

Evaluating FermOpt as a tool for teaching fermentation and optimization principles

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Abstract - Educating students about industrial biotechnology processes is resource and time intensive when practical experiments are used to convey knowledge and build skills. Fermentation, the common biotechnology process, involves growing microorganisms in a stirred tank to mass-produce a protein, enzyme or chemical compound from a substrate. Fermentations typically run over several days or weeks, after which the product is purified through a series of expensive downstream processing steps. The long time involved makes it very difficult for students to gain experience in fermentation principles and process optimization. We report a trial evaluating third year biotechnology student learning using “FermOpt”, a simulation of an industrial fermentation. Student understanding of fermentation was ascertained using pre-test and post-test questionnaires and their perceptions of FermOpt obtained through a survey.

Index Terms – Biotechnology, fermentation, simulation.

INTRODUCTION

Fermentation is a well-established process that uses microorganisms to produce products from feedstocks. Traditional fermentations include beer, wine and ginger beer using yeast to ferment sugars into alcohol and carbon dioxide. Examples of industrial fermentations include antibiotic, fuel alcohol and enzyme production. Modern fermentations may use genetically-modified organisms such as *E. coli* to produce specific proteins or chemical compounds that are recovered and purified for domestic, industrial, medicinal and pharmaceutical use. Examples include endoproteases from *Bacillus* for adding to laundry detergents, penicillin from *Penicillium chrysogenum* for antibiotics, and insulin precursors from *E. coli* to produce insulin for humans.

A typical fermenter has a stainless steel tank up to 30,000 L in volume, agitators to keep the solution well mixed, aeration to maintain dissolved oxygen concentration, pH and foam monitoring, dosing equipment to maintain constant pH and reduce foaming, and heating and cooling to maintain a constant temperature. The feedstock may be a sugar solution, with added minerals and growth factors to help the organism grow. To prevent cross contamination, the fermenter and feedstock are sterilized before adding the microorganism. The microorganism progresses through a lag phase, and then grows exponentially before entering a stationary phase. The product may be the cells themselves, or biochemicals produced by the cells. These biochemicals

may be within the cells, or excreted as secondary metabolites during the stationary phase. Fermentations may run for up to 30 days before the desired product is removed from the broth using processing steps such as filtration, centrifugation, and chromatography. These downstream processing steps can contribute up to 50% of the capital cost and 80% of the operating cost of the entire process to obtain a purified product.

Fermentations are notoriously difficult to optimize to achieve maximum product yield at minimum cost. Adding ‘factors’ that increase cell growth and product yield may increase downstream processing to purify the product. The microbes may be delicate or difficult to grow because they are being forced to produce proteins they might not normally produce. Thus, careful control of fermentation conditions is required. Obtaining optimal feedstock and growth conditions for an organism may take many months of small-scale, trial-and-error experimentation and may involve using statistical packages and neural network software. Translating this to a learning experience can be difficult.

In the Department of Engineering at the University of Waikato, we have attempted to teach fermentation using small-scale experiments by growing baker’s yeast in a sterilized yeast extract solution in a 4-L vessel. The experiment was prepared two to three days before the laboratory and the yeast inoculum added. Students then investigated the effects of air flow-rate and agitation speed on dissolved oxygen levels in the vessel. Students only had three to four hours to run the experiments so they were unable to investigate the effects of differing conditions such as feedstock, pH and temperature. Sometimes the yeast would not grow or the feedstock became contaminated by other micro-organisms that grew more successfully. The demonstrators and students were frustrated when time was wasted with unsuccessful fermentations. Hence fermentation was only addressed in lectures with no practical experience.

Software simulations are often used as a teaching tool as substitute for practical experiments. A literature search identified several packages, which were either expensive and process oriented [1,2] or for specific experimental conditions and equipment [3,4]. We wanted a simple, generic and inexpensive tool that could be easily used by students with minimum training. “FermOpt”, an industrial fermentation simulation program, was developed by Professor Conan Fee (a colleague who is now at the University of Canterbury, New Zealand). This program allows students to explore the effects of aeration, feed and growth factor concentration, pH, temperature and agitation on cell growth, product yield, downstream processing and process economics. A complete

simulation can be run in two minutes and students can see, in real time, the cell, product and by-product concentrations, dissolved oxygen levels, pH and foaming. This program allows students to gain experience optimizing a simulated fermentation within four hours rather than, for example, six months to a year for a real fermentation.

