DEVELOPMENT OF CHEMOSTAT SUPPLY SYSTEM DURING FERMENTATION PROCESS

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Abstract. At present automation of biotechnological processes is an extensive industry in which many scientists are working. New bioreactors have wide functionality and can be easily supplemented with additional modules, sensors and control systems. Older bioreactors mostly do not have such ability, therefore, to produce the desired result additional systems can be used. In this paper automatic an feeding control system is described. It consists of a programmable logic controller, digital scales and additionally gas sensor can be used to obtain online information about the process. It can be easily modified and different sensors can be included in this system. Depending on the information from the scales and sensor, the peristaltic pump speed for feeding is controlled. It allows controlling the growth rate of microorganisms during the fermentation process. The described system can be used as a standalone system or together with the bioreactor.

Keywords: biotechnology, fermentation process control, feeding control.

Introduction

Biotechnology is an industry that deals with obtaining products for humans needs, using microorganisms. Many products of biotechnological processes are used in other industries, e.g., food, medicine, chemical, biofuel, agriculture etc. Modern biotechnology is a multidisciplinary industry. It involves many sciences such as biology, chemistry, process control and optimization. Biotechnological processes control equipment is being developed in the recent years, but the process control is still a complex task. Biotechnological processes usually take place in a bioreactor, an apparatus that maintains optimal conditions for the growth of microorganisms, but it can take place also in a flask [1; 2].

Performing fermentation from the point of view of engineering realization is – providing substrate fluxes according to technological specialisation, drawoff liquid with culture, measurement and sustaining physico-chemical parameters, ensuring and controlling optimal concentration of substrates and products for specific culture. Two most popular types of fermentation processes are fed-batch and continuous processes. In the fed-batch process, a fresh substrate is supplied into the bioreactor continuously or in portions. When the bioreactor is full, it is partially or completely discharged. The process is finished or resumed. In the continuous process or chemostate, the solution cultivated in the bioreactor is continuously discharged [2; 3].

A chemostat is a bioreactor to which fresh medium is continuously added, while the culture liquid is continuously removed to keep the culture volume constant. By changing the rate with which medium is added to the bioreactor the growth rate of the microorganism can be easily controlled. The supply of fresh medium is provided via a peristaltic pump, which works with constant speed. For biomass output also a peristaltic pump is used, which speed is higher than the feed inlet pump [1].

The continuous process can proceed for a very long time, and its duration is commonly determined by the production requirements and technical factors. The most interesting phase of the process is the stationary phase. As soon as the substrate is metabolised the growth slows down or stops completely. The biomass increases only gradually or remains constant during this stationary phase, although the composition of the cells may change. New substrates are released, which then may serve as energy sources for slow growth of the survivors. Various metabolites are formed in the stationary phase and they often have great biotechnological interest [1; 2; 4].

A build in function to change the feeding profile by time in many bioreactors is implemented. It is possible to control the growth of the microorganisms using different feeding profiles. In some older models this function is not available and a peristaltic pump is used with constant speed is used for feeding. But often the need for dynamically changing profile appears – the feeding flow should change depending on some parameter of fermentation or it is often necessary to change the feeding profile. Not all bioreactors allow such functionality. At the same time during long fermentation some problems connected with chemostat support may appear, e.g., the tubes may become soft and it will change their throughput. This means that the feeding flow will change and all parameters of the process will be affected [2].

As the measurement instruments for substrate flow a scales can be used, that measure the change of weight in the substrate bottle or bioreactor. Similar systems are offered by many producers of bioreactors, e.g., *Sartorius* or *NewBrunswick* but the price for the additional equipment is quite high and that usually is a limiting factor choosing of the bioreactor. Many pump manufacturers offer pumps with a constant pumping speed but not all of them provide a possibility to set the flow speed, e.g., in ml/min, so the feeding speed stays constant all the time. This can be done only with experience or by pump calibration at the beginning of each fermentation, as this may vary depending on the tube diameter and pump settings. Some perestaltic pumps also have a function to set a time feeding profile, e.g., *Verderflex*, but such pumps have a high price and they are not widely available [2; 4-6].

