# A microsystem approach to measure the oxygen consumption of bacteria. Towards a precise evaluation of the BOD (Biological Oxygen Demand) parameter of wastewater

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# A microsystem approach to measure the oxygen consumption of bacteria. Towards a precise evaluation of the BOD (Biological Oxygen Demand) parameter of wastewater.

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**Abstract.** The quantity of organic pollutants present in wastewater is classically evaluated by measuring the quantity of dissolved oxygen during five days; it is quantified by the so called BOD5 parameter (Biological Oxygen Demand) [1]. This work constitutes the first step of an overall strategy targeting to improve the monitoring of BOD5. We focus on the development of a microsystem approach allowing monitoring the O2 consumption induced by the biodegradation process of organic matter. To evaluate the organic pollutants concentration, we use *Escherichia coli* as bacterial indicator, confined in a PDMS-glass chip. Their metabolic activity in presence of organic molecules is deduced from their oxygen consumption. These measurements are ensured by optical sensors present in each of the five instrumented chambers of the chip. The results show that the microsystem approach is suitable to measure simultaneously different concentrations of organic load, and that it is possible to reduce the analysis time. By examining the $O_2$ diffusion towards the walls of the device, we analyse the different part of the experimental results; it allows predicting, in the future, a precise evaluation of the BOD value within a few hours.

## 1. Introduction

In the wastewater treatment plants (WWTP), one of the most important tests to determine the water quality is the parameter called Biological Oxygen Demand (BOD5): it is based on the evaluation of the oxygen ($O_2$) quantity used by bacteria to degrade the organic molecules present in a representative water sample [1] [2]. The normalization of this approach imposes duration of five days for measurement, by comparing the oxygen quantities consumed on this period [2]. The drawbacks of this approach are multiple: stationary apparatus; non differentiation between organic molecules, too long measurement duration, etc… If we keep the same principle based on the use of bacteria as an indirect transducer, there are needs for the development of micro-devices, capable to give more precise indications about the water quality within a reasonable time. To that purpose, by associating microsystems technology and biological techniques, we have initiated a new approach based on an instrumented microfluidic microsystem integrating five separated compartments and used as micro-laboratories [3]. Hence, the monitoring of the oxygen consumption of different and selected bacteria immobilized in adjacent micro-wells should give complementary information about the load in organic matter of the water sample. In order to realize quick prototyping, we have designed and implemented PDMS-Glass chips. To overcome some unexpected aspects of the manipulation of 'in vivo' sensors, i.e. bacteria, we have limited our experiments to a unique type of bacteria, *E.coli*. As perspective, we planned to use other specialized bacteria.

## II – BOD principles and microsystem chip

The aerobic biodegradation consists of oxidising organic matter by biological processes. The biochemical reaction, summarized in equation (1), needs the presence of microorganisms [4] in the water sample to transform the carbonaceous matter C, through the dissolved oxygen $O_2$, in a biomass and molecules $CO_2$ and $H_2O$:

$$X_i + O_2 + C \longrightarrow X_f + CO_2 + H_2O \qquad (1)$$

Where $X_i$ and $X_f$ are the initial and final population of bacteria.

Examination of this equation shows that, for assessing the parameter BOD, it is possible to use either measurements of the concentrations of $O_2$ consumed by bacteria or that of the $CO_2$ produced by the biodegradation reaction. The first one is the most used by the water operators: it has the advantage to be direct.
The BOD value is obtained with more or less accuracy, depending on the sophistication of the methods and equipment used [5]. The reference BOD5 (ISO 5815) is hence calculated through two values of $O_2$ concentration: one, at t=0, and the other after 5 days. Many research approaches have been developed either to measure the BOD5 parameter or to predict it, using different transduction methods to convert the concentration of oxygen towards an electrical or optical signal [2] [5] [6]. None of them is entirely satisfactory because they need sophisticated equipment, long time analysis, or are affected by important measurements uncertainties [2] [7]. In addition of the technological aspect, it appears that two biological strategies are opposed. The first one, based on microbial population (recommended by the reference ISO 5815), has the advantage to provide a pertinent information, but time and space varying. On the opposite, the second one uses only one strain to assess the BOD parameter. In this case, the information lacks of representativeness but is very reproducible. To overcome the limitations of these two strategies, an approach based on the use of a set of known and controlled strains seems to be a relevant alternative.

