Research Note

Biodegradation of heavy fuel oil by newly isolated strain *Enterobacter cloacae* BBRC10061: Study of unstructured kinetic models and innovative equation

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Abstract. Modeling process is very important and valuable to predict process outcome, especially bioprocesses which are intricate. Because of complex hydrocarbon compounds and oscillations happened in the medium of process, biodegradation of mazut has not already been investigated by mathematical models. In this study, an indigenous bacterium was isolated from oil contaminated soil to investigate biodegradation of mazut at different experimental conditions. Data resulted from mazut degradation, pH, and electrical potential in the medium were recorded. Some reported kinetic models and combinations were investigated to practically model the process. In addition, a new equation that can predict various phenomena, was applied to functionalize changes of mazut concentration, pH, and electrical potential. Any of the kinetic models did not have potential for modeling behavior of mazut biodegradation at different conditions. Opposed to them, the novel equation was able to predict desirable parameter, using two variables, and functionalize data by appropriate coefficients.

1. Introduction

Mazut is the residual compound outcome by crude oil distillation high viscous, and difficult to be degraded [1]. Bioprocess is suggested as an effective and economical provident ways because of its simplicity and lower cost [2-5]. Use of each area's native microorganisms makes biodegradation as the most important process [6]. Assessment of effective parameters and conditions on biodegradation, in order to design a bioremediation system, is very significant [7].

Mathematical modelling and simulating processes can be effective tools to design and develop them via de-creasing the number of different experiments designed for a process [8,9]. Prominent models have already been introduced in biosystems field. No matter how biosystems are complex and various factors contribute to them, more parameters should be used to actualize the model in order ot make the occurred events more systematic. Although simplicity of a model is an advantage for its utilizzation, accuracy of predicted results by the model has self value, and naturally simple models are less capable of predicting more accurate results [10]. To predict and model activity and behavior in a biosystem, biokinetic models are designed so that different kinetic parameters and rate curves based on real and controlled data are assessed [11,12]. Therefore, enough knowledge of microbial growth kinetics and degradation kinetics of matters are necessary to model biokinetics [13]. Simple biokinetic models have al-

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ready suggested whatever is usually adapted for special conditions and is also suitable to particular processes. Among the models, Monod model (1949) is the simplest model which has already been reported, and other models, such as Moser model (1958), Webb model (1963), Aiba et al.'s model (1968), Haldane model (1968), Yano and Koga's model (1969), Teissier model (1970) and Levenspiel model (1980) describe kinetic behavior of complex biosystems [14,15]. Active sludge systems, such as ASM1, ASM2, ASM2d and ASM3, were designed for biodegradation of pollutant [16]. Kinetic modelling for biodegradation of materials in soil can be too complicated because of the soil environment complexity, microbes' metabolism consisting of significant factors, such as nutrient for microbial growth, oxygen demand, water content, interaction between microorganisms etc. [11]. Several kinetic models have been proposed which predict the biodegradation of some different hydrocarbon compounds [11,17,18], but heretofore kinetic modelling on biodegradation of mazut has not been carried out. Moreover, so far, there has not been a model to predict events and phenomena that were categorized in several scientific fields. On the other hand, not any general mathematical statement has been reported to modulate the effect of various changes such as mazut concentration, pH and electrical potential in biodegradation of mazut. The first general predictive equation called Ch equation was presented by Chackoshian Khorasani et al., in several fields, and was able to significantly predict phenomena and changes in biotechnology, nanotechnology and polymers modification [19-21].

There are many studies on degradation of oil, heavy oil, and other petroleum compounds, but investigation on heavy fuel oil (mazut), having enormous dissimilarity from heavy oil on components, has rarely been reported before. Furthermore, there was no study on the modified medium condition for biodegradation of mazut by one indigenous bacteria strain. In addition, mathematical investigation on biodegradation of mazut has not been reported yet.

Objectives of this study were kinetic investigation on mazut biodegradation with simple, complex and new combined unstructured models, and modelling various changes in the process by first general predictive equation. To carry out this study, a newly isolated bacterium was used for degradation of mazut, and obtained data were fitted to unstructured models and Ch equation.

