

Encapsulation of model bacteria – sensors for optical and electrochemical detection of environmental pollutants

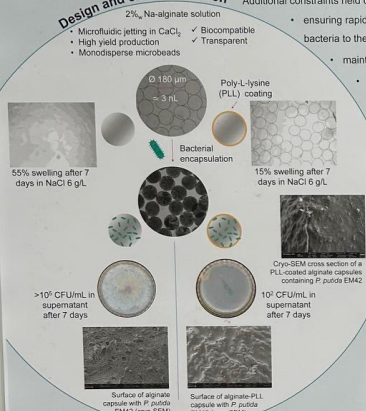
Environmental pollution poses a major threat to health and biodiversity. In line with the ZERO-POLLUTION programme of the European Union, the BioSensei project addresses this challenge by developing a real-time, multiplexed, end-to-end, on-site optical and electrochemical monitoring system. Using cellular responses, this hybrid biosensor targets both biotic and abiotic pollutants in water (i.e., nitrates and phosphates, endocrine disruptive chemicals, PFAS and toxins).

To this purpose, immobilisation of genetically engineered bacterial cells on the transducer surface is required. Moreover, this project is embedded within a Safe-and-Sustainable-by-Design (SSbD) framework, which includes rigorous risk assessments, thus requiring a null release of modified bacteria from the sensor into the environment.

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BioSensei

Design and characterization



Additional constraints held on this task include:

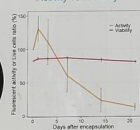
- ensuring rapid diffusion of analytes from the environment to the bacteria and from the bacteria to the optical and electrochemical transducers
- maintaining a metabolic activity
- avoiding optical or electrochemical interference

Viability and stability performances

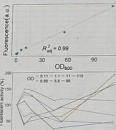
Model sensors

P. putida EM42 Rhamnose sfGFP
ROS tolerance Salicylic acid Pyocyanin

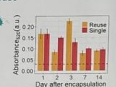
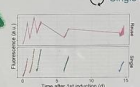
Activity vs. viability



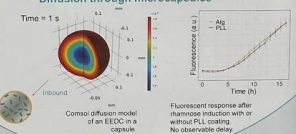
Cell load influence



Single use vs. reuse



Diffusion through microcapsules



Integration on transducer

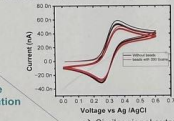
imec

Optical sensor and chamber

- No interference from capsules in chamber on optical outputs
- Use of a K-based minimal nutritive media
 - Reduces swelling
 - Avoids autofluorescence

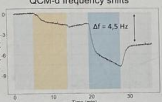
Electrochemical sensor

Detection of pyocyanin with Alg-PLL capsules



Surface immobilisation

QCM-d frequency shifts



SSbD approach

Genipin 0.5%
Poly-L-lysine 0.1%

Tyndall

- The developed model showed:
- Good microscale encapsulation of bacteria
 - Robust Lbl. protocol decreasing cell release and swelling, complying with biosafety targets
 - High viability and potential circular use of such whole-cell biosensors, enhanced by the encapsulation technique
 - No major hindrance of analytes diffusion by the polymer system

Further work will:

- Immobilise capsules on SSbD deposition
- Continue integration in sensing device
- Implement relevant synthetic biological input in bacteria (e.g. PFAS, N, P, endocrine disruptors)
- Combine genetic tweaking, allowing multiplexing
- Perform case studies in The Netherlands and Ireland

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