

Bioreactors and Bioreactor Processes

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chemical

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Bioreactors

Bioreactors are technical vessels of different design in which biological and chemical substances are converted or produced under controlled conditions. In bioreactors the knowledge about metabolic processes is applied

- to produce very different substances and products (see list below),
- to generate energy and
- to degrade or transform pollutants

Bioreactors, also called fermenters, are not specifically designed for a certain biochemical production process; as a result of the wide range of demands made by biological systems they must be, in contrary, designed for very flexible and universal applications. By far most common is the stirred-tank reactor, which, because of its optimal mixing and mass transport performance, can be used in a high number of applications.

Biochemical reactions involve biocatalysts (i.e. microorganisms, plant and animal cells, enzymes) and result in the conversion and production of biological and chemical substances. Vessels (reactors) are required for this process where living microorganisms can exhibit their activities under defined conditions. The reactors must provide the environmental conditions which allow the microorganisms to develop their optimal catalytic activity.

Regulation and control of a Bioreactors depend on exact and continuous information about typical process parameters which can be classified in (fig. 1)

- **physical parameters** such as temperature, pressure, power input, turbidity, viscosity e.a.,
- **biological parameters** such as content of proteins, DNA, e.a. and

- **chemical parameters** such as pH value, pO_2 , pCO_2 and redox potential of the process medium as well as content of O_2 , CO_2 and occ. CH_4 in the exhaust gas.

Besides optimization and stabilization of the biological conditions the measured values are also used to quantify the process steps and to scale up process data from a pilot plant to a large production plant.

Bioreactors capacities range from some liters in laboratories and some m^3 for pilot installations up to some 1000 m^3 in production plants.

The actual process involved defines the requirements on the reactor design. These range from non sterile reactors for e.g. waste water treatment to medium requirements (e.g. stainless steel vessels) in food industries up to reactors operated under totally sterile conditions with strong regulations and specifications regarding design and materials.

Process

Biotechnological processes are very versatile and therefore a general process procedure cannot be described. Basically, the following process steps can be defined:

- Feed of nutrients, acids/bases and, in aerobic processes, air and/or oxygen
- Material transfer (mixing) within the liquid phase
- Dispersion into a second phase (mostly air) for effective oxygen supply and effective heat transfer
- Biochemical reaction and cell growth process
- Heat transfer for discharge of reaction heat
- Discharge of exhaust gas
- Discharge of fermentation fluid (product).

Bioreactors are operated in batch or continuous mode.

Modern apparatus design includes mechanisms for product separation directly in the reactor vessel, e.g. through a semi-permeable membrane. Particularly important is the oxygen feed, which, because of the low solubility of oxygen in water, must be supplied in high quantity and finely dispersed.

Processes are classified by means of the type of catalysts used into processes with reactions using free enzymes and processes using aerobic or anaerobic conversions using suitable microorganisms.

Products and process objectives of biotechnology (selection)

- Pharmaceuticals (Antibiotics, vaccines, diagnostics)
- Food and food additives (Alcoholic beverages, yeast, fragrances and preservatives)
- Agriculture products (Pesticides, amino acids, feed yeast)
- Technical materials (Surfactants, organic acids)
- Raw material conversion (Splitting of starch and cellulose, conversion of saccharides)
- Energy generation (Biogas)
- Degradation of pollutants (Waste water treatment)

Process parameters

As in other production processes the Bioreactors process is optimized regarding consumption and product yield. The relevant operational parameters are divided into

- **physical parameters**
such as temperature, pressure, mass transport, viscosity, conductivity, composition of the gas phase;
- **chemical parameters**
such as dissolved gases (O_2 , CO_2), pH, ion concentration, redox potential, and
- **biological parameters**
such as growth rate, number of cells, viability, heat generation e.a.

According to the process flow these parameters may be also classified as

- **Input parameters**
e.g. air throughput, oxygen content of input air, stirrer velocity, feed of acid or lye, heating/cooling,
- **Process parameters**
e.g. pH value, gas partial pressure, temperature, substrate content in the liquid phase, O_2 and CO_2 content in the gas phase and biomass composition (C-N-O-H, proteins, enzymes, ...),
- **Optimization parameters**
to rate the process quality, e.g. growth rate, consumption, yield, foam generation; these are derived parameters: oxygen consumption and CO_2 generation, for instance, can be calculated from the air throughput and the O_2 resp. CO_2 content of the input and exhaust air.

