BIOREACTORS
PILOT AND INDUSTRIAL SCALE
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Introduction

We are an innovative company designing and manufacturing laboratory and pilot scale bioreactors.

Our bioreactors meet up-to-date requirements and include innovative solutions. Model-based control of fed-batch fermentation, as well as the use of our novel magnetic drive are an example. We test our new bioreactor solutions in our laboratory through real fermentation processes.

Our bioreactors have been designed in such a way to be easily supplemented and adapted to various applications. We manufacture laboratory bioreactors for cultivation of bacteria, mammalian cells and micro-algae, using a common basic solution. We manufacture pilot scale bioreactors made of components (vessel, technological stand, control cabinet), which can supply each of them as a product separately.

We have wide experience in the implementation of our pilot scale bioreactors in fully automated production lines for various applications (vaccines, enzymes, bio-fertilizers, bioremediation, etc.). We have installed production facilities with up to 15 bioreactors, using volumes up to 20 m$^3$. All bioreactors are equipped with novel magnetic coupling mixers.

To ensure the compliance with GMP rules, we are using SCADA software according to the requirements of CFR Title 21 Part 11 (document from US Food and Drug Administration).

We can provide our equipment with IQ (Installation Qualification) and PQ (Performance Qualification).
1. Pilot scale bioreactors

We offer stainless steel bioreactors from 10 litres up to 20 000 litres, as well as bioreactor lines, including other technological vessels.

An autonomous bioreactor consists of 3 main parts:

- The bioreactor vessel with mixer;
- The technological stand;
- The control cabinet, including PLC (Process Logic Controller).
# Technical specifications

<table>
<thead>
<tr>
<th><strong>Vessel design, volume</strong></th>
<th></th>
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</thead>
<tbody>
<tr>
<td><strong>Vessel design</strong></td>
<td>Jacketed with heat insulation. Removable lid (up to 800 litres). The hydraulic lifting device of the lid. Vessels over 800 litres have an access port of corresponding size. Side glass on the lid and in the upper cylindrical part of the vessel.</td>
</tr>
<tr>
<td><strong>Total volume (L)</strong></td>
<td>15 – 20 000 (customized)</td>
</tr>
<tr>
<td><strong>Working volume (L)</strong></td>
<td>10 – 16 000 (customized)</td>
</tr>
<tr>
<td><strong>H/D range</strong></td>
<td>1,1 – 3,2 (customized)</td>
</tr>
<tr>
<td><strong>Surface quality</strong></td>
<td>Inner vessel wall: surface roughness lower than 0.63μm. Outer vessel wall: surface roughness lower than 0.8 μm. (passivated)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Materials</strong></th>
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<tbody>
<tr>
<td>- The material of the parts, in contact with the product is stainless steel 316L;</td>
<td></td>
</tr>
<tr>
<td>- For parts, not in contact with the product (e.g. the jacket) – stainless steel 304;</td>
<td></td>
</tr>
<tr>
<td>- O-ring gaskets and membranes in contact with the product - EPDM;</td>
<td></td>
</tr>
<tr>
<td>- Insulation - mineral wool 50 mm thick;</td>
<td></td>
</tr>
<tr>
<td>- Casing insulation - stainless steel 304;</td>
<td></td>
</tr>
<tr>
<td>- Sight glass – heat-resistant glass Pyrex;</td>
<td></td>
</tr>
<tr>
<td>- Hoses peristaltic pumps - silicone rubber of pharmaceutical quality.</td>
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</tr>
<tr>
<td>All bioreactors have insulation over the jacket. The outside surface of insulation is covered by stainless steel 304</td>
<td></td>
</tr>
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</table>

