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Use of a Case on Metabolically Engineered *Escherichia coli* to Develop a Framework for the Design and Analysis of Bioprocesses*

KAJAN SRIRANGAN[†‡], LAMEES K. AKAWI, C. PERRY CHOU and MARC G. AUCOIN[†**]
Department of Chemical Engineering, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1. E-mail: maucoin@uwaterloo.ca

LYNDIA E. STACEY and CHERYL NEWTON
Waterloo Cases in Design Engineering, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1

Thematic case-based studies can be used by instructors to enhance critical thinking and knowledge in a holistic fashion, as well as improve students' cognitive and metacognitive processes. While case-based learning approaches have been long used in the teaching of business, law and medicine, they have yet to see widespread use in many engineering disciplines. Furthermore, empirical research on the effectiveness of case-based learning within engineering is still in its infancy. Herein, we describe the use and impact of case-based instruction implemented in a third-year undergraduate bioprocess engineering course at the University of Waterloo. The overall objective of our study was to link key concepts related to bioprocess engineering to ''real-world'' bioprocesses through a series of tutorial modules and research projects tied together by a common thematic case. The developed case focuses on genetically engineered *Escherichia coli* for the production of biofuels, specifically 1-propanol. The intent was to provide the students (n = 94) with the opportunity to gain a deeper understanding of biological systems by linking traditional fields of bioprocessing to an emerging field such as synthetic biology while also immersing students into situations that they could encounter while working in industry. Our results suggest that a majority of the students felt that the case was engaging and that the small-group based problem-solving exercises helped their understanding of design principles relevant to bioprocess engineering.

Keywords: chemical engineering education; case studies; thematic cases; conceptualized approaches; chemical engineering; bioprocess education; metabolic engineering education; synthetic biology

1. Introduction

Engineering is an applied discipline in which problem-solving, critical thinking and self-directed learning play an important role. Contemporary engineering classes, however, still mainly adhere to traditional lecture-based learning approaches (i.e., ''teaching by telling'') whereby students are passive recipients of information in an ''instructor-centered'' paradigm [1–3]. This method of learning often leaves graduates ill-equipped for the engineering profession, as students work on oversimplified exemplifications of real-world data, with little emphasis on representation, analysis and modeling [4, 5]. Outside the classroom, engineers face real-world problems that often transcend a number of sub-disciplines, are more complex, are unstructured and may contain incomplete data. For this reason, engineering students require a broader skillset than simply what is provided in textbooks and lectures. In the US, movements like ''Big Beacon'' (bigbeacon.org) are challenging the status quo and are advocating change in traditional ways of teaching engineering. This is also happening at the University of Waterloo (herein referred to as Waterloo) which prides itself in its cooperative learning education. Students spend up to two years in industry as part of their undergraduate degree (all engineering students must spend at least 20 months in a workplace setting, i.e., 5 × 4-month work terms, although the programs are designed for 6 work terms). Even with the success of the cooperative education, Waterloo is also rethinking how classes are being taught. Over the last 8 years, three major initiatives have helped foster the way we present material in the classroom at Waterloo. First, through the Natural Science and Engineering Research Council (NSERC) of Canada, a Design Chair was awarded in 2005 to develop a library of engineering cases that helps bring real world scenarios into the classroom (uwaterloo.ca/engineering-cases). This group, Waterloo Cases in Design Engineering (WCDE), has a large engineering case study collection with over 160 cases. Second, MINERVA (safetymanagementeducation.com), a non-profit organization dedicated to process safety management and education, has inspired an initiative to

[†] These authors contributed equally to this work.
[‡] Current affiliation: Human Health Therapeutics Portfolio, National Research Council of Canada, Montréal, Québec, Canada, H4P 2R2.
** Corresponding author.

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create modules that highlight safety and allows safety to be "first-in-mind" in the classroom [6]. Third, and most recently, the Faculty of Engineering at Waterloo started championing Engineering Ideas Clinics (uwaterloo.ca/engineering-ideas-clinic), which pushes students to realize that engineering is all around them and that all theory comes back to observation of real world situations.

One method to enhance learning in classrooms is through the use of thematic cases. Cases have been widely used in several disciplines as a complementary teaching method for students to find holistic solutions to more open-ended and multifaceted real-world problems. Proponents of this pedagogical tool believe that cases offer the potential to provide a rich framework for the understanding of pertinent concepts, themes and processes [7, 8]. It allows for learning to be relevant and meaningful, as students can appreciate the scope and scale of practical, authentic problems by actively participating in the analysis, discussions and problem-solving of cases. The case method also shifts the learning approach by placing less emphasis on rote and passive application and more emphasis on creative and meta-cognitive thinking skills. Although case-based instruction gained some traction in the engineering curriculum in the early 1970s [9], it has yet to see widespread adoption in engineering classrooms. While part of the problem is that very few faculty members know how to adequately design and deploy case studies in the classroom, another part is that engineering cases are not pervasive for implementation in a variety of courses. Herein, the implementation of a thematic, case-based learning framework used in a third-year undergraduate bioprocess engineering course at Waterloo is described. The goal of the case study was to teach the utility of engineering approaches and design principles in the area of bioprocess engineering. Furthermore, in using the case titled "Engineering *Escherichia coli* for Biofuel Production", students were able to use real-world data to validate theoretical models of microbial kinetics, use flux analysis to quantitatively probe complex metabolic networks, and gain a deeper understanding of reactor design and operation. The methods used in this course have been offered in the Department of Chemical Engineering at Waterloo since 2013 and complement the traditional lecture format used in previous course offerings.