FERMOPT

FermOpt (Figure 1) simulates growth of a hypothetical, genetically engineered microbe in a stirred tank fermenter containing a known starting concentration of substrate. The microbe has an optimum growth temperature and pH, and has a gene for producing a protein that is activated at 42°C. Dissolved oxygen levels can be controlled by adjusting aeration rate and/or agitation speed. Aeration, agitation, and adding 'factors' causes foaming, which can be controlled by adding antifoam. As the microbe grows and metabolizes, it consumes substrate and requires dissolved oxygen. Growth factors can be continuously added throughout the fermentation to enhance the bacteria's growth and/or protein production. The bacteria can also be poisoned by too much growth factors or substrate.

Throughout the fermentation, the bacteria produces by-products. After the fermentation is complete, the required protein (product) is separated from the bacterial cells, impurities (by-products), unused substrate, growth factors and antifoam in a series of downstream processing steps.

The number of purification steps is affected by the concentration of the impurities.

Once the fermentation is complete (250 hours simulated time but 2 minutes real time), FermOpt calculates the fermentation cost, based on starting substrate concentration, factor and antifoam addition, and operating parameters. It also calculates the cost of separating the protein and the protein yield, based on the number of downstream processing steps required. It then calculates the process economics in terms of total product produced, cost per mg of product and production cost ratio.

FermOpt was programmed and run using Stella/Ithink (ISEE Systems). Stella uses a visual interface to represent inputs, outputs and processes in a model. Complex models can be shown simply on a computer screen (Figure 2), and clicking on a process on the screen shows the underlying equations governing the process. The fermentation model developed mimics microbial growth using Monod kinetics, oxygen transfer with simple mass balance equations, and factor addition, substrate concentration, pH, antifoam and temperature effects using Arrhenius equations. The partial differential equations for microbial, growth factor, substrate, dissolved oxygen, product and by-product concentrations are solved by Stella using the Runge-Kutter method. The model and equations are hidden behind an interface, which provides user controls and graphs model outputs.

