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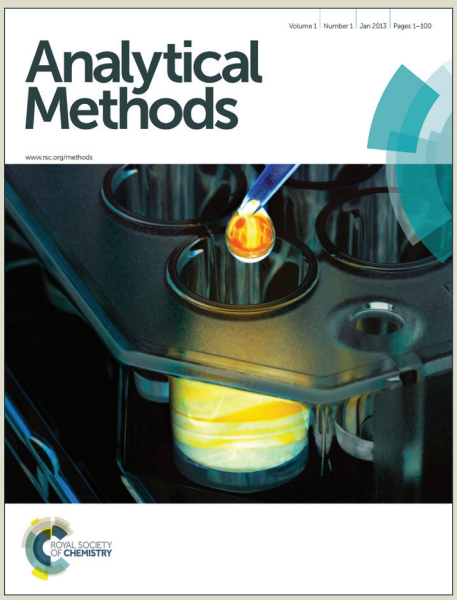

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Potentiometric and Hybrid Electronic Tongues for Bioprocess Monitoring – an Overview

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Abstract

This work summarizes and discusses the monitoring of various bioprocesses with the use of electronic tongue (ET) systems. These devices coupling sensor arrays with pattern recognition units allow for the qualitative and quantitative analysis of complex composition liquid samples based on their unique "fingerprint", i.e. sensor response pattern. Such approach offers obtaining reliable results in real time, with the use of simple, inexpensive tools – therefore ET systems seem to be an interesting alternative to classical control of bioprocess run based on chromatography or spectrophotometry. The presented attempts focused on the development of different sensors arrays based on flow-through potentiometric ETs as well as hybrid ET systems dedicated to the monitoring of biogas production, alcohol fermentation and *Aspergillus niger* fermentation.

Keywords: electronic tongue, hybrid sensor array, multisensor system, data analysis, bioprocess monitoring

Introduction

Sensor arrays whose signals are analyzed by the numerical procedures (so-called electronic tongues) have been used successfully in multiple applications ranging from food analysis, through environmental and process monitoring to medical diagnostics.[1-8] Instead of determining the selected analytes in the samples, the sensor arrays are designed to solve problems linked with their overall characteristics on the basis of unique and characteristic "fingerprint" of the sample.[1,2,9] According to the current definition, an electronic tongue (ET) is a device for automatic analysis and classification/recognition of liquid samples, comprising a sensor array and chemical pattern recognition block.[1] Various numerical procedures analyzing the unique "fingerprint" of the sample, i.e. its chemical image, provide qualitative and/or quantitative analysis.[9] ETs are often used for fast foodstuff classification, origin recognition, or estimation of complex properties of the samples.[1,3] They can be divided into few categories according to sensor type used to form an array. Most of such systems utilize electrochemical sensors: potentiometric,[10,11] voltammetric,[12] amperometric,[13] however also mass and optical sensor arrays are constructed.[14-17]

In the last few years ET systems were proposed for the monitoring of various fermentation processes (e.g. alcohol fermentation, *Aspergillus niger* fermentation, *Escherichia coli* fermentation, tea fermentation, etc.), as devices capable of automated and on-line control taking an advantage of no need of sample pretreatment.[18-23] The first review on the recent achievements and applications of electronic tongues/noses for the monitoring of biotechnological processes was published in 2008.[18] However, it must be underlined, that among ~80 publications on ET applications a year, there are only few dedicated to bioprocess monitoring.[23] It is

most likely related to an extremely complex composition of the bioprocess medium as well as a large variability of the studied samples.[24]

The first example of ET exploited for bioprocess monitoring was a device used for Miso (soybean paste) fermentation presented in 1996 by Imamura.[25] Modeling and prediction of amino-acids contents and titratable acidity of samples with different fermentation degrees was performed by potentiometric sensors containing lipid membranes coupled with Multiple Linear Regression. The same 8-channel taste sensing system was applied to the assessment of titratable acidity of samples during Kimchi (traditional Korean pickle) fermentation.[26]

The monitoring of the fermentation process carried out by the bacterium *Escherichia coli* was an interesting example of the ET application for bioprocess control.[19] An array of 21 potentiometric sensors was used to rapid off-line analysis of changes in the composition of the medium during the process. In this case, significant correlation between biomass growth and change in the composition of the samples was detected. Moreover, the ET allowed also determining the concentration of organic acids. Similar system based on an array of potentiometric sensors has been proposed for the monitoring of batch fermentation process of starting culture for light cheese production. The reliability of such device was compared with classic HPLC technique.[20] Only a single attempt was made to control the fermentation process involving *Aspergillus niger* using ET system. Nevertheless, the proposed potentiometric electronic tongue has been applied for the analysis of simulated fermentation complex media, where a simultaneous determination of ammonium, citrate and oxalate was achieved with good precision.[21] Finally, near and mid infrared spectroscopies combined with electronic nose/tongue were reported for the

monitoring of alcoholic fermentation process. It was observed that the electronic tongue enabled to detect the evolution of taste and aroma profile.[27]

In this contribution, the results of the studies carried out recently in our group, aiming at the application of electronic tongue systems for the monitoring of various bioprocesses, were summarized and discussed. The overview presents the development and the performances of different sensors arrays based on flow-through potentiometric as well as hybrid systems dedicated to the monitoring of methane fermentation (biogas production), alcohol fermentation and *Aspergillus niger* fermentation processes.

Potentiometric Sensor Arrays for Bioprocess Monitoring

The control of bioprocesses is still suboptimal, since only a few parameters can be easily monitored in situ such as pH or O_2. Samples to be analyzed with the use of HPLC or GC techniques can be collected infrequently; moreover, the relatively long time of the analysis delays the moment of eventual reaction towards abnormal process run.[23] The ideal method for bioprocess monitoring should enable rapid, on-line and reliable analysis of bioprocess samples without their pretreatment. Therefore, flow-through systems providing automation and shortening of the whole analytical procedure seem to be especially favorable for this purpose. Till now, the majority of ET systems that have been used for fermentation monitoring were based on potentiometric sensors, among which ion-selective electrodes (ISEs) with PVC membranes or chalcogenide glasses were the most common. Sensor arrays fabricated with the use of 4-8 PVC membrane ISEs with “artificial lipids” were used for Kimchi and Miso fermentation[25,26] as well as for wine production monitoring.[27] Up to 30 ISEs utilizing chalcogenide glasses or PVC membranes as chemosensitive

materials, formed sensor arrays applied to the analysis of samples from cheese production and from *E. coli* fermentation.[19,20] However, it must be noticed, that in all cases the constructed sensor arrays were adapted only for batch measurements.

