

Olivier D.T. Sessink · Hendrik H. Beeftink
Rob J.M. Hartog · Johannes Tramper

Virtual parameter-estimation experiments in Bioprocess-Engineering education

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Abstract Cell growth kinetics and reactor concepts constitute essential knowledge for Bioprocess-Engineering students. Traditional learning of these concepts is supported by lectures, tutorials, and practicals: ICT offers opportunities for improvement. A virtual-experiment environment was developed that supports both model-related and experimenting-related learning objectives. Students have to design experiments to estimate model parameters: they choose initial conditions and ‘measure’ output variables. The results contain experimental error, which is an important constraint for experimental design. Students learn from these results and use the new knowledge to re-design their experiment. Within a couple of hours, students design and run many experiments that would take weeks in reality. Usage was evaluated in two courses with questionnaires and in the final exam. The faculties involved in the two courses are convinced that the experiment environment supports essential learning objectives well.

Keywords Modeling education · Virtual experiment · Experimental design

Introduction

Cell growth kinetics and reactor concepts constitute essential knowledge for Bioprocess-Engineering students. In two courses at Wageningen University, cell growth kinetics are described with the Monod kinetics,

while the consumption of substrate is described with the Pirt linear growth law.

Traditionally, students learn about theoretical models in lectures and tutorials, and about practical issues in a wet-lab practical. In the lectures and tutorials, the students spend a considerable amount of time on the calculation of solutions to exercises. By solving exercises, students are supposed to discover various characteristics of the models. In wet-lab practicals, students learn many practical aspects of cell culturing and apply the theory to a practical situation. Because cell culturing includes many new procedures for students (e.g., reactor building, medium preparation, sterile sampling, cell counting, etc.), application of theory receives often little attention.

Among staffs there was a general agreement on two opportunities for the improvement of our traditional learning support. First, the BSc-graduates’ understanding of the integration of kinetics and reactor concepts and how theoretical models relate to reality could be improved. This opportunity is outlined under “Model-related learning objectives”. Second, BSc-graduates’ understanding of how experiments relate to modeling could be improved as well. This opportunity is outlined under [Experimenting-related learning objectives](#).

This article discusses a virtual-experiment environment that was developed to exploit these opportunities. The virtual-experiment environment was developed for two courses: “Introduction to Process Engineering” early in the second year of the BSc of students both in Food Science and Technology and Biotechnology, and “Bioprocess-Engineering” late in the second year of the BSc of students in Biotechnology.

The virtual-experiment environment should comply with generally accepted learning and instruction principles. First, retention and retrieval of knowledge improve when students actively elaborate on the knowledge [1]. The learning process is best supported if the elaboration with the experiment environment is well structured [2]. Second, the cognitive processing capacity of the student is a limiting factor in learning. By minimizing the cog-

O. D.T. Sessink (✉) · H. H. Beeftink · J. Tramper
Food and Bioprocess Engineering Group, Wageningen University,
P.O. Box 8129 6700 EV, Wageningen, The Netherlands
E-mail: Olivier.Sessink@wur.nl
Tel.: +31-317-483229
Fax: +31-317-482237

R. J.M. Hartog
Wageningen Multimedia Research Centre, Wageningen University,
Wageningen, The Netherlands

nitive load, there is more capacity left for learning [3]. In computer simulations, an important factor to minimize extraneous cognitive load is the user interface [4]. Usability guidelines (e.g., [5]) can help to minimize the cognitive load in the user interface. Last, student motivation is a key requirement for learning. The ARCS model on motivation [6] provides four components that should be included: attention (stimulate curiosity and interest), relevance (connect to the students goals and interests), confidence (stimulate sense of success), and satisfaction (stimulate sense of achievement).

In the virtual-experiment environment, students have to estimate some model parameters. The virtual-experiment environment allows the student to design and run multiple experiments. In each experiment, the student chooses the reactor setup and the initial conditions. The initial conditions are all variables, such as substrate concentration, flow rate, and initial biomass concentration. Students then run the experiment and 'take' samples. The experimental results, biomass concentration and substrate concentration, contain experimental error. From these experimental results, students estimate the parameters. Students learn from the experimental results, supported by feedback, and use the new knowledge to design a new experiment. The cycle of designing and running many experiments executed to estimate the requested model parameters now takes a couple of hours instead of a number of days or even weeks.

