**Microalgae degradation follow up by voltammetric electronic tongue, impedance spectroscopy and NMR spectroscopy**

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Highlights:

- Microalgae degradation follow up by electrochemical techniques and NMR spectroscopy
- Voltammetric electronic tongue was sensitive to evolution with a good prediction
- Impedance spectroscopy was sensitive to evolution and provided a good prediction
- ^{1}H NMR spectroscopy detected metabolical changes with microalgae evolution
- This is a proof of concept for quality control strategies on microalgae production

Abstract:

Microalgae play a fundamental role in aquatic primary production and the food chain. They are a recognized source of fatty acids and fatty acid-based lipids of potential interest in the preparation of functional health products, biofuels and renewable chemicals. The exploitation of this bioresource requires a fine monitoring of ~~the culture conditions~~ each production stage. The aim of this work is the microalgae degradation follow up after the concentration stage at the end of the production process by voltammetric electronic tongue~~, and~~ impedance spectroscopy~~, using~~ and ^{1}H NMR spectroscopy. Microalgae samples were allowed to progress along time (from 1 to 23 days). At scheduled selected times, voltammetry, impedance spectroscopy and ^{1}H NMR measurements were performed. Multivariate analysis was carried out on these data by PLSR. A model calculated in a training set was then applied to a set of validation to predict the time of evolution. For the three techniques good results in prediction for the validation set were obtained ($R^2$/RMSEP of 0.961/1.51, 0.956/1.67 and 0.969/1.25 respectively for impedance, voltammetry and NMR spectroscopy). The three techniques were sensitive to the evolution of the microalgae samples. The detection of metabolical changes in the ^{1}H NMR spectra is also included. This proof of concept could be the basis for future development of rapid and robust strategies for quality control on microalgae production plants.

## 1. Introduction:

Microalgae are small microscopic aquatic photosynthetic plants. They play a fundamental role in aquatic primary production and the food chain. Microalgae are used as feed for the cultivation of mollusks, crustaceans and fish. Microalgae chemical composition is a complex mixture of minerals, vitamins, and primary and secondary products and their use opens a huge number of applications for humans [1]. Microalgae are excellent sources of various biologically active compounds such as sterols, fatty acids, phenolic compounds, carotenoids and polysaccharides [2].

Fatty acids and fatty acid-based lipids from microalgae have been recognized of potential interest in preparation of functional health products [3–5]. Moreover, diverse studies have revealed anti-inflammatory [6], anticancer [7], hypocholesterolemic [8], antioxidant [9] and antiviral [10] activities of microalgal-derived compounds [11]. Additionally, bioactive derivatives from microalgae have been reported to improve cholinergic function, antioxidant status and prevent memory impairment in the Alzheimer Disease [11].

Besides the application in health and nutrition, microalgae have been proposed as a sustainable alternative of crop for generating renewable energy [12–15]. Microalgae can grow in poor-quality water, can utilize $CO_2$ from point sources such as coal fired power plants and use nutrients from wastewater treatment plants with a uniquely high productivity potential [16–18]. Lipids obtained from microalgae can be used for the production of biofuels and renewable chemicals [14,15,19,20].

Microalgae cover almost 75% of algae species, and at least 40,000 species of microalgae phytoplankton have been identified [21]. *Nannochloropsis oceanica* is one of six species of the genus *Nannochloropsis,* which has drawn attention as a potential feedstock for the production of nutraceuticals and industrial chemicals due to its high productivity and oil content, and has been studied as a potentially powerful candidate for biodiesel production. Three *Nannochloropsis* strains have been reported to have a lipid content of 30% or higher and lipid productivity ranging from 55 to 61 mg $L^{-1}$ $day^{-1}$, making them the best lipid producers among 30 marine and freshwater microalgae in terms of both lipid content and lipid productivity [22].

From an application viewpoint, the exploitation of this bioresource requires a fine monitoring of the microalgae culture conditions, since the accumulation of the diverse compounds that microalgae produce depends on the operational growth conditions and on nutrient supplementation [23,24]. Usually, culture monitoring is performed with macroscopic indicators such as biomass evolution or pigment quantification [25]. After the culture, the microalgae are harvested and concentrated by centrifugation. This moment is a critical step when microorganisms that are ubiquitous can proliferate. In the production plant, the performance of microbial cultures in the harvested microalgal biomass is too time consuming for the production routine. Bearing this in mind However, in this scenario, the design of low-cost, fast response and easy-to-read instrumentation is appealing for the monitoring control of this last step of microalgae production.

Electrochemical techniques are on the basis of a wide number of low-cost and fast response analytical strategies. Among them, electronic tongues are analytical systems based on low selectivity cross-sensitivity sensors that, together with pattern recognition or multivariate analysis tools allow the classification of complex liquid samples or the assessment of physicochemical parameters [26–31]. Electronic tongues can be classified according to their sensing technology:

potentiometry, voltammetry, colorimetry, acoustic waves and others. Electronic tongues based on voltammetry (VET) have been intensively investigated in recent years due to their high sensitivity and high signal-to-noise ratio. The concept of VET was first introduced by Winquist in 1997 [32]. A set of potential pulses is applied to different metallic electrodes and the resulting current is sampled; the current data are then processed using multivariate analysis tools. Analyses of food stuff [33], water quality [34], wines [35] and urine for disease detection [36] and sensing of explosive material [37] are some of the applications of this type of electronic tongues. On the other hand, electrical impedance spectroscopy (EIS) is a very powerful analytical tool. It consists on the measurement of the impedance of the sample for a certain range of frequencies. EIS has been applied to a large number of applications in many different fields such as health, [38–40] food engineering [41–44] and materials characterization [44–47]. In the context of microalgae studies, sub products from algae decomposition as 2-methylisoborneol (MIB) and geosmin (GSM) have been quantified in water samples using an electronic system based on non-specific polymeric sensors and impedance measurements [48].

