University of West Hungary

Theses of PhD dissertation

Investigation of biocatalytic processes in the environmental strategy

Zoltán Herke

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University of West Hungary
Faculty of Forestry
Kitaibel Pál Doctoral School of Environmental Science

Chief:
Prof. Dr. Márton Veress DSc.

Bioenvironmental Science Program
Program leader: Prof. Dr. Levente Albert

Supervisors:
Dr. Zsolt István Németh
Dr. Tibor Cserny

Introduction

Bioremediation technologies have been used widespread in the biotechnological field of environmental applications during the preceding decades, which are based on the degradation of contaminants by enzymes from the microorganisms. The biocatalytic or bioremediation technologies have several advantages over the other physical or chemical ways of rehabilitation, e.g. relative cost-effectiveness; using them *in situ* causes less damage to the original function of land and they can be well connected with other environmental processes, respectively.

However, it should be said that the applicability of these microbial operations are limited by numerous factors, e.g. they are time-consuming and sensitive to environmental conditions. The most important factor from the point of view of my research work is their dependency from peculiarities of the contaminated media and contaminants.

An effective execution and optimization of the biocatalytic environmental strategies requires clear understanding of the biochemical degradation processes.

The aim of my doctoral thesis was therefore to investigate the detection of substrate inhibition and activation effects that are capable of influencing the rate of enzymatic degradation using model and experimental data series.

Calculation- and experimental methods

Modelling of biodegradation

The biodegradation reactions are described by the Michaelis–Menten equation:

$$\frac{d[S]}{dt} = \left(-\frac{d[P]}{dt}\right) = -v_{max} \cdot \frac{[S]}{K_M + [S]} \quad , \qquad (1)$$

where S – the concentration of model contaminant, as initial substrate (in mmol/L), *P* – the concentration of product, K_M – the Michaelis-constant of the biodegradation step, v_{max} – the maximal reaction rate of the biochemical reaction. The results of the model biocatalytic degradation curve are depicted in *Fig. 1*. These kinetic results are involved the feasible mechanisms of various reversible inhibitions and enzyme activation during demolition of the model substrates. Different solutions of degradation model have also been generated by Scilab algorithms. A realistic noise (with different amplitude of concentration mean) was superposed to the data series of the substrates.

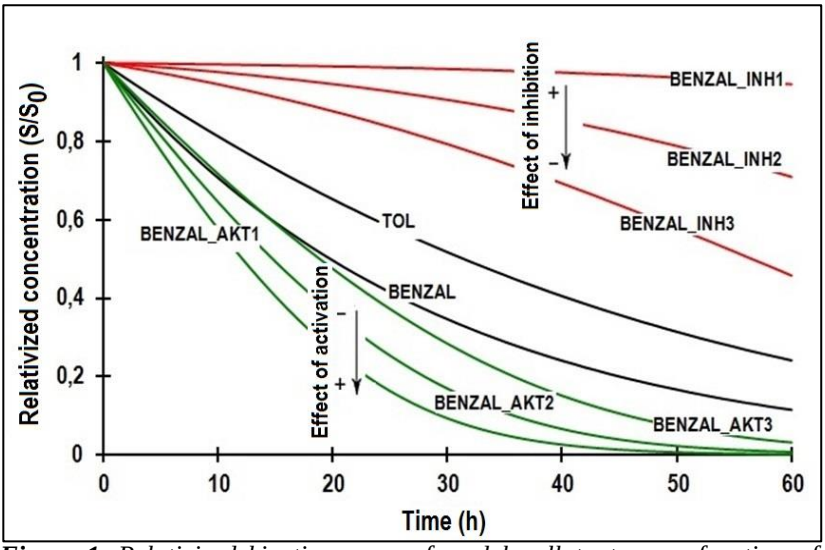

Figure 1. *Relativized kinetic curves of model pollutants as a function of time. BENZAL and TOL stand for benzaldehyde and toluene, respectively. The kinetic curves were calculated without inhibition. BENZAL_INH1-2-3 show inhibition effects of varying strength due to the interaction with toluene. BENZAL_AKT1-2-3 show activation effects of varying strength due to the interaction with* K^+ *ion.*

