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# Modeling Biodegradation Kinetics on Benzene and Toluene and Their Mixture

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***Abstract:*** *The objective of this work was to model the biodegradation kinetics of toxic compounds toluene and benzene as pure substrates and in a mixture. As a control, Monod and Andrews models were used. To predict substrates interactions, more sophisticated models of inhibition and competition, and SKIP (sum kinetics interactions parameters) model were applied. The models evaluation was performed based on the experimental data from Pseudomonas putida F1 activities published in the literature. In parameter identification procedure, the global method of particle swarm optimization (PSO) was applied. The simulation results show that the better description of the biodegradation process of pure toxic substrate can be achieved by Andrews' model. The biodegradation process of a mixture of toxic substrates is modeled the best when modified competitive inhibition and SKIP models are used. The developed software can be used as a toolbox of a kinetics model catalogue of industrial wastewater treatment for process design and optimization.*

***Keywords:*** *Biodegradation, Benzene, Toluene, Kinetics modeling, Global optimizer.*

## Introduction

The mono-aromatics hydrocarbons benzene and toluene are found in oil derivatives and are widely used in chemical industries as raw materials for synthesis of other products [10]. The benzene is involved in the production of rubber, plastics, pesticides and inks. The toluene is an important commercial chemical product generally used as a dilution agent of inks and as a solvent in the production of resins, glues and oils. These composites are considered dangerous substances to the human health mainly for being depressors of the central nervous system, besides causing damages to the respiratory, gastrointestinal and reproductive system. The benzene is proved to be a carcinogenic and mutagen substance [2, 4], being able to cause leukemia. Hence, the extreme toxicity of benzene and toluene and their frequent presence in industrial discharges and fuel spillings as environmental contaminants have accelerated the research efforts to develop the green biodegradation technologies based on the last achievements of system modeling and optimization. The main goal of bioremediation processes is the costs minimization during the purification process where toxic compounds concentrations after the treatment are in accordance with environmental regulations. It means, the key knowledge for the biodegradation process optimization of aromatic hydrocarbons must be search in the microorganisms aerobic and/or anaerobic metabolite activity.

Many pure bacterial cultures have been isolated on aromatic composites, as only carbon source, including *Pseudomonas species*. Hamed et al. [6], individually and in mixture, carried

through the experiments on biodegradation of the benzene and toluene, by using *Pseudomonas putida* (*P. putida) F1* strain. The authors have experimentally investigated biodegradation of mixture of substrates their interactions during the process. However, their efforts did not extend to formalization of these phenomena in mathematical models. The knowledge about the biodegradation kinetic preserved in models leads to the successful application of bioremediation technologies for pollutants removal.

The objective of this work was to evaluate microbial biodegradation kinetic of benzene and toluene by building different hypotheses on microbial degradation activity, and by validating them through the description of real experimental data published in the literature [6]. This goal was achieved by applying a modern particle swarm global optimizer method in the parameters identification procedure.

## Model development

### *Microbial kinetics of toxic compounds utilization*

The kinetic models of biodegradation processes of benzene and toluene have been evaluated based on experimental data published by [6]. The modeling strategy was built on gradually increased sophistication of the kinetic hypotheses about biodegradation process on population level. The simplest (Monod and Andrews) were used for evaluation of pure substrates degradation by cells. When benzene-toluene mixture were degradated by the microbial population, more complex models of competitive, not-competitive, uncompetitive inhibition, and SKIP were applied.

The Monod model for the $i^{th}$ substrate can be written as follows:

$$\mu_{x_i} = \frac{\mu_{\max_i} S_i}{\left(Ks_i + S_i\right)} \tag{1}$$

The inhibition phenomenon of the $i^{th}$ substrate on the specific growth rate can be representing by Andrews form:

$$\mu_{x_i} = \frac{\mu_{\max_i} S_i}{\left(Ks_i + S_i + S_i^2 / Ki\right)} \tag{2}$$

The mathematical modeling of the multiple substrates microbial kinetics is sufficiently complex. The microorganisms grown on multiple substrates, show preference to some composites favoring their biodegradation [9]. When microorganisms grow on substrates mixture, some phenomena such as catabolite repression, induction and enzymatic inhibition must be considered, which promote the sequential or simultaneous substrates utilization. During the biodegradation competitive inhibition reaction, the inhibitor and the substrate compete for the same active site of the involved enzyme.

The specific growth rates on benzene and toluene mixture are described by the competitive inhibition model [13] and are presented by equations (3) and (4), respectively:

$$\mu_{x_b} = \frac{\mu_{\max_b} S_b}{\left(Ks_b + S_b + S_t\left(\frac{Ks_b}{Ks_t}\right)\right)} \tag{3}$$

$$\mu_{x_t} = \frac{\mu_{\max_t} S_t}{\left( Ks_t + S_t + S_b \left( \frac{Ks_t}{Ks_b} \right) \right)} \tag{4}$$

Another model of enzymatic inhibition, evaluated in this work, corresponds to the model used by [12]. In this case, the inhibiting substrate binds to the enzyme-substrate complex, inactivating it, but not influencing the action of the free enzyme. The specific growth rates on benzene and toluene, where un-competitive inhibition phenomenon is involved, are presented by equations (5) and (6):

$$\mu_{x_b} = \frac{\mu_{\max_b} S_b}{\left( Ks_b + S_b + \frac{S_b S_t}{Ks_t} \right)} \tag{5}$$

$$\mu_{x_t} = \frac{\mu_{\max_t} S_t}{\left( Ks_t + S_t + \frac{S_t S_b}{Ks_b} \right)} \tag{6}$$