A different strategy for the analysis of samples is to explore the chemical profile to determine as much as possible all components in the sample. Nuclear Magnetic Resonance (NMR) spectroscopy is a well-known technique that provides information on the sample composition [49]. It has been extensively applied to solution and semi-liquid samples in areas of biological interest such as in food [50,51], biomedicine [52,53] or materials chemistry [54] among others. In the context of microalgae, NMR spectroscopy has been used to determine their chemical composition using diverse technical implementation to show lipophilic [55–57] and hydrophilic [55,57,58] compounds or the whole cells profile [55,59,60]. The usefulness of NMR spectroscopy for microalgae classification has been also demonstrated, showing the changes in the profiles of different microalgae classes [61]. Moreover, NMR has revealed variations in the metabolites of microalgae according to the harvesting conditions [62] and has been helpful to describe the central metabolic processes of marine microalgae [57]. Finally, NMR spectroscopy experiments have evidenced the metabolical composition related to their nutritional value [55]. However, NMR equipment imply an important money investment for installing and maintenance, and also needs the surveillance of highly qualified operators. Owing these requirements, NMR it is not easy to implement in industrial applications to provide *in situ* fast response.

The aim of this work is the monitoring of the degradation process of microalgae after the concentration stage at the end of the production process using voltammetric electronic tongue and impedance spectroscopy, using NMR spectroscopy to assess chemical changes in the solution. This proof of concept could be the basis for future development of rapid and robust strategies for quality control on microalgae production plants.

## 2. Materials and Methods

*Sample preparation*

Microalgae samples, *Nannochloropsis oceanica*, were provided by BUGGYPOWER, S. L. (San Pedro del Pinatar. Murcia, Spain). Microalgae was harvested in closed photo-bioreactors with an aeration system (air + $CO_2$), air lift. The microalgae medium was constituted by saline water supplemented with inorganic nutrients, mainly nitrates and phosphates. After harvesting, microalgae were concentrated by centrifugation (13000 g) until a content of 20% in weight of microalgae. A sample of this concentrated microalgae (3 kg) was packed in a plastic bag and was frozen at -20ºC until the moment of the experiments, one month later.

The microalgae were unfrozen overnight at 5ºC. Once unfrozen the content was poured in a big beaker and was gently homogenized. The sample was split in aliquots of 100 mL and stored in polypropylene beakers of 150 mL with screw caps. 32 samples of 100 mL were obtained. A needle was used to perform a hole in each cap to enable the gas exchange between the samples and the ambient. After this, all samples were weighted and put in a ventilated place in the laboratory. The samples were allowed to progress along different times (1, 2, 5, 6, 7, 9, 12, 14, 16, 21, and 23 days). With the evolution time out of the ideal harvesting conditions (without the appropriate nutrients, aeration and culture medium), microalgae underwent degradation. Changes in composition and cellular integrity were expected to occur accordingly. After the selected evolution time, the samples were weighted; evaporated water replaced and homogenized gently. Table 1 shows the weights and the evolution days for each sample. The samples were prepared then for impedance, voltammetry and NMR measurements.

*EIS and VET measurements*

The measurements of electronic voltammetric tongue and electrical impedance spectroscopy were performed in the polypropylene beakers were the samples had been stored. The beakers containing the samples were introduced in a bath thermostatted at 24ºC. The electrodes for EIS and VET experiments were introduced in the beakers, remaining in contact with the samples during the measurements. EIS measurements were carried out using the Advanced Voltammetry and Impedance Spectroscopy Analyzer (AVISA) developed by the Instituto Interuniversitario de Investigación de Reconocimiento Molecular y Desarrollo Tecnológico (IDM), at the Universitat Politècnica de València (UPV, Spain). Working and counter electrodes were implemented by means of a double needle electrode composed of two parallel stainless steel needles 1.5 cm long and 1 mm in diameter, separated by a distance of 1 cm (Figure 1). Both the measurement system and the double needle electrode have been previously described by Conesa et al. [63]. Preliminary measurements of impedance module and phase in the frequency range from 1 Hz to 10 MHz on microalgae samples with different evolution times were carried out in order to define the optimal

measurement configuration. These preliminary tests showed that the better discrimination results were obtained using the impedance phase value in the frequency range from 100 Hz to 10 MHz. Fifty frequencies within this range were selected based on a logarithmic distribution. Each sample was measured 3 times and the 3 measurements were averaged afterwards.

VET system used in this study was based on pulse voltammetry, and was also developed by the IDM. The electronic tongue was composed of an electronic equipment, a software application that runs on a personal computer and a set of metallic electrodes (Figure 2). The electronic equipment applied voltage signals to electrodes. The temporal evolution of the current signal was collected for each working electrode and was sent to the PC to be stored for further processing. A set of pulses was put together to form a pulse train in order to extract as much information as possible from the solution [64]. Details of this electronic equipment have already been published [65]. Following the methodology proposed by Winquist et al., [32] the electronic tongue device used in this work consisted in an array of four metallic working electrodes, Iridium (Ir), Rhodium (Rh), Platinum (Pt) and Gold (Au), with purity of 99.9 % and a 2 mm diameter. The electrodes were housed inside a homemade stainless steel cylinder, which was used at the same time as both the body of the electronic tongue system and the counter electrode. A more detailed description of the electrodes used can be found in Campos et al. 2012 and 2014 [66,67]. Electrodes were conditioned before their use by mechanical polishing and immersion in an acidic solution. Afterwards, electrodes were rinsed with distilled water before measurements. A saturated calomel electrode was used as the reference electrode. A pulse train was programmed with 42 pulses, ranging from -1 to 1 V. Each pulse was applied for 50 ms. The detail on the applied pulse sequence is shown in Figure 3. Current values collected per pulse and electrode were described by 23 measurements (1 current value every 2.17 ms). In global, 3864 current data points were obtained for each sample (42 pulses x 4 metals x 23 points = 3864). Three measurements on the same sample were performed to assess the reproducibility. Data from the 3 measurements were averaged and the average was considered representative of the measurement of each sample.