2. Materials and methods

2.1. Microorganism

Enterobacter cloacae BBRC10061, named NO_4 in this study, is indigenous bacteria strain isolated from oil-contaminated soil where buses were fixed and fuelled in Mashhad, Iran. It was procured from the Biochemical and Bioenvironmental Research Center (BBRC) that was a local culture collection in Sharif University of Technology Tehran, Iran. The microorganism was maintained in glycerol stock at -20°C for further use.

2.2. Experimental design and condition

Four experiments were designed based on 6 various parameters, having 4 levels, to investigate the models on biodegradation of mazut. This was because different experiments could demonstrate advantages and disadvantages of a model and the model would be more significant if obtained data from various experiments fitted into a model were more and scattered. All experiments in this study were conducted in duplicate.

A preculture mineral salt medium, used in this study was consisted of $MgSO_4.7H_2O$ 0.1 g, $CaCl_2$ 0.01 g, and $FeSO_4.7H_2O$ 0.01 g per liter and distributed in 50 mL centrifuge tubes, which were completed based on the designed experiments shown in Table 1. 2000 ppm mazut (0.1 g per 50 mL), purchased from NIOPDC (Oil Corporation, Iran), was added into every tube containing sterile mineral medium. Control for each experiment contained all of the components except mazut. After cooling, the NO_4 bacteria were inoculated in amount of 0.008 OD_{600} into mediums. For every 4 designed experiments, 5 similar medium were prepared, because all experiments were analyzed for 5 stages in 5 different times. All cultivations were then incubated for 2, 4, 6, 8 and 10 days at 33°C in a shaker incubator agitated with a speed of 160 rpm.

Table 1. Designed experiments used to study the models on biodegradation of mazut.

Experiment no.	Parameters					
	pH	Tween 80	Glucose	KH_2PO_4 + K_2HPO_4	$(NH_4)_2SO_4$	Sea salt
1	5.8	0 g/l	0 g/l	1 g/l	1 g/l	0 g/l
2	6.8	1 g/l	1 g/l	3 g/l	3 g/l	0.5 g/l
3	7.3	2 g/l	2 g/l	5 g/l	5 g/l	1 g/l
4	8.3	4 g/l	4 g/l	9 g/l	9 g/l	2 g/l

Table 2. Original and combined kinetic models used for biodegradation of mazut.

Model	Equation	Ref.	Model	Equation	Ref.
Monod	$r_s = r_{s\,\max} \dfrac{S}{K_s + S}$	[22]	Third order	$r_s = dS^3 + cS^2 + bS + a$	—
Teissier	$r_s = r_{s\,\max}\left(e^{-\frac{S}{K_i}} - e^{-\frac{S}{K_s}}\right)$	[14]	Aiba et al. & Moser	$r_s = r_{s\,\max} \dfrac{\left(e^{-\frac{S}{K_i}}\right)}{1 + K_s\left(S^{-\lambda}\right)}$	This study
Aiba et al.	$r_s = r_{s\,\max} \dfrac{S\left(e^{-\frac{S}{K_i}}\right)}{K_s + S}$	[23]	Aiba et al. & Contois	$r_s = r_{s\,\max} \dfrac{S\left(e^{-\frac{S}{K_i}}\right)}{\beta x + S}$	This study
Yano and Koga	$r_s = r_{s\,\max} \dfrac{S}{K_s + S + \frac{S^2}{K_1} + \frac{S^3}{K_2^2}}$	[14]	Aiba et al. & Contois, Moser	$r_s = r_{s\,\max} \dfrac{\left(e^{-\frac{S}{K_i}}\right)}{1 + \beta x\left(S^{-\lambda}\right)}$	This study
Haldane (Andrews)	$r_s = r_{s\,\max}\left(\dfrac{S}{K_s + S + \frac{S^2}{K_i}}\right)$	[24]	Webb & Moser	$r_s = r_{s\,\max} \dfrac{\left(1 + \frac{S}{K_i}\right)}{1 + K_s\left(S^{-\lambda}\right) + \frac{S^\zeta}{K_1}}$	This study
Webb	$r_s = r_{s\,\max} \dfrac{S\left(1 + \frac{S}{K_i}\right)}{K_s + S + \frac{S^2}{K_1}}$	[25]	Webb & Contois	$r_s = r_{s\,\max} \dfrac{S\left(1 + \frac{S}{K_i}\right)}{\beta x + S + \frac{S^2}{K_1}}$	This study
Levenspiel	$r_s = r_{s\,\max} \dfrac{S\left(1 - \frac{S}{K_i}\right)}{K_s + S}$	[26]	Webb & Contois, Moser	$r_s = r_{s\,\max} \dfrac{\left(1 + \frac{S}{K_i}\right)}{1 + \beta x\left(S^{-\lambda}\right) + \frac{S^\zeta}{K_1}}$	This study
Moser	$r_s = r_{s\,\max} \dfrac{1}{1 + K_s\left(S^{-\lambda}\right)}$	[27]	Levenspiel & Moser	$r_s = r_{s\,\max} \dfrac{\left(1 - \frac{S}{K_i}\right)}{1 + K_s\left(S^{-\lambda}\right)}$	This study
Contois	$r_s = r_{s\,\max} \dfrac{S}{\beta x + S}$	[28]	Levenspiel & Contois	$r_s = r_{s\,\max} \dfrac{S\left(1 - \frac{S}{K_i}\right)}{\beta x + S}$	This study
First order	$r_s = bS + a$	—	Levenspiel & Contois, Moser	$r_s = r_{s\,\max} \dfrac{\left(1 - \frac{S}{K_i}\right)}{1 + \beta x\left(S^{-\lambda}\right)}$	This study
Second order	$r_s = cS^2 + bS + a$	—	Contois & Moser	$r_s = r_{s\,\max} \dfrac{1}{1 + \beta x\left(S^{-\lambda}\right)}$	This study

