Development of Enzymatic Biosensors to Detect Biocides Disinfectants to Strengthen Self-Monitoring in Industry †

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Abstract: Biocides disinfectants are used in agro-food industries in order to limit the development of pathogens present in environment or on surfaces in contact with food for human or animal consumption. Biocides residues remaining on food surfaces may constitute a toxicological risk for the consumer. Very little feedback from the field on the rates of biocides residues in the industry exist due to a lack of simple, fast and responsive self-checking methods. The development of biosensors for the detection of biocides disinfectants represent a promising way to explore but most of this research remains limited.

Keywords: biocides disinfectants; enzymatic biosensors; optical; electrochemical; food products

1. Introduction

The implementation of Cleaning and Disinfection plans, essential elements in the control of hygiene and food safety for consumers, is a requirement of the "Food Law" (Regulation EC 852/2004) [1]. A wide variety of these products are used on a daily basis throughout the food chain to limit the development of undesirable microorganisms present in the environment or on surfaces in contact with food intended for human or animal consumption. Following these treatments, biocidal product residues may persist on the surfaces, in particular if these residues are not completely removed during rinsing operations. It has also been proven that their transfer to or to food is possible. In 2012, a European Food Safety Agency (EFSA) investigation found excessive amounts of two quaternary ammonium compounds (QACs) [2]. The greatest number of positive results (12%) was found in milk and dairy products, with contamination levels ranging from a few tens to a few thousand μg/kg. The presence of residues may be associated with i) toxicological risks and ii) emergence of resistance to biocides and cross-resistance with antibiotics. Therefore cleaning and disinfection procedures must be continuously and effectively monitored to verify their suitability and effectiveness (Biocide Regulation 528/2012) [3].

However, self-checking in food industry is very limited to date and uses insensitive methods. The performances announced by the manufacturers are not subject to any evaluation or official validation. An inventory of tests for the detection of residues of disinfectant biocides has enabled us to show the few commercial methods available for self-monitoring [4]. In the context of collaborative projects, we have established that the detection limits were high, in the order of 0.5 to more than 2000 mg/kg, i.e., 1000 times less sensitive than methods based on mass spectrometry [4]. In addition, these methods are not very selective (e.g., pH, conductivity) or too specific (e.g., ELISA). Despite the fact that their sensitivity thresholds are high, strip tests are the most used by doof industries. This is why it is necessary to develop innovative tools such as biosensors that are sensitive, fast, inexpensive,

portable, even online, to set up more efficient self-monitoring, and thus better protect consumer health. Many biosensors based on the inhibition of enzymatic activity were built for the detection of organophosphate pesticides (OPP) [5-8]. These works draw inspiration to develop biosensors based on the use of enzymes as a recognition tool for the detection of disinfectant biocides. The results of the work on pesticides make it possible to reach detection limits of the order of μg/kg, i.e., 1000 times more sensitive.

2. Enzymatic Biosensors for the Detection of Biocides Disinfectants

2.1. Development of Enzymatic Biosensors for the deTection of Biocides Disinfectants

Due to the structural resemblance of organophosphate pesticides (OPP) to the substrate (i.e., Acetylcholine) of acetylcholinesterase (AChE), these pesticides cause a reversible inhibition of this enzyme since they take the place of the substrate at the level of the active site of the enzyme [5]. The QAs have a chemical structure similar to acetylcholine, such as OPP. The reversible inhibitory effect of certain QACs (tetraethylammonium ions) on the activity of AChE (e.g., From electric eel) was demonstrated in 1952 by a manome-tric method [9].

