SCIENTIFIC BIOPROCESSING

eBook

Dissolved Oxygen (DO) Monitoring

In Microbial Bioprocessing

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What Is Dissolved Oxygen (DO)?

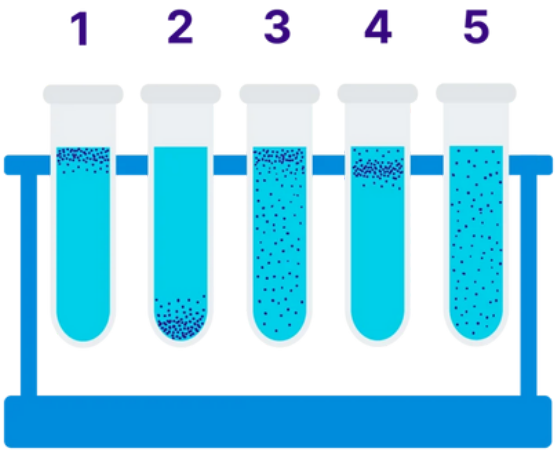
Dissolved Oxygen (DO) refers to the level of free, non-compound oxygen ions present in an aqueous solution.

In bioprocessing, the presence of dissolved oxygen is vital for the growth and metabolism of aerobic microorganisms, as it serves as a crucial substrate for their energy production. DO is typically measured and reported as

- milligrams per liter [mg-DO/L of water (mg/L)],
- percent saturation (%), or
- partial pressure (pO2).

Classification of Microorganisms

While there are many variables that are important to cellular growth, oxygen is one of the most Critical Process Parameters (CPPs) in bioprocessing. Microorganisms can be divided into three main groups based on their oxygen requirements:



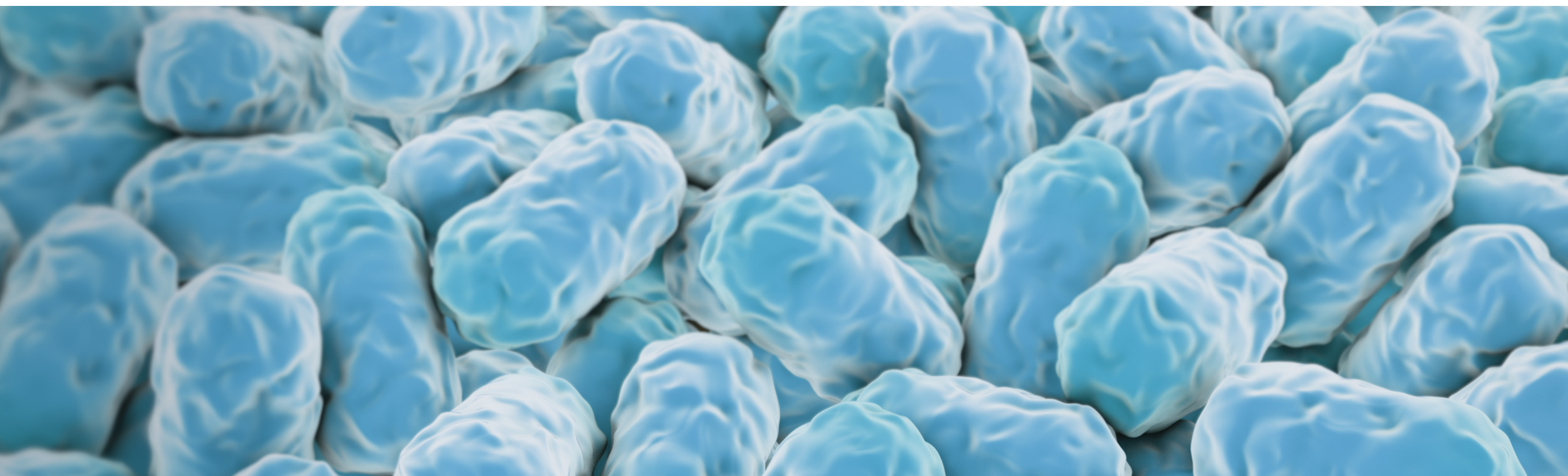
1. **Obligate Aerobes**
   Can produce energy only via aerobic respiration and require molecular oxygen to live.

