Biodegradation **4**: 101–105, 1993.

A microbial biosensor system for dihalomethanes

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Received 21 June 1991; accepted in revised form 5 January 1993

Key words: biodegradation, biosensor, dechlorination, dehalogenase, dichloromethane, Hyphomicrobium

Abstract

A biosensor system able to measure dichloromethane (DCM) and other dihalomethanes has been developed. The analysis is based on Hyphomicrobium DM2 cells immobilized in alginate. A combination of transducers consisting of a flow-calorimeter followed by a chloride-sensitive electrode has been used. By this design it was possible to monitor different aspects of the cell metabolism from one and the same pulse of substrate. The detection limit for the biosensor was 0.1 μM dichloromethane. The biosensor system can be used for continuous measurements in a sample stream.

Introduction

The use of microbiological processes for waste water treatment, bioremediation and destruction of hazardous wastes is on the increase. Microbiological growth plays an important role in such biotechnological processes and therefore it is very important to control parameters such as substrate and product concentrations. When culturing cells on toxic substrates it is important to control the substrate level very carefully since a too high concentration may kill the cells. With a reliable analytical system it is possible to maintain the substrate concentration at an optimal level. Chromatography can in some cases be used for the determination of these parameters. Lately biosensors has been shown to be an alternative in several applications even if the status of the technology today needs to be improved before on-line measurements can be done. However, analysis of a sampling stream is fully possible today.

Biosensors based on cells have an interesting potential in analysis. The use of cells instead of isolated enzymes gives certain advantages e.g. better stability of sensitive enzymes, in vivo regenerating systems for coenzymes, and possibility to induce enzyme production in the cells while immobilized (Mattiasson 1983a). Cell-based biosensors have been developed for several different microbial substrates such as acetic acid (Hikuma et al. 1979a), methane (Karube et al. 1982) and ethanol (Hikuma et al. 1979b); the latter sensor has also been commercialized. In order to deal with situations when inhibitory products are formed or inhibitory metabolites are enriched during growth, it is advantageous to utilize the same cell-strain in the sensor as in the growth reactor. In this way it will be possible to detect when the levels of inhibitory products or metabolites are too high. This gives the biosensor system a certain advantage compared to chromatography systems.

There are, however, limitations with microbial biosensor systems. The fact that whole cells are present influences the specificity of the biosensor. A broad range of substrates may be accepted by the cell and lead to a metabolic response that is regis-

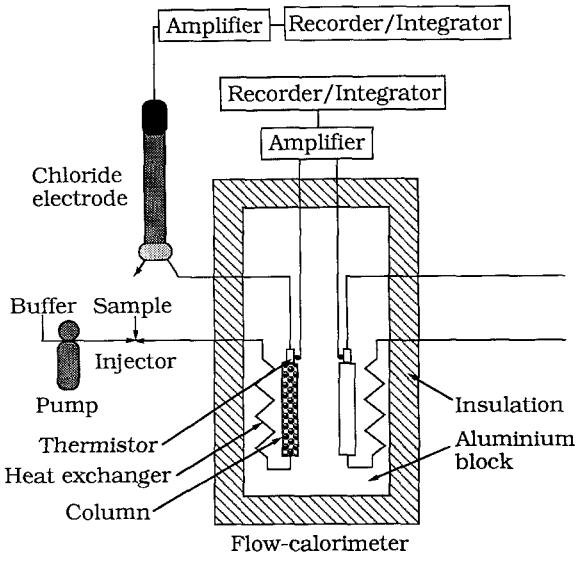

Fig. 1. Schematic presentation of the biosensor. The column with the immobilized Hyphomicrobium cells was situated inside the flow-calorimeter device. The flow-calorimeter device was a commercialy available unit (Danielsson 1990). The empty column to the right on the figure was used as a reference. A flow cell was mounted on the chloride sensitive electrode.

tered by the transducer. Furthermore, a substance may be metabolized by a sequence of enzymes thereby inducing uncertainty into the readings. Several measures have been taken to eliminate or circumvent these problems. Partial denaturation of the cells (Mattiasson 1983b), and introduction of partially selective membranes (Hikuma et al. 1979a) are two such steps. By using more than one transducer for monitoring of the cell metabolism it might be possible to differentiate between effects of different substrates and thus use these differences to increase the specificity of the analysis.

