Fully automated and long term stable biological Toxicity Sensor

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MOTIVATION AND AIMS

Aim: Enhancement of the biosensor prototype AquaBioTox for broadband detection of chemical contaminants
from a laboratory device with high maintenance effort to a fully automated prototype (targeted maintenance effort ~ 4 weeks)

Fraunhofer IGB: biological part

Fraunhofer IOSB: low cost fluorescence sensor; automation and integration

AquaBioTox prototype (Fraunhofer IGB + IOSB, 2010)
MOTIVATION AND AIMS

Motivation

- Online monitoring of water quality by measuring fluorescence from genetically engineered bacteria (e.g. Ecoli and Caulobacter)

- Industrial Fluorescence measurement systems are very costly (8 – 14 k€, e.g. Algaetorch, bbe Moldenke or FP 360, Hach Lange)

- Targeted costs for online water toxin meters are < 7.000€ for the complete system

Principle of AquaBioTox biosensor:

- Genetically constructed strains of Escherichia coli and Caulobacter vibrioides

- Non pathogenic bacteria (risk group 1)

- Adapted to the normal conditions in water
Main components of the automated sensor system:

- Pressure Reducer
- Valve
- Water Free Fall
- Filter
- Inactivating Unit
- Automated Cartridge Exchanger with Pump and Heating
- Control Units
- Fluorescence Sensor
- Electrical Power
LOW-COST FLUORESCENCE SENSOR

Principle of the low-cost Fluorescence Sensor

- Robust rugged case IP65
- 2 versions of the optical unit
  - Coaxial setup with dichroitic filter
  - 20° Angle setup
- Stable long term behavior
Software modules:

- Lab-Vision with ResiWater project
- PLC program for free fall and inactivator
- Module for fluorescence sensor and data exchange
SAFETY ASPECTS

- Prevention of reflow of contaminations into the water distribution system:
  - No direct connection to the water pipe
  - Water free fall with sensor based level control integrated in the water intake system of the demonstrator

- Prevention of contamination of the environment with micro-organisms by an inactivation unit:
  - Inactivation temperature: 90°C, transient time: 100min
  - Experimental results of microbiological testing: no augmentable bacteria leave the system
BIOSENSOR SYSTEM

β Biosensors:

Bacterial strains:

- strains react on different substances: extended application
- easily handling
- red fluorescence by genetically engineering
- Real time measurement by newly developed fluorescence sensor
BIOSENSOR SYSTEM

Biosensor immobilisation on carrier material:

- **Material:**
  - Porous glass particles (Robu Glass)

- **Advantages:**
  - High amount of biosensor cells available for a significant reaction
  - Biosensor stabilized
  - Preservation for more than 6 month under cooled conditions
  - Long term operation possible
Experimental testing of influence of toxic substances on biosensors in the automated system:
Example: Influence of pH effect on biosensor (E. coli RFP)

Addition of acetic acid pH 4 (1)
Regeneration of fluorescence by addition of water (pH 7) (2)

Reaction time: < 1 min
**Experimental Results**

**Results with selected substances and toxins:**
tested by Fraunhofer IGB and TZW

<table>
<thead>
<tr>
<th>Agens</th>
<th>Biosensor</th>
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</thead>
<tbody>
<tr>
<td></td>
<td><strong>E. coli RFP</strong></td>
<td><strong>Caulobacter vibrioides RFP</strong></td>
<td></td>
</tr>
<tr>
<td>Buffered solution</td>
<td>pH 4</td>
<td>84 %</td>
<td>80 %</td>
</tr>
<tr>
<td></td>
<td>pH 5 - 7</td>
<td>&lt; 3 - 8 %</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>30 %</td>
<td>4.5 %</td>
<td>Not tested</td>
</tr>
<tr>
<td></td>
<td>50 %</td>
<td>40 %</td>
<td></td>
</tr>
<tr>
<td>Substance #1*</td>
<td>42 mg/L</td>
<td>&lt; 2 %</td>
<td>40 %</td>
</tr>
<tr>
<td>Substance #2*</td>
<td>430 mg/L</td>
<td>54 %</td>
<td>&lt; 2 %</td>
</tr>
<tr>
<td>Substance #3*</td>
<td>1 g/L</td>
<td>26 %</td>
<td>&lt; 2 %</td>
</tr>
</tbody>
</table>

*) Name hidden (confidential)

decrease of fluorescence in percentage (%)
EXPERIMENTAL RESULTS

Characterization of the biosensors:

- Biosensor types: 2
  - *Escherichia coli* RFP
  - *Caulobacter vibrioides* RFP

- Method of application: immobilisation on carrier material

- Time for response: < 1 min — real time measurement
- Long term stability tested: 3 weeks
- Storage of biosensors under cooling conditions (8°C): > 6 months
- Availability in the demonstrator:
  - about 6 measurement cells (more are possible) — operation of system theoretically possible for about 6 months
- Before leaving the demonstrator: cells are effectively inactivated by heating (90 °C)
CONCLUSIONS

- The biosensor prototype *AquaBioTox-II* for broadband detection of chemical contaminants has been developed to a fully automated and long term stable system.
- A stable operation for more than 30 days was achieved.
- The targeted maintenance effort of 4 weeks was fulfilled.

Recommendations for future work:

- For robust continuous operation the hydraulic part of the sensor system has to be optimised (pipes, pumps).
- The size of the sensor system could be minimised.