# Novel Application of Quantitative Risk Assessment Modelling to a Continuous Fermenter

by

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**STATEMENT OF DECLARATION**

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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 ($\mu_{max}$), 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 $K_s$, $\mu_{max}$ 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 ($K_s$, $\mu_{max}$ 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 $\mu_{max}$ 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 $\mu_{max}$ 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 ($\mu_{opt}$). 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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I trust that the results of my research justify the expectations and confidence of all the people concerned, and the interest and encouragement of my family and colleagues.

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CHAPTER **ONE**

# INTRODUCTION

The food and pharmaceutical industries are generally a nation's largest manufacturing sector, and importantly, one of the most stable (Davey 2001). These industries are especially important to Australia as a major food exporter. One of the most important and widely used unit operations is fermentation.

Of particular research interest is the practical notion that no matter how good the design and operation of plant there will be an occasional failure (Davey and Cerf 2003). Failure in these industries can be a serious risk to public health. Most often there is too small a data set for detailed analyses however - especially if failure is simply put down to operator error. Additionally, data for plant failure are often not available for analyses by researchers because of "commercial in confidence" restrictions (Davey 2001). In recent years, new mathematical approaches have been pioneered that can offer insight into process operation and bioprocess plant failure (Cerf and Davey 2001; Davey and Cerf 2003). These approaches are based on the input parameter being a distribution of values selected from Monte Carlo Assessment (MCA) sampling, rather than on the traditional or Single Value Assessment (SVA).

The principal aim of the research presented here is to assess the novel application of a Quantitative Risk Assessment (QRA) methodology to a continuous fermenter. A continuous fermenter is selected because it has advantages over a batch, in that greater control over product quality and cost can be maintained. Process failure is defined as unexpected fermenter washout, or Friday $13^{th}$ Syndrome (Davey and Cerf 2003).

The justification for this research is that it will aid a greater understanding of factors that contribute to fermenter washout, and, highlight the impact of the combined effect of Uncertainty and Variability in the microbiological input parameters that define the micro organism and its growth in the fermenter.

A logical and step-wise approach to this research was adopted.

The relevant literature is reviewed in Chapter 2. This chapter introduces both SVA and MCA approaches and highlights that there is no published literature on the novel application of QRA to a continuous fermenter. This is despite the obvious importance of this unit operation to the food and pharmaceutical industries and the possible practical

insights that might be gained. Within the MCA approach the notion of Uncertainty, Variability and Chance are introduced and defined. A fermenter model based on that of Monod growth kinetics is selected for a model of a continuous fermenter. In the microbiological literature a continuous fermenter is described as a *Chemostat.* To the biochemical engineers it is a Continuous Stirred Tank Reactor (CSTR).

Chapter 3 describes in detail the development of the Monod process model for the continuous fermenter. This chapter begins with an SVA approach to the model development. The MCA model is then presented. Limited published data for the growth of *Escherichia coli* are used to specify fermenter input growth parameters. A number of illustrative simulations are presented and discussed. The role of Uncertainty and Variability in de-stabilising a well-operated continuous fermenter is illustrated. This chapter concludes with a discussion of the need for greater accuracy in input *E. coli* growth parameters.

In Chapter 4 extensive published data are collated for the growth kinetics of *E. coli.* The predictive growth model of Rosso (Rosso *et. al.* 1993) is fitted to these data using non-liner regression techniques. This growth model was selected as it is widely used and generally gives a good fit to growth data. Resulting estimates from the non-linear regression analyses are presented and discussed.

Chapter 5 presents QRA of the continuous fermenter with MCA random sampling carried out using the more accurate revised data for growth of *E. coli.* A more realistic simulation output obtained using the revised data is presented and discussed.

Chapter 6 is a summary of the findings and conclusions of this research together with the recommendations for future work.

The definition of some important terms used in this research is given in Appendix A. All notation used is listed and defined at the back of this thesis. A list of refereed publications arising from this research is presented in Appendix E.

CHAPTER **TWO**

# LITERATURE REVIEW

## 2.1 Introduction

The food and pharmaceutical industries are some of the most important industries world-wide. Fermentation as a unit operation is widely used in these important industries. Failure of fermentation in the food and pharmaceutical industries could have catastrophic impact on public health.

Mathematical models are widely used to define, control and optimise unit operations. The application of mathematical modelling to unit operations is widely used in engineering research.

In the mathematical modelling of fermentation, process parameters describing growth characteristics of the selected micro organism must be combined with other parameters such as fermenter dilution (feed) rate, yield coefficient and productivity.

In this chapter the principles in design of a continuous fermenter are first reviewed. The Monod process model for a continuous fermenter is selected as it is widely used and understood. A summary and collation of predictive growth models used in fermentation is then presented and discussed. Predictive models are conveniently classified into two groups: those simulating the number of micro organisms as a function of time, and; those simulating the growth rate as a function of temperature. The mathematical notions of Uncertainty (i.e. level of ignorance) and Variability (i.e. effect of Chance) inherent in QRA are then introduced and defined. The principles in applying QRA are then reviewed, and the potential for application to a continuous fermenter discussed. Although there are numerous examples in the literature of analyses of a continuous fermenter, it is shown that a Quantitative Risk Assessment (QRA) has not been applied however.

## 2.2 Continuous fermentation

Fermentation is defined as the enzymatically controlled transformation of an organic compound.

Foods have known to be fermented since Neolithic times (Shurtleff and Aoyagi 2004). The earliest types were beer, wine, and leavened bread (made primarily by yeasts).

Fermentation of fruits was also discovered in antiquity. The ancient Greeks believed wine had been invented by the god Dionysus (Ruck 1982). Methods for the fermentation of milks, meats and vegetables have been described with earliest records dating back to 6000 BC and the civilisations of the Fertile Crescent in the Middle East (Caplice and Fitzgerald 1999). There is strong evidence that people were fermenting beverages in Babylon as early as 5000 BC and in ancient Egypt about 3000 BC.

In the late 1700's Lavoisier illustrated that in the process of transforming sugar to alcohol and carbon dioxide (as in wine), the weight of the sugar that was consumed in the process equaled the weight of the alcohol produced (Shurtleff and Aoyagi 2004). In 1810 the fermentation process was accurately summarized by Guy-Lussac, namely as:

$$C_6H_{12}O_6 \rightarrow 2CO_2 + 2C_2H_6O \qquad (2.1)$$

By the middle of the 19$^{th}$ century, Caplice and Fitzgerald (1999) stated that two events – the "industrial revolution and blossoming of microbiology as a science", had occurred which had a very significant impact on the manner in which food fermentations were performed and understood. According to these authors, the industrial revolution resulted in the concentration of large masses of populations in towns and cities. Consequently, the ability to provide service to the new markets required products to be made in large quantities necessitating the industrialisation of the manufacturing process, as highlighted by Caplice and Fitzgerald (1999).

Caplice and Fitzgerald (1999) highlighted that the beginning of microbiology as a science from the 1850's onwards resulted in the biological basis of fermentation being understood for the first time. According to these researchers the essential role of bacteria, yeasts and moulds in the food fermentation came to be understood that resulted in more controlled and efficient fermentations.

However it was in 1857 that the French chemist Louis Pasteur (1822-1895), in a series of now classic investigations, proved conclusively that fermentation was initiated by living organisms (Snewin 1999; Bordenave 2003). Pasteur proved that alcoholic fermentation was brought about by yeasts (Bordenave 2003).

Sir Alexander Fleming was the individual who serendipitously "discovered" penicillin (Ligon 2004). In 1928, while working on influenza virus, he observed that a mould had developed accidently on a *Staphylococcus aureus* culture plate and that the mould had created a bacteria-free circle around itself. The mould culture prevented the growth of the bacterium, even when diluted many times. He named the active substance as "Penicillin". Ligon (2004) highlights that despite the determination displayed by Fleming, little notice was given to his discovery for more than a decade, and the active substance was not isolated. According to Ligon (2004), in 1939 Florey along with Chain, led a team of British scientists who successfully manufactured the drug from the liquid broth in which penicillin grows. The Australian, Howard Florey (later Lord Florey) and a graduate of the University of Adelaide, developed methods for mass production.

In 1958, the use of continuous fermentation in New Zealand sparked a revolution in the way beer was brewed around the world by (Kennedy 1996). New Zealand was the first to exclusively brew beer using a continuous fermenter.

Continuous fermentation is carried out in a *Continuous Stirred-Tank Reactor* (*CSTR*[3]). In the field of microbiology, a CSTR is referred to as a *Chemostat*. From a biochemical engineering view, a continuous fermenter is actually a bioreactor where the transformations are carried out by the action of living cells (Lee 1992).

In addition to a continuous fermenter, the transformations can be carried out in either batch or fed-batch operation. A continuous fermenter offers advantages over a batch, or fed-batch operation. In a continuous fermenter, the parameters such as cell concentration, substrate concentration, pH, viscosity and other physicochemical properties within the fermenter, remain constant with time, and permit greater control than is possible as with a batch process where these properties change with time (Aiba *et. al.* 1973; Blanch and Clark 1997). Continuous operation is used for higher production rates as compared to batch operations in which large-scale production is more difficult. Continuous operation can reduce operating costs over a batch operation, which requires higher labour costs, and therefore is widely used in most of the food and pharmaceutical industries. One disadvantage however with continuous operation is a high risk of strain mutations in comparison with batch or fed-batch operation.

[3] sometimes in the literature referred to as CSTF- Continuous Stirred Tank Fermenter.

A continuous fermentation aims to provide constant environmental conditions for growth, product formation and generally supplies uniform-quality product (Shuler and Kargi 2002).

Figure 2.1 is a schematic diagram of a continuous, stirred-tank, fermenter. In this fermenter, fresh medium is fed continuously with a feed rate ($F$) to the fermenter of volume ($V$). Fermented product is removed continuously. The products, metabolic waste and micro organisms are removed in the effluent stream, where, $x$ is the concentration of the micro organisms, $s$ is the concentration of the substrate, and $x_p$ is the concentration of the product. The feed stream ($x_f$), where suffix $f$ represents the feed, is usually sterile so that $x = 0$.

In a successful continuous fermenter, the micro organisms are always in the exponential growth phase. During the exponential growth, the entire metabolic effort of the micro organisms is directed to reproduction (McMeekin *et. al.* 1993). The exponential growth of a microbial population is preceded by a period called lag phase (Baranyi and Pin 2001; Kutalik *et. al.* 2005). A lag phase in micro organism growth model means no product i.e. by having the micro organisms in the growth phase, product formation is initiated immediately in the fermenter. A microbial growth curve shows at least four identifiable phases: the lag phase, growth (or exponential) phase, stationary phase, and death (or decline) phase (Monod 1949; Davey 2001; Zwietering *et. al.* 1991). Although the lag phase of the micro organisms is important in food microbiology, it has no importance in the operation of a continuous fermenter (Baranyi and Roberts 1994; Davey 2001).

The micro organisms in a continuous fermenter can also be immobilised to maximize their retention within the fermenter and thereby increase productivity, for e.g. yeast immobilised in thread-type, gel particles in a continuous beer fermentation (Que 1993); *Candida tropicalis* immobilised on porous glass and cultivated in a continuous fermenter to produce xylitol from xylose (Silva and Afschar 1994), yeast immobilised on alginate beads to convert ethanol to glucose (Gilson and Thomas 1994), and *Saccharomyces cerevisiae* immobilised in calcium alginate gel beads were used for continuous ethanol fermentation from cane molasses and other sugar sources (Nagashima *et. al.* 2004).

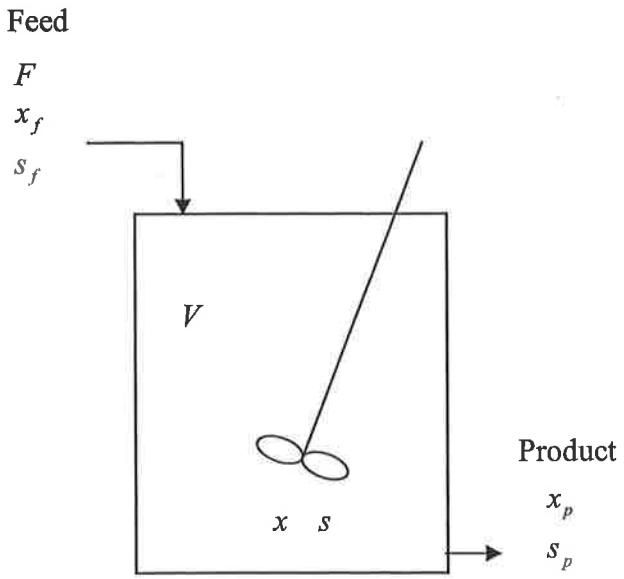


**Figure 2.1** Schematic diagram of a continuous stirred-tank reactor (CSTR), where, $V$ is the fermenter volume, $F$ is the feed, $x$ is the concentration of the micro organism, $s$ is the concentration of the substrate, suffix $f$ represents the feed, and $p$ represents the product (*after* Lee 1992).

The specific growth rate ($\mu$) of a micro organism is a measure of how fast the micro organisms grows. The units of $\mu$ are $time^{-1}$. The greater the value of the specific growth rate, the faster is the growth of the micro organisms.

The specific growth rate also depends on the substrate concentration within the fermenter. The specific growth rate ($\mu$) decreases as the substrate concentration decreases and eventually lead to no growth i.e. $\mu = 0$. The value of the specific growth rate is relatively constant during exponential growth (McMeekin *et. al.* 1993). However, according to McMeekin *et. al.* (1993), exponential growth can be inhibited either by the availability of oxygen in an aerobic fermentation, or poor mixing within the fermenter in an anaerobic fermentation. In which case, growth of micro organisms is mass transfer limited rather than limited by kinetics.

## 2.3 Modelling continuous fermentation

### 2.3.1 Monod model

In 1949, Monod published a "milestone contribution" to studies of micro organisms that gave a systematic description of the growth of the micro organisms and led to the notion that a limited number of growth constants define the behaviour of the micro organism (Ferenci 1999).