Dimensions for some of parameters in combined models are different from what has been identified in the nomenclature.

2.3. Analytical methods

Mazut concentration was measured by spectrophotometer (S2000 UV/VIS). To extract mazut from medium, 3 mL chloroform was added into medium, and centrifuge tube was shaken and blended till all mazut was dissolved in chloroform. Two separate phases were created which contained mazut dissolved into chloroform in under phase, and mineral phase (another phase) in up. Mineral part voided of mazut, and other organic components were decanted to extract organic phase. After that, organic phase was sampled and diluted by chloroform solvent. Absorbance of mazut in medium was measured at 450 nm by spectrophotometer. Sample concentration was estimated based on absorbance-concentration curve. Also absorbance of samples at 600 nm, recorded as Optical Density (OD_{600}), was used for bacterial enumeration.

Electrical potential and pH were estimated by pH meter (3020 model, Jenway) with sampling culture medium.

2.4. Unstructured kinetic models

Degraded mazut data was fitted into various biokinetic models (Table 2). Experimental data for the degraded mazut and bacterial growth was used to validate the models. The kinetic parameters of models, which are

calculated based on experimental data, were adjusted by trial and error method. Then the models were ready to be solved. For solving models, all parameters were determined; with replacing mazut concentration and bacterial optical density in each time into the model, mazut degradation rates in each time were calculated. Besides, mazut degradation rate in each time was estimated based on difference of mazut concentrations between two times. For each time, there were one calculated rate as model answer and one estimated rate as experimental answer. Model answers were able to be changed based on the assumed parameters. Solver function in Excel program numerically solved the model equation based on the assumed parameters and given experimental answers. This method operated on minimizing square of difference between model answer and experimental answer. Solver function minimized the summation of squares of differences based on adjusting parameters which were assumed at the beginning. Therefore, models with initial assumed parameters were solved by solver function to obtain linear regression. Validation of model to investigate phenomena was related to correlation regression coefficient (R^2). After computing model parameters by solver, R^2 is calculated by Eq. (1):

$$R^2 = 1 - \frac{\sum_1^n (W' - W)^2}{\sum_1^n (W - W_{\text{ave}})^2},\tag{1}$$

where W, W', and W_{ave} are values of experimental data, model data, and average of experimental data respectively.

2.5. General predictive equation

Dimensionless number named Ch number relates specific change of every output to specific change of main input. This link is functionalized by appropriate second input which affects the output. Therefore, main and second inputs have to be selected based on the relevant data.