Model-related learning objectives

The theory of both courses describes the specific cell growth rate with Monod kinetics:

$$\mu = \mu_{\max} \frac{C_s}{C_s + K_s} \quad (\text{s}^{-1}) \quad (1)$$

where μ is the specific growth rate (s^{-1}) as a function of the substrate concentration C_s (kg m^{-3}), μ_{\max} , the maximum specific growth rate (s^{-1}), and, K_s , the Monod constant (kg m^{-3}). Specific substrate consumption rate is described with the Pirt linear growth law:

$$q_s = \frac{\mu}{Y_{xs}} + m_s \left[\text{kg(S)kg(X)}^{-1} \text{s}^{-1} \right] \quad (2)$$

where q_s is the specific substrate consumption rate [$\text{kg(S)kg(X)}^{-1} \text{s}^{-1}$], Y_{xs} , the yield of biomass on substrate [kg(X)kg(S)^{-1}], and, m_s , the maintenance coefficient ($\text{kg(S)kg(X)}^{-1} \text{s}^{-1}$).

Students apply these models in various reactor configurations, most notably in ideally mixed batch and CSTR configurations. Mass balances for batch ($\phi=0$) and CSTR ($\phi_{\text{in}} = \phi_{\text{out}}$, $C_x = C_{x,\text{out}}$, and $C_s = C_{s,\text{out}}$) are then:

$$\frac{d(V C_x)}{dt} = \phi (C_{x,\text{in}} - C_x) + \mu V C_x \quad (3)$$

$$\frac{d(V C_s)}{dt} = \phi (C_{s,\text{in}} - C_s) - q_s V C_x \quad (4)$$

where C_x is the biomass concentration (kg m^{-3}), V , the volume (m^3), and, ϕ , the flow rate ($\text{m}^3 \text{s}^{-1}$).

The learning objectives supported by the virtual-experiment environment related to this model are as follows:

1. Qualitative understanding of the behavior of biomass and substrate concentrations in batch and CSTR reactors.
2. Understanding under which conditions this model is valid.
3. Intuitive knowledge of the order of magnitude of parameters and variables for a few common cell types and substrates.

With qualitative understanding, we mean that students should be able to reason about the system's behavior without knowledge of the actual values. If some parameter or variable is increased or decreased, students should be able to infer if this will lead to an increase, decrease, maximum, minimum, or asymptote of the system's output variables.

Students will spend most of their time constructing and reconstructing their knowledge of the model. To design an experiment to estimate a certain model parameter, the student needs to understand the relation between the parameter and the system behavior, and should know under which conditions the parametric sensitivity is the highest. The students should, furthermore, be capable of reasoning that certain initial conditions will allow measurement of all unknown variables in this region of high parametric sensitivity. There are too many degrees of freedom to successfully design experiments without correct understanding of the behavior and the assumptions underlying the model.

Students will have some idea about an experimental design, which results it will return, and how these results can lead to useful information about the task. Every sample the students take will result in some new information: an increase, a decrease, a constant, a local maximum, or a local minimum. Every time the students get this new information, reflective processes will be stimulated [7]. Students will consider if this result was to be expected, and, if not, they will reconsider their idea about the experiment and reconstruct their knowledge. Because the virtual-experiment environment enables a high frequency of these reflective loops, students spend most of the time reconstructing their knowledge of the model. The virtual-experiment environment is thus appropriate to support learning objectives 1 and 2.

In traditional exercises students often get a parameter value and use it in their calculations. In such exercises there is no stimulus to acquire knowledge for the order of magnitude of these parameter values. In the virtual-experiment environment, students have to estimate parameters from data with experimental error. When students know the realistic range of the parameter, they

can use this information to get an idea if their experimental design and calculation could be correct. This is a stimulus for students to acquire knowledge about the order of magnitude. The virtual-experiment environment is thus also appropriate to support learning objective 3.

