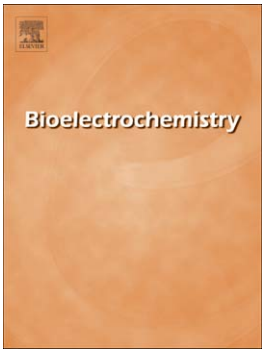

Sub-toxic concentrations of volatile organic compounds inhibit extracellular respiration of *Escherichia coli* cells grown in anodic bioelectrochemical systems

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

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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.

Keywords: VOC, *Escherichia coli*, amperometric biosensor, biofilm

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 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 from used water treatment plants in Singapore. 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, which is released from polymeric drinking water pipes, likely due to the water chlorination, has also been detected in trace amounts [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] due to their release from drinking water pipes [38]. The abovementioned VOCs and the other VOCs reported here have been observed sporadically at high concentration (>50 mg L^{-1}) in freshwater by the Public Utility Board of Singapore. In a recently published research [39], 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 waterways of n,n-dimethylacetamide (DMA), dimethyl sulphide (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 BES 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 sulphide (DMS), n-methyl succinimide (NMS), cyclopentanone (CP), n-cyclohexyl-pyrrolidone (CHP) and toluene (TOL).

Morpholinepropanesulfonic acid (MOPS) minimal media were prepared as previously described [40]. 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 air-tight to avoid VOC volatilization. Carbon felt (Alfa Aesar, 3.18 mm thick, 99% purity) with geometric area of 2 x 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 1M 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 multi-channel 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 minutes (during the growth phase) and every 60 seconds during the sensing experiments.

Following inoculum, the current increased until a stable value was achieved after 15-18 hours. 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 hours, 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) (Figure 1). This variability is consistent with bioelectrochemical experiments employing viable cells [41]. To facilitate comparison among the replicates in the VOC addition experiments (Figure 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.

[Figure 1 here]

4.2 Toluene

The first VOC tested was toluene, due to its well-known toxicity and environmental impact [29]. Toluene was added 17 hours after inoculation of the BES, when current stabilized (Figure 2a). Following toluene addition, the current output dropped stepwise. Figure 2b shows the point of addition of toluene. Figure 2c shows $j_{residual}$ at increasing toluene concentrations. The $j_{residual}$ measured 5 and 60 minutes after toluene addition were similar, indicating that toluene rapidly inhibits respiration at the concentration range used (Figure 2c). As the electrochemical cell was sealed, toluene remained in solution. The dose-response curve showed two ranges, at low and high toluene concentration. The first range (50-1000 mg L^{-1}) shows higher respiration inhibition, likely because cells are permanently damaged at toluene concentration higher than 1000 mg L^{-1}. The range for toluene determination was 50–1000 mg L^{-1} (Figure 2c), with a detection limit (LOD) of 50±2 mg L^{-1} (S/N>3).

[Figure 2 here]

4.3 Other VOCs

Dose-responses plots for various VOCs are reported in Figure 3. Figure 3a shows those VOC that have little or no effect on current output, i.e., DMA, BUT, NMS and DMS (Figure 3a). A decrease of 17±5% was detected at the highest concentration investigated (1% or 10 g L^{-1}) for DMS, BUT and DMA (Figure 3a). No effect was measured for NMS indicating low toxicity to viable *E. coli* cells (Figure 3a).

Other VOCs has stronger inhibition effect, including NMP, CP, TOL and CHP (Figure 3b). CP reduced the current output by 15±4% at 1% addition, NMP by 30±6% (at 1%), TOL by 50±5% (at 1% addition) and the most toxic VOCs tested was CHP, with a residual current of 20±4% when 1% of the compounds was added. The mechanism of extracellular respiration inhibition in presence of VOCs is not yet understood and is currently under investigation.

[Figure 3 here]

4.4 Effect of Potentials on the Current output

The effect of the applied potential was investigated for TOL and CHP (Figure 4). Following inoculation, three electrochemical cells were poised at 0, +200, +400 mV vs. Ag/AgCl and their current output was recorded (Figure 4.a). Higher working electrode potential increased current output, as previously reported for other BESs [42-43]. However, the $j_{residual}$ following VOC addition did not change with applied electrode potential for either TOL (Figure 4.b) or for CHP (Figure 4.c). This indicates that different applied potentials affect current output but not the response and that the $j_{residual}$ and consequently the quality of VOCs detection is not affected.

[Figure 4 here]

4.5 VOCs cumulative effect on current response

The cumulative effect of TOL and CHP on the current output was also investigated. Toluene and n-cyclohexyl-pyrrolidone were added concurrently to the electrochemical cell in the concentration range 50-10,000 mg L^{-1}, after a stable current output was obtained. We focused on TOL and CHP as they perturbed residual current ($j_{residual}$) to the greatest extent compared to other VOCs tested, indicating higher degrees of interaction with *E. coli* (Figure 5). CHP is more toxic than TOL at high concentration (> 0.2 % w/w), as determined by residual current (Figure 5). At low concentrations, the combined effect of simultaneous addition of the two VOCs showed a synergistic effect, while at higher VOC concentrations, the combined toxicity was lower than the sum of individual toxicities, most likely because VOCs adsorbed to dead or inactive biomass, resulting in an effective lower VOC concentration.