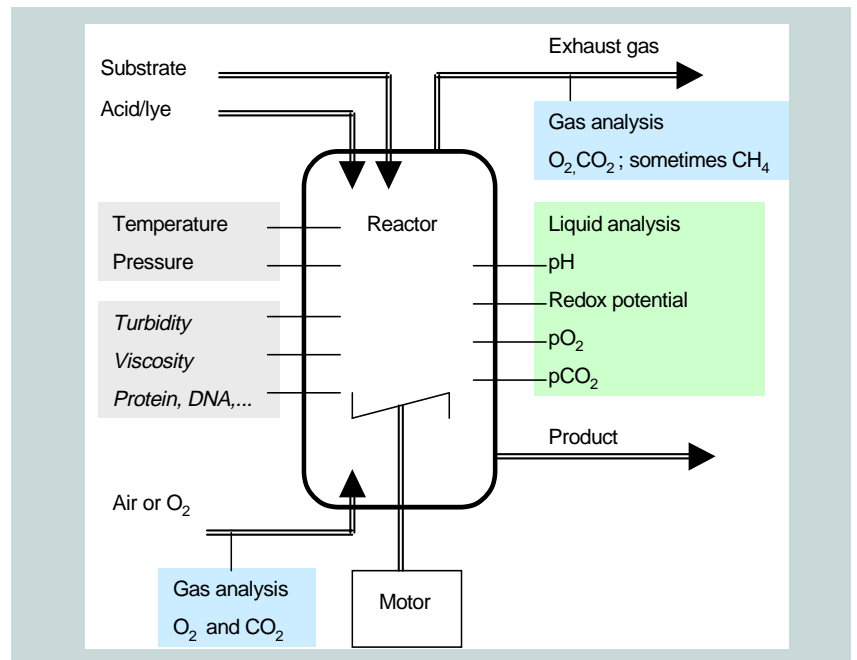


Fig. 1 Bioreactor with measuring instrumentation

Terms

Enzymes

Group of proteins which, used as biocatalysts, strongly enhance the reactivity of chemical processes by reducing the level of activation energy. They regulate very specifically the metabolic rate.

Aerobic/anaerobic conditions

Requirements for growth and multiplication of microorganisms which need oxygen to transform nutrients (aerobics). These conditions exist when the organisms are exposed to air or oxygen. Other microorganisms (anaerobics) can exist only under conditions which are free from oxygen (anaerobic conditions).

Substrate

Nutrients required by organisms, for their growth and for energy generation.

Fermentation

Especially used to describe the biotechnical production process of leather, tobacco, coffee or tea; more generally speaking fermentation includes all reactions in a microorganism culture from substrate consumption up to final product formation.

Biomass

In general biomass is the total quantity of organic material which is formed through growth and metabolism of animals, plants and microorganisms. Strictly speaking, however, biomass is the cell mass formed by growth of microorganisms, which is either the desired process result, e.g. yeast, or an unavoidable by-product, e.g. digested sludge.

Use of Process Analyzer

The number of analytical methods applied in biotechnological processes has increased considerably during the past decades. Preferred are continuous and almost delay-free measurement techniques with in situ installation of the sensors. This, however, is quite limited because of the requirement, to keep all parts contacting the process medium in sterile condition.

To measure in the *liquid phase*, all common methods of liquid analysis are used.

To determine the composition of the *gaseous phase* including the exhaust gas the process gas analysis (NDIR, paramagnetic and FID principle) is used; in certain cases also gas chromatography or mass spectrometry is used as well.

Tasks Form Gas and Liquid Analysis

For Gas Analysis

the determination of oxygen content in the exhaust gas of aerobic processes is the most important task. From this, in relation to the oxygen content in the input air, the oxygen consumption can be determined. Depending on the required measuring quality either paramagnetic oxygen analyzer or electrochemical cell analyzer are used.

In most processes also carbon dioxide (CO₂) is formed and sometimes methane (CH₄) as well; sometimes both are also measured to get a complete balance. For this task gas analyzers based on the NDIR (non dispersive infrared) principle are applied.

For certain applications very economical multi-component analyzers (e.g ULTRAMAT 23) are available which can measure all three gas components (O₂, CO₂, and CH₄) with just one device.

Liquid analysis

is used for the determination of

- pH value as one of the most important indicators for the process conditions,
- dissolved oxygen (pO₂) to determine the amount of oxygen available for the cell growth,
- dissolved CO₂ (pCO₂) to judge possible influences to the reactions and of
- the Redox potential, which as well informs about details of the process.