The work of Monod (1942) underpins CSTR theory.

The Monod process model form is one of the most widely used models for predicting the effect of the substrate concentration on the specific growth rate of the micro organism. One key reason is that it includes a term for substrate concentration.

The Monod process model is empirical. It is a functional relationship between the specific growth rate of a micro organism and the substrate concentration within the continuous fermenter.

The assumptions of the Monod model are that the:

1. Growth rate is limited by deficiency of a single chemical species

2. Fermenter is well-mixed i.e. the sterile feed is uniformly dispersed throughout the working volume of the fermenter

3. Kinetics follow Langmuir-Hinshelwood kinetics or Michaelis-Menten kinetics for enzyme reactions (Blanch and Clark 1997; Shuler and Kargi 2002).

When applied to growth of micro organisms in a continuous fermenter the Monod process model is given by:

$$\mu = \frac{\mu_{max} s}{K_s + s} \tag{2.2}$$

where, $\mu$ is the specific growth rate of the micro organism ($h^{-1}$), $\mu_{max}$ is the maximum specific growth rate ($h^{-1}$), $K_s$ is the limiting nutrient concentration at which $\mu = \mu_{max}/2$, and $s$ is the substrate concentration ($kg\ m^{-3}$).

The Monod process model, together with a number of other growth rate models can be conveniently described by a single differential equation (Shuler and Kargi 2002):

$$\frac{d\omega}{ds} = K\omega^L (1-\omega)^M \tag{2.3}$$

where, $\omega = \mu/\mu_{max}$, $s$ is the limiting substrate concentration, and $K$, $L$ and $M$ are constants.

Table 2.1 summarises those constants that apply for the Monod, and a number of other, related growth rate models. These values of the constants, given in the Table 2.1, when incorporated in the generalised differential equation describe the other forms of Monod process model such as Tessier, Moser and Contois.

Bailey and Ollis (1986) pointed out that, in some instances, these later three Monod related model forms, Tessier, Moser and Contois give a better fit to the experimental data than the Monod form.

The Monod form is however the most widely used as it is "sufficient" for most kinetic studies (Hoskisson and Hobbs 2005) and it is conveniently easy to use. Application of the Monod form requires a growth model for the particular micro organism that it is, a functional relationship of the form:

$$\mu_{max} = f(\text{environmental factors}) \tag{2.4}$$

where, the environmental factors include: temperature, pH, water activity, oxygen availability, etc. (Davey and Daughtry 1995; Davey 2001). The relationship('s) of Equation (2.4) can be substituted into Equation (2.2).

### 2.3.2 Growth models

A number of predictive modelling approaches to the growth of micro organisms in a continuous fermenter have been published in the literature. Predictive growth models are used to describe the behaviour of micro organisms under different physical or chemical conditions such as temperature, pH, and water activity. In engineering applications predictive models are widely used to approach optimal operation.

A number of categorisations and comparisons between these growth models can be made (Adair *et. al.* 1989; Davey 1989b; Davey 2001). Models of interest to this research can be conveniently classified into two groups - Models used for simulating the number of micro organisms as a function of time, and; Models used for simulating the growth rate of the micro organism as a function of temperature. These two model types are summarised in Table 2.2.

Those models used for simulating the number of micro organisms as a function of time ($\log N$) are presented in the upper part of Table 2.2. Those for simulating the growth rate ($\mu_{max}$) as a function of temperature are presented in the lower part. The growth models of the upper part of the Table 2.2 are reviewed first.

**Table 2.1** Constants for a generalised differential rate equation:

$$\frac{d\omega}{ds} = K\omega^{L}(1-\omega)^{M}$$

for different process models for fermentation (*after* Shuler and Kargi 2002).

| Model | $L$ | $M$ | $K$ |
|---|---|---|---|
| Monod | 0 | 2 | $1/K_s$ |
| Tessier | 0 | 1 | $1/K$ |
| Moser | 1-1/$n$ | 1+1/$n$ | $n/K_s^{1/n}$ |
| Contois | 0 | 2 | $1/K_{sx}$ |

The Logistic, Gompertz, Richards, Schnute and Stannard models for simulating the number of micro organisms as a function of time were extensively reviewed by Zwietering *et. al.* (1990). These researchers reported that it was difficult to calculate the 95 % confidence intervals for the microbiological parameters: lag time, asymptote and maximum specific growth rate. Therefore they reported that they found it necessary to modify these models. The Modified Logistic, Modified Gompertz, Modified Richards, Modified Schnute and Modified Stannard models are shown in Table 2.2, where, $y$ [= $\ln(N/N_0)$] is the population density, $a$ and $b$ are mathematical parameters, $\lambda$ is the lag time, $A$ is the asymptote [= $\ln(N_\infty/N_0)$] i.e. the maximal value reached, $N_\infty$ is the maximum population density and $N_0$ is the population density at time ($t$) equal to zero.

McMeekin *et. al.* (1993) reported that the Gompertz model was actually formulated for actuarial science for fitting human mortality data but it has also been applied to deterministic to organ growth.

Fujikawa *et. al.* (2004) also reviewed these models for simulating the number of micro organisms ($\log N$) as a function of time. Fujikawa *et. al.* highlighted that the microbial growth curves are generally sigmoid on a semi-logarithmic plot. However, they underscored that the Logistic model generates a convex curve that consists of a monotonically increasing portion and a stabilising portion without a lag phase at the initial period, and therefore cannot generate a sigmoid curve on a semi-logarithmic plot. Fujikawa *et. al.* (2004) actually suggested therefore that the Logistic model might not be applicable to microbial growth.

McKellar and Lu (2004b) demonstrated that the original Logistic and the Gompertz model are considered as "mechanistic", whereas the modified forms of these two models are considered as "empirical". They also highlighted that the Gompertz model is generally preferred over the Logistic model because of its asymmetric nature about the point of inflection unlike the Logistic model. However, McKellar and Lu (2004b) underscored the limitations of using the Gompertz model, such as - the generation time can be underestimated by 13 %, and a wide range of experimental growth data was required over the entire growth range (Membre *et. al.* 1999).

**Table 2.2** Summary of predictive growth models used in fermentation [where $e = \exp(1)$].

| | | |
|---|---|---|
| **1. Models used for simulating the number of micro organisms ($\log N$) as a function of time** | | |
| Modified Logistic | $y = \frac{A}{\left\{1+\exp\left[\frac{4\mu_{max}}{a}(\lambda - t)+2\right]\right\}}$ | McMeekin *et. al.* (1993)<br>Zwietering *et. al.* (1990)<br>McKellar and Lu (2004b) |
| Modified Gompertz | $y = A\exp\left\{-\exp\left[\frac{\mu_{max} e}{a}(\lambda - t)+1\right]\right\}$ | McMeekin *et. al.* (1993)<br>Zwietering *et. al.* (1990)<br>McKellar and Lu (2004b) |
| Modified Richards | $y = \left\{1+\upsilon\cdot\exp(1+\upsilon)\cdot\exp\left[\frac{\mu_{max}}{a}\cdot(1+\upsilon)^{\left(1+\frac{1}{\upsilon}\right)}\cdot(\lambda - t)\right]\right\}^{(-1/\upsilon)}$ | Richards (1959)<br>Zwietering *et. al.* (1990) |
| Modified Schnute | $y = \left(\mu_{max}\frac{(1+b)}{a}\left\{\frac{1-b\cdot\exp(a\cdot\lambda+1-b-at)}{1-b}\right\}\right)^{1/b}$ | Schnute (1981)<br>Zwietering *et. al.* (1990) |
| Modified Stannard | $y = \left\{1+\upsilon\cdot\exp(1+\upsilon)\cdot\exp\left[\frac{\mu_{max}}{a}\cdot(1+\upsilon)^{\left(1+\frac{1}{\upsilon}\right)}\cdot(\lambda - t)\right]\right\}^{(-1/\upsilon)}$ | Stannard *et. al.* (1985)<br>McMeekin *et. al.* (1993)<br>Zwietering *et. al.* (1990) |
| **2. Models used for simulating the growth rate ($\mu_{max}$) as a function of temperature** | | |
| Square-Root (Ratkowsky-Belehradek 1) | $\mu_{max} = [b_1(T - T_{min})]^2$ | Ratkowsky *et. al.* (1982)<br>McMeekin *et. al.* (1993)<br>Ross and Dalgaard (2004) |
| Expanded Square-Root (Ratkowsky-Belehradek 2) | $\mu_{max} = \left(b_2(T - T_{min})\cdot\{1-\exp[c_2(T - T_{max})]\}\right)^2$ | Ratkowsky *et. al.* (1983)<br>Belehradek (1930) |
| Modified Square-Root (Ratkowsky-Belehradek 3) | $\mu_{max} = [b_3(T - T_{min})]^2\cdot\{1-\exp[c_3(T - T_{max})]\}$ | McMeekin *et. al.* (1993) |
| Schoolfield | $\mu_{max} = \frac{\mu_{25}\frac{T}{298}\exp\left[\frac{H_a}{R}\left(\frac{1}{298}-\frac{1}{T}\right)\right]}{1+\exp\left[\frac{H_l}{R}\left(\frac{1}{T_l}-\frac{1}{T}\right)\right]+\exp\left[\frac{H_h}{R}\left(\frac{1}{T_h}-\frac{1}{T}\right)\right]}$ | Schoolfield *et. al.* (1981)<br>McMeekin *et. al.* (1993)<br>Ross and Dalgaard (2004) |
| Hinshelwood | $\mu_{max} = k_1\cdot\exp\left(-\frac{E_1}{RT}\right)-k_2\cdot\exp\left(-\frac{E_2}{RT}\right)$ | McMeekin *et. al.* (1993)<br>Ross and Dalgaard (2004) |
| Davey Linear-Arrhenius | $\mu_{max} = \exp\left(C_0+\sum_{i=1}^{J}(C_{2i-1}T_i + C_{2i}T_i^2)\right)$ | Davey (1989a)<br>Davey and Daughtry (1995)<br>Davey (2001)<br>McMeekin *et. al.* (1993)<br>Ross and Dalgaard (2004) |
| Rosso | $\mu_{max} = \frac{\mu_{opt}(T - T_{max})(T - T_{min})^2}{(T_{opt} - T_{min})[(T_{opt} - T_{min})(T - T_{opt}) - (T_{opt} - T_{max})(T_{opt} + T_{min} - 2T)]}$ | Rosso *et. al.* (1993)<br>Rosso *et. al.* (1995) |

Zwietering *et. al.* (1991) underscored that models with a greater number of parameters usually give a lower Residual Sum-of-Squares (RSS) that can be obtained using more parameters. Therefore, they added the shape parameter ($\upsilon$) in the Modified Richards and Modified Stannard models to define the shape of the growth curve. However, this extra parameter used in these two models did not provide any useful improvement in goodness-of-fit to the growth data. It can be observed that the Modified Stannard and the Modified Richards models appear to be the same. These researchers highlighted that, in some cases, the Modified Stannard and the Modified Richards models did not fit the data accurately and predicted large value of the parameter estimates that resulted in large error.

In the study conducted by Zwietering *et. al.* (1991), the Modified Logistic, Modified Gompertz, Modified Richards, Modified Schnute and Modified Stannard models were found to give a statistically acceptable goodness-of-fit those with few parameters (McMeekin *et. al.* 1993). Zwietering *et. al.* (1990) compared each of these models and concluded that the Modified Gompertz model was "statistically sufficient and easy to use" and could be regarded as the best model to describe the growth data in terms of the goodness-of-fit to the data (Whiting and Cygnarowicz-Provost 1992). However, Zwietering *et. al.* pointed out that these models are of limited use as they assess only the number of micro organisms ($N$) or a logarithm of the number of micro organisms ($\log N$) as a function of time, and do not include the substrate consumption ($s$) in comparison to that as given by Monod (1949).

Stannard *et. al.* (1985) reviewed these models for simulating the growth rate ($\mu_{max}$) as a function of temperature. These researchers highlighted that knowledge of the relationship between temperature and growth rate is important in the prediction of the likely levels of micro organisms after a known time at a specific temperature. They concluded that the Square-Root (Ratkowsky-Belehradek 1) model proposed by Ratkowsky *et. al.* (1982) is a better description of the microbial growth and temperature relationships than is the widely used Arrhenius Law[4]. However, Ratkowsky *et. al.* (1982) highlighted that the Arrhenius

[4] The Arrhenius Law was originally proposed by Van't Hoff and Arrhenius to describe the temperature dependence of the specific reaction rate constant in chemical reactions (Ratkowsky *et. al.* 1982). The Arrhenius Law is given by:

$$k = k_0 \exp(-E/RT)$$

where, $k$ is the specific reaction rate constant ($s^{-1}$ (mole $m^{-3}$)$^{-1}$), $k_0$ is the collision factor or frequency factor, $E$ is the activation energy ($J$ mole$^{-1}$), $R$ is the universal gas constant having a value (= 8.314 J mole$^{-1}$ K$^{-1}$), and $T$ is the absolute temperature (K).

Law does not adequately describe the effect of temperature on the growth of the micro organisms. Adair *et. al.* (1989) also pointed out that the Arrhenius Law has been widely applied to growth of the micro organisms as well as to the chemical reactions, however, the Arrhenius relationship is non-linear or atleast linear over only a portion of the temperature range.

The models used for simulating the growth as a function of temperature, presented in the lower part of Table 2.2 can be reviewed. The first of these is the Ratkowsky-Belehradek 1 model. In that $b_1$ is the Ratkowsky parameter ($^{O}C^{-1}$ $h^{-0.5}$), and $T_{min}$ is the minimum temperature at which growth is observed ($^{O}C$). This model gives a linear relation between the square root of the growth rate of the micro organism, and the temperature of growth. However, a number of researchers (McMeekin *et. al.* 1993; Zwietering *et. al.* 1991) demonstrated that the Ratkowsky-Belehradek 1 model does not have a biological basis and is based on the observation that at lower temperatures the square root of the specific growth rate is linear with temperature.

