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Current good manufacturing practice in plant automation of biological production processes

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Abstract

The production of biologicals is subject to strict governmental regulations. These are drawn up in current good manufacturing practices (cGMP), a.o. by the U.S. Food and Drug Administration. To implement cGMP in a production facility, plant automation becomes an essential tool. For this purpose Manufacturing Execution Systems (MES) have been developed that control all operations inside a production facility. The introduction of these recipe-driven control systems that follow ISA S88 standards for batch processes has made it possible to implement cGMP regulations in the control strategy of biological production processes. Next to this, an MES offers additional features such as stock management, planning and routing tools, process-dependent control, implementation of software sensors and predictive models, application of historical data and on-line statistical techniques for trend analysis and detection of instrumentation failures. This paper focuses on the development of new production strategies in which cGMP guidelines are an essential part.

Introduction

For many years, biologicals are produced in bioreactor systems. From bioreactor point of view, no major differences can be observed between the present production processes and the way the biologicals were produced thirty years ago. However, in the control of these production processes, major improvements have been achieved. The introduction of computerized measurement and control units has made it possible to apply on-line modeling of the production process, thereby improving both the yield and the consistency of the process. Recently, developments in the measurement and control focus on recipe-driven control systems (Büntemeyer *et al.*, 1994; Locher *et al.*, 1991b; Wieten *et al.*, 1995). These enable the development of tailor-made control strategies, allowing current good manufacturing practice (cGMP) rules to be implemented. Already in 1985 the introduction of recipe-driven

control systems which were allowed to make decisions concerning plant operation was predicted (Harris and Spier, 1985). Although it has been technically feasible for years, this type of control systems still is a scarce phenomenon. In the past five years, especially Locher *et al.* have paid much attention to advanced bioprocess automation (Locher *et al.*, 1991a,b,c, 1993a,b). Their research clearly showed that the reproducibility of bioprocesses is mainly dependent on the reproducibility of the environmental conditions for the cells and, hence, on the quality of the equipment (Locher *et al.*, 1991c). They showed that ruling out operator actions by means of extensive automation resulted in identical batch cultures of *Saccharomyces cerevisiae*. The need for identical cultivation conditions is again emphasized by Kell and Sonnleitner (Kell and Sonnleitner, 1995). They claim that 'one should not aim to seek improvements by constructing a predictor-controller to cope with fuzzy biology. Rather an appropriate Standard

Operating Procedure (SOP) should be devised, or better equipment used’.

With the increasing governmental regulations, it has become more interesting to develop recipe-driven control systems. This paper presents the most recent advancements in the development of new production strategies for biological production processes in which cGMP guidelines are an essential part.

Introduction of current good manufacturing practice in control systems

Biological production processes are obliged to obey to strict governmental regulations. Amongst others, the U.S. Food and Drug Administration has designed the rules for cGMP (FDA, 1995). These include requirements related to the methods used in, and the facilities and controls used for, design, purchasing, manufacture, packaging, labeling, storage, installation, and servicing of all finished products intended for human use. As can be seen, these rules cover a broad range of operations that are directly or indirectly linked to the production process. Amongst these rules, validation of the production process and equipment as well as the use of SOPs are essential. SOPs prescribe in detail how the production process has to be executed. Not only the bioreactor process, but all of the facilities used are subjected to the cGMP rules. This implies a huge amount of registration activities and therefore a lot of additional work. Fortunately, computers can take over most of these tasks. This is done in so-called Manufacturing Execution Systems (MES). These are dedicated systems that allow one to monitor and control all product and information flows, the production environment and all production processes in the facility. In case of batch production processes, an MES is generally built according to the ISA S88 standard for batch processes (Wieten *et al.*, 1995; ISA, 1995). This standard defines reference models for batch control as used in the process industries, and terminology that helps explain the relationships between those models and terms. This software standard was developed in conjunction with IEC and ISO standards groups. Fig. 1 shows a typical plant structure with the MES operating as the heart of the plant. It is clear that the MES operates on four levels. The production management is provided with tools for planning and routing of the production process, and is able to extract real-time and historical data from the database in order to generate batch records, (semi) annual reports and quality control

charts. On the level of plant operation, MES controls humidity, pressure household and temperature inside the production facility. Additionally, all incoming and outgoing stocks are registered and the inventory of the storehouse is kept up to the mark. On the process floor, MES controls all processes, varying from the bioreactor production process to downstream processing and packaging. Depending on the integration level, these processes are either controlled by a general MES, or by Man Machine Interfaces (MMI) that are directly linked to an MES. For all process equipment, log-books and calibration reports are archived. As soon as a new production run is started, the reliability of the equipment to be used is verified using these data. The operator uses the MES to generate daily reports, to look at real-time data, and to operate the measurement and control unit. The latter is done using a graphical user interface that interacts with the operator by means of instructions and questions. Finally, the MES collects and if necessary verifies all data generated by on- and off-line analytical equipment. If required, the MES can adjust the production process based on the outcome of these analyses.