All parameters mentioned above, together with temperature and pressure, determine the overall "climate" in the reactor, which is necessary for the required reaction.

Important and well known criteria for analyzer performance are measuring accuracy and reliability. New and with increasing influence on user benefits are state of the art features arising from digital data communication, such as self diagnostic, remote parameter setting or preventive maintenance.



Bild 2 ULTRAMAT 23

| Typical measuring ranges for gas and liquid analysis in bioreactors | |
|---|--------------------------|
| O ₂ Feed | 16 ... 21 % resp. |
| O ₂ Off gas | ...100 % |
| CO ₂ Off gas | 16 ... 21 % 0 ... 5 % |
| pO ₂ | 0 ... 100 % |
| pCO ₂ | 0 ... 100 mbar |
| pH | 2 ... 12 pH |
| Redox | -0,6/-0,3 V |

Table 1

| Measuring Parameter | Analyzer (Siemens) |
|---|----------------------------|
| O ₂ in feed line and off gas | OXYMAT 61 |
| CO ₂ and CH ₄ in off gas | ULTRAMAT 6 |
| O ₂ , CO ₂ and CH ₄ in off gas | ULTRAMAT 23 |
| pH value | SIPAN 32/34 |
| Redox potential | SIPAN 32/34 |
| Solved O ₂ (pO ₂) | SIPAN 32/34 |
| Various gases | Quantra Mass Spectrometer |
| Various gases | MAXUM II Gas Chromatograph |

Table 2



Bild 3 SIPAN Liquid Analyzer

Siemens Analyzers

Siemens offers all types of gas and liquid analyzers required for Bioreactor process control from its own development and production, see table 2.

The **OXYMAT 61** is a gas analyzer that operates according to the paramagnetic principle and is designed for high-precision measurements of oxygen concentrations in gases. The pulsating magnetic field creates minute flow pulses detected by the Siemens microflow sensor and converted into the measuring signal. Thus, the OXYMAT 61 does not contain any moving parts. The sample stream gas also does not come into contact with the microflow sensor, which ensures an extremely long life time and high operating stability.

Because of its measuring principle using a reference gas, the OXYMAT 61 is specifically capable to measure very small concentration differences in two gas streams with high accuracy.

This is the most important requirement in oxygen analysis in Bioreactor process control.



Bild 4 OXYMAT 61 Gas Analyzer

The **ULTRAMAT 6** is a gas analyzer that operates according to the non-dispersive infrared principle (NDIR). It is designed to perform highly selective concentration measurements of infrared-sensitive gases. The ULTRAMAT 6 uses the two-beam alternating light principle with a measurement and comparison cell, dual-layer detector, and optical coupler. This optical bench design produces an extremely narrow absorption curve minimizing the influence of overlapping spectra. Thus an unparalleled analytical precision is obtained.

The **ULTRAMAT 23** multi-component gas analyzer allows to measure the three gas components O₂, CO₂, and CH₄ very economically in one single instrument using two NDIR channels and an electrochemical cell. Its physical single beam design with a dual or three layer IR detector ensures a high level of selectivity and precision.

Thanks to the autocalibration principle using ambient air, the long-term stability of zero point and measuring value is extremely high without the need for expensive test gases.

| Features OXYMAT 61 | User Benefits |
|---|---|
| Simple and robust design without moving parts | High operating reliability, availability, and service life; very low maintenance and spare parts requirements |
| Strictly linear measuring principle | High precision and flexibility |
| Very short signal response time | T90 time $t < 2$ s provides optimum reaction to process variations |
| Measuring principle allows differential measurement of two different gas streams in one analyzer with one bench. No electronic zero suppression | Very small measuring ranges for high (absolute) concentrations and thus very high measuring precision |
| Minimum drift ($< 0.5\%$ /month of span) | Very high measuring precision Very little needs for recalibration |
| SIPROM GA software package for remote control and maintenance Interface to PROFIBUS PA (option) | Easy integration into automated systems |

Table 3 Features and User benefits of OXYMAT 61

| Features ULTRAMAT 6 | User Benefits |
|--|---|
| Dual-layer detector with variable optical path length setting (Optocoupler) | Maximum selectivity and thus measuring precision. Can be optimized for actual analysis task |
| Detector uses microflow sensor with no moving parts to generate the measuring signal | No microphony: Very low signal noise, high measuring accuracy |
| Extremely stable mechanical design Electronic and physical parts separated gas tight in one robust IP 65 housing | Very high operational reliability and life time |
| Easy cleaning of gas cell (on site possible) | Minimum maintenance costs |
| SIPROM GA software package for remote control and maintenance Interface to PROFIBUS PA (option) | Easy integration into automated systems |
| Can be extended for simultaneous measurement of up to 4 NDIR sensitive gas components | Lower costs due to performing several measuring task with one device |

