

## SO2SAFE-ENZYMATIC SO<sub>2</sub> BIOSENSOR FOR RAPID FOOD SAFETY MONITORING

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**Abstract** – BIOLAN is a highly innovative SME that develops and commercializes biosensors for quantitative analysis of food quality and safety parameters. BIOLAN's technology is based on its proprietary enzyme-based electrochemical biosensor technology platform. Currently, with the funding support of the SME Instrument Phase 2 (H2020-SMEINST-2-2015; SO2SAFE - GA 684026), BIOLAN is working in the miniaturization and industrialization of this technology to enable the commercialization of a portable and user friendly biosensor device and inexpensive, disposable test strips to monitor sulphite levels in foods.

**Keywords:** biosensor, sulphite, crustacean, enzyme, screen printing scale up,

### 1. INTRODUCTION

Sulphites are food additives that have antioxidant and preservative properties, but are recorded as allergens by the main international regulatory bodies on food safety because of their adverse health effect. Hence, sulphites maximum concentration in foodstuff is regulated, and must be ensured by the agro-food processing industries [1]. The methods and tools currently used for sulphite level monitoring in the agro-food industry and in the crustacean industry, such as *Monier Williams* [2], are not solving the problem of the high quality control standards required to comply with new regulation on food safety and consumer health protection. The agro-food industry demands new technology enabling fast, accurate and simple methods for sulphite level monitoring and traceability along the whole supply chain.

In the specific case of crustacean industry, sulphite additives are used in order to prevent crustacean melanosis, and are applied after harvest

and throughout the whole chain process. Melanosis is the formation of blackspots on crustaceans, being a natural mechanism caused by the enzymatic action. It has no impact on the food flavour or consumer health, but however, the blackspots can drastically affect consumer acceptability of the food and significantly diminish its market value. Legislation compliance with raising standards and regulations on food safety and consumer health protection is challenging for the crustacean industry regarding additives and allergens.

Early monitoring of sulphite in the water of the bins where shrimps are collected and mixed with water, ice and sulphite will prevent failures in the control of melanosis and moreover will prevent the presence of excessive amounts of sulphite in the final product.

The FDA optimized AOAC Method (Monier Williams. AOAC 962.16) is the most widely used method in the food industry to determine the concentration of total sulphites in food products [3], despite being time-consuming and technical labour-intensive. Other sulphite monitoring methods such as test strips, enzymatic method or methods based on titration have great limitations as false positives, reproducibility and accuracy for on-site monitoring. The European Food Safety Authority (EFSA) already includes the enzymatic biosensors as an alternative method for detection of sulphites in foods in the Scientific Opinion on the evaluation of allergenic foods and food ingredients for labelling purposes [4].

Main objective of the project SO2SAFE, financed under the H2020 SME instrument phase 2, is to develop a novel miniaturized ready to use biosensor based on disposable Screen Printed Electrodes (SPE) which will allow a highly sensitive, selective, fast and user friendly method for sulphite quantification in crustaceans and processing water.

The novel solution will have a direct impact in the food processing industry, but also in the society by providing consumers with safe and healthy food.

## 2. EXPERIMENTAL

### 2.1. Portable potentiostat design

To achieve miniaturization and portability, miniaturized electronics have been design based on a 32 bits Pic32 microcontroller to handle information coming from different areas of the device. Communications to the sensor and the HMI and GPRS/GSM and TCP/IP connections have been established. Connections to the screen printed electrode (SPE) have been established for integrating the three electrodes of SPEs.

### 2.2. Sulphite biosensor and electrochemical assay

The disposable SPE consisting of a carbon working electrode, a carbon counter electrode and a silver/silver chloride (Ag/AgCl) pseudoreference electrode were produced at IK4CIDETEC, using a Thieme 110E screen-printing machine from Thieme GmvH&Co (Teninge, Germany), an UV tabletop dryer Aktiprint T/A 40-1 from Technigraf (Hessen, Germany) and an oven UNE 200 from Memmert (Winsconsin, USA).

The sulphite biosensors were constructed by modifying the working electrode of the SPEs with potassium ferricyanide and with the Sulphite Oxidase (SOx) enzyme produced in a 15 L bioreactor Biostat C Plus from Sartorius (Göttingen, Germany). Amount of each component, as well as the working potential were optimized in order to achieve the highest sensitivity.

Amperometric measurements were performed with a PGSTAT 302N/SRA2 potentiostat-galvanostat from Autolab (KM Utrecht, The Netherlands) and the portable device developed by BIOLAN.

### 2.3. Real samples measurement

Water was collected from three shrimp farms bins located in Ecuador, where sodium metabisulphite was added to prevent melanosis.

### 2.4. Scale up manufacturing process definition

The manufacturing process is been designed in order to avoid operations that don't generate added value based on the following principles:

simplification, integration, time reducing and efeciency.

## 3. RESULTS AND DISCUSSION

Miniaturization and portability was achieved by designing miniaturized electronics and casing (Figure 1). The Measuring Device prototype includes an electrochemical cell, where the addition of the sample causes an increase of current, that the device correlates with the amount of sulphite. The software retrieves from the Measuring Device the data to be present to the user in the Human Machine Interface (HMI).



Fig.1. Portable potentiostat.

Optimization of the electrochemical response for sulphite analysis in water samples was undertaken through the definition of the composition of the miniaturized enzymatic biosensor.