Estimation of the kinetic parameters by regression models

The relation (1) is a first order difference equation, which is transformable at the $t = 0$, $S = S_0$ initial value to the following (1.1) implicit equation:

$$K_M \cdot ln\frac{[S]}{[S_0]} + S - S_0 = v_{max} \cdot t \ , \qquad (1.1)$$

On the basis of the (1) and (1.1) equation, the values of kinetic parameters (K_M and v_{max}) can be concluded from the measuring/model results of the S substrate. After the Lineweaver–Burk linearization of the (1) equation, the *dt/dS* and the *1/S* quantities correlate with each other. The K_M and v_{max} kinetic parameters can be estimated from the regression slope and the intercept of the reciprocal data. In the case of this eval-

uation the numerical derivation of the $S = S(t)$ kinetic curves is necessary. According to the S implicit term of equation (1.1) it is transformable into a quasi-multivariate, linear relation. Using the following variables, $Y = \ln(S/S_0)$ és $X = S$ we can write this formula:

$$K_M \cdot Y + X - S_0 = -v_{max} \cdot t \ , \qquad (1.1.1)$$

which determines a three-dimensional *(Y,X, t)* line. The multivariate regression coefficients of the (1.1.1) equation provide opportunity to estimate the kinetic parameters by this alternative method. The estimation accuracy of the two different methods was tested in my research using different extents of noise effect and different initial substrate concentration.

Biocatalytic experiments

Potential environmental pollutants, e.g. benzol, toluene, benzaldehyde were applied in the biocatalytic experiments, besides, they were degraded by extracts of earthworm and also by a specific breakdown product. The biodegradation of pollutants was investigated individually as well as in mixtures – in case of inhibition effects – and in the presence of KCl or glutation for the sake of the activation effects. The bioconversions of the pollutants were quantified using gas chromatography.

New scientific results

Thesis 1

The kinetic parameter estimation of the biochemical processes can be executed more precisely using the elaborated multivariate linear regression method in contradiction to the Lineweaver-Burk double reciprocal plot.

The multivariate linear approach (*1.1.1 equation*) eliminates the distortion errors of derival calculation and its results so that the extreme big outliers cannot manifest in the database. This method has an additional advantage; it can partly decrease the applied noise effect. On the basis of all this the multivariate linear approach enables to execute more precise estimation. This interplay is illustrated by Figures 2.a-2.b in case of the prediction $-(K_M/v_{max})$ kinetic parameter using the two different estimation methods.

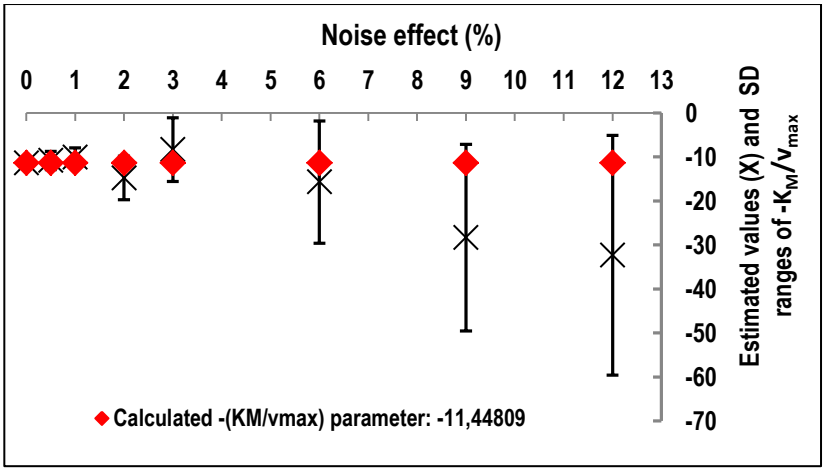

Figure 2.a. *Estimation of the $-(K_M/v_{max})$ parameter and their standard deviation renges using the multivariate linear approach with different noise effect*