*NMR spectroscopy experiments*

The supernatant of each sample (medium where microalgae were allowed to evolve and remained after centrifugation) was studied by NMR spectroscopy. 1 mL of the corresponding sample was diluted with PBS (10 mL) and the solution was filtered with a fluted filter. The filtrated solution was collected with a syringe and filtered with a PTFE syringe filter with a pore size of 0.45 μm. 500 μL of this filtered solution and 200 μL of $D_2O$ (98% deuterated) were put in a 5 mm NMR tube. NMR spectroscopy experiments were performed on a 400 MHz ~~magnet~~ NMR spectrometer (Bruker Ascend 400) equipped with an ATM 5 mm probe (BBO 400 MHz 5mm Z-Grad). [1]H 1D NOESY experiment (noesygppr1d Bruker library) with water presaturation during relaxation delay and mixing time and spoil gradient was performed for each sample. 128 scans were

performed, with an acquisition time of 2.56 sec, spectral width of 16 ppm (6402 Hz), a fid resolution of 0.390750 Hz and a size of fid of 32 k. Each spectrum was recorded in 10 min and 7 seconds. FID's were line broadened (exponential windowing function of 1 Hz), Fourier transformed, phase, base line corrected and aligned with MestReNova version 6.0.2. The areas of low and high ppm without relevant resonances and the residual water signal after water suppression were not included in the analysis.

*Data analysis*

Due to the important number of data collected during voltammetric, impedimetric and NMR measurements, complex mathematical algorithms are required to process the information. Multivariate analysis tools as Principal Component Analysis (PCA) are frequently applied to measurement data in order to classify the samples [68]. In PCA a new variable space is created from the original variables so that the maximum variance of the samples is concentrated in the first components of the new space. A classification of the samples can be obtained by representing graphically the samples in the two or three first dimensions of this new space called principal components (PC). When the objective is the generation of quantitative prediction models, multivariate regression tools as Partial Least Square Regression (PLSR) are employed [68]. In this case, the new variable space is built so that the maximum covariance between the measurement data and the parameter to be predicted is condensed in the first axes of the new space. The axes of this new space are named latent variables (LV). The prediction model is then built by applying a multiple regression to the LV containing the relevant information of the data.

Multivariate analysis was performed by the PLSR [69] method in impedance, voltammetry and NMR spectroscopy using the software PLS Toolbox Solo 8.0 (Eingenvector Research, Inc.) for chemometric analysis. Before the analysis, data were autoscaled. To evaluate the adequacy of the experimental data and to select the quantity of LV, a cross-validation was performed before building the model for each technique. Venetian Blinds was the method used in the cross-validation. The minimum value for the cross-validation error was used to select the number of LV for the model. Then, the obtained model was applied to the set of validation samples to predict the time of evolution.

EIS data consisted of 50 values that corresponded to the phase of 50 frequencies in the range from 100 Hz to 10 MHz. VET data was composed by 3864 for the whole set of metals considered in the electronic tongue (23 current data points for each pulse). NMR data were selected according to the signals relevance. Thus, the areas of low (< 0.6 ppm) and high ppm (> 8.8 ppm) without relevant resonances and the residual water signal (from 4.4 to 6.7 ppm) were not included in the analysis. The final number of data points included were 6041. The data were normalized versus the aliphatic area (0.6 – 4.4 ppm). NMR spectra were assigned. Signals in the spectra were

identified according to the chemical shift, the multiplicity and the values found in the bibliography [55,57,61,62].

## 3. Results and Discussion

Microalgae samples experienced changes in color and appearance that were more clearly observable in the samples with longer evolution. <mark>After the freezing process, some population in the microalgae biomass was expected to be damaged. But this damage was the same for all the samples at the initial moment, and the changes with respect to this initial point with the different times of evolution were observed by the three techniques.</mark>

*EIS results*

Figure 4 shows the values of the impedance phase in the frequency range between 100 Hz and 10 MHz for all samples. The color of the plots indicates the evolution time (blue short evolution time and yellow long evolution time). A good correlation between impedance phase values and evolution time can be observed in two frequency sub-ranges. From frequencies between 300 Hz and 1 kHz the higher the evolution time the higher the value of the phase is. This is the frequency range were conductivity measurements are carried out, and therefore the change in the impedance for this range corresponds most likely to changes in the conductivity of the sample as the evolution time increases. In the range from 2.5 MHz to 5 MHz the value of the phase decreases as the evolution time increases. In this case, changes observed in the impedance are attributed to changes in the sample capacitance due to the destruction of the microalgae cells body.

In order to get a better understanding of the relation between impedance measurement and evolution time, a PCA was performed. Figure 5 shows that the two first principal components include 95% of the total variance. The first principal component which contained 81% of the total variance was clearly related with the evolution time. This confirms the close relation between phase impedance and microalgae degradation.

Finally, a prediction model for the evolution time was built using impedance data. Figure 6 shows the observed *vs* predicted plot corresponding to the validation of the model. Impedance spectroscopy data allowed the generation of a very robust prediction model with an $R^2$ of 0.961, an RMSEP of 1.51, a slope of 1.04 and an intercept of -0.62. These results support the use of EIS as a valid method for the control of microalgae degradation. Although the cross-validation procedure establishes that 3 LV should be used, a model built using only one LV gave very similar results ($R^2$ 0.964, RMSEP 1.47, slope 1.06 and intercept 0.49). The robustness of the model generated using only one LV was consistent with the fact that for some frequencies phase values are ordered according evolution time (see Figure 4). From a practical point of view, this implies

that a good evolution time prediction could be achieved with only one impedance phase measurement. As mentioned before, if a low frequency (in the range between 300 Hz and 1 kHz) is selected, the prediction model will have a strong relation with the sample conductivity. On the contrary, if a high frequency is used (between 2.5 MHz and 5 MHz) the influence of conductivity would be reduced as, for these frequencies, the phase value depends mainly on changes in the cells body integrity and structure.