2. **Obligate Anaerobes**
   Cannot tolerate any molecular oxygen and they require an oxygen-free environment to grow.

3. **Facultative Anaerobes**
   Can grow in both environments – in the presence of molecular oxygen and in its absence. Within the group of facultative anaerobes, two subgroups can be defined.

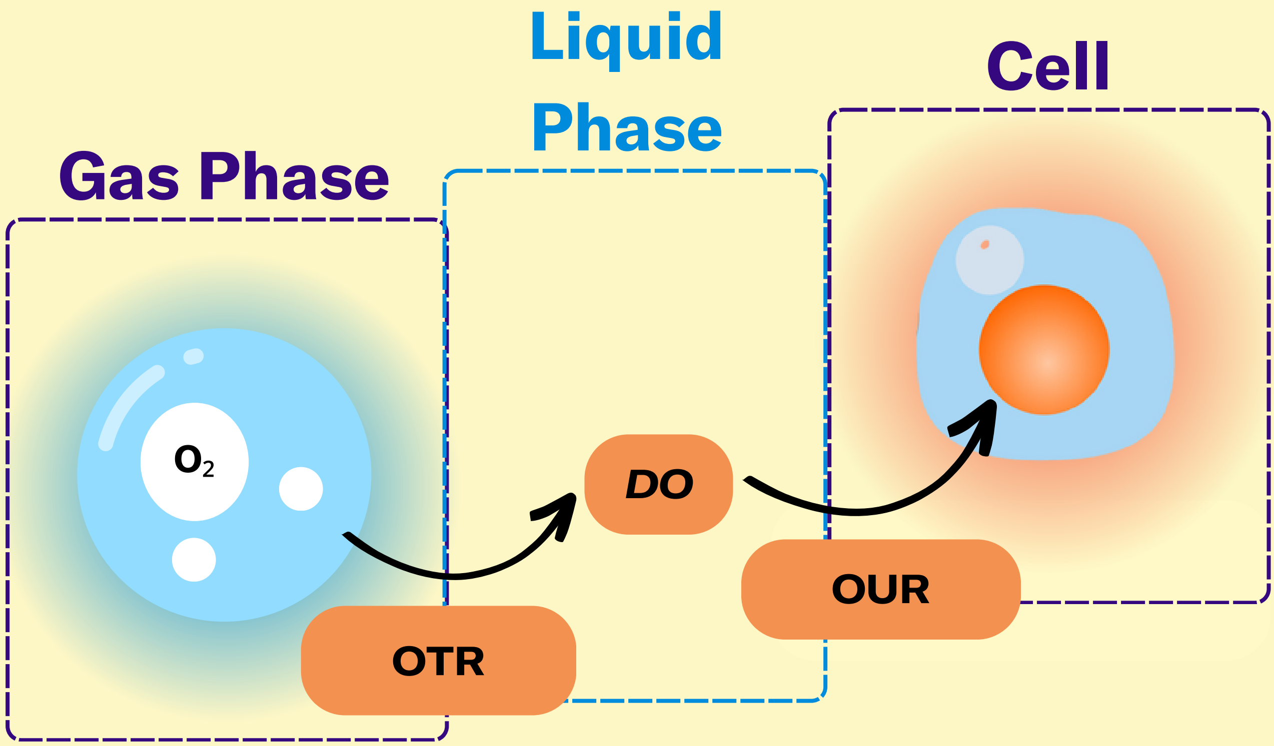
   4. **Aerotolerant Organisms**
      Can grow in the presence of molecular oxygen, but they cannot use it. They would always produce their energy via anaerobic fermentation.

   5. **Facultative Anaerobic Organisms**
      Use oxygen for energy production if present and switch to the anaerobic fermentation when oxygen is depleted.



The Basics of DO

There are several key terms used to describe the points at which oxygen transfer occurs in bioprocessing.



Oxygen Transfer Rate (OTR) refers to how fast oxygen molecules can move from a gas bubble to the liquid media. To do so, O_2 molecules must diffuse through a stagnant region called the gas-liquid interface. The OTR is influenced by the volumetric mass transfer coefficient (kLa).

