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Operator training simulation for integrating cultivation and homogenisation in protein production

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ABSTRACT

Operating training simulators (OTS) are virtual simulation tools used for training of process operators in industry in performing procedures and running processes. Based on structured mathematical models of the unit operations of a bioprocess an OTS can train a process operator by visualising changing conditions during the process, allow testing operator actions, testing controller settings, experience unexpected technical problems and getting practice in using prescribed standard procedures for a plant.

This work shows the design of an OTS where two sequential steps of a recombinant protein production process, a fed-batch cultivation and a high-pressure homogenisation, are integrated. The OTS was evaluated on a user test group and showed that the OTS promoted and developed their understanding of the process, their capability to identify parameters influencing process efficiency and the skills of operating it.

1. Introduction

Operating training simulators (OTS) are virtual simulators for training of operators in industry or other human activities in performing procedures and running processes [22,17,5,2,18]. Well-known examples are OTS for running process units, navigating airplanes and ships, performing medical surgery and training military personnel in using weapons in combat [3,16,7,15,21]. The OTS simulate the procedures of the technical system to be trained on based on established mathematical models. The typical OTS has an interface that visualizes the technical system and allows the trainee to interact with the virtual system through emulated tools and gears such as pumps, valves and controllers. By that, the OTS provides a number of opportunities for training operators by visualising different process conditions, trying alternative operator actions, solving technical problems, changing controller settings and follow prescribed standard procedures for a plant.

In particular for bioprocesses the OTS have great potential by visualizing critical and complex operating procedures in the bioprocess. So far, few efforts are made to use OTS in industrial training or education. Previous studies by us have shown how the OTS can efficiently be applied to biogas digesters [4], bioethanol fermentation [9], and distillation [13] as well as recombinant protein production in bioreactors [10].

These previous efforts in OTS development have been mainly focused on single unit operation of the bioprocesses (an ethanol fermentor, a distillation tower, an aerobic recombinant bacterial cultivation). However, good performance of bioprocess operators in industry requires that sequential process units can be operated efficiently and simultaneously. What happens in the first unit operation of a process sequence affects the subsequent unit operations in ways that require very good understanding of the process by the operators and skills in running it efficiently and without faults.

In this study we show how the two sequential unit operations in a bioprocess for production of a recombinant protein are integrated in an OTS. The intracellular recombinant protein is produced in a fed-batch bioreactor from which its suspension of protein-containing cell is transferred to a subsequent high-pressure homogeniser. The OTS with the two coupled process units allowed training of process operators in how to run the high-pressure homogeniser in relation to key conditions and parameters such as pressure, flow rate and number of passes. The OTS was tested on a user group for evaluating their ability to comprehend

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the dynamics of the process and to take appropriate actions during operation.

2. Materials and methods

2.1. Microorganism

Escherichia coli strain HMS 174(DE3) (Novagen, Madison, WI, USA) transformed with plasmid pET30a (Novagen) expressing the green fluorescent protein (GFP) mutant 3.1 (GFP-mut-3.1, Clontech, US), under control of the T7/lac promoter and a 25 bp lac operator sequence was used for GFP production.

2.2. Cultivation

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, analytical methods and media composition are described in [12] and [9].

2.3. Cell disruption

Harvested suspension of two completed *E. coli* cultivations were mixed (10 L) and stored at 4 °C until the use in homogenisation experiments. The cell suspensions were subsequently homogenised using a Gaulin-Homogeniser APV LAB 40 (SPX Flow Technology Rosista GmbH) with a horizontal three-piston pump. The AVP homogeniser had a maximum capacity of 45 L/h at a maximum pressure of 1000 bar. The experimental set-up included a tank that was connected to the homogeniser and a glass flask for collecting the homogenate. After each pass the tank was refilled with the homogenate and samples were taken for subsequent analysis. Cell suspensions of 5 L volumes were homogenised at 300 and 600 bar. The cell suspension was cooled to 5–10 °C between the homogenisation passes.

2.4. Fluorescence spectrophotometric analysis

Two-millilitre samples from homogenization were centrifuged at 10,000 × *g* for 10 min at 4 °C. For analysing the protein release the supernatant was collected and measured in a fluorescence spectrophotometer (Fluostar Galaxy, BMG Labtechnologies GmbH, Offenburg, Germany). Two hundred microliters of 1:100 diluted (PBS buffer) supernatant were measured in well-plates at an excitation/emission of 485/520 nm. Also, uncentrifuged samples from the two cultivations were analysed as controls.

2.5. Model formulation

A previously developed model for protein expression in *E. coli* [10] was modified in order to simplify its structure, to improve simulation of protein expression and to facilitate its connection to a model for high-pressure homogenisation (see Section 3). Both models were formulated in the programming language C++ and numerically solved using a 4th order Runge–Kutta algorithm for dynamic systems. Model parameter were taken from literature or adjusted by comparing simulation results with experimental data.

