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Qualitative Process Understanding Tools within Bioprocessing: A Case Study

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Abstract

Biotechnology is a key area of industrial interest and the importance of effective knowledge management for rapid bioprocess development, optimisation and operation is widely recognised as an important driver of biomanufacturing excellence. The Britest suite of tools and methodologies, designed to highlight knowledge gaps within chemical and physical processes, is explored as an approach to bioprocess knowledge acquisition and management. These tools can help identify where optimisation may be most beneficial, and also increase understanding of the process as a whole across a range of disciplines. This research identifies areas where Britest tools are not directly transferable into biotechnological applications, and formulates a whole bioprocess development methodology. The Britest tools have been considered using SuperPro Designer in relation to production of insulin using *E. coli*. Some of the existing Britest tools have been found to be directly applicable to biological processes, although adaptations were required in some cases, to account for differences between chemical and biological processing. A gap was identified relating to considering the process as a whole, and so a new tool (the Reaction/Reagent/Transformation Tracker, R2T2) was developed to address this. The Britest tools show promise within the context of a bioprocess, although further work is required to fully realise their potential in this exciting field. It is anticipated that the tools can be applied to aid in the identification of Critical Quality Attributes (CQAs) and Critical Process Parameters (CPPs), constructing a robust design space, and facilitating the development of a Quality by Design (QbD) approach to bioprocessing.

Keywords: Whole process understanding; Qualitative process understanding; Knowledge elicitation; Critical quality attributes; Critical process parameters; Quality by design

Introduction

The design and development of sustainable and innovative processes is a challenge across a broad range of manufacturing sectors, especially in the high value sectors. Key difficulties include: pressure on development lead times to reduce time to market; complex systems where chemical, physical and/or biological properties are not fully understood; poor communication of critical process information between different technical disciplines; lack of detailed understanding of whole process challenges within a process made up of a number of separate unit operations; identification of viable process flowsheet concepts, and rapid identification of the most viable options.

In recent years there has been great progress in the development of tools to support the design and development of chemical and biological processes. Many of these are based on computational simulation of the different unit operations, and the integration of these operations into whole process flowsheets. In general, however, such approaches require large amounts of quantitative data about the different process steps. While some individual steps can be modelled based solely on theoretical data, the development of a whole process model during the early stages of process design can be extremely challenging as a result of limited quantitative data availability. Computational simulation approaches are also often highly complex, requiring an expert user and significant periods of time to deliver a robust model. Furthermore, multidisciplinary communication of input and output from these models is often difficult for non-expert users.

The challenges posed by the complexity of the products/processes and highly regulated character of the industry exacerbate these issues within the bioprocessing/biopharmaceutical industry sector. Whilst the introduction of Quality by Design (QbD) and Process Analytical Technologies (PAT) [1-3] has contributed to the generation of much richer datasets through the bioprocess design and development process, it also raises additional challenges. The identification of Critical Quality Attributes (CQAs), Critical Process Parameters (CPPs) and the definition of the design and control space are frequently not straightforward, although fundamental to the process understanding and the ability to effectively control the process.

Different approaches to defining the design and control space have varying degrees of robustness, but are generally based on a combination of process understanding and experimentation. There is not currently a standard approach which is recommended, and this means there can be no guarantee of the robustness of the design space generated. Harms et al. attempted to define the design space for the bioreaction of *Pichia pastoris*. While this approach did create a design space, it did not cover the process as a whole including up- and downstream process tasks. The approach used to generate the design space relied on the use of scale down models, which may not be available for all process units, and so implementation for other unit operations could be difficult. The design space covered temperature, pH and dissolved oxygen (DO), but other parameters were also investigated. The design space definition could be more beneficial if it linked the outputs of the bioreaction to the downstream processing strategy, though there are clear benefits in being able to characterise the bioreaction step alone.

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One option for extending the design space scope could be to create a design space for each unit operation. However, as Zhou et al. have shown, adopting a Windows of Operation approach is more effective for optimising the process outcome. The Windows of Operation approach aims to balance the desired conditions and outputs from the units within the process, and from this optimise the process as a whole rather than as a set of individual units. Performing the same level of characterisation and risk analysis for a whole process, particularly for biologically based processes with their associated high complexity, would be challenging and may not generate a design space with a high level of confidence.