Experimenting-related learning objectives

The learning objectives supported by the virtual-experiment environment related to experimenting are as follows:

1. Qualitative understanding of the uncertainty in parameter estimates.
2. Understanding that there may be large differences between the uncertainty in estimates of different parameters.
3. Understanding that uncertainty is affected by the experimental design.
4. Knowledge that constraints such as operational limits of analysis equipment, parametric sensitivity, and costs have impact on experimental design.
5. Skills to design an experiment that is appropriate to answer a specific research question.

Students often assume that the models and parameter values are perfect. In BSc courses, students are seldom confronted with uncertainty in parameter values. Too often, literature and textbooks do not show the uncertainty of parameter values. In the experiment environment, students will experience experimental error as a problem during the estimation of parameters. They will find that different experiments give numerically different results. These results show various fundamentals of parameter estimation. Because this will teach students to be critical towards parameter values, the virtual-experiment environment is appropriate to support learning objectives 1, 2, and 3.

Students have, furthermore, little experience with experimental design. In a wet-lab practical, students mostly learn practical skills. Hofstein and Lunetta [8] wrote “to many students, ‘a lab’ means manipulating equipment but not manipulating ideas”. In many practical experiments, lecturers spend most of their time managing the laboratory, and many students follow recipes without a clear understanding of the purpose of each step and without a clear goal [9]. The recipe, furthermore, is seldom questioned (e.g., [10, 11]). Students expect it to be correct and efficient, and expect the data gathered to be useful for their calculations. In real research, however, experimenting is tightly linked with uncertainty. Researchers should question if their experimental design is correct, if it is efficient, and if the gathered data will be useful for their research question. In the virtual-experiment environment, students are faced with the same uncertainty. Students are confronted with a design problem, and can practice exper-

imental design. Students get feedback on their designs, and accordingly design new, better experiments. Because students design and test many experiments in a short period of time, and get much feedback, the virtual-experiment environment is appropriate to support learning objectives 4 and 5.

Description of the virtual-experiment environment

For an overview of the virtual-experiment environment, we encourage readers to visit the online demo available at <http://www.pkedu.fbt.wur.nl/webman7/demos/>, and choose “Cell growth experiment design”.

Student task

Typically, the experiment environment is used after lectures and tutorials about the model; although online theory is available, the experiment environment itself does not introduce the model theory. Students get the task to estimate one or more parameter(s) for some model. For the model described in this article, these are μ_{\max} , K_s , Y_{xs} , and m_s . In order to do so, they should design and run a number of experiments. The design choices are as follows:

1. Reactor configuration, batch, or CSTR.
2. Value of operational variables $C_s(0)$, $C_x(0)$, and, for a CSTR, also $C_{s,in}$, and ϕ .
3. Time and number of samples, as well as sample analysis method. Students will measure $C_s(t)$ and $C_x(t)$, which include experimental error.

These experimental data can be used to estimate the parameter values. To allow the environment to be used in multiple courses, the environment does not require a specific method for parameter estimation.

Because in reality, costs are often a constraint in experimental design, students are shown the costs of their experiments. The costs are, however, no constraint in the experiment environment; students have an unlimited budget. The well-defined goal and limited number of choices of this task structures the way students use the experiment environment. A structured task is required for educational simulations to support the learning process in an effective way [2].

The experiment environment may present different tasks to different students. This allows the lecturer not only to give unique tasks to each student within a course, but also to use the experiment environment in different courses that have different models.

In the course “Introduction to Process Engineering” the goals are to estimate the maximum specific growth rate μ_{\max} and the Monod constant K_s . Students in this course use the experiment environment in a computer room. To avoid students copying answers from other students, each student will get a different organism with

different target parameter values; but their model will be identical. In the course “Bioprocess-Engineering” the goals are to estimate the biomass yield Y_{xs} and maintenance coefficient m_s . Again each student will get different parameter target values and use the same model. Both the tasks are considered highly authentic by staff and students. Authentic tasks might stimulate the transfer of skills to other problem solving situations [12].