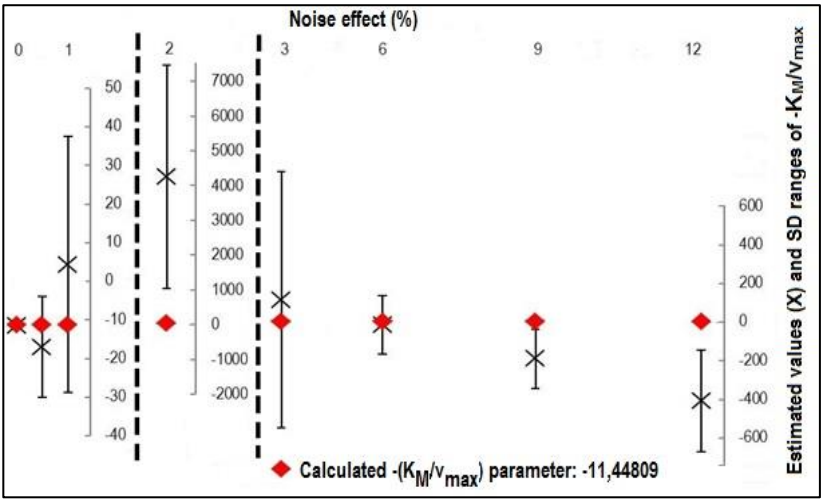

Figure 2.b. *Estimation of the -(K_M/v_{max}) parameter and their standard deviation ranges using the Lineweaver–Burk approach with different noise effect*

Thesis 2

The theoretical error analysis of *Δrv* (error of reciprocal maximal reaction rate), namely

$$\Delta \boldsymbol{rv} = \Delta \boldsymbol{h_t} \cdot \frac{\boldsymbol{K_M}}{\boldsymbol{S_0}} + \left(\frac{\boldsymbol{K_M}}{\boldsymbol{S} \cdot \boldsymbol{S_0}} + \frac{\boldsymbol{K_M}}{\boldsymbol{S_0^2}} + \frac{\boldsymbol{S}}{\boldsymbol{S_0}} \right) \cdot \Delta \boldsymbol{h_s} + \frac{\boldsymbol{K_M}}{\boldsymbol{S_0}} \cdot \ln \left(\frac{\boldsymbol{S}}{\boldsymbol{S_0}} \right) \cdot \frac{\Delta \boldsymbol{Kv}}{\boldsymbol{Kv}}$$

was verified using model data also. In case of lower substrate concentration the precision of the estimation depends on the measuring error of substrate concentration.

The dependence of the maximal reaction rate (v_{max}) estimation on the initial substrate concentration is depicted in the *Figure 3.*

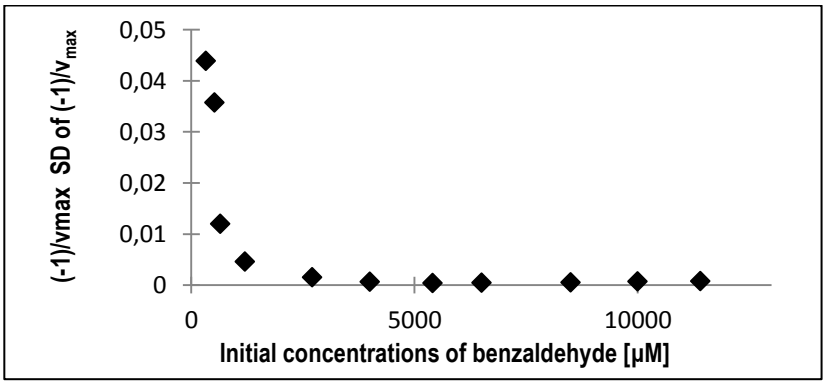

Figure 3. *The effect of benzaldehyde initial substrate concentration on the estimation of -1/v_{max} kinetic parameter standard deviation values. The noise effect is 5%.*

This conclusion indicates also one of the weaknesses of the Lineweaver–Burk linearization method, namely that the biggest measuring errors of the initial reciprocal reaction rate will occur at the smallest substrate concentration, thereby the parameters of fitting straight line will be modified and the estimation will become inaccurate.

Thesis 3

Temporal data series of pollutant concentrations that are resulted by similar degradation mechanism (single or mixed) can be linearly correlated to each other. The parameters of fitting regression straight line (slope and intercept) are changed significantly by the enzyme modification effect.

According to the alteration of reference regression straight line parameters the inhibition or activation effects can be determined. In case of inhibition the slope of straight line is increasing and the intercept is decreasing while in case of activation the alteration of the parameters occurs in the opposite direction. The alteration of the regression parameters is a measure of the strength of the modification effect (HERKE et al. 2015). *Figure 4.a and 4.b* illustrate the results in case of model inhibition and experimental data.