The not-competitive inhibition model [13] can be considered adequate when the inhibitive substrate is linked to the free enzyme and inactivates it. Hence, the specific growth rates on benzene and toluene can be written as follows:

$$\mu_{x_b} = \frac{\mu_{\max_b} S_b}{\left( Ks_b + S_b + S_t \left( \frac{Ks_b}{Ks_t} \right) + \frac{S_b S_t}{Ks_t} \right)} \tag{7}$$

$$\mu_{x_t} = \frac{\mu_{max_t} S_t}{\left( Ks_t + S_t + S_b \left( \frac{Ks_t}{Ks_b} \right) + \frac{S_t S_b}{Ks_b} \right)} \tag{8}$$

The new developments in kinetics modeling show that when the iterations between substrates differ from enzyme inhibition type, it is possible to model the system by SKIP (Sum Kinetics Interaction Parameters) model. Yoon et al. [13], to describe adequately non-specific inhibitions, proposed the SKIP model between two substrates, where the key iteration $I_{ij}$, indicates the effect of substrate $i$ on biodegradation of a substrate $j$.

Applying the SKIP model, the benzene and toluene specific growth rates can be written as follows:

$$\mu_{x_b} = \frac{\mu_{\max_b} S_b}{\left( Ks_b + S_b + S_t I_{tb} \right)} \tag{9}$$

$$\mu_{x_t} = \frac{\mu_{\max_t} S_t}{\left( Ks_t + S_t + S_b I_{bt} \right)} \tag{10}$$

Hamed et al. [6] have conducted batch experiments for degradation of single 45 mg.L-1 benzene and 46.15 mg.L-1 toluene substrates, where the initial biomass concentrations were equal. The concentration of benzene-toluene mixture was 16.36 mg.L-1.

The biomass and key substrates balance equations can be written as follows:

$$\frac{dX(t)}{dt} = \sum_{i=1}^{n} \mu_{x_i} X(t) \tag{11}$$

$$\frac{dS_b(t)}{dt} = -\frac{\mu_{x_b}}{Y_{X/S_b}} X(t) \tag{12}$$

$$\frac{dS_t(t)}{dt} = -\frac{\mu_{x_t}}{Y_{X/S_t}} X(t) \tag{13}$$

Assuming that the total specific growth rate (SGR) can be expressed in an additive form of two individual SGRs, the system description can be completed as follows:

$$SGR = \sum_{i=1}^{n} \mu_{x_i} \tag{14}$$

Based on the kinetics hypothesis about microbial behavior in a single and multiple substrate environment (see Eqs. 5 - 10, 14), the authors simulated different working conditions and analyzed sets of experimental data.

## *Models parameters identification procedure*

The identification of model parameters values can be considered as a key step in model development procedure. In this work, a *Particle Swarm Optimization* (*PSO*) global optimizer created by Kennedy and Eberhardt [7] was used.

The *PSO* method is similar to the evolutionary algorithms (EA) and is based on a population of individuals, called particle swarm [7]. However, the only difference between EA and *PSO* is in the space localization of individuals (particles) recognized by the operator speed. In genetic algorithms GA [5], the individuals pass through the operators reproduction, mutation and election.

In the *PSO* method, each population individual (particle) is a vector that corresponds to a possible solution. Each particle possesses a position and a speed in the search space with a dimension equal to the parameters number. The particles act under three influences that are vectorly combine (current vector position; better vector position determined by the particle and better vector position determined by the group), where particles' speed and space position were actualized in each iteration.

The performance of each particle is measured in accordance with statistical criterion (objective function). The end of the search is determined based on the swarm better global position.

The scheme of *PSO* algorithm is shown in Fig. 1. The following search parameters are pre-defined for a population initialization: number of individuals or particles, number of iterations, number of parameters, limit of search, factors of inertion and acceleration constants. Further step includes an evaluation of the objective function global minimum.

After a specified number of iterations, the best solution is used as information for search space restriction. It can be noticed that the parameters with high influence on global objective function are restricted.

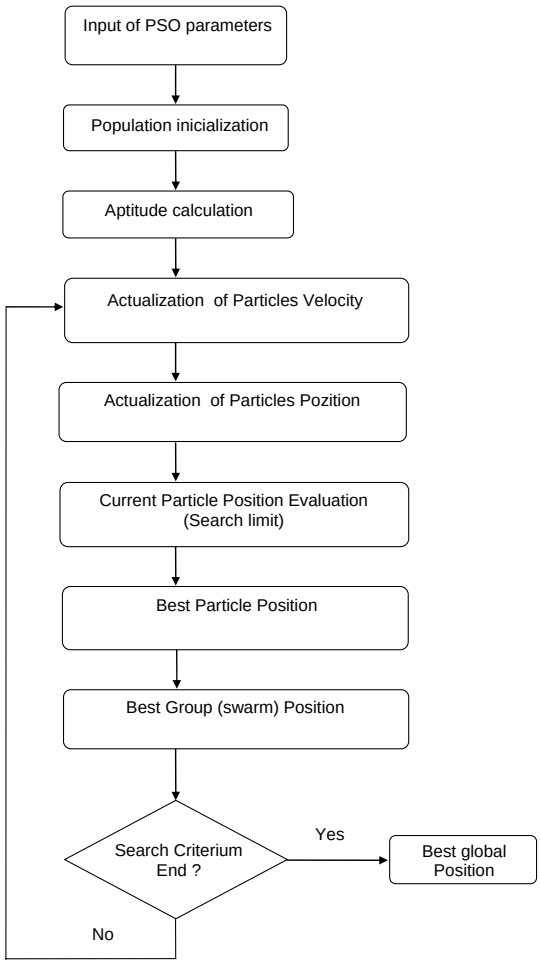


Fig. 1 Scheme of *PSO* algorithm

The above algorithm was used to evaluate model kinetics and stoichiometric parameters values based on published experimental data. The system of ordinary differential equations (see Eqs. 11-13) was solved by using RKF45 numerical method from the Maple symbolic mathematics software. For the search of an objective function (least square method) minimum, 300 vectors solution (particles) and 30 iterations were applied.

