Indication of Biocatalytic Mechanisms in Environmental Remediation by Statistical Methods

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Abstract – There is promising feasibility for environmental remediation of the soil that is called bioremediation. Its operation underlies the capability of biological systems that some enzymes are also able to demolish a number of *xenobiotic* compounds since they have such conformations which are very similar to those of natural substrates of the enzymes. Based on the dependences of biochemical reactions on the substrate and enzyme concentrations as well as on the Michaelis constants, the speed of degradation can be optimized and the bioremediation efficiency can in turn be maximized. Modelled by Michaelis kinetic is also obvious that the effects of inhibition on enzymatic reactions influence the monitoring the kinetic curves of the degradations of the pollutants. Inhibition mechanisms appearing in the enzymatic bioremediation can be detected in the parameter alterations of the regressions of the various kinetic curves. The correlations and the regressions relationships of kinetic curves of the *xenobiotic* pollutants taking part in the simultaneous biochemical degradations are potential information sources. After data pre-processing, there is a possibility to carry out principal component analysis (PCA) on the data set (object matrix) being created from kinetic data series. With its application, an alternative method is introduced for the detections of inhibition and/or activation mechanisms.

Keywords: bioremediation / enzyme inhibition / principal component analysis / correlation analysis

1. INTRODUCTION

The environmental pollutions from industrial productions are frequent all over the world. Their elimination demands a relevant effort involving expenditures of lots of time, energy and cost. The remediation of the soil and ground water caused by chlorinated hydrocarbon derivatives is one of our most important tasks. These chemicals are widespread utilized for scouring the surface of metals, cleaning and rinsing away the painted coatings. Although, their solubility in water is small nevertheless their getting out has a non negligible risk to the environment since they are able to impair the health in the range of small concentrations, too (EPA 2011). Demolition of organic pollutants by enzyme catalysis became more and more important in the environmental remediation in the last decades. Bioremediation researches

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intensively go in for the utilization of enzyme products to the degradation of hydrocarbon pollutants in groundwater (ANTON 2010). The success of enzymatic remediation depends on the potential inhibition effects of the pollutants that can reduce the enzyme activities. A key problem in the applications of bioremediation technology is originated from the large scale variety of environmental pollution since the various pollutants have specific chemical and physical properties. Designability of bioremediation technology and estimation of their degradation time call for mapping the possible inhibition and/or activation mechanisms of actual demolition. Based on Michaelis-Menten kinetic data series of degradation of some chlorinated hydrocarbons, our paper focuses on the statistical methods that are able to reveal the presence of latent inhibition or activation from the modelled biodegradations. Inhibition or activation mechanisms during enzymatic demolition can influence the correlation of kinetic curves as well as their regression. Their modifying effect appears in the monotonies of kinetic curves and in the parameters of their regressions. Inhibition can decrease the monotony while activation increases it. Application of principal component analysis (PCA) to kinetic data series of individual substrates also results in extra information that is shown in loading plot diagrams.

2. MATERIALS AND METHODS

Detection of possible enzymatic mechanisms is represented through degradations of dichloromethane (DCM) and dichloroethane (EDC). To form kinetic model of their dehalogenation, the next hypothesis has been established (LEISINGER–KOHLER-STAUB 1990, BLOCKI et al. 1994, LEISINGER et al. 1994):

- Heterolytic breakdown of C–Cl σ bond can be rendered during the enzymatic degradation of chlorinated hydrocarbons and it is the rate-limiting step, which can be model by quasi Michaelis- Menten kinetics.
- Glutathione as a cofactor is necessary to the degradation.
- The other components of reaction medium like various anions (Cl^-, F^-, SO_4^{2-}, etc) can prevent the dehalogenation.

In the case of meeting the limitations above, the decrement of concentration of chlorinated hydrocarbon (*S*) is proportional to concentration increment of the intermediate (*P*) accruing from it. Decreasing the amount of chlorinated compound in time can be expressed by equation

$$\frac{dS}{dt} = \left(-\frac{dP}{dt} \right) = -v_{max} \frac{S}{K_M + S} \tag{1}$$

, where *S* symbolizes the chlorinated substrate, K_M is the Michaelis constant of dehalogenation, v_{max} is maximal reaction rate of dehalogenation step. By applying the equipment 1 to model the enzymatic degradation of the substrates DCM and EDC, a mathematical model including differential equations 2.1 and 2.2 was used to generate kinetic curves.

$$\frac{d[S_{DCM}]}{dt} = -\frac{k_{DCM} \times E_{0DCM} \times [S_{DCM}]}{K_{M_{DCM}} \times \left(1 + \frac{[S_{EDC}]}{K_i} \right) + S_{DCM}} \tag{2.1}$$

$$\frac{d[S_{EDC}]}{dt} = -\frac{k_{EDC} \times E_{0EDC} \times [S_{EDC}]}{K_{M_{EDC}} + S_{EDC}} \tag{2.2}$$

