Mass Conversion in Bioreactors

Process Analytics controls biotechnological reactions

Case Study · July 2008

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 different substances, 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. Analyzers are used to control and optimize the reactions.

Application areas

Products gained from biotechnological processes are

- Pharmaceuticals
  such as antibiotics, vaccines, diagnostics, insuline
- Food and food additives
  such as alcoholic beverages, yeast, fragrances and preservatives
- Agriculture products
  such as pesticides, amino acids, feed yeast
- Technical materials
  such as surfactants, organic acids

Furthermore bioreactors are used for

- Raw material conversion:
  Splitting of starch and cellulose conversion of saccharides
- Energy generation (Biogas)
- Degradation of pollutants:
  Waste water treatment

Pharmaceutical Industry

Process Steps and Process Parameters

Optimal conditions

Bioreactors must provide the conditions which allow the microorganisms to develop their optimal catalytic activity. Control of bioreactors depend on exact and continuous information about typical process variables which can be classified in (fig. 1).

- **physical variables**: temperature, pressure, power input, turbidity, viscosity,
- **biological variables**: content of proteins, DNA, e.a.
- **chemical variables**: pH value, pO_2, pCO_2, redox potential of the process medium as well as content of O_2, CO_2 and occ. CH_4 in the exhaust gas.

Gas and liquid analysis methods are used to determine these parameter values. 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 m³ for pilot installations up to some 1000 m³ in production plants.

The process characteristics 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.

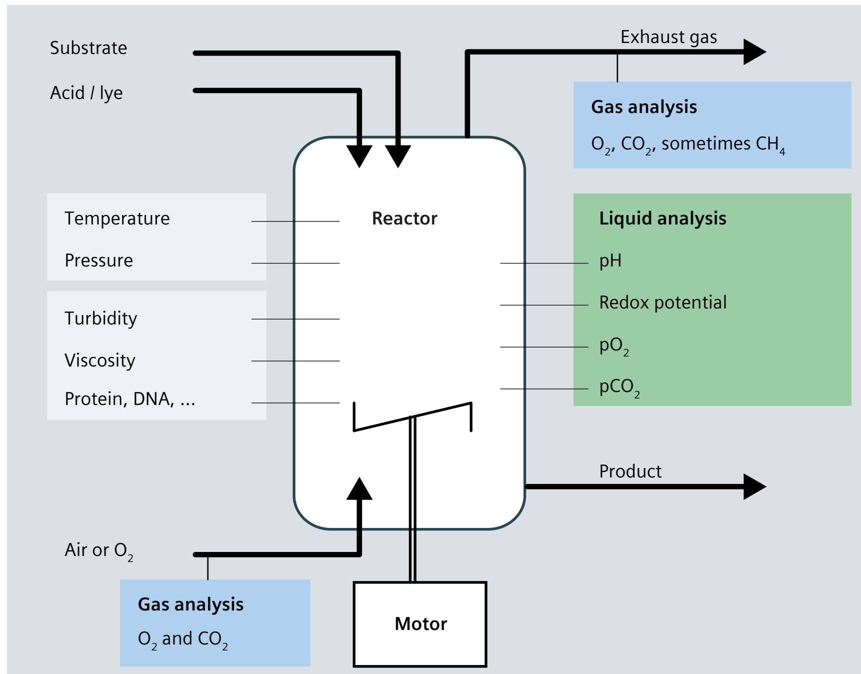

Fig. 1 Bioreactor with measuring instrumentation

Process steps

Biotechnological processes are very versatile and therefore general process cycles cannot be described. Basically, the following process steps exist:

- Feed of nutrients, acids/lyes 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 reactor design includes mechanisms for product separation directly in the reactor vessel, e.g. through a semi-permeable membrane.

Particularly important is oxygen feed, which, because of the low solubility of oxygen in water, must be supplied in high quantities and fed through fine nozzles.

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.

Use of Process Analytics

Process parameters

As other production processes, a bioreactor process is optimized regarding consumption and product yield. The relevant operational parameters are structured 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 gasphase and biomass composition (C-N-O-H, proteines, 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.

