Journal of Biotechnology 177 (2014) 53–59

Contents lists available at ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

Operator training in recombinant protein production using a structured simulator model

Inga Gerlach[a,b], Simone Brüning[a], Robert Gustavsson[b], Carl-Fredrik Mandenius[b,*], Volker C. Hass[c]

[a] Department of Environmental- and Bio-Technology, University of Applied Sciences Bremen, Neustadtswall 30, 28199 Bremen, Germany
[b] Division of Biotechnology/IFM, Linköping University, 58183 Linköping, Sweden
[c] Faculty of Medical and Life Sciences, University of Applied Sciences Furtwangen, Jakob-Kienzle-Straße 17, 78054 Villingen-Schwenningen, Germany

ARTICLE INFO

Article history:
Received 27 November 2013
Received in revised form 25 February 2014
Accepted 28 February 2014
Available online 11 March 2014

Keywords:
Training simulator
Recombinant protein production
Cultivation
Modelling

ABSTRACT

Model-based operator training simulators (OTS) could be powerful tools for virtual training of operational procedures and skills of production personnel in recombinant protein processes. The applied model should describe critical events in the bioprocess so accurately that the operators' ability to observe and alertly act upon these events is trained with a high degree of efficiency. In this work is shown how this is accomplished in a structured multi-compartment model for the production of a recombinant protein in an *Escherichia coli* fed-batch process where in particular the induction procedure, the stress effects and overflow metabolism were highlighted. The structured model was applied on the OTS platform that virtually simulated the operational bioreactor procedures in real or accelerated time.

Evaluation of training using the model-based OTS showed that trained groups of operators exhibited improved capability compared with the untrained groups when subsequently performing real laboratory scale cultivations. The results suggest that this model-based OTS may provide a valuable resource for enhancing operator skills in large scale recombinant protein manufacturing.

1. Introduction

Operator training simulators (OTS) are computer-based tools for developing and maintaining operational skills in a variety of technical systems (Mani et al., 1990; Kobayashi et al., 1995; Okapuu-von Veh et al., 1996; Dudley et al., 2008; Balaton et al., 2013; Manca et al., 2013). Successful application of OTS requires more or less complex mathematical models that sufficiently well describe the process to be operated by simulating a virtual reality of the real technical system.

So far, few efforts have been made to exploit the potential of OTS for supporting training in bioprocess manufacturing (Kuntzsch et al., 2012; Gerlach et al., 2013). Especially, recombinant protein production (RPP) processes may benefit substantially by using OTS due to their inherent biological complexity, complicated operational procedures and wide-spread use in large-scale industrial production.

In order to apply the OTS successfully the metabolic and physiological behaviour of the used recombinant organism must be accurately mirrored in the virtual reality of the simulator. For example, effects caused by the metabolic burden from the expressed recombinant protein that influence biomass yield, productivity and cellular viability model (Glick, 1995; Carneiro et al., 2013; Hoffmann and Rinas, 2004) are important to include in the OTS model. So are the impacts on the host cell metabolism from maintenance and replication of plasmids as well as from protease activity due to stress response (Sørensen and Mortensen, 2005; Silva et al., 2012).

To fully accomplish this, a structured model of high computational complexity comprising a large number of state variables and parameters, several often difficult to measure or determine, is required (Bentley and Kompala, 1989; Nielsen et al., 1991a,b; Ramírez and Bentley, 1999; Chae et al., 2000; DeLisa et al., 2001). However, for computational reasons the OTS model should preferably be kept as simple as possible without compromising the observability of relevant biological effects during simulation.

Pre-training in the OTS environment prior to practical training in the plant or laboratory environment could significantly enhance the efficiency of learning of the process operators. In particular, the operators' understanding of the complexity of the process, ability to

* Corresponding author. Tel.: +46 13 281000.
E-mail address: cfm@ifm.liu.se (C.-F. Mandenius).

