Bioelectrochemical sensors for detection of volatile organic compounds (VOCs)

Abeed Fatima Mohidin, Palanisamy Kannan, Pui Yi Yung, Carlo Santoro, Thomas Seviour, Jamie Hinks, Federico Lauro, and Enrico Marsili

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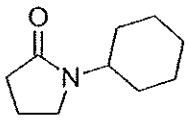
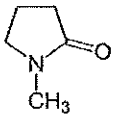
Introduction

- Volatile organic compounds (VOCs) in wastewater originate from chemical, electronic, and textile industry. While some VOCs are degraded in wastewater or in the soil, others contribute to failure of wastewater treatment plant through direct inhibition of biomass or are simply discharged in the effluent.
- Low-cost methods for detection of VOCs are needed in Singapore, due to its widely spread sewage network. Current methods (MS) for VOCs detection are very sensitive but require off-line analysis.
- Bio-electrochemical systems (BESs) measure bacterial extracellular respiration of a solid electrode. Here, we measured *Escherichia coli* and *Pseudomonas aeruginosa* respiration rate on screen printed graphite electrode coated with carbon nanotubes (CNT-SPE).
- VOC addition decreases amperometric signal, thus providing sensitive online detection of selected VOCs (10-20 ppm) in few minutes.

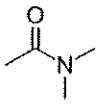
VOCs used in this work



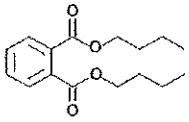
N-Cyclohexyl-2-pyrrolidone (CHP)



N-methyl-2-pyrrolidone (NMP)

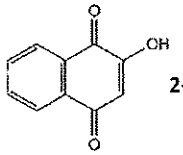


Dimethylacetamide



Dibutyl phthalate

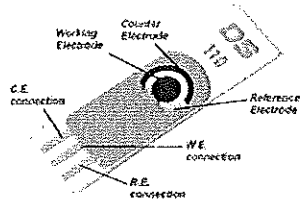
Cyclopentanone

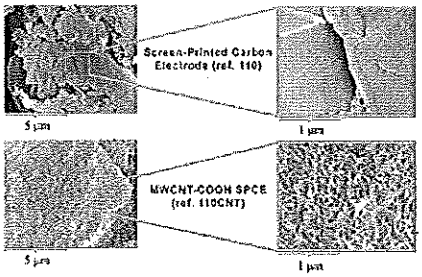


2-Hydroxy-1 4-naphthoquinone (Mediator)

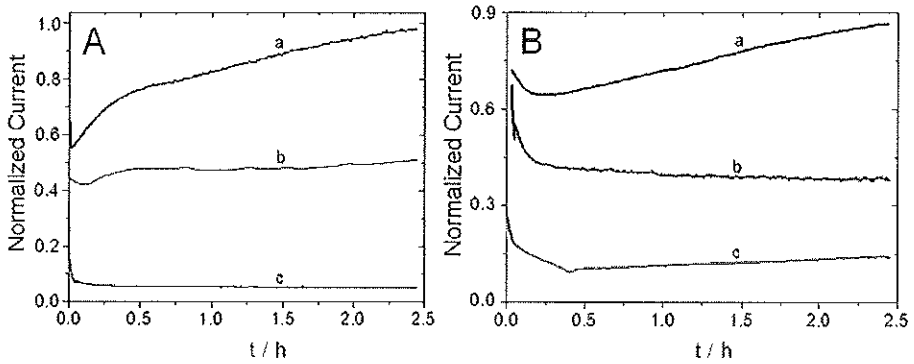
Methods

- Screen printed electrodes coated wit MWNTs
- Miniature electrochemical cell
- Small volume, low cost, highly reproducible
- Short-term experiments at oxidative potential
- *E. coli* grown in MOPS minimal medium, *P. aeruginosa* in ABTG



