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NOVEL MATHEMATICAL MODELS FOR PREDICTION OF MICROBIAL GROWTH KINETICS AND CONTAMINANT DEGRADATION IN BIOREMEDIATION PROCESS

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Abstract. Bioremediation is defined as a process, which involves decomposition of organic pollutant compounds available in soil and water resources into safe and eco-friendly materials, like water and CO2, by the microorganisms. In the present article, mathematical modeling of the bioremediation process was conducted comprehensively, and new models proposed for the microbial growth kinetics and substrate consumption (contaminant degradation). Accordingly, six kinetic models were suggested for the biomass growth and six models for the substrate consumption. Moreover, two models were considered for specific growth rate constant of the microorganisms. Then, model predictions were compared to and validated by the available experimental data in the literature. According to the obtained results, the microbial growth kinetic model, entitled as "*MVKH2*", the substrate (contaminant) consumption model, entitled as "*MVKH2s*", and the Aiba specific growth rate constant model had the best performance and the least error value in predicting the bioremediation process. Results achieved from this study are a promising beginning for practical and experimental works.

Keywords: bioremediation, environmental processes modeling, simulation, microbial growth kinetic, contaminant degradation.

Introduction

Bioremediation is defined as a process, which involves decomposition of organic pollutant compounds available in soil and water resources into safe and eco-friendly materials by the microorganisms. The process usually takes place extensively for cleaning coastal ecosystems, after leakage of oil compounds, cleaning of contaminated areas with heavy metals (such as uranium, arsenic, aluminum, tin and zinc), and also extraction of metals from their stone mine (Cristina Souza *et al.* 2014; Sheoran *et al.* 2010). Various physical and chemical methods could be employed to eliminate contaminant materials, but they are mostly expensive and incompatible with the environment. Therefore, bioremediation could be an appropriate strategy for removing pollutants, especially in places where cleanup is not physically or chemically feasible (e.g. in areas with low level of pollution) (Jeyasingh *et al.* 2011; Moscoso *et al.* 2012).

Practically, bioremediation process depends on the number and type of microorganisms. Selecting microbes also depends on the nature, chemical structure of the contaminant, and the environmental condition. Different microorganisms including bacteria, fungi, yeasts, and algae are used in bioremediation process for pollutant decomposition. Among them, bacteria, due to more adaptability with the environment, are highly considered. Contaminants are usually comprised of petroleum compounds and its derivatives, solvents, and pesticides (Khataee *et al.* 2011; Wang *et al.* 2008). Bioremediation is often a slow process that could be expedited through addition of nutrients, inoculation with bacterial consortium, and selection of suitable bioreactor. The successful completion of bioremediation depends on preparation of optimum physical, chemical and biological conditions in the polluted area (Nikakhtari *et al.* 2008).

Recently, the bioremediation process has attracted the attention of many researchers, however, few works have been conducted on modeling of bioremediation process. The following are some works done in the field of bioremediation process and modeling:

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Kindred, Celia (1989) considered simple first order kinetic for growth of biomass. Nakamura, Sawada (2000) suggested a mathematical model for prediction of chrome (VI) reduction, and its conversion to chrome (III) with less toxicity. Their model has desirable prediction only when the amount of substrate is low for bacterium growth. Tarighian *et al.* (2003) showed that glucose was a better co-substrate relative to phenol for the co-metabolic removal of 4-chlorophenol from wastewater by *Pseudomonas putida*. They mentioned that both co-substrates were metabolized by the biomass according to Monod model. Moussa *et al.* (2005) presented a model to study the effect of increasing sludge age, and the role of predators on the biomass composition in the sequenced batch reactors. Furthermore, the interaction between nitrifiers, heterotrophs and predators in wastewater treatment was considered in their model. Jankaite and Vasarevicius (2005) reviewed various remediation techniques for removing heavy metals from the contaminated soil. They pointed out that the selection of suitable soil remediation technology depends on concentration, type of pollutant, site characteristic and the final use of a contaminated medium. Van Ginneken *et al.* (2007) applied phytoremediation to clean the soils contaminated with heavy metals. They stated that uptake capacity of heavy metals can be promoted by plants via the addition of biodegradable physico-chemical agents, and stimulating the microbial community in and around the plant. Furthermore, they discussed in more detail to convert the harvested biomass crops into the biodiesel. Abbasi, Shquirat (2008) showed that the value of microbial specific growth rate constant (μ) is related to the microbial concentration and increasing that will lead to enhancement of this value. The overall bioremediation rate was modeled with the two limiting cases of zero and first order kinetics to describe biodegradation of a volatile organic compound (VOC) in a fluidized bioreactor by Clarke *et al.* (2009). Their model represented that maximum elimination capacity is limited by the microorganism growth rate and the microbial population. Also, the overall degradation of a substrate was a zero order process in the bioreactor at maximum elimination capacity. Guo *et al.* (2010) considered a first order kinetic model for the biomass growth in bioremediation of oil hydrocarbons.