Typical measuring ranges for gas and liquid analysis in bioreactors	
O_2 feed	16 ... 21 % resp. 100 %
O_2 Off gas	16 ... 21 %
CO_2 Off gas	0 ... 5 %
pO_2	0 ... 100 %
pCO_2	0 ... 100 mbar
pH	2 ... 12 pH
Redox	-0,6/-0,3 V

Table 1 Typical measuring ranges

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

Typical measuring ranges are shown in Table 1.

Gas Analysis

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

Fig. 2 ULTRAMAT 23 gas analyzer

In most processes also carbon dioxide (CO_2) is formed and sometimes methane (CH_4) as well. Very often both components are determined to get a complete balance. Gas analyzers based on the NDIR (non dispersive infrared) principle are used for this task. The multicomponent gas analyzer Siemens ULTRAMAT 23 (Fig. 2) provides a very efficient solution because it is able to measure all three gas components (O_2, CO_2, and CH_4) simultaneously in just one device.

Use of Process Analytics (2)

Liquid Analysis

Liquid analysis is used for the determination of

- pH value as one of the most important indicators for the process conditions,
- dissolved oxygen (pO_2) to determine the amount of oxygen available for the cell growth,
- dissolved CO_2 (pCO_2) 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, define the overall "climate" in the reactor, which is necessary to run the required reaction.

Alliance between Siemens and Hach Lange

Customers increasingly prefer comprehensive and customized solutions with delivery of all analyzers and other components from one hand. With this in mind, Siemens, a leading supplier of gas analyzers, and Hach Lange, a specialist in the field of liquid analytics, have formed an alliance aimed at meeting this demanding user requirement.

Hence, the two companies are in a position to offer the entire system, including measuring and control technology, as a complete, customized solution. Because there is no overlap in the companies product portfolios, the programs of Hach Lange and Siemens complement each other perfectly.

Analyzers

Table 2 shows the analyzers which are used in this application task and Fig. 3 the Hach Lange si792 transmitter for electrochemicals sensors, which is best suited for the application at hand.

Important and well known criteria for analyzer performance are measuring accuracy, operational reliability and data communication capabilities for e.g. self diagnostics, remote parameter settings or preventive maintenance. These features are state of the art in all analyzers mentioned here.

Measuring Parameter	Analyzer
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	si792 / si794[1)]
Redox potential	si792 / si794[1)]
Dissolved O_2 (pO_2)	si792 / si794[1)]
Various gases	MAXUM II Gaschromatograph

[1)] Products fromFirma Hach Lange

Table 2 Measuring parameter and analyzers

Fig. 3 si792 transmitter (Hach Lange)

Analyzers details (1)

Siemens offers all types of gas analyzers required for Bioreactor process control from its own development and production.

OXYMAT 61

OXYMAT 61 (fig. 4 and table 3) 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 a micro-flow 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 highest accuracy. This is the most important requirement in oxygen analysis in bioreactor control.

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 surpression	■ 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

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 3 and 4   Features and user benefits of OXYMAT 61 and ULTRAMAT 6

ULTRAMAT 23

The ULTRAMAT 23 multi-component gas analyzer (fig. 3 and table 5) allows to measure the three gas components O_2, CO_2, and CH_4 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.

ULTRAMAT 6

ULTRAMAT 6 (table 4) 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. Hence, an unparalleled analytical precision is obtained.

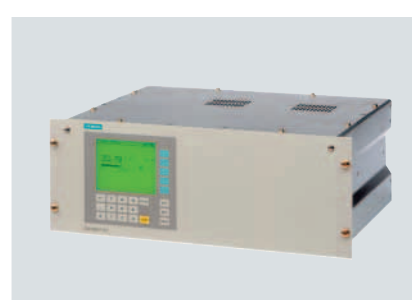

Fig. 4   OXYMAT 61 gas analyzer

Analyzers details (2)

Fig. 5 Prozess-Gaschromatograph MAXUM

MAXUM II

MAXUM II (fig. 5) is a high performance process gas chromatograph offering an outstanding broad variety of analytical possiblities. Main application field is process monitoring and control for gases and vaporable liquids in rough industrial environments. MAXUM performs a wide range of duties in refineries and chemical/petrochemical industries. MAXUM features e. g. a flexible, energy saving single or dual oven concept, valveless sampling and column switching, and parallel chromatography using muliple single trains as well as a wide range of detectors such as TCD, FID, FPD, PDHID, PDECD and PDPID.

