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DIGITAL APPLICATION FOR REMOTE CONTROL OF BACTERIAL ENDOPHYTES GROWTH IN BIOREACTOR VIA INTERNET AS A DESIGN SOLUTION TO A VIRTUAL LABORATORY

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Abstract

Defining and delimiting the notion of biotechnology is becoming increasingly complicated, as this field is connecting various branches of science and technology. Biotechnology advances are inextricably linked to the successes of bioengineering, as well as the development and implementation of equipment and means to control these processes.
The present paper aims to create a real time monitoring system for bacteral endophytes production carried out in the bioreactor. The advantage of this application allows not only locally monitoring of the process but also via INTERNET. The practical applicability of this system should be mentioned due to the effective possibility of using the bioreactor monitoring program in the educational process. Therefore, developing a software application for remote and real-time monitoring the technological process at bioreactor level is a central point of a virtual laboratory, an important element in achieving a quality educational process in the current pandemic context, a framework that has led to an increased need of digitization processes in all sectors of society.

Key words: *bioreactor, digitalisation, endophytes, INTERNET monitoring, virtual learning.*

INTRODUCTION

Biotechnology means the directed obtaining of compounds useful both in the national economy and medicine, through biological agents, such as microorganisms, animal, or plant cells, through extracellular substances and cellular components.
Defining and delimiting the notion of biotechnology is becoming increasingly complicated, this field integrating with various branches of science and technology, such as: microbiology and biosynthesis processes, molecular biology, genetics, and genetic engineering (to improve biological agents), biochemistry, physical chemistry and electrochemistry (creating biosensors), engineered technology (creating bioreactors and other equipment) (Larroche, 2016).
The main directions of biotechnology development are conditioned by two aspects: on one hand, by the demand in certain products and energy, simultaneously with the quantitative accumulations of unusable raw materials, including wastes, and on the other hand, by the appearance of new discoveries in fundamental research.
Emphasis is placed on developing technical solutions for biotechnological processes, by: (1) bioreactors design for the intensive growth of microorganisms and cell cultures, with strict observance of the homogenization in the regime of biological agent management, elaboration and choice of special materials for the construction of internal components of bioreactors; (2) elaboration of new bioreactor constructions, with incorporated membranes for the fractionation of synthetic products, for tissues cultures growth on supports, in suspensions and in films etc., in general, the necessary devices are of reduced volume; (3) development of bio-catalytic reactors, necessary due to the fact that many substrate-product transformations are achieved through immobilized biological agents; (4) a processing of fermentation products will be carried out in a manner analogous to those of the food and chemical industries, providing for the use of transducers, information processing systems and execution mechanisms (taking into account

the particularities of biotechnology); (5) the mathematical modelling of some technological processes, and especially of the fermentation process (bio-catalysis), will be directed towards the optimization of their current stage, as well as of the integral technological scheme; (6) a highly development must know the devices for measuring primary information; thus, cloud biosensors (e.g. enzymatic transducers) will be necessary and will be used to determine the concentration of substrates, semi-finished products, and, respectively, products of the new biotechnology; (7) as in other industries, the evaluation and registration of raw material and final product flows, as well as the control of technological processes are provided by computers.

The development of biotechnology is inextricably linked to the successes of bioengineering, and therefore of improved equipment and means to control these processes.

Exploring beneficial endophytes as bio-fertilizers represent a promising strategy to reduce dependency on agrochemicals (fertilizers and pesticides) in developing sustainable agriculture (Adeleke and Babalola, 2021).

We choose to cultivate endophytes in bioreactor due to their significant roles in promoting growth such as phytostimulation, biotic/abiotic stres resistance (Shen et al., 2019), their ability to secrete bioactive metabolites to control plant pathogens (Fadiji and Babalola, 2020).

There is an important aspect to translate microbial endophyte process technology from the laboratory scale to a manufacturing process (pilot) providing optimal controllable growth conditions (e.g., temperature, pH, oxygen transfer, mixing) for massive production of bio-fertilizers (Ganeshan et al., 2021).

Here, we focus our discussion on the development of a digital application to monitor, adjust, and securely control bacterial endophytes cultivation in bioreactors from anywhere at any time via internet. Also, the virtual biotech laboratory can help the students to learn and understand more easily the experiments to improve their laboratory skills for remote access without affecting the quality of learning.