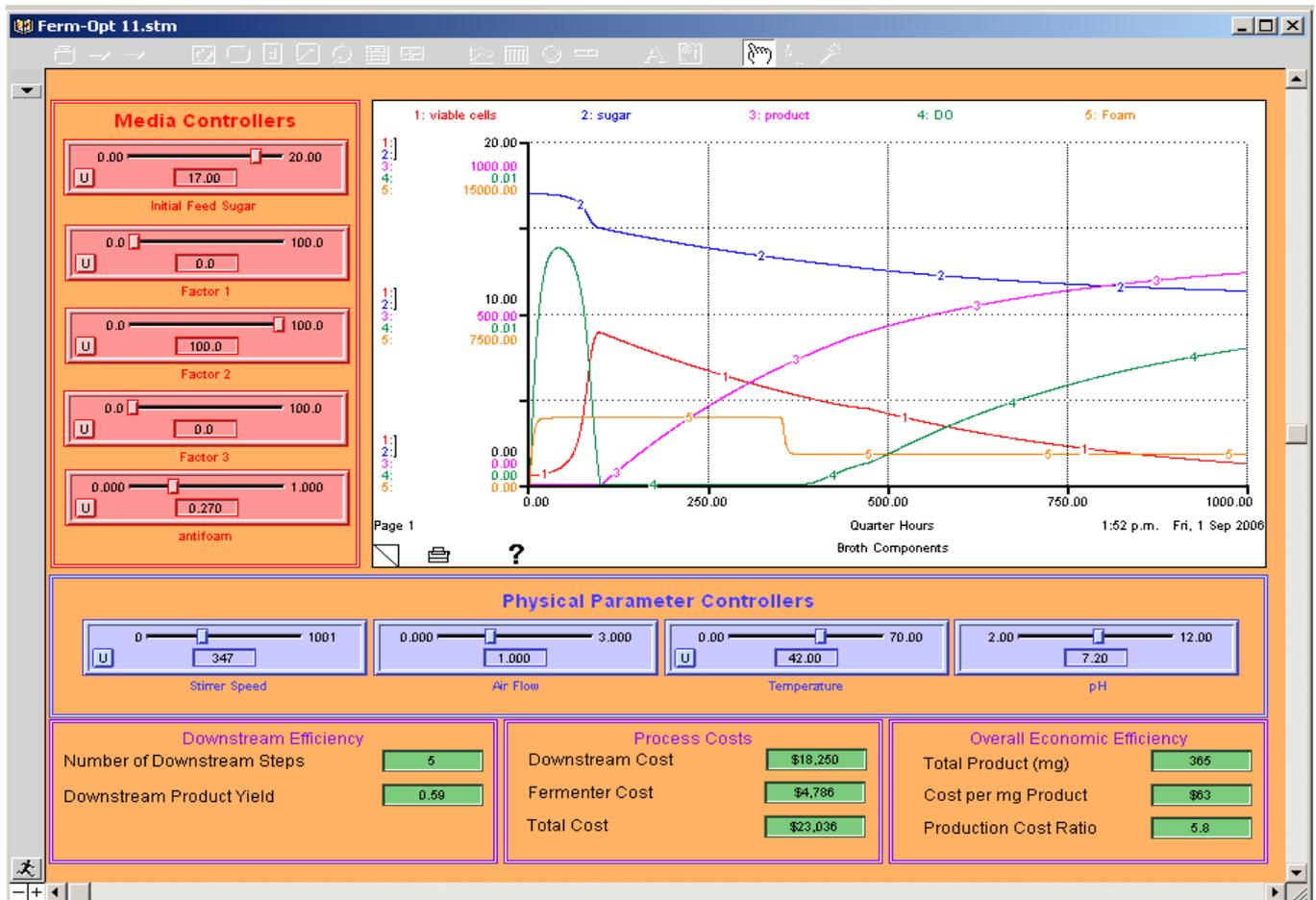


FIGURE 1
FERMOPT INTERFACE.