In addition to facilitating the QbD approach in processing, effective knowledge capture has been correlated with organisational effectiveness [4]. In order to be useful, however, it is important that any knowledge capture approach used is able to organise the information in a manner that enables its effective future use and supports process understanding.

Process understanding tools

A range of process understanding tools, both quantitative and qualitative, are available to the bioprocessing sector to aid in the adoption of a QbD approach. Quantitative tools can be highly beneficial for supporting regulatory applications, along with the definition of a design space. When combined with experimental results they can effectively show the relationships between the CQAs and Critical Process Parameters CPPs, thereby enabling accurate and rapid process optimisation, and also demonstrating the process understanding required to support a QbD approach. However, tacit knowledge cannot be incorporated easily into these models, which are often based on cost modelling [5-7]. Qualitative tools, although unable to give a quantitative answer to a problem, could be used alongside these quantitative modelling tools to enhance and support applicability. The knowledge of a process or plant, captured by the qualitative tools, could enhance the results obtained from quantitative process improvement tools, and so their value should not be underestimated. There are several examples of qualitative knowledge management tools, the most widely known of these being the Six-Sigma approach.

Six-Sigma was developed in 1986 [8], and is currently used in a range of process sectors [9-11]. The Six-Sigma process is outlined in ISO 13053:2011 [12]. The underlying principle is repeated cycles of process evaluation. The methodologies are designed to encourage continuous process evaluation and therefore improvement, in contrast to typical development where the process is considered satisfactory when targets are reached. The reliance of Six-Sigma on process understanding makes this approach strongly complementary to the Britest approach described below.

With respect to bioprocessing, Dassau et al. employed the methodologies alongside process modelling techniques to consider a penicillin fermentation. After three cycles of Six Sigma evaluation of the process, the final conditions led to a 40% reduction in batch time, a 17% increase in throughput yield and a 33% reduction in impurities. The authors attribute the success to the adoption of a plant-wide approach to process improvement, previously discussed [3], which would not have been adopted without the aid of the Six-Sigma methodologies. The adoption of a whole process view requires a shift in organisational culture, and the use of knowledge management tools to aid this shift was undoubtedly beneficial in the case presented by Dassau et al. However, Six-Sigma are not the only qualitative methodologies available which could be applied to achieve this outcome.

The britest approach

One innovative approach to the challenges outlined in previous sections, developed by Britest Ltd. (http://www.britest.co.uk), has found broad use across the chemicals using sectors such as pharmaceuticals, and fine, speciality and consumer chemicals industries [13-15]. The Britest approach uses a set of qualitative and semi-quantitative models to enable cross-disciplinary understanding of industrial processes, therefore supporting innovative whole process design. The tools are deliberately designed to be complementary to more quantitative approaches such as computational process modelling, economic modelling or fluid dynamics calculations. This approach is not an expert system, and it is intended to be usable by technologists of all disciplines.

A key feature of the Britest approach is that it can be effectively applied in very data-lean environments, for example during the early stages of process design and development. This means that the Britest tools are different from, and complementary to, the Six-Sigma approach which relies on significant quantities of real process data. The use of this type of qualitative mechanistic modelling approach can enable rapid identification of critical process parameters and key knowledge gaps, and thus support effective experimental planning and quantitative modelling studies. The tools and methodologies employed are designed to enable effective cross-disciplinary communication, and support rapid transition from high-level, whole process assessment into detailed analysis of the fundamental science that influences specific process steps. The nature of the approach also means that it has been used not only in ab-initio process design activities, but also for troubleshooting and improvement of existing processes. The graphical and/or tabular nature of the Britest toolkit also makes it a very powerful vehicle for knowledge capture, knowledge dissemination and maintenance of corporate knowledge.