The amount of oxygen currently present in the liquid medium is represented by ***DO.***

Oxygen Uptake Rate (OUR) is used to describe how fast oxygen, now present in the media, can overcome the liquid-cell interface to be available for use by the cell or aggregate.

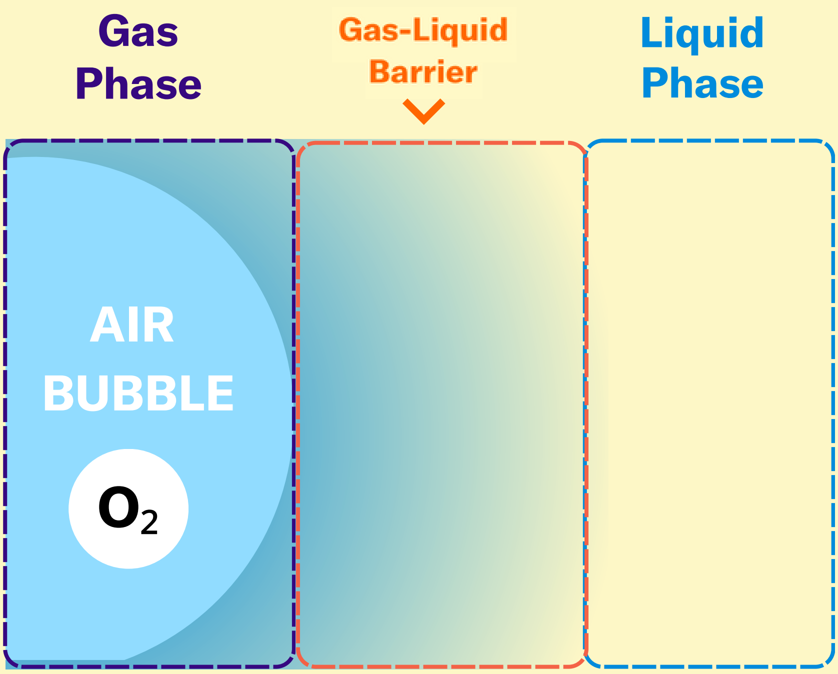
Oxygen Transfer Rate (OTR)

Efficient oxygen transfer is essential for a successful aerobic cultivation. Regardless of vessel type or application, determining the volumetric oxygen transfer coefficient (kLa) and the oxygen transfer rate (OTR) can offer some important benefits, including:

- Ensuring healthy microbial colonies
- Increasing product yields
- Reducing waste from suboptimal oxygen concentrations

What is kLa?

Consider a gas bubble containing oxygen in liquid. The volumetric mass transfer coefficient (kLa) refers to the pace at which oxygen can move between the gas phase to the liquid phase.



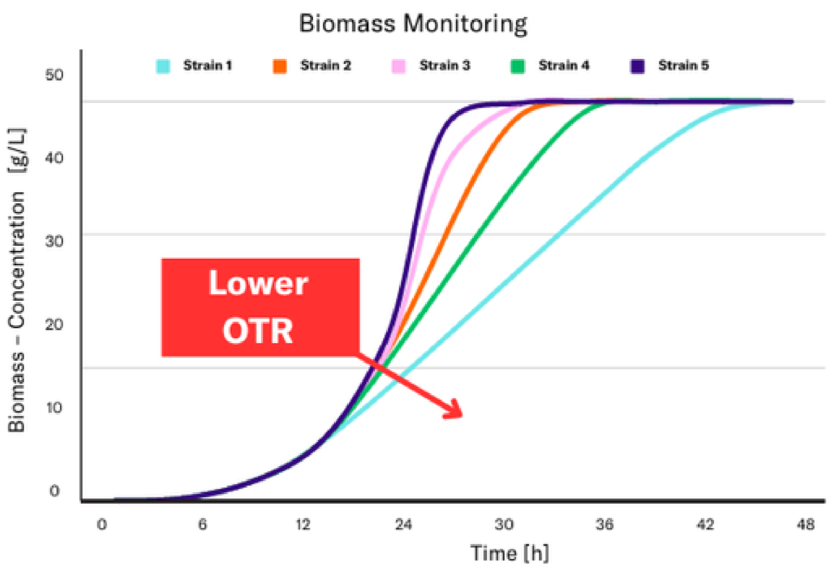
kLa

kL = mass transfer coefficient, describes the rate of molecular diffusion through the gas-liquid interface
a = the surface area available for diffusion

In most bioprocesses the most important step is the OTR, hence engineers and scientists try to affect it by changing their kLa.