<table>
<thead>
<tr>
<th><strong>Pressure and temperature</strong></th>
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<tbody>
<tr>
<td>For all vessels the maximal design pressure inside the vessels – 3,0 bar, in jacket – 3,5 or 4,0 bar (can be customized). Maximal temperature - 135° C.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Mixing, aeration</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mixing</strong></td>
<td>Novel magnetic coupling mixing with top- or bottom placed motor with reduction gear. For microbial cultures 2–3 standard Rushton turbine type agitators are used for mixing media. To improve oxygen transfer rate an extra agitator can be added. Optionally pitched blade or marine impellers for shear sensitive conditions, and special agitators for viscous media. Examples of mixer rotation speed maximal range (for standard Rushton turbine mixing system, can be customized):</td>
</tr>
<tr>
<td>20 till 50 L – 750 rpm; 2500 L – 350 rpm;</td>
<td></td>
</tr>
<tr>
<td>70 till 150 L – 650 rpm; 4000 L – 300 rpm;</td>
<td></td>
</tr>
<tr>
<td>200 till 300 L – 600 rpm; 10000 L – 200 rpm;</td>
<td></td>
</tr>
<tr>
<td>400 till 650 L – 500 rpm; 15000 L – 150 rpm;</td>
<td></td>
</tr>
<tr>
<td>700 till 1000 L – 400 rpm</td>
<td></td>
</tr>
</tbody>
</table>
### Aeration

The aeration system may be configured for both microbial and cell culture, and includes:
- Filter-Reducer with pressure gauge to set up supplied air pressure;
- Flow switch or rotameter for measuring supplied air flow in the range of 0-2 vvm (volume of air per volume of fluid per minute);
- Mass flow controller (MFC) in the range of 0 - 1 vvm (optional);
- Chemically inert PTFE membrane filter cartridges which are inherently hydrophobic used as air and gas-sterilizing filters for incoming and outgoing air (Manufactured by PALL, USA). Filters are installed in the steam sterilizable stainless steel housings;
- Automatic gas flow control system \((O_2, N_2, CO_2, NH_3)\) using a mass flow controller (the MFC) (optional);
- The measurement of the \(O_2 / CO_2\) concentration ratio in the exhaust air (optional);
- Automatic pressure control system in the fermenter vessel, mounted on the exhaust air line (optional)

### Control

#### Automation

Siemens Simatic S7 - 1500 series PLC.
Touch panel 9.7” TFT, 1024 x 768mm, 262k colours

#### Frame

Stainless steel power cabinet (Rittal, Germany)

#### Communication

Profinet connection to LAN or WAN

#### Sensors (basic)

Temperature, pH, pO\(_2\), foam, level, overpressure, hydrostatic pressure

#### Sensors (optional)

Optical density, pCO\(_2\), O\(_2\) / CO\(_2\) gas analyser, redox potential, methanol, integrated weighing system. Hamilton pH and pO\(_2\) intelligent sensors with ArcAir software

#### Control of fermentation and sterilization processes

The following processes and parameters are controlled:
- Temperature, pH, pO\(_2\) (according cascade control), foaming, high level, inlet air or gas flow (optional), overpressure (optional), methanol (optional) and ethanol (optional) concentrations;
- Substrate feeding according to adjusted profiles:
- Fully automated sterilization in place of empty and filled (with substrate) vessel, including the sterilization of inlet and outlet filters, and cooling of vessel after sterilization.
2. Automated CIP equipment

We offer two types of CIP equipment:

- Mobile
- Stationary

Mobile CIP equipment

This system is placed on a movable platform and can be easily moved to from one production unit to another. It can be connected to fermenters or other technological vessels. The maximum speed of detergent circulation is 5000 l/h. The system includes:

- 2 centrifugal pumps (for CIP circulation and discharge)
- 2 dosage pumps with side walls to concentrate detergents and other fittings
- A control board
- Valves for manual and automatic control
- Couplings and armature to provide the operation of the CIP system are placed on the mounting platform
This configuration allows to clean fermenters and other equipment with total volumes from 20 up to 1000 litres.

The given installation of CIP can use both manual and completely automatic modes of cleaning. For automatic control, CIP cleaning mode should be adjusted or selected. These operations can be initiated using the control board.

CIP cleaning mode consist of multiple stages, and each stage is divided into several steps using selected parameters (temperature, time of CIP cleaning, time of discharge output, detergent conductivity, speed of concentrate supply, speed of mixer rotation, productivity of CIP cleaning pump, duration of cleaning, etc.).

In automatic mode it is possible to perform the following operations of CIP cleaning:

- Preliminary rinsing;
- Cleaning with alkaline detergent;
- Rinsing;
- Cleaning with acidic detergent;
- Final rinsing;
- Customized operations can also be implemented.

The automatic process of CIP is carried out according to a selected recipe.

