} \]  \hspace{1cm} (28)

Fig. 6(a): Estimation of kinetic parameter \( Y_{X/S} \) using the relationship between biomass and substrate (Eq. (23))

Fig. 6(b): Estimation of kinetic parameter \( Y_{X/S} \) using the relationship between change of biomass and substrate concentration (Eq. (24))

**Differential sensitivity analysis of kinetic parameters**

The differential sensitivity analysis [15] gives the influence of parameters such as yield coefficient \( Y_{X/S} \), \( \gamma \) and \( \delta \) on the concentration of biomass, toluene and \( CO_2 \) in liquid and gas phase. Yield coefficient is defined as the incremental increases in biomass, which results from the incremental of substrate. It is one of the most important parameters in optimizing the concentration of biomass, substrate and \( CO_2 \). With the yield coefficient, material balance equations for biomass, substrate and \( CO_2 \) is straightforwardly formulated.

Fig 8 depicts the influence of the parameters on the concentration of biomass and substrate. The percentage change in concentration (Fig. 8(a)) with respect to the kinetic parameters \( Y_{X/S}, \gamma, \tau \) and \( \delta \) are 75%, 17%, 6%, 2% respectively and the percent change in concentration of substrate (Fig. 8(b)) with respect to the parameters \( Y_{X/S}, \gamma, \delta \) and \( \tau \) are 70%, 20%, 7%, and 3% respectively. From these figures, it is inferred that the yield coefficient \( Y_{X/S} \) has a significant influence in dictating efficiency of the biomass formation and substrate utilization followed by the parameters \( \gamma \) and \( \delta \).

Also, in Fig. 9, the influence of parameters in the concentration of \( CO_2 \) in liquid and gas phases are demonstrated. The percent change in concentration of \( CO_2 \) in liquid phase (Fig. 9a) and gas phases (Fig. 9b) with respect to the parameters \( Y_{X/S}, \gamma, \delta, \alpha, \tau \) and \( \beta / \mu_{\max} \) are 52%, 7%, 1%, 11%, 26%, 3% and 50%, 24%, 12%, 8%, 3%, 1% respectively. It is observed that the yield coefficient is one of the most important parameters for the production of \( CO_2 \).
Fig. 8: Influence of the parameters in concentration of (a) biomass $x(\tau)$ and (b) substrate $s(\tau)$ estimated from the sensitivity analysis.

Fig. 9: Influence of the parameters in concentration of CO$_2$ in (a) liquid phase $c_i(\tau)$ and (b) gas phase $c_g(\tau)$, $c_{i'}(\tau)$ estimated from the sensitivity analysis.

**Conclusion**

The substrate consumption and biomass growth lead to product CO$_2$ formation by the photosynthetic bacterium *Rhodococcus erythropolis* in a batch bioreactor process which can be predicted with the significance level of accuracy using the mathematical model. Kinetic parameters $\mu_{\text{max}}$, $K_S$ and $K_I$ are estimated using the method of least square. The yield coefficient $Y_{X/S}$ is also evaluated graphically.

A new analytical approach to the Homotopy perturbation method was successfully reported in this manuscript. In addition, for the first time, the method was satisfactorily applied to solve non-linear time-dependent ordinary differential equations. The proposed analytical model was in satisfactory agreement when it was successfully compared to numerical ones. Also, the relation between biomass, substrate and CO$_2$ production are reported. The sensitivity analysis of the model parameters is also presented. The theoretical results demonstrated that in order to achieve low substrate consumption and carbon dioxide production, saturation constant and yield coefficient should be maintained at their most moderate possible values.

**References**


Appendix A : A novel approach to homotopy perturbation method (HPM) and approximate analytical solution for the Eq. (14) using NHPM

The homotopy perturbation was first proposed by He [16]. This method is used to find an approximate analytical solution for linear and nonlinear problems. The homotopy perturbation method is a combination of the perturbation method and homotopy in topology. The benefit of this technique is that it does not require a small parameter in the system, thereby it finds wide application in nonlinear wave equations [17]. The homotopy perturbation method has been applied to numerous boundary value problems [18-22].

Recently, a new approach to HPM is presented to solve the nonlinear problem and this resulted in a simple approximate solution in the zeroth iteration [14].