MATERIALS AND METHODS

The bioreactor is a plant in which biological reactions and transformations take place, usually a fermentation or biotransformation. Our study was directed for the growth of bacterial endophytes widely used as inoculants in agriculture. Such endophytes are plant associated microorganisms adapted to live inside plant tissues without causing harms to their hosts. Due to the intimate-beneficial relationship between these microorganisms and plants, it is considered that endophytes are valuable agro-inoculants. The endophyte used as microbial model to design the bioreactor application is a *Bacillus amyloliquefaciens* strain wisely selected to meet the requirements of organic farming and agro-inoculants production.

Before enlarging inoculants production to bioreactor scale, endophytic bacteria were selected based on their beneficial effect on economically important plant species and various agronomic and technical qualities. Among the agronomic traits the selected endophytic strain meets various biostimulant abilities that improve plant growth and development. It solubilize and increase the uptake of important organic and anorganic nutrients, is favorize plan nutrition with macronutrients, micronutrients and oligoelements, and it produces organic acids, enzymes and plant hormones. This strain also revealed good biocontrol activity against important phytopatogens and microbial contaminants. Moreover, this train is having good technical qualities, and can easily adapt to be multiplied in controlled condition.

The growth medium conventionallyused for *B. amyloliquefaciens* is Luria-Bertani. This substrate contains tryptone1%, yeast extract 0.5%, sodium chloride 0.5 to 1%, and a pH of 7.0±0.2. The optimal temperature is 30°C but can tolerate a wide thermal range. As *B. amyloliquefaciens* is an aerobic bacterium, to reduce flocculation, a proper stirring is recommended for the liquid culture.

The bioreactor, fermentation and biotransformation are the bases in biotechnology, from the manufacture of bread to the production of interferon with the help of genetic engineering, all represent fermentation processes produced in the bioreactor (Schugerl, 2001).

Although the classification categories of bioreactors are multiple, conventionally, bioreactors are classified into 3 groups, according to capacity: (1) Laboratory bioreactors, with a capacity of up to 50 L, are used in the laboratory, for research, for starting and optimizing the fermentation process on an industrial scale; (2) The bioreactors in the pilot stations, with a capacity between 50 and 1000 L, are used as an intermediate and optimization phase in the process of raising the fermentation process to industrial scale (Komives, 2003).

Bioreactors in pilot stations require a high degree of technological flexibility to allow the optimization of the bioprocess; (3) Industrial bioreactors can have any capacity, but usually have a volume of over 1000 L and can reach 1000000 L. In general, industrial bioreactors are much more specialized than pilot station bioreactors, they are designed to be able to operate a certain process with maximum efficiency (Mandenius, 2016).

Researchers aim to create a real-time culture control system. This requires two premises: high-performance computer systems as well as the existence of adequate software, and the possibility of on-line measurement of intracellular activities. Currently, not all parameter measurements can be made on-line, some being done off-line.

On-line measurements can be made for: temperature, pH, dissolved oxygen, determined gases. The values of pH and dissolved oxygen provide extremely useful data to the system, representing practically the alarm signal of the process, any deviation in these values must be corrected on the spot.

The analysis of gases emitted by microorganisms can be done on-line and provides information on the respiration coefficient and the rate of oxygen uptake.

Monitoring and regulation systems can be grouped into three basic categories: in-line, on-line, and off-line.

Off-line systems involve collecting samples at regular intervals for further treatment and analysis. These techniques involve standard chemical analyses which, although they can be optimized using automated laboratory analysers, take too long to achieve effective feedback regulation.

On-line systems use techniques that allow the continuous collection of samples on the flow, with their rapid analysis, so that the response time is within the time required to make decisions to regulate the process (Mitra, 2021). Examples of such systems include continuous gas extraction for analysis performed with a mass spectrophotometer or gas chromatograph, continuous dialysis of a culture medium for analysis with a high-performance liquid chromatograph (Schael, 2002).

In-line systems, electrodes and sensors are in direct contact with the environment, giving a fast, continuous signal. These include temperature, pH, dissolved oxygen, pressure sensors. In-line sensors must be sterilizable.

One of the main limiting factors in the processes carried out in bioreactors with a capacity of more than a few litres is the availability of oxygen and its speed of distribution to cells.

Oxygen is slightly soluble in water and is therefore found in small amounts in the culture medium, so that at a high density of microorganisms in the environment, they deplete it in a few minutes.

In general, the distribution of gas in the culture medium is done by aeration through a bubbler. As bubbles are smaller in diameter, faster and more efficient is oxygen transferred to the cells (Chopda, 2020).

One way to reduce the size of the bubbles is to introduce air through perforated pipes, instead of using a bubbler. Another way is to stir the culture with a stirring mechanism, a stirrer that can be of several types and sizes, depending on the nature of the process and the result sought.