Methods

Process simulation

This study examines the application of the outlined process understanding tools to a simulated bioprocess to investigate their general applicability and the potential for future developments. The model process chosen, the production of insulin from *E. coli*, is a complex process, which can be carried out using two methods [16]. Either the chains could be synthesised separately and mixed, reduced and reoxidised after purification [17]. Alternatively, the bacterial culture produces proinsulin, which then undergoes extensive downstream processing to give biologically active insulin [18].

In this case, the proinsulin method was simulated using SuperPro Designer Ltd. This simulation of insulin expression in *E. coli* has been presented previously as part of Chapter 12 in Bioseparations Science and Engineering [19]. The process scheme is summarised in Figure 1. The fermentation, producing Trp-LE-MET-proinsulin precursor, is performed in bioreactors using transformed *E. coli* cells. The fermentation duration is 18 h and it is performed at 37°C. The product is formed as inclusion bodies and a total yield of 30 g/L is obtained. The primary recovery consists of cell lysis and purification of inclusion bodies, using centrifugation for cell separation, homogenisation to lyse the cells and then further centrifugation to separate the inclusion bodies from cellular debris. A detergent (Triton-X-100) is then added prior to the final centrifugation step, to aid further separation of the inclusion bodies. The reaction section of the downstream process starts with solubilising the inclusion bodies using urea and 2-mercaptoethanol to break the disulphide bonds prior to concentration through diafiltration.

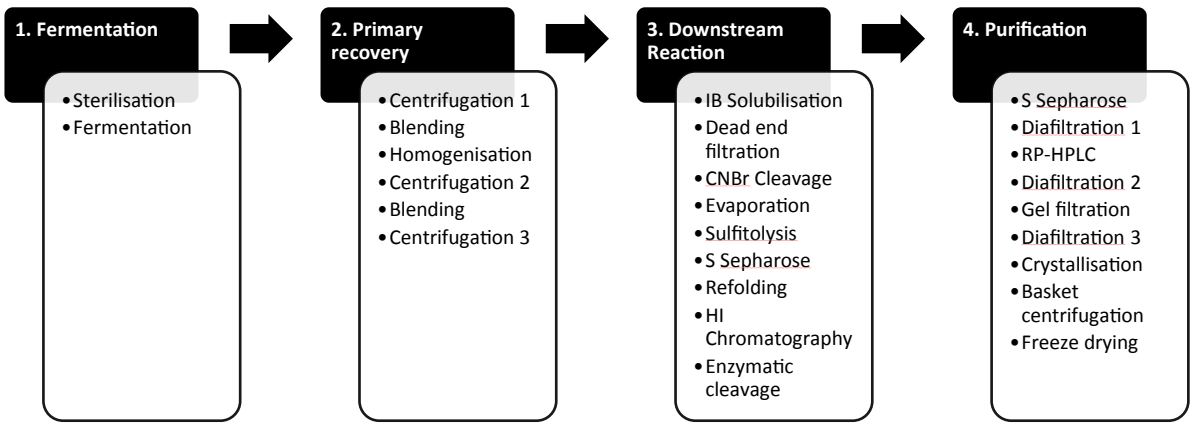

Figure 1: Process outline for insulin production within SuperPro Designer. This was the process on which the Britest study was conducted.

The solubilised inclusion bodies are then cleaved with cyanogen bromide to remove the signal sequence, and evaporated before sulfitolysis results in protein unfolding. The next stage is S-seraphose chromatography, followed by refolding, the final step, again using 2-mercaptoethanol. The resulting protein is purified with Hydrophobic Interaction Chromatography (HIC) before being cleaved enzymatically with trypsin to remove the C-terminal peptide. The final purification consists of four chromatography stages, followed by crystallisation of the insulin. Centrifugation is used to recover the crystals for freeze drying.