2.6. Operator training simulator

The development of the OTS was carried out by using the commercial software WinErs (Ingenieurbüro Schoop GmbH, Hamburg, Germany; www.schoop.de/en/software/winers). WinErs is a convenient software for development of process control and automation and includes features for visualisation, data monitoring, control and simulation of industrial processes. The WinErs software was here used for designing the graphical user interfaces (GUI), and control schemes and functions of the OTS. WinErs was also used for implementation of the C++ models as dynamic-link libraries (DLL). The in- and outputs of the models where connected to the GUIs to visualize the actual state variables in data tables or history diagrams. By that, the OTS replaced the real bioreactor and homogenizer with models that mimic their behaviour in the virtual environment of the OTS. With the WinErs

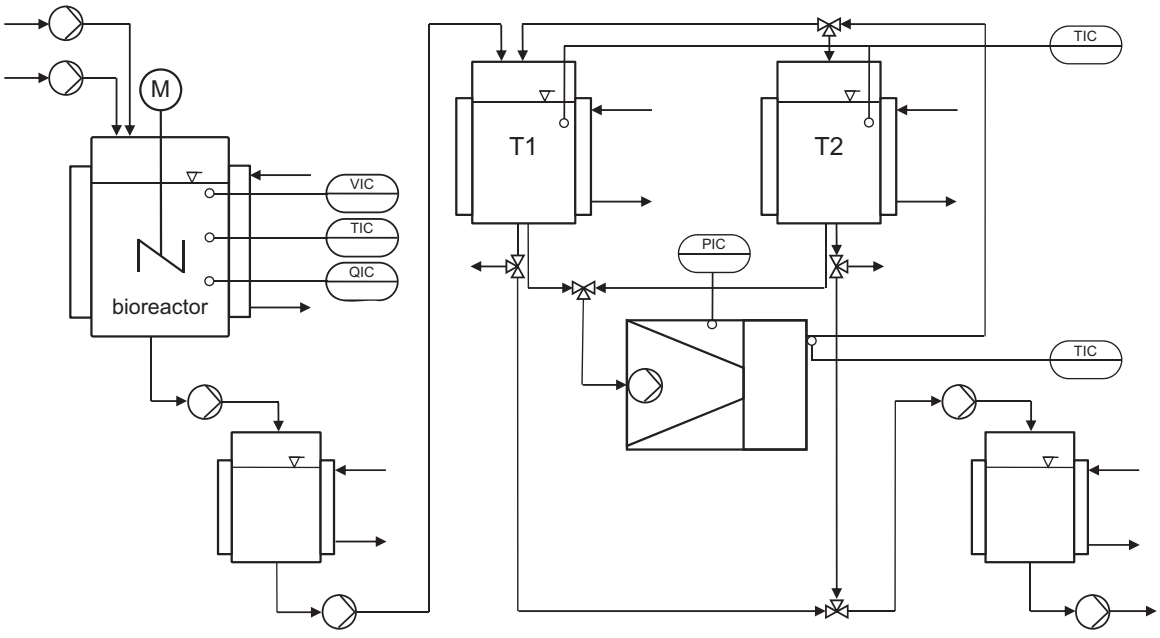

Fig. 1. Integrated process plant including a bioreactor with a product tank and a HPH system connected to two holding tanks (T1 and T2) and a product tank.

Table 1
User needs to be met in the development of the integrated OTS.

Needs	Description
(1) Efficient virtual bioprocess training in recombinant protein production	Procedures of how to carry out a recombinant protein cultivation including fermentation techniques and induction as well as a subsequent or continuous homogenisation (downstream) should be trained with high fidelity and within shorter time by using the OTS.
(2) Efficient transfer of fundamental knowledge about high-pressure homogenisation (HPH)	The transfer of fundamental knowledge about HPH such as changing pressure and temperature of the cell suspension should be facilitated by using the OTS.
(3) Efficient virtual training in cell disruption properties of different microorganisms during HPH	The analysis of disruption properties of different microorganisms during homogenisation should be accomplished easier and within shorter time by using the OTS.
(4) Efficient transfer of understanding of protein denaturation effects during HPH	The understanding of protein stability during homogenisation should to be reached within shorter time by using the OTS.
(5) Efficient transfer of understanding for applying different pass strategies for HPH	Procedures of how to run single-pass and continuous homogenisation strategies should be comprehended easier and within shorter time by using the OTS.

software the simulations in the OTS could be run in real-time as well as in accelerated time (1-, 5-, 10- or 15-times).

2.7. Virtual OTS training and evaluation

Applicability and feasibility of the OTS were evaluated with a user test group with background in bioengineering and similar biosciences. The trainees took part in a one-day hands-on training with the OTS where the accelerated mode of the OTS was used to reduce the training time. Detailed written instructions were available for the trainees beforehand. These instructions were comparable with a standard operation procedure for the integrated process and included the typical manual actions by operators and the scheduling of the process sequence. The training was subsequently assessed from questionnaires and tasks (see Supplementary data, 3.) as well as with individual interviews.

The OTS training started with virtual fed-batch cultivations for protein expression and separate homogenisation experiments using the virtual homogeniser and was continued with training the integrated process with the cultivation and homogenisation procedures including varying induction and feeding times as well as comparing single-pass or continuous homogenisation at different pressures.

3. Homogeniser model development

The homogeniser model used in this study is based on a model developed by Hetherington et al. [14] for the release of soluble protein in a Manton–Gaulin homogeniser. Hetherington et al. adapted the protein release to *Saccharomyces cerevisiae* as function of the operating pressure (P) and the number of passes through the homogenizer (N) as:

$$\log\left(\frac{R_{max}}{R_{max}-R}\right) = K \times N \times P^{a} \quad (1)$$

where R_{max} is the maximum amount of soluble protein that can be released, R the fraction of disrupted cells, K the rate constant and a a pressure exponent. The exponent a is specific for a certain microorganism and depends on the microorganism's robustness to disruption. It is related to the cell wall structure but might also be influenced by growth conditions, growth rate, cell concentration and the temperature of the suspension as suggested in other reports [11,8,19]. Subsequent development of this model has been done by Augenstein et al.; Sauer et al.; Spiden et al. [1,24,19], and Choonia et al. [6].