The Pitfalls of Oxygen Limitations

Oxygen limitations occur when the amount of available molecular oxygen falls below a certain threshold. Without close monitoring, shake flask cultures can often fall victim to oxygen limitations. While you cannot visually identify oxygen limitations, it can have severe detrimental effects on your fermentation process. The best options to identify oxygen limitations in cultivations include biomass and dissolved oxygen monitoring.

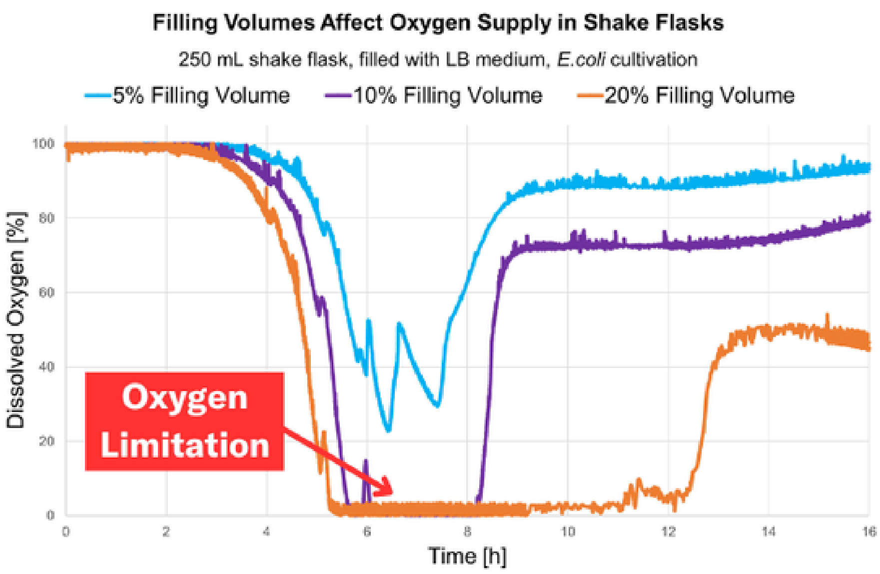


Biomass Monitoring

Lack of oxygen results in slowed growth of microorganisms, often associated with a lack of an exponential phase. While a linear curve does not correlate solely to oxygen limitation, secondary parameters can assist in determining the root cause.

DO Monitoring

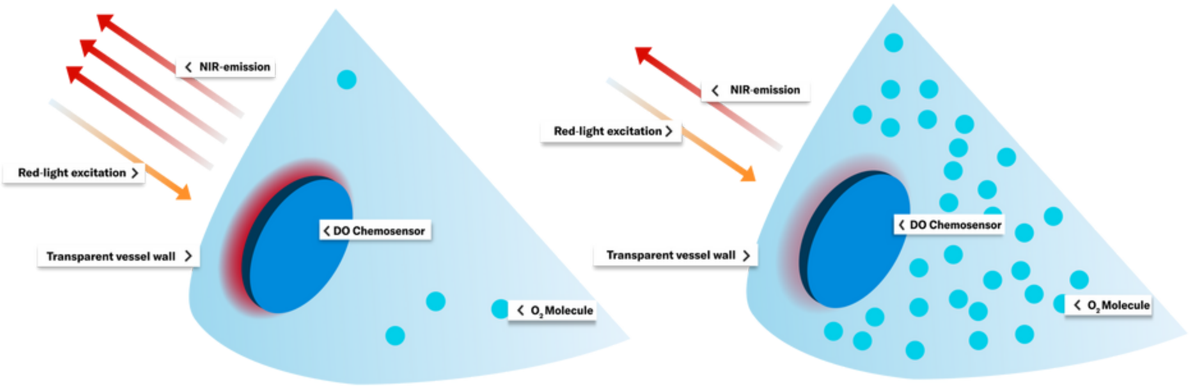
Oxygen consumption is a complex process and oxygen availability is influenced by many factors, e.g., by the filling volume of the shake flasks. Monitoring DO directly provides valuable insights into your bioprocess.