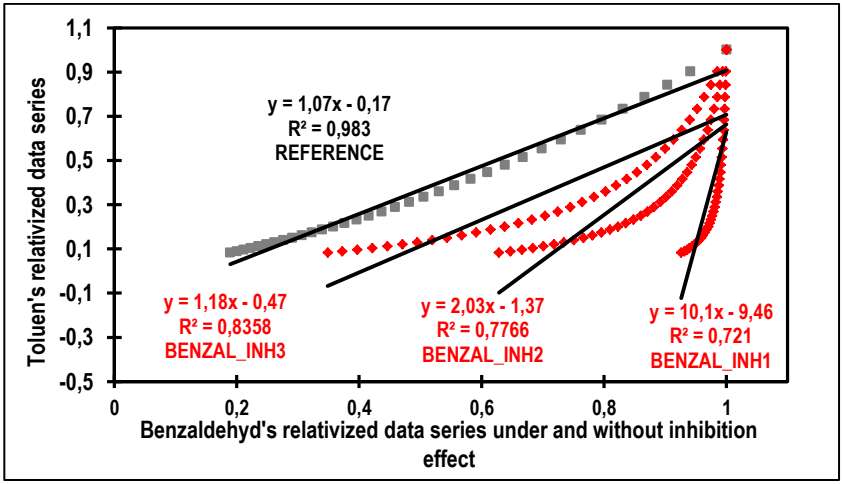

Figure 4.a. *Correlations between the normalized concentration data of toluene vs. benzaldehyde. Model data without inhibition (SINGLE) and under various intensity of competitive inhibition effects (BENZAL_INH1-3)*

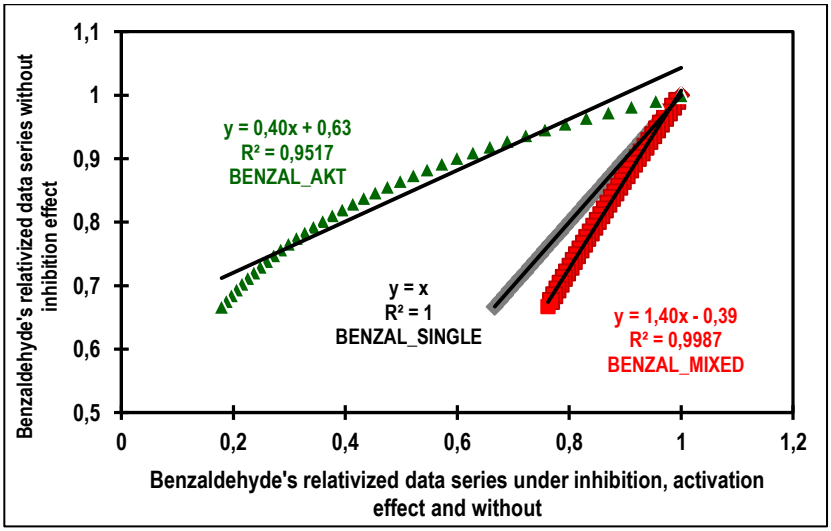

Figure 4.b. *Experimental data of independent degradation trials with single substrate degradation (SINGLE) and of experiments with mixed substrates (MIXED) and with activation compound (BENZAL_AKT).*

Thesis 4

Using an empirical exponential function between the regression parameters and the concentration of the inhibitor compounds makes it possible to originate the strength of the enzyme activity modification effect.