The present paper deals with the development of a biosensor system according to a new concept where the same strain is used in the biosensor and in the bioreactor. The biosensor is sensitive to dihalomethanes and is based on immobilized Hyphomicrobium DM2 cells and the use of two different transducers. Part of this work has been presented as a conference abstract (Henrysson & Mattiasson 1991).

Materials and methods

The bacterium used in the biosensor was the dichloromethane degrading Hyphomicrobium sp. strain DM2 (ATCC #43129). It was cultured according to Stucki et al. (1981), with the minor modifications given by Scholtz et al. (1988), on minimal medium with dichloromethane as the only carbon and energy source. The cells were harvested by centrifugation at 10 000g for 15 minutes. Directly after harvesting, the cells were immobilized in alginate essentially according to Adlercreutz & Mattiasson (1984). A 2% sodium alginate (Protanal 60 LF, Protan, Norway) solution was used and the beads were approximately 1.5mm in diameter. The cell density was approximately 1g (wet wt.)/ml. The alginate beads were placed in a column (0.7ml) situated inside a commercially available flow calorimeter device (Mattiasson et al. 1977; Danielsson 1990). A chloride-sensitive electrode (Orion 96-17B) with a flow cell was placed at the outlet (Fig. 1). The flow medium contained 10mM Pipes (Piperazine-N,N'-bis-2-ethane-sulfonic acid) buffer, (Sigma, St. Louis, USA), pH 7.2, 1.5mM $(NH_4)_2SO_4$, 1mM $MgSO_4$, 5mM $Ca(NO_3)_2$ and 1ml/l of the trace element solution used for the cultivations (Scholtz et al. 1988). Pure water was also used as flow medium where were indicated. The flow rate was 0.5ml/min. Unless otherwise indicated substrate pulses of 0.1ml were used and signals from both the flow-calorimeter and the chloride-sensitive electrode were recorded and integrated.

Results and discussion

A scheme for construction of the biosensor is shown in Fig. 1. Pulses of 0.1ml of solutions containing dichloromethane were given and the response from both the flow-calorimeter and the chloride sensitive electrode were registered (see Fig. 2).

Calibration curves for dichloromethane from the flow-calorimeter as well as from the Cl^--sensitive electrode are shown in Fig. 3. It can be seen that the peak area as measured from the Cl^--sensitive electrode gave a larger dynamic response range as compared to the case when peak height was used.

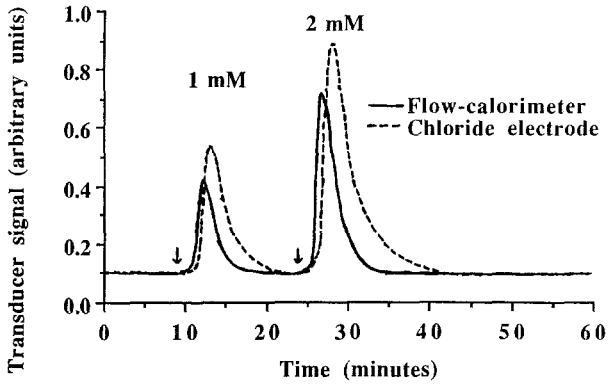

Fig. 2. Responses of immobilized Hyphomicrobium DM2 to 1 mM and 2 mM dichloromethane. The arrows indicates sample injection. The traces from both the flow-calorimeter and the chloride electrode transducers were redrawn from the integrator charts.