2. Course overview

The course listed in the Waterloo undergraduate calendar as ChE360 (Bioprocess Engineering) is a relatively new third-year course first implemented in 2008 as a successor to ChE032 (Introductory Biotechnology). The major difference is that students taking ChE360 are previously introduced to concepts in cell biology and biochemistry in their second term in ChE161 (Engineering Biology). This allows engineering concepts to be pushed further in the third-year course and enables a deeper understanding of biological systems. ChE360 is a full-credit core course with two scheduled classes and one tutorial per week (36 hours total in one 4-month period). With a class size of approximately 90, students of ChE360 gain an appreciation of engineering concerns for biological processes (see Fig. 1 for a detailed course outline) and how to address each concern through analysis and design. This may include data reconciliation, modeling, simulation and prediction.

As shown in Fig. 1, we first introduce students to the role of bioprocess engineering in biotechnology by providing a brief history on the use of biological systems for the production of value-added chemicals. Here, students are also introduced to the safety considerations for industrial and environmental applications of microorganisms. Next, students

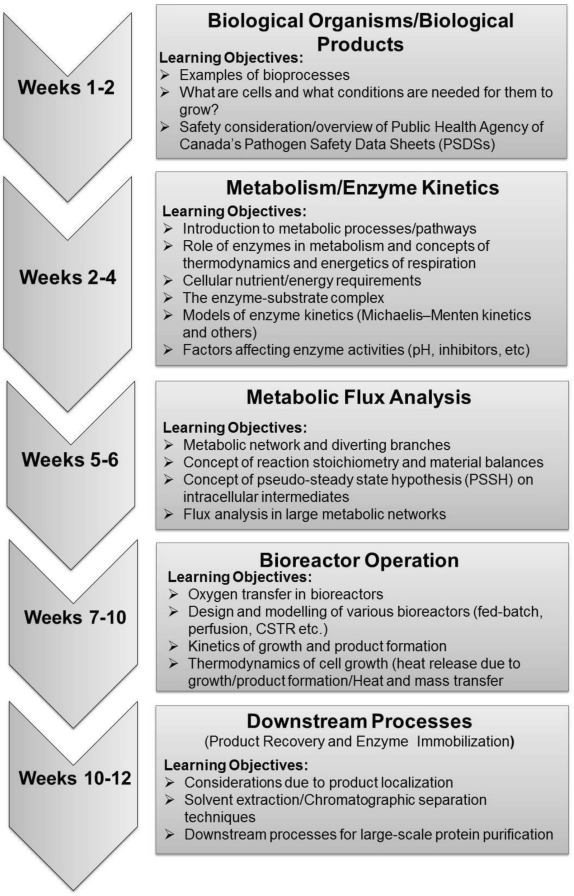

Fig. 1. Course outline and learning objectives for ChE360. ChE360 is a full credit course that runs for approximately 12 weeks and comprises of two scheduled classes and one tutorial per week (36 hours in total).