Technical implementation

The experiment environment is implemented as Java applet. In order to use it, only a WWW browser with recent Java version is required, which is commonly available. The experiment environment can thus be used from any location with Internet connection at any time.

The Java applet can be controlled from the server, such that the single Java applet can accommodate different assignments. Different assignments might contain different parameter target values, different tasks, and different kinetic models.

The models can be selected from a list of available models; only a limited number of kinetic models is currently available. Extending the experiment environment with more models requires modification of the Java applet.

Experimental designs and results from each student are regularly sent to the server. They can be restored upon restart of the experiment environment, which allows students to stop and continue later on.

To allow students to do their calculations in a familiar environment, the experimental data can be copied and pasted into spreadsheet programs from all common office suites, such as Microsoft Office and Openoffice.org.

User interface

The user interface of the experiment environment consists of three windows. The first window contains the applet running in the Internet browser, called the “Main window”. The second window is the “Experiment window” in which students spend most of their time. The last window is the “Test your answer window” in which students can test if their answer to the task is correct.

Students start in the Main window (Fig. 1). This window introduces the task to the students, and it allows them to request a new task and to reset the experiment environment. Second, it gives access to the experiments and it allows the students to start new experiments. Third, it gives the students access to the Test window. Last, it gives access to help pages that open in a new browser window.

Clicking “new experiment” results in a new experiment window, each experiment will open a separate window. The user interface for experiment setup and execution is designed to continuously adjust and limit the choices presented to the students to those who are relevant to the pertinent situation. There are, e.g., two stages in each experiment: initial setup of the experiment

Fig. 1 The “Main window” in which the assignment is presented to the students

The screenshot shows a web browser window titled "Experiment assignment - Mozilla Firefox". The address bar shows the URL "http://pkedu.fbt.eitn.wau.nl/webman7/cases/growth_exp2/experiment.php". The main content area has a purple header "Experiment assignment" and a sub-header "Design your experiment". Below this is an "Assignment" section for *Spodoptera Frugiperda*, describing the task of estimating biomass yield and maintenance coefficient. It lists costs: Startup (€270), Running (€5/hour), and Analysis (€50 for high precision, €5 for low precision). A "Navigation" sidebar on the right lists links: Library, Cell growth in batch, Cell growth in CSTR, Linear regression, and Adding trendlines. At the bottom, there is a table of experiments (1-6) and buttons for "Start new experiment", "Re-open experiment", and "Test your answer". The current cost is shown as "Current costs: € 0,-".

and running the experiment. The tabbed layout matches these stages (Fig. 2). In the “Experiment conditions” tab, students can modify the reactor setup, initial biomass concentration, initial substrate concentration, feed flow rate, and feed substrate concentration. If students enter impossible values, such as concentrations exceeding the solubility, they are notified on the status bar and starting the experiment is disabled.

The “Run and Results” tab shows the buttons to run the experiment and to take samples, a button to get feedback on the results, and the results themselves, presented both graphically and in a table.

For sample analysis, students can choose between a cheap and rough optical density analysis and a more expensive and more precise cell-counter analysis. The reason to introduce this choice in the experiment environment is to force students to think about experimental error and costs when taking a sample.

The experiment starts with the first sample and is continued by selecting when to take the next sample. An ‘incremental time’ of zero can be used for a duplicate sample. The results, substrate and biomass concentrations, are plotted against time in graphs that automatically scale to show all results. The results are also added to the table. At any moment a student may decide to copy and paste the results to a spreadsheet program to do calculations. The “Feedback” button will show feedback on the current results in relation to the task (see below). If, e.g., a student who has to find the maximum specific growth rate μ_{\max} and the Monod