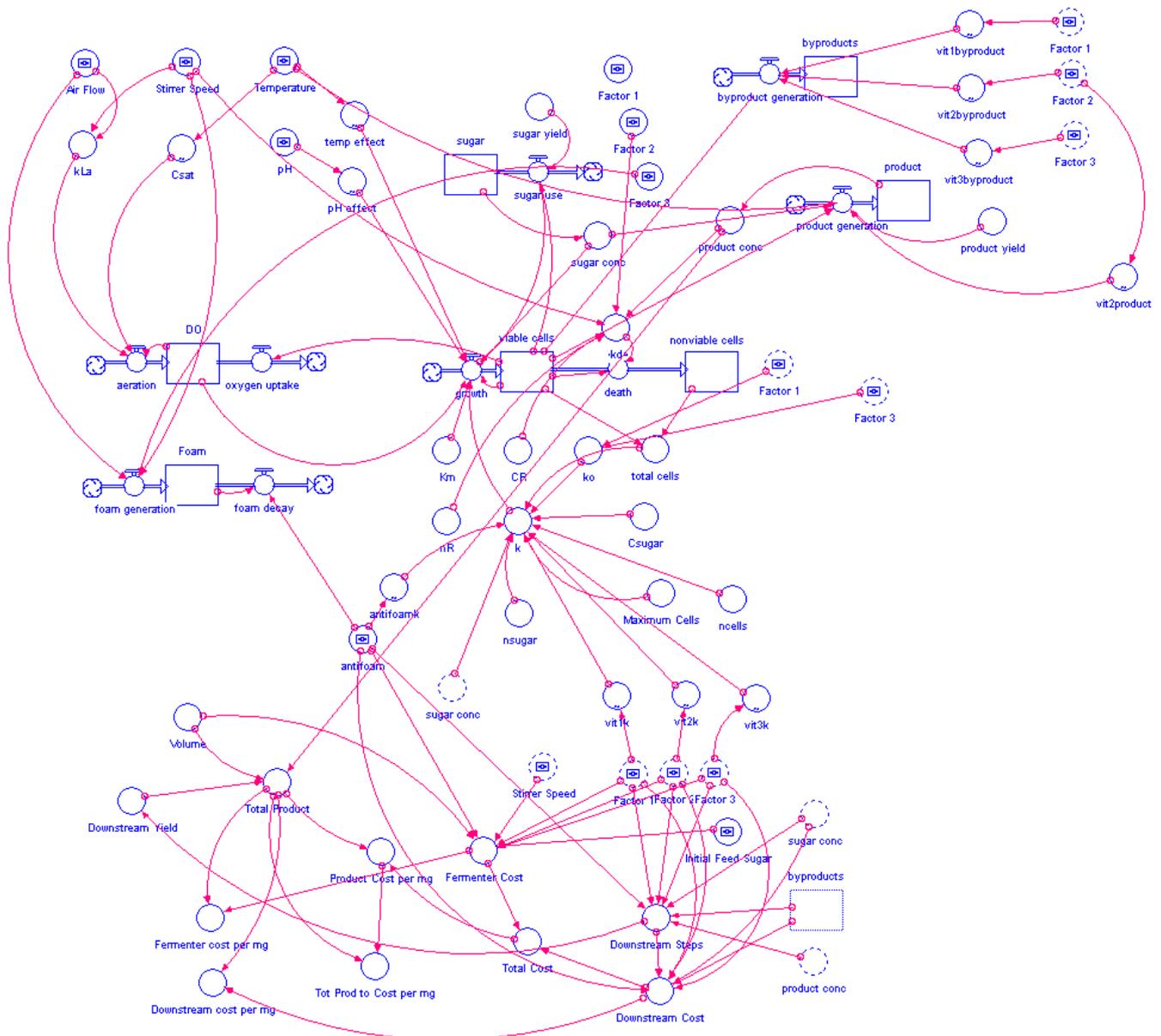


FIGURE 2
UNDERLYING PROGRAM FOR FERM OPT.

RESEARCH OBJECTIVES

We wished to evaluate FermOpt as a teaching tool to introduce third year biotechnology students to fermentation and optimization principles and to identify how the simulation software could be improved.

RESEARCHER BACKGROUNDS

Both authors are from the Department of Engineering at the University of Waikato. Mark Lay has a PhD in biochemical engineering (Waikato), teaches advanced biotechnology to third year students, and environmental technology; he also has a half time position in the Cooperative Education Unit at the University. Janis Swan is the Associate Dean and Chair of Engineering, has a PhD in chemical engineering (Waterloo); she teaches meat processing and biochemical engineering.

METHODOLOGY

Ethics approval for this study was obtained from the Science & Engineering School Ethics Committee.

Eleven third year biotechnology students, as part of their advanced biotechnology course run by the Departments of Biological Sciences and Engineering, were asked to participate in trialing the FermOpt software for the fermentation laboratory.

The students completed a questionnaire before using the FermOpt simulation, where they:

- Scored the effects of process factors (aeration, pH, temperature, agitation, foam, substrate, growth factors, antifoam) on process outcomes (cell yield, cell growth, product yield, difficulty of downstream, process cost) on a scale of 1 (not important) to 5 (very important).

- Ranked the order of the parameters students would investigate to optimize the fermentation to increase product yield and lower cost.

The students were then introduced to FermOpt, the fermentation scenario was outlined, and they were shown how to operate the software. Students were asked to plan and run a series of experiments using FermOpt. They were allowed to work together and discuss ideas for optimization, while we were available to answer questions. Students were encouraged to be competitive, and after two hours of trials, the students were asked to report their results. The students with the three best results were asked to demonstrate their optimized fermentation process to the rest of the class.

They then completed a post-simulation questionnaire, which was the same as the pre-simulation questionnaire, so we could assess their increase in knowledge. They also completed a simulation evaluation questionnaire.

Results were analyzed for differences between the pre- and post-simulation questionnaires and student comments on the software. Key areas of the FermOpt software that could be improved were then identified.