Taking into account the specificity of the bioprocess monitoring, a flow-through potentiometric sensor arrays consisting of single modules connected with each other, were elaborated (Figure 1). Horizontally combined modules allowed for easily connecting and disconnecting and formed a channel through which the sample flows.[2,24,28-30] Thanks to the adaptable design, the flow-through cell can be equipped with sensors of various architecture e.g. miniaturized ion-selective electrodes of classical architecture[28] or planar solid-state electrodes[24] based on poly(vinyl chloride) plasticized membranes. The individual sensors can be conditioned in a mixture of conditioning solutions or a single, dedicated solution (the method of the membranes preparation and the electrodes conditioning were the same as for the standard ISEs). The developed sensor arrays comprised up to 10 sensors (with an additional double junction reference electrode) and can be used for flow-through/flow injection analysis.

The constructed flow-through potentiometric sensor arrays were applied for anaerobic digestion monitoring,[24,28] and together with voltammetric cells and other electrochemical sensors formed the hybrid sensor arrays for beer fermentation[29] and *Aspergillus niger* fermentation monitoring (Figure 1).[30] It should be also emphasized that the employment of flow technique for sample handling benefited the final system performance, through the reduction of sensor drift and reproducibility improvement.[31]

Hybrid Sensor Arrays for Bioprocess Monitoring

Hybrid electronic tongue (h-ET) takes advantage of two or more measurement techniques. Usually, such systems combine various electrochemical sensors

(potentiometric, voltammetric, conductivity), but optical, mass sensors as well as enzymatic biosensors (forming in this case so-called bioelectronic tongue) can also be introduced.[32] The signals recorded for all types of sensors are processed by appropriate chemometric methods to extract the useful information. In this case, data fusion processing tools may improve the performance of the electronic tongue systems based on different sensor types.[33] Finally, the hybrid systems using sensors of various types, allow to obtain more diverse information about the sample thanks to the enrichment of its chemical image with data obtained by different measurement technique, which gives new, additional "orthogonal" information that does not duplicate information gained by the one type of sensors. This gives the opportunity for better, more reliable and more accurate sample characterization, improving the correctness of its classification.

Due to the promising performances of the hybrid sensor arrays, such type of ET system was tested for bioprocess analysis in our further studies. Potentiometric flow-through sensor arrays were coupled with an additional 3-electrode voltammetric cell (classical 3-electrode voltammetric set-up and miniaturized 3-electrode cell integrated on silicon support, Figure 1) for complementary characterization of the samples during beer fermentation.[29] A more complex electrochemical system based on potentiometric/voltammetric sensors coupled with pH, ORP and conductivity probes was introduced for the monitoring of citric acid production by *A. niger* [30] (Figure 1).

Analysis of Sensor Array Data – Data Mining, Qualitative, Semi-Quantitative and Quantitative analysis

A key step in the analysis of sensor array data is the extraction of information on overall characteristics of the sample, i.e. the creation of so-called chemical image of

the sample - a set of values that are its unique "fingerprint" or "barcode". This image is formed by the direct values of signals of particular sensors included in the array or by the features obtained by extracting significant information from dynamic sensor responses.[1,3,34] Vectors of the chemical images of the tested samples, recorded in the form of a data matrix, are then analyzed using unsupervised methods ("without a teacher") or supervised ("with a teacher"), in which class memberships of every sample or the contents of key components/characteristic features are known. The proper solution of each classification task requires obtaining sufficient, appropriate amount of information about the classified objects.

For the first look at the ET data, various data mining unsupervised procedures can be used and usually Principal Components Analysis (PCA) is the most widely applied.[1,5] PCA is a statistical procedure using an orthogonal transformation to convert a set of observations of possibly correlated variables into a set of values of linearly uncorrelated variables – principal components. Another method of unsupervised visualization of multidimensional ET data is the Self-Organizing Maps (SOM), i.e. Kohonen networks trained using a neighborhood function to produce typically two-dimensional, discretized representation of the training samples input space in a form of a map.[35] Such unsupervised numerical procedures were proposed for the signal processing of ETs during various bioprocess monitoring, e.g. Kimchi fermentation and alcohol fermentation.[26,27,36]

In contrast to potentiometric sensor array data, where steady-state responses are recorded and the investigated data matrix is relatively small resulting in direct formation of chemical images of the samples, voltammetric techniques demand additional preprocessing of voltammograms data in order to reduce huge multi-dimensionality and to extract significant information. In our case, direct analysis of

parts of voltammograms[29] or Discrete Wavelet Transform[30] was used for the feature extraction of bioprocess sample data (Figure 1).

Qualitative, semi-quantitative, and quantitative analysis of ET data can be realized with a number of various supervised and unsupervised classification/pattern recognition techniques, such as Partial Least Squares (PLS) regression, PLS-Discriminant Analysis (PLS-DA), Artificial Neural Networks (ANN), Multiple Linear Regression (MLR), Linear Discriminant Analysis (LDA), K-Nearest Neighbors (KNN).[1,9,23] PLS/PLS-DA techniques were mostly applied for the bioprocess monitoring with the use of ET systems (*E. coli* and *A. niger* fermentation, light cheese and biogas production, alcohol fermentation),[19-21,24,28-30] however ANN were also tested (*A. niger* fermentation[21]). The supervised methods require, additionally to data matrix, the formation of an appropriate target matrix, that contain coded information on sample class (e.g. phase of fermentation) or some semi-quantitative/quantitative sample characteristics (e.g. Chemical Oxygen Demand). PLS finds the relations between the two matrices, i.e. models the covariance structure of these two spaces and organizes the information in a set of latent variables so that the following new variables contain maximum covariance of the original data.[37] PLS-DA is a variant of PLS regression where categorical data in the target matrix are used.