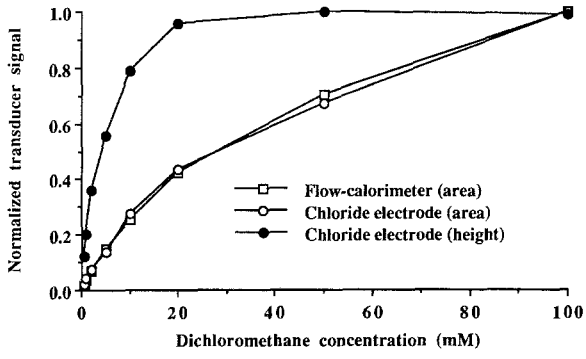

Fig. 3. Calibration curves of dichloromethane registered by the flow-calorimeter and the chloride electrode. The integrated peak areas and peak heights were normalized to the largest value for each curve.

Therefore, peak area was chosen for this study. The response of the Cl^--electrode based biosensor was linear for concentrations up to 10 mM. Concentrations from 20 µM to over 100 mM could be measured with the normal assay conditions. A more sensitive biosensor was achieved with larger sample volumes and a lower flow rate. This led to a larger peak width and a longer time to reach the baseline after each substrate pulse. Since the chloride electrode has a logarithmic response it was also possible to change the sensitivity by changing the background chloride concentration. When deionized water was used as flow medium it was possible to detect dichloromethane concentrations down to 0.1 µM (approx. 10 ppb). The peak width was also related to the substrate concentration. With the normal assay conditions, the time to reach the baseline was less than 10 minutes for a substrate pulse of 1 mM and under 20 minutes for 10 mM.

To examine the specificity of the biosensor a range of compounds was tested. When a non-chlorinated substrate, such as methanol, was used the calibration curve shown in Fig. 4 was obtained. In Fig. 5 the response to a 1 mM concentration of several different compounds can be seen. As expected it was only the dihalomethanes that gave a significant response on the chloride electrode. This is due to the substrate specificity of the immobilized cells. Somewhat surprising was the greater response from the bromomethanes compared to dichloromethane. This can, however, be explained in terms of the higher electrode sensitivity for bromide than for chloride. Chloroform and 1,2-dichloroethane gave responses at just the detection level of the chloride electrode. Chloroform gave a significantly larger response on the thermistor compared to the chloride electrode. This may be an indication of metabolism of chloroform without a concomitant dechlorination or it may be a result of unspecific interactions between chloroform and the immobilized cell preparation. It was also seen in Fig. 5 that 1,2-dichloroethane inhibited dichloromethane degradation as reported by Kohler-Staub & Leisinger (1985). The non-chlorinated compounds only gave responses on the flow-calorimeter. It is interesting to note that formaldehyde gave the largest response although it can not support growth of the organism as reported by Stucki et al. (1981).

The calibration curves in Fig. 3 based on the integrated areas of the response peaks from the Cl^--sensitive electrode and the flow-calorimeter have nearly the same shape. One interpretation is that the only active enzyme under these circumstances is the dichloromethane dehalogenase (Kohler-Staub & Leisinger 1985). If other enzymes were also involved, the response from the flow-calorimeter would have been the integrated response from all the enzyme catalyzed reactions. An alternative interpretation is that the dehalogenase or a subsequent enzyme in the metabolic sequence is the rate limiting step so that the heat response is a result of the total metabolism. As can be seen in Fig. 4 an-

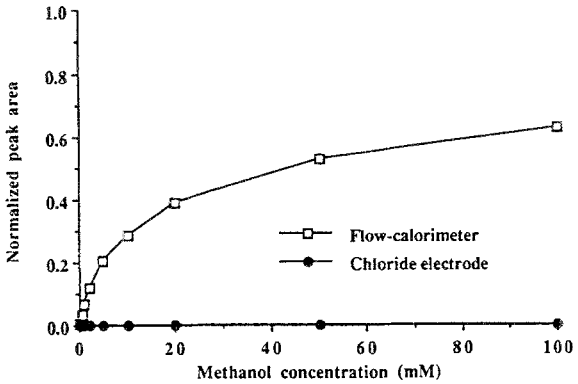

Fig. 4. Calibration curves of methanol for the flow-calorimeter and the chloride electrode transducers. The integrated peak areas were normalized to the largest peak areas from Fig. 3.