Table 4 Features and User benefits of ULTRAMAT 6

SIPAN stands for the extensive family of modern liquid analyzers, available in 2- and 4- wire technology, for hazardous and non hazardous areas, for measurements of pH, conductivity, ORP, and dissolved oxygen, as plug-in unit or in field housing. With interfaces to HART and PROFIBUS PA, SIPAN offers access to the two leading field communication technologies in process industry.

Quantra by Siemens (Fig. 5) is the first high resolution mass spectrometer that is capable to perform on-line applications.

The Quantra is well suited for a number of process applications such as trace gas analysis and ambient air monitoring in the chemical industry, or residual gas analysis in the semiconductor industry.



Bild 5 Quantra Mass Spectrometer

The Process **Gas Chromatograph MAXUM edition II** (Fig. 6) combines the analytical features of the Siemens Process Gas Chromatograph PGC 302 with the Parallel Chromatography (Separation of complex analyzing tasks into several more simple steps) and the Ethernet communication technology of the Advance MAXUM of Applied Automation in one device. The wide selection of columns and detectors allows a high selective and sensitive analysis of most different process components.

| Features ULTRAMAT 23 | User Benefits |
|---|---|
| Single beam measuring principle together with AUTOCAL ambient air calibration and multilayer NDIR detector technology | High level of selectivity and accuracy Long-term stability Very low consumption of test gas |
| Modular design with 1-3 NDIR channels and additional oxygen measurement using an electrochemical cell | Economical investment and operation |
| Easy cleaning of gas cell | Minimum maintenance requirements |
| SIPROM GA software for remote control and maintenance Interface to PROFIBUS PA (option) | Easy integration into automation systems |

Table 5 Features and User Benefits of ULTRAMAT 23

| Features SIPAN | User Benefits |
|--|--|
| Analyzers in 2- and 4-wire technology, with and without Ex-protection, sensor and fittings | Flexible engineering, low installation requirements, branch specific solutions even for critical applications and in corrosive sample streams |
| Extensive diagnostic functions Redundant measurements Logbook | Cost reduction by extended maintenance and calibration cycles. Extended reliability by alarm/pre-alarm status signals. Recording of important events |
| Individual and user specific temperature compensation | Highest measuring accuracy even for very low conductivity ranges because of non-linear temperature calibration curve |
| Remote switching of measuring parameters (4 sets) | Highest dynamic range, accuracy, and flexibility. Easy parameterization |
| HART communication and interface to PROFIBUS PA | Offline parameterization from the office, extended functionality and diagnosis. Sensor, measuring ranges etc. to be parameterized |

Table 6 Features and User Benefits of SIPAN



Bild 6 MAXUM edition II Gas Chromatograph

Example

Production of the Surfactant RHAMNOLIPID

Rhamnolipid is a very effective surfactant with antibacterial, mycoplasmaicidal and antiviral features. It is used very universally, e.g. in pharmaceutical applications or for cleaning from heavy oil pollutants.

As shown in the process flow chart (fig. 7) the production of Rhamnolipid is performed in a CST reactor (Continuous Flow Stirring Tank Reactor) equipped with an external loop, which includes two membrane stages. The microorganism used is the aerobic bacterium *Pseudomonas aeruginosa* DSM 2659. A glucose solution with nitrogen source acts as nutrient.

The external loop of the CSTR mainly helps to protect against foam generation, which is the biggest problem in bioreactor operation, especially in surfactant production. This external loop principle allows a 50% smaller reactor volume compared with the conventional method of mechanical foam destruction.

In the first stage of the loop a cell hold back is done for accumulation using an ultra filtration membrane.

The second stage is a pore free solvent diffusion membrane and causes the complete discharge of the CO₂ through gas exchange. Because of the very efficient mass transfer between CO₂ and O₂, ambient air (enriched with O₂) is sufficient here as purge gas. Therefore most of the oxygen required by the microorganisms must be supplied separately. This offers a major advantage since a much smaller gas exchange membrane can be utilized.

All gas exchange processes must be controlled by gas analyzers (MP 1-3, fig. 7 and table 7) not only during the growth reaction start phase but also during continuous operation.