## Results and discussion

The models efficiency evaluation was carried out on the objective function minimum value, which corresponds to the experimental data best fitting. The results obtained by using *PSO* global search method can be considered as good as expected. The minimum objective function values for each model describing pure substrate degradation are presented in Table 1, and for the benzene-toluene mixture in Table 2, respectively.

Table 1. Objective function values in the search of model parameters, which describe the pure substrate utilization process

| **Models** | **Objective Function (Benzene)** | **Objective Function (Toluene)** |
|---|---|---|
| Monod | 51.3004 | 79.3665 |
| Andrews | 28.0559 | 75.2279 |

Table 2. Objective function values in the search of model parameters, which describe the mixture substrates utilization process

| Models | Objective Function |
|---|---|
| Competitive inhibition | 61.6835 |
| Uncompetitive inhibition | 94.7615 |
| Non-competitive inhibition | 132.8412 |
| SKIP | 90.8251 |

It can be notice, that the modified competitive inhibition and SKIP models have best fitted experimental data. However, the other evaluated models have also shown similar experimental data representation.

The Andrews model was applied to describe set of experiments on single substrates taken from [6], where the initial 60 mg.L$^{-1}$ benzene and 55 mg.L$^{-1}$ toluene concentrations were used. The substrate inhibition effect occurred above 30 mg.L$^{-1}$ benzene and 28 mg.L$^{-1}$ toluene concentrations. Based on this observation, the authors considered to add the substrate inhibition term $(S^2/Ki)$ into the competitive inhibition model where degradation of the benzene-toluene mixture takes place. Thus, the specific growth rates on the substrates mixture are given by equations (15) and (16):

$$\mu_{x_b} = \frac{\mu_{\max_b} S_b}{\left( Ks_b + S_b + \frac{S_b^2}{Ki_b} + S_t \left( \frac{Ks_b}{Ks_t} \right) \right)} \tag{15}$$

$$\mu_{x_t} = \frac{\mu_{\max_t} S_t}{\left( Ks_t + S_t + \frac{S_t^2}{Ki_t} + S_b \left( \frac{Ks_t}{Ks_b} \right) \right)} \tag{16}$$

Following our strategy to model the single substrates degradation and to compare them with simultaneous degradation of two toxic substrates mixture, the authors applied Andrews model for description of the system where benzene is used as a single substrate (see Figs. 2 and 3).

Experimental and simulation results of toluene degradation as a single substrate are presented in Figs. 4 and 5. Analyzing Figs. 2 - 5, one can see that the deviation between the model and experimental data occurs mainly in biomass concentration profile. As a key component of the system, the biomass experimental concentrations have to be determined with special attention. It means that every assay for biomass experimental points has to be carried through duplicates, triplicates etc. to minimize standard errors from the measurement of each point. Especially important for the parameters identification procedure is the knowledge about "exact" value of initial biomass concentration $X_0$.

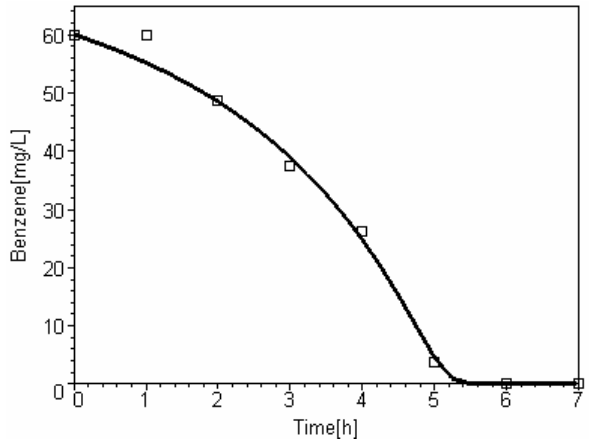


Fig. 2 Benzene utilization – experimental and simulation results obtained by using Andrews model

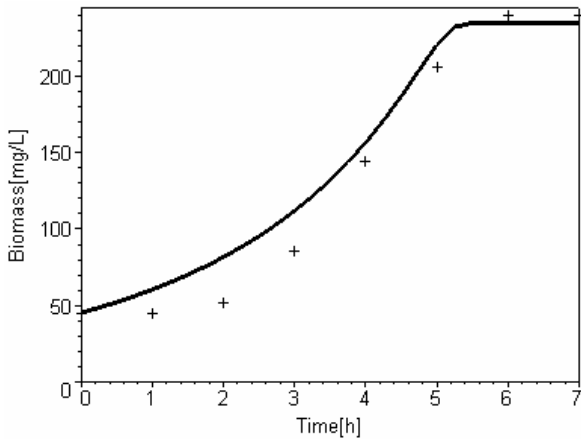


Fig. 3 Biomass growth on benzene – experimental and simulation results obtained by using Andrews model

