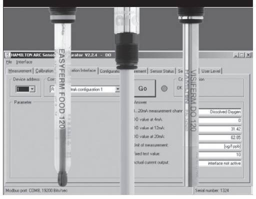
VISIFERM DO ARC and POLILYTE PLUS ARC in fermentation

Application Note



Sensor application in a scale down simulator for studying the impact of industrial scale inhomogeneities on microbial cultures

Industry: Biotechnology

Application: Bioreactor cultivation

Hamilton products: VISIFERM DO ARC and POLILYTE PLUS ARC

The use of the VISIFERM DO ARC 120 and POLILYTE PLUS ARC 120 pH sensors from HAMILTON in a plug flow reactor (PFR) is described. The PFR is connected to a common stirred tank bioreactor (STR). With this combination, the impact of inhomogeneities on *Bacillus subtilis* cultivations, which occur in industrial large-scale processes, were observed. To achieve conditions that mimic those in large scale, the determination of the hydrodynamic behavior by determining the residence time distribution is of basic interest. Therefore, a reliable measurement of the pH along the PFR is necessary. During cultivation, the distribution of oxygen and pH along the PFR allows an exact description of the process conditions and enables the user to adopt them to large scale conditions.

A **Plug Flow Reactor** (PFR), also called **Continuous Tubular Reactor** (CTR), is a continuous flowing reaction system. It can be perceived as a tube in which the reaction medium flows in distinct packets (plugs) of varying composition in an axial direction.

PFRs are typically used for continuous production.

Sensor use at the PFR module

The PFR is characterized by high inhomogeneities in the axial direction with respect to oxygen concentration. Since oxygen limitation in industrial fed-batch processes occurs near the feeding zone, the feed is introduced at the entrance of the PFR.

Unlike the first lab-scale two-compartment reactors, the system of this study is equipped with five pH and optical dissolved oxygen (DO) sensors along the height of the PFR (Fig.1). Static mixers included in the module are used for enhanced oxygen transfer and mixing, while the plug flow characteristics are maintained. Sampling is possible between each static mixer module (at the same heights where pH and DO are measured – Fig.2).

Calibration of pH electrodes was performed applying the software package ARC Sensor Configurator V2.2 in calibration solutions of pH 7.00 and 4.01; DO sensors were calibrated in controlled N_2 sparged water for 15 minutes (zero-level) and air sparged water (100 % level). After utilization, accuracy of calibration was proven by sparging water with a gas mixture of 5 % O_2 and a calibration in air. Small deviations between the responses among DO sensors was normalized to one electrode, enabling a more reliable comparison between all DO sensor signals at the

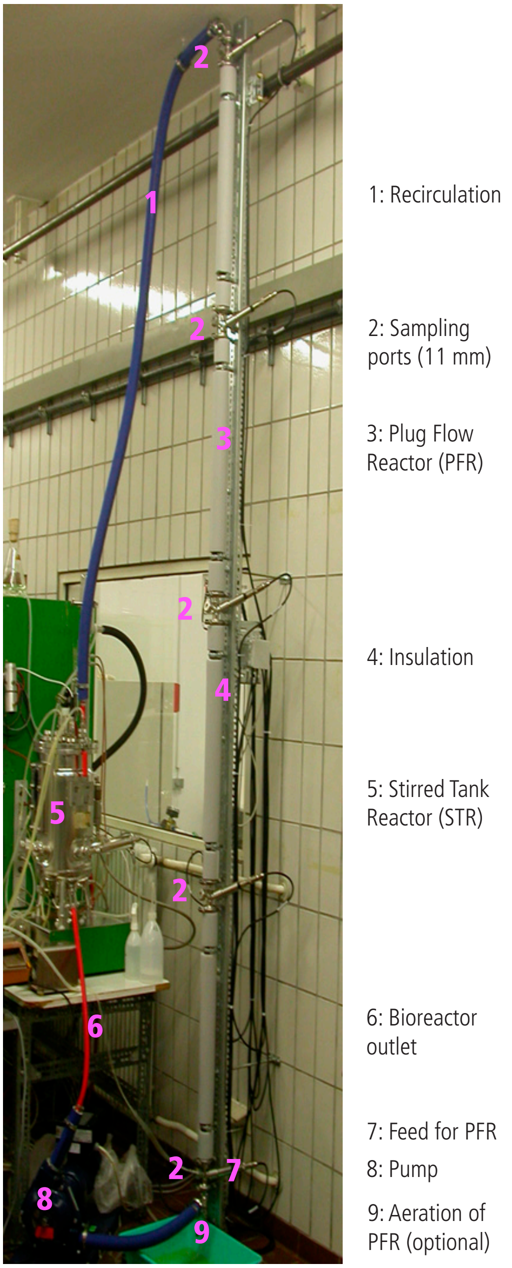


Figure 1: *Scheme of the combined STR-PFR reactor*

PFR at very low values close to zero. The sensors in the PFR were sterilized with steam for 40 min at 110 °C prior to and after each experiment. A mineral salt medium was applied for cultivation. The sensor data was transmitted via a USB Modbus Converter RS 485 to a LabVIEW™ v. 8.2 environment.