The aim of the present work was to develop a convenient and advanced feeding profile control system. The system is based on the Siemens programmable logic controller and digital scales. For the system control a touch screen panel is used. The flow speed, system configuration parameters and pump calibration can be entered on the screen. In the weighting system an automatic stabilization program is used. An additional subprogram for impulse feeding depending on the time and CO_2 parameters was developed. This information can also be used to adjust the feed rate and control growth rate. It can be useful to explore the influence of the impulse feeding on the growth rate. The described system will be used with the Infors HT Labfors 2 bioreactor. At the same time the system should eliminate loopholes in the systems which are already on the market [7].

The proposed feeding profile control system is also particularly useful in bioreactor systems where intensive research activities are taking place. It allows making experiments with the feeding impact on the culture growth. The concept of the described system may become a solution that can be implemented in bioprocess control systems that are currently available on the market.

Materials and methods

The feeding profile control system consists of *Siemens S7-224XP* programmable logic controller (PLC), *Siemens Siwarex* digital scales, *Siemens SIMATIC* device (HMI) and *Masterflex* peristaltic pump. Additionally *Bluesens* O_2 and CO_2 gas sensors were used to get information about the process. The devices are connected using different communication protocols. A control program for PLC and interface for HMI were developed. The system is compact and easily transportable.

Structure of the system

As mentioned above, the two most popular types of fermentation are continuous and fed-batch. The main difference is the duration of the process – fed-batch fermentation lasts until the bioreactor tank is full, then the process is terminated but the continuous process can last for weeks. A part of the culture is continuously removed. In Figure 1the structural schemes of these two processes are shown.

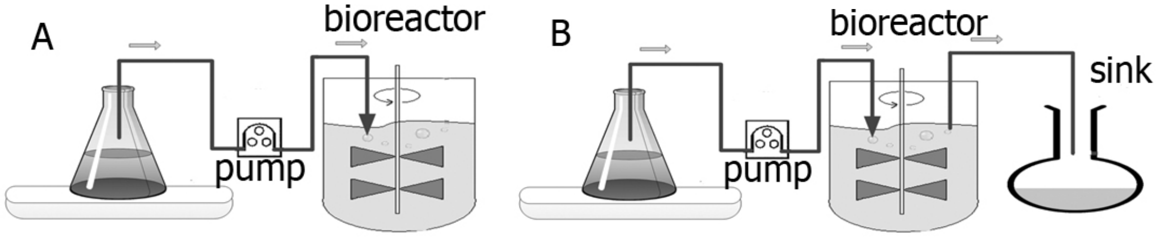

Fig. 1. **Schemes of fermetation processes:** A – fed-batch, B – continuous

Substrate from the flask is pumped into the bioreactor tank with constant speed or depending on the time schedule if the bioreactor control system or the pump have such functionality. In our case bioreactor does not have the possibility to control the feeding speed, therefore there is a need for an additional system.

Digital scales are added in the bioreactor system on which the flask with substrate is situated. The gas sensor was connected to the outgoing gas tube. To measure the data from the scales and the sensor a programmable logic controller (PLC) was added. The HMI for the user interface was used. The change of the substrate weight is measured and the feeding flow is calculated, so it can be controlled. Also a computer can be used to see the state of the process and change the software versions on PLC and HMI. The scheme of the proposed system is shown in Figure 2.

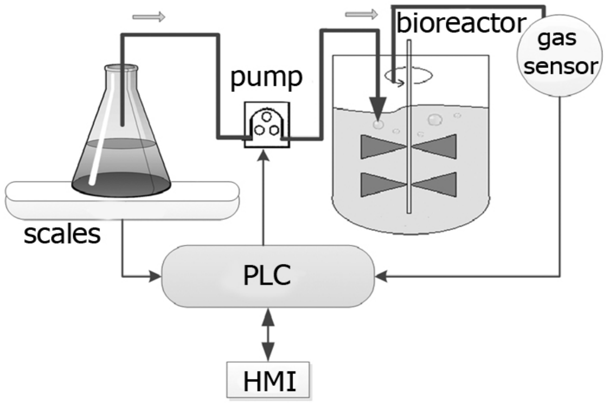

Fig. 2. **Scheme of proposed system**

Using the proposed system does not depend on the type of the fermentation process. The schemes introduced above should be supplemented with additional components (they can be present also in the existing bioreactor system)**.**

Parts of the system

The *S7-224XP* PLC series is a line of micro-programmable logic controllers (Micro PLCs) that can control a variety of automation applications. The compact design, expandability, low cost, and powerful instruction set of the *S7-224XP* PLC make a suitable solution for controlling small applications [8].