*VET results*

For VET measurements the discrimination between the evolution days was distinguishable only for some pulses. As an example, Figure 7 shows the current signals for the Ir electrode. When a pulse of -1V was applied to this electrode, the measured current clearly depended on the time of evolution; the longer the samples had evolved the higher the measured current is. This might be due to the presence of compounds that are released when the microalgae degrade and react with Ir electrode at a certain potential. Ir also shows evolution time discrimination for other positive and negative pulses. Rh and Pt electrodes exhibited a similar behavior (data not shown). For the Au electrode, the discrimination appeared only in positive pulses (data not shown). This results suggested that the VET was detecting compounds whose presence or concentration was related with the microalgae degradation process.

 A PCA of the voltammetry measurements for all electrodes is shown in Figure 8. Although samples corresponding to evolution days 21 and 23 were clearly separated from the rest, the discrimination was very poor for degradation time below 16 days. Besides the variance for the 2 first PCs was very low (22% for PC1 and 15% for PC2). This is an indicator that more PCs should be included to obtain all relevant information.

With the aim of built a prediction model for the evolution time, PLSR studies were carried out. Evolution time prediction models were built using the data of all electrodes and the data of each electrode separately. Results are shown in Table 2. All models, except for Au, presented a very good performance. The highest robustness was achieved when data from all electrodes was used, ($R^2$ 0.965, RMSEP 1.67, slope 0.85 and intercept 1.34) (see Figure 9), which confirms that the combination of information from several electrodes can improve the prediction capability of the system (in agreement with the electronic tongue concept). Four LV were necessary in this case, probably due to the complexity of microalgae samples and the variety of processes related to the electrochemical reactions that take place during voltammetric measurements.

*NMR results*

NMR spectra were obtained for the supernatant from each of the samples, and changes in the composition were clearly observed within the days of evolution (Figure 10). The spectra showed resonances in the aliphatic and in the aromatic region for all samples. The assignment of the main

resonances in the spectra is shown in the Table 3 and is detailed in the Figure 11. As can be observed (Figures 10 and 11), the intensity of some compounds as tyrosine, hydroxyphenyllactate and the resonances at 1.04 (t) and 2.2 (m) increased with the time of evolution. Moreover, a great number of compounds as lactate, alanine, acetate, γ-aminobutyric acid, succinate, trymethylamine, glycinebetaine, glycine, dihydroxyacetone and formate among others showed a drop in the intensity (Figures 10 and 11) when increasing the evolution time, which is in agreement with the degradation process. A descent in main aminoacids concentration as alanine has been observed in photosynthesis impairment in green microalgae, and glycinebetaine and choline derivatives descents have been related with decreased osmoregulation [69].

A prediction model for the evolution time was built with the NMR spectroscopy data from the supernatant of each sample. Figure 12 shows the observed vs predicted plot corresponding to the validation of the model. The NMR spectroscopy data provided a very robust model ($R^2$ 0.969, RMSEP 1.2512, slope 0.97 and intercept 0.50).

The differences in the intensity of some signals observed in NMR and the ability of the built model to predict the evolution time support the changes observed in the VET data. In fact, some of the assigned compounds in the Table 3, and whose concentration changed over time, are redox active substances, such as amino acids (alanine, glycine, glycinebetaine and tyrosine), glutathione, ethanol, lactate, acetate, succinate, choline and fumarate among others. The potentials of the redox processes involving these compounds are in the range of the potentials applied in the VET experiments [70,71] and therefore it is reasonable that the changes in the metabolite concentrations observed by NMR were also detected by voltammetric experiments.

All above results demonstrate de ability of EIS, VET and NMR techniques to detect changes in the evolution of the microalgae samples after the concentration stage at the end of the production process. The changes observed by NMR and VET are more directly related with changes in the chemical composition. The results observed by EIS are more probably related with the changes in the microalgae cellular integrity that yield changes in the sample capacitance.

## 4. Conclusions

The use of EIS and VET as analytical tools for the assessment of microalgae degradation has been evaluated in samples concentrated after harvesting and preserved frozen until the time of analysis. Both methods allow the generation of robust prediction models for the evolution time of *Nannochloropsis oceanica* microalgae samples. In the case of impedance spectroscopy, the prediction capability is related to the conductivity of the samples (for low frequencies) and probably with the microalgae cells body structure (for high frequencies). The phase spectra (where for some frequency ranges signals are ordered according to the evolution day) and the fact

that one LV suffices to generate a robust prediction model indicates that microalgae degradation can be followed by the measurement of the impedance phase at one single frequency. For VET, prediction models are based on the information corresponding to electrochemical processes occurring between the electrodes and some of the sample compounds whose concentration changes during the microalgae degradation. Moreover, changes in the composition of the supernatant solution were identified in the NMR studies. Although from a practical point of view and considering an industrial application, impedance spectroscopy seems the most convenient method for the assessment of microalgae evolution with time this study demonstrated that the three techniques used (i.e. EIS, VET and NMR) are suitable to monitor microalgae grow and evolution.

