LABORATORY AND PILOT SCALE

BIOREACTORS

◆ Complex bioreactor lines
◆ Process automation
◆ Magnetic coupling mixers

BTC

CONTENTS

Laboratory bioreactors EDF-5.4

EDF-5.4 is a glass bioreactor with working volume of 4.5 l (total volume 6.2 l). The bioreactor is mounted on stainless steel pipe legs.

It is foreseen for the cultivation of bacterial cultures, filamentous fungi, yeast, microalgae, mammalian cell cultures and steam cells.

Depending on the applied microbial culture it is possible to adapt the configuration of the bioreactor in a corresponding way.

EDF-5.4 includes the following main parts and features:

- Completed bioreactor vessel with a novel upper magnetic drive. The range of mixer rotation speed is 40 - 1000 rpm;

- Bioprocess controller BIO-4 controls pH, pO2, temperature, antifoam, level, mixer revolutions in the Basic configuration, as well as additional parameters and operations according to the customer request. pO2 cascade control and time dependent feeding profiles are also included in the Basic configuration;

- Stainless steel "monoblock" construction, which contains the bioprocess controller BIO-4, the thermostat, peristaltic pumps, power control parts, gas supply and mixing (air, O2) units;

- Thermoregulation is carried out by circulation of thermostated water trough the jacket, which covers the bottom of the bioreactor;

- 4 free configurable peristaltic pumps;

- The possibility of wireless control using smartphones (iPhone and others) and tablet computers (iPad and others);

- Accessories for upper lid (needles, sparger, chemostat tube, ports for sensors, condenser with outlet filter and valve; etc.)

- Two rotameters for air and O2, inlet and exhaust air filters;

- Valves and a program module for O2 enrichment process;

- A sterilizable pH sensor from Finesse (PHS-EFP-K8-325) or Mettler-Toledo (405-DPAS-SC-K85/325) with stainless steel protection covering and fittings;

- A sterilizable pO2 sensor from Finesse (DOS-OFF-D4-325) or Mettler Toledo (Inpro 6820/12/320) with fittings;

- Autoclavable sampling device in the bottom of the vessel;

- A frame for titration bottles (3);

- Other standard accessories to ensure full functionality of the fermentation process (bottles for titration liquids, tubes, spare parts etc.).

Optionally:

- A special mixer for mycelial and other shear sensitive microorganisms;

- A gas mixing system (O2, N2, CO2);

- A PC SCADA program for process data monitoring, data archiving and control. A special USB dongle key for seamless remote access to the SCADA system can be supplied.

- Application and integration of additional sensors (redox, O2 and CO2 in exhaust gas, gas flow rates, optical density and other);

- Automatic feeding control, using a supplement program, based on MATLAB;

- Customer specialised solutions for adapting to different microorganisms cultivation tasks.

Application of the EDF-5.4 monoblock for SUB (single use bioreactors) control

The EDF-5.4 monoblock can be adapted for single use bioreactor control. For example, we have expierence in application of single use vessels from CerCell (Danmark).


CerCell has configurable vessels of different volumes (0.5 to 75 liters). These vessels are equipped with all necessary accessories to provide fermentations of different microbial cultures. An important advantage of these vessels is the possibility to achieve high mixer rotation speeds. For example, 7.5 liter bioreactor, equipped with 3 ruchton turbines, can achieve mixer rotation speed up to 1500 rpm. We have tested 7.5 liter vessel from CerCell in fed-batch cultivation of E.Coli. Obtained results were comparable with fermentations in a laboratory scale autoclavable glass bioreactor. In SUB it is possible to apply both single use and sterilizable pH and pO2 sensors .

The application of SUB has following advantages:

- Decreases the risk of contamination;

- Eliminates the necessity to acquire and maintain the infrastructure for the processes (cleaning and sterilisation), which can be critical for validation;

- Simplifies and reduces validation costs

- Reduces the costs of labor expenditures for the preparation operations (assembling - disassembling, cleaning and sterilization);

- The possibility to develop the fermentation processes according GMP requirements.
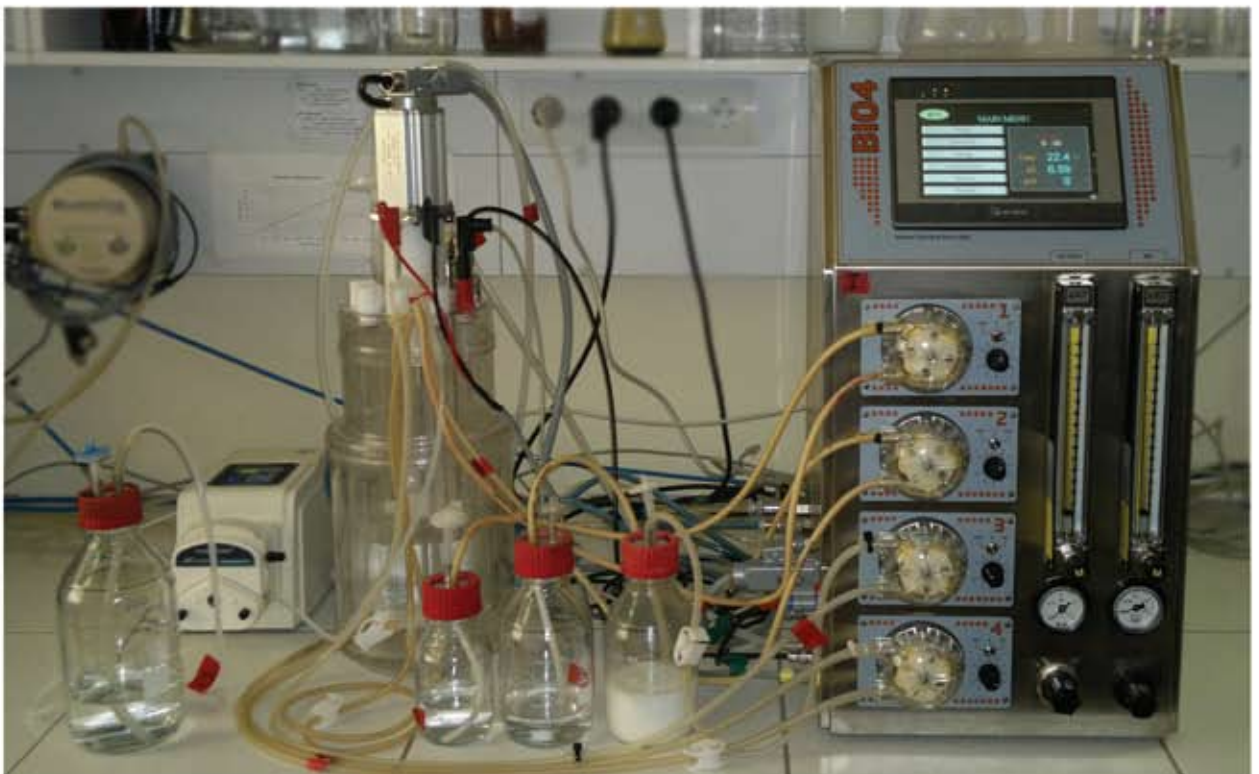
SCADA for Laboratory bioreactors BioRe

BioRe is user friendly SCADA software to control laboratory fermentation process and store the process data. Additional sensors, balances and devices can be integrated in SCADA. BioRe can operate on a single PC, network or remote via Internet. It can control one or several bioreactors. BioRe ensure the possibility to communicate with MATLAB based program via OPC server. MATLAB program ensures fed-batch automatic control by on-line comparing of mathematical model with process off-line data. The user interface acts through SCADA.