Important user benefits include

- Flexible range of oven capabilities
- Multiple detector and valve options
- Local panel and remote workstation
- Powerful software
- Extensive local and remote I/Os and serial links
- Multiple network capabilities
- Large, experienced support team

Features ULTRAMAT 23	User Benefits
■ Single beam measuring principle together with AUTOCAL ambient air calibration and multi-layer 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

Features si792 and 794 (Hach Lange)	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
■ HART communication and interface to PROFIBUS PA and FF	■ Offline parameterization from the office ■ Extended functionality and diagnosis ■ Sensor, measuring ranges etc. to be parameterized

Table 5 and 6 Features and user benefits of ULTRAMAT 23 and si792/si794 (Hach Lange)

si792 / si794 Transmitter (Hach Lange)

si792 and si 794 (fig. 3 and table 6) are transmitters for electrochemical sensors 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, PROFIBUS PA and FF including capabilities for diagnostics, remote parameter setting, preventive maintenance etc.

Application Example

Biosynthetic Production of the tenside Rhamnolipid®

Rhamnolipid is a very effective tenside with antibacterial, mycroplasmacidal and antivirial 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.

External loop to avoid formation of foam

The external loop of the CSTR mainly helps to protect against foam generation, which is the biggest problem in bioreactor operation, especially in tenside 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 at the permeate outlet.

The second stage is a pore free solvent diffusion membrane and provides the complete discharge of metabolic CO_2 through gas exchange. Because of the very efficient mass transfer between CO_2 and O_2, ambient air (enriched with O_2) is sufficient 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.

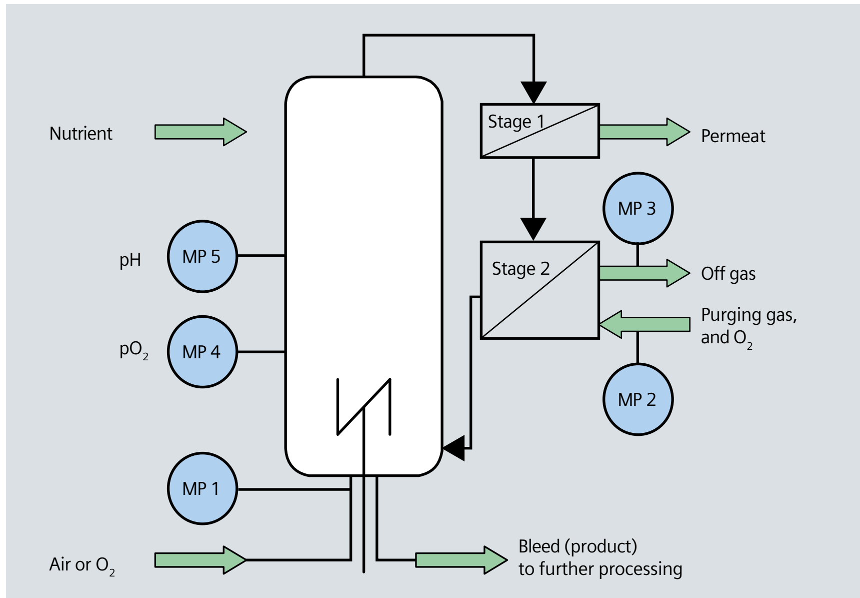

Fig. 6 Production of Rhamnolipid flow chart with measuring points

Measuring point	Measured medium	Measuring 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	Off 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

Table 7 Measuring points and measured components (reference to fig. 6)

Use of process analyzers

All gas exchange processes are to 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. Oxygen consumption (derived from O_2 differences) and the CO_2 value are the most significant characteristics for the growth of the organism.

Oxygen feed must be controlled with high accuracy. Control parameters are the value of dissolved oxygen or oxygen partial pressure (MP4) in the liquid phase, which, for optimum growth rates, must be within certain limits.

Another important parameter is the pH value (MP5), which is used to control the efficiency of CO_2 discharge and to optimize the cell growth conditions. Both measurements are performed using liquid analyzers.

User benefits Terms

User benefits

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

- 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
- the possibility to get all required analyzer from one supplier and a complete analyzer system planned, manufactured, and installed by the Siemens System House

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.

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