Any phase of cleaning consists of definite sequential steps that can be chosen according to the selected mode of cleaning. Some examples of these steps are: valve opening, start-up of CIP cleaning pump, start-up of mixer and other processes, connected with switching on/off the working appliances. The parameters of steps are given in the instruction and can be changed according to the user’s needs.
Stationary CIP equipment

Stationary CIP equipment is customized depending on the requirements of the production process. This equipment is provided for CIP/SIP cleaning of bioreactors and other technological vessels, as well as for the pipeline connections. CIP/SIP customized systems can be installed with more than one vessel.

There are two working regimes of stationary CIP/SIP equipment: cleaning and sterilization. The regimes can be adjusted from the operator panel or using the SCADA.
The connections of steam line, cooling water, compressed air and discharge can be provided according to the following diagrams (variants with one and four vessels).
CIP / SIP processes can be carried out in two variants:

1) Locally by direct control from the operator panel of CIP/SIP;
2) Centrally by control from a remote operator panel or SCADA.

CIP / SIP processes are fully automatic, but can also be controlled manually if necessary.

The automatic CIP-process is carried out according to the selected recipe. The recipe includes the stages and steps with corresponding parameters.

Any phase of cleaning consists of definite sequential steps. According to the selected mode of cleaning process, steps can differ.

In automated mode it is possible to perform the following phases of process:

- Preliminary rinsing with cooled water;
- Preliminary rinsing with hot water;
- Cleaning with alkaline detergent;
- Rinsing with cooled water;
- Cleaning with acidic detergent;
- Final rinsing with cooled water;
- Final rinsing with injection solution;
- Cleaning and sterilisation of pipelines.

It is possible add new phase according the requirements of concrete production. In each phase can be adjusted the parameters to provide optimal working of CIP/SIP.

Stationary CIP / SIP gives the possibility carry out the processes according GMP requirements.
3. Industrial application examples of bioreactor lines

We have experience in the application of complex bioreactor lines for biotechnological production. We develop technical requirements, project and qualification documents, we manufacture the equipment and provide the delivery, montage and starting up.

Our experience covers the design of the following application plants:

1. Substances for bioremediation of soil from oil pollution
2. Ferments for dairy industry
3. Vaccines for animals
4. Probiotics from whey lactose
5. Bio-fertilizers
6. *Pichia pastoris* fermentation line for manufacturing of enzymes and proteins
7. Bio-leaching

The technical solutions are customized depending on the requirements of the concrete production process.
**Substances for bioremediation of soil from oil pollution**

The application of this fermentation system is bioremediation of soil, contaminated with oil over a long period. In this system a bacteria consortium has been cultivated, which has been isolated from oil-contaminated soil.

The fermentation systems consist of 30-, 100- and 800 litre bioreactors. Another 800 litre reactor is used for storage of a substrate. Every bioreactor includes temperature, pH, pO2, foam and level control, as well as time profiles of automatic substrate feeding rates, which can be adjusted. Sterilization is available for all fermentation system including pipelines.

**Ferments for dairy industry**

This bioreactors line system is used for the production of ferments for dairy industry, using the following microorganisms: *Lactococcus lactis*, *Streptococcus thermophilus* and *Candida* strains.

The line consists of seeding-fermenters with working volume of 10 and 100 litres and one production fermenter with a working volume of 1000 litres. Three vessels of 100, 150 and 1000 litres are intended for medium preparation, as well as aseptic technological solution and product storage. All fermenters are connected in a technological line via pipelines to ensure cleaning and sterilization with CIP/SIP equipment. Technological line cleaning is performed with two mobile CIP devices. Data collection, processing and documentation is performed with the help of SCADA software, which can be installed on a user’s computer.
Vaccines for animals

We manufactured and installed 2 lines of bioreactors designed for the cultivation of mammalian cells used in the production of vaccine preparations for farm animals.