An important part of the production process of most biologicals is done in bioreactors. Since the measurement and control unit that is attached to the bioreactor controls every action regarding the bioreactor, it makes sense to implement the SOPs concerning this part of the process in the software that controls the measurement and control unit. This way it is assured that all instructions described in the SOP are carried out. It can be verified whether or not a production run is done according to the SOP by logging all operator actions by the computer. Additionally, the data obtained by the measurement and control unit has an increased value, since each dataset is derived under identical conditions. Comparison of different batches becomes relatively easy this way. Finally, the use of SOPs ensures that all procedures are executed identically. This way highly consistent production results can be obtained that mainly depend on the quality of the raw materials. Therefore the batch-to-batch variation caused by small variations in the operations during the production period will be minimized. This is clearly shown in a paper by Locher *et al.* (1991c). They show that in six repetitive batch cultures of *Saccharomyces cerevisiae* differences in batch cultivations with respect to duration and amplitudes of CO₂ did not exceed 5%. The main reason for this small deviation could be found in the irreproducible way the amount of inoculum was weighed. Fig. 2 shows the results of two Vero-cell

Figure 1. Typical plant design with the Manufacturing Execution System serving as the heart of the production facility. (cGMP = current Good Manufacturing Practice; (ISA) S88 = Software standard for batch processes; MES = Manufacturing Execution System; MMI = Man Machine Interface; M&C = Measurement and Control).

Figure 2. Example of two simultaneously performed batch cultures of Vero cells that followed the same recipe. The inoculum of both cultures originated from the same primary Vero-cell culture. The cultures were performed in two different bioreactors, using two different measurement and control units.

batch cultures, run simultaneously in different cultivation units. Both were cultivated according to the same recipe. The inoculum originated from a primary batch culture of Vero cells. During both cultivations, operator actions like sampling were still done. This figure shows that, although the cultures were executed

in two different cultivation units, their characteristics are virtually identical. This concerns not only the controlled parameters such as pH, DO (dissolved oxygen) and stirrer speed. Even the controller action of the dissolved-oxygen controller (DOCO) that is required to maintain setpoint is almost identical.

Figure 3. Internal structure of ISA S88-based control systems.

The man-machine interface

MMI can be seen as the interface that translates existing SOPs into instructions for both the measurement and control unit and the operator. It is either embedded or directly linked to an MES, or it operates stand alone. Just as an MES, an MMI-based system is built according to ISA S88 standards for batch processes (Wieten *et al.*, 1995; ISA, 1995). This enables the validation of the software code, including that of the implemented features (software sensors, event-based control, ...). The basis is formed by the Recipe. A schematic representation of the linkage between the different levels of the Recipe is presented in Fig. 3. It shows that a recipe consists of four different parts. The Header contains information about the authors, the tags used, the history of modifications and the version number. The Procedure contains the control algorithms, the software code for the different production phases, and the safety interlock system. The latter prevents the occurrence of hardware conflicts such as simultaneous heating and cooling of the bioreactor. Formula is used to store process constants like setpoints of controllers and trigger values for certain event-based procedures such as taking a sample every 5 hours. The Equipment requirement contains information about the hardware components. For a bioreactor system, this includes the measurement and control unit and the service unit (water and steam supply). In the Recipe all parts are combined to form a complete set of information that

specifies the control requirements for manufacturing a batch.

Recipes can be built that focus on specific steps of the production process. Therefore, in each production step, the response of the measurement and control unit can be adapted to the current needs. For example, the temperature inside the bioreactor can be controlled at a fixed value during the cultivation procedure, whereas during the inactivation of the product, the temperature is forced to follow a predefined profile. MMI can also be given the authority to decide at what point actions have to be taken. The cultivation process can for instance be stopped as soon as the biomass activity starts to decrease (Fu *et al.*, 1995). This ensures identical cell conditions at the end of each cultivation. Depending on the time at which the culture ends (e.g. midnight), MMI then decides if the harvest has to be cooled until the next day, or if an operator has to be warned that the cultivation has ended. In fully automatized production facilities, the harvest will be pumped to a clarification or a concentration unit, where the next step in the production procedure will be started automatically. From cGMP point of view, MMI-based control systems offer increased control over the operator actions. It is for instance possible to command an operator to finish a complete calibration sequence before the next step of the process is executed. On the other hand, MMI can provide assistance by informing the operator about the actions that have to be taken during important operations. Additionally, the presence of different access levels enables decisions to be made at

the right level of authority. An operator is only allowed to strictly follow the recipe, whereas the production manager is allowed to change setpoints, start and stop controllers, design his own recipes, etcetera.