Fallgren *et al.* (2010) used respirometric measurements of CO_2 due to microbial activity in a soil contaminated with diesel to derive empirical models. The presented empirical equations described the effects of environmental factors such as temperature, water content, nitrogen and phosphorus content on microbial hydrocarbon degradation via CO_2 data analysis.

Vaezihir *et al.* (2012) presented transport model and a field-scale three-dimensional numerical flow to simulate the destiny and transport of benzene, toluene, ethylbenzene and xylenes (BTEX) from six source regions of light nonaqueous-phase liquids (LNAPLs). They investigated the effect of natural debilitation and enhanced remediation procedures on the destiny of BTEX. Their results indicated that the remediation time for BTEX was more than 60 years, under natural debilitation only, but using enhanced bioremediation methods and LNAPL removal could decrease this time to about 30 years for BTEX.

Das Saha (2013) studied the effects of operational variables such as adsorbent dosage, contact time, dye concentration, initial pH, and agitation on the dye removal from wastewater using rice husk in a stirred tank reactor. She applied response surface methodology (RSM) to investigate the interaction between various process parameters, and the optimization of the variables on dye removal. In addition, the artificial neural network was used to model process parameters in her work.

Mohajeri *et al.* (2013) concluded that the bioremediation kinetic modeling of contaminated soil is complicated due to the existence of numerous factors. Despite this fact, they have used the first order kinetic to predict the biological decomposition of oil hydrocarbons.

Vasiliadou *et al.* (2013) applied a kinetic model to predict the effect of biomass growth and nutrients consumption on the sorption and bioremediation of the pollutants (pharmaceutical compounds) in individual and simultaneous treatment. In their work, the bioremediation process was modeled by a pseudo-first-order kinetic type with a double-Monod kinetic expression. Koreiviene *et al.* (2014) applied microalgae consortia for bioremediation of wastewater and their biomass potential to produce the biofuel. Their results revealed that: a) *Chlorella/Scenedesmus* consortium removed up to 99.7–99.9% of inorganic phosphorus and up to 88.6–96.4% of inorganic nitrogen from the wastewater within three weeks, b) The ammonium elimination was more efficient than that of nitrate, c) *Chlorella* algae grew better in diluted, while *Scenedesmus* in the concentrated wastewater, d) The consortium treated wastewater more efficiently than a single species.

Since the experimental study of bioremediation for evaluating different aspects of the process (such as microbial growth and contaminant degradation trends), is extremely time-consuming and expensive, process modeling and simulation are greatly contributed to achieve the desired objectives. In the present article, comprehensive modeling of microbial growth trend and substrate consumption, as two key parameters in bioremediation were accomplished. In this regard, novel kinetic models have been proposed and compared to predict the biomass growth behavior and the substrate consumption (pollutant degradation). Moreover, different models were considered to determine the value of μ.