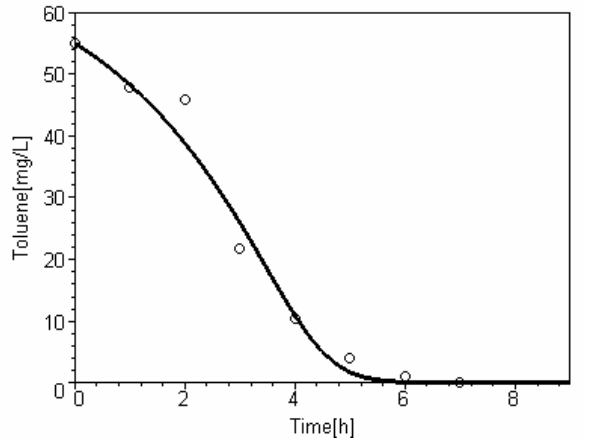


Fig. 4 Toluene utilization – experimental and simulation results obtained by using Andrews model

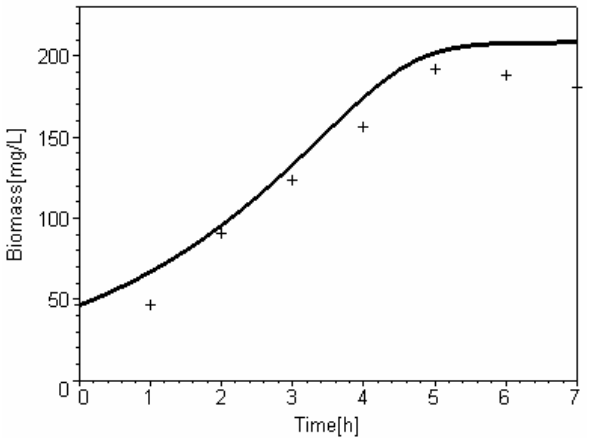


Fig. 5 Biomass growth on toluene – experimental and simulation results obtained by using Andrews model

Applying different and more sophisticated models for description of substrate mixture biodegradation does not change the understanding about the system behavior. Experimental and simulation results of benzene-toluene microbial degradation by using the modified competitive inhibition model are shown in Figs. 6 and 7. As one can see, the model performs very well when describes benzene-toluene utilization. The simulated biomass and substrates profiles differ from the experimental ones because of prolonged lag phase (up to 3$^{d}$ hour) which description is not taken into account in kinetics models. Usually, in wastewater treatment, microbial adaptation to the toxic environment takes time and if the lag phase is long, the modeling efforts are concentrated on the point where the process starts. The indicator of the start-up has to be search in the substrates profile where the measurements of initial substrates concentrations are more correct than the initial biomass concentration measurements.

Based on this understanding, one is able to evaluate more precisely the differences between models and experimental data. All the applied kinetics models to describe experimental data published by Hamed et al. [6] have faced difficulties to fit biomass concentration profiles.

The evaluated parameters values by *PSO* method are shown in Table 3 for the biomass growth on single substrates, and in Table 4 for benzene-toluene mixture.

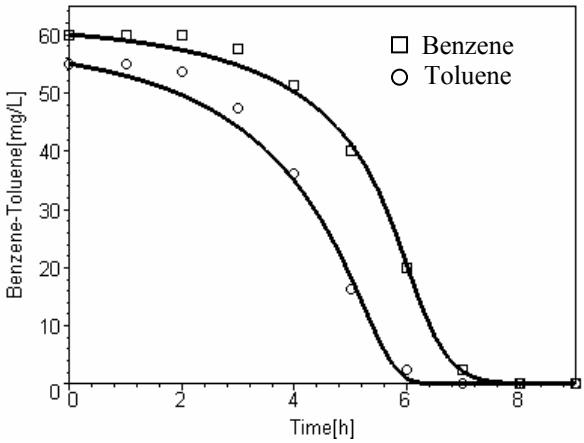


Fig. 6 Benzene-toluene utilization – experimental and simulation results obtained by using modified competitive inhibition model

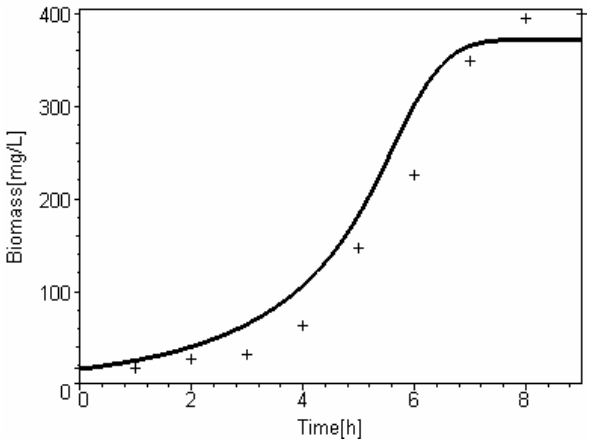


Fig. 7 Biomass growth on benzene-toluene mixture –experimental and simulation results obtained by using modified competitive inhibition model

Table 3. Estimated parameters values on the base of experimental data for pure substrate (benzene and toluene) utilization

| Substrates | Models | $\mu_{max}$ ($h^{-1}$) | *Ks* (mg. $L^{-1}$) | $Y_{x/s}$ (mg.$mg^{-1}$) | *Ki* (mg. $L^{-1}$) |
|---|---|---|---|---|---|
| Benzene | Andrews | 0.4825 | 3.1000 | 3.18 | 97.00 |
| Toluene | Andrews | 0.5800 | 15.8800 | 2.70 | 117.35 |
| Benzene | Monod | 0.3398 | 3.0000 | 3.15 | - |
| Toluene | Monod | 0.4565 | 12.1083 | 2.95 | - |