Data handling in ISA S88-based MMI-systems

The use of MMI-based control systems enables the use of sophisticated features. For instance software sensors and advanced stat-based control strategies can be implemented easily. Stats, such as pH-stats or chemostats, combine control and physiological parameters to regulate the feed rate of substrates in fed-batch bioreactors or the dilution rate in chemostats. Software sensors are models, implemented in software of the MMI, that use information already available to predict parameters that are not measured on-line (Dorresteyn *et al.*, 1995). This information generally consists of common parameters like stirrer speed, dissolved-oxygen concentration and pH. The use of software sensors is far from a new idea. As early as in 1978 papers about software sensors have been published (e.g. Zabriski and Humphrey, 1978). In literature different applications of software sensors have been described. Roughly they can be subdivided into three categories. Software sensors based on metabolic activity are mostly based on the measurement of the oxygen uptake rate, carbon-dioxide evolution rate, acid production rate and redox potential (Agrawal, 1989; Dorresteyn *et al.*, 1996; Dorresteyn *et al.*, submitted; Dorresteyn *et al.*, 1995; Eyer *et al.*, 1995; Eyer and Heinzle, 1996; Graindorge *et al.*, 1994; Konstantinov *et al.*, 1994; Nam and Kim, 1994; Park *et al.*, 1983; Pugh, 1988; Takagi and Ueda, 1994; Yamada *et al.*, 1990; Yoon and Konstantinov, 1994). Another type of software sensors uses artificial neural networks, fuzzy logic and knowledge-based supervision systems (Eerikäinen *et al.*, 1993; Kitsuta and Kishimoto, 1994; Konstantinov and Yoshida, 1992; Lübbert and Simutis, 1994; Montague and Morris, 1994; Montague *et al.*, 1992; Oishi *et al.*, 1992; Sonnleitner *et al.*, 1992). These do not require specific knowledge of the characteristics of the organism that is being cultivated. Instead, they rely on historical data of previous production runs and experience of process experts. Finally, software sensors have been developed based on component balancing and predictive models, often in combination with Extended Kalman Filtering techniques (Acuña *et al.*, 1994; Chattaway and Stephanopoulos, 1989; Liao, 1989; Montague *et al.*,

1986; Pelletier *et al.*, 1994; Shimizu and Takamatsu, 1989; Stephanopoulos and San, 1982; Swiniarski *et al.*, 1982).

With the aid of these software sensors it is possible to estimate parameters like biomass concentration or activity, and the consumption and production rates of important metabolites like glucose, glutamine, ammonium and lactate on-line. Control algorithms based on these software sensors are possible too. Fig. 4 shows an example of the course of glucose and glutamine during a glucose-controlled batch culture of Vero cells (Dorresteyn *et al.*, submitted). In this case, both glucose and glutamine have been estimated using software sensors based on metabolic activity. To estimate these concentrations, the dissolved-oxygen concentration, the stirrer speed, information from the mass-flow controllers and the base utilization were used as input parameters for the software sensors. All these parameters can easily be measured on-line, whereas both glucose and glutamine are very difficult to determine on-line. As can be seen, the glucose control is started after 50 hours of cultivation. In this case, the glucose is controlled using a peristaltic pump. Since the flow rate of such a pump is not very accurate, a small deviation occurs between measured and estimated glucose concentration. This example clearly shows the strength of software sensor applications in an MMI-based environment. Provided the knowledge about software sensors is available, it will take only a few days of programming to incorporate them into the MMI-software. Hence, without investments in analytical equipment, it is possible to extend the optimization possibilities significantly.

Looking at the amount of publications about software sensors, it is clear that a lot of research is being done in this field. The most important fields of interest are biomass determination, nutrient consumption, production of ammonium and lactate, and product formation. However, in addition to these common applications, software sensors are used for detection of contaminants in bioreactors (Chattaway and Stephanopoulos, 1989) and for the automation of the *sake* brewing process (Oishi *et al.*, 1992). Especially if software sensors are used for the optimization of complete production processes, this requires a software platform that is completely adjusted to the individual process steps. MMI-based systems then become an essential tool in the optimization process (Konstantinov and Yoshida, 1992; Wieten *et al.*, 1995).