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**Table 1**

Initial weight, weight after evolution, evaporated weight and evolution time for each sample.

| Sample number | Initial weight[a] | Weight after evolution[a] | Evaporated weight[a] | Evolution time[b] |
|---|---|---|---|---|
| 1 | 115,99 | 115,95 | 0,04 | 2 |
| 2 | 116,37 | 116,35 | 0,02 | 2 |
| 3 | 116,37 | 116,33 | 0,04 | 2 |
| 4 | 116,15 | 115,99 | 0,16 | 5 |
| 5 | 115,98 | 115,81 | 0,17 | 5 |
| 6 | 117,13 | 116,96 | 0,17 | 5 |
| 7 | 115,90 | 115,71 | 0,19 | 6 |
| 8 | 116,70 | 116,48 | 0,22 | 6 |
| 9 | 116,95 | 116,72 | 0,23 | 6 |
| 10 | 116,95 | 116,67 | 0,28 | 7 |
| 11 | 117,76 | 117,44 | 0,32 | 7 |
| 12 | 114,94 | 114,65 | 0,29 | 7 |
| 13 | 115,94 | 115,61 | 0,33 | 9 |
| 14 | 116,37 | 116,01 | 0,36 | 9 |
| 15 | 116,16 | 115,80 | 0,36 | 9 |
| 16 | 116,18 | 115,65 | 0,53 | 12 |
| 17 | 115,78 | 115,27 | 0,51 | 12 |
| 18 | 114,85 | 114,40 | 0,45 | 12 |
| 19 | 115,78 | 115,24 | 0,54 | 14 |
| 20 | 116,32 | 115,78 | 0,54 | 14 |
| 21 | 116,31 | 115,75 | 0,56 | 14 |
| 22 | 114,12 | 112,90 | 1,22 | 16 |
| 23 | 117,19 | 116,39 | 0,80 | 16 |
| 24 | 116,38 | 115,95 | 0,43 | 16 |
| 25 | 116,75 | 116,01 | 0,74 | 21 |
| 26 | 116,66 | 115,98 | 0,68 | 21 |
| 27 | 116,22 | 115,46 | 0,76 | 21 |
| 28 | 116,20 | 115,47 | 0,73 | 23 |
| 29 | 116,03 | 115,23 | 0,80 | 23 |
| 30 | 116,05 | 115,28 | 0,77 | 23 |
| 31 | 115,66 | - | - | 1 |
| 32 | 116,64 | - | - | 1 |

[a] Weights are expressed in grams, [b] Evolution time is expressed in days.

**Table 2**

Voltammetric data adjusting parameters and number of LV for PLSR prediction models in the validation set.

| Electrodes | Data number | LV | $R^2$ | p1 | p2 | RMSEP |
|---|---|---|---|---|---|---|
| Ir Rh Pt Au | 3864 | 4 | 0.956 | 0.848 | 1.34 | 1.67 |
| Ir | 966 | 3 | 0.947 | 0.877 | 0.94 | 1.69 |
| Rh | 966 | 2 | 0.911 | 0.782 | 2.95 | 2.40 |
| Pt | 966 | 3 | 0.913 | 0.891 | -0.20 | 2.41 |
| Au | 966 | 3 | 0.860 | 0.724 | 2.59 | 2.82 |

**Table 3**

Assignment of the main resonances in the NMR spectra.

| Compound | $^1H\ \delta$ [a] | Multiplicity [b] | Group |
|---|---|---|---|
| Aminoacids and lipids | 0,87 | x | $CH_3$ |
| Aminoacids and lipids | 0,98 | x | $CH_3$ |
| Ethanol | 1,17 | t | $\beta CH_3$ |
| Lactate | 1,33 | d | $CH_3$ |
| Alanine | 1,47 | d | $CH_3$ |
| γ-aminobutyric acid | 1,89 | q | $3CH_2$ |
| Acetate | 1,91 | s | $CH_3$ |
| γ-aminobutyric acid | 2,29 | t | $4CH_2$ |
| Succinate | 2,4 | s | $(CH_2)_2$ |
| Trimethylamine | 2,89 | s | $(CH_3)_3$ |
| γ-aminobutyric acid | 3,01 | m | $2CH_2$ |
| Creatine | 3,02 | s | $CH_3$ |
| Choline | 3,19 | s | $(CH_3)_3$ |
| Glycinebetaine | 3,26 | s | $N\text{-}CH_3$ |
| Glycine | 3,56 | s | $CH_2$ |
| Alanine | 3,78 | q | CH |
| Glycinebetaine | 3,9 | s | $\alpha CH_2$ |
| Creatine | 3,91 | s | $CH_2$ |
| Choline | 3,98 | m | $CH_2$ |
| Lactate | 4,11 | q | CH |
| Dihydroxyacetone | 4,36 | s | $CH_2$ |
| Fumarate | 6,51 | s | $\alpha CH$ |
| Hydroxyphenyllactate | 6,84 | d | CH |
| Tyrosine | 6,90 | d | C3,5H ring |
| Tyrosine | 7,17 | m | C2,6H ring |
| Hydroxyphenyllactate | 7.21 | d | $(CH)_m$ |
| Glutathione | 8,19 | d | NH Cys |
| Formate | 8,45 | s | CH |

[a] Chemical shift ($\delta$) is expressed in ppm.

[b] Multiplicity patterns are defined as: singlet (s), doublet (d), triplet (t), quadruplet (q) and complex o broad signal (x).

**Captions to illustrations**

**Figure 1:** Impedance spectroscopy measurement system

**Figure 2:** Scheme of the voltammetric electronic tongue

**Figure 3:** Applied pulse sequence for all the electrodes

**Figure 4:** Impedance Spectroscopy measurements for all the samples. Time scale is in the color bar in days.

**Figure 5:** PCA of the impedance spectroscopy measurements. Each sample is labelled with the time of evolution in days.

**Figure 6:** Predicted *vs* Measured evolution days plot of the PLSR model validation using impedance spectroscopy measurements

**Figure 7:** Voltammetry measurements for Ir electrode for all the samples. Time scale is in the color bar in days.

**Figure 8:** PCA of voltammetry measurements for all the electrodes. Each sample is labelled with the time of evolution in days.

**Figure 9:** Predicted *vs* Measured evolution days plot of the PLSR model validation using voltammetry measurements for all the electrodes

**Figure 10:** NMR spectra: aromatic and aliphatic regions are shown. One sample for each evolution time is displayed. The intensity of Aromatic region is scaled x10 compared to the Aliphatic part.