Ratkowsky *et. al.* (1983) noted that the Square-Root model was especially useful for modelling the growth rate of the micro organisms below the optimum growth temperature. An Expanded Square-Root (Ratkowsky-Belehradek 2) model was therefore proposed.

The Expanded Square-Root Ratkowsky-Belehradek 2 model is shown in Table 2.2, where, $c_2$ is the Ratkowsky parameter ($^{O}C^{-1}$), and $T_{max}$ is the maximum temperature at which some very limited growth can still be observed ($^{O}C$). This model describes the growth of the micro organisms throughout the entire temperature range (Ratkowsky *et. al.* 1983). However, Zwietering *et. al.* (1991) highlighted that at temperatures above $T_{max}$ this model will predict values of growth rate greater than zero - and therefore it cannot be reliably used. The Expanded Square-Root Ratkowsky-Belehradek 2 model was then modified by Zwietering *et. al.* (1991) to avoid positive values of growth rate above $T_{max}$.

The readers should note that the Ratkowsky-Belehradek 3 has as the square term $[b_3(T-T_{min})]^2 \cdot \{1-\exp[c_3(T-T_{max})]\}$, in contrast to the square term of Ratkowsky-Belehradek 2, $(b_2(T-T_{min}) \cdot \{1-\exp[c_2(T-T_{max})]\})^2$.

In the Schoolfield *et. al.* (1981) model of Table 2.2, $\mu_{25}$ is the growth rate at 25 $^{O}$C ($h^{-1}$), $T_l$ is the temperature (K) at which the enzyme is 50 % inactivated due to low temperature, $H$ is the enthalpy of activation (J $mole^{-1}$), and $T_h$ is the temperature (K) at which the enzyme is 50% inactivated due to high temperature. Adair *et. al.* (1989) demonstrated the effect of temperature on the growth of the micro organisms by comparing both – the Schoolfield model and the Square Root model - and recommended that the Schoolfield model makes realistic predictions of growth and is more reliable at low temperatures. Adair *et. al.* (1989) recommended that the Schoolfield model should therefore be adopted to develop predictive models from the growth data. However, McMeekin *et. al.* (1989) argued that the procedure used by Adair *et. al.* (1989) to compare the models, which contradicted the use of the Square-Root model, was inappropriate and was then justified by McMeekin *et. al.* (1989).

The Hinshelwood model (Table 2.2) was elaborated from a mechanistic approach by Rosso *et. al.* (1993) to the growth of the micro organism based on a single growth rate-determining reaction. Zwietering *et. al.* (1991) in their study on modelling of microbial growth as a function of temperature discussed the fact that the Hinshelwood model shows an Arrhenius type of temperature dependency, where, $k_1$ and $k_2$ are frequency factors ($h^{-1}$) and $E_1$ and $E_2$ are the activation energies (J $mole^{-1}$). McMeekin *et. al.* (1993) underscored that, in comparison with the other growth rate models, the Hinshelwood model does not fit the data well at low temperature due to the lack of a cold denaturation term in the equation.

Zwietering *et. al.* (1991) compared the growth models as a function of temperature, Ratkowsky-Belehradek 1, Ratkowsky-Belehradek 2, Ratkowsky-Belehradek 3, Schoolfield and Hinshelwood models, and concluded that the modified forms of Ratkowsky model are the most suitable model for both the growth rate of the micro organism and the asymptote ($A$) as a function of temperature.

Davey (1989a) proposed a linear Arrhenius (DL-A) model to describe the effects of temperature and water activity on microbial growth rate in the exponential phase. He also applied this model to describe the lag phase of the microbial growth in situations where either temperature or water activity or both were the rate controlling factors. Ross and Dalgaard (2004) highlighted that Davey (1994) extended his earlier linear Arrhenius model

(Davey 1989a) to account for the effect of multiple environmental factors affecting the microbial growth rate. The DL-A model is shown in Table 2.2, where, $T$ is the temperature, and $C_0$ to $C_{2i}$ are the coefficients of the DL-A model to be determined with $j$ environmental factors. The proposed DL-A model gives a better fit when compared to the Square-Root model and the Arrhenius relationship (Davey 1989b). Although successfully applied to a wide range of growth data, the DL-A model has not been used in continuous fermentation.

Rosso *et. al.* (1993) showed that the popular Ratkowsky-Belehradek 2 model is badly conditioned as it is difficult to estimate the parameters accurately. Therefore, these researchers proposed a new model. They pointed out that the three cardinal temperatures were found to be independent of specific growth rate at the optimum temperature, and a very strong and an unexpected linear correlation was observed between the cardinal temperatures for the specific growth rate of the micro organisms as a function of temperature. These three cardinal temperatures are: the temperature below which growth is no longer observed $T_{min}$ ($^{O}$C), the temperature above which no growth occurs $T_{max}$ ($^{O}$C), and the temperature $T_{opt}$ ($^{O}$C) at which the maximum specific growth rate $\mu_{max}$ ($h^{-1}$) is equal to its optimum value $\mu_{opt}$ ($h^{-1}$).

The Rosso model is easy to use. It seems to satisfy an intuitive feel for the environmental envelope, of the effect of temperature, inside which growth can be observed.

It should be added that throughout the 1990's the literature reveals that there was a lively debate between those researchers who approached microbial growth from a biochemical engineering and fermentation viewpoint, and, those who viewed microbial growth from a statistical or microbiological aspect, on the correct use of associated terminologies, definitions and assumptions for predictive modelling that could be reliably made - *see* for e.g. the correspondence between Baranyi and Roberts (1992), Davey (1992) and Whiting and Buchanan (1993). In fermentation, it is the biochemical engineering process terminology that is widely used. In predictive growth models, the microbiological terminology appears entrenched. It is after-all the microbiologists that produce the more fundamental data for predictive growth models.

The solution to the growth models that are coupled with the continuous fermenter process model can be carried out effectively using one or two ways. The first of these is the SVA or Single Value Assessment modelling. This is widespread in the literature where a single "best guess" estimate or a mean value of an input parameter is used. The alternate way is MCA or Monte Carlo Assessment modelling. In this approach, the input parameter values take the form of a distribution.

The SVA or Single Value Assessment modelling is reviewed first in the following section.

## 2.4 SVA or Single Value Assessment modelling

The usual solution to the Monod process model taken by biochemical engineers (and others) is a single point stochastic and deterministic approach. Cerf and Davey (2001) defined this approach as a Single Value Assessment (SVA).

SVA involves using a single value or "best guess" estimate of the value of each parameter such as growth rate, yield coefficient, substrate concentration and feed concentration within a process model to obtain a single value predictive outcome for the output parameter such as maximum dilution rate, dilution rate at maximum output, and maximum productivity. It can be seen that this is another way of saying the usual approach is using a sensitivity analysis for each input parameter - in which a small amount of variability (say $\pm$ 1%) is introduced around the mean value.

In the SVA, the model input parameters are linked with each other as well as with the output parameters via the usual mathematical functions e.g. multiplication, addition, subtraction, exponentiation etc. The equations are then solved. This can be done using software for mathematical modelling for e.g. Microsoft Excel™ spreadsheet.

## 2.5 MCA or Monte Carlo Assessment modelling

In contrast to the SVA is the Monte Carlo Assessment (MCA). This takes into account all possible values that the input parameters may take. Input parameters for the MCA are a distribution therefore of possible values (with the probability of each occurring). These are linked via the usual mathematical functions as is the case in SVA. This is because a

distribution of values together with the probability of each occurring is given. The mean value of MCA will often be the SVA. Cerf and Davey (2001) used MCA sampling in a QRA of a *sterilisation* unit operation.

## 2.6 Quantitative Risk Assessment (QRA)

A Quantitative Risk Assessment (QRA), can be defined as "A stepwise analysis of hazards that may be associated with a particular type of food product, permitting an estimation of the probability of occurrence of adverse effects on health from consuming the product in question" (Notermans and Mead 1996).

QRA is a relatively new field - and one that is almost certain to grow rapidly in chemical and biochemical engineering (K R Davey *pers. comm.*)[5]. QRA was tried out in 1960's - but because of limitations in available software and computer programming, and necessary hardware, it all but died out (Vose 2000). However, it re-emerged in the mid 1990's when computing became more widespread and available (Vose 2000).

Importantly for engineers and applied researchers, QRA is not to be considered as the same thing as HAZOP Studies or HACCP.

Hazard and Operability Studies (HAZOP) is a "systematic, structured approach to questioning the sequential stages of a proposed operation in order to optimise the efficiency and the management of risk" (Swann and Preston 1995). However, Swann and Preston (1995) underscored the problem with HAZOP actions is that they are created at a stage when detailed design is under way, and to make a number of changes at this stage is inevitably expensive and causes potential delay, and these changes could be expensive. Whiting and Buchanan (1997) highlighted that HACCP is the more widely recognised. It focuses on identifying and controlling the key process steps that most significantly affect the safety of production.

[5] There are significant opportunities for biochemical engineers – not least because of a strong background in mathematical and process orientation that bioengineers can bring.

HACCP, Hazard Analysis Critical Control Point is a systematic approach to produce acceptable, safe product based on identification and management of critical control points[6] (Notermans *et. al.* 1996). HACCP was apparently developed by NASA in the 1960's to help prevent food poisoning with astronauts (Backeburg *et. al.* 2005). However, Whiting and Buchanan (1997) pointed out that as HACCP has become more widely adopted, it has become evident there are areas within this approach that could be strengthened if researchers were better able to quantitatively link product attributes with public health concerns.

In 1999, Nauta assessed both HAZOP and HACCP. He highlighted that potential microbial growth can be incorporated into both by applying predictive microbial modelling methods. Nauta suggested that new types of predictive models that incorporate modelling of Uncertainty and Variability in microbial growth are needed.

The fundamental principle of QRA is that Uncertainty and Variability are the two components that will enable precise prediction of future events.

### 2.6.1 Uncertainty

This is defined as a lack of knowledge, or level of ignorance, about the parameters that characterise the physical (process) system. Uncertainty is sometimes reducible through further measurement or careful study, or through consulting more experts.

Uncertainty is essentially a statement with which any logical person should agree - given the same information.

### 2.6.2 Variability

This is the effect of Chance - and is a function of the system. Variability is not reducible through further study or careful measurement, and can be only be reduced by changing the physical system (Vose 2000).

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[6] A *Critical Control Point* is defined as any point or procedure in a specific food system where loss of control may result in an unacceptable health risk. A *Control Point* is a point where loss of control may result in failure to meet (non-critical) quality specifications.

### 2.6.3 Total Uncertainty

This is a combination of the two ideas i.e. Uncertainty and Variability, which influence the ability to predict future events.

Uncertainty and Variability might be called *Fact* and Chance respectively (Vose 2000).

Why separate the two? The two components - Uncertainty and Variability are separated in risk modeling so as to observe how both contribute to the risk model (Vose 2000). Separating Fact and Chance is therefore important to understand process behaviour - and to avoid large errors that could easily result in unexpected process failure.

### 2.6.4 Insight offered by QRA

The key insight offered through QRA modelling of a unit operation is the idea that the process output can be significantly affected by the combined effect of a series of small changes in the input parameters and attaches a practical likelihood, or probability, of the Chance occurring. These will be allocated to Chance in this way the probability, however small, of an unexpected failure of the process plant.

QRA approach accounts for Uncertainty and Variability in the model input parameters by using repeated sampling from a distribution of values of an input parameter, and provides a framework to evaluate the influence of a variety of input parameters on the process efficacy.

Models that account for Uncertainty and Variability in a system are referred as *stochastic* models (McKellar and Lu 2004a). In *microbiological process modelling* (Davey 1993), the stochastic predictive models are used to define the growth kinetics of the micro organism and predict behavior of the micro organism under various environmental conditions.

The different components and stages of risk assessment are linked together by the usual mathematical relationships and Variability in inputs at each stage is propagated throughout to the final output.

The output is also expressed in the form of a probability distribution. This may give a better and more practical representation of the risk being assessed than is the current use of a SVA or "best guess" estimate.

Repeated sampling of values of the distribution of input parameters is carried out using the Monte Carlo sampling method.

## 2.7 Quantitative Risk Assessment (QRA) using Monte Carlo Assessment (MCA) random sampling techniques

Within a QRA, Monte Carlo approach is used as a random sampling technique for solving deterministic equations. MCA originated during 1940's at Los Alamos from the work of Ulam, von Neumann and Fermi (Cullen and Frey 1998). MCA replaces single value inputs with probability distributions, of the input parameters. This involves random sampling of each probability distribution within a parameter to produce hundred's or even thousand's of iterations. Each probability distribution is sampled in a manner that reproduces the shape of the distribution. The distribution of the values calculated for the parameter outcome therefore reflects the probability of the values that could occur practically in plant operation. The effect of distributions of the values in each of the model parameters is therefore highlighted through MCA with a consequent distribution of practical values. The characterisation of Uncertainty using MCA allows the decision-makers to choose whether to actively reduce an exposure or to conduct an additional research to study the impact of Variability in the risk factors on the output (Cullen and Frey 1998). The main advantage of using MCA is that the simulations are carried out in a repeated manner. This yields important insights into the sensitivity of the model to the variations in the model input parameters, as well as into the likelihood of occurrence of any particular outcome. It is therefore possible to represent Uncertainty in the output of a model by generating sample values for the model inputs, and running the model in a repetitive manner.

There are a number of examples of QRA with MCA sampling in the literature. These however, almost invariably deal with either animal health (Canon and Roe 1982), human health (Burmaster and Anderson 1994) or economics (Cramer 1971) and forecasting methods in management (Makridakis and Wheelwright 1989). Further similar examples can be found in Vose (2000) and McKellar and Lu (2004).