BioRe is develop according the requirements of 21 CFR Part 11 (document from US Food and Drugs Administration). That means that process can be evaluated, reviewed, approved and archived without a single sheet of paper and without the possibility to falsify process data and signatures. Software development is based on ARC Informatique PC Vue industrial development package.

The examples of screen shots explaining the possibilities of BioRe:

The main part includes important current process information and ensures easy access to another windows

The report of all alarms

Data visualisation (graphics, table) for current and archivated process, including off-line data

Adjusting of set points, process configuration and another settings

Interactive control of bioreactor

The user friendly recipe interface

The report of events and operator actions

Easy generating of reports from archive

SCADA communicate with MATLAB based program to ensure fed-batch control

Access level of every operator is defined through passwords

The program drivers ensure the communication between the software and many popular PLC. The communication can be provided also with the help of OPC server, which gives the possibility to connect software with control units in wide range. We are open to adapt the program for special requirements of Customer.

Pilot scale bioreactors

We offer stainless steel bioreactors from 20 to 15000 liters, as well as bioreactor lines, including other technological vessels.

An autonomous bioreactor consists of 3 main parts:

1) Bioreactor vessel;

2) Technological stand;

3) Power cabinet, including PLC.
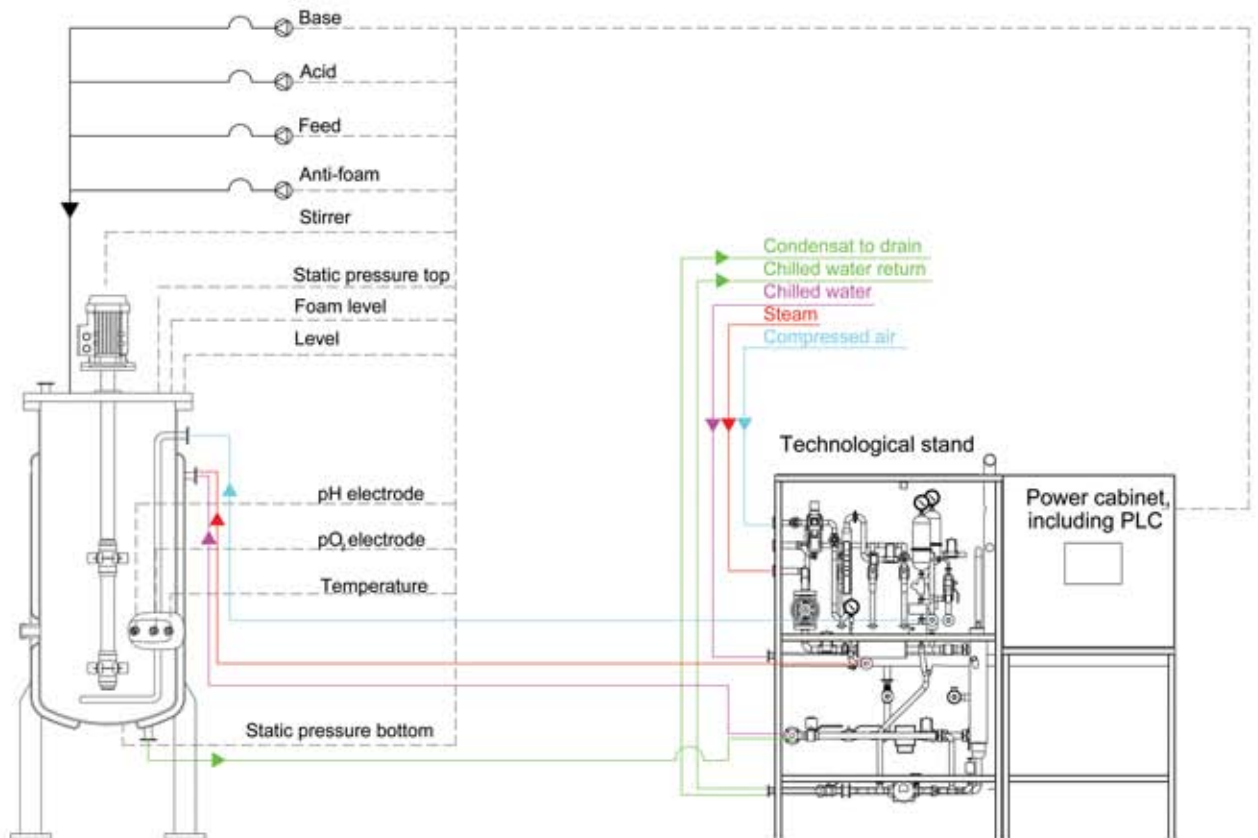
The connections between the bioreactor vessel and the technological stand are implemented with the help of flexible parallel wrapped stainless steel tubes.

To ensure fully automated in-situ sterilization process, it is necessary to install pneumatically controlled membrane valves in the vessel:

1) In the bottom;

2) In the inlet of a sparger;

3) In the lid.

The bioreactor vessel

The fully equipped stainless steel bioreactor vessel (sterilizable in place) with upper or lower magnetic drive for agitation with 2 – 3 standard rushton turbines (pitched blade or marine mixer optionally). For all vessels H:D is in the range of 1:1.5 to 1:3. It can be specified in certain limiting sizes. For all vessels the maximal pressure inside the vessels – 3.0 bar, in jacket – 3.5 bar. Maximal temperature – 140°C.

Inner vessel wall: surface roughness lower than 0.4μm.
Outer vessel wall: surface roughness - 0.63 μm (pickled & passivated).

The material of parts, contacting with product is stainless steel 316L. For parts, not contacting with product (for example, jacket), − stainless steel 304. All bioreactors have insulation over the jacket. The outside surface of insulation is covered by stainless steel 304.

The aeration system includes a ring sparger with maximal flow rate of 2 vvm. DN=25mm ports according to Ingold standard are installed in the lower cylindrical part for inserting sterilizable pH, pO2 and temperature sensors, as well as a DN=25mm port according to Ingold standard for the sterilizable sampling device.

A stainless steel membrane pressure sensor (Endress+Hauser) and a pneumatically controlled membrane valve are placed in the bottom of the vessel.

In the upper cylindrical part there is a longsided sight glass.

Following accessories are placed in the lid: Membrane pressure manometer, safety valve, needles with membrane valves for titrating (2-4), sight glasses with lighting, overpressure sensor with control signal (Endress + Hauser), foam and level sensors, CIP washing head, additional sanitary tri-clamp based connections.

Technological stand

Technological stand includes pipeline connections for supply of air, purified water, steam, as well as for filtration of air, thermostating, sterilization, sampling and discharging. A group of pneumatically controlled valves ensures fully automatic control of sterilization and thermostating. Inlet and outlet air supply filters (PALL MCY4463PFRPJ), as well as air pressure control system and stainless steel air supply control rotameter (MFC and system of O_2 enrichment - as options) are also installed in the stand.

The technological stand is connected to the control cabinet, providing the following fully automated processes:

1) Temperature control in the bioreactor vessel through jacket by heating or cooling. The heating is possible with the help of electrical heating or steam injection;

2) Pre-heating of the vessel with the help of steam injection;

3) Cooling of the vessel after sterilization;

4) Sterilization of the empty vessel, including sterilization of inlet and outlet air filters;

5) Sterilization of the vessel containing substrate;

6) Separate sterilization of the air filters.

For example, sterilization of the empty vessel is provided by the following main steps in automatic mode:

1) Filling of the jacket and circulation contour;

2) Ventilation of the vessel and air filters;

3) Water heating of in the circulation contour;

4) Interruption of the vessel ventilation when achieving the adjusted temperature in the vessel;

5) Sterilization of air filters;

6) Sterilization of the sparger and pipelines;

7) Holding of the sterilization temperature for the adjusted time;

8) Starting of gradual cooling;

9) Discharging of condensate from the vessel and filters;

10) Starting ventilation of the vessel and filters;

11) Cooling until the adjusted final temperature is achieved.