Agitation and aeration of the culture medium can produce foaming especially in environments with a high cell density or with the addition of nutrients such as yeast, soy extract, etc. Foaming can be detected by attaching a sensor above the culture medium. When the foam produced touches the sensor, an electrical circuit closes that activates a pump connected to a source of antifoaming agent.

In addition to these aeration and agitation devices, bioreactors must have sensors to control and maintain oxygen, temperature, pH. Temperature control, for example, is important for many fermentation processes that occur with the release of heat into the system, and the participating microorganisms may not tolerate this excess heat as the system needs to be cooled (excess heat dissipated).

It is recommended to monitor the intracellular activities and to achieve an optimal standardized technological profile of the fermentation in the bioreactor so that if deviations from it are observed to intervene by modifying the environmental conditions to correct them (Ferrero, 2012).

The equipment descripted above is contained in the reactor tank (Figure 1). The material of manufacture is stainless steel, and it must be of a high degree of purity in order not to corrode and to prevent the leakage of toxic metal salts into the growing medium.

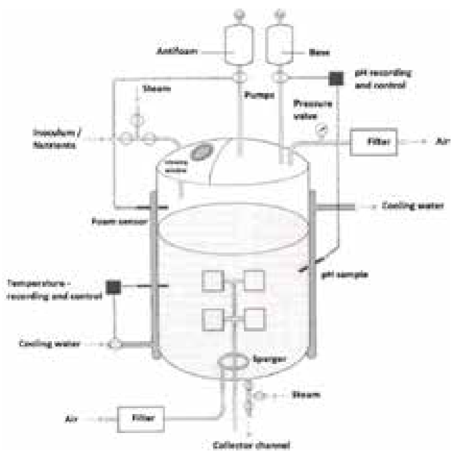

Figure 1. Schematic representation of a bioreactor

The autoclavable bioreactor used in this paper (Figure 2), for real-time monitoring of the main culture parameters, is of a benchtop type.

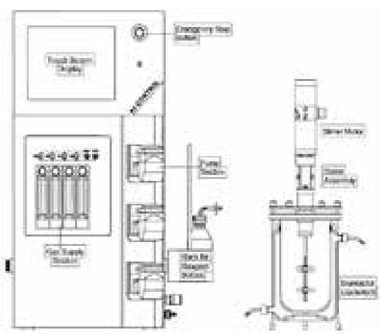

Figure 2. Diagram of Applikon autoclavable bioreactor

The bioreactor has a digital control and measurement system typeez Control, produced by Applikon Biotechnology B.V., the Netherlands (Figure 3).

Figure 3. EzControl interface of the bioreactor

The bioreactor has the connection with the sensors necessary for the proper functioning of the internal processes, as previously described. We refer to the sensor for temperature, pH, aeration, foaming, stirring (Figure 4).

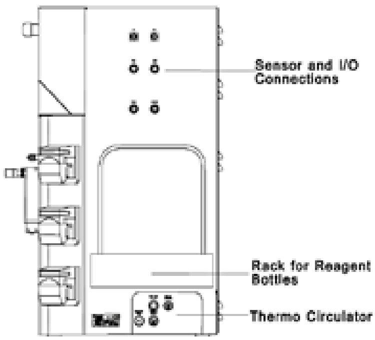

Figure 4. Sensor connectors

It offers the possibility to connect both through the ETHERNET interface and through USB type connectors, USB-Print port and RS232 (Figure 5).

Figure 5. Control panel with inputs/outputs

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For an efficient use all the main routes relate to the bioreactor and with its sensors, according to the following scheme for connecting the gasses output of the ezControl to an air inlet sparger (Figure 6).

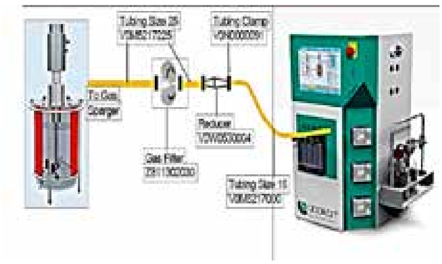

Figure 6. Connecting the gasses output of the ezControl to an air inlet sparger

Also, there is an Adding liquids scheme through a pump and the medium inlet triple (Figure 7).

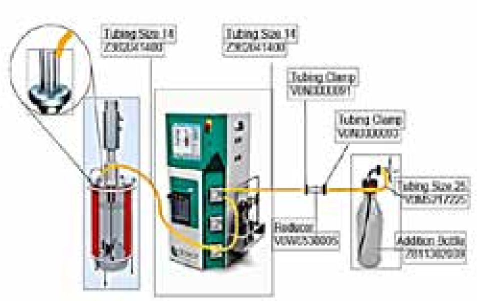

Figure 7. Adding liquids via a pump and the medium inlet triple

Connecting a PC to the bioreactor control system, ezController, allows on-line monitoring and control of on-going fermentation, and any unexpected events can be detected and corrected.