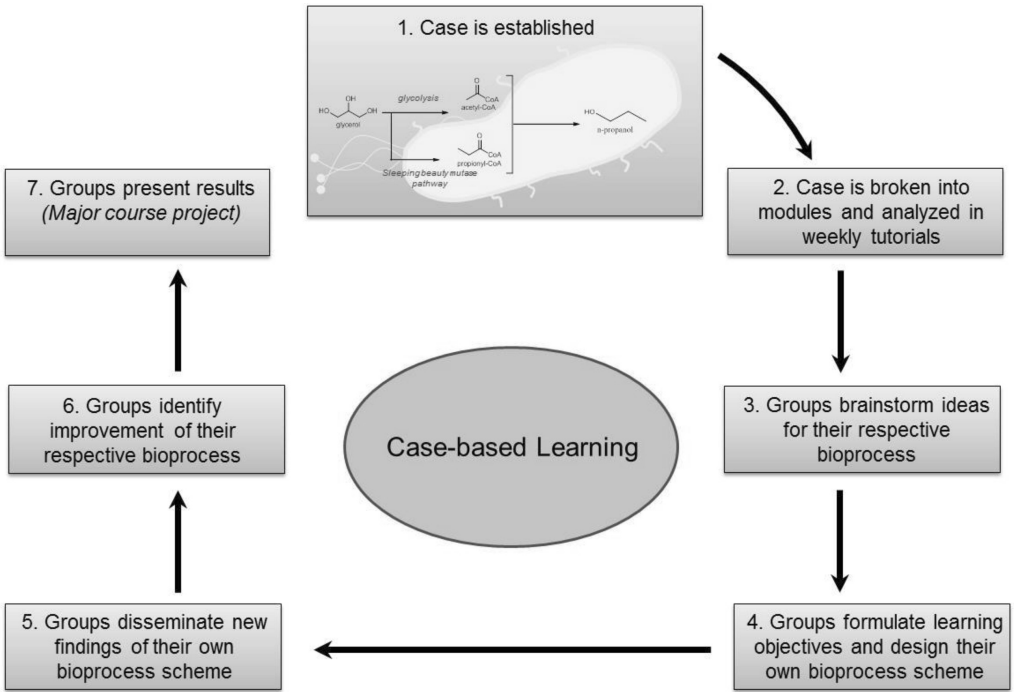

Fig. 2. The case-based learning process used in ChE360. The case architecture was as follows: A case study on the use of metabolically engineered *E. coli* was established and broken into tutorial modules to be analyzed on a weekly basis. Students were then provided the opportunity to design and implement their own bioprocess schemes, disseminate new findings and identify ways of improving their schemes.

are given an overview of cells, conserved metabolism and the importance of enzymatic reactions in the conversion of nutrient influx into balanced amounts of energy, biomass precursors and metabolites. While some of these concepts are introduced in ChE161, a major goal in ChE360 is to reinforce and extend a deeper understanding of concepts. For example, given a student's understanding of a near universal genetic code, protein production "machinery" and intracellular environment, it becomes possible to talk about the "design" of organisms imbibed with new features obtained from the addition of heterologous genes. As such, the known catalytic properties of enzymes from one organism can be exploited in a different setting to produce a target product. Furthermore, students can then look at metabolic maps with an eye for potentially modifying organisms. ChE360 further explores the concepts of metabolic flux analysis (MFA), which requires students to be able to generate mass balances around a cell and solve sets of linear equations. This strengthens the students' ability to generate mass balances around a system as well as obtain a solution for a set of algebraic equations. It also adds to the students' critical analysis of data and existing metabolic maps. The next two modules of the course introduce bioreactor operation (i.e., batch, fed-batch, perfusion, and continuous stirred tank reactors (CSTR)) and downstream processing (i.e., centrifugation, cell lysis and precipitation, and ion exchange chromatography). Finally, enzyme immobilization is presented and discussed, thus allowing the overall course to mimic an actual process train, from choosing a microorganism or cell factory and culturing it, to the recovery and use of a bioproduct.

3. Case architecture

3.1 Case overview and objectives

The case study that was developed for ChE360 was based on research conducted at Waterloo on the production of 1-propanol in engineered *Escherichia coli* [10, 11]. In this work, it was demonstrated that a silent, yet functional pathway (termed the Sleeping beauty mutase pathway, or Sbm pathway) in *E. coli* can be genetically activated to dissimilate endogenously produced succinate into the intracellular precursor propionyl-CoA. Briefly, genetic activation of the Sbm pathway was performed by placing a strong, regulatable promoter upstream of the Sbm genes using a phage-encoded recombination system. The synthesized propionyl-CoA can then be reduced via a variety of endogenous and heterologous alcohol dehydrogenases to enable production of 1-propanol. *E. coli*, as a model system, is an excellent way to teach engineering students the core concepts of biochemical and bioprocess engineering since the organism can grow under aerobic and anaerobic cultivation conditions. In this way, we

Table 1. List of case study modules* developed and implemented in ChE360

Module Number[1]	Title
Module 1	Case Overview
Module 2	Benchmarking with 1-Butanol in Microbes
Module 3	Organism Design
Module 4	Metabolic Pathway and Theoretical Yields
Module 5	Metabolic Flux Analysis of Propanogenic *E. coli*
Module 6	Growth and Production Kinetics
Module 7	Reactor Simulation and Operation
Module 8	1-Propanol Extraction and Downstream Processes
Module 9*	Benchmark Solutions
Module TN*	Teaching Note

[1] Modules 1-8 available as online: https://uwaterloo.ca/engineering-cases/cases/engineering-escherichia-coli-biofuel-production

can discuss, compare and contrast metabolic pathways that are active under respiratory and fermentative conditions. Furthermore, the use of real cultivation data of the propanogenic strains cultivated in lab-scale, shake-flask growths and higher cell-density fermentations (batch and fed-batch) provided us the opportunity to discuss central tenets of reactor design. Examples of this include oxygen transfers, growth kinetics, limitation of nutrients and reactions, calculation of theoretical maxima of biomass and product yields.

The overall deployment of the case study is schematically illustrated in Fig. 2. The case modules are also in Table 1. All case materials, tutorial modules and teaching notes are available online (see "Supporting Information").