By introducing the microsystem technology and by adapting it to the micro biology field, we have developed an original approach to obtain a micro-device capable to give a new dimension to the measurement of pollutants through the use of bacteria [8]. By adopting a multi sensing approach, our principal objective has been to develop an experimental protocol to follow-up the $O_2$ quantity present in each of the some instrumented micro-wells. These measurements could be carried out in a reasonable period of time, allowing afterwards assessing the load of organic pollutants in analyzed samples.
Based on a PDMS/Glass technology [8], we have designed and implemented chips having five wells to test different bacteria strains or, for a given bacteria, different nutrients concentrations [3]. Each of these micro chambers is equipped with optical sensor ‘optode’ whose fluorescence intensity changes with the quantity of oxygen present in the water sample [3].
Figure 1 shows the schematic representation and the photography of a chip realized in our laboratory. The device used for experiments contains five wells, for an individual volume capacity of ~20 µL, and is provided for receiving one bacteria type or a predefined pollutant. Knowing that the PDMS is partially permeable to gas [3], we have coated the walls of the micro-chambers with a SU8 polymer film.

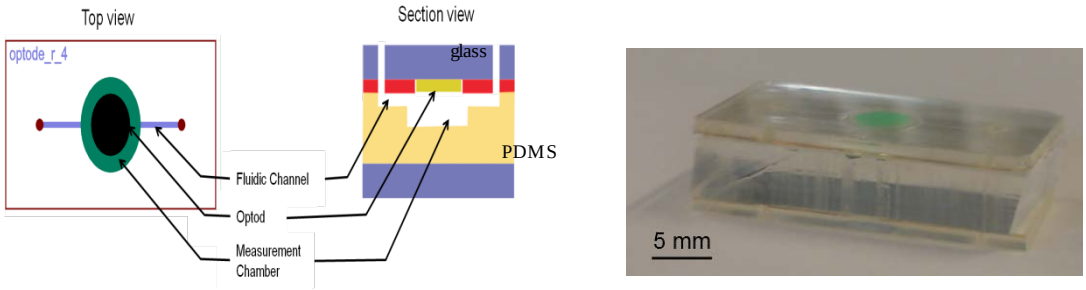


Figure 1: Schematic representation of a one-well instrumented microfluidic device and the photography of a chip ready to use. Before sealing the micro chambers, the inner walls are coated with a SU8 polymer film.

## III. Measurements

The experimental protocol can be summarized as follows: preparation of lyophilized *E.coli* bacteria; injection of solutions containing living bacteria and standardized nutrients *Luria-Bertani* (LB buffer) at different concentrations in the micro-chambers maintained at 30°C. The measurement of bacteria activity is performed with optodes electrically polarized and activated every five minutes, for total measurements duration of 10 hours. Our first tests have been performed to compare the results obtained with our device (‘micro’) with those obtained by a classical process conducted by biologists (‘macro’) with their specific material and apparatus. Other experiments have been done to study the effects of different nutrients concentration on a fixed bacteria population.

### a.Macro-micro comparison:

The dissolved oxygen are measured simultaneously in the two types of device (macro and micro) for a fixed bacteria and nutrients concentration (9.2 $10^7$ cells per mL in LB buffer). The results reported in Figure 2 show that there are two remarkable parts, each of them linked to many aspects of the protocol used in the experiments.

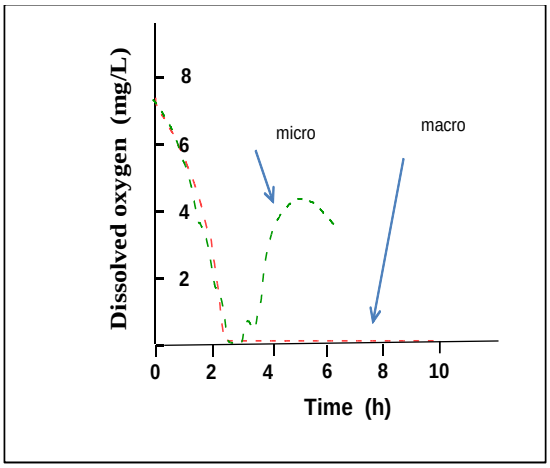


Figure 2: Experimental curves representing the consumption of oxygen by 9.2 $10^7$ cells per mL, in LB buffer, respectively in a ‘ macro’ device and our microsystem chip.

When the time measurement is less than three hours, it can observed that there is quasi-perfect concordance between the curves ‘micro’ and ‘macro’; after that, we observe an important divergence

of the curves. Thus, we can give some confidence to experiments conducted with our micro-device, while seeking tout understand the source of the difference between the two curves in the last part.

**b. Experiments with microsystem device**

By using a test bench developed in our laboratory [5], we have measured the $O_2$ consumed by a population of ~$10^7$ cells par /mL for five dilutions of LB buffer (1, 1/10, 1/100, 1/300 and 1/500).
The experimental results for simultaneous measurements are reported in Figure 3.