**Figure 11:** NMR spectra assignment. Stacked NMR spectra for all the samples. Aliphatic (a) and Aromatic (b) regions of the spectra included in the PLSR analysis. The intensity of Aromatic region is scaled x10 compared to the Aliphatic part. Keys: AA & lip, Amino Acids and lipids; EtOH, ethanol; Lac, lactate; Ala, alanine; Ac, acetate; Succ, succinate; TMA, trimethylammonium; Cho, choline; Cr, creatine; GB, glycinebetaine; Gly, glycine; DHA, dihydroxyacetone; HPL, hydroxyphenyllactate; TYR, tyrosine; GABA, γ-aminobutyric acid; GSH, glutathione. The color scale is according to the days of evolution.

**Figure 12:** Predicted *vs* Measured evolution days plot of the PLSR model validation using NMR spectroscopy data.
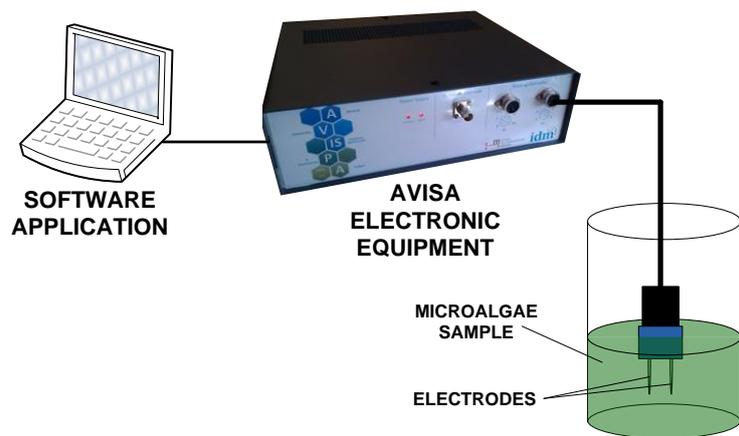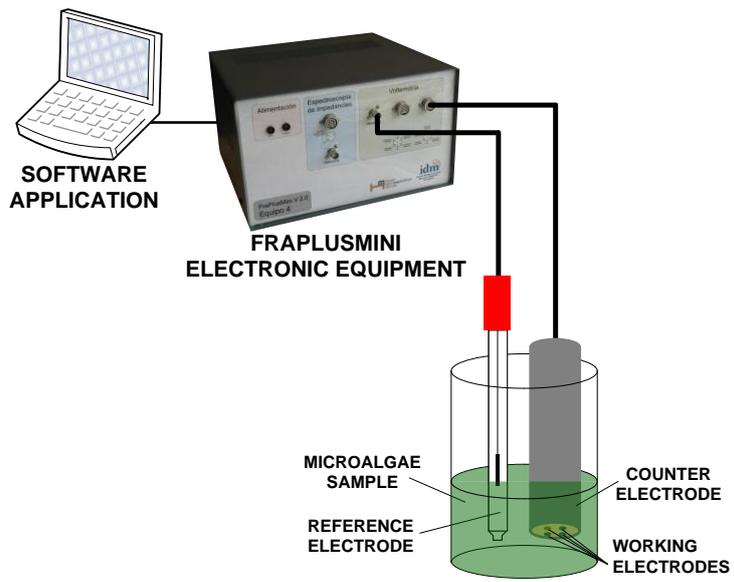
**Figure 1**