Ch number can be used for several objects and phenomena, because each object has one or some creators which result object by self changes. Therefore, by Ch number, output behavior can be determined based on the main and second inputs behavior. Eq. (2) shows Ch number based on output Y and main input X:

$$Ch = \frac{dY/Y}{dX/X}.\tag{2}$$

Here, Y is replaced by pH, electrical potential and mazut concentration, and for all of them, X is replaced by optical density (OD_{600}) of NO_4 bacteria. Second input is time for all outputs and Ch function is fourth order linear equation based on it. All of the coefficients in the statement are functions of system conditions. Ch

number estimated by time is given in Eq. (3):

$$Ch = A + Bt + Ct^2 + Dt^3 + Et^4.\tag{3}$$

To approach Ch equation, it should be integrated as follows:

$$Ch = \frac{dY/Y}{dX/X} \Rightarrow \frac{dY}{Y} = Ch\frac{dX}{X} \Rightarrow \int \frac{dY}{Y} =$$

$$Ch \int \frac{dX}{X} \Rightarrow \ln Y = Ch \ln X + K.\tag{4}$$

With replacing Eq. (3) to Eq. (4), Eq. (5) was resulted as Ch equation.

$$\ln Y = (A + Bt + Ct^2 + Dt^3 + Et^4)\ln X + K.\tag{5}$$

In this equation, K is a constant appeared because of integrating. Six points were considered for each assay. Ch equation had 6 parameters which could be evaluated by 6 points or more. Therefore, 6 equations, provided with 6 points, were solved to calculate 6 parameters of Ch equation. All of the parameters in the Ch equation are determined by fitting experimental data into the equation. The equation to evaluate constants was solved by the method mentioned for solving unstructured models. This method is easier to be employed than other mathematical methods, such as direct solving that operates on equation with number of variables being equal to constants.

For using Ch equation, we had to notice simplifier assumption. According to the data fitted into the equation, curve fitting resulted in identified conditions and was depended on certain parameters. Consequently the equation was able to predict changes based on the parameters, but naturally using the equation based on other parameters was not able to respond. In definition of Ch number, specific change of output and main input was assumed positive. In trend of integrating, it was assumed that the fourth order linear equation, being function of time, was constant; so it was only integrated on main input and output, and Ch equation as a constant number exited from integral.

3. Results

3.1. Mazut degradation

In this case, various bacteria were isolated from oil-contaminated soil to identify powerful strain, because indigenous strains, isolated from environment contaminated by petroleum compounds, are able enough to efficiently degrade mazut without modifying it genetically. NO_4 bacteria with other bacteria strains were isolated, but according to experiments, clearly investigated in Institute of Biotechnology, Ferdowsi University of Mashhad, this strain was detected as

more efficient strain to degrade mazut. Although employing only one microorganism is not able to degrade mazut quickly, but modifying culture medium, which is cheaper and more facilitative than genetically changing, can compensate this deficiency.

Experiments 2-4 were designed to improve experiment 1. Results show that adding sufficient ingredients, which are necessary to bacterial growth or helpful to dissolve mazut, caused high yield of mazut degradation. Amount of elements in assay 2 did not satisfy assays 3 and 4. Therefore, the addition of necessary ingredients cannot always increase efficiency; rather proper amount of them is able to increase the yield. According to Figures 1 and 2, until 4th day, degradation rate in assay 2 is more than assay 1, but after that, it reduces, because, at the beginning, Tween 80 dissolved mazut into medium of assay 2, but after 4 days, the bacteria interned into stationary phase; production of biosurfactant as secondary
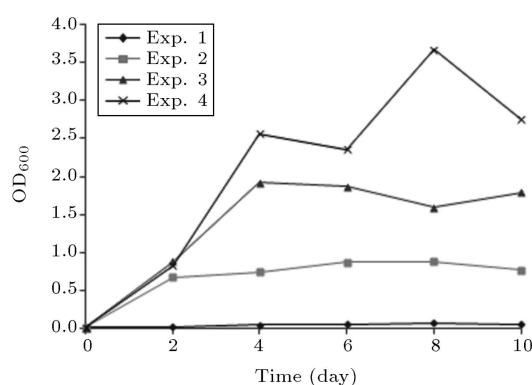