A QRA was first applied to a unit operation in the chemical - biochemical engineering literature by Cerf and Davey (2001) to explain the unexpected failure of a well-operated Ultra-High Temperature (UHT) process plant (This appears in fact to be the only example applied to process plant in the chemical - biochemical engineering literature as at February 2006). Failure was defined as a non-sterile milk pack. *Bacillus stearothermophilus* and *Bacillus thermodurans* were used as contaminant spores. Failure of sterilisation with these micro organisms could be a serious risk to public health. The concentration of contaminant spores, thermal resistance of the spores, heating temperature and the residence time of the milk in the steriliser were identified as the process input parameters. Davey and Cerf (2003) illustrated the effect of distributions of values in each of those in the UHT process. This was highlighted with a distribution of practical values of the process input parameters.

In 2003, Davey and Cerf described unexpected failure as Friday $13^{th}$ Syndrome. By this was meant that there will be failures despite all efforts in a well-operated plant due to Chance. Davey and Cerf noted that one reason that these Friday $13^{th}$ Syndrome events are rare is that most commercial sterilisations involve over-treatment (which is not only wasteful in terms of cost and energy, but also diminishes the nutritional and sensory qualities of the product).

A practical upshot of the QRA was that the predictions showed that a higher proportion of the number of milk packs would be non-sterile than was predicted by the SVA. This number of non-sterile packs was more or less the number that was anecdotally known to be found non-sterile in well-operated UHT process plant, between 1 and 4 in $10^4$. Davey and Cerf (2003) concluded that the occurrence of a fixed number of non-sterile milk operations associated worldwide with the UHT process plant, and greater than that predicted by the SVA, is the failure to take into account a distribution of values for each of the process parameters.

## 2.8 Summary

From a critical review of the literature, the following important factors emerge which are relevant to this research:

1. Fermentation is one of the most widely used unit operations worldwide. A continuous fermenter is usually preferred over a batch, or, a fed-batch, fermenter.

2. The Monod process model for the process performance of a continuous fermenter is one of the most widely used and readily employed. The Monod model requires integration of a predictive model for micro organism growth. These micro organism predictive growth models can be categorised into two groups: Models used for simulating the number of micro organisms as a function of time, and; Models used for simulating the growth rate of the micro organism as a function of temperature. The Rosso form has the advantages of giving a good fit to the growth data and therefore is widely used.

3. In biochemical engineering, the solution of process models for continuous fermentation are usually via a Single Value Assessment (SVA) in which "best guess" estimates or a mean value of input parameters are used. The output is a single value. In contrast to an SVA is the Quantitative Risk Assessment (QRA). The Quantitative Risk Assessment (QRA) accounts for the Uncertainty and Variability in the input parameters and uses Monte Carlo sampling, or Monte Carlo Assessment (MCA). The output prediction is a probability distribution of values in which the mean is nearly always equal to the SVA value. The mathematics for both the approaches - SVA and MCA - are similar i.e. all the mathematical formulations such as addition, subtraction, multiplication, and exponentiation remain the same - except in MCA the inputs are required to be a distribution of values. For the QRA in the MCA, output is also a distribution of likely values, in contrast to a single, or a mean, value from SVA

4. Despite the apparent utility of a QRA for gaining potential insights into the practical operation of a continuous fermenter, none has been reported.

In the next chapter, a novel QRA is applied to a continuous fermenter based on the Monod process model. The importance of Uncertainty and Variability in the microbiological input parameters in de-stabilising a continuous fermenter is highlighted.

CHAPTER **THREE**

# DEVELOPMENT OF A QRA MODEL OF A CONTINUOUS FERMENTER

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## 3.1 Introduction

A review of the literature (Chapter 2) showed that continuous fermentation is an important unit operation used worldwide. Failure in product efficacy can be catastrophic to public health - and costly. Attempts have been made by a number of researchers to model continuous fermentation with varying levels of sophistication.

What is of particular interest, however, is why a well-operated process plant fails unexpectedly - and sometimes catastrophically.

Davey and Cerf (2003) illustrated that a Quantitative Risk Assessment (QRA) can give insight into unexpected process failure. They titled this unexpected failure of an otherwise well-operated process plant as "Friday 13$^{th}$ Syndrome". This expression strongly conjures the reality of the notion of the sum of the combined effect of small changes in the value of process parameters having an adverse effect, i.e. failure, on the process. Cerf and Davey (2001) demonstrated the practical insights into failure that can be gained using an alternate modelling approach, that of QRA. QRA, in contrast to Single Value Assessment (SVA), is based on a distribution of input and output values and the probability of these occurring. The probability distribution is used to highlight the likelihood of occurrence of failure in a unit operation.

For a continuous fermenter, failure is defined as washout. Cerf and Davey (2001) defined failure of continuous sterilisation of milk as non-sterile packs.

In this chapter a new Quantitative Risk Assessment (QRA) of a well-operated continuous fermenter based on Monod kinetics is carried out. The Monod process model is selected as it is widely used, and of a form that can be readily employed.

A continuous fermenter process model is developed and initially established using the traditional or SVA approach. The QRA is then presented. The Rosso model is selected for the predictive growth kinetics. The traditional SVA approach is contrasted with the new MCA approach within the QRA.

## 3.2 Methodology

A logical and step-wise methodology to the Quantitative Risk Assessment (QRA) is used:

1. A Monod process model of a generalised continuous fermenter is developed in Microsoft Excel™ spreadsheet using the Single Value Assessment (SVA) modelling approach

2. The micro organism selected for this study is *E. coli* - a Gram negative, vegetative and non-spore forming bacterium that is widely used in fermentation

3. A Monte Carlo Assessment (MCA) model of a continuous fermenter is then developed in a Microsoft Excel™ spreadsheet with an add-in *@Risk*™ version 4.5 (Palisade Corporation)

4. The outputs of the MCA simulations are assessed to predict the probability of failure (i.e. washout) in a continuous fermenter. This is done to evaluate the combined effect of Uncertainty and Variability in the *E. coli* microbiological growth parameters in de-stabilising an otherwise a well-operated continuous fermenter.

This methodology was adapted from Vose (2000), namely:

Identify the risk to be analysed and potentially controlled; Qualitatively describe the risk (Why it might happen? What one might do to reduce the risk?); Quantitatively analyse the risk (What is the optimal strategy for controlling that risk?); Implement the risk strategy; and, Predict the probability of failure.

## 3.3 Monod process model of a continuous fermenter

Consider a continuous fermentation process as shown in Figure 3.1 - in which *viable* microbial cells consume substrate and grow and divide to produce daughter cells. The continuous fermenter is assumed to be well-mixed and to operate at steady state (Bailey and Ollis 1986; Blanch and Clark 1997).

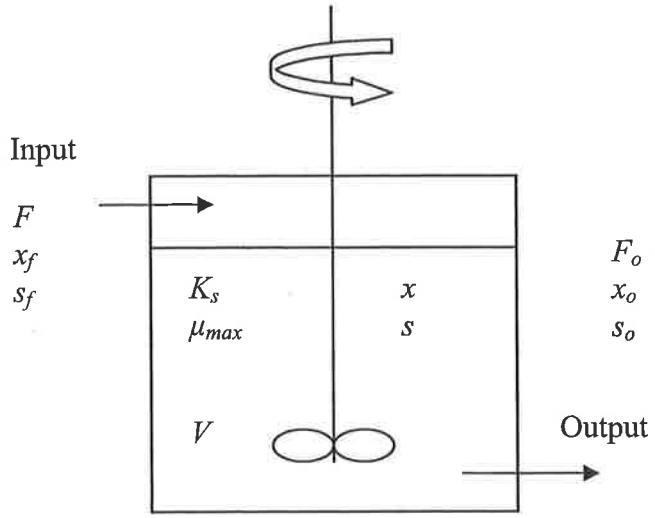


**Figure 3.1** Schematic of a generalised continuous fermenter, where $F$ is the feed, $V$ is the fermenter volume, $x$ is the concentration of the micro organism, $s$ is the concentration of the substrate, $K_s$ is the Monod constant, $\mu_{max}$ is the maximum specific growth rate of the micro organism, and suffix $f$ represents the feed and $o$ represents the output. It is usual for $x_f$ to be zero. This is to indicate a sterile feed stream to the continuous fermenter.

Mass balances can be established using the general conservation statement:

$$Rate\ of\ accumulation = Input\ rate - Output\ rate + Generation \tag{3.1}$$

For the Monod process, the relationship between the specific growth rate and substrate concentration is given by (Aiba *et. al.* 1973):

$$\mu = \frac{\mu_{max} s}{(K_s + s)} \tag{3.2}$$

where, $\mu_{max}$ = maximum specific growth rate achievable when $s >> K_s$ and, $K_s$ is the value of limiting nutrient concentration at which the specific growth rate is half its maximum value.

The Monod (CSTR) model can be stated as:

$$[\frac{\mu_{max} s}{(K_s + s)} - D]x + Dx_f = 0 \tag{3.3}$$

where, $x_f$ is the concentration of the cells, and, $D$ is the dilution rate ($= F/V$) at which the continuous fermenter operates.

In a continuous fermenter, the steady state dependence of the cell concentration ($x_f$) and the substrate concentration in the feed ($s_f$) on the dilution rate ($D$) yields:

$$x_f = Y_{x/s}(s_f - \frac{DK_s}{\mu_{max} - D}) \tag{3.4}$$

For sterile feed i.e. $x_f = 0$, Equation (3.4) reduces to:

$$s_f = \frac{DK_s}{\mu_{max} - D} \tag{3.5}$$

Equations (3.4) and (3.5) show that when the value of the dilution rate increases, the value of the substrate concentration initially increases linearly with $D$ and then increases more rapidly as $D$ approaches $\mu_{max}$. The cell concentration exhibits an opposite behaviour; it decreases first linearly in $D$ and then diminishes rapidly as $D$ approaches $\mu_{max}$.

When the dilution rate surpasses the maximum possible growth rate, cell washout occurs i.e. failure.

The maximal cell output ($D_{maxoutput}$) is obtained from:

$$D_{maxoutput} = \mu_{max}(1 - \sqrt{\frac{K_s}{K_s + s_f}}) \tag{3.6}$$

The maximum dilution rate ($D_{max}$) at which a complete washout occurs from the fermenter is given by:

$$D_{max} = \mu_{max} \frac{s_f}{K_s + s_f} \tag{3.7}$$

If $s_f >> K_s$ the value of $D_{max\,output}$ approaches $\mu_{max}$ - and consequently is near fermenter washout. Intriguingly, washout is sometimes observed to occur at values of $D < D_{max\,output}$. This could be because of variability in the values of microbiological parameters $K_s$, $\mu_{max}$ and $Y_{x/s}$ - and also importantly because of the effect of chance (Aiba *et. al.* 1973; Blanch and Clark 1997).

### 3.4 Selected micro organism for fermentation

The micro organism selected for this research was *Escherichia coli*. This is a Gram negative, vegetative and non-spore forming bacterium widely used in fermentation. Advantages of this micro organism for study include that it is very well documented and

exists as discrete cells (not filaments). Importantly, it is readily homogenised for extraction of intra-cellular product.

## 3.5 Single Value Assessment (SVA) solution to a continuous fermenter model

The Monod–based model with the usual Single Value Assessment (SVA) solution (Davey and Cerf 2003) was written in Microsoft Excel™ using values for the process model parameters from Bailey and Ollis (1986): $K_s = 0.2$ g L$^{-1}$, $s_f = 10$ g L$^{-1}$, $\mu_{max} = 1.0$ h$^{-1}$ and $Y_{x/s} = 0.5$. The SVA solution to a continuous fermenter model is shown in Figure 3.2. From the figure it can be seen that with an increase in the value of the dilution rate ($D$), there is an increase in the value of the substrate concentration ($s$) and a decrease in the value of the cell concentration ($x$). The productivity ($xD$) of a continuous fermenter is also observed to increase with an increase in the value of the dilution rate. However, in reality, the input microbiological process parameters are not single values. This can be due to Uncertainty and natural biological Variability in the microbiological process parameters.

## 3.6 Quantitative Risk Assessment (QRA) model of a continuous fermenter

A Quantitative Risk Assessment (QRA) uses Monte Carlo Assessment (MCA) modelling to express the Uncertainty and Variability in an input parameter as a probability distribution. This distribution can be thought of as a frequency diagram of all the possible values of a parameter in relation to the probability of each value occurring. MCA therefore contrasts with the traditional or SVA approach.

A stochastic-predictive model was written over the SVA solution using a Microsoft Excel™ spreadsheet with an add-in *@Risk*™. The model was formed by linking together the mathematical relationships and the Variability in the inputs at each stage. Variability propagates throughout to the final output parameter, which is also expressed in the form of a probability distribution. This might give a better description of reality of the risk than the usual practice of SVA single value.

A number of types of distributions can be used, for example, Binomial, Beta, BetaSubjective, Chi Squared, Cumulative, Exponential, Logistic, Lognormal, Normal,

Pearson, Pareto, Pert, Poisson, Triangular, Uniform, and Weibull (Vose 2000). The Normal distribution can be seen in a wide range of applications due to the results of Central Limit Theorem (CLT)[7] (Vose 2000). The amount of Variability in a Normal distribution can be varied easily. It is therefore simple and convenient to use. The other distribution types converge to a Normal distribution as the coefficient of Variability that is a ratio of standard deviation to the mean approaches zero (Vose 2000). Therefore, a Normal distribution has been used to represent the mean and the standard deviation in the microbiological input process parameters.

Experience with the *@Risk*™ software for these simulations showed that stable outputs were always obtained with 100,000 iterations.

### 3.7 Results and Discussion

Table 3.1 presents a comparison between the SVA and MCA of risk. The input process parameters of continuous fermentation are given in the Column 1 of Table 3.1. The SVA values calculated are shown in the Column 2 of Table 3.1. Column 3 of the table lists the MCA value of each process parameter. However, the MCA values in bold in Columns 3 and 4 show an assumed standard deviation of 15 % *RiskNormal* on the mean value of the input process parameters. The MCA input therefore are not single values but are a probability distribution.

Figure 3.3 are the MCA simulations of the fermenter with a 15 % random variation in the value of the microbiological input parameters - $K_s$, $\mu_{max}$ and $Y_{x/s}$. It can be seen from the figure that as a result of this Variability, the productivity varies with a factor of 2.16 for different values of the microbiological input parameters.