This project will focus on enzymatic biosensors (ie. AchE) for the detection of QACs, commonly used in the dairy industry (e.g., benzalkonium chloride (BAC), didecyldimethylammonium chloride (DDAC)) (Figure 1). Three transduction modes (colorimetric, fluorimetric, electrochemical) were selected because they have common advantages: simplicity, speed, low cost, little or no investment in hardware, miniaturization and portability [10]. The main challenges will be selectivity, accessibility of the active site of the immobilized enzyme, its stability and repeatability.
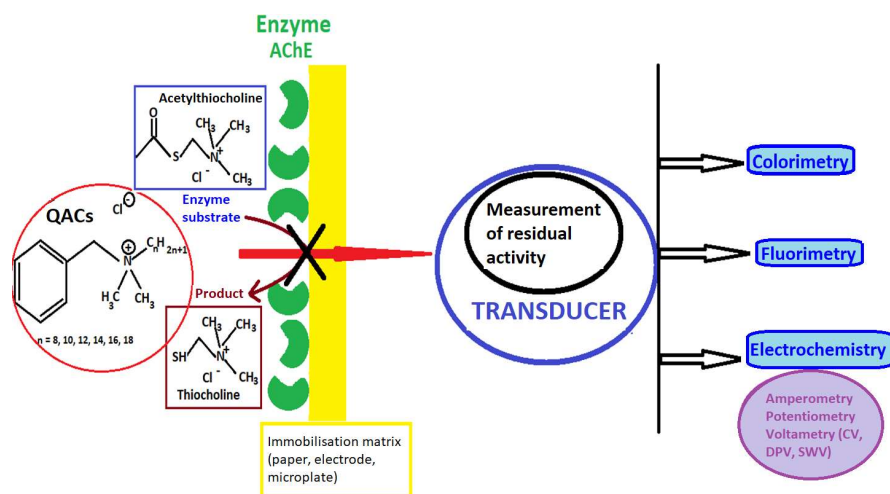
Figure 1. Description of the principle of biosensors based on the enzymatic inhibition of acetylcholinesterase (AChE) by quaternary ammoniums compounds (QACs).

One work focused on developing an electrochemical (conductimetric) biosensor based on AChE for the detection of two QACs (BAC, hexadecylpyridinium bromide) in aqueous solution [11], while hundreds of articles have been published on the detection of pesticides in this way. So the strategies to be investigated for the detection of QACs by enzymatic biosensor and the recent development of nanotechnologies (i.e., Nanomaterials) makes it possible to predict a sensitivity in line with our expectations [12].

2.2. Intra-Laboratory Characterization of the Method Developed and Its Validation with Regard to the Expected Use

Intra-laboratory characterization consists in determining each performance characteristic (e.g., Sensitivity, selectivity, repeatability, robustness). Validation is the comparison of performance

characteristics to expected performance criteria [13]. The validation of the developed methods will be carried out in accordance with the European regulations for veterinary medicinal products (European decision EC/2002/657 [14], European guideline for the validation of screening methods [15]) since there are no regulations for biocidal tests. In fact, this guideline which concerns the so-called screening methods (first instance of control) is applicable to disinfectant biocides since the analytical me-thods are the same.

The objective is to develop tests able of detecting QACs below the Maximum Residue Limit (MRL) in milk (0.1 mg/kg) and with the lowest possible detection capabilities, i.e., g/kg in rinsing water (by comparison with current tests which tend to have detection thresholds of the order of mg/kg).

2.3. Use of Biosensors Developed and Validated for Sample Analysis (Rinsing Water, Milk)

Finally, the developed methods will be used to analyze rinsing water collected during cleaning and disinfection operations to see if residual concentrations of QACs are detected. Regarding food products, the project will be focused on milk samples and dairy products following the results of the EFSA survey in 2012. Given the low sensitivities of the current tests used in industry, the question addressed is whether it is possible, with more efficient methods, to detect low quantities (0.1 μg/kg) of biocidal residues in milk and dairy products for supplying exposure data needed to risk assessment. A quantitative physicochemical method based on liquid chromatography coupled to mass spectrometry in tandem (LC-MS/MS) for the detection of QACs to confirm positive results obtained with the biosensors [16].

3. Conclusions

In a sector where very little research has been carried out and published, this project will make it possible to provide the dairy industry with more efficient self-control methods while remaining economically interesting. The knowledge and results acquired during this project will make it possible to open up enzymatic biosensors to other biocides disinfectant subsequently, and other food matrices than milk, or even to develop multiplexed biosensors for the simultaneous analysis of several families of disinfectants.

The study on milk samples and dairy products will provide us a better understanding of the residual concentrations of QACs that may be present in these foodstuffs, following use or misuse of these biocidal products during cleaning / disinfection procedures. This will allow the procedures to be corrected if necessary to ensure better control of the chemical risk. These self-monitoring tools will ultimately make it possible to generate data on the residual concentrations of disinfectant biocides which may be useful in the context of exposure risk assessments for consumers carried out by the Risk Assessment Department of ANSES.

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