Case study

Quantification of Urease Activity via High Throughput Conductivity Measurements

Tools:
CardioExcyte 96

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featured by Nanion Technologies

Frédéric Maurice Lapierre, Munich University of Applied Sciences. His focus is the development of a bioprocess for microbial induced calcite precipitation (MICP)-relevant microorganisms.

Microbial urease catalyzes the hydrolysis of urea to ammonium and carbonate, which results in an increase of the environmental pH value. Addition of calcium ions then leads to calcium carbonate (= calcite) precipitation. Microbial Induced Calcite Precipitation (MICP) is successfully applied for, e.g., restoration of construction materials, ground improvement or inhibition of weed growth. Urease activity is considered a key factor for MICP performance. Multiple studies use different techniques to adjust the urease activity of a bacterial culture, or isolate new ureolytic microorganisms. Consequently, quantification of urease activity plays an important role for MICP research and application [1].

The conductivity assay for urease activity determination, first published by Chin and Kroontje [2] and later established for MICP-relevant microorganisms by Whiffin [3], is widely used in MICP research. The conductivity assay is easy to perform; an urease-positive sample is added to an urea solution, resulting in the formation of ions, increasing the overall conductivity of the solution. In general, the conductivity change over five minutes is monitored and the slope of the conductivity signal correlates with the urease activity [3]. As the determination of enzymatic activity is a time dependent parameter, it inherently takes several minutes for the data to be aquired. Measuring one sample after another with conventional methods can consequently be considered as time consuming.

To our best knowledge, no commercial high-throughput conductivity measurement is available for such enzyme assays. Here, the CardioExcyte 96 (Nanion Technologies) was used for parallelized urease activity determination, and this system proved to overcome the hurdles met with conventional measurement setups.

Nanion´s impedance-based cell monitoring technology CardioExcyte 96 was originally developed to investigate electrophysiology, contractility and viability recordings of adherent cells. In general, reading the impedance of planar

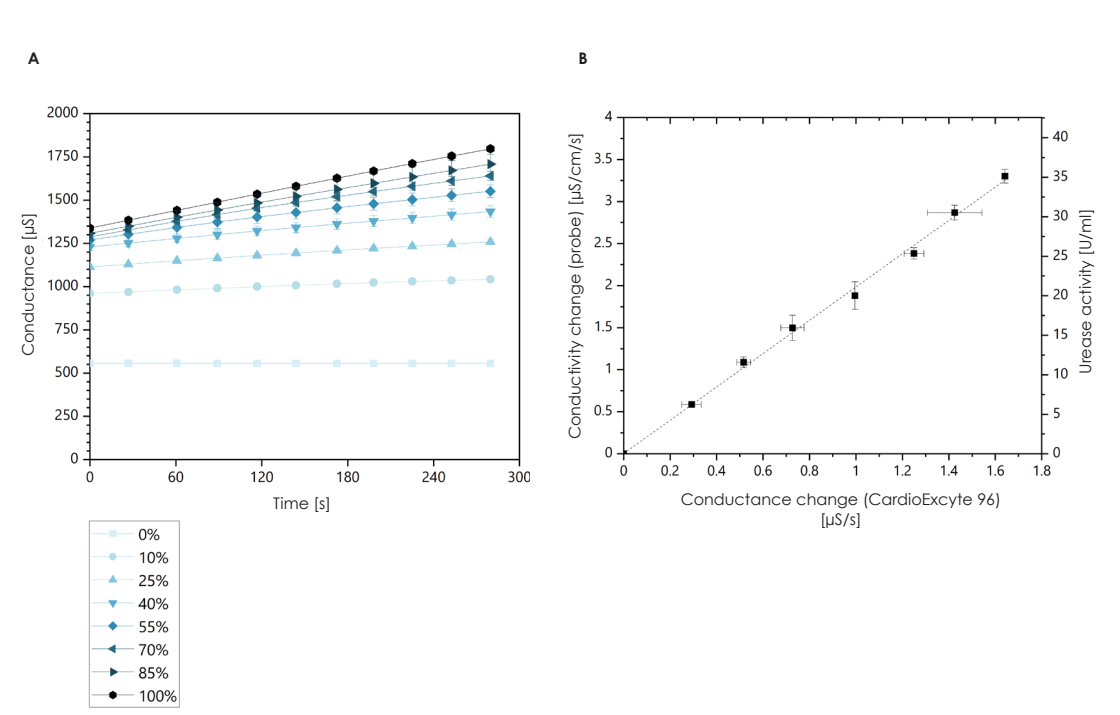
Nanion's CardioExcyte 96 platform
The conductance is continuously and automatically monitored as a measure of urease activity. The CardioExcyte 96 is an automated device, recording from 96 wells at a time.

> **"The CardioExcyte has dramatically improved our ability to determine the urease activity. The ease-of-use and high throughput allows for a drastic time saving when measuring large numbers of samples."**
>
> **Robert Huber**, Prof. Dr., Munich University of Applied Sciences

gold-film electrodes that are used as growth substrate for adherent cells reveals changes in electrode coverage or cell behavior.

Here, the CardioExcyte 96 was used to measure the conductance of solution which is containing urease-positive bacteria. In principle, an urease-positive sample is added to an urea solution, resulting in the formation of ions. In this study, a culture of urease-positive bacterium *Sporosarcina pasteurii*, the most used microorganism for MICP in published research, was prepared by overnight shake flask cultivation at 30 °C and 250 rpm in chemically defined medium as described by Lapierre *et al.* [4]. A dilution row was pipetted (100%, 85%, 70%, …, 0%) using culture medium. For comparison, 1 ml of each dilution was measured traditionally with conductivity probes (InLab 751, Mettler Toledo, Giessen) in a stirred beaker containing 24 ml of 1.1 M urea solution as described in Lapierre *et al.* [1].