The developed biosensor shows good sensitivity (62 nA/mg/L) and reproducibility (RSD=4%, n=5), with a linear range of 15-1000 mg/L (Figure 2).

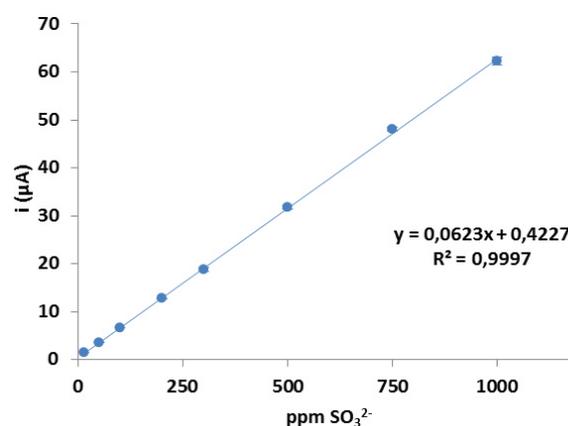


Fig.2. Calibration curve of sulphite obtained with the developed biosensors. Each point represents

the current value recorded at 60 seconds.  $E_{app}=225$  mV vs. Ag/AgCl pseudo reference electrode.

Stability of the biosensors is a key factor in order to achieve commercialization. The long-term stability of the developed biosensors was evaluated. A batch of biosensors was fabricated and stored at 30 °C for 45 days. Calibration curves in the range 250-1000 mg/L were recorded every 7 days (Figure 3)

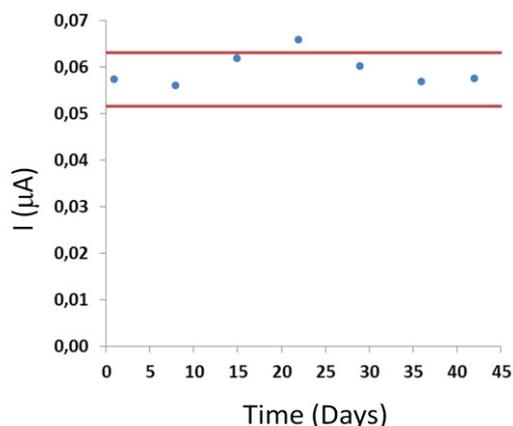


Fig.3. Stability assay obtained for sulphite biosensors stored at 30 °C. Upper and lower control limits are 10% of the slope value obtained the first day.  $E_{app}=225$  mV vs. Ag/AgCl pseudo reference electrode.

The developed biosensor was applied for the detection of sulfite in real samples, in particular, in the water collected from three shrimp farm bins located in Ecuador, where sodium metabisulphite is added to prevent melanosis. The real samples were diluted 100 times with 0,1M carbonate buffer pH = 10,2 before performing the amperometric measurements and the sulfite concentration was determined by using the calibration curve shown in Figure 1. The sulfite concentrations in water samples containing 0, 50 and 100 g/l of sodium metabisulphite were found to be  $0,2 \pm 0,07$ ,  $52,2 \pm 0,4$  and  $100 \pm 7$ , respectively (n = 7). These results demonstrated that the developed sulfite biosensors can be used for monitoring sodium metabisulphite in shrimp farming.

Scale up manufacturing process necessary to face the challenge of increasing the production capacity to cover SO2SAFE biosensor sales demand

is been defined and first steps for its implantation have been taken.

For SOx enzyme production, culture conditions have been defined in order to obtain high growth rates in a 15 L bioreactor by fed batch culture media obtaining a 30-fold optical density comparing with batch cultivation.

The SPE design is been optimized in order to simplify manufacturing process, packaging, shipment and handling operations by the user. A plastic cover with a dispensing area around the working electrode has been designed (Figure 4).

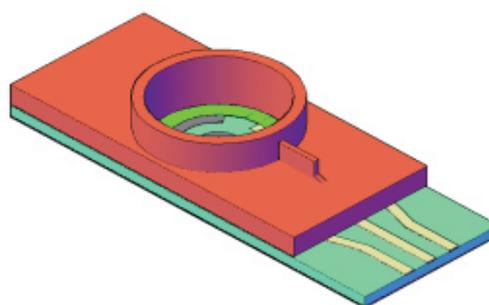


Fig.4. Strip or Biotest designed that integrates the SPE and a cover or holder.

The biofunctionalization procedure is been optimized in order to adapt the actual protocol, which is done manually to automatic dispensing with a BIODOT equipment.

On the basis of the previously explained processes (enzyme production, SPE printing, holder placement and dispensing) a scale up manufacturing process has been defined.

#### 4. CONCLUSIONS

A miniaturized biosensor has been developed for sulphite on site monitoring in water samples where crustaceans are harvested. The developed biosensor shows good sensitivity (62 nA/mg/L) and reproducibility (RSD=4%; n=5), with a linear range of 15-1000 mg/L. The biosensor is stable at 30 °C for 45 days. A portable measuring device prototype has been developed for sulphite biosensor lecture. Scale up manufacturing process is been defined including enzyme production, SPEs printing, biofunctionalization and packaging.

### ACKNOWLEDGMENTS

The research leading to these results has received funding from the European Union's Horizon 2020 Research and Innovation programme 2016-2017 under grant agreement N° 651556.

- [2]<http://www.cfsa.net.cn:8033/UploadFiles/news/upload/2015/2015-04/6486dfae-d8c6-4f3e-82fa-61442540b4bb.pdf>
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