Digital scales *SIWAREX* R type SP were used. This is a sensor that converts the mechanical variable (i.e., weight) into an electrical signal. The basic element is a special type of spring body to which friction-locked strain gauges are attached. The strain gauges are made of a thin insulation material in which a resistance film has been integrated. Under the influence of the weight force F, the spring body is deformed and as a result the strain gauge deforms elastically. Due to the change in the external shape of the strain gauge, the ohmic resistance of its conductor also changes and the electrical signal to PLC module is passed. The maximal weight is 6 kg and the accuracy is ±0.02 %. The reaction time is less than 100 ms [9].

The HMI system represents the interface between the operator and process (machine/plant). The HMI system assumes such tasks as process visualization, operator control of the process, displaying alarms and process and machine parameter management. The Simatic OP 177B DP operator panel is used [6].

A peristaltic pump, or roller pump, is a type of positive displacement pump used for pumping a variety of fluids. The fluid is contained within a flexible tube fitted inside a circular pump casing. In this model remote speed control also is available using analog signal 2-10 volts.

Bluesens gas sensors were used to get online information about the process from outgoing gas. The CO_2 and the O_2 gas sensors in PA6 housing were integrated with the analog-to-digital converter (ADC) by *BTC* company. It allows making online monitoring on the PC screen. Also the analog signal from the sensors is connected to the PLC's ADC. It allows using the data from the sensors in the control system. The concentration range of the sensor is 0-10 Vol. %, the accuracy is ±2 % of the value and the reaction time is 1 minute.

Communication types

In the system all elements are connected to the PLC and use different types of communication. The scales are connected using Siemens Siwarex interface, which analog signal from the scales is converted to the digital one. To use it to PLC Siwarex MS extension module should be connected. *SIWAREX* MS (Micro Scale) is a versatile and flexible weighing module, which can be used wherever the scales are to be used in the *SIMATIC S7-200* automation system. The outgoing signal from the gas sensor is analog signal 4-20 mA but PLC *224XP* have only one analog input 0-10 volts. For this reason additional analog-to-digital converter *EM235* should be used. The HMI is connected to the PLC using the MPI protocol. It uses the RS485 ports to exchange the data. The data from the PLC are automatically updated on the HMI and the data entered from the HMI are updates on the PLC. The delay in data transfer is about 100ms. The PLC controls the peristaltic pump using the analog signal 2-10 volts from the built-in digital-analog converter (Fig. 3).

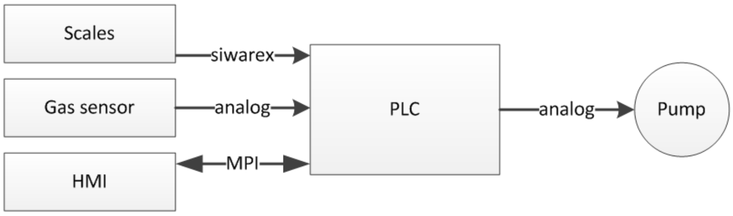

Fig. 3. **Communication scheme in system**

Using the computer it is possible to monitor the process and change software versions. The PLC can be connected to the computer trough the PPI or MPI interfaces and using the software *STEP-7 Micro/Win* the control program is developed and installed on the PLC. The HMI can be connected to the computer trough the MPI or Ethernet interfaces. The easiest way is to use the Ethernet interface - it is possible to connect to the HMI remotely using the *WinCC flexible* software. The scales module is connected to the computer trough the Siwarex MS interface. For the calibration and configuration the *Siwatool MS* software is used. The gas sensor is connected to the EM235 analog-digital-converter and trough the Ethernet interface the *Adam* software made by the *BTC* company can read the measurements [9-10] (Fig. 4).