The working protocol includes the following elements: (1) date and time of registration; (2) the values of the monitored parameters; (3) alarm messages.

The connection of the computer to the command-and-control system of the bioreactor is made either through the ETHERNET interface or through the RS 232 serial interface.

After connecting the peripheral (computer) to the automated bioreactor unit, it can take over the monitoring and control functions of ezController.

The advantage of this control switch (taking control through another device) is that the monitoring of the process can be done outside the room where the bioreactor is located and implicitly the ezController system.

Thus, the monitoring can be done, through the computer, in another room (office), another building within the institution (via INTRANET) or even another city, country, continent (via INTERNET) (Lisci, 2021).

The practical applicability of this system should be mentioned due to the possibility of effective use of the bioreactor process monitoring program in the educational process by developing a software application for remote, real-time monitoring of the technological process at bioreactor level, as a central point of a virtual laboratory, an important element in achieving a quality educational process in the current pandemic context, a context that has determined an accentuation of the digitization processes in all sectors of society.

RESULTS AND DISCUSSIONS

INTRANET monitoring of the bioreactor requires, in addition to the existence of an adequate part of hardware (both high-performance computers, digital sensors and its own automated bioreactor control system) an adequate software component (Alford, 2006).

This element is an essential component in the bioreactor monitoring process. Through the software component the computer can. “Understands” and can tell us what is happening in the bioreactor (can “read” the fermentation parameters). The non-existence of this component makes unusable the constructions and hardware connections made to integrate the computer in the monitoring process.

To create the software necessary to "read" the information coming directly from the bioreactor - via its control and command system, ezController - several programming languages can be used, as well as several presentation variants both as a graphical aspect (interface) and as a system presentation of information: on-line or off-line or as a complexity of the data (setting the number and type of parameters read).

Both ways of presenting information (on-line or off-line) have advantages. Thus, the on-line model allows direct monitoring of the process carried out in the bioreactor both for the purpose of verifying the smooth running of the process and for teaching purposes, the possibility of tracking processes via INTRANET, anywhere inside the University, allows virtual presentation of fermentation via computer, at various laboratories, where the result of such a process is monitored, without the need to travel to the bioreactor. The offline model allows the storage of process parameters in the archive, thus making it possible to post-evaluate the process, compare various batches, and find solutions to improve the whole process by evaluating different data archived in the computer belonging to different batches obtained in the bioreactor.
In addition, this can be done in any department interested in the respective fermentation process, it is not necessary to "walk" the observation sheets from one laboratory to another or from one building to another, the INTRANET system allows searching all this data in the archives of the system.
The software made in this paper is written in the language of JAVA, one of the most modern object programming languages (Bloch, 2017). The monitoring program is called BioMon.BioMon is a client-server type application. Both the server component and the client component have a modular structure (Figure 8).

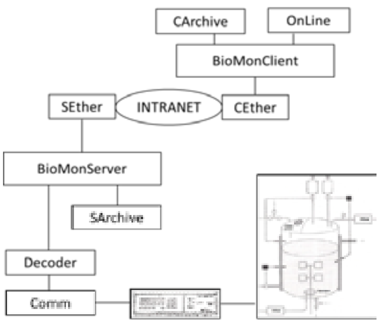

Figure 8. Modular structure of BioMon software

BioMonServer is a servlet program and was designed to run on the computer connected to the bioreactor via the RS232 serial interface. The Comm module provides the connection, setting the parameters of the serial interface and controlling the data flow from the bioreactor to the computer.
The Decoder module extracts useful information from the data stream: values of temperature, pH, pO_2, stirring speed, etc. This information can be stored locally in files using the S Module Archive or can be sent to clients, the connection with potential clients is provided by the SEther Module which implements the TCP/IP protocol using the 8060 ports for communication.
BioMonClient is an applet program and can be run from any computer in the laboratory's INTRANET network. If this network is connected to the INTERNET, the BioMonClient applet can be launched from any computer connected to the INTERNET using a browser.
The client program has two ways of working: on-line and off-line.
The On-line module performs a real-time monitoring of the processes carried out in the bioreactor. The monitored parameters are: pH, temperature, pO_2, stirring speed, amounts of antifoam, base and acid added.
The CArchive module allows access to the database stored on the server, which contains information about previous experiments (Figure 9).