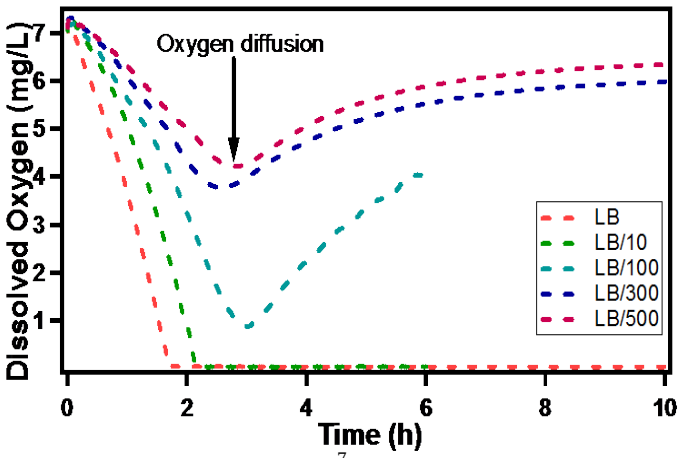


Figure 3: Oxygen consumption by E.Coli (~$10^7$cells per mL) present in a water sample for five dilution of LB buffer (5 concentrations of nutrients).

It can be observed that on one side, the quantity of oxygen consumed by bacteria is non-constant, indicating the temporal variability of their metabolism; on the other side, the variations of the $O_2$ concentration in the five chambers, follow a reproducible, but non-identic evolution.

## IV. Analysis and prospective

The global analysis of results reported on Figure 3 shows that there are two remarkable zones:

1) The first one shows a regular decrease of oxygen during the period 0 to 3 hours : the observed kinetics vary according to the carbonaceous concentration and present a non-linear rate variation ;
2) The second zone includes two specific cases:
   - Either, all nutrients were degraded by cells inducing a stop of the biological $O_2$ consumption. The $O_2$ concentration in the microchip increases due to a parasitic oxygen contamination (results obtained with LB/100, LB/300 and LB/500).
   - Or, the available dissolved $O_2$ concentration is insufficient to oxidize all organic matter. The measured concentration is null (results obtained with LB and LB/10). In this case, the parasitic oxygen allows feeding the biodegradation process by cells.

For a fixed bacteria concentration, the first period can constitute an important indicator to assess the evolution of the BOD: it shows that the bacteria adapt their temporal oxygen consumption to the richness of their living environment. Hence, the tangent attached to these curves will be used as a powerful tool to evaluate the quantity/quality of pollutants present in a water sample.

By examining the topology and the physical properties of the device, we have identified two possible sources of this parasitic oxygen:

- Air imprisoned at the top of the micro wells: when the wells are not entirely filled, their effect is negligible because the $O_2$ quantity is very small and not renewable;
- PDMS properties: knowing that this material is partially permeable to the oxygen gas, we have estimated that the $O_2$ quantities flowing towards the protected walls of the micro chambers imbalance those present in the sample.

This rapid prototyping with PDMS/glass chips confirm, at least for the first hours of the measurements, that it is possible to follow correctly the $O_2$ consumption by bacteria. In the second period, although the cells activity can be differentiated according to their alimentation, the influence of external source of $O_2$ can disturb the results.
For very low nutrients quantity, it can be necessary to extend the measurement duration beyond six hours. For such long time, it becomes necessary to be sure that the chamber has a total tightness. To that purpose, it can be suggested to increase the SU8 coating of the wells; or to adapt the PDMS chips to a resazurin protocol; or to use other microsystem material like SU8 or DF.

## IV. Conclusion:

To reduce the BOD time measurements in wastewater, we have introduced a microsystem approach by associating bacteria and micro-devices. By using a PDMS-glass technology, we have demonstrated that this miniaturization is suitable, and it opens promising perspectives to enhance many of the indicators of the water quality. By developing a poly-wells instrumented PDMS device, we have demonstrated that it is possible to conduct different measurements either to detect the presence of organic molecules at different concentrations.
By using a PDMS/glass technology, it has been demonstrated that the optical signal, thanks to optodes, allows determining some significant indicators of the water quality: concentration of organic molecules, rate and duration of their consumption etc…. We have conducted some manipulations and experiments which led to conclude that the microsystem approach is suitable to reduce the time measurements of BOD, at least for wastewater containing a lot of organic molecules. This capability responds fully to the multisensorial approach (several microbial strains) of the overall concept of BOD5 measurement in which this study is integrated.

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