Control cabinet

Power control cabinet is made of stainless steel (RITTAL, Germany) and includes the following elements:

1) Siemens Simatic seriesS7-300 PLC;

2) Touch screen display (7" or 12");

3) Pneumatic pilot valves;

4) pH and pO2 sensors signal transmitters;

5) Power protection and switching devices;

6) UPS;

7) Network communication equipment.
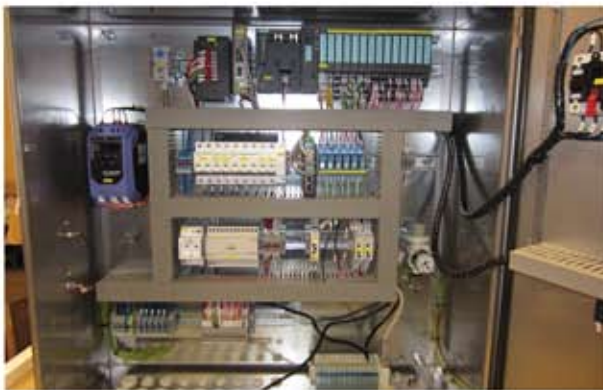
The following parameters are controlled in basic version:
- Temperature;
- pH;
- pO2;
- lower and upper static pressure;
- foam;
- upper emergency liquid level.

There is an additional possibility to connect:
- Exhaust gas flow O2 and CO2 measuring;
- Overpressure control of in the vessel;
- Additional peristaltic pumps;
- Mass flow controllers (MFC);
- Additional gas supply and mixing (O2, N2, CO2).

The PLC can be connected to Profinet and Ethernet networks for communication with other devices (bioreactors, CIP/SIP, SCADA and etc.). The PLC allows selecting of the following processes control:

1) Fermentation;
2) Different variants of sterilization (SIP);
3) Pre-heating;
4) Cooling;
5) CIP;
6) Loading and discharge of the substrate or biomass.

With the help of user friendly operator interface the following process preparation and control operations can be provided:

- Adjusting of set points;
- Sensor calibrations;
- Process and devices Configuration;
- Graphical presentation of the process;
- Adjusting and displaying the state of alarms;
- Start / stop of selected processes;
- Working mode of devices (manual, automatic or the profile according to time)
- Adjusting of substrate feeding profile;
- The determination of access level for every user.
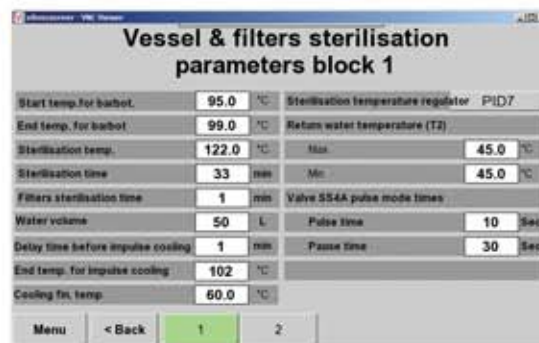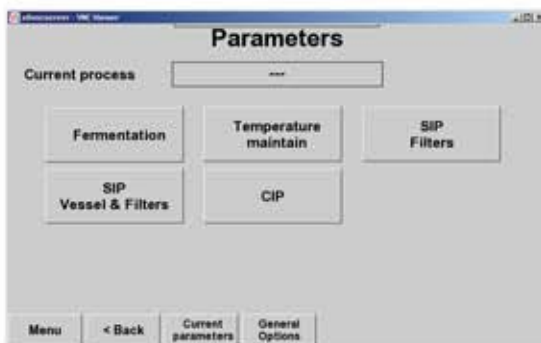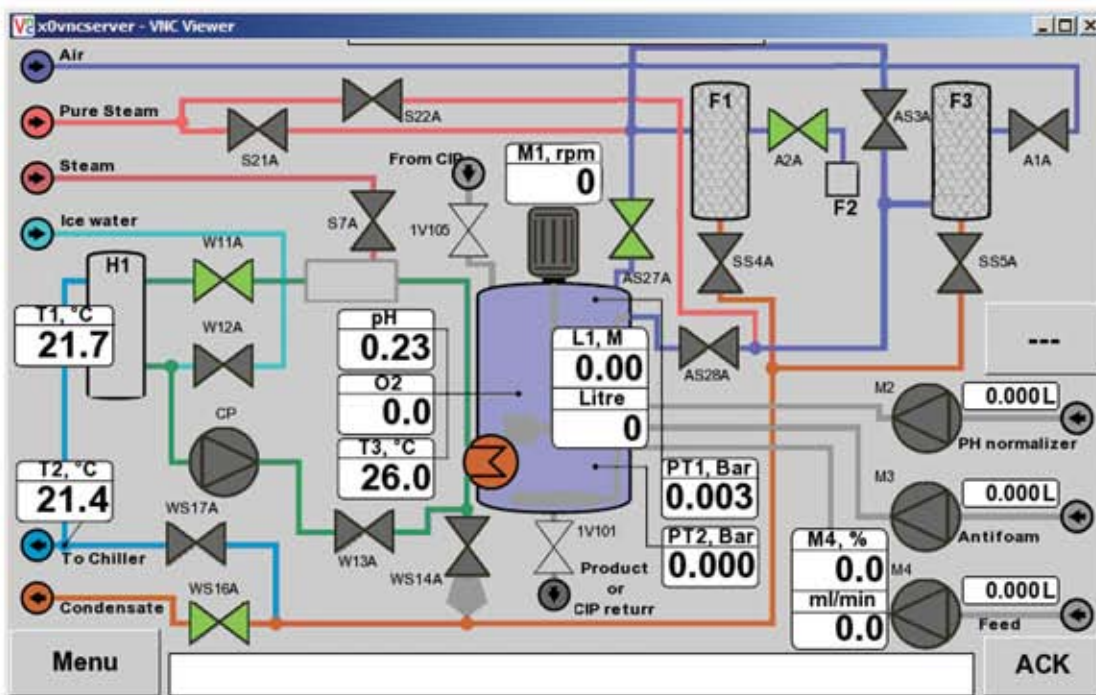
Magnetic mixers for bioreactors and other aseptic reactors

