Towards a portable magnetoresistive biochip for urease-based biocementation monitoring*

D. C. Albuquerque, R. Cardoso, G. A. Monteiro, S. O. Duarte, V. C. Martins, S. Cardoso

Abstract—In recent years there has been an exploitation of sustainable and environmental-friendly technologies for civil engineering applications. One of the most studied and promising technologies is soil improvement using biocementation through microbially induced calcite precipitation (MICP). MICP occurs due to the enzymatic activity of microbes living or added to the soil, with the analysis of this enzyme allowing a possible method for quantifying the amount of biocement (calcite) produced. This work presents and validates a novel detection tool to quantify and monitor urease in situ by employing a lab-on-a-chip (LOC) device. The device uses magnetoresistive bioships as biosensors in tandem with a read-out electronic set-up, magnetic labels and an integrated microfluidics system. A calibration curve for jack bean urease concentrations of 0.5 mg/mL to 70 mg/mL was obtained, serving as proof-of-concept for future in situ monitorization of biocementation. Experiments comparing direct urease assay detection versus sandwich assays were also conducted, with no difference being found between the two methodologies.

I. INTRODUCTION

Population and consequently civil infrastructure continue to expand at unprecedented rates. As such, the availability of competent soils for construction, as well as the rehabilitation of existing civil infrastructure are a major concern to meet ever-growing societal needs at minimal environmental impact [1]. Traditional soil and infrastructure reinforcement solutions usually involve the use of cement which is not an environmentally-friendly technique. As such, the harnessing of biological processes in civil engineering emerges as a promising approach, a “green technology”. In case of being used for soil improvement, it can be used to reduce ground permeability and increase stiffness and strength [2].

Biomineralization, in particular microbially induced calcite precipitation (MICP), has been the primary focus of research in biogeochemical engineering to date. MICP is the creation of calcium carbonate (calcite) as a consequence of microbial metabolic activity [3]. Calcite precipitation may be achieved by many different processes, of which enzymatic hydrolysis of urea by urease being the most energetically efficient of all [1]. MICP is uplifted by injecting the soil or medium to treat with nutrients (calcium chloride and urea) and urease producing bacteria, such as Sporosarcina pasteurii [4], to fill pores, flaws and cracks of those materials [5, 6].

The calcite produced is the biocement and has already been used in applications concerning soil stabilization, earth construction, restoration, sealing and remediation [7] [8]. There are, however, some drawbacks of using this technique. The main adversity comes on obtaining a homogeneous distribution of bacteria (and, consequently, calcium carbonate) over the soil [9]. The microbial activity is also dependent of environmental factors like temperature and pH, which can also be a problem. Both affect the amount of biocement produced, and therefore the treatment efficiency.

In this context, one of the challenges of biocementation is centered around the performance monitoring, which mostly lies on the assessment of enzymatic activity of urease. Standard enzymatic assays include spectrophotometry, fluorimetry, potentiometry and amperometry. However, these traditional assays are mostly time consuming, require extensive sample pre-treatment, and are unsuitable for in situ implementation [10]. This creates a need for an alternative enzymatic monitorization method for urease, that despite reliable is also able to give in situ information. Lab-on-a-chip (LoC) devices, consisting on integrated systems to achieve automation on sample preparation and user-friendliness for point-of-use operation, could be the answer to this need [11]. In this paper a LoC device consisting of a magnetoresistive platform is tested on the quantification of urease. The LoC apparatus consists then of four main components: biochips with magnetoresistive sensors, magnetic labels (magnetic nanoparticles MNPs), electronic set-up, and a reusable microfluidics system is used as a proof-of-concept.

II. MATERIALS AND METHODS

A. Biological Reagents

All reagents used in the biochemical tests were of analytical grade and the water deionized. The anti-Canavalia ensiformis urease rabbit polyclonal antibody biotin-conjugated was purchased from Rockland. Sulfo-LC-SPDP (Sulfosuccinimidyl 6-[3'-[2-pyridyldithio]-propionamide] hexanoate) and BSA (Bovine Serum Albumin) were acquired from Pierce, and urease C. ensiformis (Jack bean) from Sigma-Aldrich. Nanometer sized magnetic particles (250 nm, Nanomag-D) were from Micromod, Germany. The magnetic nanoparticles (MNPs) are 75-80% (w/w) magnetite, coated with dextran and streptavidin-modified, presenting about 1000 binding sites for biotin. They present a magnetic moment of 1.6 x 10-16 A.m² for a 1.2 kA/m magnetizing field and a susceptibility of $\chi \sim 5$ [12].

