10th International IFAC Symposium on
Computer Applications in Biotechnology
Preprints Vol.1, June 4-6, 2007, Cancún, Mexico

DEVELOPMENT OF A NEW PROBE FOR IN-SITU OXYGEN UPTAKE RATE (OUR) MEASUREMENT IN MAMMALIAN CELL CULTURE PROCESSES

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Abstract: We have developed a new probe that measures the oxygen uptake rate (OUR) of mammalian cells, an important indicator for the metabolic state, inside the reactor (in-situ) and in real-time without the need for sampling or complicated analytics. The probe isolates a known volume of cell culture from the bulk inside the bioreactor, monitors the oxygen consumption over time and releases it again. The sample is mixed during the measurement with a new agitation system to keep the cells in suspension and prevent oxygen concentration gradients. The OUR measurement system also doubles as a standard DO probe for process monitoring when it is not performing OUR measurements. This new probe was successfully tested in BHK perfusion cell cultures.

Keywords: Automatic Process Control, Biomedical Control, Fermentation processes, Biotechnology, Sensor systems, Sensor integration

1. INTRODUCTION

Low specific growth rates as well as usually low cell and product concentrations often characterize cell culture processes. Therefore, it is necessary to maintain optimal growth conditions during cultivation, requiring sophisticated control systems. The measurement of metabolic states and fluxes is a good tool to ensure optimal culture conditions and the oxygen uptake rate (OUR) is a good indicator for metabolic rates. It has also been used successfully to design feeding and process scale up strategies. Robust oxygen uptake rate measurements are particularly important for processes with high oxygen transfer requirements such as high cell density perfusion systems given the low solubility of oxygen.

2. SYSTEM DEVELOPMENT

2.1 Established Methods

There are several commonly used methods to measure the oxygen uptake rate.

Global (Gas) Mass Balance: The use of mass flow controllers is common in cell culture, so the composition and flow rate of the in-going gas is known. The oxygen content of the off-gas is measured to close the balance (Ruffieux et al., 1998). The high accuracy of this measurement needed to get useful results usually requires a Mass Spectrometer. However, these are complex and sensitive to moisture, so elaborate drying systems need to be installed, or measurements can only be performed for short periods at a time.
Additional complexity is added in a perfusion system by in- (medium, base) and outgoing (Harvest, cell purge) fluid streams also supplying and/or removing oxygen, although these streams can typically be neglected in high cell density cultures due to the low solubility of oxygen.

Dynamic method: Another common method of OUR measurement is to monitor the oxygen consumption over time after stopping all gas supplies to the reactor. This is also called the dynamic method (Singh, 1996; Kyung 1994). However, performing this measure-ment in an actual (production) bioreactor will create a significant and undesirable perturbation of the biological system.

Analysis of an offline sample: The usual procedure is to transfer a sample from the reactor to an external vessel equipped with a monitoring and control system and to record the decrease in dissolved oxygen (DO) (Fig. 1a). An example for a commercial system is the YSI 5300 A (YSI, Yellow Springs, USA). The conditions (pH, Temperature, DO, Agitation) in reactor and external vessel have to be identical to obtain accurate results.

Bypass reference probe: An additional DO probe is installed in a bypass and the oxygen consumption is

calculated based on the residence time of the cells in the bypass before reaching the 2nd probe and the DO difference between bypass and reactor probe (Yoon and Konstantinov 1994).

2.2 Measurement Integration Concept

Duplicating the conditions of the main vessel in an external measuring vessel is time consuming and can be quite challenging. Therefore, performing the OUR measurement directly in the reactor without system perturbation is very desirable. Figure 1 shows the concept of this integration.

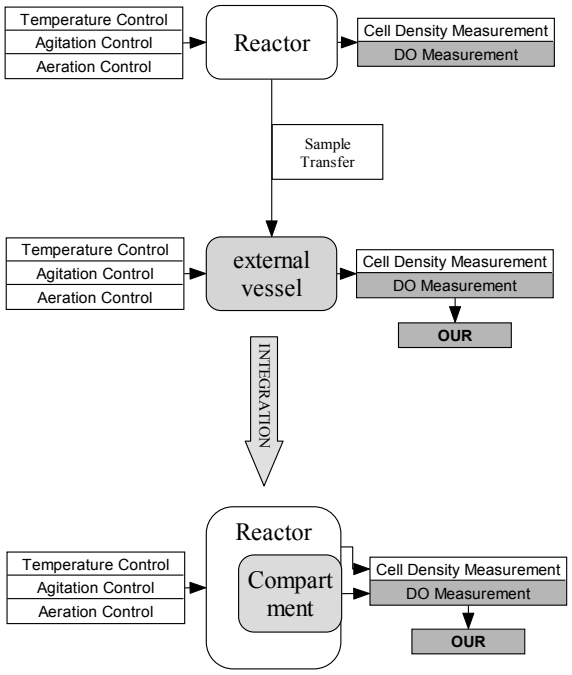

Fig. 1. Concept of the measurement integration

An ideal OUR measurement system would also function as a standard DO probe during the culture and double as an OUR probe in predetermined intervals without the need for sampling or complicated analytics. This is especially important for long-term processes as the number of probes that can be installed in a bioreactor is limited and redundant probes are frequently used.