1. Modeling of bioremediation process

Modeling is a useful tool for design, scale-up, and process control in bioremediation. Also, it could cause the realization of effective strategies and removing of misguided policies. Some factors such as biomass concentration and the amount of substrate (contaminant) have an important role in optimum performance of the bioremediation. Thus, various models have been proposed and compared for predicting the biomass concentration, the amount of substrate consumption, and the value of μ in the present study. In proposed biomass concentration models, both growth and death kinetics of microorganisms were considered, because the amount of biomass initially increase by passing of the time and substrate consumption and then will decrease due to reduction of substrate and cells' death.

1.1. Microbial growth kinetic models

As can be seen in Table 1, various kinetic models are suggested to predict the behavior of microbial growth through the bioremediation process (growth kinetic models identified by *MVKH*). In all developed growth models, the related terms of growth and death of microorganisms were considered (death of microorganisms will occur after the stationary phase).

In existing mathematical models in Table 1, X is the biomass concentration, μ the microbial specific growth rate constant, γ stationary constant, k_d death or biomass degradation constant, and X_m the maximum biomass concentration. In these models the term $k_d\left|\int_0^t X(t)dt\right|$ describes death kinetic of the cells.

1.2. Substrate consumption models

As can be seen in Table 2, different mathematical models have been designed to predict the rate of substrate consumption (contaminant degradation) in the bioremediation process (substrate consumption models defined by *MVKHs*).

In presented models of Table 2, S is the substrate (contaminant) concentration and, Y is the yield factor. It is worth mentioning that in substrate consumption models the corresponding terms of growth and stationary phases from growth curve were only considered, and the related terms of death kinetics have been ignored, because the amount of substrate consumption (contaminant biodegradation) is negligible in the death phase.

1.3. Models of microbial specific growth rate constant (μ)

The value of specific growth rate constant of microorganisms has a significant role in correct prediction of growth kinetic model. In most previous studies on bioremediation, the amount of this parameter (μ) was assumed as constant. So, the effect of contaminant concentration on μ will be ignored with this assumption (Mohajeri *et al.* 2013; Geng *et al.* 2013; Li *et al.* 2013; Louati *et al.* 2013). In order to overcome this shortcoming, two models were considered for predicting the value of μ in the present study. These models are shown in Table 3. In Table 3 μ_{max} is the maximum specific growth rate constant, K_s biomass affiliation to the substrate, and K_i substrate dissociation constant.

Table 1. The microbial growth kinetic models proposed in the present study

Model	Name
MVKH1	$\frac{dX}{dt}=\mu X(1-\gamma X)+k_d\left\|\int_0^t X(t)dt\right\|$
MVKH2	$\frac{dX}{dt}=\mu-\gamma+k_d\left\|\int_0^t X(t)dt\right\|$
MVKH3	$\frac{dX}{dt}=\mu X\left(1-\frac{X}{X_m}\right)-\gamma+k_d\left\|\int_0^t X(t)dt\right\|$
MVKH4	$\frac{dX}{dt}=\mu X\left(1-\frac{X}{X_m}\right)-\gamma X+k_d\left\|\int_0^t X(t)dt\right\|$
MVKH5	$\frac{dX}{dt}=\mu X-\gamma+k_d\left\|\int_0^t X(t)dt\right\|$
MVKH6	$\frac{dX}{dt}=\mu X-\gamma X+k_d\left\|\int_0^t X(t)dt\right\|$

Table 2. The substrate consumption models proposed in the present study

Model	Name
MVKH1s	$\frac{dS}{dt}=\mu X(1-\gamma X)/Y$
MVKH2s	$\frac{dS}{dt}=\mu-\gamma/Y$
MVKH3s	$\frac{dS}{dt}=\mu X\left(1-\frac{X}{X_m}\right)-\gamma/Y$
MVKH4s	$\frac{dS}{dt}=\mu X\left(1-\frac{X}{X_m}\right)-\gamma X/Y$
MVKH5s	$\frac{dS}{dt}=\mu X-\gamma/Y$
MVKH6s	$\frac{dS}{dt}=\mu X-\gamma X)/Y$