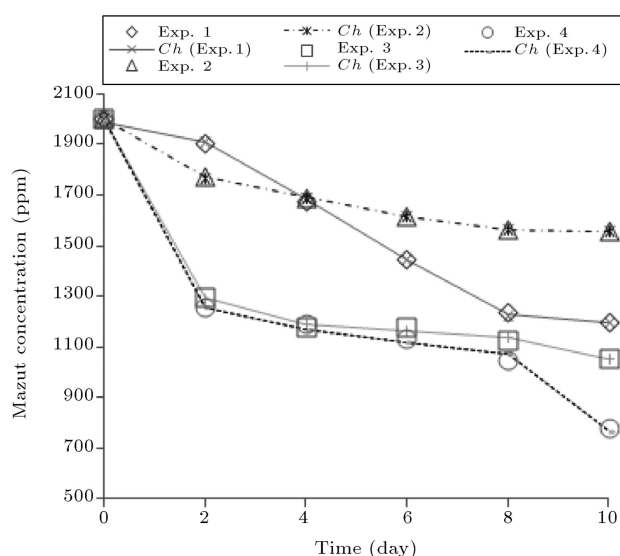**Figure 1.** Growth of NO_4 bacteria during degradtion of mazut in experiments 1-4.

Figure 2. Experimental and Ch equation data for mazut concentration changes during biodegradation by NO_4 bacteria in experiments 1-4.

metabolite, which is stronger than Tween 80, began in assay 1. Tween 80, during assays, inhibited production of biosurfactant, which is a prominent reason for the differences between results of assays 1 and 2 after 4th day of the experiment. Although, Tween 80 prohibited production of biosurfactant, its high concentration in assays 3 and 4 was able to dissolve high concentration of mazut, which compensated biosurfactant famine. Moreover, high concentration of Tween 80 (the is high concentration of glucose and mineral elements) rapidly increased bacterial growth, and caused fast production of biomass in assays 3 and 4. The more the biomass as biocatalyst degrading mazut increases, the higher the degradation rate becomes. Consequently, assay 4 was the best medium to biodegrade mazut by NO_4 bacteria. In this study, glucose as a secondary source of carbon helped bacteria to grow, which became a negative factor for degrading mazut. Addition of glucose enriched the bacteria to gain needed carbon, which increased reluctance among bacteria to degrade mazut as carbon source. Due to this fact, there were fabricated growths for bacteria in assays 2-4, and efficiency of mazut biodegradation confirms this phenomenon. Therefore, prominent reason of bacterial growth difference between assay 1 and other assays was originated from secondary carbon source that was glucose.

Finally, production of biosurfactant was the main reason of high degradation of mazut in assay 1. In assay 2, the prominent causes of not satisfying degradation were lack of necessary elements and not producing biosurfactant. Enough ingredients determined the well-known reason of high degrees of biodegradation in assays 3 and 4.

3.2. Unstructured models

Analysis of the results of the original kinetic models is presented in Table 3. Calculated correlation coefficients perform accuracy of the models.

According to Table 3, the ability of the models varied based on the experiments, and naturally dominant conditions on the assays caused vicissitude of degradation behavior. Therefore, the original models differently responded to several degradation behaviors. Among the models, second and third order models accurately correlated with mazut degradation data. Among experiments, experiments 2 and 3 were fitted into the models. Therefore, any of the models could not significantly predict all of the designed biodegradation assays.

Experimental data were assessed by the combined kinetic models (Table 4). Different linear correlations were shown no model predicted the assays, although experiments 2 and 3 were fitted in the all combined models. An appropriate model has to modulate a

Table 3. Correlation coefficients (R^2) for original kinetic models.