2.3 From Concept to Prototype

We have developed such a in-situ oxygen uptake rate (iOUR) probe that performs this in-situ measurement by forming a measurement compartment around the dissolved oxygen (DO) probe that allows temporary separation of a sample from the bulk in the reactor. The principle of operation is shown in Figure 2. This sample will be isolated under the known bioreactor conditions eliminating the need for all the additional controls shown in Figure 1. Agitation is the only function that the probe has to take over from the reactor, as the cells in the sample will start to settle after isolation from the bulk. This problem was solved by using the DO probe as an agitator to keep the cells in suspension and prevent oxygen concentration gradients from forming.

The temporary compartment mentioned above is formed by a divider tube moving back and forth on a stepper motor driven linear stage [Fig.2 and Fig.3 A]. The DO probe is mounted off-center in a carrier tube. Rotation of that carrier results in an oscillating movement of the DO probe tip in the sample chamber that mixes the sample very effectively [Fig 3 B]. With the divider retracted, the system measures the dissolved oxygen concentration in the bulk, therefore acting as a regular DO probe.

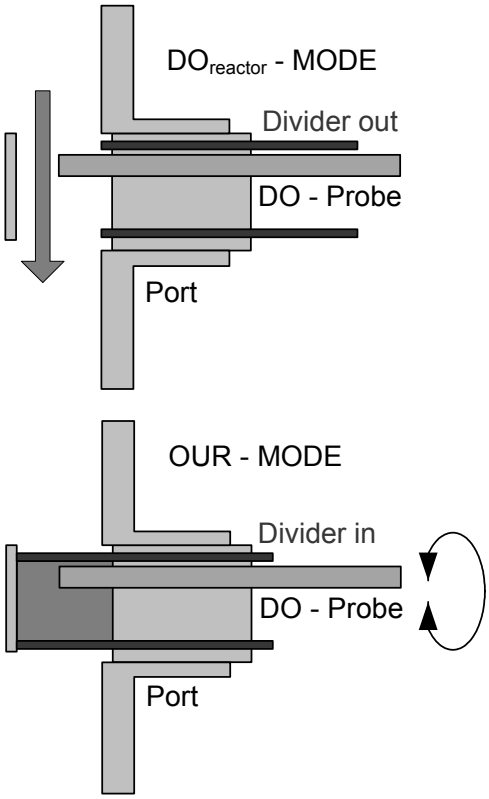

Fig. 2. Operating principle of the iOUR probe

The system uses a fluorescence-based optical dissolved oxygen probe [Fibox 3, Presens GmbH, Heidelberg, Germany]. These optical probes have some advantages over the conventional clark-type probes since they consume no oxygen during the measurement, are independent form the fluid flow velocity and have usually have a very good long-term stability. They are also easier to miniaturize, a requirement for this application.

Data acquisition and probe operation are performed by two dedicated controllers (TIMS 0201, JoVa Solutions, San Francisco, USA) and a program written in Labview (National Instruments, Austin, USA).

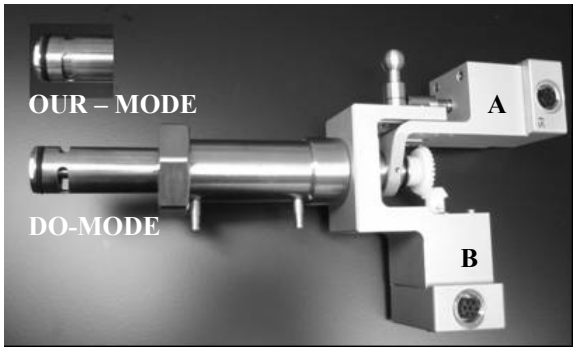

Fig. 3. The iOUR probe prototype

The control unit of the probe that operates the agitation and divider movement can be replaced by a steel cap for autoclaving or steam-in-place SIP sterilization (Fig. 4).

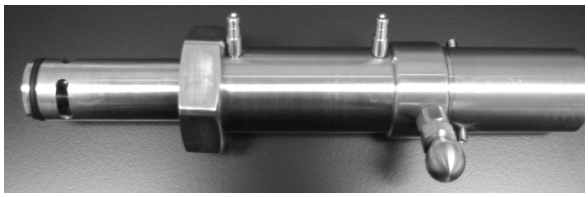

Fig. 4. The iOUR probe prepared for SIP or autoclaving

The system was designed for long-term perfusion processes that typically run for 3 months or more. A retraction system, based on the industry standard Intrac, was incorporated to allow replacement of the oxygen sensing patches and system cleaning without process interruption. Figure 5 shows the retracted probe. The system is reinserted into the reactor after servicing and steam sterilization.

