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Sub-toxic concentrations of volatile organic compounds inhibit extracellular respiration of *Escherichia coli* cells grown in anodic bioelectrochemical systems

Carlo Santoro [a], Abeed Fatima Mohidin [a], Letizia Lo Grasso [a], Thomas Seviour [a], Kannan Palanisamy [a], Jamie Hinks [a], Federico M. Lauro [a,b], Enrico Marsili [a,c,*]

[a] Singapore Centre for Environmental Life Sciences Engineering (SCELSE), Nanyang Technological University, 60 Nanyang Drive, SBS-01N-27, Singapore 637551, Singapore
[b] Asian School of the Environment, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798, Singapore
[c] School of Chemical and Biomedical Engineering, Nanyang Technological University, 42 Nanyang Drive, Singapore 637459, Singapore

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ABSTRACT

Low-cost and rapid detection of volatile organic compounds (VOCs) is important for the control of water quality of used water and protection of downstream used water treatment processes. In this work, the effect of sub-toxic concentration of VOCs on the current output of *Escherichia coli* in bioelectrochemical systems (BES) is shown, in light of environmental sensing applications for sewage and used water networks. *E. coli* cells were grown on carbon felt electrodes in artificial used water, to increase sensitivity and decrease response time for detection. Extracellular electron transfer was promoted by the addition of a biocompatible redox mediator, 2-hydroxy-1,4-naphthoquinone (HNQ). Among the eight VOCs investigated, toluene is the most toxic to *E. coli*, with a detection limit of 50 ± 2 mg L^{-1} and current output of 32 ± 1 nA mg^{-1} L^{-1}. This work offers a straightforward route to enhance the detection of organic contaminants in used water for environmental applications.

1. Introduction

Bioelectrochemical systems (BESs) have applications in energy recovery from used water [1–3], bioelectrosynthesis of hydrogen [4–5], and other organic compounds [6–9] and biosensors for environmental and biomedical applications [10–12]. In contrast to conventional electrochemical systems, BESs avail of electrochemically active biofilms or respiring planktonic cells to catalyze redox reactions at or near to electrode surfaces [13–14]. Electrochemically active microorganisms at the anode oxidize organic compounds (e.g., acetate and glucose), releasing electrons directly to the anode. At the cathode, other electrochemically active microorganisms accept electrons from the electrode and use soluble or insoluble oxidizing agents (e.g., oxygen, sulfates and nitrates) as terminal electron acceptors [15]. Electron donors or acceptors in BESs can also be organic or inorganic contaminants. For example, metals and metalloids can be reduced or oxidized from soluble into a less-toxic, insoluble form [16–17]. As for organic pollutants, BESs have been used for removal of organofluorines [18] and attenuation of trace organic compounds [19]. In certain cases, BESs can be used as redox amperometric sensors, providing that the concentration change of a redox-active anolyte can be isolated from the change in the background signal, although strategies can be implemented to deconvolute signals [20]. Recent examples include volatile fatty acids detection [21], recalcitrant organic chemicals [22] and BOD [23–24].

However, BES-based sensors are mostly based on organic or inorganic species that take part in the electron transfer process, either as electron donors or electron acceptors or that perturb the anodic community. Recently, "shock" sensors have been developed as in-situ water warning system. Specifically, small microbial fuel cells with mixed culture anodic biofilm have been used as sensors that respond rapidly to low concentrations of nickel and chromium in the water [25–26].

To our best knowledge, BESs have not previously been used to detect toxic organic pollutants not serving as electron donors or acceptors. Organic pollutants that are toxic to electrochemically active microorganisms should inhibit extracellular respiration rate, thus providing a sensitive mean of detecting organic compounds of interest. In this study, we focus on volatile organic compounds (VOCs) due to their high toxicity to microorganisms [27]. VOCs contribute to failure of used water treatment processes either through direct inhibition of biomass or because they are not degraded during biological treatment and are discharged in the effluent. The direct utilization of VOCs (i.e., benzene and toluene) as a carbon source [28] and their conversion to electricity was previously demonstrated [29–30]. However, the time required for the anodic biofilm to adapt to toluene as carbon source is too long for environmental sensing, particularly if the detection of pollution events in the form of sudden VOC peaks is desirable. Instead, a

* Corresponding author.
E-mail address: emarsili@ntu.edu.sg (E. Marsili).

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direct and rapid approach to VOC detection exploiting respiratory inhibition is promising.

Several VOC contaminants have been detected both in used water and exhaust air. 1-methyl-2-pyrrolidone (NMP) was recently listed as an emerging water contaminant [31] and was detected in activated sludge [32], where it was found to be readily biodegradable under normal process conditions. NMP degrading microorganisms were later identified in activated sludge (*Pseudomonas*, *Paracoccus*, *Acinetobacter* and *Rhodococcus* genera) [33] and surface water (*Pseudomonas*) [34]. Cyclopentanone has been detected in treated wastewater from refineries [35]. Toluene is a common contaminant of air [36] and water [37]. Butanol is a common contaminant of indoor air [36] and various phthalates are listed as emerging contaminants [31]. The requirements for discharge of trade effluents into the public sewers in Singapore (http://www.pub.gov.sg/general/usedwater/Documents/requirements UW.pdf) also list several prohibited VOCs, including toluene and dimethyl sulfide (DMS). In a recently published research [38], we showed that the VOCs reported in this study slow down microbial growth at concentration > 100 mg L^{-1}. To our best knowledge, no previous studies are available on the bioelectrochemical detection in water of n,n-dimethylacetamide (DMA), (DMS), n-methyl succinimide and n-cyclohexyl-pyrrolidone.