It can be seen from the figure that there are a number of simulations that end before the dilution rate at which maximum productivity is obtained ($D < 0.86$ $h^{-1}$) i.e. productivity ($xD$) falls to a value below zero. The MCA results therefore highlight the number of fermentation failures or washouts with a 15 % Variability in the microbiological input process parameters.

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[7] CLT states that the mean will be Normally distributed in a set of variables, when the number of variables is large.

Figure 3.4 shows MCA simulations for a 5 % random variation in the value of the microbiological input parameters. It can be seen from the figure that the productivity varies with a factor of 1.25. When compared with Figure 3.3 it can be highlighted that as the Variability in the input process parameters increases, the range of productivity in a fermenter widens, however with an increase in the number of failures.

The simulation results of Figure 3.4 show that the decrease in the natural biological Variability in the microbiological input process parameters reduces the number of simulated failures over a 15 % variation (Figure 3.3) in the process parameters - as might be expected.

Table 3.2 summarises the predicted failure rates in a continuous fermentation when the microbiological input process parameters have the selected values of variability shown. The table highlighted that as the Variability in the input process parameters increases, so too does the number of predicted failures. With a value of Variability equal to zero, MCA reduces to SVA. The MCA productivity is expressed as the expected value, with a range of $90^{th}$ percentile values shown in the parentheses as ($5^{th}$ percentile, $95^{th}$ percentile).

Figures 3.5 and 3.6 present the correlation coefficients for a 15 % and 5 % random variation respectively in each of the key input parameters - the Monod constant ($K_s$), maximum specific growth rate ($\mu_{max}$), and yield ($Y_{x/s}$) from *@Risk*™ software. These correlation coefficients determine the influence of each of the key input parameters on the maximum productivity that is obtained from the continuous fermenter. The correlation coefficients range in the value between -1 and 1.

A value of 0 indicates that there is no correlation between the two variables - i.e. they are independent. A value of 1 is a complete positive correlation between the input variables whereas -1 indicates a complete inverse correlation. When the input parameter shows a positive correlation, an increase in the value of that input parameter increases the value of the output parameter; whereas, with a negative correlation coefficient the value of the output parameter decreases with an increase in the value of that input parameter.

It can be seen from Figures 3.5 and 3.6 that for a random variation of 15 % and 5 %, respectively, the correlation coefficient for $\mu_{max}$ is greater than that of $Y_{x/s}$, and $K_s$. That is $\mu_{max}$ has a highly significant influence on the predicted number of failures and the productivity ($xD$) of a continuous fermenter. The Monod constant ($K_s$) is of less significance because of its negative correlation with $\mu_{max}$ and $Y_{x/s}$.

Table 3.3 presents a summary of simulations that show the MCA predicted failure rate in a continuous fermenter as a result of inclusion of the Variability in the true value of $\mu_{max}$, the maximum specific growth rate of *E. coli*. It can be seen from the table that MCA reduces to SVA when the Variability in *E. coli* is 0 %. It should be noted also that the mean value of $\mu_{max}$ obtained from MCA is observed to be the same as that of the SVA value.

Variability associated with the three key input parameters i.e. $K_s$, $\mu_{max}$, $Y_{x/s}$ is important. The more accurate the random Variability in each of these is known, the better is the quality of the predictions of the QRA model for failure of a continuous fermenter i.e. washout.

Therefore, research resources should be allocated to determining an accurate Variability in the true value of the growth rate to enhance the predictive value of the QRA model for a continuous fermenter.

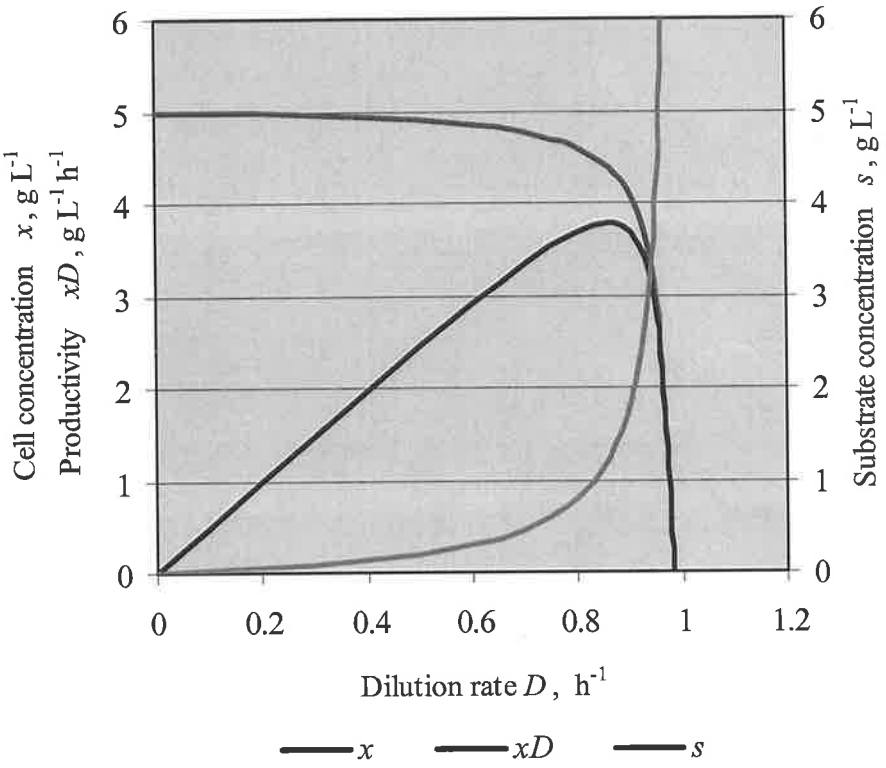



**Figure 3.2** SVA simulations of substrate concentration ($s$), cell concentration ($x$), and cell production rate ($xD$) on continuous culture dilution rate ($D$) from the Monod process model for *E. coli* plotted on Microsoft Excel™ spreadsheet with: $K_s = 0.2$ g L$^{-1}$, $s_f = 10$ g L$^{-1}$, $\mu_{max} = 1.0$ h$^{-1}$ and $Y_{x/s} = 0.5$.

**Table 3.1** Comparison of SVA with MCA for *E. coli* using initial growth data from Bailey and Ollis (1986). Column 2 gives SVA values and Column 3 gives MCA values for each of the process parameters. The bold values in Column 4 give selected distributions used in calculations for MCA. The distribution is defined as: *RiskNormal*(*mean*, *standard deviation*). The value of the standard deviation shown in bold in Column 4 is 15 % on the mean.

| Process Parameters | SVA[8] | MCA[9] | |
|---|---|---|---|
| $x_f$, g $L^{-1}$ | 0.00 | 0.00 | |
| $K_s$, g $L^{-1}$ | 0.20 | **0.20** | ***RiskNormal*(0.2, 0.03)** |
| $s_f$, g $L^{-1}$ | 10.0 | 10.0 | |
| $\mu_{max}$, $h^{-1}$ | 1.00 | **1.00** | ***RiskNormal*(1.0, 0.15)** |
| $D_{max\ output}$, $h^{-1}$ | 0.86 | 0.86 | |
| $Y_{x/s}$ | 0.50 | **0.50** | ***RiskNormal*(0.5, 0.075)** |
| $x_{max\ output}$, g $L^{-1}$ | 4.39 | 4.39 | |
| $(xD)_{max\ output}$, g $L^{-1}$ $h^{-1}$ | 3.77 | 3.77 | |
| $D_{max}$, $h^{-1}$ | 0.98 | 0.98 | |

[8] Single Value Assessment of risk.
[9] Monte Carlo Assessment of risk.

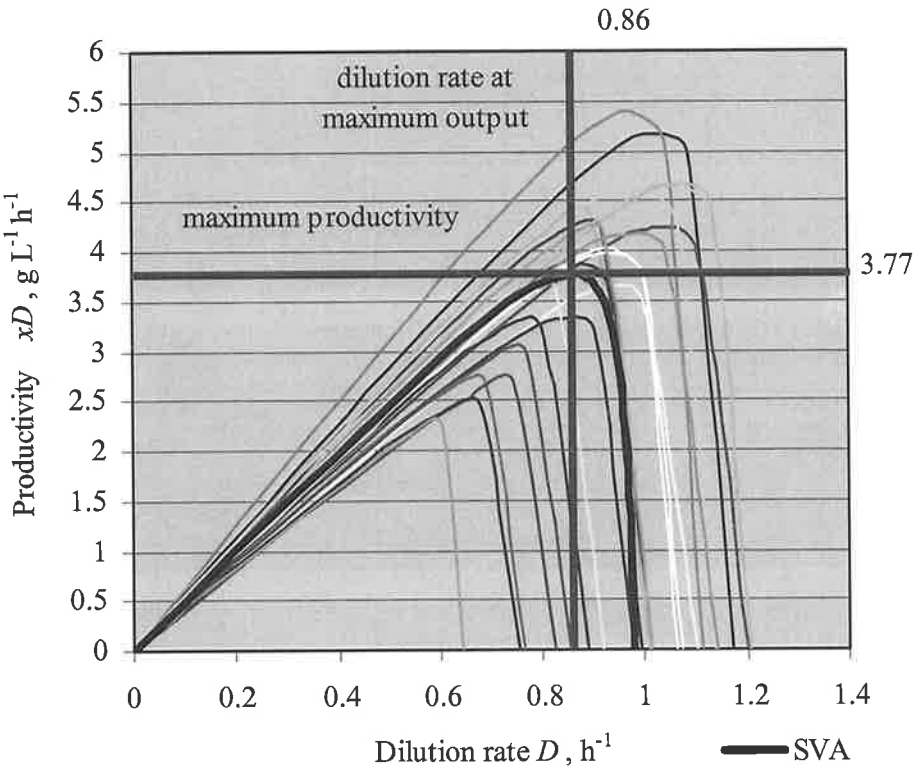



**Figure 3.3** 20 from 100,000 MCA simulations of a generalised fermenter with a 15 % random variation in the value of the microbiological input parameters ($K_s$, $\mu_{max}$ and $Y_{x/s}$).

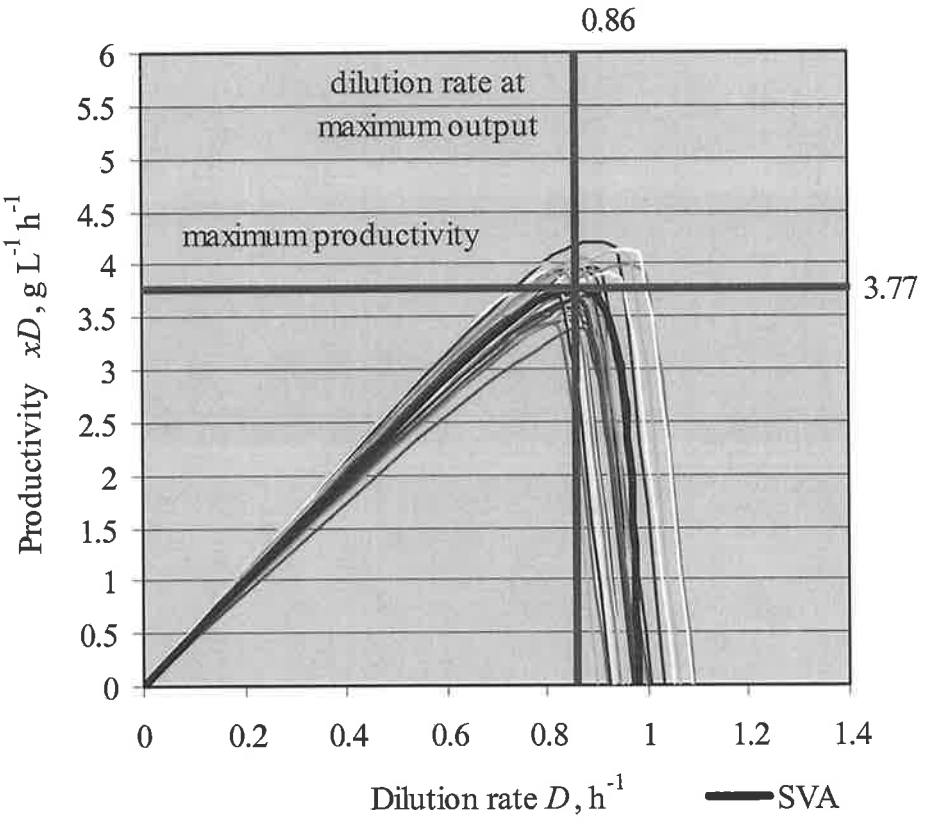



**Figure 3.4** 30 from 100,000 MCA simulations of a generalised fermenter with a 5 % random variation in the value of the microbiological input parameters ($K_s$, $\mu_{max}$ and $Y_{x/s}$).

**Table 3.2** Summary of predicted failure rates in a continuous fermentation unit operation when key input microbiological parameters - $K_s$, $\mu_{max}$ and $Y_{x/s}$ have 1, 5, 10, 15 % Variability in each respectively.

| Variability, % | Productivity[1], $gL^{-1}h^{-1}$ | Failure, % |
|---|---|---|
| 0 (SVA)* | 3.77 | 0 |
| 1 | 3.77 (3.68, 3.86) | < 0.001 |
| 5 | 3.74 (2.97, 4.15) | 0.71 |
| 10 | 3.64 (0.00, 4.47) | 11 |
| 15 | 3.50 (0.00, 4.78) | 21 |

* with a Variability of zero, MCA reduces to SVA.

[1] The MCA productivity is expressed as: expected value ($5^{th}$ percentile, $95^{th}$ percentile).

