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A biosensor platform for soil management: the case of nitrites

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ABSTRACT

Conventional farming faces unprecedented pressures and challenges related to climate change, resources constraints, and food security. Cleaner production involves environmental-friendly and knowledge-based agricultural practices that extend natural recycling and minimize the input of chemicals. Precision agriculture and site-specific management drive a new era in farming technology based on the direct measurement of soil properties on-the-go. Although a variety of sensing technologies have been proposed for a variety of chemical compounds, the use of different technologies, each with its own protocol of measurement, operating requirements, and handling expertise, to span one field might fail to support decision making. The idea, on the other hand, of employing a common detection platform, readily modified and adjusted to assess different species as needed, might turn into a key concept in soil management. Towards that end, this paper reports on a versatile lipid nano-platform that allows the easy customization of sensor's selectivity and sensitivity through the adjustment of macro-parameters. As a pertinent case example, the development of a soil nitrite biosensor is presented, including a detailed design rationale, optimization of the chemical and physical variables, and the preliminary validation results on simulated soil and real soil samples. The sensor exhibits high tolerance to interfering ions, a detection limit of 2.1 μg/L for soil extracts and a lowest detectable field nitrite ion concentration of 1 ppm.

1. Introduction

Crop production efficiency in both, economic and environmental terms, raises concerns about the sustainability of current agricultural practices and debates over the updating of standards and procedures towards cleaner production (Kubota and Da Rosa, 2013). The ever increasing need for agricultural products led conventional farming to become aggressive with serious medium- and long-term negative effects on crop yields, environmental quality and natural resources (Tilman et al., 2002). The development of a suitable farm traceability system has received much attention lately (Bellon-Maurel et al., 2014, 2015), supporting the concept of precision agriculture (Stafford, 2000) and highlighting the need for monitoring technology to aid management (Srbinovska et al., 2015).

Precision agriculture and site-specific management involves monitoring of the spatial and temporal variability in soil and crop factors within a field (Stafford, 2000). Special emphasis is given to macro-nutrients such as nitrogen (N), phosphorus (P) and potassium (K), which drive fertilizer application for optimizing crop yields and input costs (Wu et al., 2013). On-the-go measurements become indispensable, raising the need for developing reliable field sensors to assess a variety of parameters. Although a challenging task, progress is considerable for a number of technologies, mainly spectrophotometric and electrochemical, that could turn into viable on-the-go detectors in the near future (Sinfield et al., 2010).

Soil is a complex matrix where macro-nutrients may be present in various interchangeable chemicals forms, each representing a different kind of concern that calls for discrete monitoring. For example, nitrogen may be present in three forms: ammonia, nitrate and nitrite. Ammonia is oxidized to nitrite, at an extent depending on the mineralization and decomposition capacity of soil; nitrite is readily converted into nitrate. Ammonia accumulation warns against an increasing dominance of soil-feeding insects (Ji and Brune, 2006), whereas high nitrate levels point towards strong carryover effects, neglected nitrogen reservoirs and microbial imbalances (Nettleton and Peterson, 2011). Nitrites are only expected to be found in very dilute concentrations, unless a variety of factors have been tangled with simultaneously, including fertilizer load,

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organic matter, water availability and biocidal treatment (Gelfand and Yakir, 2008). Toxic to both, plant and soil microorganisms, nitrite becomes a major concern for agriculture (Laanbroek et al., 2002) and turns into a serious health risk when reaches drinking water and the food chain (Pawełczyk, 2012). The WHO nitrite guideline value for water is 3 mg/L for short-term exposure and 0.2 mg/L for long-term exposure (World Health Organization, 2008); the European limit is 0.5 mg/L (European Communities, 2007). Low detection limits and reliable monitoring still remain an on-going research effort.

Numerous detectors and detection systems have been proposed for nitrites based on spectroscopic (UV/vis, fluorimetric, IR, Raman), chromatographic (gas, liquid) and electrochemical (ion-selective electrodes, voltamperometric, amperometric and culometric) principles; for a comprehensive review see Moorcroft et al. (2001) and Dutt and Davis (2002). Regardless, sensitivity and selectivity issues have yet to be resolved for all technologies proposed. Spectroscopy offers good precision and high sampling frequency at a high cost; detection limits range between 20 μg/L (Ensafi et al., 2004) and 22 μg/L (López Pasquali et al., 2010), although lower detection levels (15 μg/L) have been reported (Sreekumar et al., 2003). Chromatography gives slightly higher detection limits, ranging from 30 μg/L (Kodamatani et al., 2011) to 100 mg/L (Niedzielski et al., 2006), and a better performance that is limited to routine laboratory analyses (see Michalski and Kurzyca, 2006, for a comprehensive review). Electrochemistry provides detection limits comparable to chromatography with less complicated and time-consuming formats that have higher chances of developing into field instruments despite the low tolerance to common ions. Reported values vary with sample matrix and detection method, ranging between 46 μg/L (Salimi et al., 2008) and 92 μg/L (Pietrzak and Meyerhoff, 2009); Pham et al. (2011) proposed a carbon nanotube thin film system that decreased detectability to 10 μg/L.