We manufacture, deliver and install patent pendeding novel magnetic mixers. Compared to other magnetic drive mixers, these ones are developed to have following advantages:
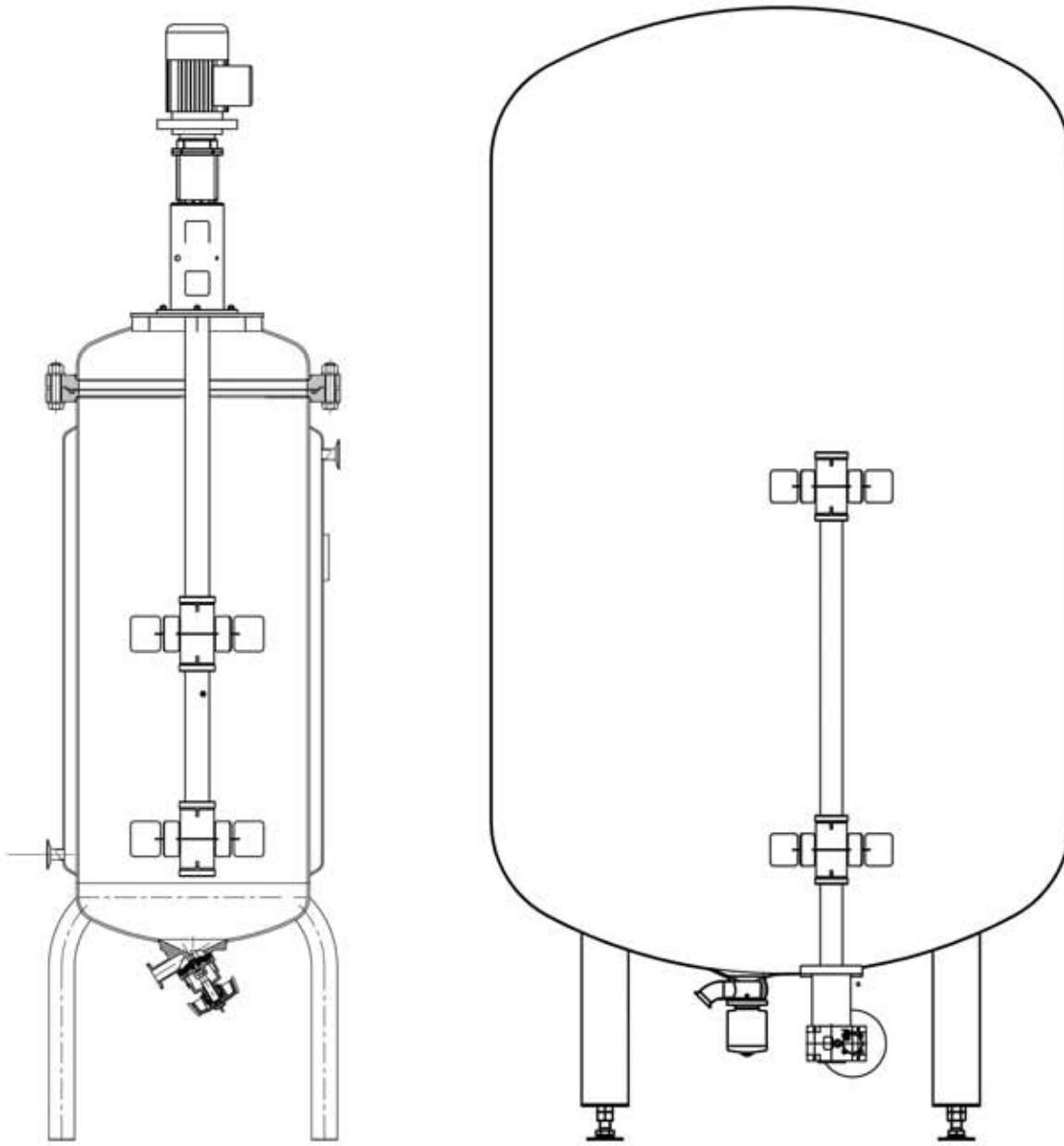
1) Decreased losses of energy due to the transfer of the torque through magnetic coupling to mixers;

2) Simpler mounting in the bioreactor vessel and maintenance during exploatation.

The novel magnetic mixers can be used with top or bottom motor drives.

These magnetic mixers can be installed in newly developed bioreactors, as well as in already existing bioreactors vessels and other reactors. We have experience in application of the offered magnetic drive both in laboratory and industrial bioreactors with volume up to 15000 liters.

Automated CIP equipment

We offer two kinds of CIP (Cleaning In Place) equipment: mobile and stationary.

Mobile CIP equipment

This system is an equipment unit, placed on a movable platform. The platform is easy to move around in the production facility to connect it to the CIP washed fermenter or other technological vessel. Maximum speed of detergent circulation – 5000 l/h (can be varied).

The CIP platform contains two centrifugal pumps (for CIP supply and return), two dosage pumps with two plastic vessels, pipes and fittings for detergent concentrate, a control cabinet with a touchscreen operator panel, manual and automatic valves, couplings and armature to provide the washing operation.

The mobile CIP unit allows washing of fermenters and other vessels with total volume of 20 to 1000 liters.

The mobile CIP unit allows implementing of both manual and completely automatic modes of washing process. The automatic mode of operation has to be adjusted (or selected) in each case. These operations can be done from the operator panel.
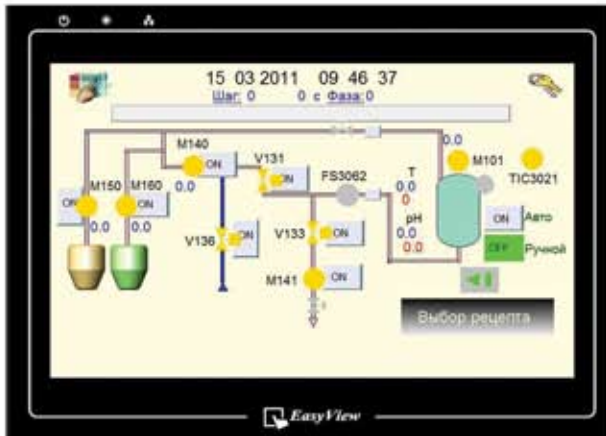
CIP washing mode consists of stages, but each stage – of steps. In fact, a stage of the washing process is considered as realization of definite type of washing process with selected parameters (temperature, time of CIP washing, time of discharge output, detergent pH, speed of concentrate supply, fast speed of mixer rotation, slow speed of mixer rotation, speed of CIP washer pump, duration of washer impulse and a pump interval at impulse working mode etc.).

In automatic mode it is possible to perform the following operations of CIP washer:
- Preliminary rinsing;
- Washing with alkaline detergent;
- Rinsing;
- Washing with acidic detergent;
- Final rinsing.

The automatic process of CIP is carried out according to a selected recipe. The recipe includes the defined stages, and steps with corresponding parameters. Any phase of washing consists of definite sequential steps. According to the selected mode of washing process, steps can differ. These steps can be, for example, valve opening, start of a pump, start of the mixer and other events, connected with switching on or switching off the working appliances. Parameters of steps are given in the instruction and according to the necessity can be changed. In that way the instruction consists of a definite sequence of washer phases. The maximum amount of washer phases is– 12. In the automatic mode of CIP washer it is possible to automatically stop and finish the work process.

Stationary CIP equipment

Stationary CIP equipment is customized depending on the requirements of particular production. This equipment is foreseen for CIP/SIP washing of bioreactors and other technological vessels, as well as for CIP/SIP washing of the pipelines of the technological line.

There are two working regimes of stationary CIP/SIP equipment: washing and sterilization. These regimes differ with settings, adjusted from operator panel or SCADA.

The connections of steam line, cooling water, compressed air and discharge can be provided according to the following diagram (one of the possible variants is introduced here).