The oxygen feed must be controlled very exactly. Control parameter is the measuring value of the dissolved oxygen or oxygen partial pressure (MP4) in the liquid phase, which, for optimum growth rates, must be within certain limits.

An important parameter as well is the pH value (MP5), which is used to control the efficiency of CO₂ discharge. and to optimize the cell growth conditions.

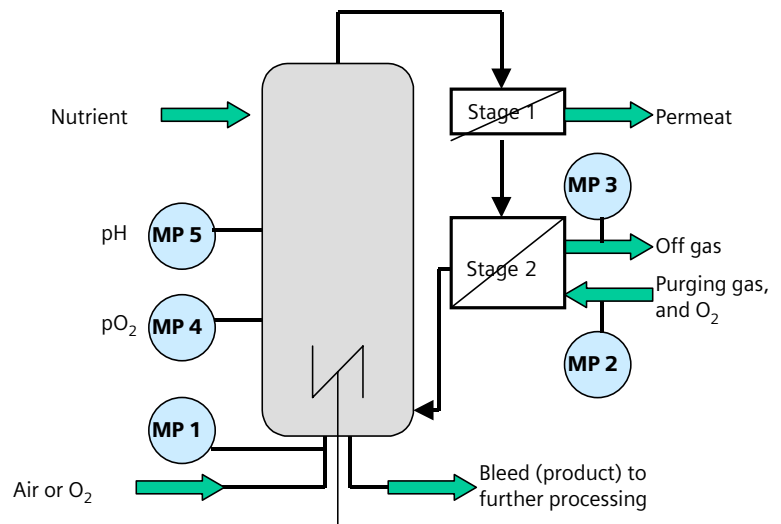


Fig. 7 Rhamnolipid production flow chart with measuring points

| MP | Measured medium | Meas. component | Comment |
|----|--|-----------------|---|
| 1 | Oxygen feed Air (pure or enriched with O ₂) or pure oxygen | O ₂ | Oxygen as nutrient for aerobic processes |
| 2 | Purge gas Air, enriched with O ₂ | O ₂ | For efficient CO ₂ discharge |
| 3 | Exhaust gas | CO ₂ | Metabolic product |
| | | O ₂ | For comparison with oxygen content of input air to calculate oxygen consumption |
| 4 | Bioculture fluid | pO ₂ | For oxygen feed control |
| 5 | Bioculture fluid | pH | For optimization of growth conditions e.a. |

Table 7 Measuring points and measured components

Case Study

User Benefits

Remarkable user benefits arise from using Siemens process analyzers in bioreactor applications because of

- all required analyzers are available from one single supplier,
- the OXYMAT 61 is the only analyzer available for very accurate measurements of small Oxygen content differences in two gas streams with one bench
- the ULTRAMAT 23 is the most economical analyzer by measuring the three most important components in just one device, and the possibility to get a complete analyzer system planned, manufactured, and installed by the Siemens System House.

| Features Quantra | User Benefits |
|--|--|
| Very high Mass Resolution (20 000 at 100 amu) | No interferences of components of similar mass |
| Exact mass determination Mass Accuracy +/- 0,0004 amu | Correct identification of components Identification of unknown components |
| "Ion Ejection" application tool | Discharge of high concentrated components for improved detection capability |
| Robust design of measurement cell, filament, and vacuum system | Suitable for operation in harsh process environment |
| No moving parts contained | Long service life, very low maintenance needs |
| Ion pump | Cell vacuum 10 ⁻¹⁰ Torr No mechanical vacuum pumps required |

Table 8 Features and User Benefits of Quantra

| Features MAXUM edition II | User Benefits |
|---|--|
| Single or dual oven design, with 1 or 2 channels each, for use in areas with or without explosion hazards | Optimum adaption to actual analysis task Extremely cost efficient operation |
| Retrofit possible to include second oven later | Low-cost expansion capability |
| Wide range of detectors available, including highly sensitive HID (Helium detector) for trace analysis in high purity gases | Most versatile for optimum adaption to analysis task |
| Valveless Live Column Switching technology | Increased analysis capabilities Lower maintenance requirements |
| Parallel Chromatography: One complex application is split into a number of simple and parallel running applications | Dramatically reduced cycle times Simplified maintenance |
| Electronic pressure controller | No needle valves required |
| TCP/IP communication via Ethernet | Compatible to many networks |

Table 9 Features and User Benefits of MAXUM edition II

If you have any questions, please contact your local sales representative or any of the contact addresses below.

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