For integrating the HPH with the preceding cultivation step we modified Eq. (1) to describe the release of recombinant protein (c_{RPn}) after homogenisation:

$$c_{RPn} = c_{RPn,int} \times \left(1.0 - 10.0^{(-K \times P^{a})}\right) \quad (2)$$

where n refers to tank 1 (T1) or tank 2 (T2) of the HPH system, c_{RPn} is the released protein concentration (comparable to R (Eq. (1))) and $c_{RPn,int}$ the intracellular recombinant protein concentration (comparable to R_{max}) (Supplementary data, Eqs. S 1:3 and S 1:4).

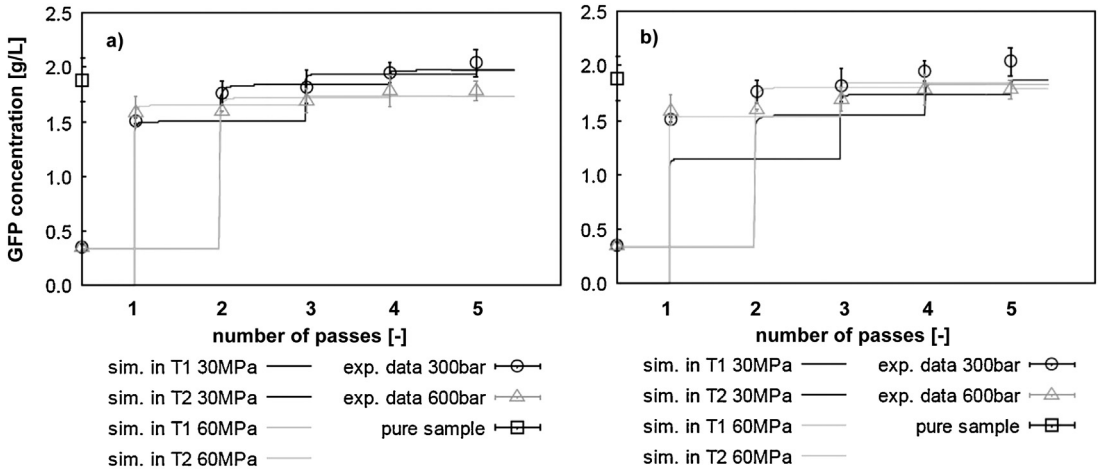

Fig. 2. Comparison between experimental data from high-pressure homogenisation runs (dots) and simulation (sim.) with the homogeniser model (lines) showing the released protein concentration after homogenisation at: (○) 300 bar (30 MPa) and (△) 600 bar (60 MPa). Simulation results show the released protein concentration in tank 1 (T1) and tank 2 (T2) for each pass by transferring the suspension from T1 through the homogeniser to T2 and vice versa. The total recombinant protein concentration from a pure sample of the mixed cultures used in the homogenisation experiments was around 1.7 g/L (□) (other parameters are given in Table 2).

Table 2
Parameters applied in simulations.

Fig. 2	K	a	K_d	b	$c_{RP0,int}$ (g/L)	$c_{RP0,rel}$ (g/L)
(a)	0.009	1.2	0.1E-05	0.0	1.65 (30 MPa) 1.4 (60 MPa)	0.34
(b)	0.01	1.0	0.2E-04	1.5	1.65	0.34

The number of passes of the cell suspension through the homogeniser (N in Eq. (1)) was included as shown in Fig. 1.

The total release of recombinant protein from the homogeniser is calculated from initially released protein in tank T1 or T2 ($c_{RPn,rel}$, Supplementary data, Eqs. S 1:5 and S 1:6), released protein due to homogenisation (c_{RPn}, Eq. (2)) diminished by protein denatured during homogenisation ($c_{RPn,deg}$, Eq. (5)):

$$c_{RPHn,rel} = c_{RPn,rel} + c_{RPn} - c_{RPHn,deg} \quad (3)$$

The intracellular protein concentration left after homogenisation is calculated from initial intracellular protein in tank T1 or T2 ($c_{RPn,int}$, Supplementary data, Eqs. S 1:3 and S 1:4) diminished by released protein due to homogenisation (c_{RPn}, Eq. (2)):

$$c_{RPHn,int} = c_{RPn,int} - c_{RPn} \quad (4)$$

The change of protein concentration due to denaturation during homogenisation is described as:

$$c_{RPHn,deg} = c_{tn,rel} \times k_d \quad (5)$$

where $c_{tn,rel}$ is given by:

$$c_{tn,rel} = c_{RPn,rel} + c_{RPn} \quad (6)$$

and k_d is a denaturation factor depending on the pressure:

$$k_d = 1.0 - 10.0^{(-K_d \times P^b)} \quad (7)$$

where K_d is the denaturation constant and b a pressure exponent.

Augenstein et al. [1] assumed that protein denaturation increases with pressure. Using Eq. (2) for deriving the release rate and Eq. (7) for the specific denaturation rate they predicted the total protein yield. Moreover, Choonia et al. [6] added observations of decrease of protein concentration at higher pressure and Engler and Campell [8] did observations on disruption stress effects.

Based on these findings, we defined the parameters K, K_d, and a in Eqs. (2) and (7) and as a function of suspension temperature during homogenisation. For clarification these parameters are written as K', K_d' and a′ (Supplementary data, Table S1.2). A double sigmoidal function as described by Gerlach et al. [10] is applied in which the suspension temperature is the argument x of the function (Supplementary data, Eq. S 1:22).