The buffers used were phosphate buffer 0.1 M pH 7.4 (PB) and phosphate buffer 0.1 M pH 7.4 Tween-20 0.02% (v/v) (PB-Tween20).
B. Microfabrication

The fabrication of the spin-valve magnetoresistive biochip (MR-biochip) comprises several steps of microfabrication, as described in (Martins et al., 2009) [13]. The MR sensor (spin-valve) was microfabricated with the stack Si/Al₂O₃ 100/Ta 1.5/NiFe 2.8 /CoFe 2.7 /Cu 2.7 /CoFe 3.3 /MnIr 7.5 /Ta 5 (thicknesses in nm) deposited by ion beam sputtering (Nordiko UK). The spin valve sensors used were characterized, showing a minimum resistance of 390-440 Ω and a magnetoresistance of ~8 %. The chip layout used has 30 U-shaped spin valve sensors arranged in 6 distinct sensing regions (Figure 1). Each region comprises 4 biological active sensors plus a reference sensor. The biological active sensors are coated by sputtering (Alcatel450, France) with a gold film (Cr 5nm /Au 40nm) for biological immobilization purposes. The same gold surface was deposited over a silicon wafer and diced (automatic dicing saw DISCO DAD) onto 5 x 7 mm² test samples, for the functionalization tests.

C. Surface Activation

The substrates (gold substrates and MR-biochips) surface functionalization started by stripping the photoresist layer, used to protect the surface during the dicing process, with Microstrip 3001, Fujifilm for two hours at 65ºC. Then the substrates were rinsed with isopropanol (IPA) and deionized water and blown-dried with a compressed air gun. After, the substrates were exposed to ultraviolet light/ozone plasma for 15 min at 28 mW/cm² inside an UVO cleaner machine from Jelight, USA.

D. Immunoassays

The immunoassays were first optimized on gold substrates, which mimic the chip surface while avoiding to expend MR-biochips in optimization tests. The chosen immunoassay were the direct and sandwich strategies (Figure 1a-b).

The direct assay starts by manually spotting 1 μL of sulfo-LC-SPDP linker at 2 mg/mL of linker over the substrate [14]. After 20 minutes of incubation, the substrates were washed with PB 0.1 M pH 7.4, to remove any unbound crosslinker molecules, and left to dry out at room temperature. A volume of 1 μL of urease in PBS at a desired concentration is then spotted over the linker and incubated for 1 hour at RT inside a Petri dish (humid chamber). After another cleaning step with PB, 20 μL of BSA 0.1% (w/v) was spotted on the substrate and incubated at RT for 1 hour. In the meantime, the MNPs - antibody solution was prepared. The supernatant of 10 μL of MNPs stock solution was removed with the aid of a magnetic concentrator (DynaMag-2, Invitrogen), and resuspended in 100 μL of PB Tween 20. This washing process was repeated twice. After, 10 μL of antibody solution at 10μg/mL in PBS 0.1 M pH 7.4 was added. The solution is left to react for 45 minutes in a rotator stirrer (model 3000445, JP SELECTA). The supernatant of this solution was removed and resuspended in 100 μL of PB Tween 20.

A volume of 20 μL of the former solution was manually spotted on the substrate surface and left to react for 30 minutes in a humid chamber at RT. After washing with PB Tween 20 was observed under an optical microscope (DFC 300 FX, Leica). In parallel a negative control, without urease (only PB) was also performed (Figure 2a-b).

The sandwich assay is similar to the direct assay, differing in an additional reaction step for probe antibody immobilization before the urease. After the linker, 1 μL of antibody at 10 μg/mL was spotted and left to react for 1 hour in a humid chamber at RT. After washing the unbound molecules with PB, a blocking step with BSA for 1 hour took place. From this step onwards, both tests proceeded in the same way.

E. MR-biochip detection

The electronic read-out setup used in this work was the same reported by Martins et al. [15]. The measurement conditions used were 1 mA DC current for sensors biasing, an in-plane transverse external AC excitation magnetic field of 1.1 (kA/m)rms (211 Hz) and a DC field of -2.4 kA/m for MNPs magnetization.