Finally, the fitness and reliability of the models established by supervised method can be evaluated by the calculation of parameters such as percent of correct classifications, sensitivity, and specificity (for qualitative analysis), or parameters characterizing linear regression between real values and values predicted by the model (determination coefficient, slope, bias, RMSE). Moreover, the classification ability of the ET system and of particular sensors in the array can be described by the so-called F factor.[38] Factor F is equal to the ratio of feature variances: variance

between different classes and variances in particular classes and when it increases, the discrimination between different classes is greater.

ET Systems for Bioprocess Monitoring – Selected Applications

Monitoring of methane fermentation

Methane fermentation is the process of the microbiological decomposition of organic matter carried out by microorganisms under anaerobic conditions, with methane as the main product. In the first step the hydrolysis of the substrates occurs, in which polycarbonates, fats and proteins decompose to simpler compounds. Next, carboxylic acids are produced from the hydrolyzed products (mainly propionic acid, valeric acid, formic acid). After this acidogenesis step, acetogenesis takes place, in which acetates are a characteristic product, which in turn are substrates for methanogenesis ending the process of fermentation biogas production. The process has a great industrial importance, since organic wastes (sewage, municipal waste) are used and combustible gas, whose main component is methane, is obtained.

Methane fermentation occurs spontaneously in landfills, but increasing its efficiency requires the use of bioreactors and appropriate types of bacteria. Their population is in dynamic equilibrium state, which can be disrupted by a number of chemical and physical factors. For example, too big amount of Volatile Fatty Acids (VFA) and the lowering of the pH lead to the inhibition of methanogenesis, which in turn causes a decrease in the process efficiency. Therefore, the process can be optimized to obtain higher yields controlling the main process parameters i.e. the appropriate dilution of the medium, VFA concentration, chemical oxygen demand (COD) and pH. A more detailed process control could be carried out by sensor arrays recording comprehensive chemical image of samples, moreover, allowing for on-line

monitoring.[24,28] Till now, only two reports on the application of multisensor systems for the analysis of anaerobic digestion can be found in the literature.[18]

The ET system for methane fermentation monitoring developed in our group was based on modular flow-through potentiometric sensor array described above. Upon conducing several biogas production sequences (in the case of sequencing methane fermentation[24]) or production batches (periodic methane fermentation[28]), part of them was selected on the basis of maximally differentiated maximum/minimum and final values of key parameters to be analyzed with the use of sensor array. After centrifugation, the suspension samples taken from the bioreactor were analyzed with the use of classical methods (determination of VFA, COD, pH and methane/CO_2 monitoring in the gaseous phase) and potentiometric sensor array (Figure 2A and 3A). Two modular potentiometric sensor arrays based on planar solid-state electrodes (6 types: AC, CS, AS, H^+, K^+, Na^+)[24] and miniaturized ion-selective electrodes (7 types: AS, CS, AC, AM, H^+, Na^+, NH_4^+)[28] were tested (Figure 1).

The sensor signals were measured in the samples collected during biogas production. Comparison of the sensor array responses and the reference values – key parameters values analyzed by classical methods – was carried out. PLS and PLS-DA models correlating the potentiometric data matrix and target data matrix were created. Chemical images of the samples were easily differentiated according to the fermentation phase (see exemplary results of qualitative analysis in Figure 2B) and COD levels (see exemplary results of semi-quantitative analysis in Figure 3B). The possibility of VFA and COD level control in a complex fermentation medium is very important – COD value decreases during the process, and the lowest values for this parameter are observed only in the last days of batch fermentation, if only it was run properly. Its high value in turn is an evidence of inappropriate composition of the

medium and following low COD reduction. Samples of the highest VFA content were also distinguishable from the others so that it was possible to detect the inhibition of methanogenesis due to a high content of fatty acids in the reaction medium.

Quantitative ET analysis of both VFA and COD in the reaction medium was attempted in the next step. All data was divided into two parts: the train set (for the establishing of the model) and the test set (to validate it). Signals from the sensor array were correlated with the reference values by PLS, and then correlation plots were constructed showing the relationship between the real values of the parameter and the values provided by the model. High slope values and correlation coefficients for the prediction of COD and VFA content for the training data were noticed, and relatively reduced but still satisfactory for the test data (Figure 3C).

The results indicated the possibility of process monitoring using the developed sensor array for semi-quantitative/quantitative determination of VFA and COD, as the key parameters of methane fermentation process. The relatively simple system of on-line control provided the monitoring of the process run, maintaining an adequate technological regime.

Monitoring of alcoholic fermentation

Continuous monitoring of alcoholic fermentation, i.e. an enzymatic anaerobic reaction in which saccharides degradation products are obtained, is extremely important because of the rapid changes occurring throughout the process. Usually, the control of certain parameters during a particular stage of e.g. beer production is carried out using various analytical techniques: spectrophotometry (colorimetric calcium assay), potentiometric titration (total water hardness), hydrometry (alcohol

concentration), amperometric sensor (concentration of oxygen), turbidimetric and pH measurements.[39]

Taking into account the composition of the reaction medium, a hybrid electronic tongue based on miniaturized potentiometric and voltammetric sensors (Figure 1) was developed in our group for the homemade beer fermentation monitoring.[29,36] Malt, wheat, barley, top fermenting yeast, and Lublin hops were used for the beer production. Crushed malt was added to water and after the malt break (saccharification) and following dextrin temperature break, iodine test was performed. After negative result, showing the lack of starch in the mash, the latter was heated and filtered on braiding filter with water addition. The final wort was boiled with hops, and then cooled. The next step in the production of beer was the fermentation with yeast. Stormy fermentation was conducted for 7 days, quiet – 3 days, and aging step – 15 days. One sample was collected before the addition of yeast, whereas fermentation samples were collected twice a day (samples “1 day” – “4 days”) and samples from aging step – every third day (samples “9 days” – “29 days”).