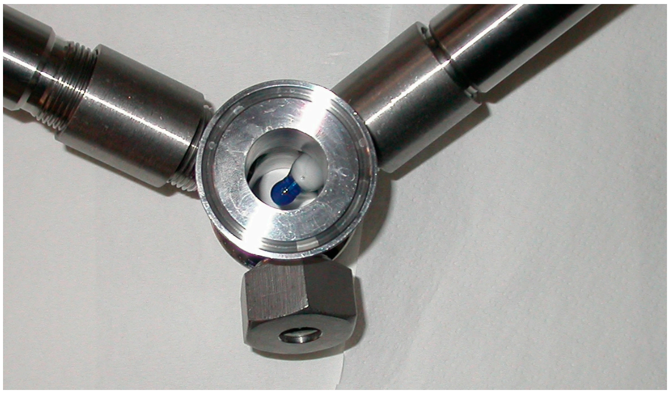


Figure 2: *DO sensor (left), pH sensor (right) and sample port (in front) mounted between static mixer modules of the PFR*

Application of VISIFERM DO and POLILYTE PLUS sensors in cultivations

In the above system, the metabolic response of non-sporulating *Bacillus subtilis* mutant strains to oscillating substrate and oxygen availability were successfully examined.

With the help of the DO sensors, the dissolved oxygen concentration was excellently monitored along the PFR. When air was supplied at the bottom, the dissolved oxygen concentration was higher at the second monitoring port (DO sensor 2) than at the first one (DO sensor 1) due to gas dispersion (fig.3).

When no air was supplied at the bottom of the PFR, a weak signal was observed at the first electrode (DO sensor 1) (fig.3). Based on this sensor, the oxygen content in the STR was successfully adjusted so that not all oxygen in the liquid phase was consumed by the bacteria on the way from the STR to the PFR. No oxygen could be detected at the two top sample ports in the PFR (DO sensor 4 and 5). If the air supply relied only on the oxygen that was dissolved in the STR, a gradient of aerobic to anaerobic conditions in the PFR was observed, indicated by O_2 depletion in the top phase.

The pH in the PFR and STR was monitored as well (fig.4). In this case, the pH measurement along the PFR module did not indicate acid release as confirmed by HPLC analysis. pH monitoring is even more useful at cultivations, where acids are formed in the PFR module (e.g. *Escherichia coli* cultivations).

The application of the sensors was successful, fulfilled the customer's needs and showed an excellent measurement performance throughout all experiments.

User benefits of ARC sensors:

- Stable and reliable measurement of DO and pH gradients in a plug flow reactor
- Fast calibration
- Easy integration into LabVIEW™ environment
- Excellent sensor robustness against sterilization procedure

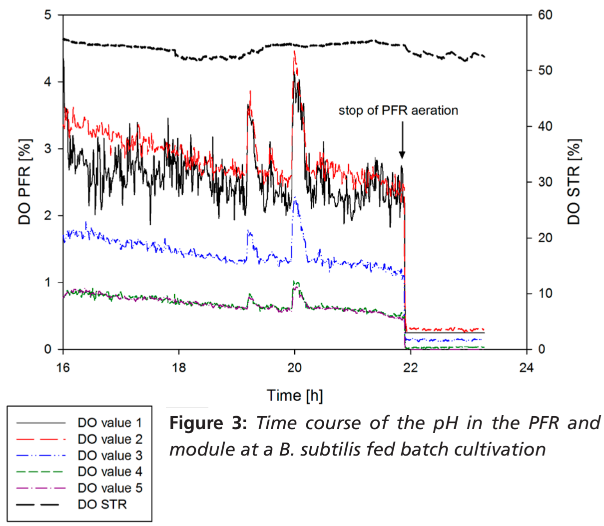


Figure 3: *Time course of the pH in the PFR and STR module at a B. subtilis fed batch cultivation*

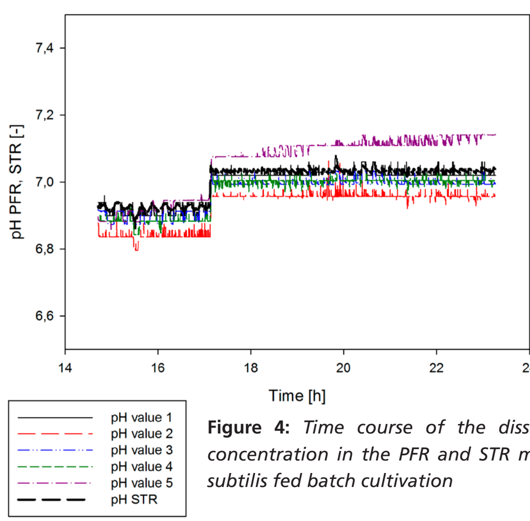


Figure 4: *Time course of the dissolved oxygen concentration in the PFR and STR module at a B. subtilis fed batch cultivation*

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