The homogenisation temperature of the suspension is defined as T_{H1} and T_{H2}. T_{H1} and T_{H2} are modeled according to the assumption that the temperature of the suspension increases by 2.5 °C per 10.0 MPa of operating pressure [20]:

$$T_{Hn} = 0.25\frac{K}{MPa} \times P + T_{Tn} \quad (8)$$

where T_{Tn} defines the temperature of the suspensions from tank 1 or tank 2.

Furthermore, we observed decreased flow rates at higher pressure as also noted by Choonia et al. [6]. Thus, a linear equation is used to describe the change of the flow rate (F) at different pressure (P):

$$F = -0.25 \times P + 45.0\frac{L}{h} \quad (9)$$

Applied model parameters are defined from own data where 45.0 L/h is the maximum flow rate without homogenisation pressure.

The described kinetic expressions were integrated in dynamic mass balances that can be found in the Supplementary data.

4. Results and discussion

In order to develop the integrated "Bioreactor-High-pressure-homogeniser" OTS five critical needs were set up as goals for the design (Table 1). The needs cover the capability of the OTS to accomplish (1) efficient virtual interaction with a recombinant protein production process, (2) efficient transfer of fundamental knowledge about HPH including influence of pressure gradient and temperature of the cell suspension, (3) efficient virtual training in cell disruption properties of different microorganisms during HPH, (4) efficient transfer of understanding of protein denaturation

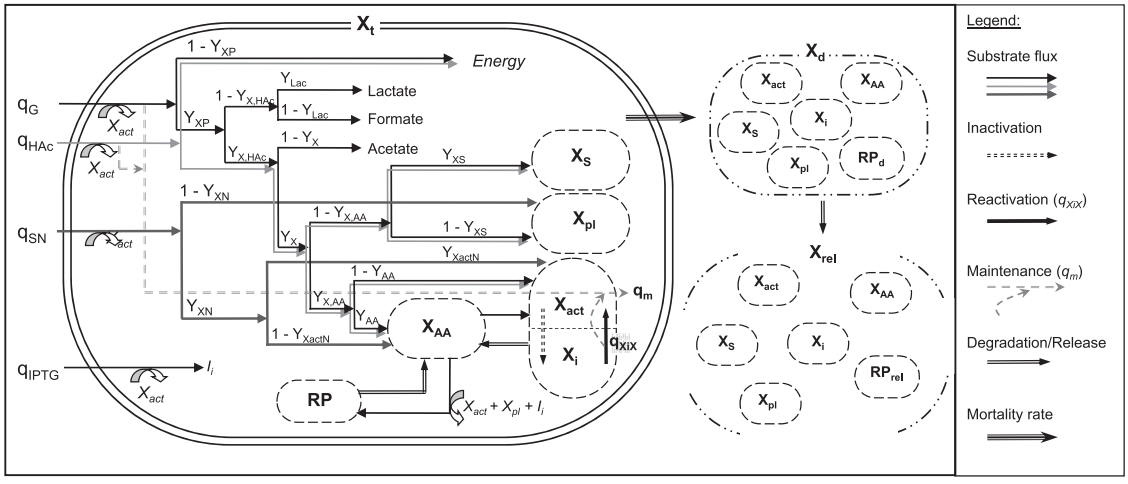

Fig. 3. Simplified model scheme of the protein expression model showing fluxes of substrate to different metabolic pathways (anabolic and energy pathway), in- and reactivation processes of active/inactive biomass (q_{XiX},q_{XXi}), the maintenance metabolism that is related to the reactivation process, the degradation of recombinant protein and inactive biomass and the mortality rate as well as the compartment structure of total viable biomass (X_t), dead biomass (X_d) and released biomass (X_{rel}). q_G–glucose uptake rate; q_{SN}–nitrogen uptake rate; q_{HAc}–acetate uptake rate; q_{IPTG}–IPTG uptake rate; X_{act}–active biomass; X_{AA}–amino acid biomass; RP–recombinant protein in X_t; RP_d–recombinant protein in X_d; RP_{rel}–released recombinant protein; X_{pl}–plasmid biomass; X_S–structural biomass; X_i–inactive biomass; I_i–intracellular IPTG. The modelling principle is shown *e.g.*, with q_G the glucose flux within the cell towards anabolic pathway (Y_{XP}) and energy (ATP) producing pathway (1-Y_{XP}). The types of arrow illustrate the respective reaction (legend).

effects during HPH and (5) efficient transfer of understanding for applying different pass strategies at HPH. These needs guided the design of the integrated OTS into a functional support.

4.1. Virtual homogeniser design

The layout of the graphical user interface of the OTS was designed close to the piping and instrumentation diagram (P&ID) of the units of the integrated process. As shown in Fig. 1, the cell suspension coming from the protein harvest tank of the bioreactor is pumped into holding tank T1 of the HPH. The layout of the HPH includes two storage tanks, by that allowing the use of different pass strategies through the homogenizer, for example by transfer of the cell suspension from holding tank T1 to T2 or *vice versa*, or for continuous homogenisation, by using additional pumps. Sampling ports allow collection of off-line data from both tanks. The off-line data are presented together with on-line signals in separate sub-interface diagrams. Furthermore, cooling jackets control the temperature of both holding tanks and sensors monitor temperature (TIC), operating pressure (PIC) and tank volumes (VIC) for controlling the process. These OTS features relate to Need 1 as well as Need 5 in Table 1.

Furthermore, by using the HPH-part of the OTS separately (without connection to the bioreactor) the initial concentrations of intracellular and released recombinant protein into the cell suspension were defined by the trainees. Different HPH experiments were carried out by changing the operating pressure that affects the flow rate capacity (cf. Eq. (9)), thereby addressing Need 2.