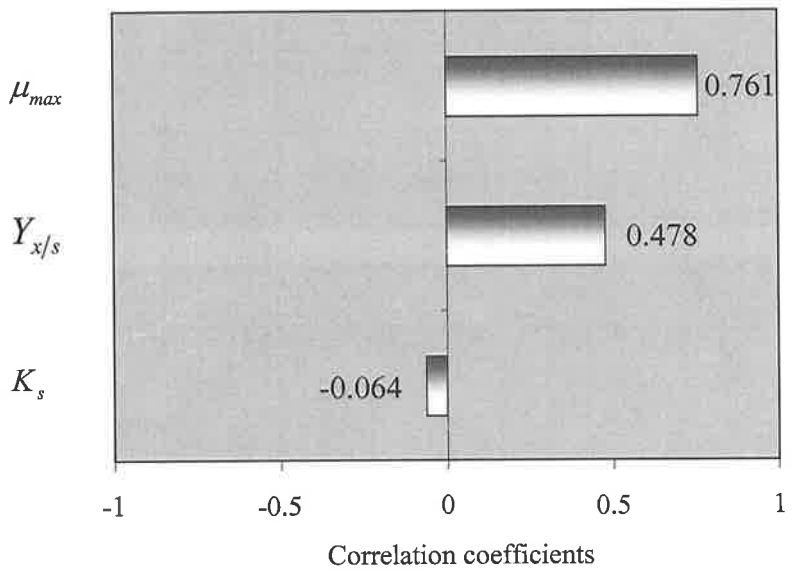



**Figure 3.5** Correlation coefficients between key input parameters - $K_s$, $\mu_{max}$ and $Y_{x/s}$ for maximum productivity with a 15 % random variation in the input values.

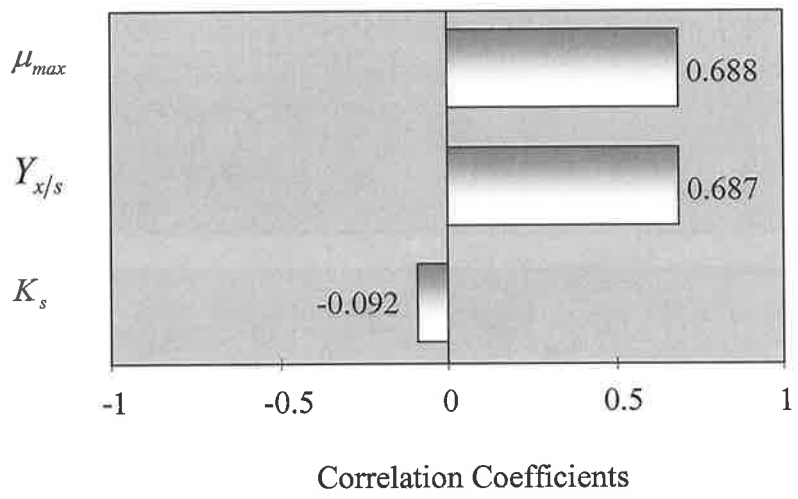



**Figure 3.6** Correlation coefficients between key input parameters - $K_s$, $\mu_{max}$ and $Y_{x/s}$ for maximum productivity with a 5 % random variation in the input values.

**Table 3.3** Summary of predicted failure rates in a continuous fermentation unit operation where a random Variability in $\mu_{max}$ is stepped from 0 to 15 % - with $K_s$ and $Y_{x/s}$ having a 1 % random Variability in each.

| **Variability, %** | $\mu_{max}$ [11], $h^{-1}$ | **Failure, %** |
|---|---|---|
| 0 (SVA)* | 1.00 | 0.00 |
| 3 | 1.00 (0.95, 1.05) | < 0.01 |
| 6 | 1.00 (0.90, 1.09) | 2.04 |
| 9 | 1.00 (0.85, 1.15) | 8.62 |
| 12 | 1.00 (0.80, 1.19) | 15.33 |
| 15 | 1.00 (0.75, 1.24) | 21 |

* with a Variability of zero, MCA reduces to SVA.

[11] The MCA productivity is expressed as: expected value (5th percentile, 95th percentile).

## 3.8 Summary and Conclusions

1. Simulations from a novel Quantitative Risk Assessment (QRA) model of a continuous fermenter developed from Monod process kinetics for growth of *E. coli* showed that in some instances the naturally occurring and combined effect of small changes (i.e. random Variability) in key microbiological input parameters, $K_s$, $\mu_{max}$ and $Y_{x/s}$, led to a catastrophic failure - this is defined as washout.

2. With an assumed natural Variability in each of the key microbiological input parameters of 15 %, the predicted number of failures from the model of the continuous fermenter is 21 in every 100 unit operations. For a 5 % Variability, the predicted number of failures is less than 1 in every 100 unit operations.

3. Findings highlighted that the more accurate the value of the natural microbiological Variability in the maximum specific growth rate ($\mu_{max}$) of *E. coli* was known, the closer the true number of actual practical failures of the continuous fermenter could be predicted using the novel QRA modelling.

4. An "accurate value" of Variability in $\mu_{max}$ for *E. coli* can be gleaned from appropriately collated data from published sources.

In the next chapter, extensive data for growth of *E. coli* is collated from various published sources. The widely used cardinal temperature model of Rosso (Rosso *et. al.* 1993) is selected to predict $\mu_{max}$ for the Monod process model of a continuous fermenter. A non-linear regression analysis of the collated data is used to fit the Rosso predictive model for an "accurate value" of $\mu_{max}$.

CHAPTER **FOUR**

# A PREDICTIVE MODEL FOR GROWTH OF *ESCHERICHIA COLI* IN A CONTINUOUS FERMENTER

Parts of this chapter are being prepared for publication as:

Patil, R. A., Davey, K. R. and Daughtry, B. J. 2006. Assessment of cardinal - temperature predictive model for growth of *Escherichia coli* in a Monod continuous fermenter, *Food Research International* - in preparation.

## 4.1 Introduction

Findings from Chapter 3 highlighted that a more accurate value of the natural microbiological Variability in $\mu_{max}$ for *E. coli* was needed for more realistic predictions from the novel QRA for a continuous fermenter.

In this chapter, appropriate extensive and published data (n = 191) for *E. coli* growth, over a range of temperature are collated. The Rosso *et. al.* (1993) predictive model for bacterial growth is assessed against these data. The Rosso model is selected because it is widely used and generally gives a good fit to growth data. In this model $\mu_{max}$ 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 ($\mu_{opt}$). The range of temperature to fit the Rosso model must cover these cardinal temperatures. Therefore data for entire range of growth temperature from 10 $^{O}$C to 45 $^{O}$C will be needed.

An estimate of the maximum specific growth rate ($\mu_{max}$) is obtained from the Rosso model using the mean value of each of the four input parameters obtained from the non-linear regression analyses of 18 independent growth data sets for growth of *E. coli*. This value is rejected, however, in favour of a value that is determined by including Uncertainty from a defined *RiskNormal* distribution for $\mu_{max}$ in each of the four Rosso model parameters, and including Variability by addition of the residual standard error (RSE) to $\mu_{max}$. This value is the best estimate or "accurate value" of $\mu_{max}$.

In the next chapter, the Rosso predictive model is used with this "accurate value" of $\mu_{max}$ in a QRA of the Monod process model of a continuous fermenter. Results are presented and discussed.

## 4.2 Methodology

The methodology adopted for research in this chapter is as follows:

1. Collate extensive data for growth of *E. coli* from various, published sources

2. Assess a best fit Rosso predictive growth model for $\mu_{max}$ for *E. coli* from extensive non-linear regression analyses of the collated data

3. Define a *RiskNormal* distribution to each of the four Rosso model parameters to include the Uncertainty and add the residual standard error to $\mu_{max}$ to include the Variability to obtain a best estimate or "accurate value" of $\mu_{max}$ for each data set for growth of *E. coli*.

## 4.3 Collation of growth data

A summary of the extensive growth data for *E. coli* collated from published and appropriate literature is tabulated in Table 4.1. As shown, there are 18 data sets from 10 researchers. These data cover a range of appropriate liquid media that can be reasonably assessed to be applicable for fermentation broths. Growth data for solid media, e.g. unblended meat, was rejected as inappropriate.

The *E. coli* growth data collated from various published sources is shown in Appendix B.

## 4.4 Model selection

Various predictive models to describe the effects of temperature on microbial growth have been proposed - these have been critically reviewed in Chapter 2.

The predictive growth model selected for growth is that of Rosso *et. al.* (1993). This is because it is widely used and generally gives a good fit to growth data (McMeekin *et. al.* 1993; Ross and Dalgaard 2004; Rosso *et. al.* 1993).

The model is given by:

$$\mu_{max} = \frac{\mu_{opt}(T - T_{max})(T - T_{min})^2}{(T_{opt} - T_{min})[(T_{opt} - T_{min})(T - T_{opt}) - (T_{opt} - T_{max})(T_{opt} + T_{min} - 2T)]} \qquad (4.1)$$

where, $T_{min}$ is the temperature below which growth is no longer observed, $T_{max}$ is the temperature above which no growth occurs, $T_{opt}$ is the temperature at which the maximum specific growth rate $\mu_{max}$ is equal to its optimum value $\mu_{opt}$.

The Rosso model 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 ($\mu_{opt}$). Rosso *et. al.* (1993) underscored that the three cardinal temperatures were found to be independent of specific growth rate at the optimum temperature. Also, an unexpected linear correlation between these three cardinal temperatures was observed by these researchers.

Although the Rosso model assists in estimating the three cardinal temperatures and the optimum specific growth rate, the justification of the linear correlation between each of the model input parameters is unknown (Rosso *et. al.* 1993).

## 4.5 Non-linear regression analysis of data

A non-linear regression analysis was used for fitting the growth data and to estimate accurate values of the model cardinal temperatures and optimum specific growth rate (Snedecor and Cochran 1969). Software used for this was R software (R Foundation for Statistical Computing) version 2.2.0 - this produces an optimum goodness of fit between the data and the function. An advantage of this software is that it fits the input functions to the data.

As an example of the generalised R program that was written for the non-linear regression analyses of the published data sets is presented in Appendix C for the data of Ross *et. al.* (2003). This same program was used for all data sets of Table 4.1.

The Rosso model fit for each of the collated data for *E. coli* growth was plotted - and is presented in Figures 4.1 through to 4.11 as $\mu_{max}$ (mu.max) *vs* growth temperature (Temp). Each of these figures consists of: a fit to the original growth data; a histogram; a residuals plot, and; a *quantile*-quantile plot for each of the 18 growth sets.

**Table 4.1** Description of the *strain* of *E. coli* and its growth in various media.

| Micro organism | Strain | Media | $T$, $^0C$ | Source |
|---|---|---|---|---|
| *Escherichia coli* | C-600-1 | Minimal medium (supplemented with glucose) | 15 - 37 | (O'Donovan *et. al.* 1965a) |
| | K-I-01 (high temperature mutant) | Minimal medium (supplemented with glucose) | 20 - 37 | (O'Donovan *et. al.* 1965a) |
| *Escherichia coli* | C-600-1 | (Nutrient broth) Complex medium | 9.5 - 44 | (O'Donovan *et. al.* 1965b) |
| | K-II-27 | (Nutrient broth) Complex medium | 10 - 44 | (O'Donovan *et. al.* 1965b) |
| *Escherichia coli* | C-600-1 | glucose-minimal medium | 11.8 - 44 | (O'Donovan *et. al.* 1965b) |
| | K-II-27 (high temperature mutant) | glucose-minimal medium | 20 - 44 | (O'Donovan *et. al.* 1965b) |
| | K-II-27 + histidine | glucose-minimal medium plus 10 $\mu g\ mL^{-1}$ of histidine | 11.8 - 44 | (O'Donovan *et. al.* 1965b) |
| *Escherichia coli* | ML 30 replicate1 | Basal medium 56 (Monod *et. al.* 1951) | 10 - 37 | (Ng 1969) |
| *Escherichia coli* | ML 30 replicate2 | Basal medium 56 (Monod *et. al.* 1951) | 9 - 35 | (Ng 1969) |
| *Escherichia coli* | ML 30 G | Minimal medium (Basal medium supplemented with 0.2% glucose) | 9 - 35 | (Shehata and Marr 1975) |
| | ML 30 G | Complex medium (Basal medium supplemented with 0.2% glucose and 0.8% nutrient broth) | 14 - 40 | (Shehata and Marr 1975) |
| *Escherichia coli* | B/r | MOPS medium (Neidhardt *et. al.* 1977) supplemented with 0.4 % glucose (wt/vol), amino acids (minus leucine; 0.12mM valine and 0.08 mM isoleucine), five vitamins, and four bases in concentrations given previously by Wanner *et. al.* (1977). | 13.5 - 46 | (Herendeen *et. al.* 1979) |
| *Escherichia coli* | (Not defined) | Nutrient broth (Oxoid, London) | 21 - 47 | (Ratkowsky *et. al.* 1983) |
| *Escherichia coli* | SF | Meat blended | 8.2 - 40 | (Smith 1985) |
| *Escherichia coli* | O157:H7 | Complex medium (Brain Heart Infusion) | 10 - 42 | *pers. comm.* (Buchanan 1992) |

**Table 4.1** *continued...*

| | | | | |
|---|---|---|---|---|
| *Escherichia coli* | ML 30 | Mineral medium supplemented with glucose (100 μg $L^{-1}$ in a chemostat; 500 μg $L^{-1}$ in batch culture) or with a mixture of glucose and galactose (each 50 μg $L^{-1}$ in chemostat). | 17.4, 28.4, 37 and 40 (dilution rate of 0.2, 0.3, 0.4 and 0.5 $h^{-1}$ resp.) | (Kovarova *et. al.* 1996) |
| *Escherichia coli* | M 23 and SB 1 | Complex medium | 7 - 48 | (Ross *et. al.* 2003) |
| *Escherichia coli* | O157:H7 | Brain Heart Infusion (BHI, Difco Laboratories, Detroit, MI) broth diluted in peptone water and then added to ground beef for a final concentration of 3-4 log cfu $g^{-1}$. | 5 - 46 | (Tamplin *et. al.* 2005) |

It is not usual to put so much information into a diagram of a model fit to the experimental data shown in Figures 4.1 - 4.11. This was done to achieve the best and accurate values for $\mu_{max}$ for *E. coli* to predict accurate number of actual practical failures in a continuous fermenter.