Biosensor technology packs natural chemoreception processes into sensitive and selective miniature detectors using less reagents and sample volumes, justifying its strong ecological-relevant and environment-friendly character (Purohit, 2003). Either electrochemical or optical, nitrite reducing enzymes or complexes seem to be the biological species (bioelements) of choice, giving detection limits of 10 μg/L or less: cytochrome c (Chen et al., 2009), horseradish peroxidase (Liu et al., 2009), and nitrite reductase, either in mediated (Tepper, 2010) or non-mediated (Silveira et al., 2010) amperometric platforms. Other species have been also considered, such as heme proteins (Liu and Ju, 2003; Dai et al., 2008; Ding et al., 2010), although not sufficiently characterized. Promising indeed, none could demonstrate its working principle in real samples due to electrode fouling, as reported by Larsen et al. (2000) for a field study and verified experimentally for lipid membrane biosensors (Batzias and Siontorou, 2005) and field effect transistors (Siontorou et al., 2010). Furthermore, some of the enzymes and modifiers used are commercially unavailable; e.g., the method proposed by Dai et al. (2008) makes use of cadmium sulfide nanospheres produced in-house. Considering, also, that nitrite has to be extracted from soil samples for analysis, low field concentrations are further diluted, often to levels below the detection limits of the methods.

Notwithstanding, on-the-go assessment of ion concentrations requires a different way of thinking in developing strategies and devices (Sinfield et al., 2010). Using a number of different technologies, each with its own protocol of measurement, operating requirements, and handling expertise, to span one field might fail serve the intended purpose. The idea of employing a common detection platform, readily modified and adjusted to assess different species as needed and provide comparable results to work out total constituent analyses could become a key concept in soil management.

Towards that end, this paper reports on the development of a soil nitrite biosensor, using a versatile lipid nano-platform that allows the easy customization of sensor's selectivity and sensitivity through the adjustment of macro-parameters (e.g., lipid composition, ionic strength, and pH).

2. The lipid biosensor platform

Lipid membranes are two-dimensional fluid nano-structures where two, preferably, lipid layers are held together by non-covalent hydrophobic interactions of amphipathic molecules. The films have a thickness of about 5 nm, varying with the lipid tail length. Bilayer lipid membranes (BLMs) spanning an aperture that separates two electrolyte solutions (suspended BLMs) exhibit a resistance of 100 MΩ cm^2 allowing for a transmembrane ion current of a few picoamperes (pA) (Bright et al., 2013) that supports ion channel recordings (Zakharian, 2013) and electrochemical switches (Cornell et al., 1997). Selectivity towards a given analyte is induced with the addition of a biological moiety with a known affinity for that analyte: any biorecognition event that involves the specific analyte–bioelement interaction modifies the physicochemical state of the membrane (e.g., surface charge density, dipolar potentials or molecular packing and fluidity), altering the transmembrane ion flux (transduction); this is manifested as transient ion current signals with a magnitude directly correlated to the concentration of the analyte (Siontorou and Batzias, 2013).

Compared to other types of biosensors, BLM platforms provide a nature-mimicking environment for most bioelements (e.g., enzymes, ion carriers, receptors, DNA, etc.) that spontaneously guides their orientation and thermodynamic stability, prevents loss of biological function and integrity, and favors biomolecular interactions (Siontorou and Batzias, 2013). Physisorption is used to attach the bioelement to the sensor with a simple, one-step, successful every time and repeatable procedure, without the need for largely intractable, cumbersome and multi-step schemes that involve crosslinkers and stabilizers (Putzbach and Ronkainen, 2013). The bioelement is simply added into one of the electrolyte solutions separated by the BLM. Upon the addition, high-magnitude transient signals appear, indicative of the adsorption of the bioelement on the membrane; these signals decrease in both, magnitude and frequency of appearance, and within 10–20 min the membrane–bioelement complex stabilizes at a residual (background) current of 5–10 pA (Nikolelis and Siontorou, 1995). The complex remains stable and functional, i.e., with no observable bioelement leakage or sensitivity decrease, for flow rates up to 20 mL/min (Nikolelis and Siontorou, 1995), rapid gel-to-liquid phase shiftings (Nikolelis et al., 1995) and temperatures up to 40 °C (Siontorou et al., 1998).

These platforms have been a very successful laboratory approach, even achieving chromatography-like separation of complex mixtures, such as triazines (Nikolelis and Siontorou, 1996) and hydrazines (Siontorou et al., 2000). Yet, there exists a major drawback that prevents their manufacturability and applicability: the collapse of the bilayer structure (with total loss of function) at voltage-induced stress, increased protein loading or mechanical shock (Siontorou and Batzias, 2013). As a result, there has been an increasing emphasis on developing solid supported membrane platforms (s-BLMs) to enhance mechanical robustness (Richter et al., 2006).

Tien and Salamon (1989) presented a very simple technique for the preparation of s-BLMs: a silver wire with a freshly-cut tip is immersed in lipid solution and then dipped into electrolyte solution; upon immersion, the lipid droplet attached to the wire is self-assembled into a bilayer that has one layer adsorbed on the metal surface (the cut tip) and the other facing the aqueous solution. s-