Now using the Eq. (13), Eq. (8) can be written as

$$\frac{dx(\tau)}{d\tau} = -1 \left[ \frac{(x(\tau) - x_s) x(\tau)}{Y_{x15} \left[ \gamma + (x(\tau) - x_s) + \delta (x(\tau) - x_s)^2 \right]} \right]$$

(A.1)

In order to solve the above equation, we construct the homotopy as follows:
\[
(1 - p) \frac{dx(\tau)}{d\tau} + \frac{1}{Y_{X/S}} \left[ \frac{(x(\tau) - 1 - Y_{X/S} k) x(\tau)}{[\gamma - Y_{X/S} k + \delta (x_{ss})^2]} \right] + p \frac{dx(\tau)}{d\tau} + \frac{1}{Y_{X/S}} \left[ \frac{(x(\tau) - 1 - Y_{X/S} k) x(\tau)}{[\gamma + (x(\tau) - x_{ss}) + \delta (x(\tau) - x_{ss})^2]} \right] = 0
\] (A.2)

The approximate solution of (A.1) is as follows:

\[ x = x_{zeroth} + p x_{first} + p^2 x_{second} + \ldots \] (A.3)

Substituting (A.3) in Eq. (A.2) and equating the like powers of \( p \)

\[ p^0 : \frac{dx_{zeroth}(\tau)}{d\tau} + A[x_{zeroth}(\tau) - 1 - Y_{X/S} s_0] x_{zeroth}(\tau) = 0 \] (A.4)

The initial condition for the above equation is

At \( \tau = 0 \), \( x_{zeroth}(0) = 1 \) (A.5)

Solving the Eq. (A.4), \( x_{zeroth}(\tau) \) can be obtained as follows:

\[ x_{zeroth}(\tau) = \frac{x_{ss}}{1 + Y_{X/S}s_0 e^{-\sigma x_{ss}}} \] (A.6)

where \( \sigma = \frac{x_{ss}}{Y_{X/S} [\gamma - 1 - Y_{X/S} s_0 + \delta (-1 - Y_{X/S} s_0^2)]} \), \( x_{ss} = 1 + Y_{X/S} s_0 \), \( s_0 = \frac{S_0}{X_0} \) (A.7)

Substituting (A.6) in Eq. (A.2), and by taking \( x(\tau) \approx x_{zeroth}(\tau) \), we obtained the Eq.(14) in the text. Similarly we can find next iteration to improve the accuracy of the solution.

**Appendix B: Numerical coding for biomass and substrate concentration (Eq.(14)and Eq.(17))**

```matlab
function main1
    options= odeset('RelTol',1e-6,'Stats','on');
    Xo = [1;0.5];
    tspan = [0,20];
    tic
    [t,X] = ode45(@TestFunction,tspan,Xo,options);
    toc
    figure
    hold on
    plot(t, X(:,1))
    figure
    hold on
    plot(t, X(:,2))
    return
function [dx_dt]= TestFunction(t,x)
    m=01;ks=0.39;ki=054;y=0.9;
    m1=m*x(2)/(ks+x(2)+(x(2)/ki));
    dx_dt(1) =(m1)*x(1);
    dx_dt(2) =-(m1*x(1))/y;
    dx_dt = dx_dt';
    return
```