CIP system P&ID
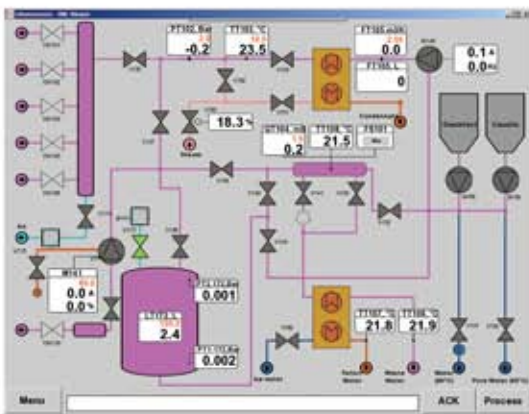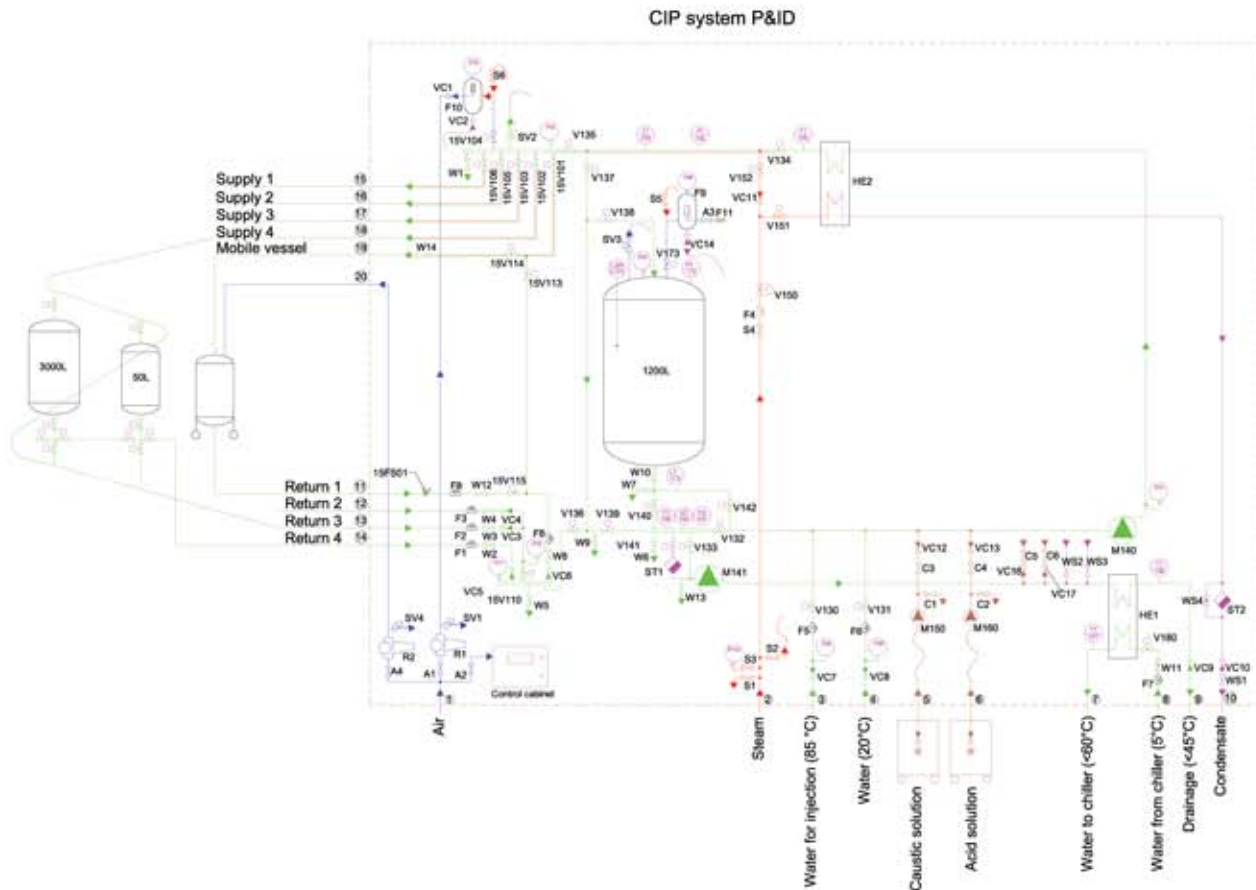
CIP/SIP allows carrying out processes in two variants:

1) Locally by operator control directly from the operator panel of CIP/SIP;

2) Centrally by operator control from a remote operator panel or SCADA system.

The process of CIP/SIP is fully automatic. But, if necessary, a particular device or process can be switched to manual control.

The automatic process of CIP is carried out according to the selected recipe. The recipe includes stages and, steps with corresponding parameters. Any phase of washing consists of definite sequential steps. According to the selected mode of washing process, steps can differ.

In automated mode it is possible to perform the following phases of the process:
- Preliminary rinsing with cooled water;
- Preliminary rinsing with hot water;
- Washing with alkaline detergent;
- Rinsing with cooled water;
- Washing with acidic detergent;
- Final rinsing with cooled water;
- Final rising with injection solution;
- Washing and sterilization of pipelines.

It is possible to add new phases according to the requirements of particular production. Parameters of each phase can can be adjusted to provide optimal working of CIP/SIP.

Stationary CIP/SIP gives the possibility to carry out the processes according GMP requirements.

Complex bioreactor lines for biotechnological production

We have experience in application of complex bioreactor lines for biotechnological production. We develop technical requirements, project documentation and qualification documents, manufacture equipment and provide the delivery, mounting and start up.
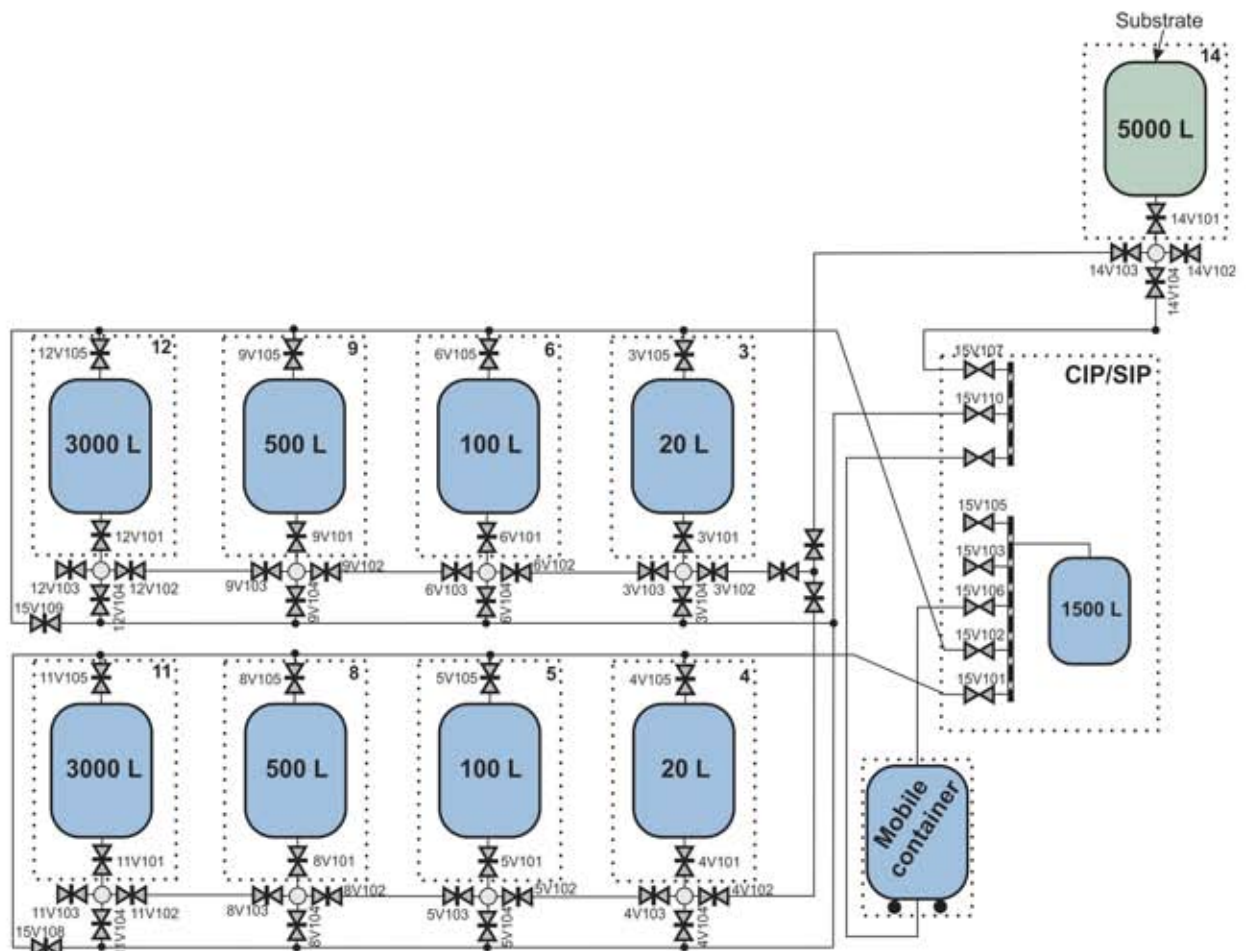
The technical solutions are customized depending on the requirements of specific production.

