New biosensors for early detection of arsenic in water bodies and edible fish

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Background

Arsenic (As) is a toxic metalloid widely distributed in the environment, occurring in organic and inorganic forms. According to the WHO, over 220 million people are at risk of chronic exposure, mainly through drinking water contaminated with inorganic As(III) and As(V). Long-term exposure can cause severe health effects, including cancer. The EU Drinking Water Directive (2020/2184) and World of Health Organization (WHO) establishes a maximum value of 10 µg/L. Dietary exposure through aquatic organisms is also a concern, as highlighted by EFSA (2024), which established a BMDL05 of 0.06 µg/kg body weight/day. Although EU Regulation 2023/915 sets maximum inorganic arsenic levels for certain foods, no general maximum level is established for fish, although it is currently being revised.
Conventional analytical methods require sophisticated equipment and skilled personnel, limiting field application.



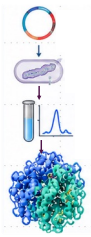
Chronic arsenic toxicity commonly affects the hands through arsenical keratosis, and pigment changes.

Aim

Develop innovative electrochemical biosensors based on Arsenite Oxidase (Aio) enzymes and their genetic variants for the detection of toxic arsenite and arsenate in water and fish samples intended for human consumption in Portugal

Multidisciplinary Approach

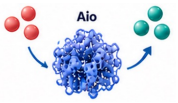
1 Enzyme production and characterization



- Production, purification and biochemical characterization
- Selection of variants with improved stability and activity

2 Enzymatic recognition reaction

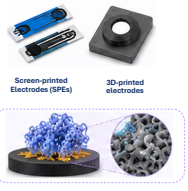
Aio catalyzes the oxidation of As(III) to As(V), enabling selective analyte recognition



$As(III) + H_2O \rightarrow As(V) + 2H^+ + 2e^-$

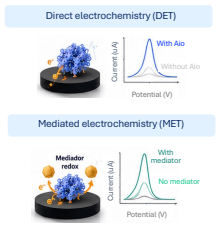
- The enzymatic reaction generates electrochemical signals
- Basis for selective As(III) detection and, indirectly, As(V)

3 Immobilization on screen-printed electrodes



- Immobilize the selected variant
- Optimize electron transfer between enzyme and transducer

4 Bioelectrocatalytic study



- Assess bioelectrocatalytic performance of different variants
- Select the enzyme with the best performance (sensitivity and selectivity)

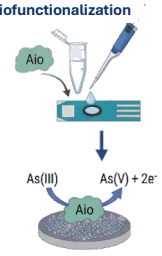
5 Application to real samples



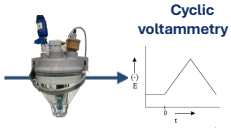
Detection of As(III) and As(V) in drinking water and fish samples

Method

Electrode biofunctionalization



- Aio from *Alcaligenes faecalis*
- Carbon SPEs (Dropsens)
- Enzyme adsorption
- Potentiostat (Palmsens)
- Technique: Cyclic Voltammetry, 20 mV/s
- Electrolyte: MES 50 mM, pH 5.5, KCl 100 mM

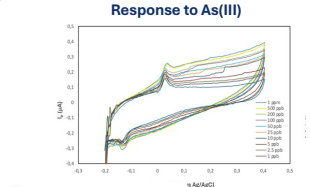


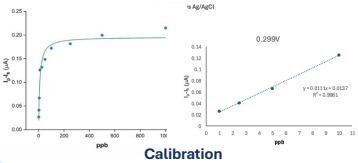
Cyclic voltammetry

DET

Preliminary Results

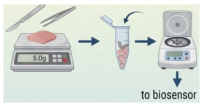
Response to As(III)





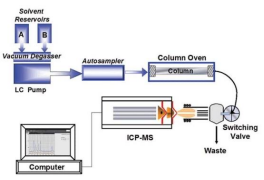
Calibration

Next steps



Analysis of fish samples

Validation (HPLC-ICP-MS)



Challenges

1- Purification yield
2- Enzyme purification consistency
3- Stability
4- Reproducibility

Acknowledgments

This work is financed by national funds from FCT - Fundação para a Ciência e a Tecnologia, I.P., in the scope of the project UIDP/04378/2020 and UIDB/04378/2020 of the Research Unit on Applied Molecular Biosciences - UCIBIO, the project LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy - i4HB, and the research Project 2023.18077.ICDT.

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