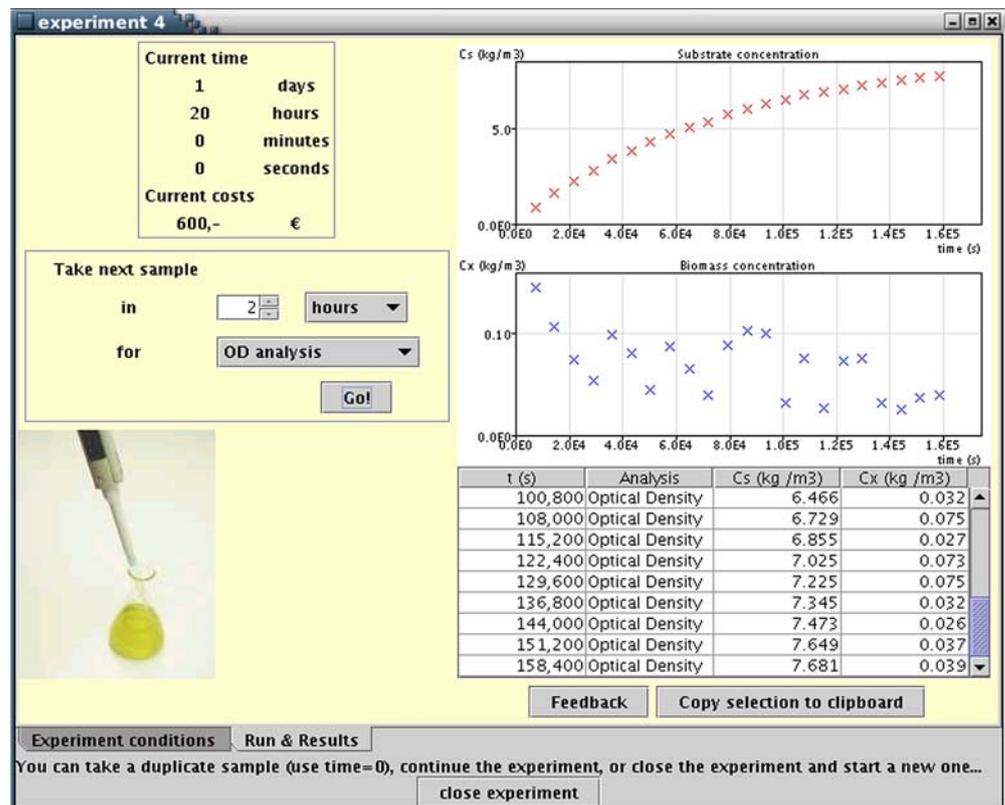
constant K_s would click “Feedback” after running a batch experiment for 5 min, the experiment environment will inform this student that these results are not very useful to find the maximum specific growth rate μ_{\max} , because there is experimental error and only little difference between the lowest and the highest biomass concentration. It will also inform this student that a batch experiment is not useful to find the Monod constant K_s because estimates of specific growth rate at a low substrate concentration in batch are inaccurate.

The experiment window is not attractive to “click without reasoning”. It stimulates a more rational analysis [13], because changing initial conditions does not immediately result in new output. Students first need to run the experiment and take samples. Badly chosen initial conditions or sample times result in useless data, while at the same time increase costs.

If manipulation of conditions is unlimited, easy accessible and immediately resulting in new output, students tend to change conditions without reasoning and work very inefficiently [14]. The delay between the experimental setup and the results is furthermore a form of asynchronous feedback, which could induce the deeper thought processes demanded by the task [4].

In a couple of hours, students may use the experiment environment to design and run many experiments with different results. Whenever students have found an answer to the task, i.e., a parameter estimate, the “Test your answer” button in the main window is clicked to open a new window, in which the estimate is submitted

Fig. 2 The “Run and Results” tab, in which students choose when to take samples, and see the results of their experiment. These results are from a CSTR, which is not yet in steady state



and judged if it is within the satisfactory range defined by the lecturer. If the value is within range, the cost score will be sent to the server and shown to other students.