RESULTS AND DISCUSSION

Students had a very clear understanding from class lectures of how process parameters would affect cell yield and growth rate. This was demonstrated by the small difference in responses to these two questions in the pre- and post-simulation questionnaires (Figure 3 and 4). The exception was for the effect of growth factors. The pre-simulation response ranked this factor fourth equal, with pH, on cell yield. After completing the laboratory, this factor became first in importance for effect on cell growth rate and second in importance for cell yield (Figure 3). This was due to their findings from the simulation exercise. We surmise that while microbial growth principles are covered very well in microbiology courses, growth factors may not be as well covered due to the complexity of the subject.

Pre- and post-simulation responses for the effect of process parameters on product yield and downstream processing cost were significantly different (Figures 5 and 6). This can be attributed to limited knowledge with downstream processing principles (a subject taught in the fourth year) and the nature of this fermentation. Substrate, aeration, pH and temperature were rated (in descending order) as very important for product yield (Figure 5), In the simulation scenario, the microbe only began producing the desired product when the operating temperature was switched from the growth temperature of 30°C to 42°C, so temperature played a fundamental role in product yield. Before carrying out the simulation exercise, students thought agitation, antifoam and growth factors had similar importance to other processing factors on downstream processing. After the exercise, they decreased the importance of agitation and gave antifoam and growth factors much higher importance (Figure 6). They realized that adding antifoam and growth factors increased the ‘contaminants’ in the broth, so more downstream processing steps would be required to obtain a pure product.

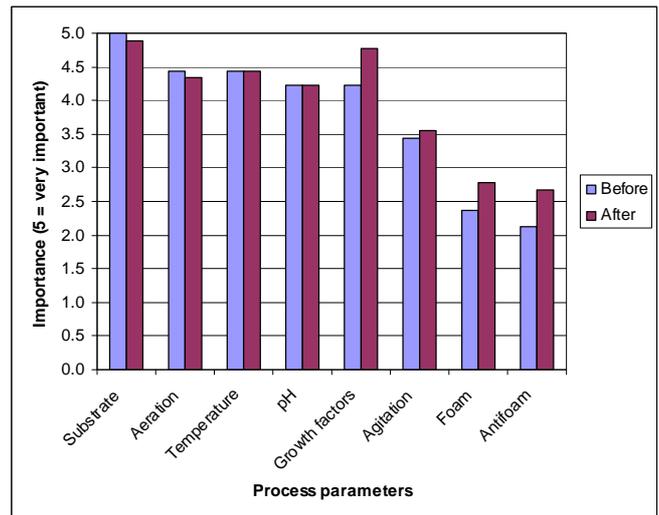


FIGURE 3
PERCEPTIONS OF THE EFFECT OF PROCESS PARAMETERS ON CELL YIELD BEFORE AND AFTER THE SIMULATION.

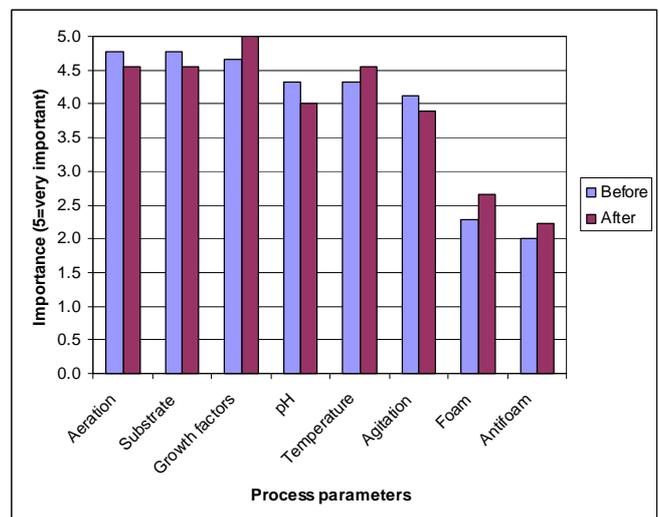


FIGURE 4
PERCEPTIONS OF THE EFFECT OF PROCESS PARAMETERS ON CELL GROWTH RATE BEFORE AND AFTER THE SIMULATION.