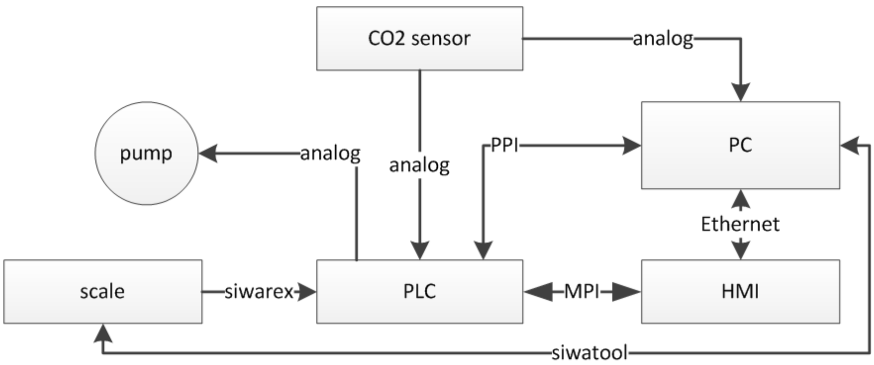

Fig. 4. **Communication scheme in system with PC**

Results and discussion

Four control subprograms for the system were developed, that control the feeding speed. The first one provides the constant feeding flow, e.g., can provide the chemostat state. It can be used for long term fermentations. It automatically adjusts the flow speed and can keep constant flow for a long time. The user sets the timer period for each calculation of the flow; the optimal time is 5-10 seconds. In the same way the flow setpoint and the volume of the substrate are set. The system variables and the pump speed are automatically calculated and the pump is switched on. After each time period the system parameters are recalculated and the pump speed is corrected. The adjustment error of the flow is ±3 % of the flow (Fig. 5, A).

The second subprogram is fed by the impulses of the substrate with the delay set by the user. The user sets the delay and the amount of the substrate. For example, every 10 minutes 5 mg of the substrate should be fed (Fig. 5, B).

The third subprogram is fed by the impulses of the substrate when the concentration of the gas is lower than the setpoint. It feeds the set amount of the substrate. Using of these program impacts of the substrate concentration change can by investigated (Fig. 5, C).

The fourth subprogram allows controlling of the growth rate of the microorganisms by controlling the outgoing gas concentration on the set level by changing the feeding flow. In our case it is the CO_2 gas. The gas concentration setpoint is set and the system feeds the substrate to the bioreactor until the needed gas concentration will be reached and then it switches off the pump. When the gas concentration is below the setpoint, the feeding pump is switched on. In this case a lot of experimental data are needed to adjust the setpoint and the flow speed (Fig. 5, D).