Principles of Measurement

CHEMOSENSORS

A chemosensor containing an oxygen-sensitive luminescent dye indicator is embedded in a matrix. A sensor emits light at one wavelength, exciting the chemosensors which show luminescence in another wavelength. Depending on the concentration of oxygen present in the solution, the amount of luminescence changes. The sensor measures this phase shift which is then calculated to represent a DO value.



LOW OXYGEN

HIGH OXYGEN

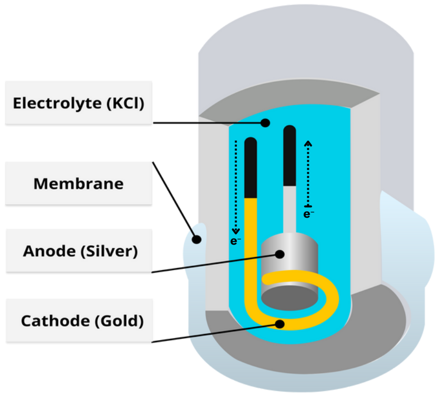
(+) Does not consume oxygen
(+) Little to no maintenance
(+) Can be stored dry
(+) On-line & in-line options
(+) Can be automated (measurement and data handling)
(+) No warm up period needed

(-) Sufficient flow is needed for accurate measurements
(-) Running costs for consumables (OPEX)
(-) Not compatible with all setups

Principles of Measurement

PROBES

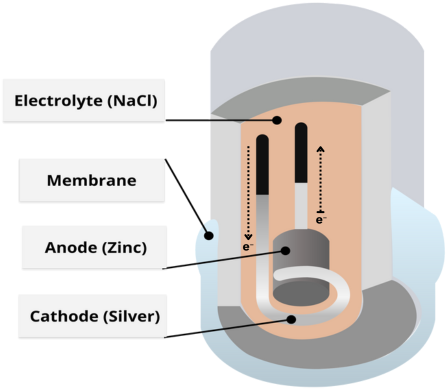
Probes consist of a cathode and anode, present in an electrolyte-containing chamber with a gas permeable membrane. A voltage is applied between the electrodes (cathode and anode). As oxygen diffuses across the membrane, it undergoes a reduction reaction at the cathode, where it is given an electron, while the anode undergoes an oxidation reaction, releasing electrons into the solution. The electron flow creates a current that is used to determine the dissolved oxygen concentration.



POLAROGRAPHIC

(+) Well-known technology
(+) Less frequent maintenance (vs galvanic)
(+) On-line & in-line options
(+) Can be automated (measurement and data handling)

(-) Requires a warm-up period
(-) Consumes oxygen
(-) Media must be in motion
(-) Requires frequent calibration



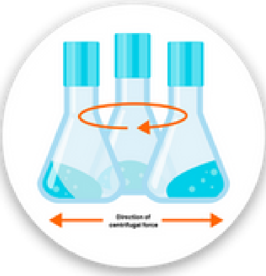
GALVANIC

(+) No warm-up period needed
(+) Very stable at low DO levels
(+) Fast response time
(+) On-line & in-line options
(+) Can be automated (measurement and data handling)

(-) Requires regular maintenance
(-) Media must be in motion
(-) Requires frequent calibration

Factors Affecting OTR in Shake Flasks

In shake flasks, oxygen transfer from the headspace to the cells is not driven by bubbles but by the thickness of the liquid film.



SHAKING SPEED

OTR increases with shaking speed.

FILLING VOLUME

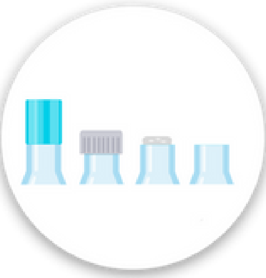
Lower filling volumes result in higher OTR.

MEDIA VISCOSITY

Higher viscosities will have lower OTR.