Potentiometric measurements were carried out in the flow-through cell equipped with miniaturized ion-selective electrodes (6 types: AM, AC, CS, AS, H^+, CO_3^{2-})[29] and miniaturized silicon-based redox electrode, whereas silicon-based 3-electrode cells were used for voltammetric measurements (Figure 1).[38] Steady-state signals of the potentiometric sensors and voltammograms registered using various techniques (CV, DPV, SWV) were used to create chemical images of samples. Voltammograms recorded in beer samples were transformed by discrete wavelet transform using biorthogonal wavelet. Data matrices containing sensor signals/features were correlated with the target matrices representing the encoded information on sample classes. In the case of fermentation samples, the target matrix contained data on

fermentation time, while in the case of maturation samples it contained data on their age. The analysis of the data obtained from each technique – potentiometry or voltammetry used separately – did not provide visual discrimination of various samples. However, it was found out, that the use of hybrid system (h-ET) allowed improving the ability to differentiate between the samples (Figure 4A). The factor F was used for objective analysis of the discrimination ability of various sensor arrays;[29,38] mean values of F for each pair and for all samples globally were calculated. The obtained F values for various sensor array configurations and for samples from aging phase of beer production were presented in Figure 4B. Generally, the highest F values for the h-ET data were observed, whereas the worst for ET systems based on separately used techniques. According to our expectations, the greater amount of not correlated data describing the analyzed samples, which can be obtained from h-ET, allowed enhancing the classification accuracy of the ET system.

Next, the errors of classification were calculated to analyze the differentiation ability of h-ET in comparison with potentiometric ET and voltammetric ET. Root Mean Squared Error (RMSE) was studied for the classification of all samples – in this way the difference between target vector and PLS-DA output vector could be compared. Much smaller values of RMSE were noticed using two techniques (e.g. potentiometry and cyclic voltammetry) – their values decreased by the order of magnitude compared to RMSE values in the case of separately used techniques. The results obtained for all data (“ALL” h-ET) showed much better fit – in this case, RMSE decreased by following two orders of magnitude. The results confirmed that the combination of various analytical techniques in one ET system allowed for the best characterization of the samples.

Monitoring of fermentation process by *Aspergillus niger*

The monitoring of the citric acid production in fermentation process by *Aspergillus niger* in standard and infected fungal culture was attempted. It should be emphasized that this bioprocess is strongly influenced by the composition of the fermentation medium and by the selection of optimal parameters (optimum pH range, oxygen content, cultivation temperature and time)[21] Moreover, the contamination by other microorganisms may limit the efficiency of citric acid production.[20] Therefore, various analytical methods of culture monitoring are involved to ensure the proper breeding of *Aspergillus niger*, whereas pH, acidity, CO_2 and O_2 are the basic parameters controlled during the fermentation.[19]

Mycelium of *Aspergillus niger* cultures was cultivated on synthetic medium.[30] Before vaccination, the samples containing only the medium were collected, and then the medium was inoculated with *A. niger* mycelium. Breeding had been carried out for 3 weeks. After 4 days one of the cultures was infected with baker's yeast. The samples were collected in 3 successive weeks, 1 sample/day during 5 days a week.

A more complex sensor array was constructed for this task i.e. pH, ORP and conductivity electrodes were added to the potentiometric sensor array (5 types of electrodes: AC, CS, Na^+, H^+, CH_3COO^-)[30] and classical voltammetric set-up, with glassy carbon disk electrode as the working electrode. The data recorded by various electrochemical techniques were merged in a data matrix of the hybrid electronic tongue. The compression of the initial voltammetric signal (cyclic voltammograms) involving discrete wavelet transform (biorthogonal wavelet, decomposition level a=5) was applied to reduce the amount of data, while maintaining waveform characteristics. A classical breeding monitoring, involving the measurement of the pH

changes and the determination of the total acidity of the sample solutions collected from both fermentation cultures, was carried out.

PCA technique provided the preliminary evaluation of the recognition ability of the h-ET, i.e. the system was able to differentiate between standard and infected fermentation process. Nevertheless, PLS-DA technique was applied for the qualitative monitoring of citric acid production. A data matrix containing chemical images of samples and its corresponding four-column target matrix were created: the first two columns indicated the process duration, whereas the two subsequent columns informed about the occurrence of infection. The results obtained by processing of the data by PLS-DA proved the suitability of the hybrid ET to assess the correct course of fermentation and to detect the occurrence of yeast infection (Figure 5A). Moreover, the estimation of the fermentation progress (process duration) was achieved by the hybrid system.[30]

The hybrid sensor array combined with PLS technique was employed for the quantitative monitoring of the fermentation process by *Aspergillus niger*. In this case, a target matrix embracing the data on the duration of the process as well as the value of the total acidity (enabling to estimate the correctness of the process) was built. The model performance was evaluated by studying of the coefficients of the linear fitting of the expected data versus the data predicted by the PLS model. Exemplary results provided by the h-ET during the standard culture, comparing the determined total acidity and the values provided by the PLS model, were presented in Figure 5B. The satisfactory values of the parameters: slope (a), intercept (b) and determination coefficient (R2) confirmed a correct quantitative modeling of the fermentation run. Moreover, the detailed analysis of the contribution of the individual electrochemical techniques to the final result indicated that the processing of the separate

voltammetric data did not ensure a proper quantitative monitoring of the fermentation process. Significantly better results were obtained for separate potentiometric data, whereas the fusion of various electrochemical techniques improved the classification ability of the system for both standard and infected culture (similar dependences were noticed for the prediction of process duration and total acidity of the samples).[30]

Conclusions

Several attempts of electronic tongue monitoring of selected fermentation processes were summarized in this work. The studied bioprocesses differed significantly in terms of mode/conditions of fermentation and composition of the reaction medium. Therefore, different electrochemical sensor arrays were designed, ranging from solely polymer membrane ion-selective electrodes to complex hybrid systems based on potentiometric/voltammetric devices coupled with additional pH, ORP and conductivity probes. The effectiveness of various numerical procedures analyzing the chemical images of the samples and providing qualitative and/or quantitative monitoring of the processes was compared.