To stop students from guessing the parameter values to get the cheapest design without serious experiments, the experiment environment will notify the students if their experiments are inadequate. For example, if the Monod maximum specific growth rate μ_{\max} is to be estimated, students should have at least an experiment that has at least two samples at a substrate concentration ten times higher than K_s and a biomass concentration within the virtual detection range, and the highest and lowest biomass samples should have a two times difference. If not, the notification message is, e.g., “This batch experiment is not very useful to find the maximum specific growth rate, because the biomass concentrations are all very low and hard to measure accurately”.

Experimental-data generation

The experimental data that are obtained by the student is generated from a set of differential equations. The experiment environment solves these equations using a Runge Kutta integrator. Experimental error is added to the exact output. The experimental error of student measurements in a real practical were evaluated and copied into the experiment environment. The experimental error contains absolute and relative parts in such a way that students are confronted with a relatively small error above a certain threshold and a relatively large error below this threshold.

The courses “Introduction to Process Engineering” and “Bioprocess Engineering” both use the same set of differential equations. All data generated by these equations should follow the Monod kinetics and the Pirt linear growth law when substrate is available. Because of the zero-order nature of maintenance, the Pirt linear growth law predicts substrate consumption in the absence of substrate. To prevent negative substrate concentrations, a different equation is needed. The Runge Kutta integrator cannot handle a model with non-continuous equations. Beftink et al. [15] describe a continuous model that follows Pirt if substrate is available and that has realistic results if no substrate is available:

$$\mu_{\text{obs}} = \mu - m_s Y_{\text{xs}} \left(1 - \frac{C_s}{C_s + K_{\text{shift}}} \right) (\text{s}^{-1}) \quad (5)$$

$$q_{\text{s,obs}} = \frac{\mu}{Y_{\text{xs}}} + m_s \frac{C_s}{C_s + K_{\text{shift}}} (\text{kg}(S)\text{kg}(X)^{-1}\text{s}^{-1}) \quad (6)$$

These equations are used to generate experimental data for both the courses. Because students use the original Pirt equation, K_{shift} value chosen is very small ($K_{\text{shift}} = K_s/1000$), such that the deviation from the Monod kinetics and the Pirt linear growth law is negligible in the substrate range in which students do their experiments.

Feedback

Because students should learn how to design experiments themselves, there is no recipe provided with the experiment environment. There is, however, feedback provided to help students with the most common problems, and induce verification, elaboration, and concept development.

The “Get help” function from the main window gives feedback how to approach experimental design and how to measure in cell-culture systems. This feedback focuses on concept development. The experimental design feedback is, e.g., to split up the problem into smaller sub-problems and to measure under conditions that minimize experimental error. Feedback on measuring in cell-culture systems is, e.g., which concentration ranges can be analyzed well, that CSTR experiments can be used to measure under constant conditions, and that batch experiments can be used to measure maximum specific rates.

In some situations, however, feedback is given that is much more focused on verification and elaboration. In situations where the experimental design is clearly flawed, the student is immediately notified. For example, if the initial biomass concentration is zero, or the feed flow rate is extremely high, a message on the status bar will notify students. To allow students to acquire intuitive knowledge about substrate inhibition, without the need to include it in the model, feedback is shown if they enter high substrate concentrations. A message on the status bar will notify them that their concentration might in reality lead to substrate inhibition.

Students can use the “feedback” button in the “Run and Results” tab to get feedback on their experimental results. Because it is unknown to the experiment environment what the intentions of the student are (e.g., to get a rough idea or to do precise measurements), it is often impossible to give a detailed feedback on the results. The feedback, therefore, shows how the experimental results compare to the task (e.g., the results useful to estimate the maximum specific growth rate), and how the results compare to the measuring system (e.g., the CSTR in steady state).

Discussion

Lessons learned

During the development of the virtual-experiment environment several preceding designs have been created and tested with formative evaluations. “Formative” evaluation refers to the structured evaluation that takes place during the use of the learning material with the intention to improve the material [16]. The results of these evaluations have, to some degree, shaped the current virtual-experiment environment.

First, formative evaluations of a prototype virtual-experiment environment that included many practical aspects of cell culturing showed that mixing theoretical and practical learning objectives in a virtual-experiment is not effective. If experimental results were unexpected, students resorted to practicalities to solve the problem. Students did not consider if their approach was theoretically sound at all.