Table 4. Estimated parameters values on the basis of experimental data of mixture substrates (benzene - toluene) utilization

| Substrates | Models | $\mu_{max}$ ($h^{-1}$) | *Ks* (mg.$L^{-1}$) | $Y_{x/s}$ (mg.$mg^{-1}$) | *Ki* (mg.$L^{-1}$) | $I_{tb}$ | $I_{bt}$ |
|---|---|---|---|---|---|---|---|
| Benzene | Competitive inhibition | 0.8375 | 28.5000 | 3.23 | 141.1500 | - | - |
| Toluene | Competitive inhibition | 0.8440 | 7.6070 | 2.82 | 35.7050 | - | - |
| Benzene | Uncompetitive inhibition | 0.8315 | 31.0000 | 2.12 | - | - | - |
| Toluene | Uncompetitive inhibition | 0.8045 | 9.4500 | 2.02 | - | - | - |
| Benzene | Non-competitive inhibition | 0.8500 | 38.6132 | 1.55 | - | - | - |
| Toluene | Non-competitive inhibition | 0.8500 | 12.4236 | 1.85 | - | - | - |
| Benzene | SKIP | 0.8500 | 15.0000 | 3.20 | - | 5 | - |
| Toluene | SKIP | 0.4087 | 2.0000 | 2.87 | - | - | 0.2 |

During the search of stoichiometric and kinetics constants values by *PSO* method, the authors were guided by the microbiological meaning and on this bases the range of parameters changes were determined and the best current constants values were preserved.

The descriptions of experimental data of the biodegradation process on pure substrate by using controls models of Monod and Andrews show that Andrews model gives more

information about the system response, when initial concentration of toxic substrate is close to the toxic critical values (see Table 1 as an example).

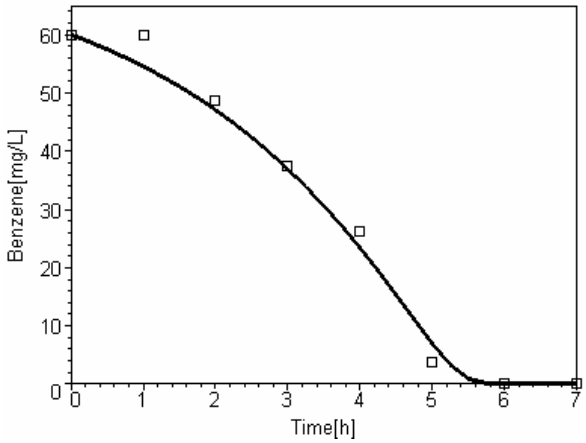


Fig. 8 Benzene utilization – experimental and simulation results obtained by using Monod model

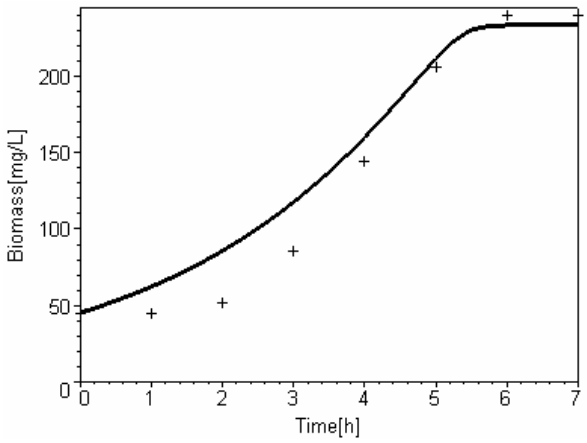


Fig. 9 Biomass growth on benzene – experimental and simulation results obtained by using Monod model

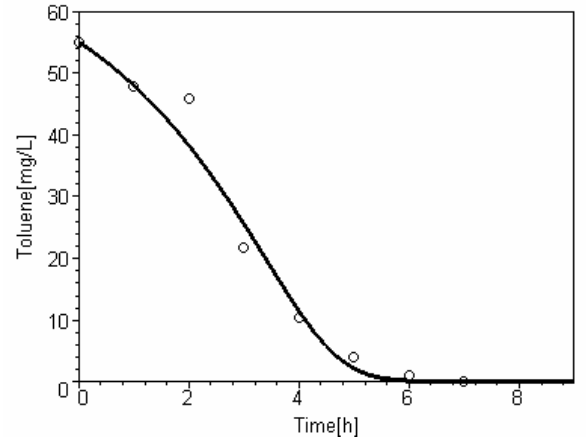


Fig. 10 Toluene utilization – experimental and simulation results obtained by using Monod model

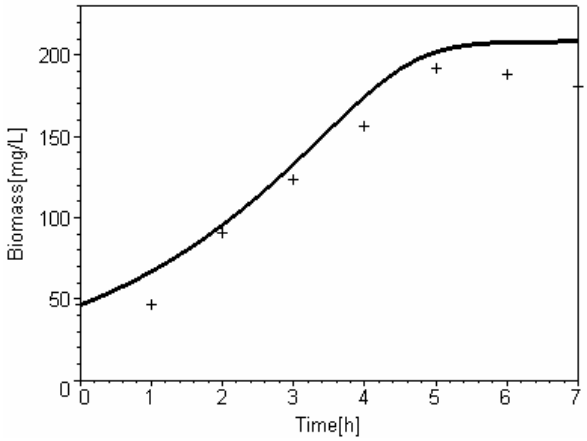


Fig. 11 Biomass growth on toluene – experimental and simulation results obtained by using Monod model

Analyzing the system behavior represented in Figs. 12, 13, 14 and 15, one may highlight some phenomena to explain the differences after the 6$^{th}$ process hour between experimental and simulated data. We will consider the two-step analysis, which is based on the system response during the substrate metabolization, and the second it response, which connected with the biomass growth as a function of substrate degradation.