[Figure 5 here]

5. Conclusions

In this study, we report for the first time the bioelectrochemical detection of VOCs using viable cells as the detection element. *E. coli* cells were used as transducer element, coupled with HNQ, a biocompatible redox mediator, which enhanced current output of the sensor and improved the detection limit for VOC. Each VOC resulted in a different effect on the *E. coli* current output, reflecting the different toxicity of VOCs to *E. coli* cells and suggesting that VOC-specific detection can be implemented. Toluene and n-cyclohexyl-pyrrolidone showed the highest inhibition of the current output. At low concentrations, the combined effect of simultaneous addition of these two VOCs was higher than that of the two VOCs added separately, indicating a synergistic effect. Further work with nanostructured electrode is needed to improve the sensitivity of the proposed BES, to achieve selective and specific detection of VOCs relevant to environmental pollution.

Acknowledgements

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Figure Captions

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

Figure 2. Current output of three independent biological replicates with the addition of TOL (a). Current produced between 16 and 24 hours with indication of the toluene addition point (b). Residual current detected after the addition of TOL (c). Red color indicates the current measured after 5 minutes, while blue color indicates the current measured after 60 minutes (c).

Figure 3. Residual current detected after VOC addition having low or no effect (a). Residual current significant affected by VOC addition (b). Results from three independent biological replicates.

Figure 4. Normalized current output trend for cells poised at different potentials (0, +200 and +400 mV vs Ag/AgCl) with addition of TOL taking place after 15 hours (a). Residual current after addition of TOL (b) and CHP (c). Results from three independent biological replicates.

Figure 5. Residual current for incremental addition of TOL only (blue), CHP only (red) and their combined addition (orange). The green line shows the theoretical residual current, calculated assuming that the effect of VOC inhibition are linearly additive. Results from three independent biological replicates.

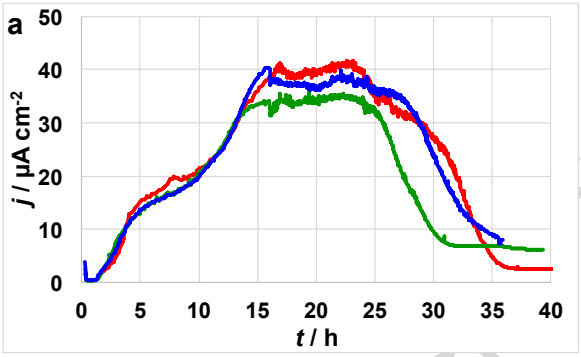

Figure 1. Current output of viable *E. coli* cells with 50 µM 1,2-HNQ. Each color line is and independent biological replicate.

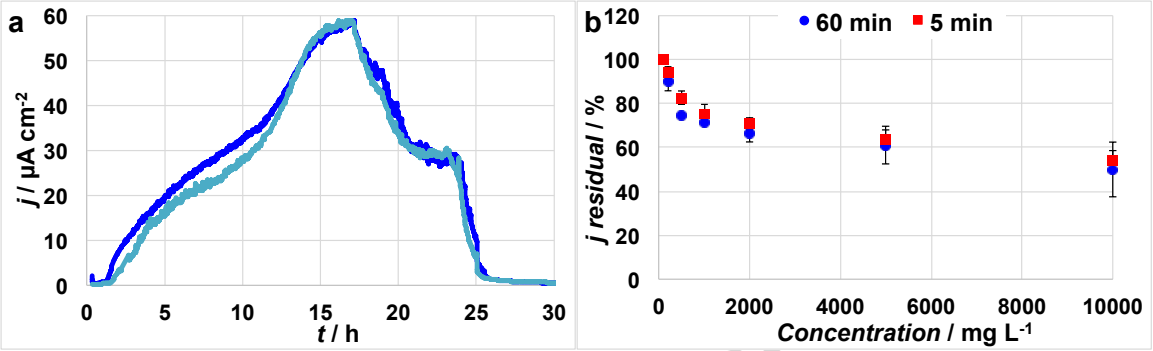

Figure 2. (a) Current output of two independent biological replicates with the addition of toluene. (b) Residual current detected after the addition of toluene. Red and blue marks indicates the current measured 5 and 60 minutes after toluene addition, respectively.