3.2 Modules 1 and 2—Relevant background information and introduction to metabolic engineering

In the first week of ChE360 lectures, the students were provided with the first two modules (see "Supporting Information" for online link). The case study (Module 1) describes the background information and problem statement as well as introduces the use of biological systems for the production of biofuels. Here, students are also first introduced to two pertinent concepts that will create a foundation for designing cell factories: *endogenous pathways* and *heterologous pathways*. In the discussion-based interactive lecture using this module, we also introduced the concept of recombinant DNA technologies and how biological pathways can be intentionally rewired in organisms to produce chemicals and fuels of interest.

Once these concepts were established, students were provided Module 2 (Benchmarking with Butanol in Microbes) which overviews the work done at Waterloo on using *Clostridium acetobutylicum* and *E. coli* for 1-butanol production. This module also explores the concept of native metabolic pathways by providing an overview of acetone—butanol—ethanol (ABE) fermentation, a process that typically uses Clostridia to produce acetone, 1-butanol, and ethanol from low-value feedstock. These anaerobic bacteria, such as *Clostridium acetobutylicum*, have a specialized metabolic pathway for extended metabolism of acetyl-CoA for ABE fermentation and therefore, have served as the major biological system to produce 1-butanol for decades [12, 13]. Nevertheless, there are technical disadvantages associated with the use of strict anaerobes as the production host. First, conducting anaerobic cultivation is tedious, inconvenient, and expensive, particularly for large-scale production. Second, techniques for genetic manipulation of Clostridia are immature, particularly compared to *E. coli*. Third, economic production of butanol using Clostridia can be limited by high-cost feedstock.

It was then possible to show how butanol production in ABE fermentation consists of first condensing two acetyl-CoA moieties to form the C4 biogenic precursor acetoacetyl-CoA, which is then reduced through a series of enzymatic reactions to form the 1-butanol molecule [14]. The native clostridial metabolic pathways for ABE fermentation were then compared and contrasted to the genetically altered heterologous pathways found in butanogenic and propanogenic *E. coli*. In contrast to ABE fermentation, heterologous production of 1-propanol in *E. coli* is via the extended dissimilation of the glycolytic intermediate phosphoenolpyruvate.

The implementation of Module 2 was followed by a discussion of different fuels and the potential of 1-propanol as an alternative fuel. For the remainder of the semester, discussion and operations-based problems pertaining to aspects of the case (i.e., theoretical yield calculations) are based on the aim of producing 1-propanol and are presented as small modules during weekly tutorials (Modules 3 to 8). The series of modules were developed in such a way so that they aligned with course material and could run parallel with the concepts presented during lectures. While a major portion of the case was presented as tutorial modules in a didactic format (approximately 60%), students also completed two major group research projects throughout the course, which were intended to scaffold the students' understanding of complex biological systems. In these projects, students build upon concepts learned in class and tutorials and then

apply their knowledge to their respective research project. Further details are provided in Module TN, a Teaching Note which summarizes implementation methods and assignment ideas.

3.3 Overview of subsequent tutorial modules

3.3.1 Module 3—Organism design

This tutorial module was presented in the second week of class. Having read Modules 1 and 2, the students were asked three study questions in Module 3 as a follow-up. The first question asks students to differentiate between biobutanol and 1-butanol. Here, the goal was to help students understand that, while physicochemically biobutanol and 1-butanol are one in the same, their mode of production is vastly different. More specifically, the environmental, socioeconomic and safety concerns of traditional butanol production from crude oil versus the microbial fermentation platforms are discussed. The next question was the central problem of this tutorial case module. Students were asked what *design choices* would influence their decision between one microbial cell factory versus another. The hope here was that students were able to choose a microbial platform based on a number of factors, such as the nature of the target product and the organism itself. For example, if the metabolite that was produced is a fermentative end-product, then students may want to consider anaerobic hosts such as a clostridia; however, if the product is growth-associated and produced simultaneously with cell growth, then a facultative aerobe such as *E. coli* is perhaps more suitable. Students should be (or become) aware, for example, that, while anaerobic production hosts (such as *C. acetobutylicum*) are excellent producers of reduced fermentative end-products, their doubling (or generation) time is approximately 1.2 hours. This is significantly slower than the 20 minute doubling time of wild-type *E. coli*. We also wanted students to take media considerations into account. For example, while clostridia are native producers of 1-butanol, they are also fastidious microbes and require unusual and/or complex media components (e.g., reducing agents). Without these components, it is often difficult to achieve strict anaerobic culture conditions required for optimal growth and metabolite production. Moreover, the goal was for students to consider the formation of by-products and how this might affect production cost. While engineered *E. coli* is a suitable choice for 1-propanol production, several unwanted fermentative-end products, such as organic acids (acetate, succinate, and propionate) and ethanol, are concomitantly secreted by the host cell. While there is no correct answer for which type of cell factory to choose, the goal was to drive home the point that the choice of a host organism in parallel with the bioprocess operation is crucial to design choices. Given that the students were also introduced to the *Canadian Biosafety Standards and Guidelines* and the Human Pathogens and Toxins Act, students were also expected to contextualize their choice with respect to these guidelines and laws. In some course offerings, students have also been asked to consider patent restrictions and overall accessibility to organisms (biological repositories). Lastly, the third question asks students to identify any other sources of information that can be used in evaluating a suitable production host. The intention here was to see if students can extract information outside of the textbooks and class materials, such as enzymatic databases, strain collections (i.e., the American Type Culture Collection) and other bioinformatics databases and resources in the public domain to aid their search.