, where k is the reaction rate constant of given substrate, E_0 is total enzyme concentration, K_M is the Michaelis constant and K_i is the constant of competitive inhibition. Because of the inhibitor in equation 2.1 is also the substrate in equation 2.2 the inhibitor concentration is decreasing during the demolition. The solutions of the model were equally reproduced to the conditions with and without inhibition. In inhibition free case, the term including K_i was omitted from equation 2.1. The kinetic data series of the substrates (S=$S(t)$) were supplied by ODE solver inserted in SCILAB 5.3.3 software. To allow for stochastic effect of analytical acquisitions of kinetic curves, a randomized error as modelled noise in the range of about 2.5 % of the average concentration was superposed to the primary solutions of the differential equations (*Figure 1.*).

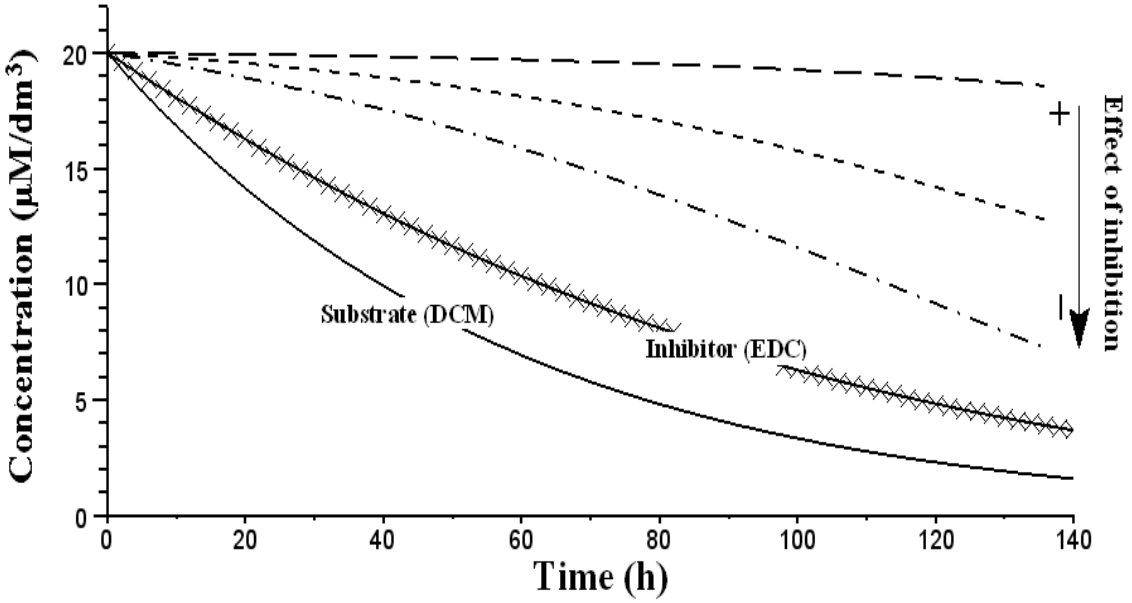


Figure 1. Effect of inhibition on the degradation of substrate (DCM), (Kinetic curves under inhibitior EDC with broken lines)

3. RESULTS

3.1. Regression analysis of kinetic curves for detection of inhibition

Temporal data series of substrate concentrations that are resulted by similar degradation mechanism linearly correlate to each other. Both the correlation of the substrates and their regression straight line are potential indicators of inhibition or activation effects. For example, the effect of the inhibition on the parameters of linear regressions can be detected by covariance analysis. Significant alterations of the slope and the intercept can reflect the appearance of inhibition mechanism in an exact way. Temporal alteration of substrate concentration can modify. The monotony of kinetic data under inhibition deviates from that of inhibition free. In the case regression of DCM and EDC kinetic curves, inhibition effects on the slope and the intercept are depicted in *Figures 2.a.* and *2.b.*