Figure 9. BioMon - CArchive module

The snapshot image of the main window (HOME) is shown in Figure 10. As can be seen, this is the “gateway” in the BioMon program. It allows the selection of the mode of

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work desired by the user, who can opt for the on-line or off-line (archive) variant depending on needs.

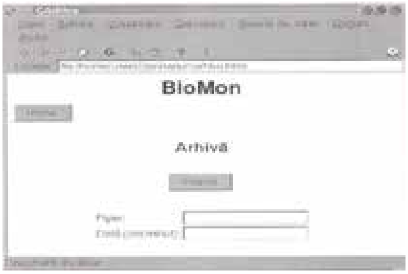

Figure 10. BioMonMaind window

The on-line monitoring program of the bioreactor is shown in figure 10 in the form of a snapshot image captured at the time of using the program.

The image shows the monitoring of a fermentation of *B. amyloliquefaciens*. The process takes place in optimal conditions, the process parameters (temperature, pO_2, pH, etc.) being within the limits provided in the working protocol.

In case of a problem (increase/decrease pH value, foaming, etc.) a warning message is displayed in the rectangle at the bottom of the image, in parallel with changing the values of the parameters that measure the amount of acid/ base/antifoam added automatically, to rectify the course of the process, in the upper right corner is monitored the time at which the reading is made.

The program is designed to read the parameters tracked at 60-second intervals.

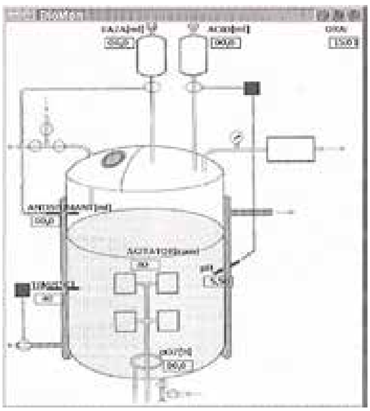

Figure 11. BioMon Server

The BioMon Server program has an extra window. It is shown in Figure 11 and is addressed exclusively to the Server because the Server is the one that directly monitors the bioreactor, receiving data from its control unit and at the same time being the place of storage of the information recorded during the monitoring process.

As can be seen in Figure 12, the Server window is basically the trigger/switch for the computerized monitoring of the bioreactor process and at the same time being the place of storage of the information recorded during the monitoring.

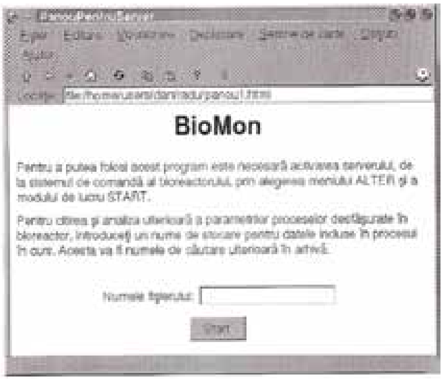

Figure 12. BioMon Server Interface

For the BioMon program to run, it is necessary to activate the server, from the bioreactor control system, by choosing the ALTER menu and the START operating mode.

For further reading and analysis of the parameters of the processes carried out in the bioreactor, a storage name must be entered for the data included in the on-going process. This will also be the name of the next search in the archive.

CONCLUSIONS

Both ways of presenting the information obtained by monitoring the industrial bioreactor (on-line or off-line) have advantages. Thus, the on-line model (direct, real-time monitoring interface of the bioreactor) allows direct monitoring of the process carried out in the bioreactor both in order to verify the smooth running of the process and for teaching purposes, the possibility of tracking processes via INTRANET, anywhere inside the University, it allows the virtual presentation of the fermentation through the computer, at various laboratories, where the result of such a process is followed, without the need to travel to the bioreactor.

The off-line model allows the storage of process parameters in the archive, thus making it possible to post-evaluate the process, compare various batches, and find solutions to improve the whole process by evaluating different data archived in the computer belonging to different batches obtained in the bioreactor.

In addition, this electronic monitoring system allows collaboration between universities in various fields of research through the INTERNET.

Thus, collaboration and research programs can be carried out between virtual teams located in different universities and even in different countries, the distance handicap being recovered through virtual access in the laboratories of teammates, through the computer. In this way, remote monitoring can be done, off-line re-verifications of the process can be done, or data can be taken from the working archive of the bioreactor, the collaboration between specialists being much easier in the current pandemic context, a context that has determined an accentuation of the digitization processes in all sectors of society.

A variant of this monitoring software specially adapted for the Android system is also being considered and is being prepared.

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