Figure 1. Schematics of the (a) Direct and (b) Sandwich detection methods employed. In the direct assay the urease is directly attached to the gold covered sensors, while in the sandwich there is specif binding to the antibodies immobilized on the gold. Features not to scale.
The immunoassays performed in the platform uses a microfluidic channel made of PDMS as described in Dias et al. [16] to transport the sample over the sensing area. After the biochip is inserted in the reader platform and let to acquire a baseline of the sensors for 5 -10 minutes, a syringe pump (New Era Pump systems) is set to run at the flow rate of 5 μL/min until the MNPs solution fills the PDMS channel. The MNPs are left to settle down over the sensing area until a saturation signal is reached. Finally, a washing step with PB Tween 20 to remove the unbound MNPs is performed at a flow rate of 10 μL/min and a binding signal recorded for another 5 - 10 minutes. The difference between the baseline voltage and the binding voltage results in a voltage variation (ΔV) which corresponds to the detection signal that is then normalized by the sensor output (baseline voltage) (ΔV/V).

III. RESULTS AND DISCUSSION

Surface functionalization tests using direct assays were performed on gold covered substrates and biochips with varying concentrations of urease, between 0.1 and 100 mg/mL (Figure 2a-b). A negative control taking PB instead of urease was also performed.

In these assays, urease was initially immobilized on the surface, and then reacted with antibody-modified MNPs. The observable difference between the negative control and bioactive sensing areas demonstrates the efficient and specific recognition of the urease by the selected antibody, proving that the used protocol was working for the tested range of urease concentrations (see Figure 2a-b).

The functionalized biosensors where also read in the MR-biochip platform. Two examples of detection curves are shown in Figures 3a-b, for a bioactive sensor, with 50 mg/mL of jack bean urease immobilized, and a negative sensor without urease. While in the negative control the signal obtained after washing practically returns to the initial baseline signal, the one with urease presents a significative difference between the initial and final voltage values due to the presence of the magnetic particles attached to the urease.

Using concentrations of urease between 0.5 mg/mL and 70 mg/mL, a calibration curve for quantification of urease was obtained (Figure 4). Since the concentration of urease is unknown (for the real samples obtained in the field), the range was chosen by taking into account that at 70 mg/mL the urease starts to precipitate in solution giving inconsistent results, and that 0.5 mg/ml is close to the detection limit.

Figure 3. Voltage signal acquired in the MR platform with respective surface microscope visualization (40x magnification) from (a) negative control direct assay, (b) direct assay with 50 mg/mL urease immobilized. (V<sub>baseline</sub>= resistance value from the initial baseline; V<sub>binding</sub>= resistance value obtained correlated to the number of magnetic particles remaining in the sensor surface after washing; ΔV= Resistance difference between V<sub>baseline</sub> and V<sub>binding</sub>).
In the calibration curve, a stabilization seems to be occurring around 60 mg/ml, corresponding to the saturation threshold.

Finally, sandwich assays were also performed to see if less non-specific binding occurred and read in the biochip platform. Direct assay with the same urease concentration was used as positive control and no urease was used as a negative control. The two detection methods did not yield different results, meaning urease is still attaching to the surface in the sandwich assay methodology (Figure 5). When using a sample containing proteins other than urease, the sandwich assay should, however, be the preferred method, as they would less likely attach to the surface since they aren’t recognized by the immobilized antibody.

Figure 5. Bioassays using 40mg/mL of urease (200x magnification). (a) Direct assay (b) Sandwich assay.

IV. CONCLUSION

The work developed in this paper aimed at optimizing a magnetoresistive platform for urease quantification that intends to act in the future as a LoC for in situ operation. This was accomplished by testing a direct immunoassay method and obtaining a calibration curve for urease concentrations between 0.5 and 70 mg/mL. The direct method was compared with a sandwich assay and although both lead to similar results, the latter will be the preferred method for analyzing environmental samples.

A microfluidic sample preparation system is under development to combine with the detection platform and allow the fully automation of the analysis.

As a next step, real samples of Sporosarcina pasteurii urease obtained from fluids extracted from soils under treatment, in field operation, will be assayed in the platform to demonstrate its capacity to monitor biocementation in situ.

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