Exp. no.	Monod	Teissier	Aiba et al.	Yano and Koga	Haldane (Andrews)	Webb	Levenspiel	Moser	Contois	First order	Second order	Third order
1	0.06	0.1	0.1	0.1	0.11	0.08	0.1	0.34	0.00	0.03	0.91	0.87
2	0.93	0.93	0.93	0.93	0.93	0.94	0.92	0.93	0.93	0.93	0.95	0.96
3	0.94	0.94	0.94	0.94	0.94	0.97	0.94	0.94	0.99	0.94	0.99	0.98
4	0.53	0.53	0.53	0.53	0.53	0.66	0.53	0.5	0.85	0.53	1	1

Table 4. Correlation coefficients (R^2) for combined kinetic models.

Exp. no.	Aiba et al. & Moser	Aiba et al. & Contois	Aiba et al. & Contois, Moser	Webb & Moser	Webb & Contois	Webb & Contois, Moser	Levenspiel & Moser	Levenspiel & Contois	Levenspiel & Contois, Moser	Contois & Moser
1	0.31	0.00	0.28	0.08	0.03	0.04	0.03	0.00	0.03	0.42
2	0.93	0.93	0.92	0.93	0.93	0.93	0.93	0.93	0.93	0.92
3	0.95	0.99	1	0.97	0.99	0.99	0.95	0.99	0.95	0.99
4	0.55	0.85	0.86	0.71	0.85	0.86	0.53	0.86	0.53	0.86

process at various conditions. However, none of the models were capable of modulating behavior of the experiments prepared at different conditions.

3.3. Ch equation

Mazut concentration, pH and electrical potential in the medium were investigated by Ch equation.

Figure 1 shows that the rate of bacterial growth (OD_{600}) in the designed assays was different, but trends of growth were similar to the standard growth curve. The bacterial growth increased with increasing components concentrations in the medium. Experimental data was completely fitted into Ch equation (Figure 2). As shown in Table 5, in experiment 1, correlation between model and experiment was less than others, but it was accurate enough. In general, Ch equation could appropriately predict behavior of biodegradation of mazut in various conditions; consequently, Ch equation performed better than other unstructured models.

Various trends of pH changes in the assays (Figure 3) indicate expectedly different behaviors. Modulating data by Ch equation follow the fact that the 4 assays were different in behaviors and conditions, because coefficients estimated for the equation were various altogether (Table 6). Therefore, the dominant behavior of the experiments, which were dependent on the behavior of the model was evaluated by them. By correlation coefficients estimated for pH data, enough accuracy and sufficiency of Ch equation were demonstrated to predict pH behavior in mazut biodegradation.

Nevertheless, changes of electrical potential in the medium were more during biodegradation of mazut, shown in Figure 4, but they adhered to a certain behavior, because Ch equation could model them by a fourth order linear equation. Correlation coefficients calculated in Table 7, described the amount or degree of the equation accuracy.

Tables 5, 6 and 7 present equation's coefficients obtained from curve fitting assays into Ch equation. Normally, the coefficients for each assay were different, because they depended on conditions not on behavior.

**Table 5.** Ch equation's coefficients and correlation coefficients (R^2) for mazut concentration data.

Experiment no.	Ch equation's coefficient						Correlation coefficient (R^2)
	E	D	C	B	A	K	
1	-0.00016	0.002626	-0.01071	0.019207	-0.02926	7.459189	0.99
2	-0.00093	0.01254	-0.02798	0.002318	-0.02924	7.459701	1
3	-2.6×10^{-5}	0.000725	-0.00971	0.026649	-0.09279	7.152896	1
4	-0.00105	0.020638	-0.13299	0.270808	-0.09147	7.159107	1

Table 6. Ch equation's coefficients and correlation coefficients (R^2) for pH data.

Experiment no.	Ch equation's coefficient						Correlation coefficient (R^2)
	E	*D*	*C*	*B*	*A*	*K*	
1	0.00014	-0.00241	0.011608	-0.01295	-0.00831	1.714582	0.99
2	0.000494	-0.00505	-0.00207	0.014209	-0.042	1.716339	0.99
3	-2.4×10^{-5}	-7.5×10^{-5}	0.001557	0.022507	-0.05165	1.738363	1
4	0.001095	-0.0241	0.16255	-0.30086	-0.0789	1.733329	0.96

Table 7. Ch equation's coefficients and correlation coefficients (R^2) for electrical potential data.