3.3.2 Module 4—Metabolic pathway and theoretical yields

Module 4 was implemented after the lectures on microbial metabolism (weeks 2 to 4). In this module, the major learning objective was theoretical yield calculations of the 1-propanol fermentation process. Students were provided with a comprehensive metabolic map thought to best describe the metabolism of the propanogenic strain of *E. coli* in Module 1. Using the metabolic pathways and within the map, students were first asked to calculate the maximum theoretical yield from glycerol for each of the fermentation end-products (i.e., 1-propanol, ethanol, succinate, acetate, and propionate). We focus on glycerol for two reasons: (1) students are familiar with glycolysis starting from glucose and do not always understand how other carbon sources fit in to overall metabolism; (2) waste glycerol—either from cooking waste or from biodiesel production—is thought to be a commercially viable feedstock. The second question in the problem statement asks students what assumptions are made to achieve these theoretical yields. One of our goals in developing this module was that students learned that certain underlying assumptions are made in order to simplify complex biological systems. Some assumptions that could be made include: (1) there is no flux through pathways other than the major fermentative pathways; (2) the system is at pseudo-steady state such that there is no accumulation of precursors or intermediate metabolites; and lastly (3) there is an infinite supply of energy carriers and redox equivalents (i.e., ATP and NADH). In the last question of the problem statement for Module 4, students are asked

to compare the actual yields obtained from the fermentation with the theoretical yields they determined.

3.3.3 Module 5—Metabolic flux analysis of the propanogenic E. coli

Following a lecture on metabolic flux analysis (MFA), students are asked to revisit the metabolic map of propanogenic *E. coli* (provided in Module 1) and apply the concepts discussed to the analysis of real data. In this module's problem statement, students are asked to reduce the metabolic map to the bare essentials (elimination of linear sequences and retention of branch points assuming no intracellular accumulation of metabolites) and determine based on raw data (Table 1 of Modules 1, 5 and 6), the quality of the data. In class, prior to receiving Module 5, students are shown the similarities between solving an over-determined system of linear equations and multiple linear regressions. These topics were covered in a third-year core course in statistics and experimental design offered in their undergraduate degree in Chemical Engineering. Furthermore, students are provided with MATLAB code that can be used for the solution of an overdetermined system of linear algebraic equations. Students are familiar with MATLAB having been exposed to it in a second year course on computer programming.

3.3.4 Module 6—Growth and production kinetics

A lecture is provided on linking the consumption of nutrients to the growth of micro-organisms as well as the development of traditional balances around a bioreactor consisting of biomass, limiting nutrient (substrate) and products. After this lecture, we again returned to the case study modules. Based on the data in Module 6 associated with the production of 1-propanol, students were asked to extract key parameters to allow for the mathematical simulation of the culture in MATLAB in a subsequent Module (Module 7). Given that very little biomass is produced in this system; students are faced with non-growth associated product formation and no links between growth and substrate consumption (which they have also seen from the metabolic flux analysis in Module 5). Students are therefore asked to establish appropriate relationships between the various variables.

3.3.5 Module 7—Reactor simulation and operation

Module 7 addressed reactor operation with practical applications for microbial fuel production. The learning scopes of this module consisted of: (1) obtaining basic knowledge of bioreactor operation; (2) learning good modeling practices for simulating a reactor; and (3) identifying ways of increasing a target compound during cultivation. Using the time course of anaerobic 1-propanol fermentation cultivation (see Module 1), we asked students to suggest a mode of operation that could extend the fermentation and optimize solvent production. While there is no correct solution to this problem, students should identify the advantages and disadvantages for each mode of operation. For example, while a simple fed-batch operation can be used to extend the fermentation, this may generally lead to further accumulation of unwanted by-products, which complicate purification or inhibit growth, production and purification. Alternatively, a continuous operation can facilitate the removal of inhibitory metabolites and thus improve overall cell growth and production. However, this mode of operation is often laborious, energy intensive and costly. In addition, it is not likely to be best for systems with extremely low growth rates. Students were asked to carry out a material balance around the various components of the cell (i.e., volume, biomass, substrate and products) then apply the material balance equations to create a simulated extension of the culture using MATLAB. Lastly, we asked students to delineate potential reasons as to why their simulations may deviate from reality. Inevitably, all simulations may deviate due to uneven mixing (i.e., dead zones), product inhibition and toxicity and also whether or not cell growth kinetics and cell maintenance requirements are accurately represented in the reactor performance equations. This final question therefore requires students to critically interpret concepts of reactor modeling and make an assessment of the broader implications of their analysis.