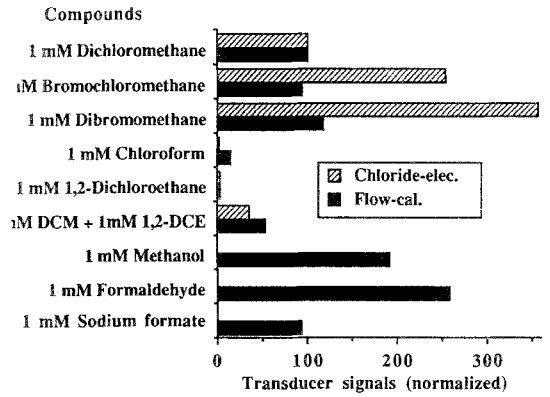

Fig. 5. Transducer signals for 1 mM of different compounds. All signals were normalized to 1 mM dichloromethane for each transducer, respectively. DCM; Dichloromethane, 1,2-Dichloroethane.

other substrate such as methanol gave a different shape of the calibration curve. The shape of these substrate calibration curves can be analyzed by a Lineweaver-Burk plot. When the data from the flow-calorimeter calibration curve for dichloromethane of Fig. 3 has been analyzed in this way an apparent Km value of approximately 30 mM dichloromethane was obtained (not shown). This value should be compared to the Km value for the purified enzyme which is 30 µM (Kohler-Staub & Leisinger 1985). The present value seems to be very high but reflects the fact that the measurements are carried out under special conditions. The enzymes are located in the cells and the substrate has to be transported over the cell membrane. At present it is not possible to say which is the rate limiting step, the permeation process or the enzyme mediated process. Furthermore, the cells were immobilized in 2% alginate beads. Severe diffusion restrictions were introduced by this arrangement. The fact that the beads were packed in a column was a further potential complication because of gradient formations along the packed bed. There was, however, no risk that such effects were of any importance since a high flow rate was used and a very low percentage of substrate was converted per passage.

The standard deviation was approximately 5% when nine repeated pulses of 1 mM dichloromethane were measured. The response was stable in the range of the standard deviation for at least 5 hours and this fact leads to both the possibility of using such a sensor for analyzing dichloromethane content in a waste stream or utilizing the sensor signal for process control. Furthermore, the column with the immobilized bacteria could be stored for 24 hours without losing more than 10% of the capacity at 4° C and 30% at 30° C.

For several applications a continuous flow system could probably be used. Figure 6 shows the traces from a one hour continuous flow experiment. Concentrations of 10 mM or greater inhibited the dechlorination in the column as can be seen by the increased dechlorination when the substrate concentration was decreased. Therefore, concentrations of 1 mM or below had to be used for continuous measurements.

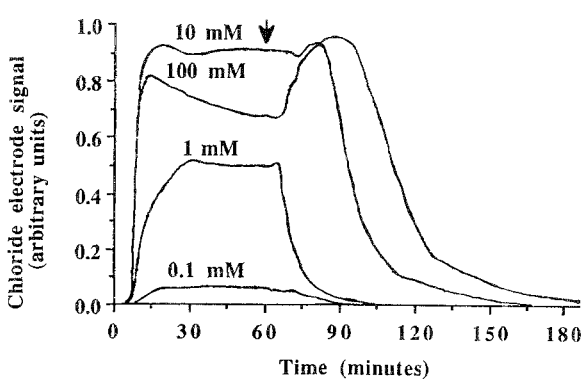

Fig. 6. Chloride electrode signals for a 60 minutes long pulse of different concentrations of dichloromethane. The arrow indicates the end of the pulse.

The results show that immobilized cells of Hyphomicrobium DM2 are useful in analysis. The combination of two different transducers helps to give a better evaluation of the signal. This may be a first step towards multitransducer-based cell biosensors for evaluation of metabolic responses to complex media. This may prove to be a strategy in fabrication of miniaturized biosensors with integrated evaluation units. In such cases multivariate analysis will help to evaluate the signals. However, in the present study conventional transducers were used.

Acknowledgement

This work was supported by the Swedish National Board for Technical and Industrial Development (NUTEK).

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