First, the benzene concentration in the mixture is exhausted (see Figs. 12 and 14) and the remaining toluene concentration at 6$^{th}$ hour is about 20 mg.L-1. It means, that the biomass growth after the 6$^{th}$ hour continuous only by utilizing this remaining toxic compound. We assumed that the catabolite repression mechanisms or other internal cell control tools and these mechanisms are not involved in this case, and the specific growth rates and yield coefficients on benzene and toluene are similar (see Table 3 and 4). Hence, the biomass growth after the 6$^{th}$ hour is exclusively based on toluene remaining concentration and accordingly, the biomass concentration values are expected to be lower. The both utilized models (see Figs. 13 and 15) clearly show this trend.

The overall response of the SKIP model is better (see Fig. 17) which can be explained by an influence of the interaction parameters $I_{tb} = Ks_b / Ks_t$ and $I_{bt} = Ks_t / Ks_b$ .

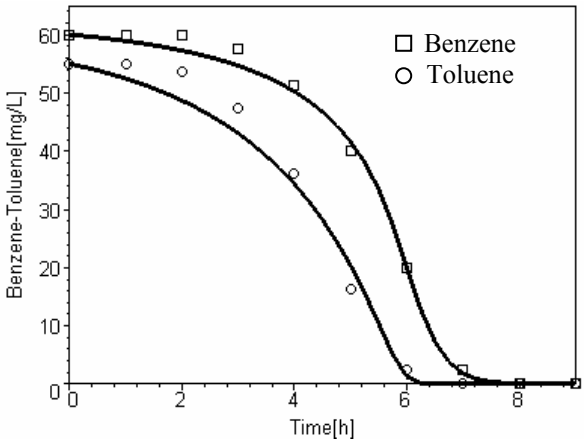


Fig. 12 Benzene-toluene utilization – experimental and simulation results obtained by using uncompetitive inhibition model

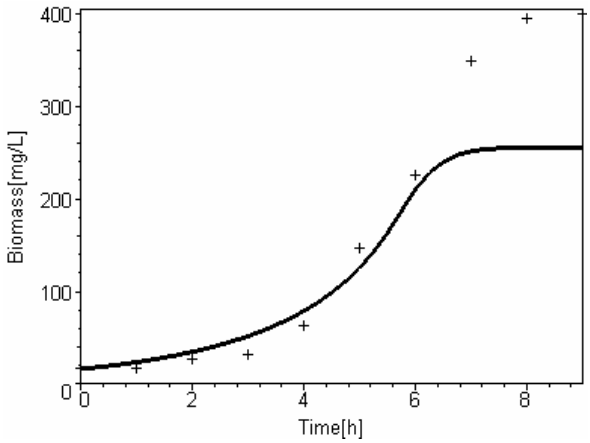


Fig. 13 Biomass growth on benzene-toluene mixture – experimental and simulation results obtained by using uncompetitive inhibition model

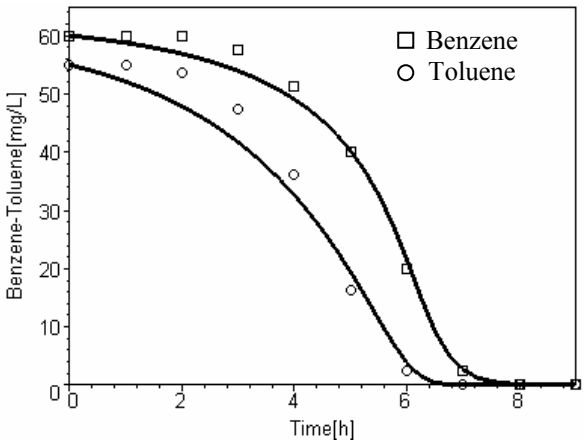


Fig. 14 Benzene-toluene utilization – experimental and simulation results obtained by using non-competitive inhibition model

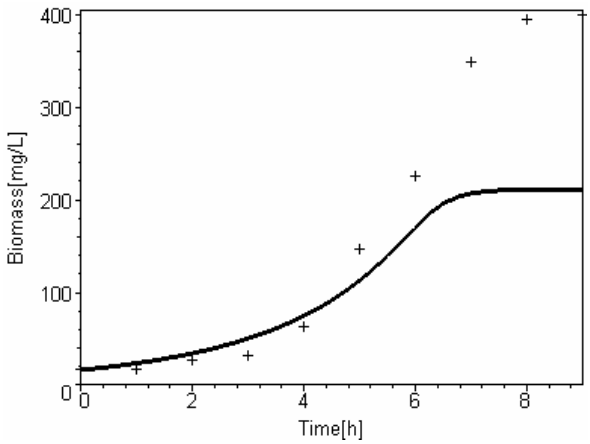


Fig. 15 Biomass growth on benzene-toluene mixture – experimental and simulation results obtained by using non-competitive inhibition model