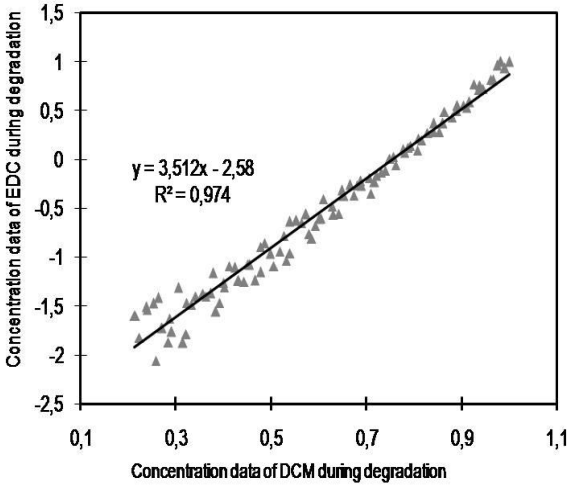


Figure 2.a. Correlation between DCM and EDC without inhibition

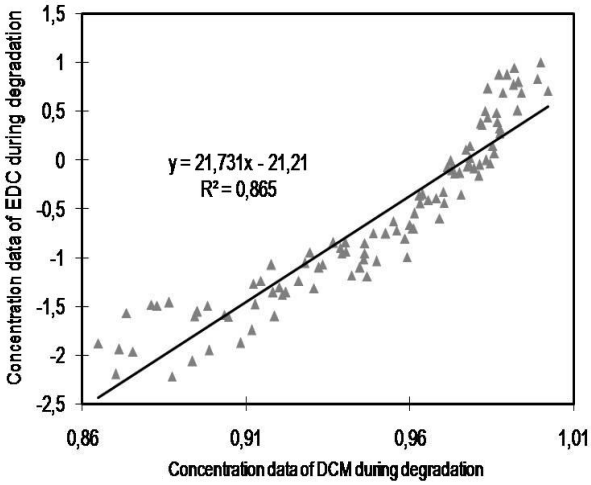


Figure 2.b. Correlation between DCM and EDC data data under inhibition

3.2. Detection of enzymatic mechanisms by principal component analysis

The data matrix created from kinetic curves of substrate concentrations can also be investigated by principal component analysis (PCA) after such data pre-processing methods as the centering or standardization. The columns of data matrix contain the concentration values of the actual substrates while the rows as the objects of given temporal sampling determine the concentration data of the various substrates at the given sampling times. PCA is able to map the structure of the total variance of object swarm in the coordinate systems of various substrate concentrations (score space). Moreover, the PCA can reveal the importance of substrate concentrations as data properties in the object space (loading space). The PCA method is an matrix decomposition method where the pre-processed data matrix, for example, by centering is decomposed to the product of two matrices, namely, to that of score and loading matrices ($\mathbf{X}_c = \mathbf{T}\cdot\mathbf{P}^T$). The columns of data matrix as variable properties (substrate concentrations) determine points in the space spanned by the column vectors of **P** matrix. The projection of the points of substrate concentrations into the plan fixed by the first two vectors of **P** matrix provides the loading plot of the PCA method. Since the inhibition decreases substrate concentrations at all sampling times the steric positions of data properties will necessarily change in the loading plot of first two principal components. There can be established some regularity in the shifting the points of various substrate concentrations under inhibition in the loading plot.

In the case of the inhibition, the points of substrate concentrations in the loading plot have smaller contributions to second principal component ($\mathbf{p}_2$) than the reference points (S_1, S_2) without inhibition (*Figure 4.a.*). Furthermore, their positions in the fourth quadrant come more and more closer to the origin with the decrement of inhibition constant K_i, that is, with the increment of the effect of competitive inhibitor (S_2). On the contrary, the activation enhances the contribution of the points to $\mathbf{p}_2$ component (*Figure 4.b.*).The points of reference substrate concentrations (S_1 and S_2) and the points of substrate concentration (S_3 to S_8) under various measures of competitive inhibition or activation effects are depicted in loading plots of *Figures 3., 4.a.* and *4.b.*. S_1 is symbolizes the substrate DCM while substrate S_2 (EDC) in the second degradation process (equation 2.2) has an inhibitor role in the equation 2.1. The points of S_3 to S_8 represent the DCM demolition under various extent of inhibition carried out by substrate S_2.