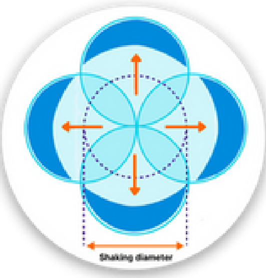
BAFFLED VS NON-BAFFLED

Baffles can be used to increase aeration.



CLOSURE TYPES

Some closure types allow for more oxygen in the vessel than others.



SHAKING DIAMETER

A larger shaking diameter can increase the mixing and aeration of the liquid.

Baffled Flasks

Baffled flasks are designed with indentations, or baffles, along the inner surface, aiming to improve oxygen transfer rates (OTR) within the liquid phase. Baffled flasks facilitate higher oxygen transfer rates, ensuring microbes receive an ample supply for growth and production.

ADVANTAGES

- Baffles create a more favorable surface/volume ratio, optimizing the contact between the liquid phase and ambient air for efficient oxygen uptake.
- Baffles aid in preventing stratification and promote better mixing, reducing the likelihood of oxygen-deprived zones within the flask.
- Can be advantageous for disrupting unwanted cell agglomerations.

DISADVANTAGES

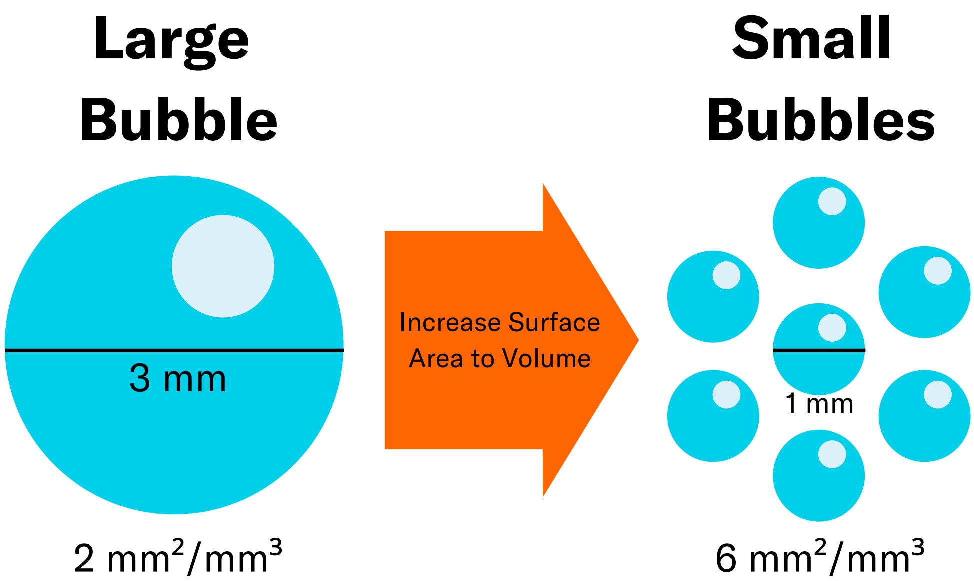
- Achieving consistent results across multiple experiments can be challenging due to variations in flask design, baffling patterns, and manufacturing processes.
- Increased turbulence may lead to increased foam formation, necessitating additional measures to control foam and prevent potential cell damage.
- Mixing in baffled flasks may introduce shear stress, affecting the integrity of delicate microbial cells and potentially impacting their performance.
- Baffled flasks present difficulties in applying mathematical models due to their complex design, making it challenging to predict and control oxygen transfer accurately.
- Can increase the likelihood of a culture becoming "out of phase".

The “Out of Phase” Phenomenon

Under normal operating conditions, the liquid in shaken flasks rotates “in phase” with the movement of the shaker table. Under certain conditions, the liquid inside the flask can become “out of phase”, meaning that different regions of the liquid move independently of each other. This phenomenon can result in uneven distribution of oxygen and nutrients to the microorganisms, impacting their growth and productivity. Factors such as improper flask size, shaking speed, or viscosity of the culture medium can contribute to the out-of-phase behavior.

Factors Affecting OTR in Bioreactors

In bioreactors, the oxygen transfer rate is heavily influenced by process parameters as well as the physical capabilities of the equipment used.