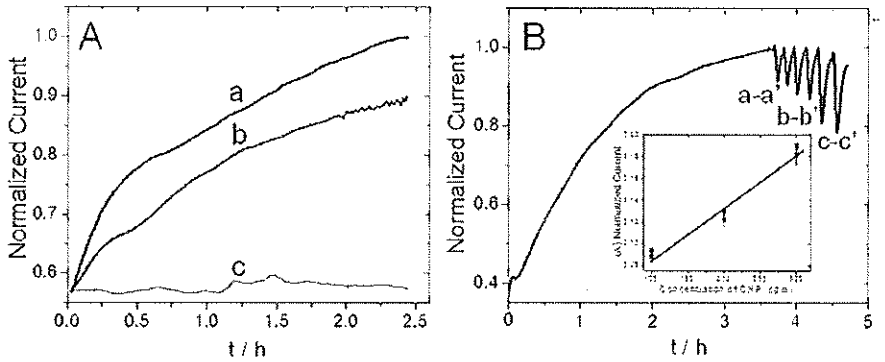


Optimization of current output for E. coli and P. aeruginosa at CNT-SPE



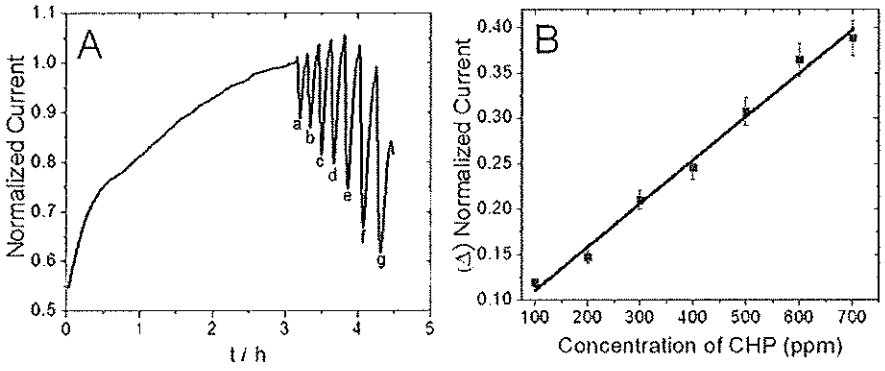
(A) Effect of of bacterial inoculum vs. medium: 1:1 (a), 4:1 (b), and 1:4 (c) on current output for *E. coli* (A), and *P. aeruginosa* (B) inoculated into the electrochemical cell containing a CNT-SPE poised at E = 0.4 V vs. Ag. **The ratio 1:1 produces the highest current output, so it was chosen for further experiments.**

Amperometric detection of CHP at E.Coli/CNT-SPE



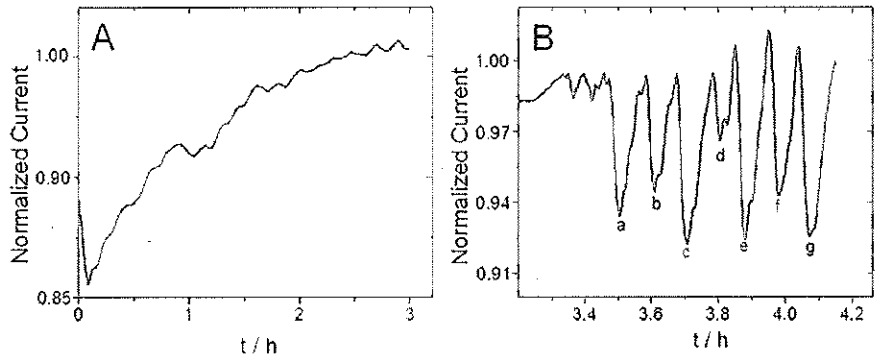
(A) Current output for *E. coli (1:1) on* CNT-SPE at an applied potential of 0.4, 0.2 and 0 V vs. Ag (a-c). The bacterial attachment was completed at 2 hr. (B) CHP addition causes current output drops (a-a' to c-c') that increase with CHP concentration. The calibration plot in the inset is obtained with the difference in current output (current before CHP addition-minimum current) vs. concentration of CHP.