Table 3. Microbial specific growth rate constant models

Model	Name
Haldan (*P1*)	$\mu = \frac{\mu_{max} S}{S + K_s + \frac{S^2}{K_i}}$
Aiba (*P2*)	$\mu = \frac{\mu_{max} S \exp\left(\frac{-S}{K_i}\right)}{K_s + S}$

2. Process simulation

In order to evaluate the performance of the proposed models of the previous section, growth behavior simulation of prokaryotic communities were carried out on aliphatic and polycyclic aromatic hydrocarbons (as contaminant) in two cases. In the first case, each microbial growth kinetic model was simultaneously solved with the related substrate consumption (contaminant degradation) model, and Haldan's specific growth rate constant model. Similarly, each microbial growth kinetic models with corresponding substrate consumption model were solved through the second case by specific growth rate constant model of Aiba, simultaneously. Then, the obtained results from simulations were compared with the experimental data of Beolchini *et al.* (2010) for growth of prokaryotic cells on aliphatic and polycyclic aromatic hydrocarbon contaminants. As it will be shown in the next section, the predictions of some models are extremely in line with the experimental results, which is indicative of high reliability of the proposed models.

Table 4. The parameters value calculated by nonlinear regression in the first simulation case

Parameter	Value	Unit
K_s	38.5	μM
K_i	9840.2	μM
γ	0.04	–
k_d	–0.006	day^{-2}

Table 5. The values of μ_{max} and *Y* calculated by nonlinear regression in each six various combinations of the first simulation case

Model	μ_{max} (day^{-1})	*Y*(gr^{-1})
MVKH1-MVKH1s-P1	5.6	70.96
MVKH2-MVKH2s-P1	3.46	45.16
MVKH3-MVKH3s-P1	0.488	5.8
MVKH4-MVKH4s-P1	0.9	12.9
MVKH5-MVKH5s-P1	0.162	2.09
MVKH6-MVKH6s-P1	0.17	2.25

In order to solve the microbial growth kinetics and substrate consumption models (mentioned in section 2), which are ordinary differential equations, the forth order Runge–Kutta method (using *ode45* function in MATLAB software environment (version of 2013a)) has been employed with variable time step size. The mentioned solving method is called "linear method". The initial value of biomass concentration is 4×10^8 cells gr^{-1}, and the initial value of substrate concentration is 500 μgr gr^{-1}.