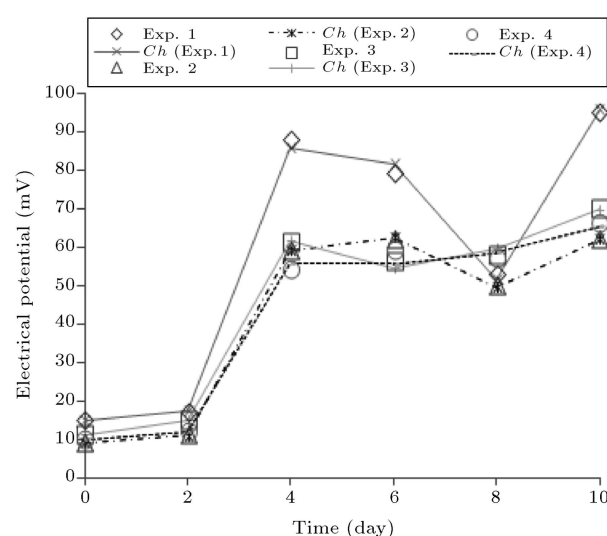| Experiment no.	Ch equation's coefficient						Correlation coefficient (R^2)
	E	*D*	*C*	*B*	*A*	*K*	
1	-0.00181	0.034829	-0.20425	0.368923	0.94235	7.253932	1
2	-0.01898	0.421703	-2.90006	5.571242	0.280926	3.554029	1
3	-0.00616	0.134381	-0.9547	2.472116	0.120818	2.981668	1
4	0.003959	-0.07602	0.410914	-0.31483	0.058836	2.588544	1
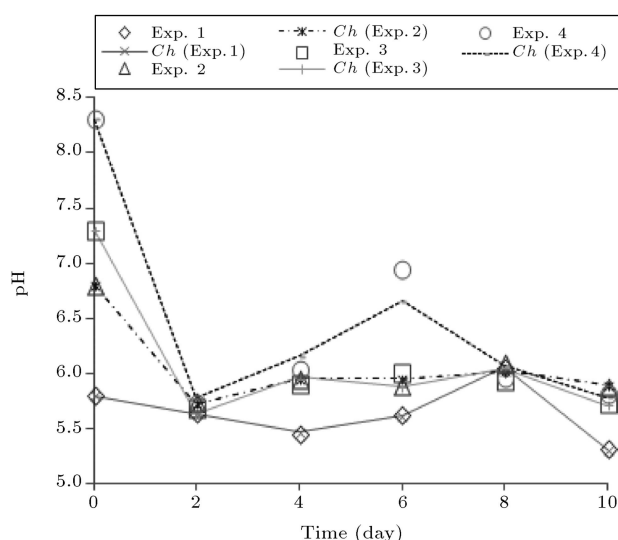
Figure 3. Experimental and Ch equation data for pH in the medium during biodegradation of mazut in experiments 1-4.

Figure 4. Experimental and Ch equation data for electrical potential in the medium during biodegradation of mazut in experiments 1-4.

So a mathematical statement predicted process behavior, while its coefficients described process condition.

In conclusion, for the first time, one mathematical statement was capable of functionalizing various phenomena happened in a bioprocess.

4. Discussion

Basically, trends of changing in a microbial system are fluctuated in the beginning of a growth. After that, as far as the process proceeds into the end, medium changes, such as pH and electric potential changes, constantly follow a specific trend. Consequently, to decrease the number of assay analysis, experiments must be considered to assign a specific trend by limited data. Therefore, to model a bacterial growth and change in its medium, using limited experimental data, which demonstrate reasonable trends, can be satisfying. According to the Ch equation with six parameters, the least experimental points which must be six, were noticed; however, more number of experimental data could cause accurate modeling. The six points were sufficient to calculate parameters of the equation. Trends of changing in the medium were reasonably obtained by the six data. Thus, in this study, more data were not necessary to evaluate the parameters of the equation.

Capability of mathematical statement to predict

phenomena is reasonably demonstrated by various experimental data at different conditions. In this case, 4 assays with dissimilar medium were prepared. The experimental data consisted of 24 points which were elicited from various conditions. Correlation coefficient (R^2) represented quality of fitting data on model. If all of the correlation coefficients computed for 4 assays were close to 1, ability of model to predict real results is confirmed; because values of four R^2 indicate accuracy of fitting 24 data on the model. However, in similar studies, curve fitting were reported based on less than 20 experimental data [13-15,23].