3.3.6 Module 8—1-propanol extraction and downstream processes

The last module of the case study was used to assess the students' conceptual understanding of downstream separation and purification of biological products. For this module, we posed a scenario where the students take on the role of an engineer whose supervisor wants them to recover 1-propanol from the fermentation culture of engineered *E. coli*. In this module, students were permitted to work in small groups of four or five to strategize and devise downstream strategies (i.e., extractive distillation) and the steps and technologies involved to extract 1-propanol. We then asked the groups to list reasons why they chose a specific downstream process and provide some insights regarding the energy requirements of the scheme. Generally, a high recovery cost is the main bottleneck in most bioprocess platforms and, as the design process progresses, interdisciplinary factors such as cost of materials and energy efficacy affect the overall design concept. By evaluating the techno-economic aspects of the down-

stream applications, students start to gain some understanding of factors involved in the strategic planning of industrial bioprocesses. The case was brought full circle by asking students what aspects of the upstream processes would influence their downstream operations. Here we wanted to gauge whether students could connect the new information learned in Modules 6 and 7 to the previously discussed modules. For example, students might now have better insight into the amount of biomass concentrations of 1-propanol and other by-products, as well as reactor operating conditions (i.e., the aeration regime, pH, temperature, etc.) required for optimal extraction and purification.

4. Results and discussion

Within engineering curricula, instructors struggle to develop practical pedagogical methods that promote critical thinking while adding significant realism to the classroom. With traditional ''instructor-led'' learning methods, student learning is generally comprised of listening to lectures and reading materials from textbooks and other course resources [15, 16]. While this form of instruction is a major tenet of most engineering classrooms with a number of benefits, there are also several drawbacks to this approach. For example, given that students are often purely relying on the instructor as a source of knowledge, they easily slip into an automatic ''pilot'' mode [17, 18]. Therefore, there is a lack of active learning as students do not take responsibility for their own learning. Previous studies [19, 20] have found that the use of these traditional methods often leads to lower student attendance and retention in most engineering programs. The same studies also concluded that a major cause of student attrition in engineering is due to poor and/or outdated teaching methods. Advocates of active or ''student–led'' learning processes suggest that the use of thematic cases offer an effective means of addressing the concerns associated with traditional lecture-based approaches. Several empirical and quantitative studies investigated the use of cases in teaching and found that the use of thematic cases enhance learning by allowing students to inquire freely about the problem at hand, develop multi-faceted hypotheses, acquire information on their own and take charge of their learning [21–23]. Also, since students work with problems and datasets that they would encounter in real-life experiences, they learn these skills more proficiently by using cases. It should be noted that, while a majority of Waterloo students work with companies (in their co-op terms), getting companies to support development and provide material for case-studies can be difficult as companies typically try to protect their trade secrets and data. Accordingly, we used a thematic case that is based on the utilization of recombinant *E. coli* for the production of 1-propanol. The advantage of this pedagogical tool is that it allows us to reinforce concepts on biochemistry, cell metabolism, cell physiology and various aspects of upstream and downstream bioprocessing technologies in a coherent fashion. As shown in Fig. 2, the case-based approach was introduced as small tutorial modules where students worked in groups to complete case problem statements specifically related to various aspects of the overall thematic case (Module 1). It should be noted that students also completed various small assignments and two major projects in which they were responsible for identifying, designing and analyzing a microbial cell factory for the production and purification of a target product.

Although ChE360 has only been offered three times with its revised curriculum, students were still given the opportunity to submit a critique of the course. Our results suggest that the students had an overall positive attitude toward the use of the case study and its modules (see Table 2 for student feedback from 2015). For instance, a vast majority of the students felt that the case study helped them to develop an informed understanding and deep appreciation for the various concepts presented in the course. Students also seemed to favor the use of small group discussions used in the various tutorial

Table 2. Student responses to Case-based learning in ChE360 from 2015 (n = 94)

	% Strongly disagree ⟵⟶ % Strongly agree				
Questions	**1**	**2**	**3**	**4**	**5**
1. The case study was an engaging application of the specific course topics.	2.2	7.5	11.8	69.9	9.7
2. The case study improved my appreciation of the relevance of the specific course topics.	2.2	3.2	18.3	67.7	9.7
3. This case study helped me understand the specific course topics.	4.3	15.1	21.5	49.5	10.8
4. Small group discussions of the case helped me understand the specific course topics.	5.4	16.1	22.6	46.2	10.8
5. I enjoyed and benefited from case based learning more than traditional classroom teaching methods.	6.2	16.9	33.8	35.4	7.7
6. The case study helped me better prepare for the midterm/final exam.	9.2	29.2	35.4	18.5	4.6
7. I would recommend the use of case studies in other chemical engineering courses	1.5	10.8	24.6	47.7	13.8

modules. In the survey, students commented that the tutorial modules "facilitated a new way of thinking about bioprocess schemes" and provided "a good extension of the topics presented during the lecture component". More specifically, students reported that the small group discussions were "an excellent platform to brainstorm new ideas and learn from each other" and that interacting with other group members allowed them to "see different ideas and viewpoints that they had not considered previously". Interestingly, students were also positively inclined to the idea of offering more case-based instruction in other chemical engineering courses at Waterloo. Lastly, students also felt that the case was also beneficial in preparing for the mid-term and final examinations.