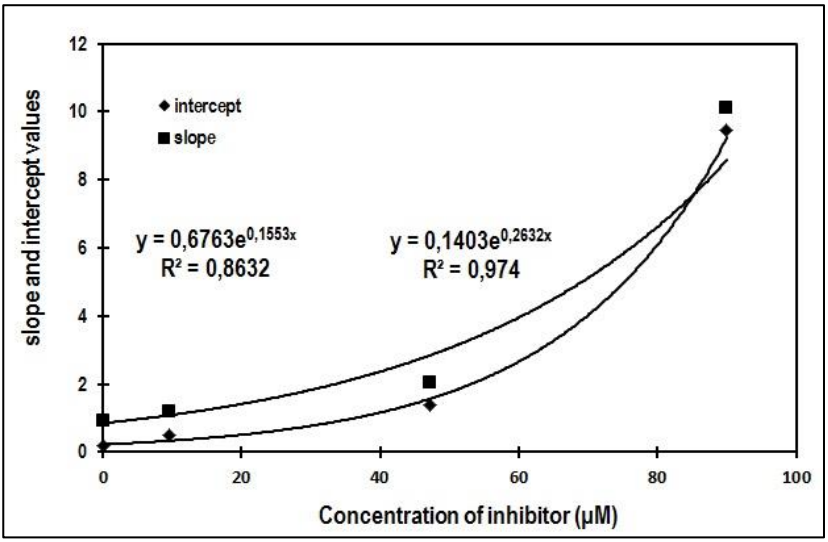

Figure 5. *The relationship between the concentrations of the inhibitor compound and the regression parameters, fitting an experimental function*

Using this approach further information is available in the field of the exploration of the inhibition effect. Activator- instead of inhibitor concentration is also applicable to measure the strength of the activator effect in the same way.

Thesis 5

Application of PCA method provides an alternative way to indicate the enzyme mechanisms. In consequence of this data representation, the most important information about the kinetic curves appearance on the loadings plot of PC1 and PC2 components.

The processing is applicable to visualize the enzyme activity modification. Using the reference kinetic dataset and its substrate point (without inhibition or activation effect) is always necessary while it is compared to the position of the other substrate points (HERKE et al. 2015).

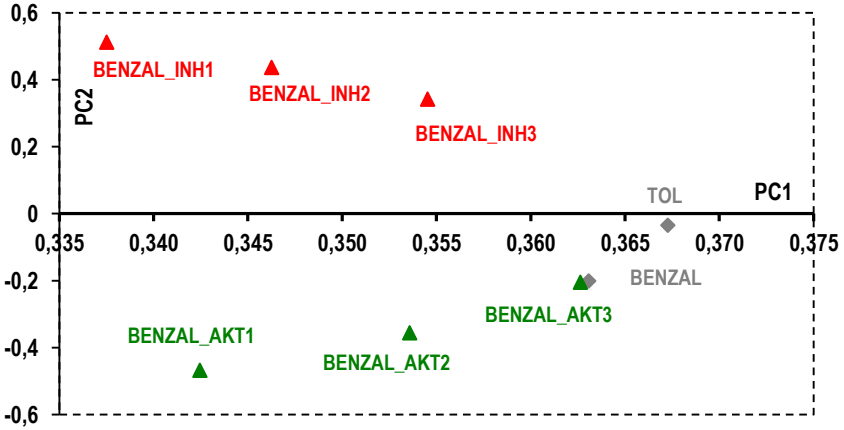

Figure 6. *PCA of model biodegradation data. BENZAL and TOL for kinetics without inhibition and BENZAL_INHI1-3 for inhibition of different strength, respectively, BENZAL_AKT1-3 for activation of different strength*

Related publications of Zoltán Herke

Journal article

HERKE, Z., MASKOW, T., NÉMETH ZS.I. (2015): New method for detection of cross inhibition effects in the environmental biocatalytic processes. BioTechnologia (A kézirat benyújtásra került: 2015. 07. 12.)

Conference proceeding / Abstract book

HERKE Z., CSERNY T., MAGYAR B., NÉMETH ZS. I. (2014): Inhibíciós és aktiválási mechanizmusok érzékelési lehetőségei biokatalitikus kármentesítésének alkalmazása során. /Opportunities for indication of inhibition and activation mechanisms in the course of application biocatalytic environmental technologies/ IN: Bidló A., Horváth A., Szűcs P. (szerk.) (2014): Nyugat-magyarországi Egyetem, Erdőmérnöki Kar, IV. Kari Tudományos Konferencia. NymE Erdőmérnöki Kar, Sopron pp. 95-99.

Z. HERKE, T. CSERNY, B. MAGYAR, ZS. I. NÉMETH (2014): Exploration of enzyme activity change based on correlation and principal component analysis IN:L. ALBERT, P. SZABÓ (editors): Kémia, Környezettudomány, Fenntarthatóság pp. 23-29.