In general, potentiometric sensor arrays seem to be reliable and simple tools for proper controlling of the fermentation process. However, the development of hybrid systems, combining various electrochemical sensors in one ET device, led to the distinct improvement of its classification ability and provided effective quantitative control of the conducted bioprocesses.[24,28-30,38] The stability of potentiometric electrodes forming the arrays was investigated during 3 months.[24] Only small variations or slight decrease of the slope values and mean values of selectivity coefficients have been noticed, which assured appropriate long-term stability. However, it must be underlined that during this period the sensor arrays were not

continuously exposed to harsh bioprocess samples, which can influence their performance. This effect should be deeply studied for on-line implementations of sensor arrays to bioprocess monitoring in order to avoid misleading results.

Concluding, despite the complex and time dependent composition of the medium samples the developed systems were able to estimate the progress and correctness of the fermentation process. Therefore, the proposed approach can replace the commonly performed off-line process monitoring, using high-performance liquid chromatography or gas chromatography. It should be also stressed, that to date, our results are the first examples of the application of hybrid ET systems for the fermentation monitoring.

Further studies on the on-line monitoring of bioprocesses using chemical multisensor systems should be focused on the optimization of the signal stability and durability of the sensors working in such complex media. Moreover, the fusion of an electronic tongue with electronic nose and electronic eye tools may improve the recognition capabilities of the system for enabling the advanced follow-up of the fermentation processes.

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Figure Captions

Figure 1. Flowchart of electronic tongue analysis of bioprocess samples.

Figure 2. Monitoring of sequencing methane fermentation: A) classical control; B) exemplary results of ET analysis. L-COD, H-COD – low and high levels of COD (below 700 mg O_2 dm^{-3} and above, respectively); L-VFA and H-VFA - low and high levels of VFA (below 2,0 mM CH_3COOH and above, respectively).[24]

Figure 3. Monitoring of periodic methane fermentation: A) classical control; B),C) exemplary results of ET semi-quantitative (B) and quantitative (C) analysis. H-COD, M-COD, L-COD – high (>7000 mg O_2 dm^{-3}), medium, and low (<5000 mg O_2 dm^{-3}) levels of COD, respectively.[28]

Figure 4. Monitoring of beer fermentation: A) classification of samples sampled 9-29 days after yeast addition; B) classification performance of potentiometric, voltammetric, and hybrid ETs. POT – potentiometric data; CV, DPV, SWV – voltammetric data (3 different techniques, details in the text); ALL – fusion of all data, i.e. POT, CV, DPV, SWV.[29]

Figure 5. Monitoring of *Aspergillus niger* fermentation: A) PLS-DA plot of chemical images of the samples collected during the standard and infected fermentation process; B) linear fitting of the real values and the PLS predicted values of the total acidity in the standard culture.[30]

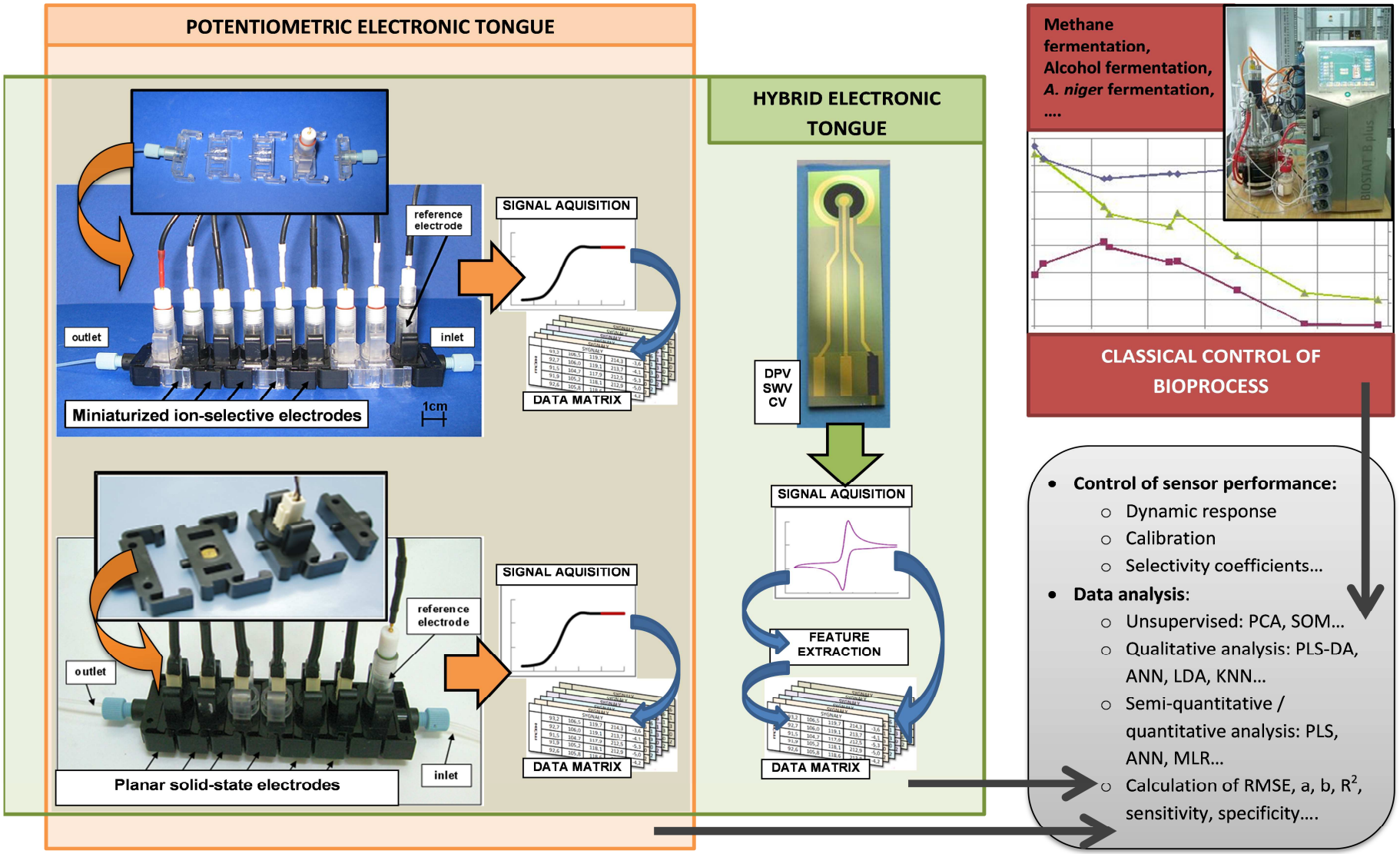
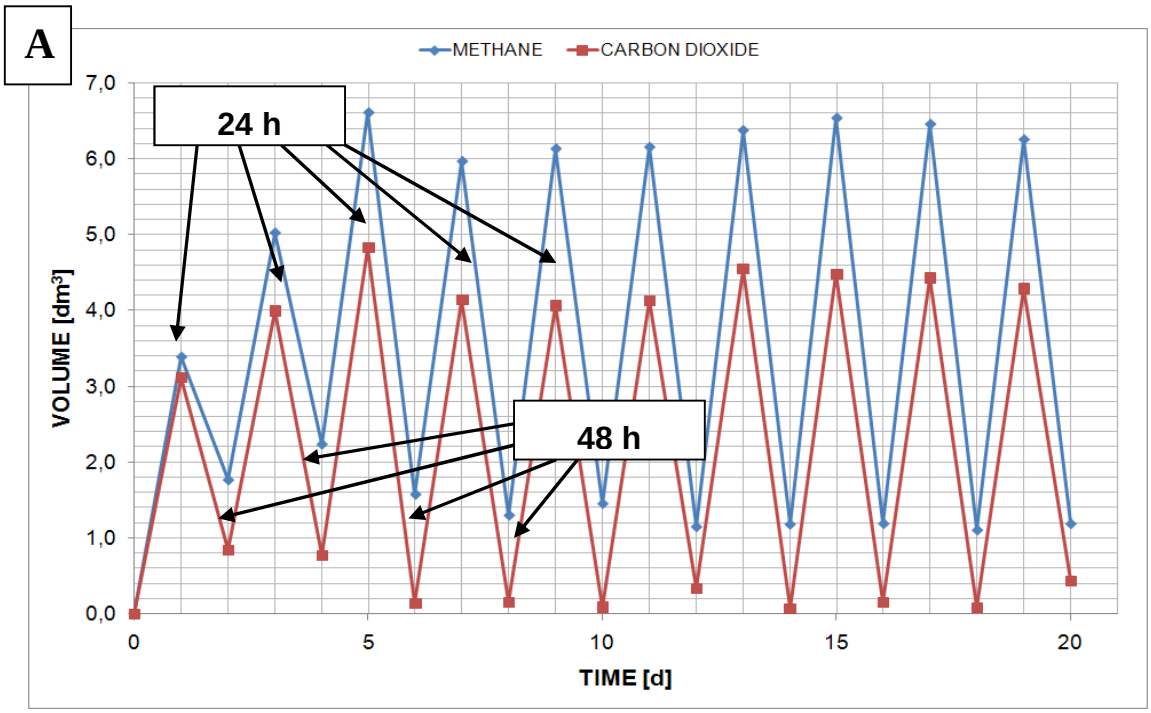