Fig. 2 shows the simulation results for release of green fluorescent protein (GFP) after homogenisation with five passes in comparison to data from experimental HPH runs. The concentration of GFP in the pure sample is around 1.7 g/L. Two different sets of temperature-independent parameters (K, a, K_d, b) are used for adjusting the model (Table 2). The release of GFP at 60 MPa in the experimental run is not significantly higher than the release at 30 MPa after the first pass. Additional passes release slightly more GFP at 30 MPa while at 60 MPa the GFP is either constant or show a tendency to decrease.

The lower concentration obtained at 60 MPa might be a result of the homogeneity of the culture in the experiments. Since the concentration at 60 MPa might be higher, a lower initial intracellular recombinant protein concentration ($c_{RPO,int}$) was used (Fig. 2a and Table 2). Compared to previous results from others

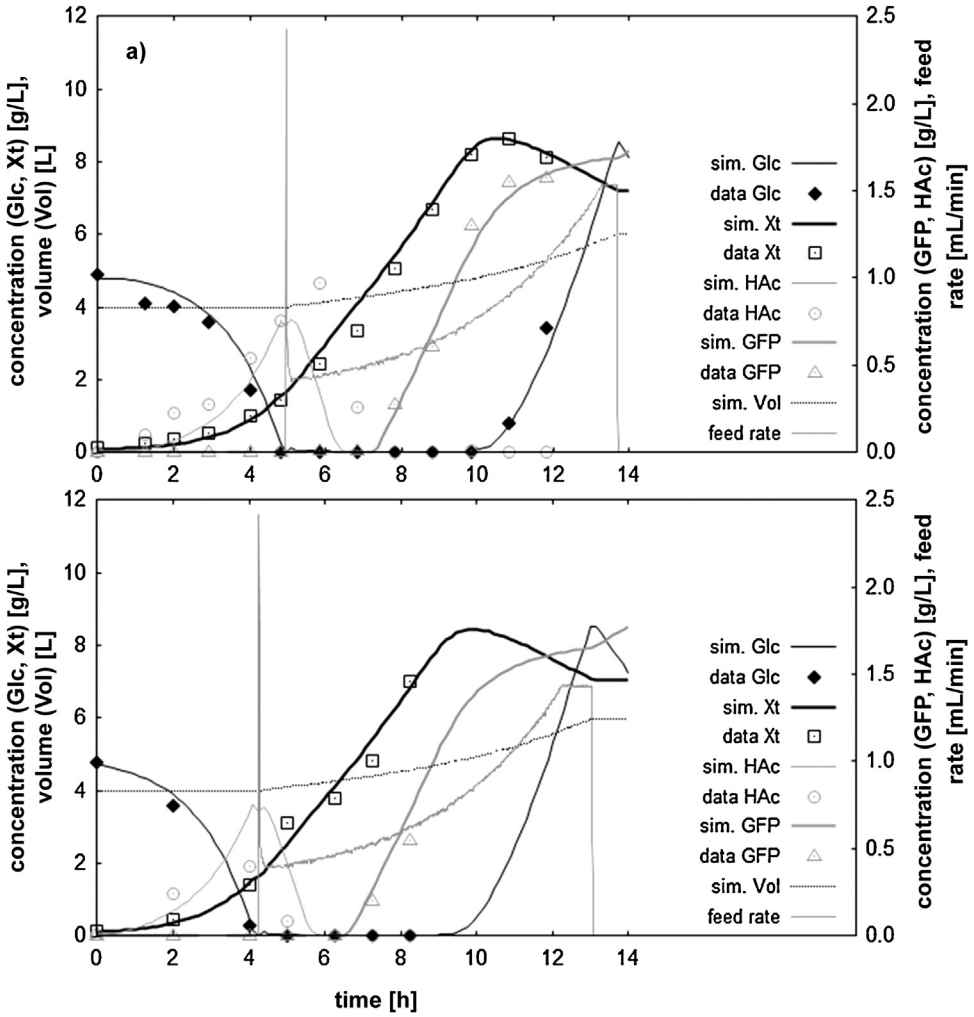

Fig. 4. Comparison between experimental data from two *E. coli* laboratory fed-batch cultivations producing recombinant GFP (dots) and simulation with the protein expression model (lines) showing relevant biological effects. These effects can be specifically trained within the OTS. Glucose (Glc, ◆), biomass (X, □), acetate (HAc, ○), GFP (△) concentrations, the volume (·····) and the applied feed rate profiles (——) are shown. (a) Cultivation process including batch and fed-batch phase with induction at 7 h. (b) Cultivation process including batch and fed-batch phase with induction at 6.5 h.