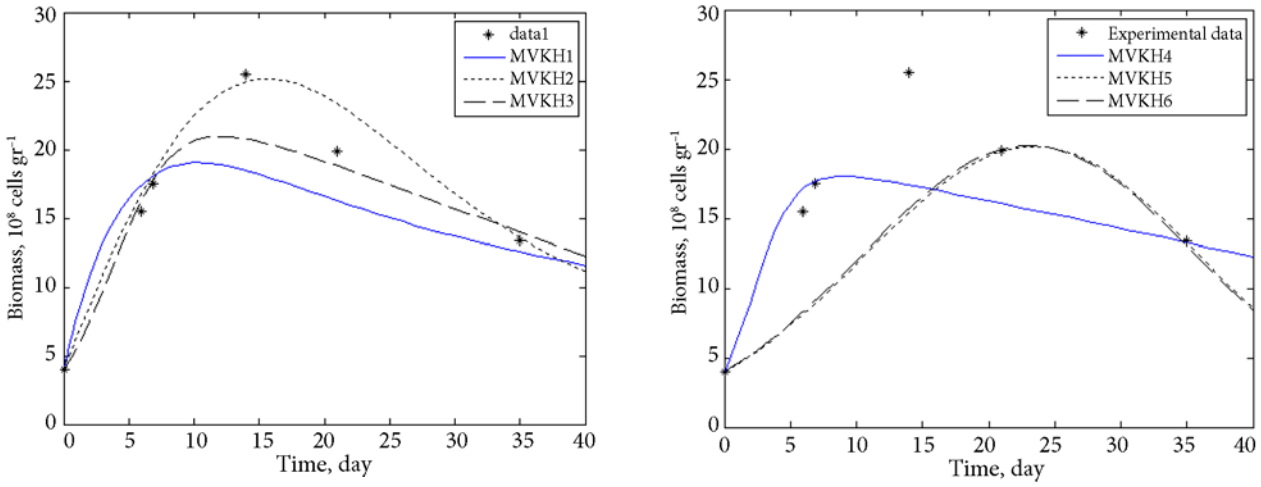
3. Results and discussion

As mentioned before, in the first case of simulation, each growth kinetic models (*MVKH1-MVKH6*) was considered with the corresponding substrate consumption model (*MVKH1s-MVKH6s*) and Haldan's specific growth rate constant model (identified as *P1*). For the best fitting with experimental data, some of the adjustable parameters in above mentioned models were computed through nonlinear regression in MATLAB environment (version 2013a) using '*nlinfit*' function and presented in Tables 4 and 5.

The values of maximum specific growth rate constant (μ_{max}) and yield factor (Y) (calculated by nonlinear regression) in six different combinations of the first case simulations were presented in Table 5.

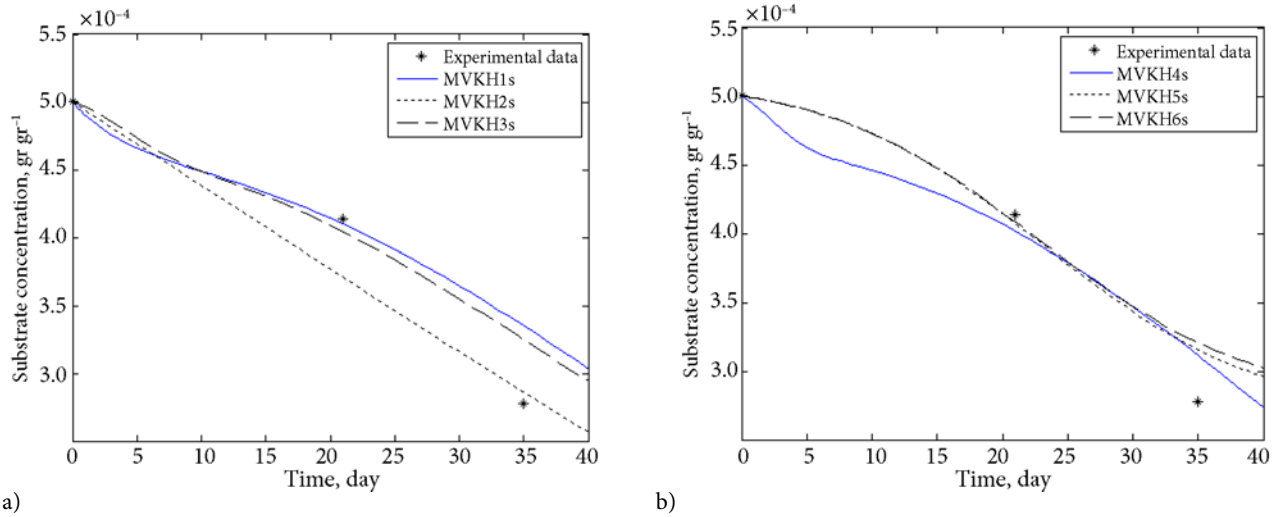
The results achieved from the simulations of the first case, and the experimental data extracted from Beolchini *et al.* (2010) work are illustrated in Figures 1 and 2 for growth of prokaryotic communities on aliphatic and polycyclic aromatic hydrocarbon contaminants. As can be seen in Figure 1 (a and b), the variation of biomass in the bioremediation process shows an increasing-decreasing trend. This is due to the fact that the microorganisms will initially grow, and the amount of biomass will increase by passing of the time and substrate consumption. After passing specified time, the microorganisms will inter the death phase and the amount of biomass will take a decreasing trend. The variations trend of substrate (contaminant) consumption is shown in Figure 2 (a and b). It is clearly evident that, the substrate is consumed during the time and its value is decreased. According to the obtained results in Figures 1 and 2, accessibility of microorganisms to substrate has been reduced by decreasing the amount of substrate from a certain value, which is a vital factor for stopping the cells growth.