Figure 1



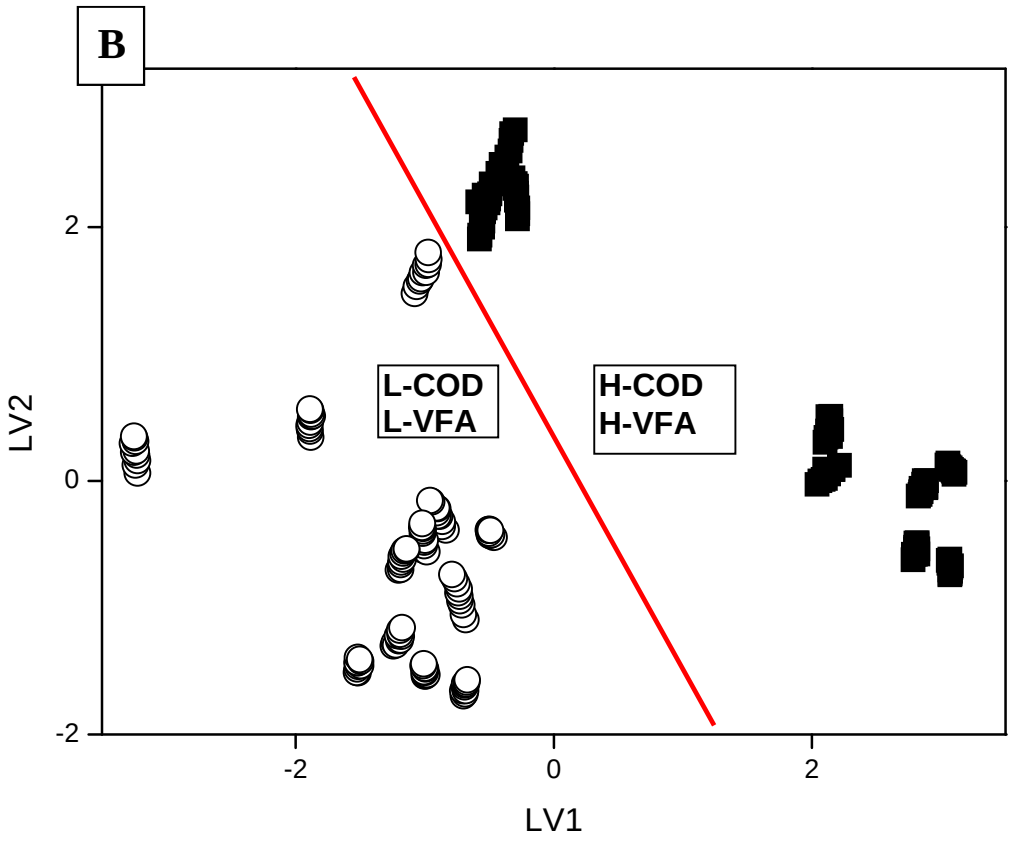
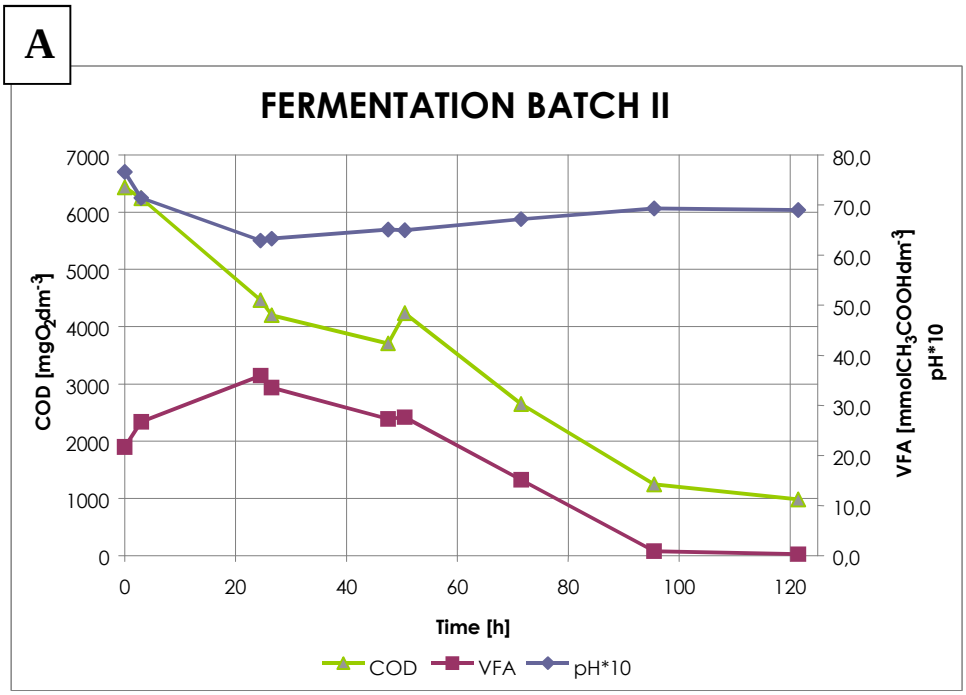
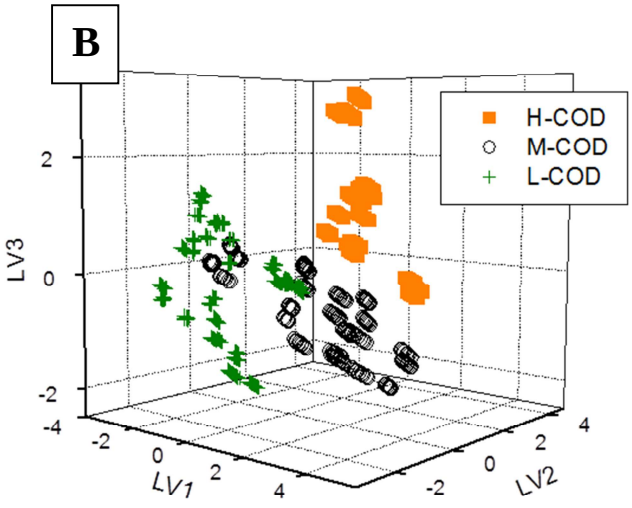