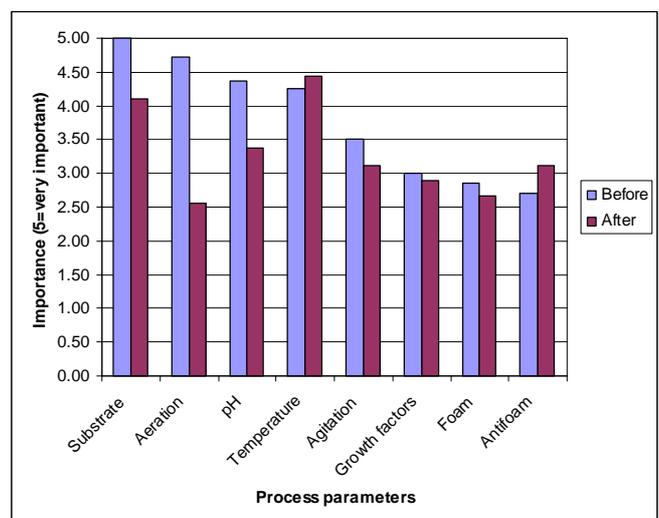


FIGURE 5
PERCEPTIONS OF THE EFFECT OF PROCESS PARAMETERS ON PRODUCT YIELD BEFORE AND AFTER THE SIMULATION.

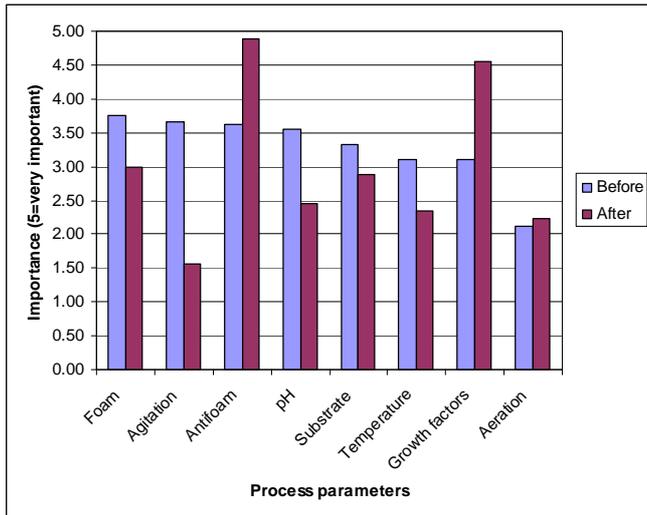


FIGURE 6

PERCEPTIONS OF THE EFFECT OF PROCESS PARAMETERS ON DOWNSTREAM PROCESSING BEFORE AND AFTER THE SIMULATION.

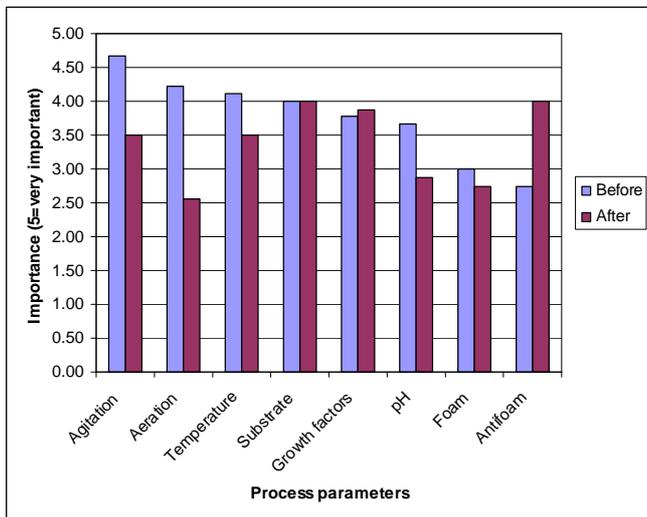


FIGURE 7

PERCEPTIONS OF THE EFFECT OF PROCESS PARAMETERS ON PROCESS COST BEFORE AND AFTER THE SIMULATION.