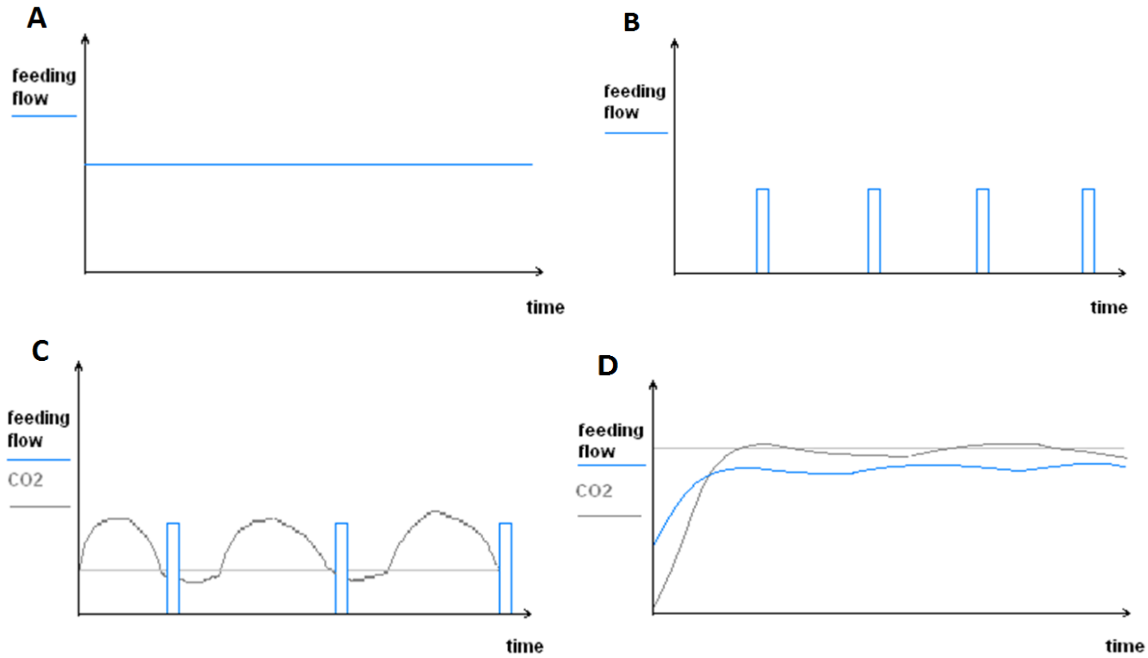

Fig. 5. **Time diagrams of feeding processes**

The PLC software and the HMI software were developed. The PLC has the possibility be programmed in three languages – *FBD*, *STL* and *Ladder Logic*. They are equivalents but in this system *Ladder Logic* was used.

The HMI software was developed in *WinCC flexible* software. In Figure 6 the user interface for the first subprogram is displayed. It consists of two parts – the configuration of the process and the system configuration. The scales should be set to zero, the tara also should be set with the empty bottle of the substrate to calculate the correct weight and the flow. Also the maximum throughput of the tubes should be set.

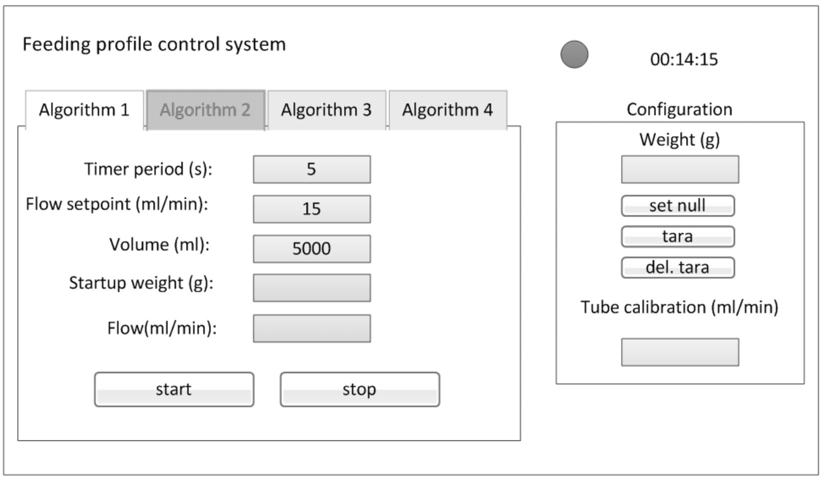

Fig. 6. **User interface on HMI**

On the display the indicator of the process state and the time of the process are present. In the program configuration area the timer period, the flow setpoint and the volume can be set. The system calculates itself the start up weight, the real flow and corrects it if necessary.

The system at the present time is tested in the laboratory of the Institute of Microbiology and Biotechnology of the University of Latvia. During the testing phase experiments in the flasks were done. It allows decreasing the costs of fermentation. The experiments were made with *Zymomonas mobilis* microorganisms in buffer in anaerobic conditions. The anaerobic conditions were provided with the N_2 gas supply into the flask. During the process *Z.mobilis* releases the $CO_{2\ gas}$ witch concentration is measured with the gas sensor.

Conclusions

1. The automatic feeding profile and the chemostat providing system were developed with four subprograms.
2. In the realization the Siemens microautomatic *S7-224XP*, Siwarex MS and *EM235* ADC hardware were used for the process automation. Additionally the Bluesens gas sensor was used.
3. The proposed system can be used like the stand alone system or together with the existing fermentation system.
4. The system can be used for different proposes, e.g. for the continuous fermentation or the fermentation in flask.
5. In the system additional sensors can be implemented and the control software can be easily edited and configured to adapt for the particular needs of the project.
6. The developed system bioreactor process controller with appropriate tooling was tested in real fermentations.

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