Qualitative process understanding tools

The Britest tools were applied according to a framework developed for a chemical processing study. The main objectives of applying the tools in this case study were:

- To capture the purpose of each stage of the process and how it works
- To identify the potential for improvement within the process
- To outline experiments required to further understand and optimise the process

While the purpose of the work presented within this case study was to identify gaps within the toolkit in relation to bioprocessing, the study was designed to mirror the typical aims of a study supported by the Britest tools. Were the process not simulated, the study would be used to capture process understanding in each stage, in addition to exploring the underlying science of the process and identifying potential opportunities for process improvement. The Britest tools are also particularly useful for facilitating interdisciplinary knowledge transfer, by providing a visual approach to knowledge capture, which is nonetheless based on the fundamental science under investigation. Such an approach is particularly pertinent to the bioprocessing sector, where many different disciplines can be involved in a single process, and effective communication of information between different disciplines can be extremely challenging.

The key tools are outlined in Table 1. Each tool was considered in turn, and relevant advantages and disadvantages used to determine which tools would be most appropriate for application to this particular bioprocess to achieve the intended knowledge outcomes. This study focussed on the Process Information Summary map (PRISM) and the Process Definition Diagram (PDD). The Transformation Map and Driving Force Analysis (DFA) are targeted at developing understanding of the chemical reactions occurring within a single process task. This was deemed too complex to consider for the fermentation step, and these transformations were not investigated in further detail with respect to downstream processing during this study. In the course of this work, a new tool was developed (the Reaction/Reagent Transformation Tracker (R2T2)) and it was employed to further enhance process understanding.

The PrISM captures key stages within a process, along with the key inputs and outputs for each stage. This tool helps the team to focus their activities on the most appropriate parts of the process by providing an overview of the most critical material, time and energy dependencies.

The Process Definition Diagram [15] is a tool that enables process technologists to describe a process independently of scale and equipment. It is a form of State Task Network, describing the process as a sequence of tasks that are performed to transform starting materials into products. The PDD provides an information rich summary of part or all of a process, which has been used for purposes such as cross-disciplinary knowledge sharing, whole process design, process technology transfer, and troubleshooting. The PDD uses a pre-defined set of symbols to denote the number and type of phases present in each process task as the presence of multiple phases can add significant complexity and risk to the scale-up of chemical and biochemical processes.

Results

The PrISM for the insulin model process considered in this research is shown in part in Figure 2. In this representation, the process has been split into four high-level stages: fermentation, primary recovery, reactions and final purification. The most expensive reagents were the enzymes, and the main waste was generated at the reaction stage within the downstream processing (stage 3). This was also the longest stage of the process and additionally generated the highest contribution to the product cost. In a traditional Britest study, the next step would be to complete a PDD for this section of the process. However, the PDD has already been shown to be applicable to chemical reactions similar to downstream processing. In light of this, the PDD was constructed for the upstream processing (fermentation) stage, to investigate its applicability to a biochemical transformation, rather than chemical or physical transformations as has been its primary application to date.

Tool	Purpose	Resulting Detail Level	Strengths	Drawbacks
Process Information Summary Map (PRISM)	A high level overview of the key stages in a process, summarises process inputs and outputs, records key information [associated with each process stage, input and output]	Overview	Easy to understand, reduces process complexity, quick to apply	Can oversimplify, no intermediates captured
Process Definition Diagram (PDD)	Task-based whole process representation, showing where process materials are introduced and/or removed from the process, the phases present throughout each task, phase changes (e.g. dissolution, gas evolution, etc.), key energy balances	Medium	Independent of scale/equipment, cross-discipline, information rich	Time consuming to construct in high detail, less beneficial in single phase processes
Transformation Map	A graphical portrayal of the network of (bio)chemical and/or physical transformations that convert raw materials into products within a process task. They should include both desired and undesired transformations, to support the use of other tools (e.g., Driving Force Analysis) to identify operating strategies favouring the desired transformations.	High	Forces user to consider all potential reactions, applicable across scale	Time consuming if lots of detail required, multiple unknowns limits benefits
Driving Force Analysis (DFA)	A qualitative model of the competing driving forces within a process to enable the identification of potential operating strategies.	High	Systematic application, helps understand impact of process changes, structured output	Requires completed Transformation Map, limited scope for inclusion of complex relationships
Transformation, Entities, Properties, Physics, Parameters, Order of Magnitude (TE3PO)	A tool used to record and analyse knowledge about transformations where the presence of parallel rate processes means that rates need to be balanced in order to deliver the optimum outcome	Medium	Information rich, breaks down process, macro/micro scale	Difficult to interlink transformations, could be time consuming

Table 1: The Britest tools, purposes and relative strength and drawbacks.