Amperometric detection of CHP at E.Coli/CNT-SPE



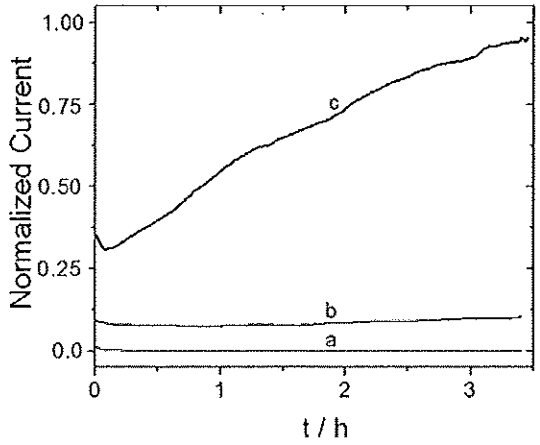
(A) Current output following CHP addition in the range 100-700 ppm (a-g) to *E. coli* on SPE-CNT poised at 0.4 vs. Ag. (B) The calibration plot in the inset is obtained with the difference in current output (current before CHP addition-minimum current) vs. concentration of CHP (a-g = 100-700 ppm). The limit of detection (LOD) for CHP in *E. coli* culture is 10 ppm.

Amperometric detection of CHP at E.Coli/SPE



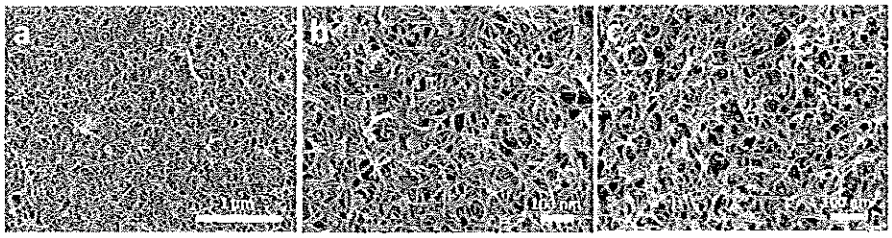
(A) Current output for *E. coli* inoculated into the electrochemical cell containing a unmodified screen printed electrode (SPE) at an applied potential of 0.4 V vs. Ag. (B) The current output following CHP addition does not decrease proportionally to the CHP concentration, indicating that unmodified SPEs are not suitable for CHP detection.

Effect of exogenous redox mediator on current output



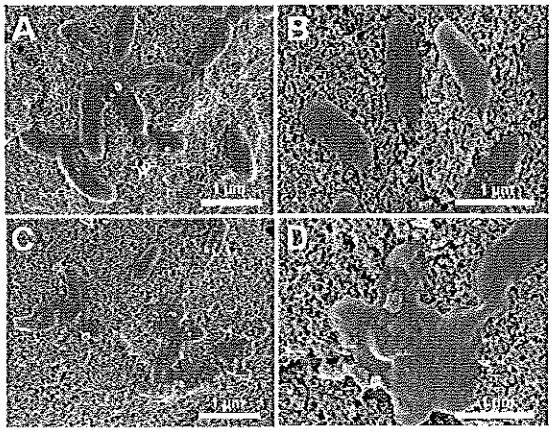
Current output for *E.coli* inoculated in electrochemical cell with 5-hydroxy-1,4-naphthoquinone (5-HNQ) (a), no mediator (b), and hydroxy-1,4-naphthoquinone (2-HNQ) (c) at an applied potential of 0.40 V vs. Ag.