As can be seen from Figures 1 and 2, *MVKH2* growth kinetic model along with its related substrate consumption model (*MVKH2s*) has the best agreement with experimental data. The sum of squared error (SSE) of each proposed models for the biomass growth and substrate consumption along with the total SSE of each six existing combinations are given in Table 6. Clearly, the combination of *MVKH2–MVKH2s* has the least total error in the first case of simulations.



a) b)

Fig. 1. a) – predictions of the biomass variations versus time using *MVKH1* to *MVKH3* growth kinetic models accompanied with Beolchini *et al.* (2010) experimental data for growth of prokaryotic communities on aliphatic and polycyclic aromatic hydrocarbon contaminants in the first case of simulation; b) – predictions of the biomass variations versus time using *MVKH4* to *MVKH6* growth kinetic models accompanied with Beolchini *et al.* (2010) experimental data for growth of prokaryotic communities on aliphatic and polycyclic aromatic hydrocarbon contaminants in the first case of simulation



a) b)

Fig. 2. a) – predictions of the contaminant variations trend versus time using *MVKH1s* to *MVKH3s* substrate consumption models accompanied with Beolchini *et al.* (2010) experimental data for growth of prokaryotic communities on aliphatic and polycyclic aromatic hydrocarbon contaminants in the first case of simulation; b) – predictions of the contaminant variations trend versus time using *MVKH4s* to *MVKH6s* substrate consumption models accompanied with Beolchini *et al.* (2010) experimental data for growth of prokaryotic communities on aliphatic and polycyclic aromatic hydrocarbon contaminants in the first case of simulation

Table 6. The SSE of microbial growth kinetic and contaminant consumption models, as well as total SSE obtained from simulations of the first case

Model	SSE of growth kinetic model	SSE of contaminant consumption model	Total SSE
MVKH1-MVKH1s-P1	64.22	1.12	65.34
MVKH2-MVKH2s-P1	15.77	0.6	16.37
MVKH3-MVKH3s-P1	22.02	0.84	22.86
MVKH4-MVKH4s-P1	87.81	0.46	88.27
MVKH5-MVKH5s-P1	326.63	2.02	328.65
MVKH6-MVKH6s-P1	209.45	0.59	210.04

In the second case of the simulation, each growth kinetics (*MVKH1-MVKH6*) and corresponding substrate consumption model (*MVKH1s-MVKH6s*) was considered with Aiba's specific growth rate constant model (identified as *P2*). Like the fist case, for the best fitting with experimental data, some of the adjustable parameters in proposed models were obtained through the nonlinear regression in MATLAB environment and presented in Tables 7 and 8. Similar to the previous stage, the values of μ_{max} and Y (calculated by nonlinear regression) in six different combinations of the second case simulations were given in Table 8.

Table 7. The parameters value calculated by nonlinear regression in the second simulation case

Parameter	Value	Unit
K_s	43.9	μM
K_i	9137.7	μM
γ	0.04	–
k_d	–0.006	day^{-2}

Table 8. The values of μ_{max} and Y calculated by nonlinear regression in each six various combinations of the second simulation case