Figure 2





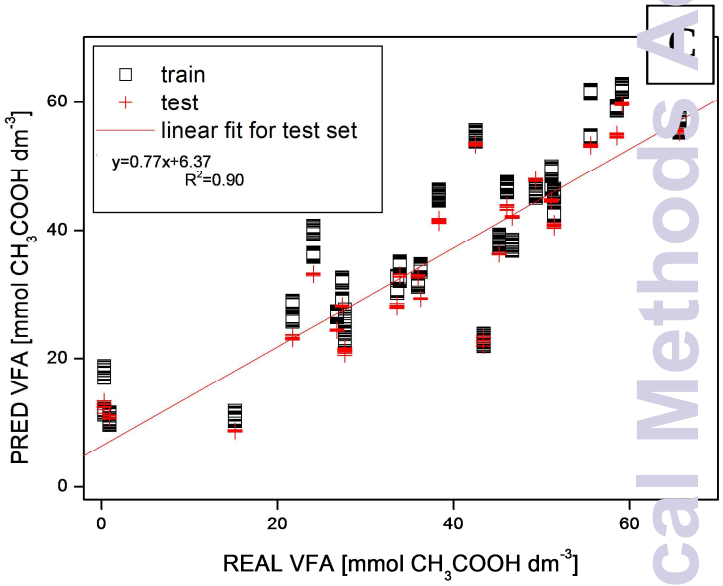


Figure 3

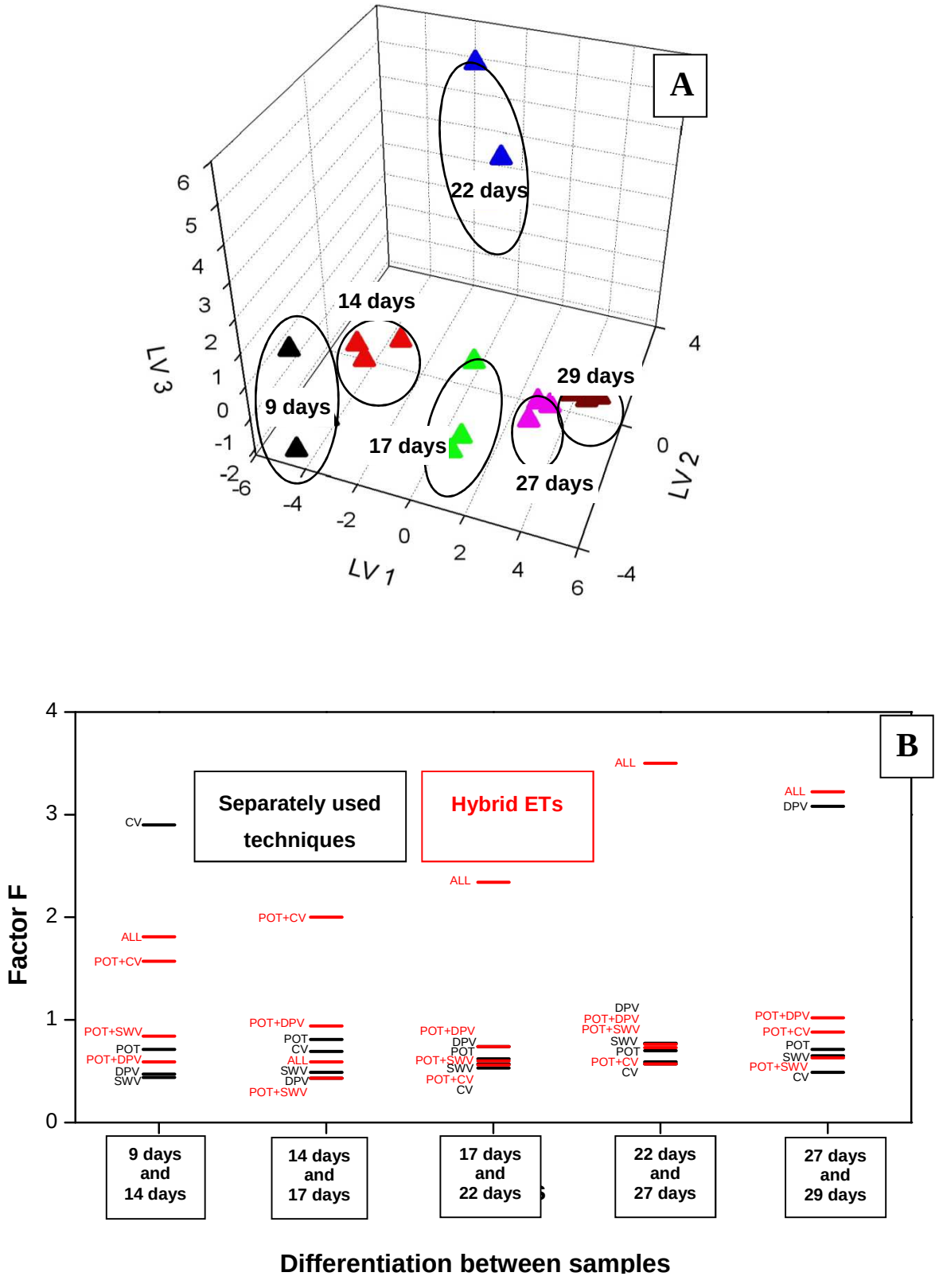