While the majority of students were positively disposed to the use of the case study, several students also raised concerns over the consistency of the course. The most frequent student comments on this topic pointed to the fact that there was not enough time allotted before each tutorial session for students to comprehend the concepts introduced in the previous lecture. Students suggested that it would have been beneficial to first introduce the tutorial modules in the lecture component and then expand upon them later in the tutorials. Students also suggested that the case should be expanded in the years to come to include other aspects of bioprocess engineering, namely the production of therapeutic proteins, cosmetics and biopharmaceuticals in animal cell lines. Lastly, the student responses also recommended that, when organizing groups for the various tutorial activities, it is important to consider the number of group members as well as time allocation. Some students felt that the groups were too large (at 4 to 5 members per group), thus there was not enough time to organize their thoughts and solutions to the module problem statements. In the same vein, students also wanted more time in the tutorial sessions to present their group work. It is also important to note that, although we have only designed one major case (with several modules) thus far, more cases and modules revolving around the use of genetically engineered strains for production of value-added products can be designed in future years. In 2016, ChE360 will be redesigned and will be offered in the second half of third-year instead of first half as ChE361. There is intent to continue with a combination of a lecture-based approach combined with several case-based learning modules. Based on the feedback received in this study, there is a goal to introduce students to three different (yet smaller cases) on (1) the use of microbial cell factories for fuel production; (2) production of a biologic in animal cell culture; and (3) recovery of a biologic in animal cell culture. The last two cases are a reflection of work-term reports created by current Waterloo students. Teaching material and notes for these new series of cases are currently being developed. Additionally, students will not only work in groups to solve smaller-sized tutorial modules relevant to the cases, but will also have a larger milestone of presenting their major research project as a poster at a symposium.

5. Conclusion

As we think about the future of the engineering profession and the rapidly changing technological world, it is crucial for instructors to reshape and redesign teaching methods to handle more novel and unstructured problems. It should be noted that this case only measured students' perceptions, specifically to ChE360, and not their ability to transfer these skills to other engineering courses. Future research will examine whether thematic cases can be applied to other chemical engineering courses (and even across other engineering disciplines). It is also important to note that there was no parallel research found in the use of case-based learning for teaching bioprocess engineering. However, we believe that, through the use of thematic case studies, the gaps between theory and practice in bioprocess engineering can be bridged. Our results show that case-based instruction using various modules that run parallel to the course allows students to use cognitive and meta-cognitive strategies at adequate levels. For example, beyond traditional concepts, students start to see the power of recombinant DNA technology and genetically engineering cells to produce novel products. Students start to understand at a conscious level about the steps involved in obtaining cells, cultivating novel strains and purifying the target product. The final results from this study, although limited in scale and scope, also suggest that students are capable of building on conceptual frameworks that were introduced in past tutorial modules and applying them to new concepts and scenarios.

6. Supporting information

Case overview and relevant background information and tutorial modules can be online:

https://uwaterloo.ca/engineering-cases/cases/engineering-escherichia-coli-biofuel-production

