



Faculty of Civil, Geo and Environmental Engineering
Chair of Urban Water Systems Engineering

Mass spectrometry measurement of enzymatic activities Miniaturization and application for environmental samples



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Referees	

Background

Numerous inorganic and organic contaminants are ubiquitously present in the environment and wastewaters. These substances originate from agricultural or industrial practices, e.g. pesticides or phenolic compounds. In addition, there is an increasing concern regarding the discharge of trace organic contaminants (TOC) to the environment including pharmaceuticals, personal care products or illicit drugs. The incomplete removal of these compounds during wastewater treatment leads to TOCs residues in the effluent and finally in surface waters. Due to their persistence, bioaccumulation and toxicity, the extent of the contamination and moreover development of removal techniques are of emerging interest. Therefore, cost-efficient screening methods as well as effective and economic elimination processes have to be established [1, 2].

For these purposes the target use of enzymes can be a convenient approach for biological water treatment processes. Enzymes often possess a broad substrate spectrum and are able to catalyze versatile reaction types. Thus, enzymes offer a widespread application spectrum and open up new possibilities for environmental technologies.

Usually, enzymatic reactions have been analyzed spectroscopically (e.g. photometry, fluorescence) or offline via LC-MS techniques using the inactivated reaction solution. Today, these enzymatic reactions can also be designed in a manner to couple them direct and online to mass spectrometric detection (MS). The apparent advantage of MS detection is the possibility to use physiological substrates as well as the cost-effective measurement due to the low consumption of components. This sensitive technique further enables the simultaneous and online detection of all ionizable assay components, i.e. substrate, product(s) and potential intermediates [3].



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Objective & Experimental Approach

With regard to environmental purposes enzymes are usable as biosensors for the detection of pollutants. To enhance cost-efficiency as well as sensitivity of those biosensors a microfluidic chip seems to possess high potential. For that reason such a chip device will be established for the analytical, zero-death-volume analysis of enzymatic activities and their respective regulation. It will be designed in a manner to enable direct coupling to a mass spectrometer. The work is conducted in cooperation with the University of Leipzig and the Institute of Energy and Environmental Technology e.V. (IUTA). By now, the final chip layout has been determined. In the next step, enzymatic reactions have to be adapted to the chip. Different enzymes will be tested with regard to a screening tool for regulators.

Besides the application as biosensors, enzymes may provide versatile opportunities for economic and ecologic degradation of TORC's. The potential of oxidative enzymes like peroxidases and laccases to degrade TORC's have already been investigated in managed aquifer recharge systems (MAR) implying the involvement of even more enzymes like monooxygenase in TORC degradation [4]. To take advantage of these enzymatic activities, first there is a need to measure their activity in environmental samples. Currently, work focusses on the establishment of enzymatic assays with commercial enzymes including cytochrome P450, laccases and peroxidases. For that purpose photometric measurements were applied. Additionally, the enzymatic reactions were directly hyphenated to

mass spectrometry, which provides the opportunity to measure those reactions in multiplex experiments, i.e. simultaneous measurement of two or more enzymes. With regard to the measurement of enzymatic activities in environmental samples, in which a variety of enzymes are present usually at low concentrations the main focus is set on establishing sensitive and multiplex capable enzymatic assays. Furthermore, the capability of enzymes concerning the degradation of TORCs will be examined. Finally, the established assays can be optimized for the application in environmental samples.

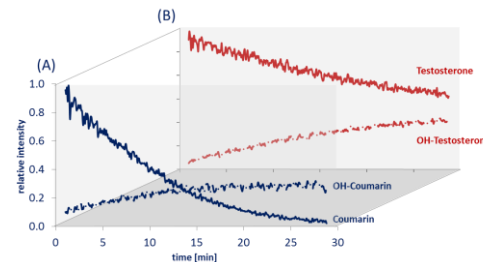


Fig. 1: Extracted ion chromatogram of substrates and products when measuring multiplex assay with (A) Testosterone and CYP3A4 and (B) Coumarin and CYP2A6

References

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