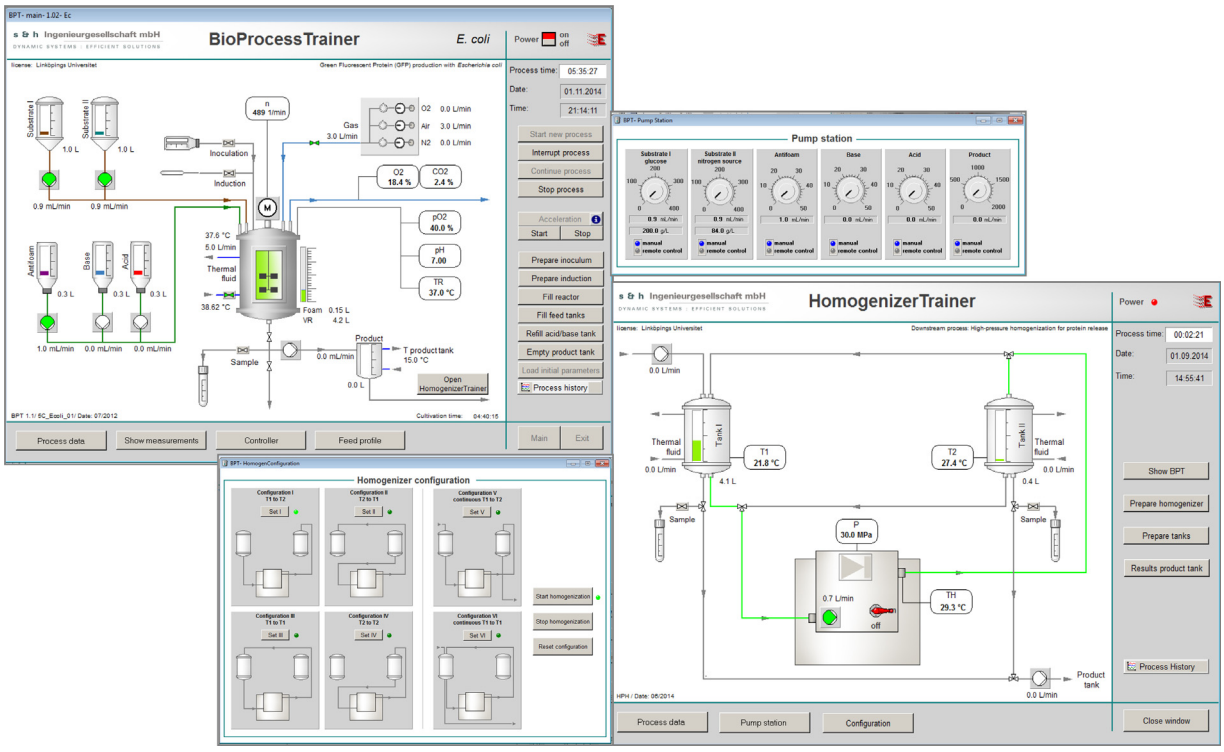

Fig. 5. Graphical user interfaces designed for the integrated OTS. Left side: Graphical user interface for virtual recombinant protein production with *E. coli* in a bioreactor. Right side: Graphical user interface for virtual homogenisation using a small-scale homogeniser. Smaller sub-windows showing the different configuration settings for the homogenizer (left side) and the pump station where different pumps can be controlled manually (right side).

(*e.g.*, Refs. [14,23,6]) where the release of protein increases with pressure, our second simulation at 30 MPa shows less release than the data from the experimental run (Fig. 2b). The results in Fig. 2 show the flexibility of the model. By tuning the constant K and the exponent a the release of recombinant protein can be adapted to the applied HPH system or microorganism. Furthermore, the denaturation constant K_d and the pressure exponent b can compensate for denaturation effects of the recombinant protein if it is instable at the pressures and temperatures used in the homogeniser.

To train Need 3, four microorganisms of different sensitivity to cell disruption were included in the simulations. The constraints used in these simulations were based on studies showing how protein release and cell disruption depend on temperature, pressure and number of passes in the homogeniser [23,19,6]. In particular the dependence of the type of microorganism and suspension temperature was applied where the parameter K, K_d and a were described as function of suspension temperature using a sigmoidal function (Supplementary data, Eqs. S 1:22 and S 1:23, Table S1.2), yielding K', K_d' and a'. In addition, when disrupting protein containing microorganisms of different stability Need 4 could also be included in the OTS.

4.2. Integration of homogeniser model with a modified protein expression model

The two processing steps were connected by using the output of the protein expression model as input for the homogeniser model (Fig. 1). Intracellular recombinant protein was taken as the sum of recombinant protein in total viable biomass (X_t) and dead biomass (X_d) while released recombinant protein was part of the released biomass (X_{rel}). Both were used as inputs as initial protein concentration values ($c_{RPO,int}$, $c_{RPO,rel}$; Supplementary data, Eqs. S 1:3 and S 1:5) in the HPH system when a certain volume was transferred into tank 1 (T1).

The integrated process was simulated in accelerated time mode allowing a faster training and time for testing several processing strategies within a limited time. This in particular is related to Need 1 described above.

In this study a previous developed structured model for recombinant protein expression [10] was modified for facilitating its integration with the HPH. The modification was necessary to improve the accuracy of the simulation and to simplify parameter adjustment, in particular for modeling of substrate consumption, energy pathway flux, recombinant protein expression yield and effects on expression due to stress response. The scheme of the protein expression model is shown in Fig. 3. The modifications included the modelling of (1) substrate uptake, (2) compartmental structure, (3) recombinant protein expression, (4) maintenance metabolism and (5) diauxic growth on acetate. As we previously described the substrate flux was split into separate pathways for anabolism (Y_{xp}) and energy metabolism ($1 - Y_{xp}$) [10]. Also, the use of a double sigmoidal function to describe a shift between pathways, inhibition of growth and overflow metabolism was used for metabolic changes in the OTS. By that, the modified model uses separate glucose (q_G), acetate (q_{HAc}) and nitrogen (q_{SN}) uptake rates that are split into one anabolic and one energy pathway. This simplifies the parameterisation of the model.