A quantile-quantile (Q-Q) plot is a graphical technique for determining if two data sets come from populations with a common distribution. It is a plot of the quantiles of the first data set against the quantiles of the second data set. A quantile means the fraction (or percent) of points lying below the given value i.e. 0.5 (or 50 %) quantile is the point (*median*) at which 50 % percent of the data fall below and 50 % fall above that value. A quantile-quantile plot has two components - the quantile points themselves and a $45^{O}$ reference line. A Q-Q plot checks for the fit of a theoretical distribution to the observed data, where, the observed values of a variable are plotted against the theoretical quantiles. A good fit of the theoretical distribution to the observed values would be indicated by this Q-Q plot if the plotted values fall on a straight line.

It can be seen from Figures 4.1 - 4.11 that overall the Rosso model gives a good fit to the growth data.

For example, Figure 4.1 shows that a good fit is obtained using the growth data over the entire temperature range 9 $^{O}$C - 45 $^{O}$C. The residual plot, the histogram, and the Q-Q plot, and highlight that there are two outliers i.e. the points located far away from the line of zero error. The residual plot shows the difference between the observed values and the predicted values of growth rate as function of temperature. The histogram of residuals shows that the residuals are "skewed left" i.e. most of the frequency counts are clustered on the right side and the tail is on the left side. The Q-Q plot highlights the "goodness" of the fit. The fit is "good" if most of the points lie on the $45^{O}$ reference line. A deviation from this straight line indicates the deviation from linearity.

Similarly in Figures 4.2 - 4.11, the growth data gives a good fit over the entire temperature range as shown in the Column 4 of the Table 4.1 respectively. The corresponding residual plots, the histograms, and the Q-Q plots show how the residuals are distributed with a fixed location and scale, and highlight the number of outliers. The histogram of residuals shows that

the residuals are either "skewed left", "skewed right", or normally distributed. It can be seen that the histogram of residuals for the Figures 4.1, 4.2, 4.4, 4.6 and 4.8 are "skewed left", and those for the Figures 4.3, 4.5, 4.7, 4.9, 4.10 and 4.11 are "skewed right".

Figures 4.1 - 4.11 highlight that the Rosso model gives a good fit to the collated growth data and therefore accurate estimates of the model input parameters can be obtained using non-linear regression analyses.

(The results for the data of O'Donovan *et. al.* (1965a) (for *E. coli* C-600-1 and K-I-01 growth in a minimal medium), Ng (1969) (for *E. coli* ML 30 replicates 1 and 2), Shehata and Marr (1975) (for *E. coli* ML 30 G growth in a complex medium), Smith (1985) (for *E. coli* SF), and Buchanan (1992) (for *E. coli* O157:H7 growth in a complex medium) are not presented. This is because these were unsatisfactory data in that either there were too few data, or what data there was, did not include $\mu_{max}$ for temperatures greater than $T_{opt}$).

A summary of the non-linear regression analyses is presented as Table 4.2. This table gives the value for each of the four parameters of the Rosso model, $\mu_{opt}$, $T_{min}$, $T_{opt}$ and $T_{max}$, for each of the 18 data sets for growth of *E. coli* of Table 4.1. These growth data cover a wide range of media, from minimal media supplemented with glucose to complex media, such as nutrient broths, and blended meat.

Column 2 of Table 4.2 shows the range of $\mu_{opt}$ - this varies from a minimum value of 0.565 $h^{-1}$ to a maximum value of 2.578 $h^{-1}$, with an overall mean of 1.256 $h^{-1}$ on all data sets. Column 3 gives the range of $T_{min}$ - this is seen to vary from a minimum value of 3.47 $^{O}C$ to a maximum value of 16.65 $^{O}C$, with an overall mean of 7.12 $^{O}C$. $T_{opt}$ is presented in Column 4. These values vary from a minimum value of 37.67 $^{O}C$ to a maximum value of 42.47 $^{O}C$, with an overall mean of 40.16 $^{O}C$. Column 5 shows the value of $T_{max}$ - varying from a minimum value of 41.19 $^{O}C$ to a maximum value of 48.30 $^{O}C$ - with an overall mean of 45.14 $^{O}C$.

For completeness, the reader is directed to Table D.1 of Appendix D where the initial estimates of the value of the model parameters required for the regression analyses for each of the four parameters of the Rosso model are presented. Table D.2 of this Appendix gives the

mean and standard deviation for each of the four parameters of the 18 data sets. Table D.3 of the Appendix presents residual sum-of-squares and residual standard error (RSE) for each of these 18 data sets. The correlation estimates that provide an empirical measure of association between the four parameters of the Rosso model for each of these 18 data sets are presented in Table D.4 of Appendix D.

## 4.6 Results and Discussion

Substitution of the value of the parameters from Table 4.2 into the Rosso *et. al.* (1993) model, Equation 4.1, and solving, gives the value of $\mu_{max}$. This value is summarised in Table 4.3 for each of the 18 data sets.

However, the values of $\mu_{max}$ in Table 4.3 do not account for Uncertainty and Variability in the four input parameters, $\mu_{opt}$, $T_{min}$, $T_{opt}$ and $T_{max}$ of the model. The Uncertainty can best be quantified by defining a *RiskNormal* distribution for each of the four parameters. The *RiskNormal* distribution for each of the four model input parameters can be defined using the mean and the standard deviation from Table D.2 of Appendix D. The Variability can be best quantified by adding the RSE to the value of $\mu_{max}$.

Results of $\mu_{max}$ for *E. coli* growth for each of the 18 data sets are summarised in Table 4.4. Column 2 of the table shows these values of $\mu_{max}$ expressed as: expected value ($2.5^{th}$ percentile, $97.5^{th}$ percentile) for all data sets. Column 3 gives the standard deviation in the expected value of $\mu_{max}$ for the corresponding 18 growth data sets. Total Uncertainty that is a combination of Uncertainty and Variability in the value of $\mu_{max}$, and is calculated as the percentage of the ratio of standard deviation to the expected value. These values of % Total Uncertainty that are presented in the Column 4 are comparable with the assumed values of the % Variability ranging from 1 % to 15 % in Chapter 3 (*see* Table 3.3). The inclusion of Uncertainty and Variability therefore give a best estimate or "accurate value" of $\mu_{max}$ for *E. coli* growth for all independent data sets.

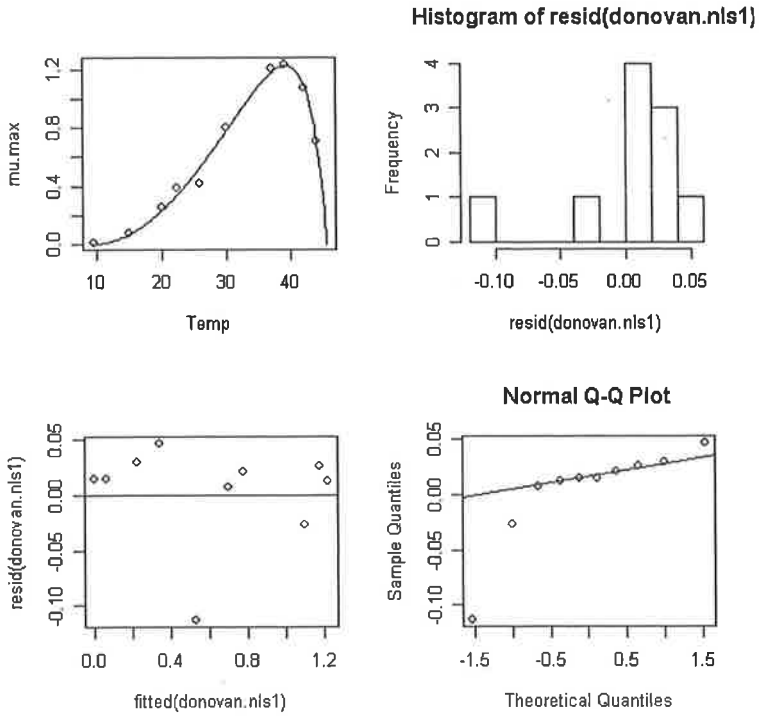



**Figure 4.1** Rosso model fit for *E. coli* C-600-1 growth in a (nutrient broth) complex medium (O'Donovan *et. al.* 1965b).

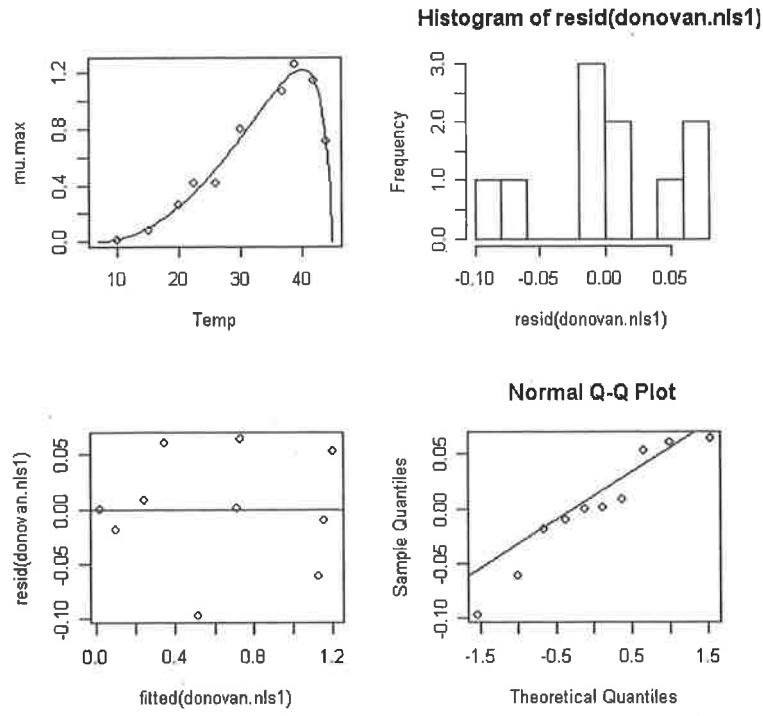



**Figure 4.2** Rosso model fit for *E. coli* K-II-27 growth in a (nutrient broth) complex medium (O'Donovan *et. al.* 1965b).

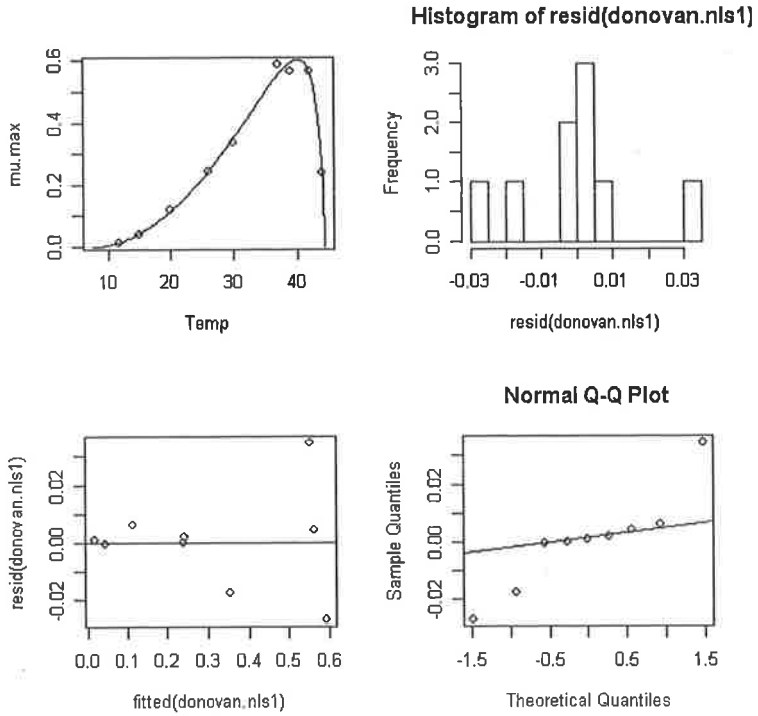



**Figure 4.3** Rosso model fit for *E. coli* C-600-1 growth in a glucose-minimal medium (O'Donovan *et. al.* 1965b).

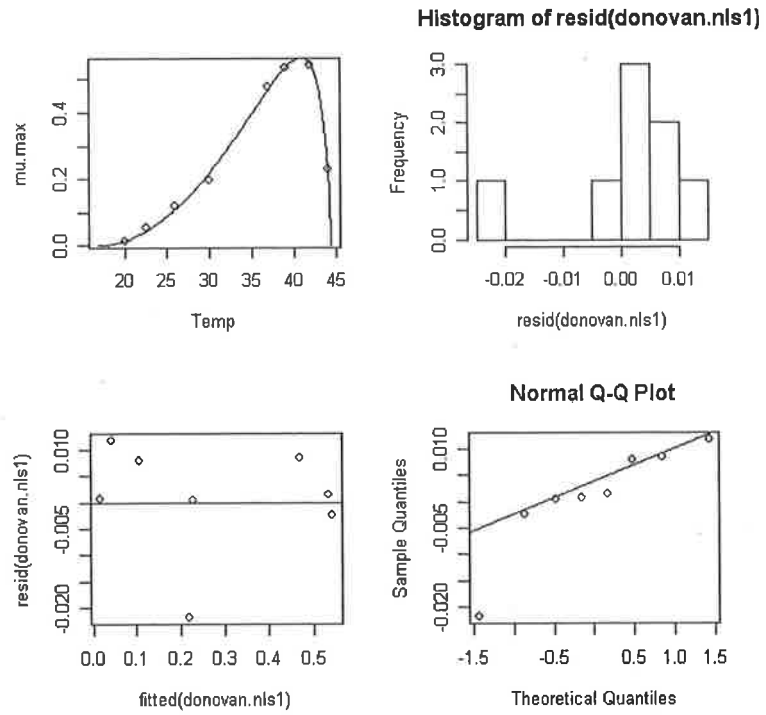



**Figure 4.4** Rosso model fit for *E. coli* K-II-27 growth in a glucose-minimal medium (O'Donovan *et. al.* 1965b).

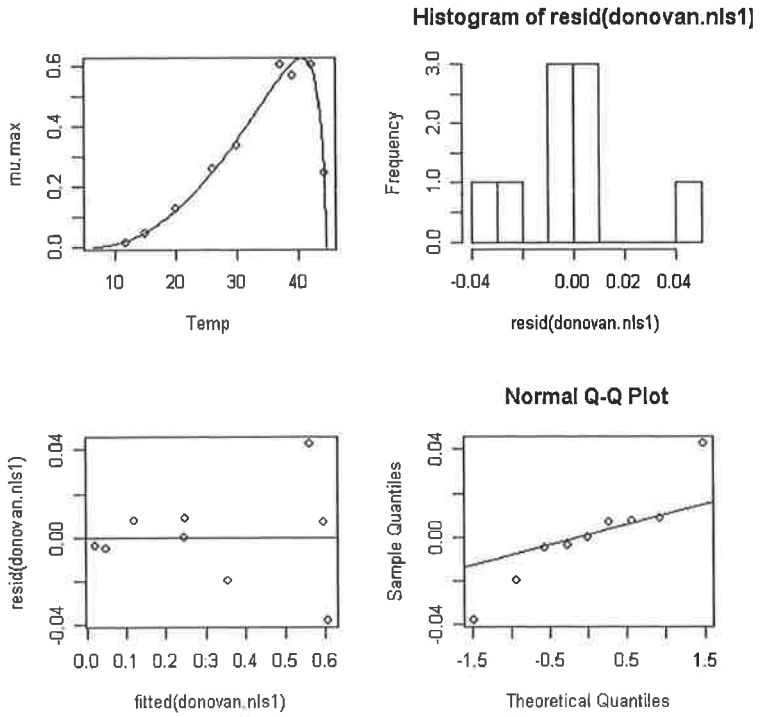



**Figure 4.5** Rosso model fit for *E. coli* K-II-27 growth in a glucose-minimal medium plus 10 μg $mL^{-1}$ of histidine (O'Donovan *et. al.* 1965b).