As an example, a following block diagram of a biotechnological production facility is shown here.

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

1) Two identical production lines, comprising bioreactors with volumes of 20, 100, 500 and 3000 liters;

2) A system of connecting heat-insulated pipelines and groups of membrane valves which connect the referred bioreactors;

3) Stationary CIP/SIP systems for automated cleaning and sterilization of both bioreactors and connecting pipelines and valves;

4) Nutrient media preparation and transfer area;

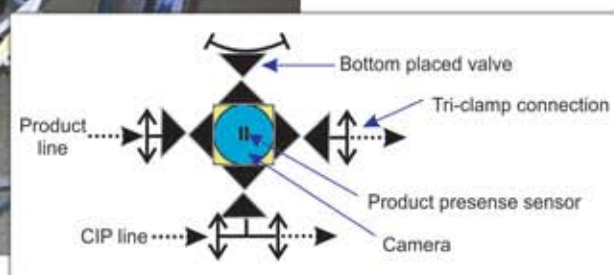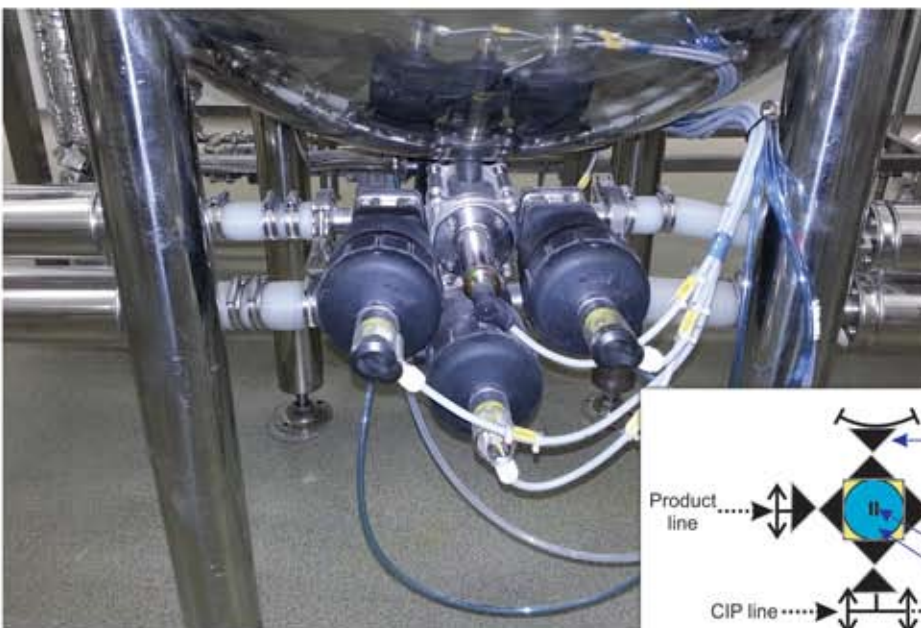5) Mobile containers with junction points to the CIP/SIP systems.

All bioreactors and other technological equipment are connected with the help of heat-insulated pipelines and groups of aseptic membrane valves.

The group of pneumatically controlled membrane valves has been developed according to the following diagram:

Each valve has an installed position sensor, and each group of valves has a vibration sensor for media presence detection. That design of the membrane valve group ensures sterile transport of liquids - substrate, product and CIP solutions.

For convenience, the automated control system can be divided into 3 parts:

1) Bioreactor and CIP/SIP local automation systems;

2) Central integrating cascade control system;

3) Computerised control system SCADA.
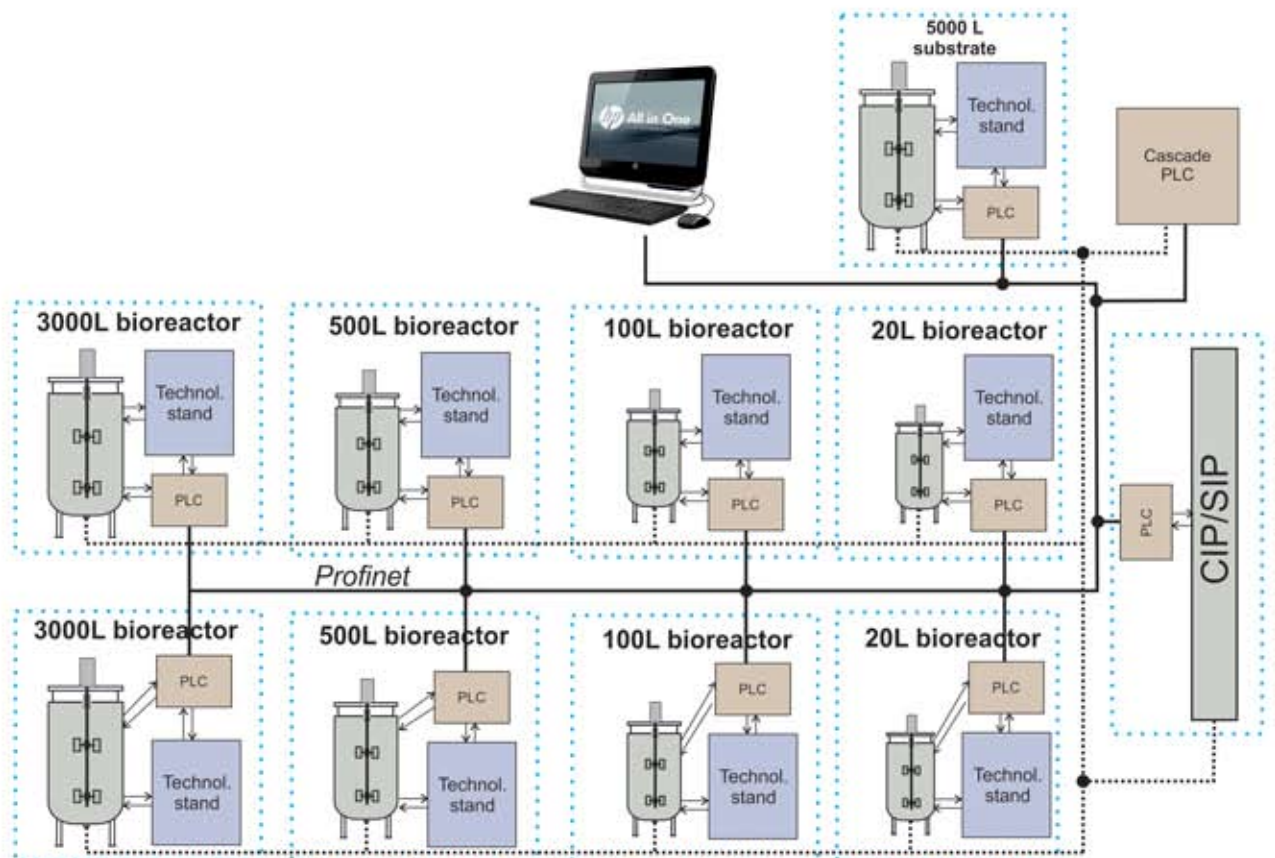
Each bioreactor is equipped with a process stand for ensuring the automated fermentation, cleaning, sterilization, heating and cooling processes. Each bioreactor vessel and process stand is equipped with pneumatic elements, mixer, circulation pump and peristaltic pumps. The bioreactors have built-in pH, pO2, temperature, maximum liquid level, foam level and pressure sensors. All sensors and control elements are connected to a corresponding local bioreactor control system, where the local control is performed from a colour touch screen display (with 7" or 12" diagonal). In the given example, the bioreactor control systems used Siemens Simatic S7 line industrial controllers.