Second, the same formative evaluations showed that simulating practical issues such as equipment peculiarities should be done very well or should be avoided. Although we spent a considerable amount of time to simulate the equipment correctly, students indicated that the relation between the peculiarities of the simulated equipment and the peculiarities of real equipment is not clear to them. Students indicated this affected their motivation in a negative way.

Last, in several tests students indicated that it is frustrating if a computer environment makes them do work manually that is easily done by a computer. Manually calculating concentrations from computer generated HPLC results are, e.g., considered more frustrating than manually calculating concentrations from real HPLC results.

Because of these three observations, all practical learning objectives were removed from the virtual-experiment environment.

Extending the learning objectives

The experiment-related learning objectives all have a statistical background. The virtual-experiment environment may be extended with more statistical learning objectives. The model as described in this article is particularly interesting to introduce statistical theory. The model's behavior is non-linear in time, and linearizing or non-linear fits are thus required for parameter estimation. This allows the lecturer to introduce aspects such as error propagation and the statistical impact of linearization (e.g., [17, 18]). There are, furthermore, many publications specific for this model. For example, about optimal experimental design for parameter estimation [19, 20, 21], as well as optimal experimental design for model discrimination [22].

Depending on the statistical background of the students and the learning objectives of the course, three extensions are envisioned for the virtual-experiment environment.

First, the calculation of the confidence interval could be added to the assignment. Currently the experiment environment does not include error estimates as part of the assignment. The experiment environment only tests if the difference between the model parameter values and student parameter estimates are within a certain range. In reality, students should calculate the confidence interval of their estimate, and decide if it is sufficiently small. The experiment environment should thus check if the confidence interval found by the students is correct.

Calculating the confidence interval from the data of multiple different experiments, however, requires advanced statistical knowledge.

Second, the experiment environment could be modified to include model discrimination, i.e., the determination of the best model for a certain set of data. Students can use the virtual-experiment environment to practice experimental design for model discrimination, and practice methods for model discrimination (e.g., [23, 24]).

Third, the experiment environment could be used to introduce the concept of optimal experimental design. An experimental design is optimal if it allows the most precise calculations of the parameters for a given model. Optimal experimental design is only possible if it is known that the experiment object can be described with a certain model, and if the experimental error is known. Because the experiment is simulated, and thus the model and experimental error are known, the environment can thus be used to introduce optimal experimental design to students.

Conclusion

The virtual-experiment environment successfully exploits the opportunities in model-related learning objectives and experimenting-related learning objectives. Students get an authentic task: students are asked to estimate model parameters. In order to do so, students can design and run experiments. Experimental results contain experimental error, which is an essential constraint for the design.

It is argued that the experiment environment supports qualitative understanding of the behavior of biomass concentrations and substrate concentrations in batch and CSTR reactors, understanding under which assumptions this model is valid, and intuitive knowledge of the order of magnitude of parameters and variables, for a few common cell types and substrates. It is, furthermore, argued that the experiment environment supports qualitative understanding of the uncertainty in parameter estimations, understanding that there may be large differences between the uncertainty in estimates of different parameters, understanding that uncertainty is affected by the experimental design, knowledge that constraints such as operational limits of analysis equipment, parametric sensitivity, and costs have impact on experimental design, and skills to design an experiment that is appropriate to answer a specific research question.

Design and development of the experiment environment as digital web-based learning module was successful. The experiment environment is implemented as java applet, and the user interface is designed to structure the learning process. The environment gives feedback that supports the learning objectives to the student.

The experiment environment is used by 134 students in two different courses in the second year of the BSc

curriculum Biotechnology and the BSc curriculum Food Science and Technology. Usage is evaluated with questionnaires and in the final exam. Students rate the environment's usefulness 3.9 on a 1–5 scale. Students indicate that the learning objectives are well supported by the experiment environment. The faculty involved in these courses, furthermore, is convinced that the experiment environment supports essential learning objectives, and that it supports those learning objectives well.

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