The system is fully closed and includes the following key elements:

1) Two identical manufacturing lines comprising bioreactors with volumes of 20, 100, 500 and 3000 litres;
2) A system of connecting heat-insulated pipelines, the group of membrane valve that connect the referred bioreactors;
3) Stationary CIP/SIP systems for automated cleaning and sterilization of the bioreactors and connecting pipelines;
4) Substrate preparation reactor;
5) Mobile containers with junction points to the CIP / SIP systems;
6) Control software package (SCADA), developed in compliance with the requirements of 21CFR Part11 (USFDA).
Probiotics from whey lactose

Nowadays, the processing technology for obtaining high added value products from whey (e.g. whey protein) is being mastered. During the processing of whey, as a side product accrues whey lactose. The utilization of this product, without polluting the environment, is problematic. This unresolved issue allows for the development of a profitable technology of whey processing. The system of bioreactors created by us is used for the production of probiotics using whey lactose as a nutrient medium.

Main equipment configuration of the fully automated system:

- Bioreactors with total volumes 35, 350, 4000 and 2 x 15000 liters;
- CIP / SIP with 4 x 4000 liter vessels;
- SCADA, including 2 PC.
Bio-fertilizers

Two bioreactor lines were installed to manufacture biological products that increase soil fertility, restore its natural balance and increase plant productivity. The main products consist of bacteria that fix nitrogen, release phosphorus, increase potassium absorption and restrict plant diseases that are caused by fungi.

Main equipment configuration of the fully automated system:

- Two bioreactor lines:
  - First line – Bioreactors with total volumes of 50, 500, 5000 and 20000 litres;
  - Second line – Bioreactors with total volumes of 50, 500 and 5000 litres;
- 500 litre mixing reactor for preparation of substrate;
- CIP / SIP with 4 x 4000 litre vessels;
- Circulation steriliser;
- SCADA, including 2 PC.
**Pichia pastoris fermentation line for the manufacture of enzymes and proteins**

This bioreactor line is for the cultivation of *Pichia pastoris* to manufacture enzymes and proteins.

The system is fully closed and automated, consisting of the following key elements:

1) 50, 500 and 2 x 5000 litre bioreactors (total volumes);
2) Stationary CIP / SIP for automated cleaning and sterilization of bioreactors, reactors and connecting pipelines;
3) Circulation sterilizer for sterilization of substrate;
4) Feeding reactors for glycerol and methanol;
5) Connecting heat – insulated pipelines and the group of pneumatically controlled membrane valves.
The following parameters in bioreactors are controlled: temperature, pH, DO, overpressure, hydrostatic pressure, level, methanol concentration and foaming.

The central cascade control system includes SCADA developed in compliance with the requirements of 21 CFR Part 11.
Bio-leaching

Bioleaching is the extraction of metals from their ores with a help of the living microorganisms. This method can be applied for example, to recover copper, zinc, lead, arsenic, antimony, nickel, molybdenum, gold, silver, cobalt.

We have developed and manufactured laboratory and pilot scale systems of bioreactors for bioleaching according to the following configuration:

1. Two 6 litre bioreactors for preparation of the inoculate and four 6 litre bioreactors with feeding reactor connected in sequence by peristaltic pumps. All inner parts of bioreactors are from glass, PTFE or covered with special chemical resistant coating.

2. Three 30 and 80 litre bioreactors with an 80 litre feed reactor connected in sequence according to two options: by peristaltic pumps or gravity. All inner surfaces are covered with special chemical resistant coating.

The systems of bioreactors can be applied for the development of bioleaching technologies. The configuration of bioreactors can be customized.

All equipment was constructed for the EU project, in cooperation between AS “Biotehniskais centrs”, Mining and Metallurgy Institute “Bor” (Serbia) and “Mintek” (South Africa).
4. Process automation

We carry out customer-specific automation projects for biotechnology-related and other production processes.

The automation can be performed on our own manufactured lines of bioreactors, as well the devices and units of other manufacturers. The application of a specialized version of SCADA according the requirements of 21 CFR Part 11 (document from US Food and Drugs Administration) is possible (including remote control via Internet).

We do the whole cycle of work required for turnkey finishing of complex automation systems:

- Development of project documentation (electrical circuit diagrams of control cabinets and external wiring, description of the control algorithms, user interfaces, etc.);
- Programming controllers (PLC) and visualisation systems (SCADA);
- Mounting control cabinets;
- FAT (Factory acceptance test);
- Installation and cabling of control cabinets and electrical equipment at site;
- SAT (Site acceptance test);
- Start-up operations;
- Staff training.

Our company is a certified partner of Siemens AG and is authorized to use the name “Siemens Partner Automation”.