HERKE Z., CSERNY T., MAGYAR B., NÉMETH ZS. I. (2013): Enzimkatalitikus mechanizmusok indikálási lehetőségei bioremediációs kármentesítések alkalmazása során, /Opportunities for indication of the enzyme catalytic mechanisms in the course of application the bioremediation technologies/, Környezetvédelmi Analitikai és Technológiai Konferencia, Absztrakt könyv, Hajdúszoboszló, 2013. október 2-4.

Z. HERKE, T. CSERNY, B. MAGYAR, ZS. I. NÉMETH (2013): Evaluation of enzyme kinetic data based on correlation and principal component analysis, Conferentia Chemometrica, Abstract book, Sopron, pp. 203.

Z. HERKE, T. CSERNY, B. MAGYAR, ZS. I. NÉMETH (2012): Exploration of enzymatic degradations by chemometric methods, IN: K. HÉBERGER & T. L. PAP (editors): XIIIth Conference on Chemometrics in Analytical Chemistry, Programme & Book of Abstracts Budapest 25-29. June 2012.

Z. HERKE, T. CSERNY, B. MAGYAR, ZS. I. NÉMETH (2012): :Indication of Biocatalytic Mechanisms in Environmental Remediation by Statistical Methods, IN: M. NEMÉNYI, B. HEIL, A. J. KOVÁCS, F. FACSKÓ (editors): International Scientific Conference on Sustainable Development & Ecological Footprint, Sopron, University of West Hungary Press.

HERKE Z., CSERNY T., MAGYAR B., NÉMETH ZS. I. (2011): Inhibíciós mechanizmusok indikálása szennyező komponensek enzim kinetikai adatsorainak korreláltatásával, /Rectification of inhibition mechanisms by correlation of pollutant compounds data series/ IN: LAKATOS F. és SZABÓ Z. (szerk.): Nyugat-magyarországi Egyetem, Erdőmérnöki Kar, Kari Tudományos Konferencia Kiadvány Nyme Kiadó Sopron.

Z. HERKE, T. CSERNY, B. MAGYAR, ZS. I. NÉMETH (2011): Detection of enzyme inhibition by regression analysis of kinetic curves, Conferentia Chemometrica, Abstract book, Sümeg.

HERKE Z., NÉMETH ZS. I (2011): Inhibíciós hatások kimutatása komplex enzim rendszerekben statisztikai módszerekkel, /Indication of inhibition effects in complex enzyme systems by statistical methods/ Lakatos F., Polgár A., Kerényi-Nagy V. (szerk.): Tudományos Doktorandusz Konferencia, Konferencia-kötet, Nyugat-magyarországi Egyetem Erdőmérnöki Kar, NymE Kiadó.

Conference and symposium presentation

Z. HERKE, T. MASKOW, ZS. I. NÉMETH: An alternative approach to detect the inhibition effects ont he environmental biocatalytic processes. 1ST International Symposiumfor Students and PhD Students „Environmental Biotechnology" 21-22. 05. 2015, Gliwice, Poland.

HERKE Z., CSERNY T., MAGYAR B., NÉMETH ZS. I.:

Inhibíciós és aktiválási mechanizmusok érzékelési lehetőségei környezetszennyező anyagok biodegradációs vizsgálatakor, /Opportunities for indication of the enzyme catalytic mechanism by investigation of environment pollutants biodegradation/ KeMoMo-QSAR 2014, 2014. május 22-23., Szeged

HERKE Z., CSERNY T., MAGYAR B., NÉMETH ZS. I.: Inhibíciós és aktiválási mechanizmusok érzékelési lehetőségei biokatalitikus kár-

mentesítésének alkalmazása során, /Opportunities for indication of inhibition and activation mechanisms in the course of application biocatalytic environmental technologies/ Kari Tudományos Konferencia, NymE-EMK, Sopron, 2013. december 10.

HERKE Z., CSERNY T., MAGYAR B., NÉMETH ZS. I.: Enzimkatalitikus mechanizmusok indikálási lehetőségei bioremediációs kármentesítések alkalmazása során, /Opportunities for indication of the enzyme catalytic mechanisms in the course of application the bioremediation technologies/ Környezetvédelmi Analitikai és Technológiai Konferencia, Hajdúszoboszló, 2013. október 2-4. (*Poster*)

Z. HERKE, T. CSERNY, B. MAGYAR, ZS. I. NÉMETH: Evaluation of enzyme kinetic data based on correlation and principal component analysis, Conferentia Chemometrica, Abstract book, Sopron, 8-11. September 2013. *(Poster)*

HERKE Z., CSERNY T., MAGYAR B., NÉMETH ZS. I.: Enzim kinetikai adatsorok feldolgozása korrelációs és főkomponens elemzési módszerekkel, /Exploration of enzyme activity change based on correlation and principal component analysis/ Nyugat-magyarországi Egyetem, Erdőmérnöki Kar, Kémiai Intézet, Sopron, 2013. augusztus 29.