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<table>
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<td>Concentration of $CO_2$ in gas phase</td>
<td>$[g \ m^{-3}]$</td>
<td>$\alpha$</td>
<td>Constant of the Luedeking-Piret model</td>
<td>$[g \ CO_2 \ g \ X^{1/1}]$</td>
</tr>
<tr>
<td>$CO_{2L}$</td>
<td>Concentration of $CO_2$ in liquid phase</td>
<td>$[g \ m^{-3}]$</td>
<td>$\beta$</td>
<td>Constant of the Luedeking-Piret model</td>
<td>$[g \ CO_2 \ g \ X^{1/1}h^{-1}]$</td>
</tr>
<tr>
<td>$K_S$</td>
<td>Toluene half-saturation constant</td>
<td>$[g \ m^{-3}]$</td>
<td>$s$</td>
<td>Dimensionless concentration of substrate</td>
<td>[none]</td>
</tr>
<tr>
<td>$K_I$</td>
<td>Toluene inhibition constant from the Haldane-Andrews model</td>
<td>$[g \ m^{-3}]$</td>
<td>$x$</td>
<td>Dimensionless concentration of biomass</td>
<td>[none]</td>
</tr>
<tr>
<td>$S$</td>
<td>Concentration of Toluene in liquid phase</td>
<td>$[g \ m^{-3}]$</td>
<td>$c_s$</td>
<td>Dimensionless concentration of $CO_2$ in liquid phase</td>
<td>[none]</td>
</tr>
<tr>
<td>$X$</td>
<td>Concentration of biomass (Bacteria)</td>
<td>$[g \ m^{-3}]$</td>
<td>$c_g$</td>
<td>Dimensionless concentration of $CO_2$ in gas phase</td>
<td>[none]</td>
</tr>
<tr>
<td>$H_{CO_2}$</td>
<td>Dimensionless Henry’s constant for $CO_2$</td>
<td>[none]</td>
<td>$x_{ss}$</td>
<td>Dimensionless steady state concentration of biomass</td>
<td>[none]</td>
</tr>
<tr>
<td>$S_0$</td>
<td>Initial concentration of substrate</td>
<td>$[g \ m^{-3}]$</td>
<td>$\tau$</td>
<td>Dimensionless time</td>
<td>[none]</td>
</tr>
<tr>
<td>$X_0$</td>
<td>Initial concentration of biomass</td>
<td>$[g \ m^{-3}]$</td>
<td>$s_0$</td>
<td>Dimensionless initial concentration of substrate</td>
<td>[none]</td>
</tr>
<tr>
<td>$CO_{2L_0}$</td>
<td>Initial concentration of $CO_2$ in liquid phase</td>
<td>$[g m^{-3}]$</td>
<td>$c_{s_0}$</td>
<td>Dimensionless initial concentration of $CO_2$ in liquid phase</td>
<td>[none]</td>
</tr>
<tr>
<td>$CO_{2g0}$</td>
<td>Initial concentration of $CO_2$ in gas phase</td>
<td>$[gm^{-3}]$</td>
<td>$c_{g_0}$</td>
<td>Dimensionless initial concentration of $CO_2$ in gas phase</td>
<td>[none]</td>
</tr>
<tr>
<td>$Y_{X/S}$</td>
<td>Biomass-to-toluene yield</td>
<td>$[g \ X \ g \ S^{-1}]$</td>
<td>$\sigma$</td>
<td>Dimensionless parameter</td>
<td>[none]</td>
</tr>
<tr>
<td>$t$</td>
<td>Time</td>
<td>$[h]$</td>
<td>$\gamma$</td>
<td>Dimensionless parameter</td>
<td>[none]</td>
</tr>
<tr>
<td>$\mu$</td>
<td>Specific biomass growth rate</td>
<td>$[h^{-1}]$</td>
<td>$\delta$</td>
<td>Dimensionless parameter</td>
<td>[none]</td>
</tr>
</tbody>
</table>
\[
\mu_{\text{max}} \quad \text{Maximum specific biomass growth rate} \quad [h^{-1}]
\]

Table 2: Comparison of normalized biomass concentration \( x(t) \) (Eq. (14)) with numerical simulation for values of \( \gamma \) and other experimental values of parameters \( Y_{x/\delta} = 0.9, k = 0.3, \delta = 10^{-3} \).

<table>
<thead>
<tr>
<th>( \tau )</th>
<th>Eq. (14)</th>
<th>Simulation</th>
<th>% of error of deviation</th>
<th>Eq. (14)</th>
<th>Simulation</th>
<th>% of error of deviation</th>
<th>Eq. (14)</th>
<th>Simulation</th>
<th>% of error of deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.0000</td>
<td>0.9999</td>
<td>0.0010</td>
<td>1.0000</td>
<td>0.9999</td>
<td>0.0001</td>
<td>1.0000</td>
<td>1.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>20</td>
<td>1.2514</td>
<td>1.2495</td>
<td>0.0015</td>
<td>1.1929</td>
<td>1.1906</td>
<td>0.0019</td>
<td>1.1498</td>
<td>1.1481</td>
<td>0.0014</td>
</tr>
<tr>
<td>40</td>
<td>1.2689</td>
<td>1.2685</td>
<td>0.0003</td>
<td>1.2506</td>
<td>1.2495</td>
<td>0.0008</td>
<td>1.2206</td>
<td>1.2193</td>
<td>0.0010</td>
</tr>
<tr>
<td>60</td>
<td>1.2699</td>
<td>1.2698</td>
<td>0.0000</td>
<td>1.2653</td>
<td>1.2649</td>
<td>0.0003</td>
<td>1.2504</td>
<td>1.2497</td>
<td>0.0004</td>
</tr>
<tr>
<td>80</td>
<td>1.2699</td>
<td>1.2697</td>
<td>0.0000</td>
<td>1.2688</td>
<td>1.2687</td>
<td>0.0000</td>
<td>1.2623</td>
<td>1.2620</td>
<td>0.0002</td>
</tr>
<tr>
<td>100</td>
<td>1.2700</td>
<td>1.2697</td>
<td>0.0002</td>
<td>1.2697</td>
<td>1.2699</td>
<td>0.0005</td>
<td>1.2670</td>
<td>1.2669</td>
<td>0.0000</td>
</tr>
<tr>
<td>Average error%</td>
<td>0.0016</td>
<td>Average error%</td>
<td>0.0017</td>
<td>Average error%</td>
<td>0.0030</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3: Comparison of normalized subtract(toluene) concentration \( s(\tau) \) (Eq.(17)) with numerical simulation for various values of \( \gamma \) and other experimental values of parameters \( Y_{ss}=0.98, k=0.3, \delta=10^{-3} \)