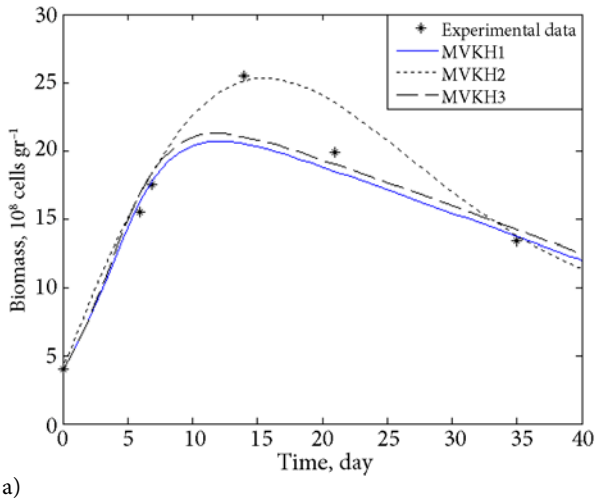
Model	μ_{max} (day^{-1})	$Y(gr^{-1})$
MVKH1-MVKH1s-P2	0.48	3.87
MVKH2-MVKH2s-P2	3.24	20.96
MVKH3-MVKH3s-P2	0.51	4.83
MVKH4-MVKH4s-P2	0.705	5.8
MVKH5-MVKH5s-P2	0.165	1.45
MVKH6-MVKH6s-P2	0.172	1.45

The predictions of the second case modeling and the experimental data of Beolchini *et al.* (2010) work were indicated in Figures 3 and 4 for growth of prokaryotic cells on aliphatic and polycyclic aromatic hydrocarbon contaminants. As can be shown, the trend of boimass variations and contaminant degradation is almost similar to the first case of simulation.

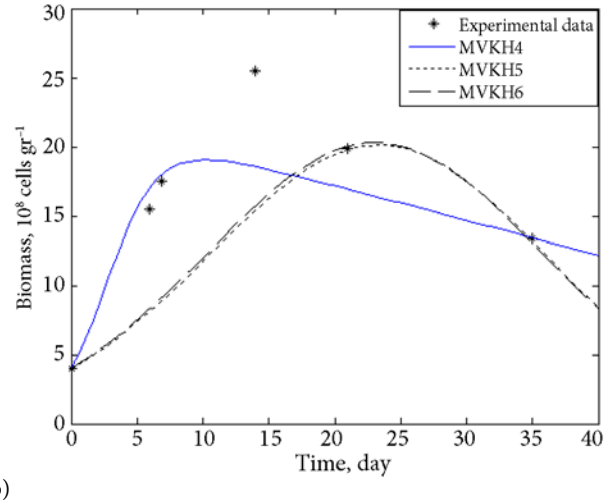
As depicted in Figures 3 and 4, *MVKH2* growth kinetic model with corresponding substrate consumption model (*MVKH2s*) has the best agreement with experimental data similar to the first case of simulations. The SSE of each proposed models for biomass growth and substrate consumption along with the total SSE of each six available combinations are given in Table 9. Again, the combination of *MVKH2-MVKH2s* has the least total error in the second simulation case.

According to the presented results in Tables 6 and 9, the total SSE value in combination of *MVKH2-MVKH2s-P1* is 16.37, while this value in combination of *MVKH2-MVKH2s-P2* is 14.45. Consequently, by comparing the achieved results from the first and second case of simulations, and the calculated error values it can be specified that the Aiba model has better performance in predicting specific growth rate constant of the microorganisms.

It is worthy to mention that the presented models in this paper are general, and they can be applied to different contaminants and bioremediation processes, but the focus of this study is on aromatic hydrocarbons degradation, because this group is one of the most important environmental contaminants.



a)



b)

Fig. 3. a) – predictions of the biomass variations versus time using *MVKH1* to *MVKH3* growth kinetic models accompanied with Beolchini *et al.* (2010) experimental data for growth of prokaryotic communities on aliphatic and polycyclic aromatic hydrocarbon contaminants in the second case of simulation; b) – predictions of the biomass variations versus time using *MVKH4* to *MVKH6* growth kinetic models accompanied with Beolchini *et al.* (2010) experimental data for growth of prokaryotic communities on aliphatic and polycyclic aromatic hydrocarbon contaminants in the second case of simulation