The PDD [15] provides a task-based process overview, which also includes a notation that captures the states present during the course of a process (Figure 3). As noted in the previous section, the focus is not on equipment but rather process tasks, allowing changes to be considered independently of the "unit operation" thinking. The second level of detail is the capture of the phases present in each task, which can be critical in determining the complexity of many chemical and physical processes but can under-represent the complexity of many bioprocesses, owing to the presence of multiple components within both solid and aqueous phases. An attempt was made to supplement the state representation with symbols to represent each major process component, and while this proved useful in supporting understanding of the changing makeup of the liquid phases, the time taken to complete the tracking meant this tool was not necessarily the most appropriate to fill the gap. Alternative notations to capture the different constituents of the aqueous phase within the existing PDD structure proved time-consuming and somewhat confusing. Based on this analysis, there was a clear need for an alternative tool that allowed the components of a process to be tracked, thus giving scope for understanding potential for process variability and improvement.

A new tool called the Reaction/Reagent Transformation Tracker (R2T2) was conceived to fill this gap. This tool aims to show how the amount of each process component changes through the course of the process, to provide a high level view of the whole process. Colour coding is employed to capture the inherent variability when considering a biological system, allowing for understanding of the challenges involved in development of a process that delivers a consistent output. Incorporation of the variability in this manner helps to tackle the second aim of understanding the potential for improvement in the process. Each of the process stages, and the process as a whole, can be viewed in relation to the best and the worst case scenarios, akin to a cost benefit analysis.

In this case study, the R2T2 generated the process overview shown in Figure 4. From this, it is evident that the biomass is eliminated completely during the primary recovery stages of the process. It is also clear that the insulin is only produced within the final stage of the process, and the requirement for the production of precursors is more apparent. The extent of reagents required to produce the insulin is easier to comprehend, and this highlights the required focus on downstream processing for process improvement. When considering the process using conventional methods, it may be tempting to focus on improving the yield from the fermentation, however the output from R2T2 makes it clear that the process improvement effort would be better expended on improving the downstream conversion reactions and purification scheme. The R2T2 took less time to complete than the PDD, and provided a whole process view that was more appropriate than the PDD for a bioprocess of this type. Additionally, the tool is simple to understand and apply, which are key criteria for delivering a new tool

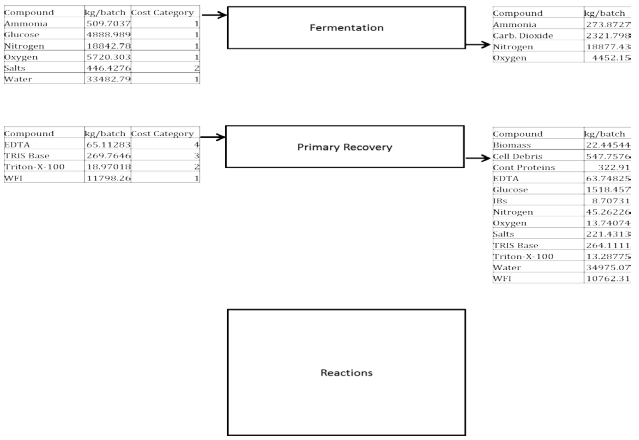

Figure 2: Extract from the PRISM for the Insulin production process covering the fermentation and primary recovery stages. The central box is sized relative to the duration of each step. The box on the left identifies additions to the process at each stage, the box on the right identifies waste leaving the process.