Morphology of CNT in presence and absence of CHP



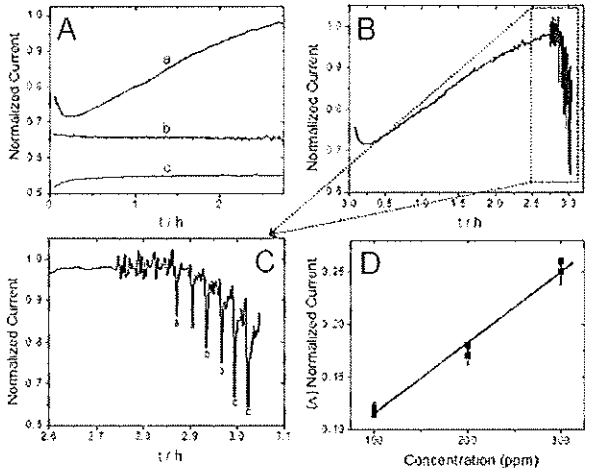
FESEM images of CNT-SPE before addition of 500 ppm CHP (a, b) and at the end of the CHP detection experiments (c), showing that CNT were not damaged/collapsed or dissolved following addition of CHP to the bacterial suspension.

Morphology of E.Coli in presence and absence of CHP



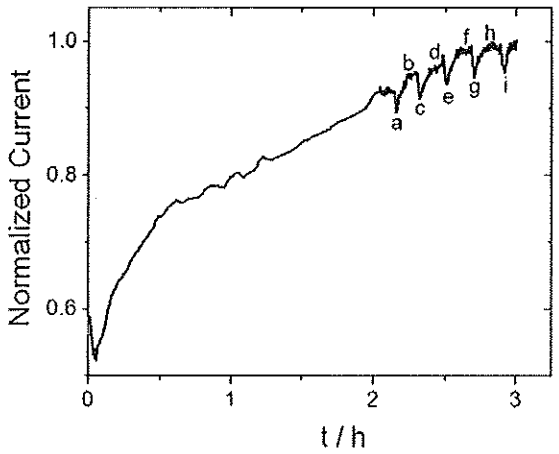
FESEM images of *E. coli* on CNT-SPE before (A, B) and after (C, D) addition of 500 ppm CHP. Cellular damage resulting from CHP exposure can be observed.

Amperometric detection of CHP at PA/CNT-SPE



(A) Current output for *P. aeruginosa (ratio 1:1)* inoculated into the electrochemical cell containing a CNT-SPE electrode at an applied potential of 0.4, 0.2 and 0 V vs. Ag (a-c). The bacterial attachment was completed at 2 hr. (B-C) amperometric current drops (a-a' to c-c') due to CHP addition to the electrochemical cell. (D) The calibration plot is obtained with the difference in current output (current before CHP addition-minimum current) vs. concentration of CHP (a-g = 100-700 ppm). The limit of detection for CHP in PA system is 10 ppm.

Interference of other VOCs on CHP determination in *E. coli* grown on CNT-SPE



Current output for E. coli at 0.40 V vs. Ag obtained for the addition of 100 ppm CHP (a, c, e, g and j) and each 100 ppm of DMA (b), MB (d), CPN (f), DP (h), respectively, showing no significant interference of other VOCs. **LOW SELECTIVITY and SPECIFICITY.**