BUBBLES

More, smaller bubbles have two advantages for oxygen transport compared with fewer, larger bubbles:

- Smaller bubbles increase the surface area of contact between water and oxygen molecule, resulting in increased diffusion of air into the media.
- Smaller bubbles spend more time in the medium, allowing more oxygen to be transferred into the liquid

PARTIAL PRESSURE

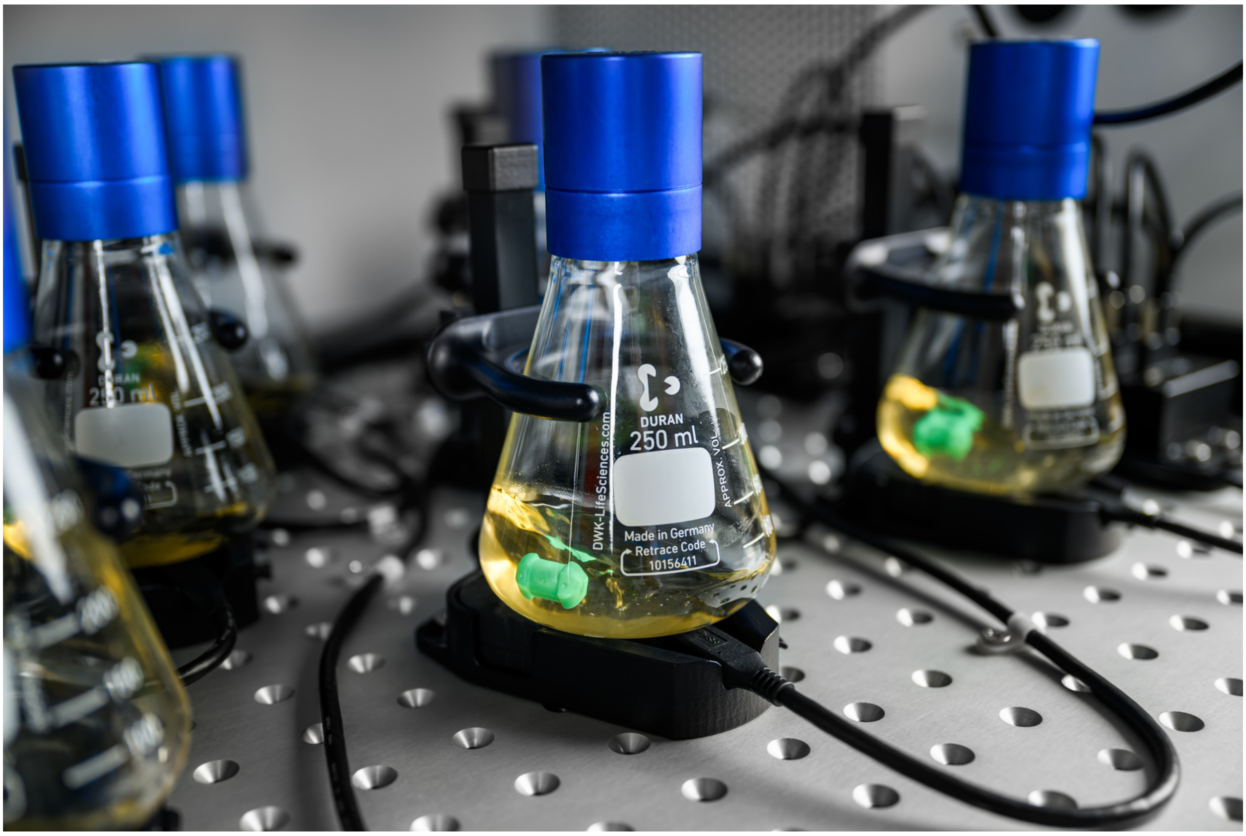
Higher pressure increases the rate of oxygen diffusion to the liquid medium.