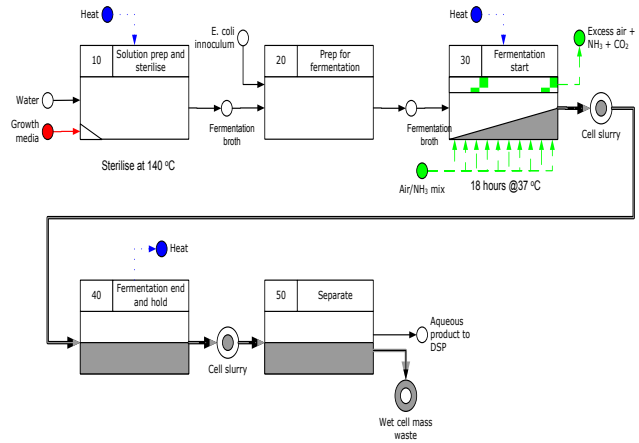

Figure 3: Process Definition Diagram for the upstream stages of the Insulin production process. The different colours present in each box represent a different phase. In this PDD the cells are considered as a solid.

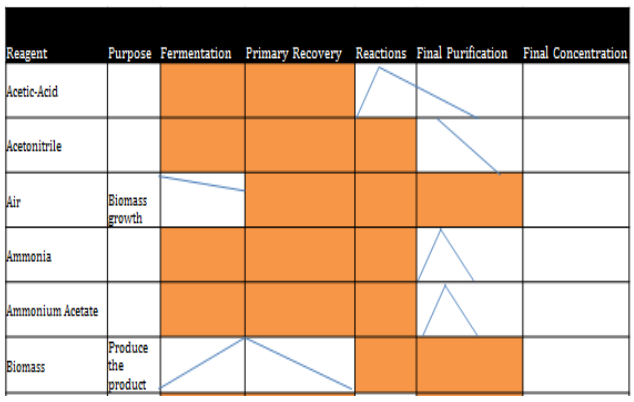

Figure 4: Extract from the R2T2 of the process. Each reagent and its purpose is captured in the column on the left. The process stages make up the remaining columns. The reagent's presence is then tracked through the process with the blue line. Orange boxes indicate the absence of the reagent.

that will find broader application. The R2T2 fills a performance gap that cannot easily be addressed using the PDD tool. These tools are complementary in nature, and the decision on whether to use PDD, R2T2, or both will depend on the problem being considered, the timelines, and the data available to the team.

Discussion

This qualitative study of the insulin production process found results at each stage of the study. Initially, the PrISM was employed. Within the completion of this tool, the highest waste stream was identified, along with the most time-consuming stage of the process. The most expensive reagents were the enzymes. The tool gives a basic overview of the process in a clear and efficient manner, thus demonstrating its applicability to bioprocessing. The underlying concept of the tool is beneficial to a bioprocess, and the simple format in which it is employed is not so simplistic as to reduce the value of the contained information.

Within a QbD process, the ability to demonstrate clearly process understanding is invaluable when applying for regulatory approval for a product. The PrISM tool has been demonstrated as an efficient way to summarise a process into a succinct format without losing crucial information about how the process operates. The PrISM could be used as a means to identify the section of the process with the most potential for improvement; from here efforts to decrease waste or enhance reaction efficiency can be investigated, either experimentally or theoretically through further tool application. The clear explanation of why a change to a process could be required and where the efforts for change would be focussed could be crucial in justifying the changes. Additionally, if a PrISM was constructed for multiple scenarios it could be used to support the varying action required within the QbD approach to facilitate the same end result. Quality Attributes with respect to cost could be identified, but these could not be related to the CPPs of the product.

Following this, the PDD was tested on the simulated process. While this tool can be extremely useful as a means of reviewing all or part of a process in detail, in the case of the fermentation stage it proved difficult to achieve a balance between too much and insufficient detail. When used in its conventional form, where states present within each task are captured, the prevalence of a dominant liquid phase meant limited information could be gained from this aspect of the tool. However, when the liquid was split into components, the content of the liquids meant that the resulting tool was highly complex and therefore could be difficult to understand. Knowledge transfer tools are most effective when easy completion and understanding enable effective knowledge capture. In the case of the PDD, the changes which were predicted to add benefit to process understanding negated this through the added complexity. It was concluded that within a biological process, the ability to track individual reagents would provide greater benefit than representing the phases present.