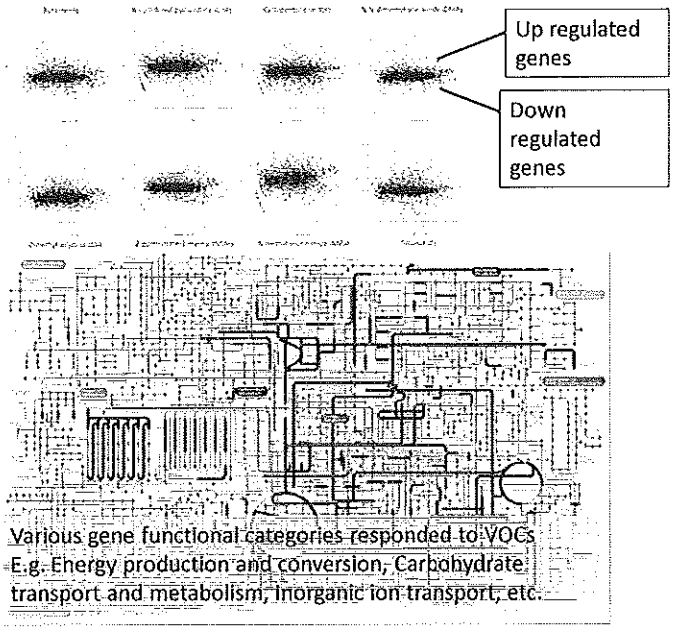
Microbial response to VOC toxicity

- VOCs are toxic and have low bioavailability for degradation
- VOCs present a challenge to receiving environments and wastewater treatment processes
- Microorganisms are adapted to sense and respond to their surroundings (e.g. nutrient content, presence of toxins)

Approach

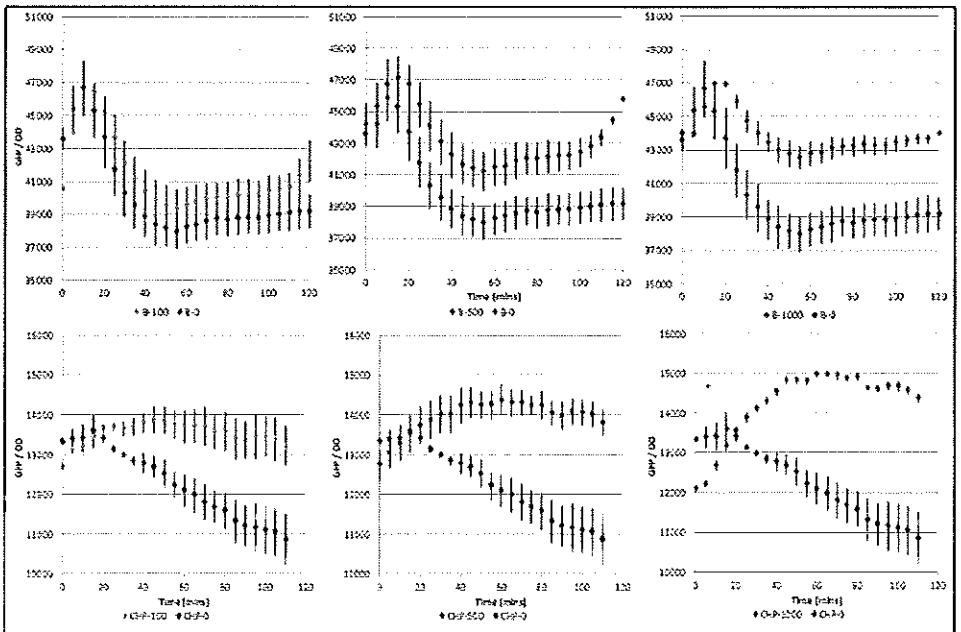
- We investigated the response of the model laboratory microorganism *E. coli* to industrially relevant VOCs (and related metabolites) using transcriptomics (RNAseq)
- Non-inhibitory concentrations of VOCs were chosen (to increase sensitivity and reduce toxicity)

Transcriptomic responses of *E. coli*



Specific response to VOCs

- Based on transcriptomics results, several clones sensitive to VOCs were prepared and an optical reporter (GFP) was attached.
- Response to CHP and butanol was tested
- Response are VOC-specific



Dose-response assay of the optical biosensor over time.
Different concentrations (100, 500 and 1000 ppm) of butanol (top) and CHP (bottom) were exposed to selected candidate *E. coli* clone

Conclusions

- Nanostructured surface increase extracellular respiration rate and current output in non-electroactive microorganisms.
- VOC addition decrease current output, thus providing rapid and sensitive detection
- Transcriptomics of *E. coli* exposed to selected VOC shows specific response, thus selective sensors for multiple VOC detection can be designed.
- Integration of electrochemistry and genetic engineering can be applied to detection of trace toxic compounds in water.