http://dx.doi.org/10.1016/j.jbiotec.2014.02.022
0168-1656/

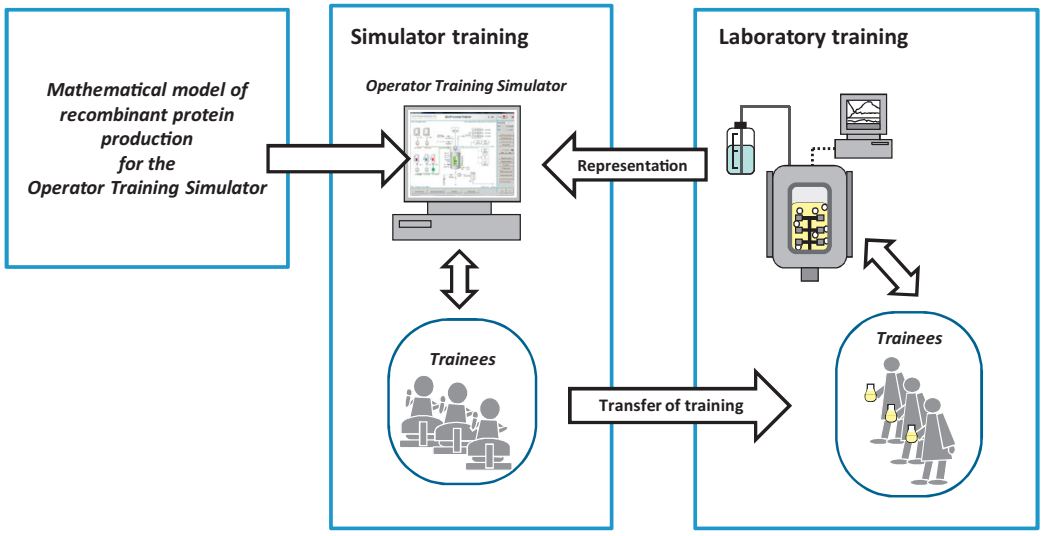

Fig. 1. Virtual pre-training with an OTS for recombinant protein production with *E. coli* in a fed-batch bioreactor can support the training effectiveness in a subsequent real cultivation experiment.

make correct operational decisions and adhere to standard routines could be expected to be improved (Lee, 2005). Thus, applying virtual bioreactor training as a part of an operator learning and training programme would have potential for increasing the efficiency and enhance capacities and skills of bioprocess operator personnel as is the case in other OTS applications (Fig. 1).

In this article we present a new OTS tool based on a refined structured RPP model tailored for the physiological effects described above. The model was adapted to a fed-batch process with a recombinant *Escherichia coli* producing Green Fluorescent Protein (GFP). Subsequently the RPP-OTS tool was evaluated in operator training of bioengineering apprentices where it showed profound effects in a number of important performance criteria.

2. Materials and methods

2.1. Microorganism

Escherichia coli strain HMS 174(DE3) (Novagen, Madison, WI, USA) transformed with plasmid pET30a (Novagen) containing GFP-mut3.1 (Clontech, US), under control of the T7/lac promoter and a 25 bp lac operator sequence was used for GFP production.

2.2. Cultivation conditions and media

Cells were cultivated in a 10 L in situ sterilized bioreactor (Model LMS 2002, Belach Bioteknik AB, Solna, Sweden) equipped with standard instrumentation. The procedure and media described in Gustavsson and Mandenius (2013) were used for batch and fed-batch as well as pre-culture preparation. The initial batch cultivation was carried out in 4 L medium whereas a 1 L fed-batch medium was fed to the bioreactor when glucose was consumed. An exponential feed profile was used for 6.2 h with an initial feed rate of 100 g h^{-1} and an end feed rate of 250 g h^{-1} while the rate was controlled by a PID-controller. For expression of GFP the cells were induced with 0.44 g isopropyl-β-D-1-thiogalactopyranoside (IPTG) (Sigma) dissolved in 10 mL deionized water 2 h after starting the feed (approx. 0.11 g/L IPTG) at an optical density (600 nm) of approximately 12 absorbance units.

2.3. Analytical methods

2.3.1. Off-line analysis

Optical density (OD) was measured at 600 nm. Fluorescence was measured using the fluorimeter Fluostar Galaxy (BMG Labtechnologies GmbH, Offenburg, Germany). Two hundred microlitres of the 1:100 diluted (PBS buffer) samples were measured in well-plates at an excitation/emission of 485/520 nm.

2.3.2. On-line analysis

Prepared samples were injected into a high performance liquid chromatography (HPLC) system (Shimadzu, Tokyo, Japan) for the measurement of glucose and acetate concentration.

2.4. Training simulator

The OTS system BioProcessTrainer (BPT) developed by s&h Ingenieurgesellschaft mbH (Bremen, Germany) in cooperation with University of Applied Sciences Bremen (Germany) was used for the model application (Gerlach et al., 2013). The RPP model was integrated as Dynamic Link Library (DLL) into the BPT.

2.5. Virtual pre-training and assessment of training

The effect of the OTS training was evaluated on bioengineering students with and without prior OTS training. The OTS training period was limited to 2 h per trainee. The test group was divided into four subgroups with five trainees per group that were pre-trained with the OTS and one reference group with five trainees had no pre-training and was only prepared by reading written instructions and listening to verbal guidance from the instructor prior to the practical experimental training. The OTS-trained subgroups as well as the reference group performed two cultivations. All subgroups followed a standard operation procedure (SOP) that covered the technical operations for the bioreactors with its auxiliary equipment. The training effectiveness was evaluated using a standardized observation protocol that included important aspects of the training objectives (Table 1).

3. Model development

The primary purpose of the biological part of the model in the OTS is to ensure effective training of relevant growth and