<table>
<thead>
<tr>
<th>( \tau )</th>
<th>Eq. (14) Simulation</th>
<th>%of error of deviation</th>
<th>Eq. (14) Simulation</th>
<th>%of error of deviation</th>
<th>Eq. (14) Simulation</th>
<th>%of error of deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.3000</td>
<td>0.3000</td>
<td>0.3000</td>
<td>0.3000</td>
<td>0.3000</td>
<td>0.3000</td>
</tr>
<tr>
<td>20</td>
<td>0.0106</td>
<td>0.0646</td>
<td>0.8359</td>
<td>0.1210</td>
<td>0.1234</td>
<td>0.0194</td>
</tr>
<tr>
<td>40</td>
<td>0.0106</td>
<td>0.0117</td>
<td>0.0940</td>
<td>0.0442</td>
<td>0.0458</td>
<td>0.0349</td>
</tr>
<tr>
<td>60</td>
<td>0.0017</td>
<td>0.0020</td>
<td>0.1500</td>
<td>0.0155</td>
<td>0.0163</td>
<td>0.0490</td>
</tr>
<tr>
<td>80</td>
<td>0.0002</td>
<td>0.0006</td>
<td>0.6667</td>
<td>0.0053</td>
<td>0.0057</td>
<td>0.0701</td>
</tr>
<tr>
<td>100</td>
<td>0.0000</td>
<td>0.00079</td>
<td>0.4287</td>
<td>0.0018</td>
<td>0.0019</td>
<td>0.0526</td>
</tr>
</tbody>
</table>

Average error\% 2.1753  
Average error\% 0.2260  
Average error\% 0.1494

Table 4: The maximum and minimum values of the biomass formation, toluene utilization and \( CO_2 \) production in terms of kinetic parameters are summarized.

<table>
<thead>
<tr>
<th>Product</th>
<th>Maximum</th>
<th>Minimum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomass</td>
<td>( x_{ss} = 1 + Y_{X/S} k )</td>
<td>1</td>
</tr>
<tr>
<td>Toluene (Substrate)</td>
<td>( \frac{s_0}{x_0} = k )</td>
<td>0</td>
</tr>
<tr>
<td>Carbon dioxide (Liquid phase)</td>
<td>( c_i = \frac{(x_{ss})^2}{6} \left{ 3 \frac{\beta}{\mu_{max}} - \frac{1}{(Y_{X/S} + 1)^2} \left{ 3kY_{X/S} \frac{\beta}{\mu_{max}} - \frac{2x_{ss}kY_{X/S} \alpha}{Y_{X/S}(\gamma + k + \delta k^2) + 3\frac{\beta}{\mu_{max}}} \right} + k_i \right} + k_i )</td>
<td>( \frac{c_i}{x_0} = k_i )</td>
</tr>
</tbody>
</table>
Carbon dioxide
(Gas phase)

\[
c_s = \frac{H_{CO_2} \left( x_{ss} \right)^2}{6} \left( \frac{3 \beta}{\mu_{max}} - \frac{1}{(Y_{X/S} + 1)^2} \right) \left( 3kY_{X/S} \frac{\beta}{\mu_{max}} - \frac{2x_0 k Y_{X/S} \alpha}{Y_{X/S} (\gamma + k + \delta k^2)} + 3 \frac{\beta}{\mu_{max}} \right) + k_2
\]

\[
\frac{c_{s,0}}{x_0} = k_2
\]