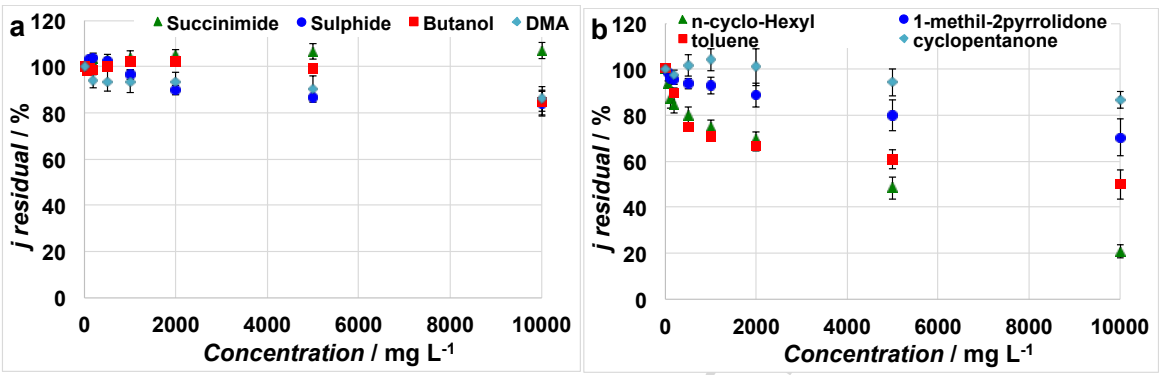

Figure 3. (a) $j_{residual}$ after addition of n,n-dimethylacetamide, dimethyl sulphide, n-methyl succinimide and butanol, which did not affect the extracellular respiration (b) $j_{residual}$ after addition of toxic VOCs: 1-methyl-2pyrrolidone, cyclopentanone, n-cyclohexyl-pyrrolidone and toluene.

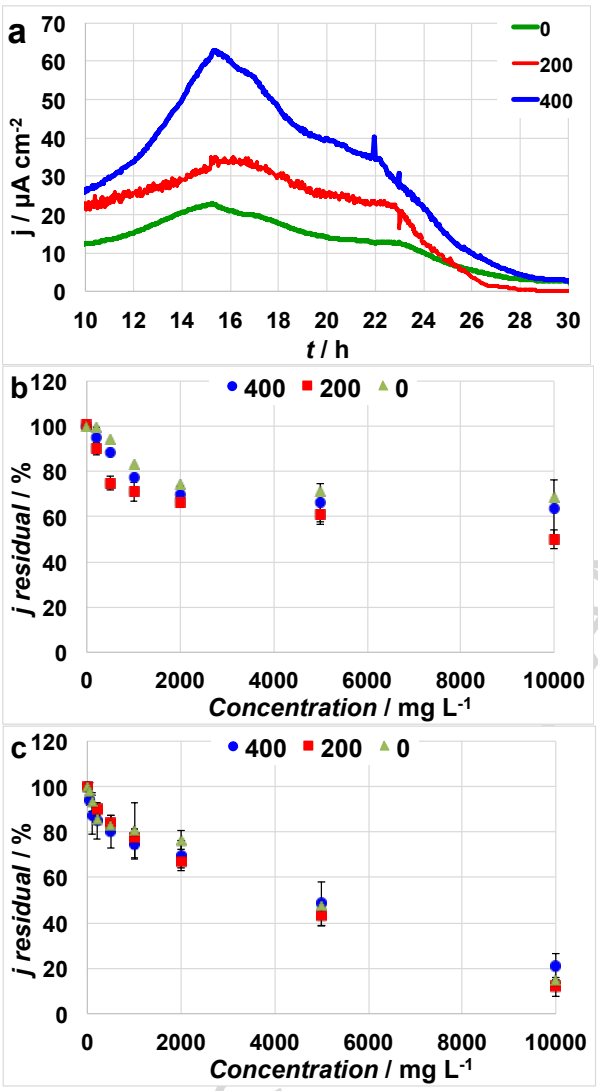

Figure 4. (a) $j_{residual}$ for cells poised at different potentials (0, +200 and +400 mV vs. Ag/AgCl) with addition of (b) toluene and (c) n-cyclohexyl-pyrrolidone after 15 hours.

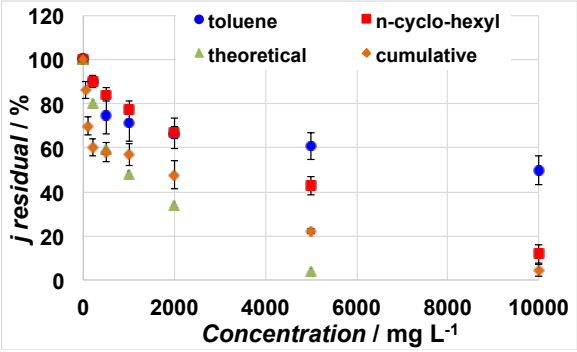

Figure 5. $j_{residual}$ for incremental addition of toluene only (blue marks), n-cyclohexyl-pyrrolidone only (red marks) and their combined addition (orange marks). The green line show the theoretical residual current, calculated assuming that the effect of VOC inhibition are linearly additive.

Highlights:

- A novel bioelectrochemical sensor for VOC detection is reported
- The detection limit for 1-methyl-2-pyrrolidone and toluene is 50 ppm
- The sensor is applicable to water quality control and wastewater management