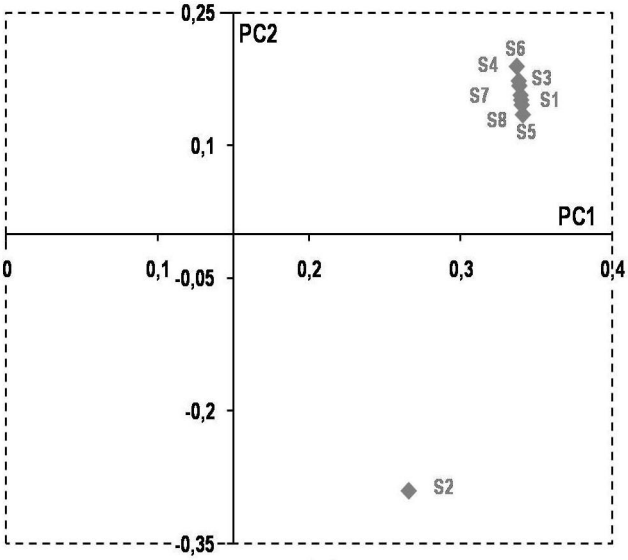
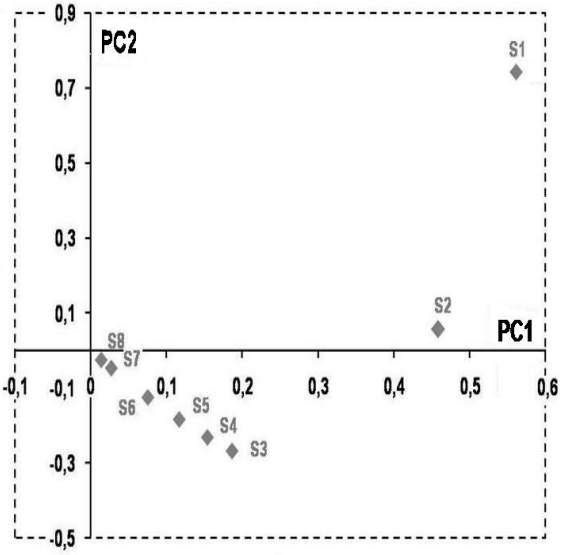
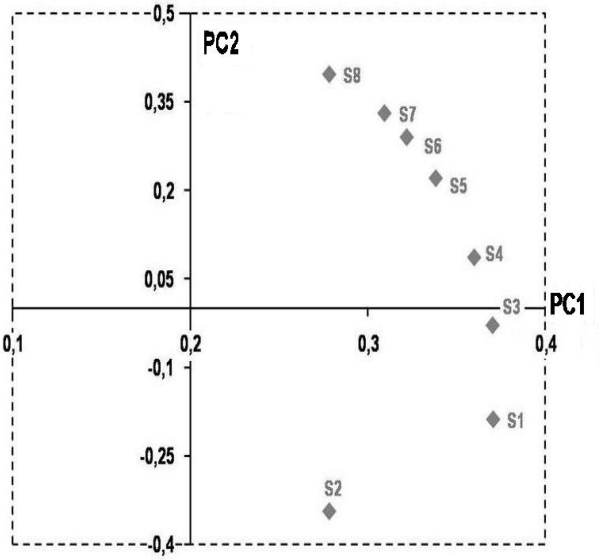


Figure 3. Substrate points without inhibition and activation in the loading plot of PC1 and PC2



Figures 4.a. Substrates under inhibiton effects (S_3 to S_8) in the loading plot of PC1 and PC2



Figures 4.b. Substrates under activation effects (S_3 to S_8) in the loading plot of PC1 and PC2

4. CONCLUSION

Modelled the enzymatic demolition of concrete pollutants, applications of some statistical methods have been developed to the recognition of the potential mechanisms modifying the velocities of enzymatic degradations. Appearance of inhibition effects can be made to be detected by assessing the correlations of kinetic data series belongig to different substrates. In the case of inhibition, the regressions of these data series reflect the decrement of enzyme activities. Presence of the inhibition become visible in the increment of the slope and in the decrement of the inercept. Application of PCA method provides an alternative way to indicate the enzyme mechanisms. This evaluation is based on revealing extra information out of loading plot diagrams where the substrate concenctrations free from or under inhibition can be separated from each other. The effects modifying the enzyme activities can change the positions of substrate concentrations in the loading plot in significant manner.

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References

ANTON A. (2010): Kármentesítési kézikönyv 5. Bioremediáció: Mikrobiológiai kármentesítési eljárások. [Manual for Biological Remediation Techniques.] Környezetvédelmi és Vízügyi Minisztérium, Budapest. 53-61 p.

BLOCKI F. – LOGAN M.S. P. – BAOLI C. – WACKETT L. P. (1994): Reaction of rat liver glutathione S-transferases and bacterial dichloromethane dehalogenase with dihalomethanes. Journal of Biological Chemistry 269 (12): 8826-8830.

EPA (2011): Basic Information about Regulated Drinking Water Contaminants and Indicators 2012. United States Environmental Protection Agency, Washington, D. C., USA. Online: http://water.epa.gov/drink/contaminants/basicinformation/

LEISINGER T. – BADER R. – HERMANN R. – SCHMID-APPERT M. – VUILLEUMIER S. (1994): Microbes, enzymes and genes involved in dichloromethane utilization. Biodegradation 5 (3-4): 237-248.

VUILLEUMIER S. – LEISINGER T. (1996): Protein engineering studies of dichloromethane dehalogenase/glutathione S-transferase from Methylophillus sp. strain Dm 11. Ser12 but not Tyr6 is required for enzyme activity. European Journal of Biochemistry 239 (2): 410-417