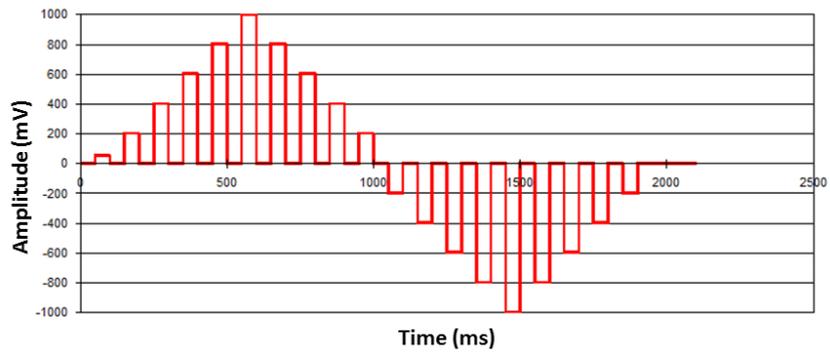SOFTWARE
APPLICATION

AVISA
ELECTRONIC
EQUIPMENT

MICROALGAE
SAMPLE

ELECTRODES

**Figure 2**



SOFTWARE
APPLICATION

FRAPLUSMINI
ELECTRONIC EQUIPMENT

MICROALGAE
SAMPLE

REFERENCE
ELECTRODE

COUNTER
ELECTRODE

WORKING
ELECTRODES

1

**Figure 3**

**Figure 4**

**Figure 5**

**Figure 6**



Predicted evolution time (days)
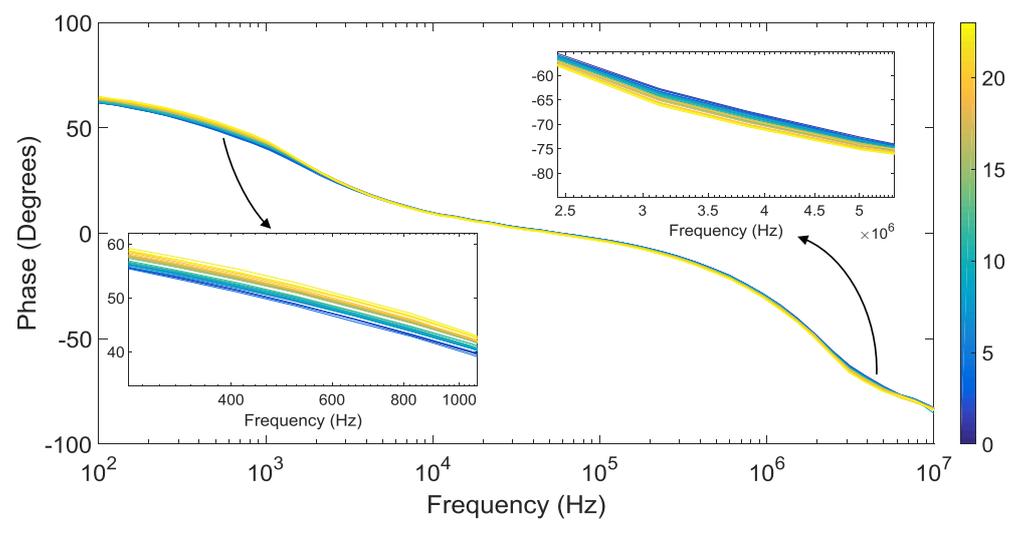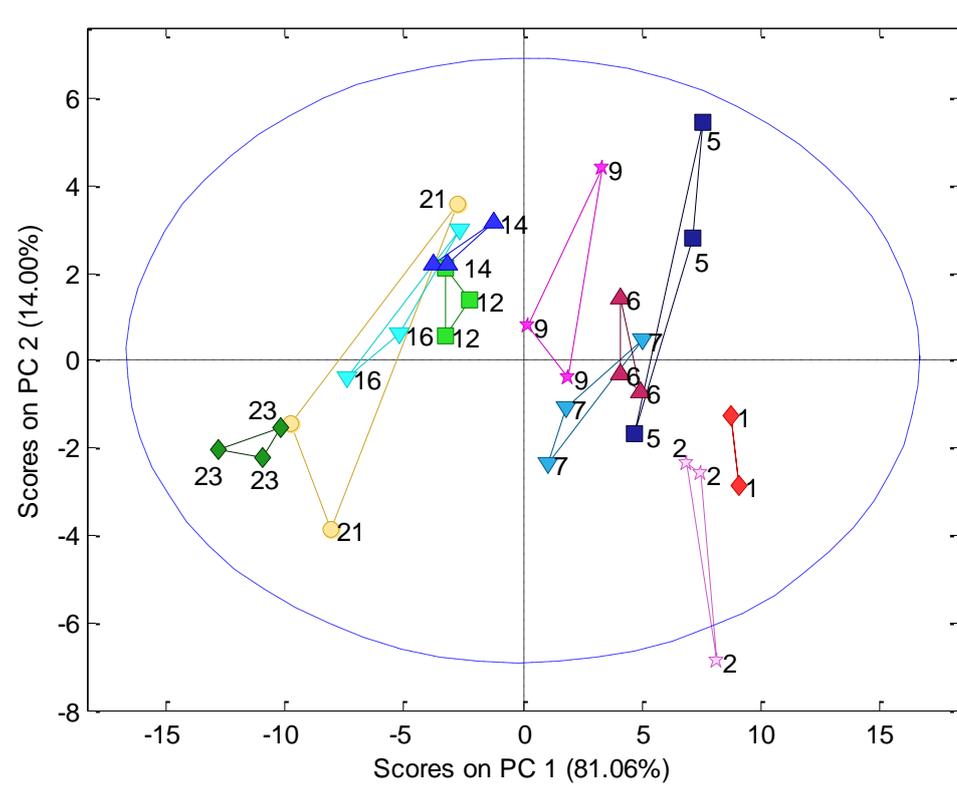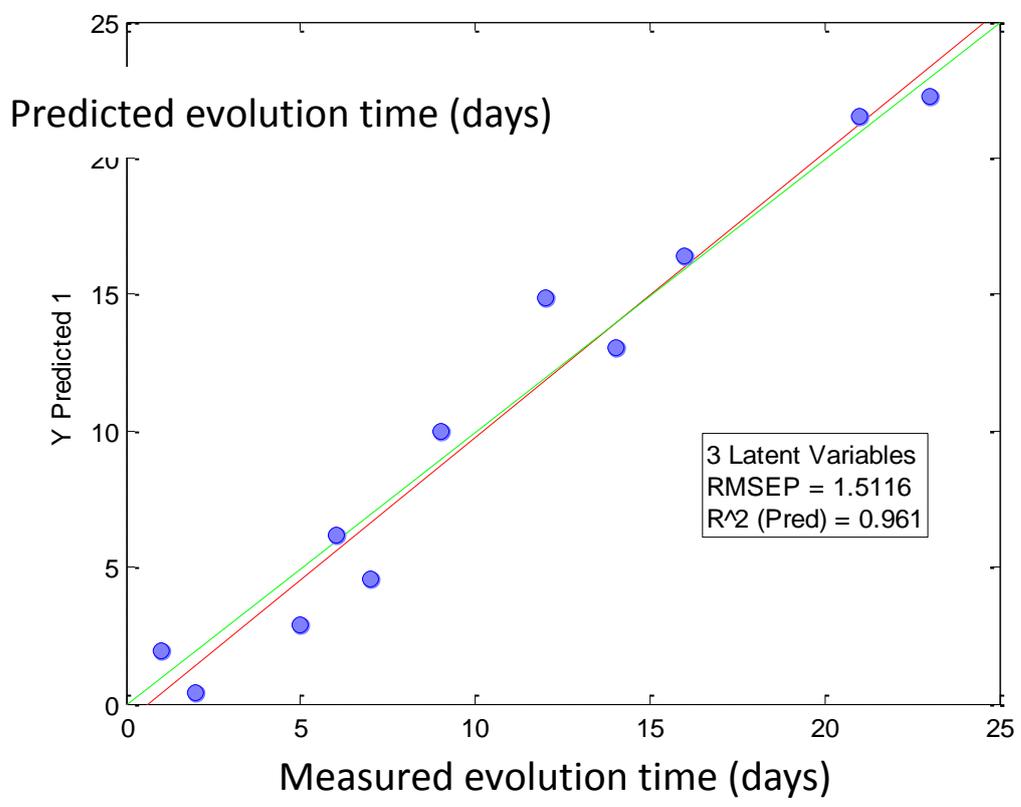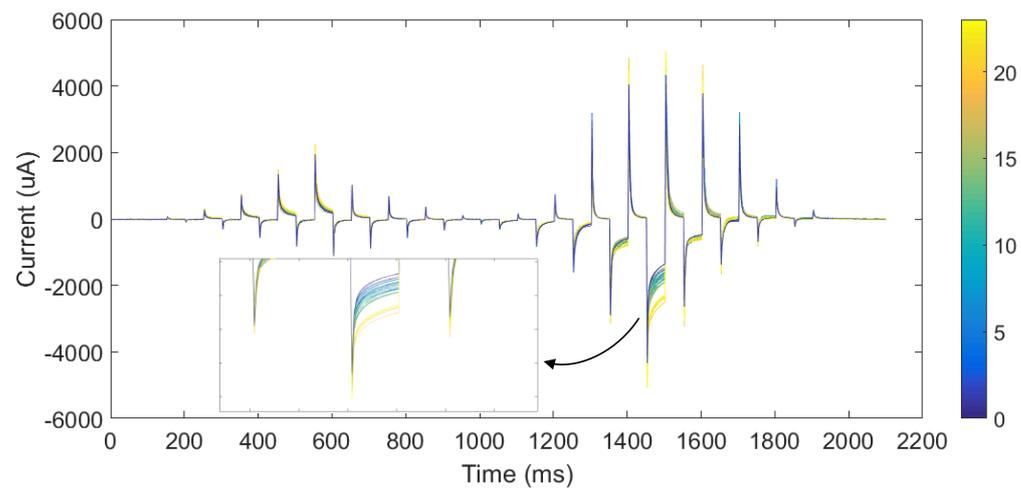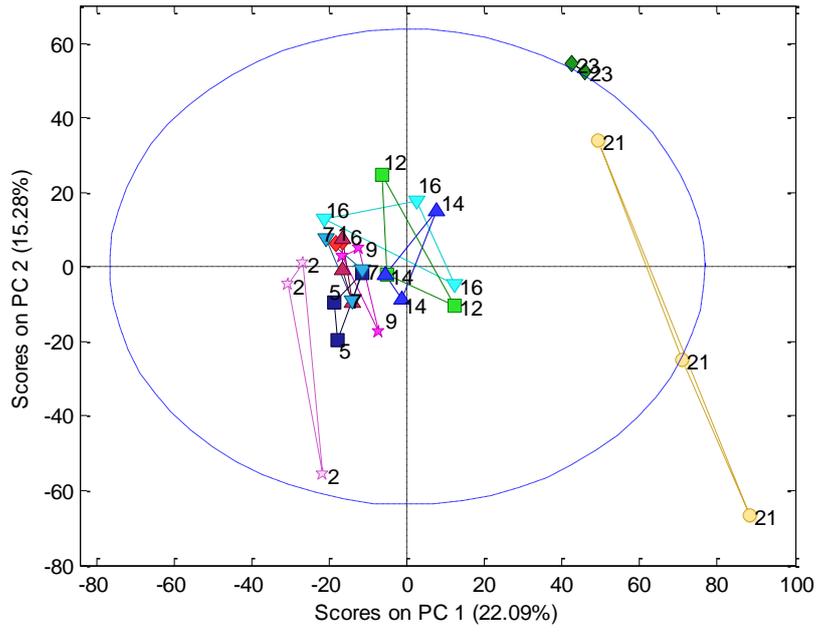
**Figure 7**