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References

1. P. S. Chinowsky and J. Robinson, Enhancing civil engineering education through case studies, *Journal of Engineering Education*, **86**(1), 1997, pp. 45–49.
2. A. E. Flynn and J. D. Klein, The influence of discussion groups in a case-based learning environment, *Educational Technology Research and Development*, **49**(3), 2001, pp. 71–86.
3. J. F. Vivas and V. Allada, Enhancing engineering education using thematic case-based learning, *International Journal of Engineering Education*, **22**(2), 2006, p. 236.
4. P. Raju and C. S. Sankar, Teaching Real—World Issues through Case Studies, *Journal of Engineering Education*, **88**(4), 1999, pp. 501–508.
5. A. Yadav, M. Lundeberg, M. DeSchryver, K. Dirkin, N. A. Schiller, K. Maier and C. F. Herreid, Teaching Science with Case Studies: A National Survey of Faculty Perceptions of the Benefits and Challenges of Using Cases, *Journal of College Science Teaching*, **37**(1), 2007, pp. 34–38.
6. L. A. Bryon Jr, Educating engineers on safety, *Journal of Management in Engineering*, **15**(2), 1999, pp. 30–33.
7. R. E. Dupuis and A. M. Persky, Use of case-based learning in a clinical pharmacokinetics course, *American Journal of Pharmaceutical Education*, **72**(2), 2008, pp. 1–7.
8. S. Lambert, Sustainable design throughout the curriculum using case studies, *International Journal of Engineering Education*, **26**(2), 2010, p. 401.
9. K. H. Vesper, *Engineers at work: a casebook*, Boston, MA, USA: Houghton Mifflin Harcourt, 1975.
10. K. Srirangan, L. Akawi, X. Liu, A. Westbrook, E. Blondeel, M. G. Aucoin, M. Moo-Young and C. P. Chou, Manipulating the sleeping beauty mutase operon for the production of 1-propanol in engineered *Escherichia coli*, *Biotechnology for Biofuels*, **6**(1), 2013, pp. 1–16.
11. K. Srirangan, X. Liu, A. Westbrook, L. Akawi, M. E. Pyne, M. Moo-Young and C. P. Chou, Biochemical, genetic, and metabolic engineering strategies to enhance coproduction of 1-propanol and ethanol in engineered *Escherichia coli*, *Applied Microbiology and Biotechnology*, **98**(22), 2014, pp. 9499–9515.
12. K. Srirangan, L. Akawi, M. Moo-Young and C. P. Chou, Towards sustainable production of clean energy carriers from biomass resources, *Applied Energy*, **100**, 2012, pp. 172–186.
13. P. Dürre, Fermentative production of butanol—the academic perspective, *Current Opinion in Biotechnology*, **22**(3), 2011, pp. 331–336.
14. E. M. Green, Fermentative production of butanol—the industrial perspective, *Current Opinion in Biotechnology*, **22**(3), 2011, pp. 337–343.
15. B. Williams, Case based learning—a review of the literature: is there scope for this educational paradigm in prehospital education?, *Emergency Medicine Journal*, **22**(8), 2005, pp. 577–581.
16. J. Garcia, J. Sinfield, A. Yadav, and R. Adams, Learning through entrepreneurially oriented case-based instruction, *International Journal of Engineering Education*, **28**(2), 2012, p. 448.
17. P. S. Arvidson and T. A. Huston, Transparent teaching, *Currents in Teaching and Learning*, **1**(1), 2008, pp. 4–16.
18. G. R. Norman and H. G. Schmidt, The psychological basis of problem-based learning: a review of the evidence, *Academic Medicine*, **67**(9), 1992, pp. 557–565.
19. M. Besterfield-Sacre, C. J. Atman and L. J. Shuman, Characteristics of freshman engineering students: Models for determining student attrition in engineering, *Journal of Engineering Education*, **86**(2), 1997, pp. 139–149.
20. E. Seymour and N. M. Hewitt, *Talking about leaving: Why undergraduates leave the sciences*, First ed., Boulder, CO, USA: Westview Press, 1997.
21. S. Bennet, B. Harper and J. Hedberg, Designing real life cases to support authentic design activities, *Australian Journal of Educational Technology*, **18**(1), 2002, pp. 73–82.
22. L. Carder, P. Willingham and D. Bibb, Case-based, problem-based learning: Information literacy for the real world, *Research Strategies*, **18**(3), 2001, pp. 181–190.
23. M. G. Aucoin and M. Jolicoeur, Is There Room in the Graduate Curriculum to Learn How to Be a Grad Student? An Approach Using a Graduate-Level Biochemical Engineering Course, *Chemical Engineering Education*, **43**(4), 2009, pp. 306–312.

Kajan Srirangan is a Research Associate at the National Research Council Canada. He received his PhD in Chemical Engineering from the University of Waterloo (Waterloo, Canada). At Waterloo, he also completed his teaching certification in university teaching with the Centre for Teaching Excellence. He primarily works on engineering cellular metabolism and development of synthetic tools and methods for production of value-added chemicals and recombinant therapeutic proteins.

Cheryl Newton is a Laboratory Instructor in the Department of Chemical Engineering at the University of Waterloo (Waterloo, Canada). She previously held a role as Design Engineer and Case Study Specialist in the Department of Mechanical Engineering also at the University of Waterloo (Waterloo, Canada). Her research area included engineering case study development to enhance undergraduate curriculum and student experience.

Lyndia Stacey is a Case Study Specialist in the Department of Mechanical Engineering at the University of Waterloo (Waterloo, Canada). She received her Bachelors in Environmental Engineering from the University of Guelph (Guelph, Canada) and has experience in engineering education and outreach. More specifically, her work examines the use of case studies as a pedagogical tool to enhance student learning and engagement.

Lamees Akawi received her Master's in Chemical Engineering at the University of Waterloo (Waterloo, Canada) and also served as a teaching assistant in 2013–2014 for ChE360.

C. Perry Chou is a professor of Chemical Engineering with a cross appointment in Biology at the University of Waterloo (Waterloo, Canada). He regularly teaches ChE360: Bioprocess Engineering. He serves as an editor for *Biotechnology Advances* (Elsevier) and an editorial board member for *Scientific Reports* (Nature Publishing Group). Professor Chou held a Canada Research Chair from 2005 to 2015. He primarily works on developing novel strategies to enhance biomanufacturing through the integrative application of bioprocess engineering, molecular biology, and microbial biotechnology.

Marc G. Aucoin is an associate professor of Chemical Engineering at the University of Waterloo (Waterloo, Canada). He is a regular instructor of ChE360: Bioprocess Engineering and a strong believer in experiential learning. He undertook this initiative to support students' understanding of the limitations of applying common bioprocessing concepts. His research interests include viral process dynamics, virus and therapeutic protein production and purification, animal cell culture engineering, and insect and mammalian cell culture metabolomics.