In the model the compartments of the cell's structural biomass (X_S), active and inactive biomass (X_{act}, X_i), plasmids (X_{pl}), amino acid pool (X_{AA}) and recombinant protein (RP) are portions of the total viable biomass (X_t). The amino acid compartment (X_{AA}) is included for describing stress response during recombinant protein expression. The recombinant protein compartment (RP) uses available amino acids when the culture is induced, while expression rate and the rate for formation of X_{act} are limited by the

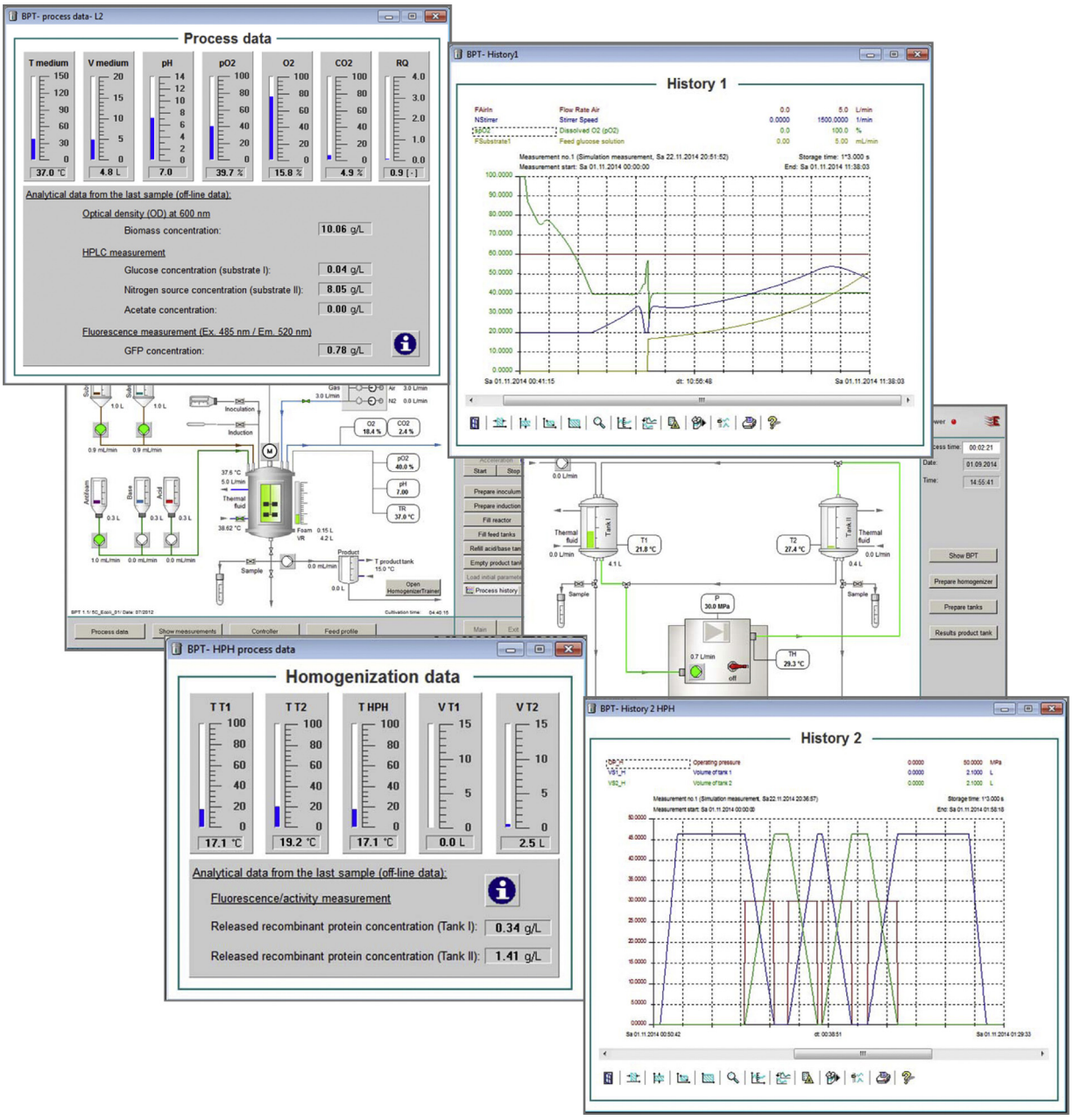

Fig. 6. Graphical user interfaces and sub-windows designed for the integrated OTS. *Via* the graphical user interfaces (Fig. 5) different sub-windows can be opened to check off-line data from the samples taken such as biomass and recombinant protein concentration and observe on-line signals such as dissolved oxygen tension (DO), temperatures and volumes from both process units. History 1: on-line signals (DO, stirrer speed, gassing rate and feed rate) from the virtual bioreactor; History 2: on-line signals (operating pressure, volume of tank 1 (T1) and tank 2 (T2)) from the virtual homogeniser.

amino acid level in the total viable cells (X_{AA}/X_t) (Fig. 3; Supplementary data, Eqs. S 2:5 and S 2:6). The rate of protein expression is related both to the plasmid copy number, represented by X_{pl}, and to the active biomass, X_{act}, after induction by in intracellular IPTG (I_i). The degradation of inactive biomass (X_i) and RP into X_{AA} (*e.g.*, degradation of misfolded and recombinant protein) is described by a separate rate. The maintenance metabolism is connected to the reactivation of inactive biomass (X_i) to active biomass (X_{act}). For instance, when glucose is available the reactivation rate is high and more active biomass is formed in the cell. Thus, the activity of the cell depends on the ratio of active biomass to the total viable biomass (X_{act}/X_t). When the reactivation rate (q_{XiX}) is lower than the inactivation rate (q_{XXi}) X_{act} decreases while X_i increases. The reactivation rate affects the glucose uptake rate because it is connected to the energy metabolism of the cell. For example, active biomass components, such as enzymes and RNA, are required for performing metabolic reactions and the activation of biomass requires energy. When the energy source is depleted these biomass components become inactive while still being part of the observed biomass in the model.