Next to software sensors, stat-systems can be implemented. These stats respond to changes in physical parameters like the oxygen uptake rate (DO-stat)

Figure 4. Example of a glucose-controlled batch culture of Vero cells (♦ = Glucose; ● = Glutamine).

or the acid production rate (pH-stat). If the value of such a parameter falls outside certain predefined limits, the feed rate is adjusted. This feed either consists of complete cultivation medium, or of one or more specific nutrients. Depending on the amount of knowledge about the physiology of the organism that is incorporated in the control system, stats can be developed that are either based on simple assumptions, or on physiological responses due to the complex interactions in the metabolic pathways. Especially stats for fed-batch systems rely on physiological knowledge to prevent waste of substrate and thereby the production of waste products that can be harmful to the organism. With this combination, a fed-batch cultivation with an optimal biomass or product yield is achieved. Several examples of stats are mentioned in literature. A DO-stat is used to control the acetic acid excretion by *Escherichia coli* (Konstantinov *et al.*, 1990; Seo *et al.*, 1992). By suppressing the acetic acid excretion the yield of recombinant human interleukin-2 was increased (Seo *et al.*, 1992). An example of a pH-stat is the ethanol production by a pH-mediated fermentation of *Zymomonas mobilis* (Ishizaki *et al.*, 1994). When glucose is exhausted, the organism begins to metabolize amino acids. By changing its metabolism, the concentration of ammonia and as a consequence

the pH increases. The increase of the pH-signal was converted in an increase of the feed rate of fresh medium. A more complex stat is used in the cultivation of *Alteromonas putrefaciens* (Hibino *et al.*, 1993). In this stat, the yield of biomass on substrate was imposed on the culture. Fresh substrate was added when based on this yield and the biomass profile, all nutrients were theoretically spent. This stat resulted in an increase in biomass by a factor 1.5 and a reduction of the nutrient feed by a half.

In the development of both stat systems and software sensors and in the control of fermentation conditions, reliable sensor reading is a prerequisite. This may seem trivial but up to now, only sensors which measure physical parameters like temperature, gas or liquid flows and pressure function highly dependable during the complete course of a fermentation. Basic chemical sensors like pH and DO sensors whose employment is widespread and which have been used for many years for the production of biologicals have to be treated with great care to obtain reliable values (Locher *et al.*, 1992). Problems with sensor reading can occur at several stages of the processing of the raw signal. The characteristics of the sensor (e.g. sensitivity to the parameter to be measured, sensitivity to other environmental conditions, signal to noise ratio, linearity),

the electrical processing of the signal (wiring, electrical connections, grounding, AD/DA-conversion), the calibration, validation and maintenance of the sensor play an important part in obtaining sound data. The situation is even more troublesome with sensors which measure biologically relevant parameters such as biomass, nutrient and product concentration. These sensors are still very delicate (sensitive towards fouling and influenced by other chemical components) and not yet suited for exploitation in an industrial environment.

Besides the use of sophisticated software sensors and stats, historical data of previous production runs can be used to detect deviations in the characteristics of the current production process. This is possible since for each production run a complete batch record exists, containing culture conditions, operator actions and trends of all sensor signals. These data are accessible at any time during the production process. If compared to the historical data a deviation occurs, any predefined action can be triggered. Other deviations of the process characteristics such as a bacterial or a fungal infection can be detected in a very early stage too. This will save both time and production costs. Finally, it is possible to apply on-line statistics to the process signals. This can be used to detect failures in the measurement equipment (Van der Heijden *et al.*, 1994) or deviations in the process characteristics. Especially in combination with historical data, on-line statistical analysis offers an important tool in the optimization of production processes.

Conclusions

The current trend for the production of biologicals is directed towards the automation of the complete production plant. This means that all operations and conditions inside the production facility are controlled by a Manufacturing Execution System (MES). Next to implementation of current good manufacturing practice (cGMP), an MES aids in increasing the reproducibility and consistency of the production process. To achieve this, standard operating procedures (SOPs) are implemented in the operator interface of the measurement and control systems for the different production steps. This interface is either the MES itself, or a Man-Machine Interface that is linked to an MES or functions stand alone. The incorporation of SOPs on the process floor ensures identical process conditions during all production runs. This is beneficial for both the production department and the research department.

The production department benefits since the production process is executed in a very reproducible way, thereby obtaining consistent production results. This is essential for obtaining a production license. On the other hand, the research department is able to obtain large quantities of easily accessible process data that are obtained from identical processes. This enables a thorough analysis of the complete production process. This issue is often neglected but very important. Since all process steps interdepend, analysis and optimization of a production process should include the complete process and not just isolated steps like a fermentation or purification step.

A recipe-driven control system such as an MES or an MMI-based system that follows ISA S88 standards for batch processes is an important tool to accomplish the above. Next to highly consistent production runs, it offers additional features that will be useful in the optimization of the production process. The most important control features are process-dependent control, the use of software sensors, stats and predictive models, the application of historical data and on-line statistical techniques for trend analysis and detection of instrumentation failures.

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