HERKE Z., CSERNY T., MAGYAR B., NÉMETH ZS. I.: Enzim kinetikai adatsorok feldolgozása korrelációs és főkomponens elemzési módszerekkel. /Analysing of the enzyme kinetic data series by correlation and principal component analysis/ KeMoMo-QSAR 2013, 2013. április 29-30., Szeged

HERKE Z., CSERNY T., MAGYAR B., NÉMETH ZS. I.: Statisztikai módszerek alkalmazása enzim mechanizmusok indikálására, /Application of statistical methods to indication of enzyme mechanisms/ VIII. Regionális Természettudományi Konferencia, Szombathely, 2013. január 24.

HERKE Z., CSERNY T., MAGYAR B., NÉMETH ZS. I.: Exploration of enzymatic degradations by chemometric methods, XIIIth Conference on Chemometrics in Analytical Chemistry, Budapest 25-29. June 2012. *(Poster)*

HERKE Z., CSERNY T., MAGYAR B., NÉMETH ZS. I.: Indication of Biocatalytic Mechanisms in Environmental Remediation by Statistical Methods, International Scientific Conference on Sustainable Development & Ecological Footprint, Sopron 26-27. March 2012. (*Poster*)

HERKE Z., CSERNY T., MAGYAR B., NÉMETH ZS. I.: Inhibíciós mechanizmusok indikálása szennyező komponensek enzim kinetikai adatsorainak korreláltatásával, /Rectification of inhibition mechanisms by correlation of pollutant compounds data series/ Nyugatmagyarországi Egyetem, Erdőmérnöki Kar, Kari Tudományos Konferencia, Sopron 2011. október 5.

HERKE Z., CSERNY T., MAGYAR B., NÉMETH ZS. I.: Detection of enzyme inhibition by regression analysis of kinetic curves, Conferentia Chemometrica, Sümeg 18-21. September 2011. (*Poster*)

HERKE Z., NÉMETH ZS. I.: Inhibíciós hatások kimutatása komplex enzim rendszerekben statisztikai módszerekkel, /Indication of inhibition effects in complex enzyme systems by statistical methods/ Tudományos Doktorandusz Konferencia, Sopron, 2011. április 13.

Other publications of Zoltán Herke

Conference proceeding / Abstract book

Zs. I. NÉMETH, R. RÁKOSA, **Z. HERKE**: Revealing Factor Effects on NIR Spectra of Alcohol-water Mixtures by Principal Component Analysis. Conferentia Cemometrica, Abstract book

MÉSZÁROS I., OLÁH V., VERES SZ., LAKATOS Á., RASZTOVITS E., **HERKE Z.** (2010): Analysis of ecophysiological plasticity of European beech provenances in the Hungarian provenance trial. In: Book of Abstracts. COST E52 "Evaluation of beech genetic resources for sustainable forestry" Final Meeting, Burgos, Spain, 4-7 May 2010.

Z. HERKE (2015): Untersuchung biokatalytischer Produkte zum Fremdstoffabbau, /Investigation of biocatalytic products for degradation of environment pollutants/ DBU-ösztöndíj záró kutatási beszámoló

Conference and symposium presentation

Zs. I. NÉMETH, R. RÁKOSA, **Z. HERKE**: Revealing Factor Effects on NIR Spectra of Alcohol-water Mixtures by Principal Component

Analysis. Conferentia Cemometrica, 2015. szeptember 13-16., Budapest

SÁRAINÉ RAUCH R., **HERKE Z.**: Talajszennyező szénhidrogének bontása biokatalitikus technológiával, /Degradation of soil contaminants by biocatalatic technology/ „Kármentesítés aktuális kérdései szimpózium 2013", Budapest, 2013. március 21-22.