OXYGEN LEVELS

Increasing the amount of oxygen in the gas mix increases OTR.

sbi DO Monitoring Solutions

For Shake Flasks: DO Sensor Pills



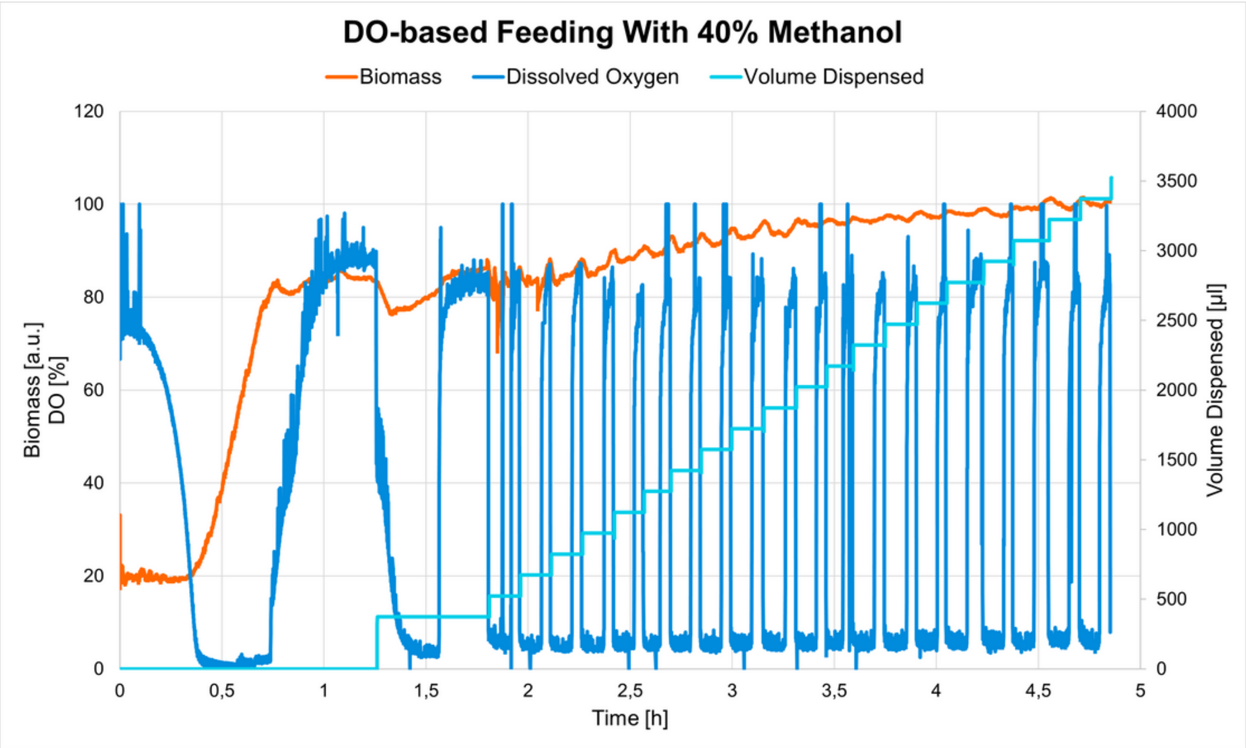
First pill-based optical sensor for online dissolved oxygen (DO) monitoring in shake flasks.

- Single-use pill: Factory-calibrated and pre-sterilized for immediate use
- Drop & Go: Easy handling and fast experiment setup
- Combine with the Liquid Injection System (LIS) for DO-based feeding in shake flasks

Learn More: DO Sensor Pills

sbi DO Monitoring Solutions

For Shake Flasks: DO-based Feeding



Close the loop: Combine reliable, online DO monitoring with automated liquid feeding.

- React to oxygen limitations or start the feeding process when oxygen has recovered
- Select the feeding profile that most benefits your process
- Advanced control options: Program reocurring liquid injections with the PID Controller

sbi DO Monitoring Solutions

For Bioreactors: DO Flow Cells

Continuously monitor dissolved oxygen (DO) in flow loops using fiber optic sensors.

- DO range: 0-50% O_2 (gas), 0-100% O_2 (liquid)
- Factory-calibrated and pre-sterilized
- Luer-lock connectors make it easy to install the flow cells, regardless of tubing size
- Ideal for use with perfusion bioreactors, custom benchtop bioreactors, and small-scale fermenters

Learn More: Flow Cells

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Have Questions?

Let's work together to find a solution that works best for you.

SPEAK WITH AN EXPERT