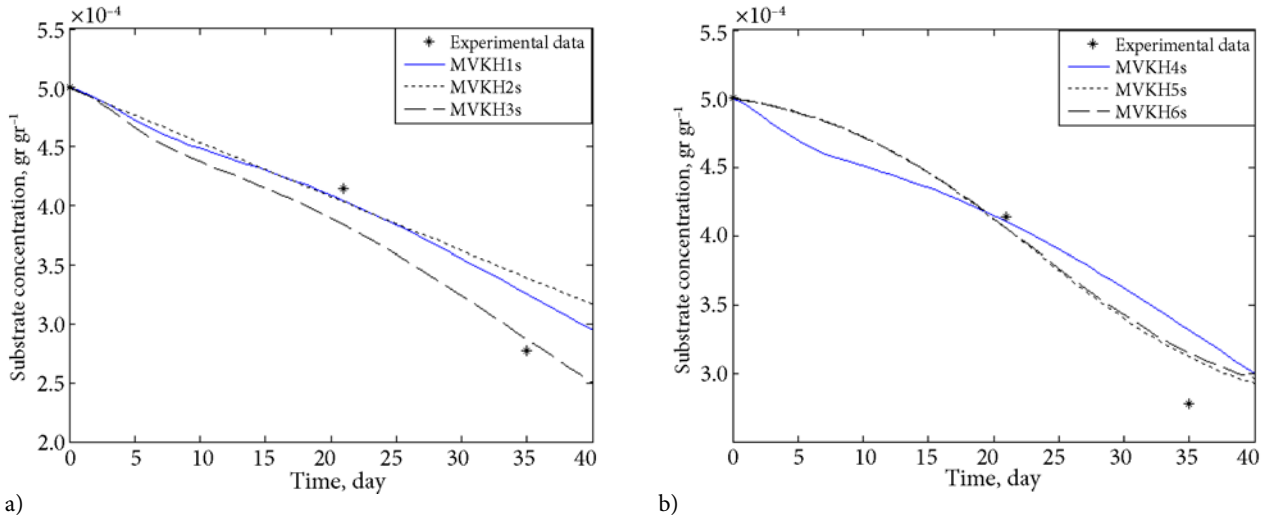


Fig. 4. a) – predictions of the contaminant variations trend versus time using *MVKH1s* to *MVKH3s* substrate consumption models accompanied with Beolchini *et al.* (2010) experimental data for growth of prokaryotic communities on aliphatic and polycyclic aromatic hydrocarbon contaminants in the second case of simulation; b) – predictions of the contaminant variations trend versus time using *MVKH4s* to *MVKH6s* substrate consumption models accompanied with Beolchini *et al.* (2010) experimental data for growth of prokaryotic communities on aliphatic and polycyclic aromatic hydrocarbon contaminants in the second case of simulation

Table 9. The SSE of microbial growth kinetic and contaminant consumption models, as well as total SSE obtained from simulations of the second case

Model	SSE of growth kinetic model	SSE of contaminant consumption model	Total SSE
MVKH1-MVKH1s-P2	27.28	0.98	28.26
MVKH2-MVKH2s-P2	13.18	1.27	14.45
MVKH3-MVKH3s-P2	23.41	6.36	29.77
MVKH4-MVKH4s-P2	59.34	0.89	60.23
MVKH5-MVKH5s-P2	18.94	0.44	19.38
MVKH6-MVKH6s-P2	222.9	0.014	222.914

Conclusion

Bioremediation is an effective, simple, and economic method for cleaning the contaminated soils and waters with petroleum compounds, industrial solvents, pesticides, and heavy metals by microorganisms. The experimental implementation of the bioremediation for evaluating different aspects of the process including biomass growth trend and contaminant degradation is extremely time-consuming and expensive. Therefore, modeling and simulation of the process could play a significant role in achieving the desired objectives. Among the presented models in the current study, *MVKH2*, *MVKH2s* and *P2* (Aiba) were introduced as the best models for predicting biomass growth, contaminant degradation and the specific growth rate constant, respectively. The achieved predictions from combination of mentioned models (*MVKH2–MVKH2s–P2*) had an apropriate agreement and the least errors to the experimental data, which indicated their efficient performance. Results obtained from this investigation could be applied to design, optimize and scale-up the bioremediation units.

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