Substrate uptake rates depend on the RP level in the cells (RP/X_t). In order to improve the simulation of stress response effects also the reactivation rate depends on (RP/X_t) (Supplementary data, Eq. S 2:7). According to the transformation of glucose to biomass the transformation of acetate to biomass was improved without forming lactate and formate. Furthermore, the maintenance metabolism was expanded for the growth on acetate (diauxic growth).

Also the compartment of dead biomass (X_d) was specified into native protein (X_{act}, X_i, X_{AA}), structural biomass X_S, plasmid

Table 3
Evaluation of the OTS *vs.* user needs.

Needs	Evaluation[a]
(1) Efficient virtual bioprocess training in recombinant protein production	++
(2) Efficient transfer of fundamental knowledge about HPH	+++
(3) Efficient virtual training in cell disruption properties of different microorganisms during HPH	+
(4) Efficient transfer of understanding protein denaturation effects during HPH	+++
(5) Efficient transfer of understanding for applying different pass strategies for HPH	+++

[a] Successfully/agreeable +++; satisfactory ++; less satisfactory +.

biomass X_{pl} and recombinant protein RP_d to model the released biomass compartment (X_{rel}). This facilitates integration of the HPH assuming the whole cell suspension (including viable/dead biomass, intra/extracellular recombinant protein) is processed.

Fig. 4 shows simulations with the model in comparison to data from two cultivation experiments. It shows the concentrations of glucose, biomass, acetate and GFP. In both simulations the same model parameters were applied while only the initial active and inactive biomass composition was changed as well as the feed rate and induction times were adjusted to the experiments. When all initial glucose was consumed the feeding was started to continue growth. Induction with IPTG was done at 6.5 h (Fig. 4a) and 7 h (Fig. 4b). In the second experiment (4b) samples were taken until 8 h cultivation time while the simulation is shown until 14 h. Both experiments had a final GFP concentration (26 h) of approximately 2.0 g/L that correspond to the concentration measured for the pure sample for homogenization experiments (Fig. 2). The model manages to simulate all on-line and off-line data from the experimental run with high precision.

4.3. Visualisation of the homogeniser operation and its integration with the bioreactor in the OTS

The models for the integrated HPH system were implemented on the OTS platform and two user interfaces with sub-windows were designed and linked (Fig. 5). Through additional sub-windows off-line data such as biomass and protein concentration as well as on-line signals such as dissolved oxygen tension, feed rate, stirrer speed, operating pressure and temperatures from the virtual process were visible (Fig. 6, History 1 and 2). The interfaces visualised animated pumps and pipelines of the homogenisation unit (*e.g.*, single-pass from tank 1 (T1) to tank 2 (T2) where pipelines become green if the user activates the virtual homogeniser).

4.4. Applicability

The applicability of the integrated OTS was evaluated in training with an inexperienced test group *versus* the needs defined in Table 1. After the training the trainees answered a set of questions and solved tasks that were related to these needs (see Supplementary data, 3.). The evaluation illuminated the efficiency and transfer of understanding and skills in the training (Table 3).

Questions and tasks related to Need 1 which were based on on-line signals shown in graphs were solved satisfactory by the majority of the trainees. Tasks related to Need 2 were solved successfully by the majority. The tasks related to Need 3, where four microorganisms of varying robustness to disruption were to be combined with graphs from runs with different settings, were less satisfactorily solved. From observations and interviews with the trainees it became clear that they understood differences in disruption properties but could not apply this on a microorganism level. That probably indicates that running a simulation without sufficient pre-knowledge about cell physiology and structure severely hinders transfer of skills. Tasks related to Need 4 and 5 were also solved satisfactorily.

Moreover, two critical prerequisites were necessary to pay attention to:

- The requirement of having a correct pre-understanding of engineering terminology and its precise definition through proper professional language command
- Not to underestimate the understanding of molecular and physiological properties of biological systems.

A previous evaluation by us on the use of the same type of OTS tools has highlighted the transfer effect concerning action related skills, *e.g.*, adjustment of dissolved oxygen tension and improvement of heat transfer using the stirrer [9]. In another study we assessed psychological effects during OTS training such as confusedness and ability to recollect specific events afterwards [10]. With the observations from these previous studies together with this study with its focus on understanding and conceptualisation of the more demanding and advanced operator task of integrating two entirely different unit operations, the benefit of using OTS for training and transferring operator skills is unambiguous. The OTS could be demonstrated in a one-day simulation training. All needs defined for the OTS (Table 1) were fulfilled based on the software development.

5. Concluding remarks

An integrated OTS was developed for training of the operators' skills of running a bioprocess consisting of a recombinant protein cultivation followed by a high-pressure homogenisation step. Two established models were used in the OTS: (1) a structured model for protein expression extended with accumulation of the target protein and acetate overflow, and (2) a model for cell disruption in a high-pressure homogeniser extended with protein denaturation. Both models could easily be embedded in a shell structure of an OTS previously developed by us.

The OTS for the integrated process presented here was able to fulfill critical training needs when evaluated with untrained volunteers. By that, it was convincingly shown that the design of the OTS allowed transfer of fundamental understanding and skills for operation of the integrated bioprocess sequence. Future development of the OTS could include influence of cell concentration and specific growth rate on the efficiency of disruption in the homogenizer. Also, the integration of subsequent down-stream steps, such as centrifugation and chromatography, would be of value. Finally, adaption to other bioprocesses would expand the utility of OTS in the biotechnological industry.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.btre.2015.03.002.

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