A conversion of conductivity change over time (unit: µS/cm/s) to urease activity (unit: U/ml) is known from previous findings. Urease activity is defined as the amount of substrate hydrolysed in 1 minute. 10 µl of each dilution of the prepared urease-positive culture is added to each well of the CardioExcyte 96 plates. Afterwards, 190 µl of 1.1M urea solution is added to each well. The plate was shaken in double orbital mode for 30 seconds using a microplate reader (SpectraMax iD3, Molecular Devices, United States). Afterwards, the conductance of each well is measured using the CardioExcyte 96 over the course of 5 minutes.



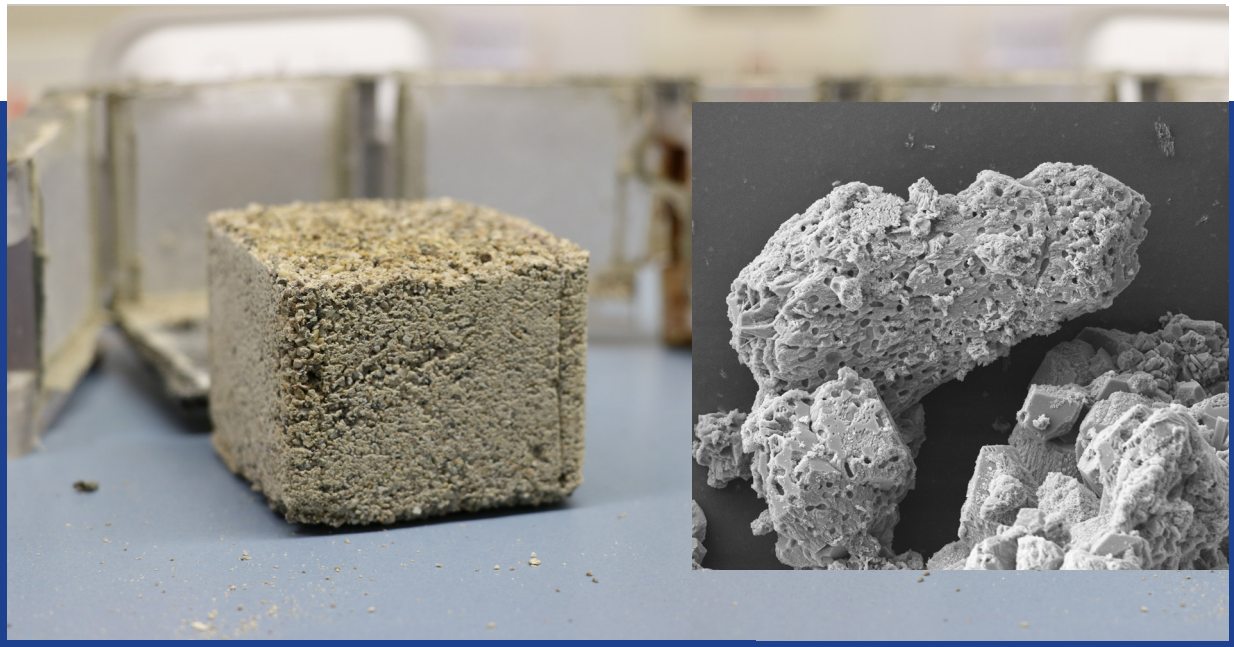
CardioExcyte 96 recordings. A *Conductance signals for a dilution row of an urease-positive culture. More urease-positive culture results in steeper curves. Only average values are shown (N =3, N = 1 for 100% due to pipetting errors).* ***B*** *Conductance change per second measured with the CardioExcyte 96 (X-axis) and InLab 751 (Y-axis) correlate nicely (R^2 = 0.9967). Only average values are shown (N =3, N = 1 for 100% due to pipetting errors). The relationship Urease activity [U/mL] = 21.097 × Conductance change (microplate) [µS/s] was determined. The error bars depict SD.*

Addition of urease-positive culture to an urea solution results in a linear conductance signal over the period of 300 seconds (Figure 1A). The conductance change measured in the microplate wells correlates nicely with measurements executed in a standard conductivity probe setup (Figure 1B). Consequently, high-throughput small-scale urease activity measurements can be performed using the CardioExcyte 96.

High-throughput screening of conductance kinetics for enzyme activity determination allows for drastic time savings when measuring a large number of samples compared to the standard method using probes, e.g. when screening for new urease positive strains.

Another advantage of the CardioExcyte 96 system compared to the standard method is that the required sample volume is much lower (20 µL instead of 1 ml).

Taken together, the CardioExcyte 96 allows for automated enzyme activity measurements, enabling fast bioprocess development. Overall, using MICP for soil stabilization reduces the environmental impact of ground improvement, since conventional ground improvement techniques often have a high impact on the environment.



Microbial Induced Calcite Precipitation (MICP) is successfully applied for restoration of construction materials. *Sand-derived Biocement (Photo: Alexander Bayerhoff) and, right, scanning electron microscope photo revealing microbially precipitated calcium carbonate. The holes in the lime structure indicate bacteria (Photo: Constanze Eulenkamp).*

"My experience with the Nanion team was 100% positive, bringing a new technology like CardioExcyte 96 on board in our lab and achieving my research goals in a very short time was striking to me."

Frédéric Maurice Lapierre, Munich University of Applied Sciences

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References

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