The R2T2 is a novel knowledge management tool which was developed as a direct result of this study. The ability to view a snapshot of how each process component changes over the course of the process is envisaged to be beneficial in both knowledge capture and process improvement. The resulting process snapshot aims to provide a method for the capture of reagent purpose, gain/loss and final concentration. With respect to this process, those aims were met through the R2T2 in a manner in which was found to be both user friendly and information rich. The ability to use colour coding to capture potential variability within a process was found to be of particular interest to biologically

based processes, where reducing variability can be a key concern.

The ability to pinpoint the source of variability within a process, and consider the options available for reduction would be highly beneficial in a QbD process. In this tool, criticality of process components could be ascertained, but like with the PrISM tool, this could not be related to the CPPs through the R2T2 tool alone. The identification of variability and the attribution of this to a cause is the first step a company could take in effective process control. Without knowing why the resulting product from a process varies, it is impossible for the company to attempt to control this. In this case the process was simulated, and so no robust assessment of variability could be made. It is hypothesised that one important source would be the fermentation. If this was found to be the case, the company could increase monitoring efforts in the reactor to more tightly control the resulting broth and therefore reduce the variability for the primary recovery. If the variability could not be controlled within the reactor, then it is possible that the conditions for the biomass removal could be altered to accommodate the output from the fermentation and obtain the optimum results regardless. This is the underlying principle of QbD, and the R2T2 has been shown in this example to be of benefit in the early phases of implementing this approach.

The weakness of this study was the inability to correlate the CQAs with their controlling CPPs, facilitating the application of the QbD approach. A new tool would be required to fill this knowledge management gap in a simpler format. Whilst a new tool was not developed as part of this study, future studies will investigate how this could be achieved.

The techniques employed for this qualitative understanding study originated from the Britest toolkit, which was developed for enhancing process understanding of chemical and physical processing. The study aimed to investigate the applicability to bioprocesses, and to overcome any potential gaps within the toolkit. It was clear from the PDD that the increased complexity within a biologically based process was the most significant barrier to application. The development of the R2T2 from this shows that the implementation can be critical to the capture of knowledge. The PDD could be used to capture the same information but was difficult to interpret. This demonstrates clearly the requirement for structured knowledge capture and management, rather than reliance on regulatory or internal documentation.

This study established the possibility of applying the current Britest tools to bioprocessing to enhance process understanding. While not all of the tools were directly transferable, it is envisaged that through further tool development, to allow for the complexity of a biological process to be captured, a user friendly qualitative toolkit for bioprocess understanding could be constructed. The value of such a toolkit is challenging to quantify. However, the requirement for enhanced process understanding underlies the QbD initiative, a growing driver in industrial bioprocess development.

Conclusion

This work considered the application of the Britest qualitative knowledge capture tools to a simulated bioprocess to ascertain the potential for employing the tools within the bioprocessing sector. It is anticipated that the requirement for methods such as those presented within this research will increase as the QbD approach becomes more widespread within bioprocessing. Some of the Britest tools were found to be directly transferable, particularly the Process Information Summary Map, while the Process Definition Diagram has a clear gap in capturing the complexity of bioprocesses. More specifically, this relates to effective capture of the complexity of homogeneous phases containing multiple components. In light of this challenge, a novel knowledge capture tool (the Reaction/Reagent Transformation Tracker) was developed to provide a means of tracking multiple components through a whole process.

Overall, our study highlights the value of using qualitative tools such as those developed by Britest to support whole process understanding and knowledge transfer for complex biological processes. However, it also flags the limitations of the existing tools, and demonstrates the requirement for new or amended tools to be developed to fill the current gaps, in particular the linking of CQAs to CPPs. With the increasing pressures to improve process understanding [1] to comply with the Quality by Design initiative, tools such as these can play an important role in enhancing cross-disciplinary process understanding in complex biological systems. Qualitative tools of this type can also provide an invaluable means of identifying the depth of knowledge and understanding of a process, and thus support targeting of more detailed experimental and/or modelling studies.

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