Novel Application of Quantitative Risk Assessment Modelling to a Continuous Fermenter

by

Mr. Rohit A. Patil

School of Chemical Engineering

The University of Adelaide

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ABSTRACT

The food and pharmaceutical industries are generally a nation's largest manufacturing sector – and importantly one of the most stable. *Fermentation*[1] will continue to grow in importance as a *unit operation* as the range of potentially bio-engineered micro organisms (for either extra-cellular or intra-cellular product) increases. In these industries plant *failure* can be costly - and sometimes catastrophic to public health with survival of unwanted pathogenic micro organisms in the plant or product. Plant failure can't always be attributed to human error, sometimes the failure can be a result of changes inside the system itself. Davey and Cerf (2003) introduced the notion of *Friday 13th Syndrome,* i.e. the unexpected failure of a well-operated plant, by novel application of Quantitative Risk Assessment (QRA) to a UHT milk bioprocess.

In this thesis the notion of Friday 13th Syndrome is used to develop a new and rigorous mathematical model of a generalised, continuous fermenter to gain insight into the likelihood of bioprocess failure in an otherwise well-operated plant. Unexpected failure is defined as *washout* of microbial cells from the fermenter. This new model is developed for a continuous, anaerobic fermenter based on widely employed Monod process model. All the cells in fermenter are in their exponential growth phase. Continuous fermentation has a number of advantages over batch, or batch-continuous, such as reduced operating costs.

The model developed requires input values of maximum specific growth rate (μ_{max}), yield coefficient ($Y_{x/s}$) and Monod constant (K_s) for a selected micro organism. The output values of particular interest from the model include: the productivity of the continuous fermentation (xD), the maximum dilution rate (D_{max}) and the dilution rate at maximum productivity ($D_{maxoutput}$). Simulations for continuous operation from the fermenter model are carried out using a Microsoft Excel™ spreadsheet with an add-in *@Risk*™ (pronounced *at risk*) version 4.5 (Palisade Corporation) with some 100,000 iterations[2] from Monte Carlo sampling of input parameters. Values of the input parameters took one of two forms. The first was a traditional or *Single Value Assessment* (SVA) as defined by Cerf and Davey (2001) in which a single, "best guess" or *mean* value of the parameter is used. The

[1] *see* Appendix A for a definition of some important terms used in this research.
[2] experience with the model highlighted that stable output values were obtained with 100,000 iterations.

simulation output therefore is a single value of a required parameter. A sensitivity analysis is carried out with SVA values abstracted from the published literature plus each of: 1, 5, 10 or 15 % Variability. The alternate form was a *Monte Carlo Assessment* (MCA) (Cerf and Davey 2001) in which the "best guess" values take the form of a *probability distribution* around the mean value. Many thousands of randomly sampled values for each input parameter are obtained using this Monte Carlo sampling. In other words, in the QRA the input values of the parameters take the form of a distribution of values. The output therefore is a distribution of values with each assigned a *probability* of actually occurring.

The micro organism selected for this study was *Escherichia coli*. This is a Gram negative, vegetative and non-spore forming bacterium is widely used in fermentation. Values for the model input parameters K_s, μ_{max} and $Y_{x/s}$ were selected from the published bioprocess literature and used to define a *RiskNormal* distribution for each.

A comparison of simulation results from SVA and QRA with MCA sampling underscored that the combined effect of small variations in the bacterial growth parameters (K_s, μ_{max} and $Y_{x/s}$) has a highly significant effect on de-stabilising a well-operated fermenter - and sometimes can lead to catastrophic failure i.e. washout. These findings highlighted that a more accurate determination of the natural microbiological Variability in μ_{max} for *E. coli* was needed for a more realistic simulation. To do this, extensive published data (n = 191) for *E. coli* growth, over a range of temperature 10 ^{o}C < T < 45 ^{o}C, were collated. The predictive model for growth of *E. coli* that was selected was the cardinal temperature model of Rosso (Rosso *et. al.* 1993) - because it is widely used and generally gives a good fit to growth data. In this model μ_{max} is a function of four parameters. These are the three cardinal temperatures (T_{min}, T_{opt} and T_{max}) and the optimum specific growth rate (μ_{opt}). Non-linear regressions to fit the Rosso model to the data for *E. coli* growth were carried out using R software version 2.2.0 (R Foundation for Statistical Computing). Resulting estimates and standard deviations of each of the four parameters of the Rosso model for growth were used to define a *RiskNormal* distribution for each data set collated. With the *E. coli* growth now more accurately defined, a more realistic MCA simulation of the fermenter model was carried out.

Findings of the resulting MCA simulations of the continuous fermenter underscored that the fermenter could exhibit Friday 13th Syndrome - i.e. failure due to washout - despite a better knowledge of the value of the input fermenter parameters for *E. coli*. This is because of the naturally occurring *Uncertainty* and *Variability* in the microbiological input parameters for any micro organism. This practical insight into an otherwise well-operated continuous fermenter contrasts sharply with the frequently adopted traditional or SVA analysis in which the natural Variability in microbiological parameters is simply not accounted for. The sensitivity analyses used with SVA does not account for the combined effect of changes in the input parameters. A QRA with MCA sampling therefore gives the more realistic, and indeed, practical, insight into fermenter operation.

The results of simulation of continuous fermentation suggest that Variability in the input microbiological parameters has a highly significant effect on productivity of the fermentation process - and sometimes can lead to washout. However, a low rate of failures may be obtained if the relative importance of input variables on the process performance can be accurately identified.

This research is the first application of its kind using an QRA, and although only one micro organism and one model for micro organism growth on a particular medium within the fermenter is used, a general principle has been illuminated - i.e. despite the best possible estimates of growth rate parameters, *Chance* can lead to failure of well-operated plant.

The potential of applying this approach to a *global food process* has been glimpsed through this research. A global food process is one in which there are two or more process unit operations combined (*pers. comm.* K R Davey). The analyses and approach outlined here could, in principle, be applied to a range of single or connected unit operations such as the sterilisation of the fermentation media (and equipment surfaces), and downstream processing operations of fermented products - or perhaps more widely - to the pressure vessels. What will be required is a measure, or very clear definition, of what constitutes failure in the unit operation - together with realistic values of all operating parameters.

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