Degradation of various hydrocarbons has been predicted by kinetic models. Michaelis-Menten modeled degradation of aromatic compounds that carried out by mixture of filamentous bacteria [29]. Degradation of 4-chlorophenol was modeled by Edward [14] and phenol degradation [13,30,31], degradation of 4-aminobenzene sulfonate [32] and degradation of TNT [33] were predicted by kinetic model of Haldane. Aerobic degradation of olive oil industry wastewater was modeled by Contois model [34]. Biodegradation of phenanthrene was significantly predicted by first, second and third order linear models, and among them, the third model was better [35]. Bioremediation of oil contaminated soil in biopile system was predicted by first order model [11]. Also some of bioprocesses were predicted by combined kinetic models. Alcohol production was modeled by combined models such as Haldane and Levenspiel, Haldane and Luong, Moser and Levenspiel, and Moser and Luong [23]. So capability of kinetic models to describe biodegradation of some hydrocarbons was acceptable, but mazut, because of complexity in its components, was not completely modeled by the unstructured kinetic models. Therefore, to model mazut biodegradation, novel models have to be provided and investigated. Except kinetic models, there were other models which were stated based on mass transport. They were usually more complex and described phenomena with special parameters and variables. Changes of hydrocarbons concentration from point of water pollutant were modeled by mass transport, isothermal adsorption and first order degradation rate equations. In this model, hydrocarbon concentration was calculated based on initial concentration, water flow rate and hydrocarbons place [36]. Intricacy of these models causes their application and usefulness get less than kinetic models; due to this fact, simplifying assumptions which lead to the decrease of accuracy are used. On the other hand inability of original and combined kinetic models in modeling biodegradation of mazut caused the first general predictive equation, called Ch equation, which was offered to cover estimating biodegradation of mazut at various conditions. Supremacy of Ch equation against other models is that it can model events in a process which is somehow complex and unrelated to each other. There was not a similar equation that can accurately perform curve fitting into different fields, and this is the first general mathematical statement.

5. Conclusion

Experimental data to study the fitness of a mathematical statement have to be tested at different conditions. The biokinetic models were not able to predict biodegradation of mazut at various conditions, but an appropriate equation was utilized, which was able to predict pH and electrical potential changes in the medium beside mazut biodegradation. Ch equation instead of various models can be sufficient to functionalize phenomena, prevent wasting time for finding out relationships between natural phenomena, and derive confusing equations and models. Application of this equation to other fields can also be promoted, and we are optimistic that as the first general predictive equation, it would predict more intricate processes.

Nomenclature

a	Constant of linear models (mgl^{-1} day^{-1})
A	Coefficient of Ch equation (dimensionless)
b	Constant of linear models (day^{-1})
B	Coefficient of Ch equation (day^{-1})
c	Constant of linear models (mg^{-1} l day^{-1})
C	Coefficient of Ch equation (day^{-2})
d	Constant of linear models (mg^{-2} l^2 day^{-1})
D	Coefficient of Ch equation (day^{-3})
E	Coefficient of Ch equation (day^{-4})
K	Constant of integral (dimensionless)
K_s	Half-saturation constant of mazut (mgl^{-1})
K_i	Inhibition constant (mgl^{-1})
K_1	Inhibition constant (mgl^{-1})
K_2	Inhibition constant (mgl^{-1})
r_s	Rate of mazut degradation (mgl^{-1} day^{-1})

$r_{s\,max}$	Maximum mazut degradation rate $(\mathrm{mgl}^{-1}\,\mathrm{day}^{-1})$
S	Mazut concentration (ppm)
t	Time (day)
x	Microbial optical density (dimensionless)
X	Main input such as microbial optical density (dimensionless)
Y	Output such as mazut concentration (ppm), pH (dimensionless), electrical potential (mV)
β	Constant of contois model (mgl^{-1})
γ	Constant of Moser model (dimensionless)
ζ	Constant of some of the combined models (dimensionless)

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