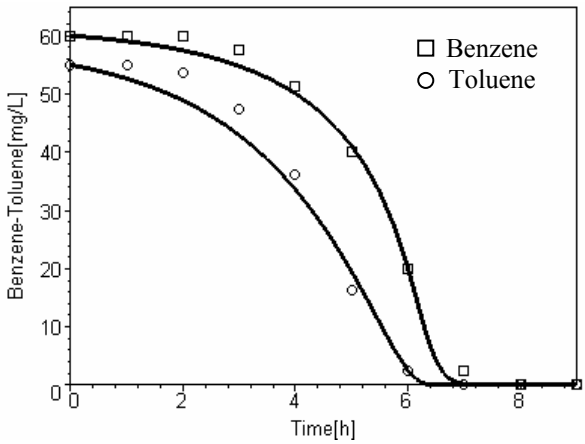


Fig. 16 Benzene-toluene utilization – experimental and simulation results obtained by using SKIP model

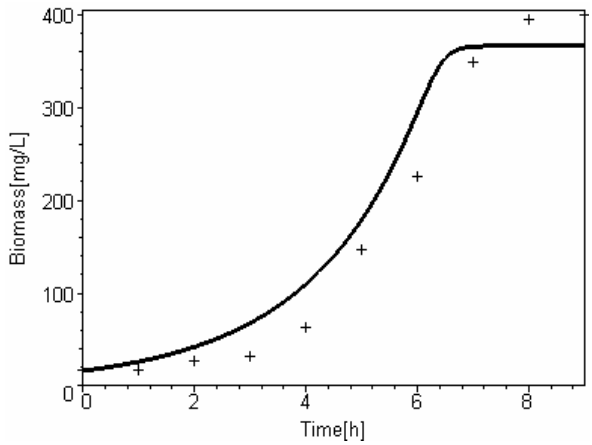


Fig. 17 Biomass growth on benzene-toluene mixture – experimental and simulation results obtained by using SKIP model

A support for our hypothesis about microbial degradation of benzene-toluene mixture by *P. putida F1* [6] is that the involved induced enzymes of this species may catalyze simultaneous utilization of similar substrates. The experimental data obtained by these authors show trend for simultaneous utilization of benzene-toluene mixture. The preferable substrate species was benzene. Hence, the interpretation of $I_{ij}$ iteration coefficients has to be directed on phenomenon – internal metabolite interactions in multi-substrate environment [1, 3, 8, 11].

In Fig. 18, one can see the metabolic pathways of aerobic biodegradation of toluene and benzene by *P. putida F1*.

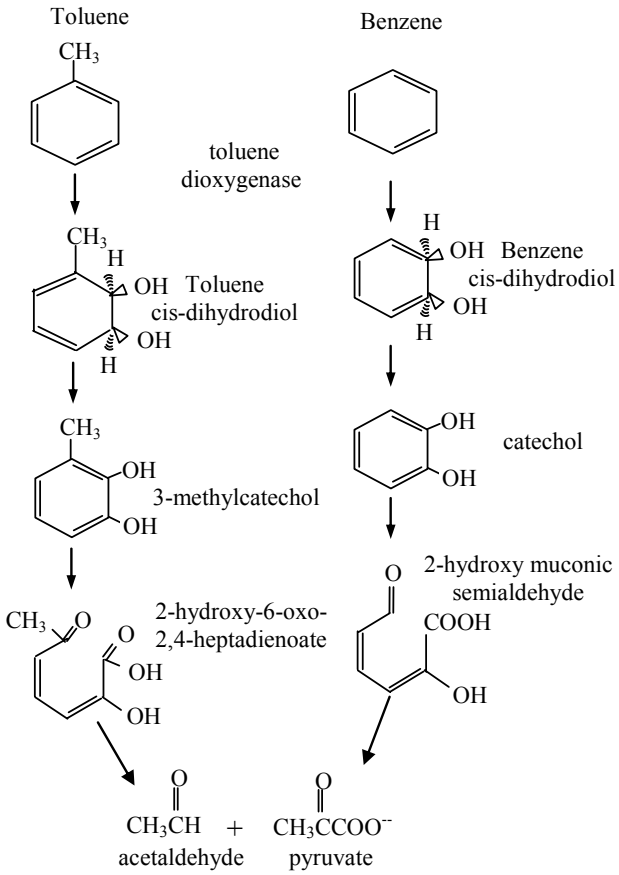


Fig. 18 Metabolic pathways of toluene and benzene in *P. putida F1*

The metabolic pathways show that the common enzyme toluene dioxigenase (TD) is involved in the catabolism of these compounds [14], and based on this fact, a simultaneous biodegradation of toluene-benzene mixture is possible as well .It means that a competition between substrates for the active sites of the enzyme can be involved, and this competition will depend on the substrates concentration ratio. For some particular ratios of substrates concentrations competition can be involved, for other ratios – simultaneous utilization of substrates will take place. The term which considers substrate inhibition in the competitive inhibition model shows good results, which is an evidence for substrates interactions and influence on microbial metabolite activity.

Two examples of Andrews' model response to the pure substrate concentration influences are shown in Figs. 19 and 20.