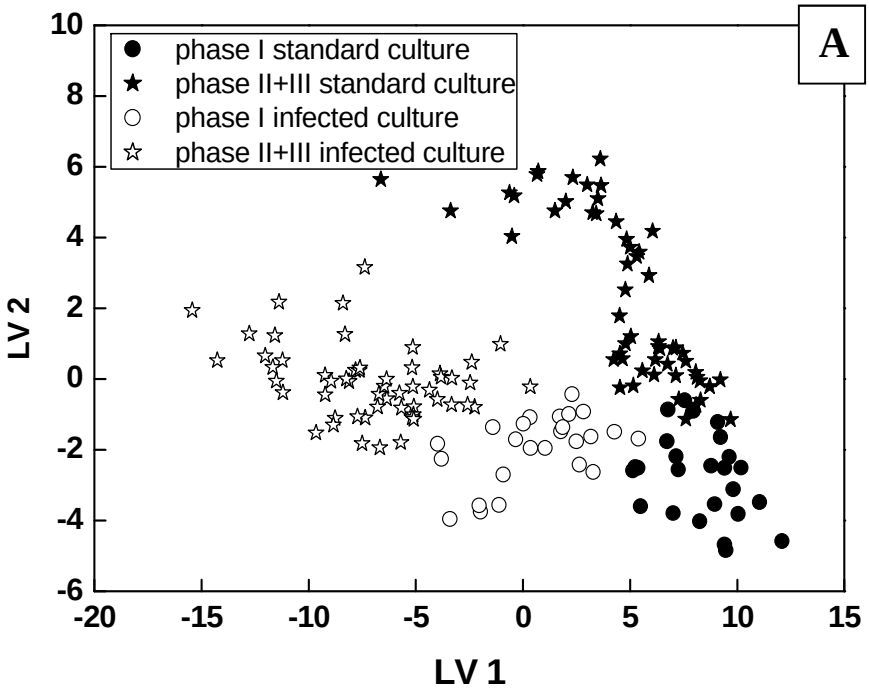


Figure 4



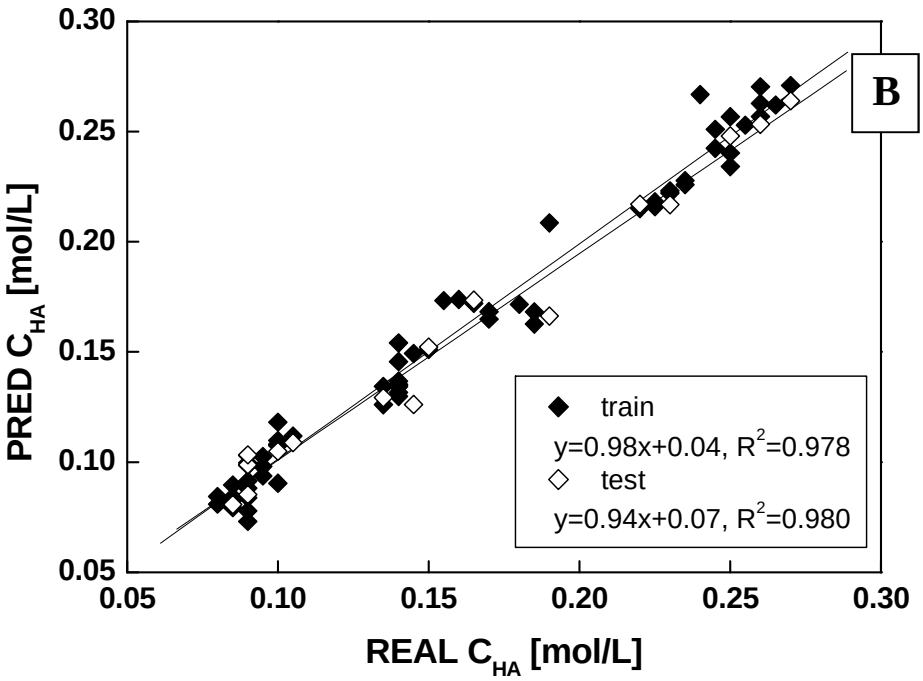


Figure 5