Thus the local control of each particular bioreactor is ensured separately from other bioreactors. The central cascade control system ensures the interaction between different bioreactors and the CIP/SIP system, as well as with the computerised SCADA system. For that purpose a more powerful Siemens Simatic controller is used in the system than in each biorector. Actually, the central cascade control system controls certain groups of valves, depending on the selected recipe and the status of the respective sensors in the bioreactors, as well as in the CIP/SIP system and pipelines (medium presence sensors are built in in the valve groups).

The central cascade control system directly controls the following processes in the selected bioreactors or pipelines: CIP cleaning, SIP sterilisation, nutritive medium feed, biomass transfer from one bioreactor to another, and bioreactor emptying.
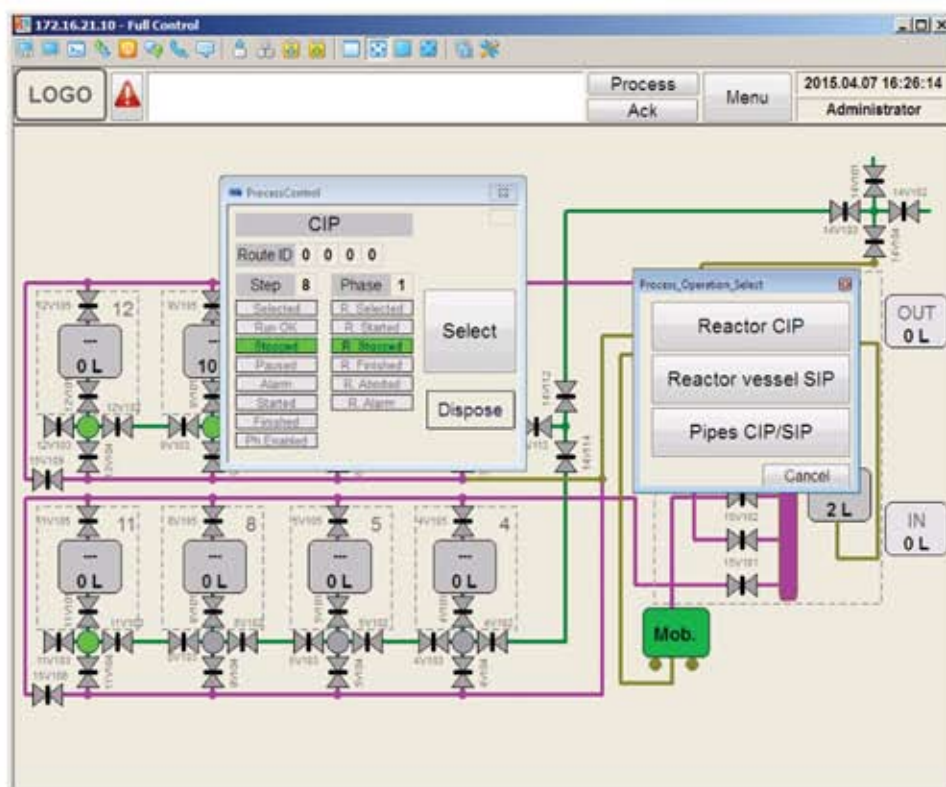
The best compliance to the GMP requirements for biopharmaceutical production is provided by the visualization and control software pack (SCADA), developed in compliance with the requirements of 21CFR Part11 (US FDA). In addition to basic control and visualisation functions, in this case the SCADA software also ensures complying with the requirements for electronic records, authorised system access sign-in and operator activity registration log. Our company has developed its own russified SCADA software, that allows creating an efficient biosynthesis process control system. The software is designed on the basis of an industrial software package in compliance with 21CFR Part 11.

The software (SW) SCADA performs the following key functions:

1) Process selection;

2) Recipe composition and storage selection;

3) Process and device condition control visualisation;

4) Graphical process and parameter display;

5) Control system data archiving;

6) Operator activity log;

7) Alarm log;

8) Several access levels to the SCADA system;

9) Remote access to the system via Internet.

Such an automated process structure in the biopharmaceutical production is simple and easy to work with. Combining the local control in separate bioreactors with the central cascade control system of the entire process line enables fast access to separate devices, without losing control over interrelated processes in the whole system. As a result, the operator mistake risk is minimised and automated management of the process is ensured along with documentation in compliance with the up-to-date GMP requirements.

Process automation

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

Automated can be our manufactured lines of bioreactors as well as the devices and units of other manufacturers. The application of specialized version of SCADA according to 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 that is required for finishing turnkey 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 visualization systems (SCADA);
- Mounting of control cabinets;
- FAT (Factory acceptance test);
- Installation and cabling of control cabinets and electrical equipment - on site;
- SAT (Site acceptance tests);
- Start-up operations;
- Staff training.

We have experience in working with different types of controllers and visualization systems.

A) Controllers (PLC)
- Siemens Simatic S7, Pcs7;
- General Electric – Series 90, Versa Max;
- Schneider Electric – Modicon, Twido;
- BTC – BIO3;
- Mitsubishi Electric – Fx series;
- VIPA – Speed7;
- FATEK – FBs series.

B) Visualization systems (SCADA):
- Siemens – WinCC;
- General Electric – Cimplicity;
- ARC Informatique – PcVue, Plant Vue;
- Beijer – Citect.

Our company is a certified partner of Siemens and is authorized to use the name "Siemens Partner Automation".
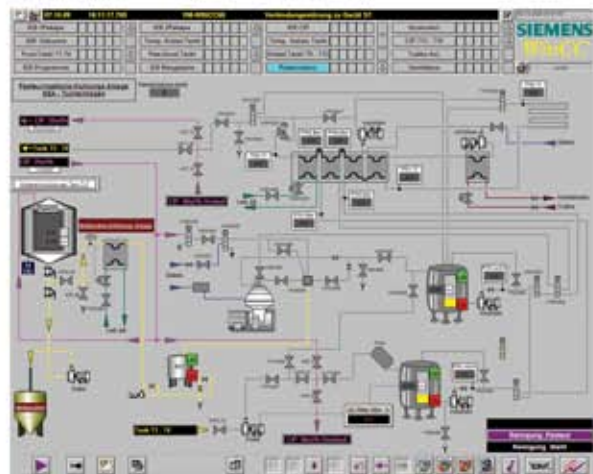
Automatic Substrate Feeding

In fermentations, where it is necessary to provide increasing high density biomass (for example, it is typical for recombinant protein biosynthesis, using E.coli strains), the choice of optimal substrate feeding profile is substantial. Scientific publications describe specifically created experimental systems for implementation of fermentations with automatically controlled feeding, based on mathematical models. Despite this, commercially available bioreactor controllers only provide possibilities for operators to set feeding profiles by time and to control substrate feeding speed depending on the pO2 sensor readings.

A particularity of the fermentation processes is the impossibility of providing fully repeatable fermentations, even under absolutely identical cultivation conditions. This is both due to the properties of the strain of cultivated microorganism used and due to other not always known factors. As a result, for ensuring optimal feeding profile it must be constantly corrected depending on the particular fermentation process implementation. It means that the definition and input of updated feeding profiles directly depend on the skill of the operator. Considering that this operation requires a very high level of training and skill of the operator, the risk of error is relatively large, which is contrary to the modern requirements of GMP. It means that solutions must be sought in order to reduce dependence on the operator, when performing the above technological operation.