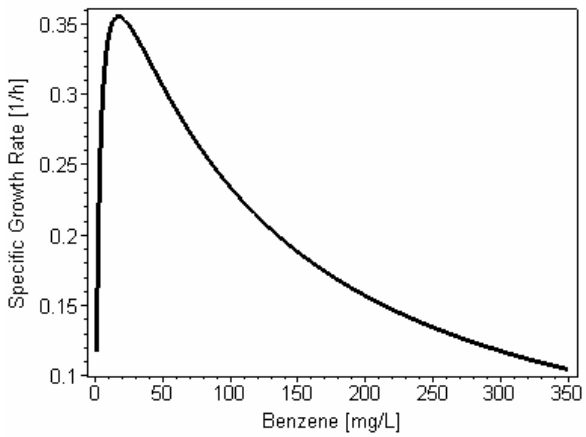


Fig. 19 SGR simulations by Andrews model as a function of benzene concentration

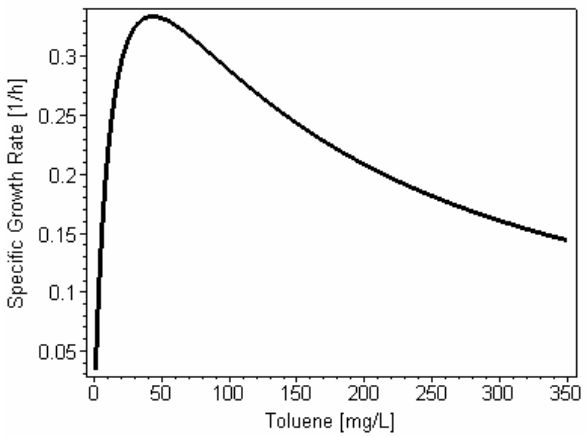


Fig. 20 SGR simulations by Andrews model as a function of toluene concentration

Taking the first derivative of Eq. 2 and equalizing it to zero gives the critical substrate concentration and SGR maximum values (see Eqs. 17 and 18).

$$S^* = \sqrt{Ks\,Ki} \tag{17}$$

$$\mu^* = \frac{\mu_{\max}}{\frac{2\sqrt{Ks\,Ki}}{Ki} + 1} \tag{18}$$

The maximum SGRs on benzene were determined to be 0.3554 $h^{-1}$ and 0.3341 $h^{-1}$ for toluene, and their corresponding critical substrate concentrations' values were 17.34 $mg.L^{-1}$ and 43.17 $mg.L^{-1}$, respectively.

Finally, the SGR models development was analyzed by using response surface analysis methodology. In Figs. 21 and 22 one can see the SGR response as a function of toluene-benzene concentration changes where the modified competitive inhibition model is applied.

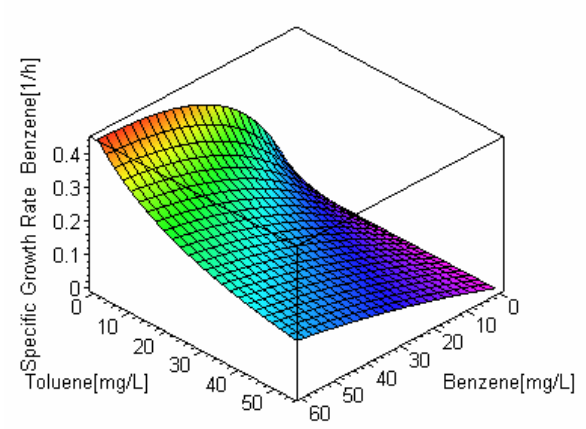


Fig. 21 SGR simulation by modified competitive inhibition model as a function of benzene and toluene concentrations (benzene is an inhibitor)

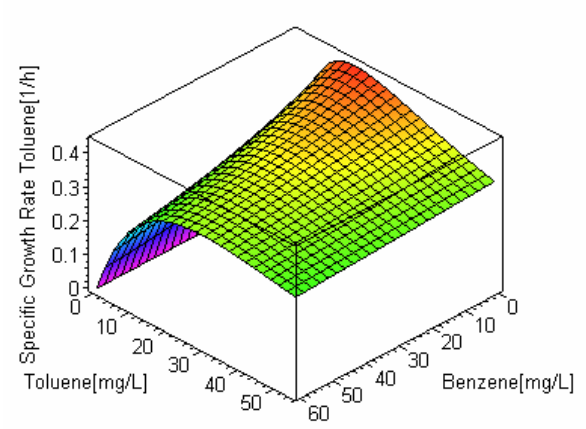


Fig. 22 SGR simulation by modified competitive inhibition model as a function of benzene and toluene concentrations (toluene is an inhibitor)

The analysis shows the influence of the benzene-toluene mixture on the SGR and shows faster decreasing compare with the growth on pure substrates.

## Conclusions

In this work, the biodegradation kinetics models were applied to describe SGR and microbial physiology on toluene and benzene toxic compounds as pure substrates and in a mixture. To evaluate pure substrate influence on microbial physiology, the simple Monod and Andrews models were used as controls. Substrates inhibition of toxic compounds and their interaction in mixture were modeled by using more sophisticated kinetic hypothesis where non-competitive, competitive and SKIP models were applied. The models evaluation was performed based on the experimental data from *P. putida F1* activities [6]. In parameter identification procedure, the global method of particle swarm optimization (*PSO*), created by [7], was applied. The simulation results show that the better description of the biodegradation process of pure toxic substrate can be achieved by Andrews' model. The biodegradation process of a mixture of toxic substrates is modeled the best when modified competitive inhibition and SKIP models are used. Evaluation of SGR models capacity to predict microbial behavior in the range of operational conditions was performed by using surface response analysis. The developed software can be used as a toolbox in a kinetics model catalogue of industrial wastewater treatment for process design and optimization.

## Nomenclature

$S$ – substrate concentration ($mg.L^{-1}$)
$\mu_x$ – specific growth rate ($h^{-1}$)
$\mu_{max}$ – maximum specific growth rate ($h^{-1}$)
$X$ – biomass concentration ($mg.L^{-1}$)
$Ks$ – saturation constant ($mg.L^{-1}$)
$Ki$ – inhibition constant ($mg.L^{-1}$)
$I_{ij}$ – interaction parameter between two toxic substrates (-)
$Y_{X/S}$ – yield coefficient (mg cells/ mg substrate)

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