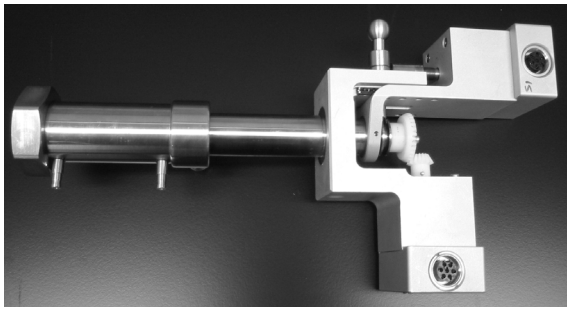

Fig. 5. The iOUR probe retracted for servicing

3. EXPERIMENTAL RESULTS

3.1 Cell Culture System

BHK cells were cultivated in a perfusion system as shown in Figure 6 in a protein free medium formulation. Experiments were conducted in 15-L bioreactors (Applikon, Foster City, USA) with a 10-L working volume. The temperature was maintained at 35.5 °C. The dissolved oxygen (DO) concentration was maintained at 50% air saturation by silicone tubing aeration with a mixture of oxygen and nitrogen. The pH was maintained at 6.8 by the automatic addition of 0.3 M $NaHCO_3$. The Experiments were performed at cell densities between $3x10^6$ and $20x10^6$ cells/mL and varying perfusion rates.

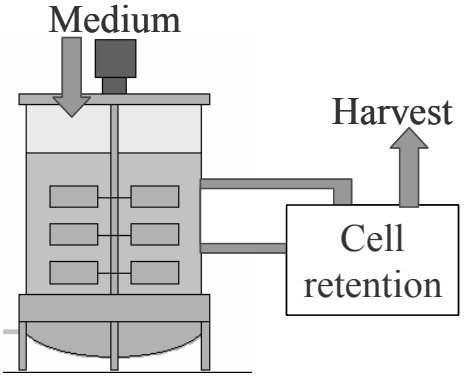

Fig. 6. Simple schematics of a perfusion system

3.2 Analytical Methods

The cell density was measured using the CEDEX system (Innovatis GmbH, Bielefeld, Germany). The accuracy of the online pH and DO concentrations was verified through off-line analysis with a blood gas analyzer (Rapidlab 248, Bayer Healthcare, USA).

3.3 insitu-OUR Measurements

All experiments were performed during a 4 week period of a perfusion process. Table 1 summarizes the results of the OUR measurements performed during a typical perfusion cultivation during which several cell specific perfusion rates (CSPR) were tested.

Table 1: Calculated OURs on 13 days during the test period and the relative error for each measurement

Cell Density 1x10e6 cells/mL	CSPR condition	OUR pmol/cell*d	Error [%]	Datasets
3.26	B	2.7	6.7	2
9.55	C	2.47	1.6	4
10.3	A	3.14	4.7	4
11.17	A	2.74	4.7	4
12.13	A	2.81	2.5	2
13.46	A	2.51	10.8	3
13.58	A	2.45	6.5	4
13.76	A	3.9	4.6	3
15.26	B	2.17	0.0	2
15.5	B	2	9.5	4
17.11	C	2.64	14.4	3
17.77	B	2.14	3.0	5
20.2	A	2.28	3.5	2

Figure 7 shows four consecutive oxygen consumption curves measured during the perfusion process as an example for the reproducibility of the measurement. Cell density and cell specific perfusion rate (CSPR) were kept constant.

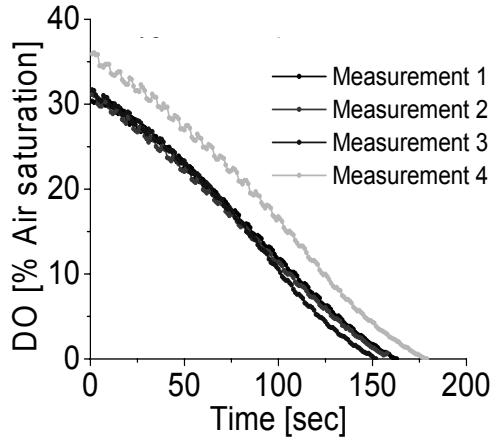

Fig. 7. Reproducibility of the OUR measurements.

Table 1 also shows the relative error for each measurement. The average relative error of all measurement is 5.5 %. The OUR was also estimated by the global mass balancing (GMB) method for one of the experiments at 15.5x106 cells/mL. The GMB Method calculated the OUR as 2.2 pmol/cell·d compared to 2.0 pmol/cell·d measured by the in-situ OUR probe. Both methods show good comparability.

4. SUMMARY

We developed and successfully tested a new system for the in-situ measurement of oxygen uptake rates. It showed good reproducibility, long-term stability and compares well to other OUR estimation methods. In addition, the system functions as a normal DO probe when not used for OUR estimation. It can therefore be implemented as a functional enhancement of an existing DO measurement sensor.

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