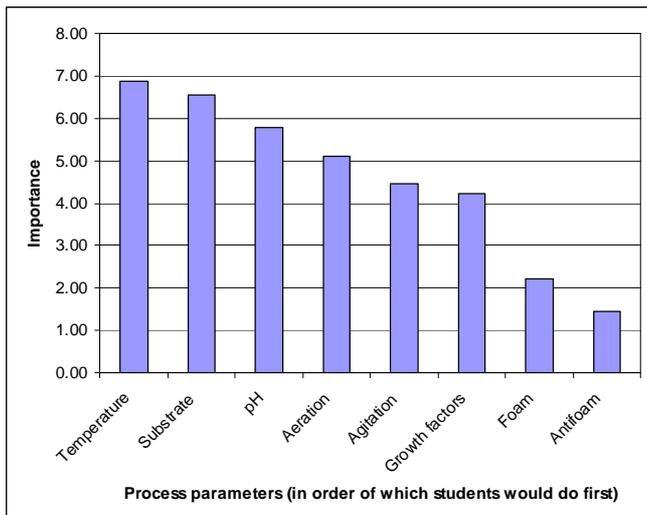


FIGURE 8

PERCEPTIONS OF MOST IMPORTANT PARAMETERS THAT STUDENTS WOULD OPTIMISE FIRST.

After the simulation, students ranked aeration and agitation as having less importance and antifoam more importance on process cost (Figure 7). Aeration and agitation are cheap operations and have little effect on process cost but adding antifoam is very expensive.

Students chose temperature, substrate, pH, aeration, agitation and growth factors (in descending order) as the most important factors to investigate first to optimize the fermentation (Figure 8). Foam and antifoam were not ranked highly as they had little effect on cell growth. This demonstrated that students had a good understanding of microbial growth and were able to choose a sensible regime to optimize the fermentation.

Data from the simulation were evaluated using a production cost ratio (total product produced divided by the cost of producing that product). Students gave an oral report at the end of the class. Those that had high production cost ratios (20 to 30 compared with initial values of less than 1) were asked to demonstrate their fermentation system to the class. Interestingly, students who obtained low production cost ratios during the class repeated the laboratory in their own time and achieved much higher ratios (30-53). This demonstrated to us that the exercise had caught the students' interest and stimulated their competitive nature. They were prepared to spend additional personal time to obtain better results.

On a scale of 1 = very effective, 2 = effective and 3 = average, students gave the simulation an average ranking of 1.6. Most thought the laboratory would be useful in teaching general optimization principles to students. Feedback on the laboratory showed students enjoyed the competitive aspect. They found the visual interface easy to use and thought the laboratory was very practical and quickly demonstrated ideas behind fermentation and downstream processing. They preferred to work individually as this allowed them to learn and understand the principles behind fermentation and process cost at their own pace. FermOpt allowed them to develop and test their different theories to try and optimize the fermentation, compared with typical laboratories where they follow a method but may not understand what they are doing.

Several students wanted the simulation to be more complex by introducing more controls and have it simulate different fermentations with thermophilic and anaerobic microorganisms. One student suggested learning would be increased if pop-ups explained why different factors have different effects. An example would be a pop-up explaining why increased foaming reduces product yield. Another suggested that the lab could be developed further by allowing them to trial different downstream processing options.

We found FermOpt an easy way to engage student interest and enthusiasm. The male students particularly enjoyed the computer game aspect of FermOpt. We could discuss with individuals their understanding of fermentation and downstream processing. It was an easy way to quickly investigate and observe the effect of many factors, trial theories, make improvements, and get immediate feedback.

For a successful simulation of a complex process the developer needs a sound understanding of fermentation and

processing principles and be able to mathematically describe each operation (Figure 2). But once created, the simulation can be easily changed by changing the constants used in the underlying equations. This means a unique set of conditions can be used for each laboratory.

We are investigating how to link FermOpt learning outcomes with those from the statistical experimental design course. One way would be to get the students to develop a statistically based strategy to optimize the fermentation simulation within a minimum number of runs.

CONCLUSIONS

FermOpt was found to be effective teaching tool for fermentation laboratories. We found that students had a good understanding of the general microbial growth principles behind fermentation but a poor understanding of downstream processing before carrying out the laboratory. Their understanding of downstream processing after completing the laboratory was much improved. FermOpt allows principles behind optimization to be rapidly taught, compared with traditional real-life fermentations that are time consuming and expensive to run. Overall, students thought the fermentation laboratory was effective and enjoyable. An added bonus was that students who initially did badly in the laboratory came back and repeated it in their own time.

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