**Figure 8**

**Figure 9**
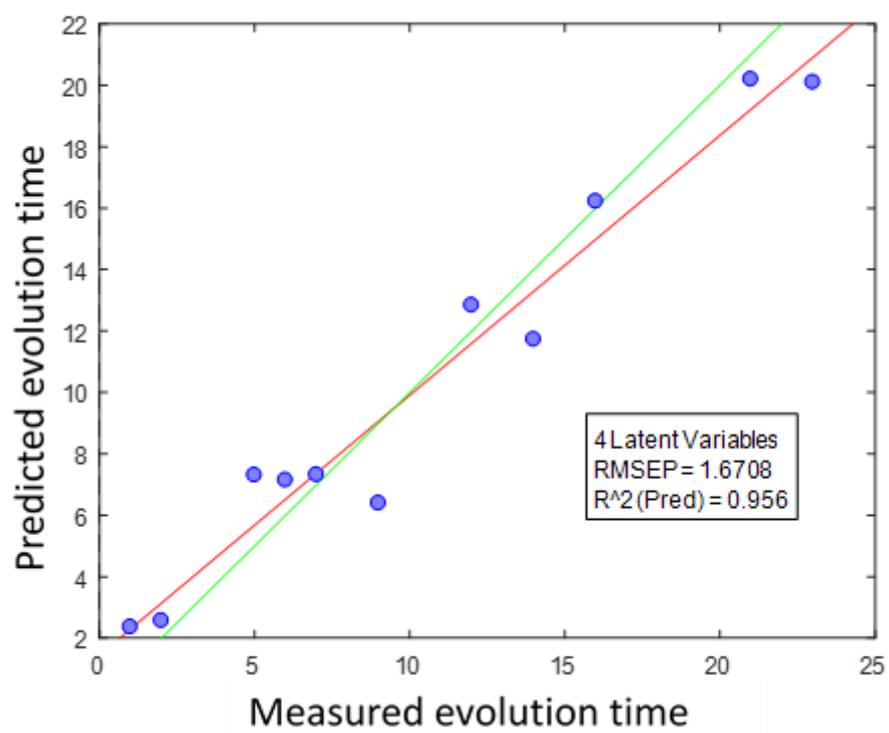
**Figure 10**

**Figure 11**

**Figure 12**