At certain stages of the fermentation process, it is possible to successfully perform the automatic feeding based on the pO2 sensor readings. But there are several problems here. Firstly, in this event it is impossible to control the pO2 concentration by rotation speed and/or oxygen-enriched air supplied to the aeration, and secondly, when relatively high biomass densities are reached, it is impossible to implement this control in an optimal way.

Summarizing the above, it should be noted that the limited capacities of controller (PLC) and process visualization software system (SCADA) lie at the base of the automatic feeding implementation problem during the fermentation processes. The above limitations are due to the compulsory use of the differential control system solutions in order to calculate the optimal feeding profiles.

In order to solve this problem, we have created a fermentation control system according to the following flowchart.

Automatic Substrate Feeding
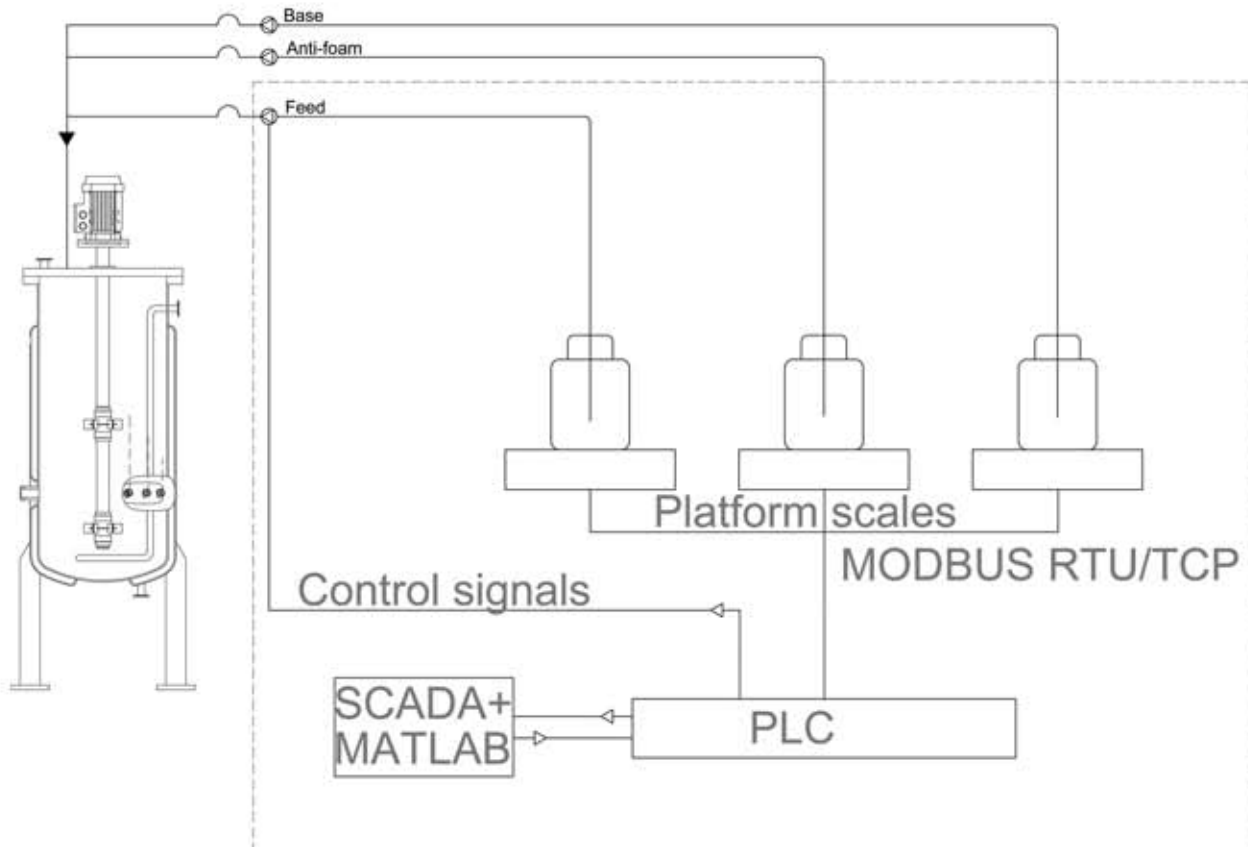
This flowchart assumes that the user already has a complete bioreactor in order to implement fermentations. The system operates as follows. Additional equipment and accessories for automatic substrate feeding are highlighted by a dashed line in the flowchart. Placed on the platform scales are the bottles for storing of alkalis and/or acids, the antifoam, the substrate used for feeding and other substances, because it is necessary to know rather precisely the current weight of fermentation medium for analysis and simulation of the process. The platform scales are connected to the controller (based on Siemens Simatic) via serial port RS485 using exchange protocol Modbus RTU. The software SCADA for substrate feeding control must run simultaneously (up to several minutes) when starting the fermentation process.
In our control system, the software SCADA for substrate feeding control must run simultaneously (up to several minutes) when starting the fermentation process.

In our control system, the software SCADA is connected to the feeding simulation software developed on the MATLAB base. Before starting the fermentation process, the initial data must be put into the software SCADA — the volume of the culture medium, the biomass and glucose (or other source of sugars) concentration, and also the initial feeding profile must be set (calculated using the MATLAB).

During the fermentation process, samples are taken in order to input the current results of the tests on biomass and glucose (or other sugars) in the software SCADA. Performed the input of the results of the tests, the software MATLAB performs

automatic comparison of these results with the results of calculations according to the adopted mathematical model. If the deviations are above the set standards, the software MATLAB performs calculation of the new feeding profile. The updated feeding profile is automatically uploaded into the controller, and the substrate feeding is further implemented according to the new profile (up to a following update).

E.coli fermentations were carried out using this control system. Automatic substrate feeding cultivation processes were compared to the processes whose optimal feeding profiles were set by the operator, before starting the cultivation.

The chart below shows the automatic feeding biomass concentration in green color, while the operator-set feeding biomass concentration is in brown. The chart shows approximately 20% higher biomass concentration in fermentations with automatic feeding. In fermentation without automatic feeding, increases in glucose concentration (brown triangles) are observed, in the end of the fermentation, causing accumulation of acetate, which has an inhibitory effect on the growth of the culture.
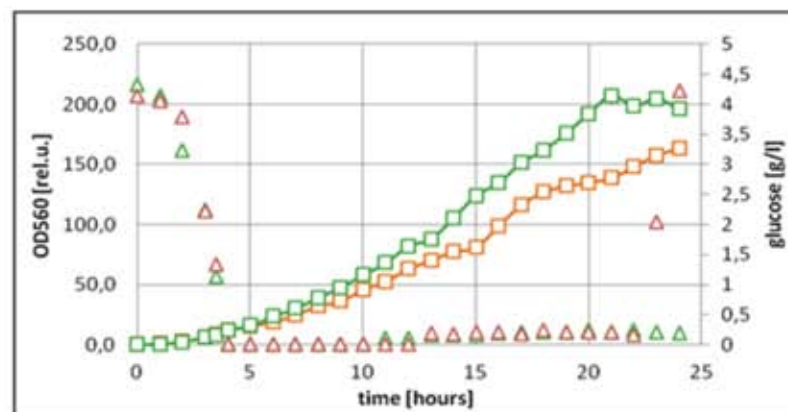
As practice shows, the application of this control system not only makes it possible to substantially increase the efficiency of fermentation, E.coli in particular, but it is also user friendly. This is due to the circumstance that all calculations are automatically performed, and therefore the human factor is practically completely eliminated in the choice of optimal feeding profiles.

The above fermentation process control system can be successfully used at the stage of technology development, as well as in the industrial fermentations that meet the modern requirements of GMP.