Continuous, distributed and automated field analysis of VOCs is desirable both to protect water reclamation plants and to identify polluters. Current methods for VOC detection are costly and often require off-line analysis. These shortcomings make current technology incompatible with distributed environmental VOC sensing. In this study, we used anodic BESs to detect VOCs. Viable *Escherichia coli* cells were used as sensing element, together with the exogenous redox mediator 2-hydroxy-1,4-naphthoquinone (HNQ) to increase current output. The proposed system has sufficient sensitivity for field application where unspecific VOC detection is needed as a mean of early detection of pollution events.

2. Materials

E. coli K12 (ATTC 10798) was maintained on Luria-Bertani (LB) agar plates whereas the culture was cultivated overnight in LB medium at 37 °C with shaking at 150 rpm. 2-hydroxy-1,4-naphthoquinone (HNQ) was used as redox mediator to facilitate electron transfer in *E. coli*. All the VOCs were purchased from Sigma-Aldrich, and used as received. The VOCs have been abbreviated in the text as following: butanol (BUT), 1-methyl-2-pyrrolidone (NMP), n,n-dimethylacetamide (DMA), dimethyl sulfide (DMS), n-methyl succinimide (NMS), cyclopentanone (CP), n-cyclohexyl-pyrrolidone (CHP) and toluene (TOL).

Morpholinepropanesulfonic acid (MOPS) minimal media were prepared as previously described [39]. The medium was buffered with 200 mM HEPES and 1.5% *w*/w glucose was used as carbon source. 126 mL volume of glass bottle (Pyrex) was used as electrochemical cell for the amperometric experiments. The electrochemical cell was sealed and autoclaved in order to avoid bacterial contamination. The electrochemical cell was filled with 118 mL of MOPS, 6.25 mL of 1 mM HNQ (final concentration 50 μM) and 1.25 mL of overnight *E. coli* culture. The glass bottle was covered with aluminum foil in order to prevent photobleaching of the redox mediator.

The working, reference and counter electrodes were accommodated in the plastic cap of the bottle. The electrochemical cell was sealed airtight to avoid VOC volatilization. Carbon felt (Alfa Aesar, 3.18 mm thick, 99% purity) with geometric area of 2 × 1 cm was used as working electrode. The carbon felt was attached to a titanium wire current collector through nylon screw and nut. A glass tube (Φ = 4 mm) ending with a vycor glass frit was filled with autoclaved agar, amended with 1 M KCl to serve as salt bridge, to avoid contamination of the microbial culture and to protect the reference electrode. Ag/AgCl 1 M NaCl was used as reference electrode. A long titanium wire coil (~50 cm) was used as counter electrode.

3. Methods

The electrochemical measurements were performed with a multichannel potentiostat (VSP Biologic, France) at a constant applied potential of + 200 mV (vs. Ag/AgCl), unless otherwise stated. The amperometric current output data was recorded every 5 min (during the growth phase) and every 60 s during the sensing experiments. Following inoculum, the current increased until a stable value was achieved after 15–18 h. After that, the required concentration of VOCs was added with a micro-syringe (range 0–25 μL, 0–100 μL, 0–1000 μL), in the concentration range from 0.005% *v*/v (6.25 μL) to 1% *v*/v (1.25 mL). To allow current output stabilization, increasing VOC concentrations were added every hour. The current output corresponding to each VOC addition was calculated as the average of the current output immediately before the subsequent VOC addition and averaged on ~10 points. A minimum of three independent biological replicates were used for each condition.

4. Results and discussion

4.1. Current generation over time

The current output with time of viable *E. coli* cells in presence of 50 μM HNQ and without VOCs was measured to determine the stability interval of the BES, allowing for precise VOC determination. The current output increased steadily for ~15 h until it stabilized and remain constant for at least 10 h, before a rapid drop, due to either exhaustion of organic carbon or acidification of the media resulting from glucose fermentation by *E. coli*. The current output was reproducible, with a standard deviation of 5–10% (number of biological replicates n = 3) (Fig. 1). This variability is consistent with bioelectrochemical experiments employing viable cells [40]. To facilitate comparison among the replicates in the VOC addition experiments (Fig. 2-5), the current output is reported as $j_{residual} = j/j_{max} * 100$, where j_{max} is the maximum current density before the addition of VOCs.

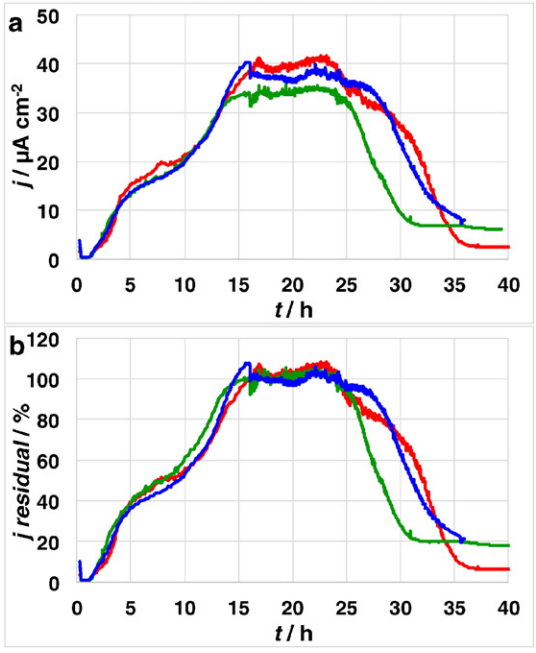

Fig. 1. Current density trend during a cycle without addition of VOC (a). Red, blue, and green lines indicate the replicates. Normalized current is also showed considering the current at 16 h as initial current and equal to 100%(b).