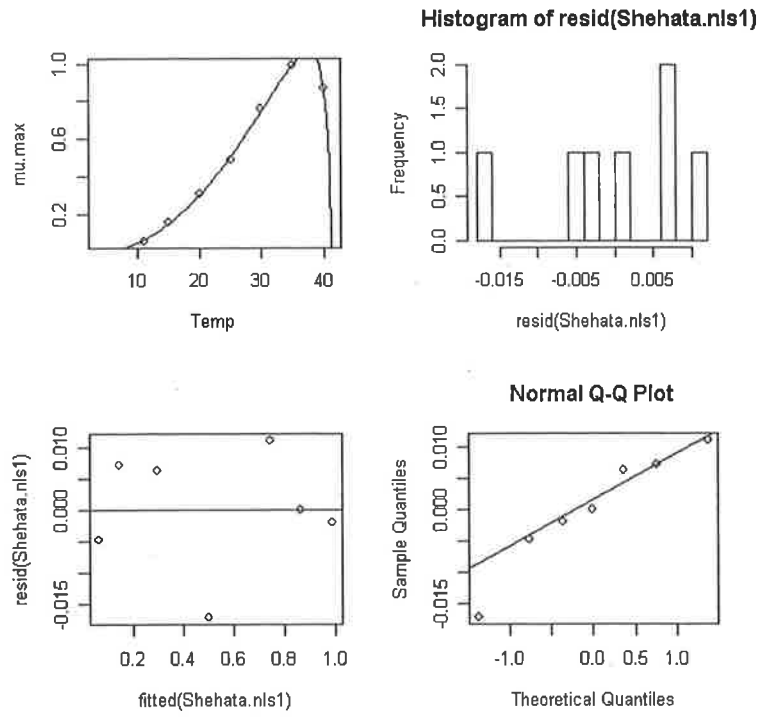



**Figure 4.6** Rosso model fit for *E. coli* ML 30 G growth in a minimal medium (Shehata and Marr 1975).

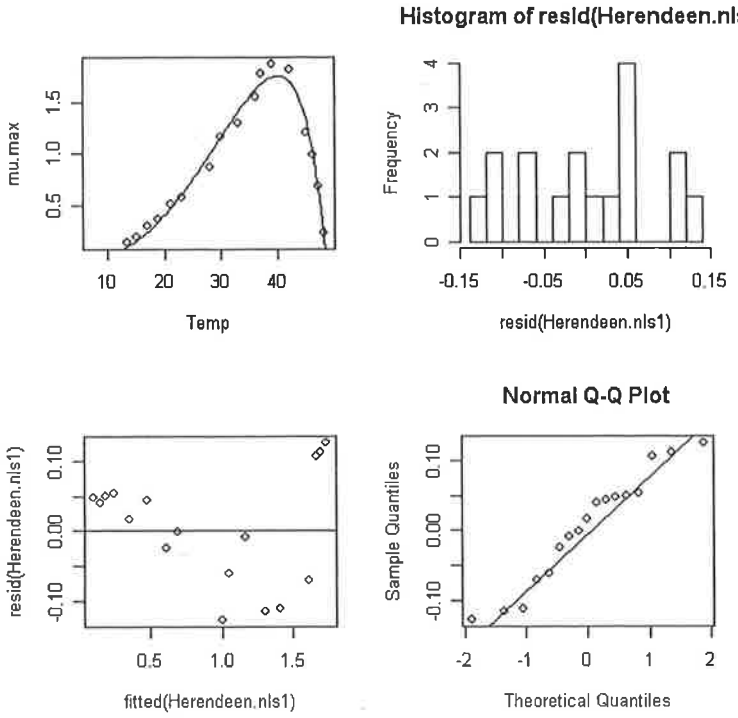



**Figure 4.7** Rosso model fit for *E. coli* B/r growth in a complex medium (Herendeen *et. al.* 1979).

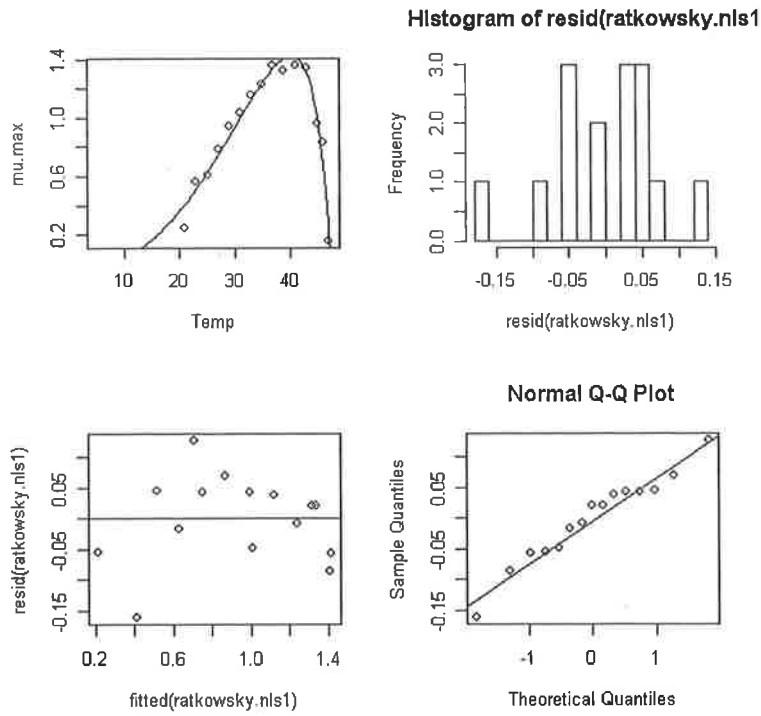



**Figure 4.8** Rosso model fit for *E. coli* growth in a (nutrient broth) complex medium (Ratkowsky *et. al.* 1983).

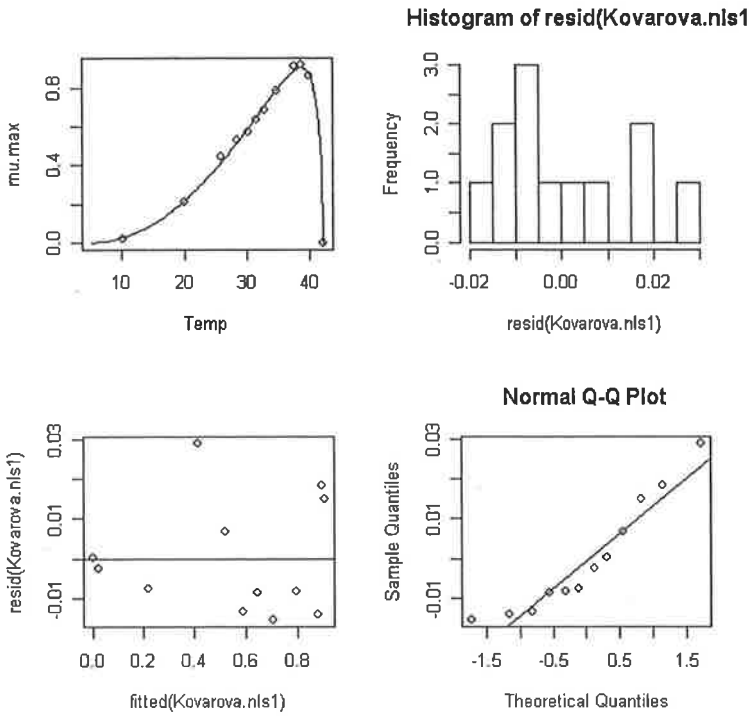



**Figure 4.9** Rosso model fit for *E. coli* ML 30 growth in a minimal medium (Kovarova *et. al.* 1996).

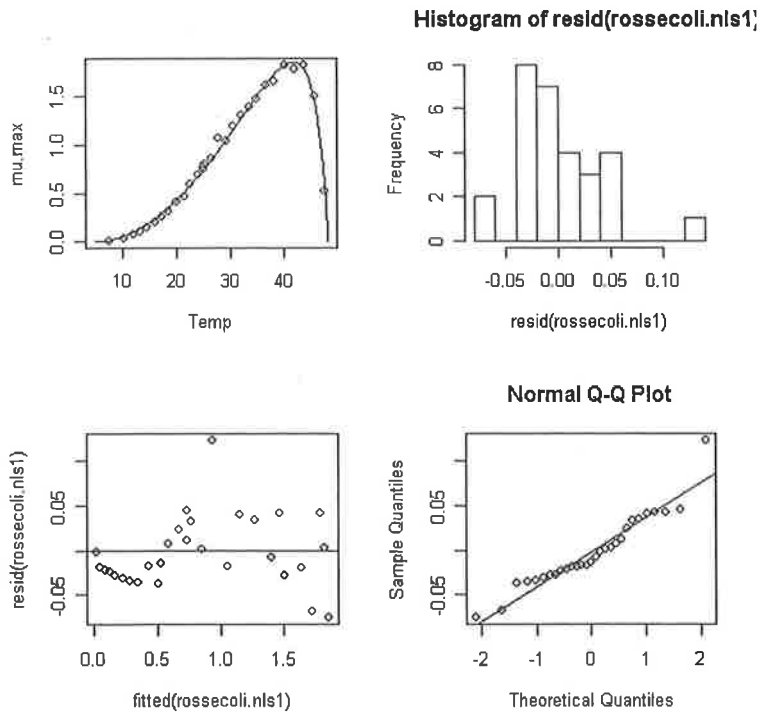



**Figure 4.10** Rosso model fit for *E. coli* M 23 and SB 1 growth in a complex medium (Ross *et. al.* 2003).

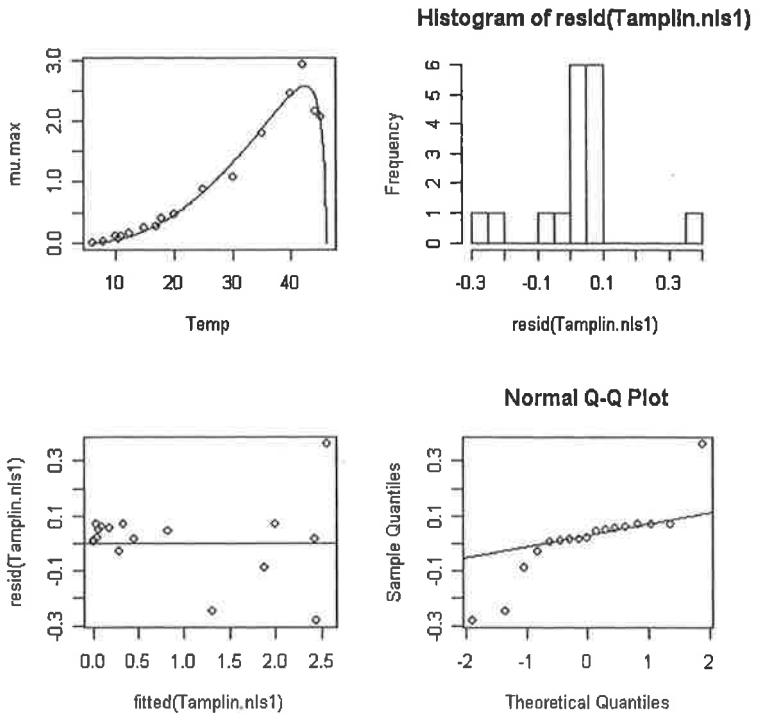



**Figure 4.11** Rosso model fit for *E. coli* O157:H7 growth on meat (Tamplin *et. al.* 2005).

**Table 4.2** Summary of values of the parameters of the Rosso *et. al.* (1993) model from the non-linear regression analyses of 18 data sets for growth of *E. coli*:

$$\mu_{max} = \frac{\mu_{opt}(T - T_{max})(T - T_{min})^2}{(T_{opt} - T_{min})[(T_{opt} - T_{min})(T - T_{opt}) - (T_{opt} - T_{max})(T_{opt} + T_{min} - 2T)]} \quad (h^{-1})$$

| *E. coli*, medium (source) | $\mu_{opt}$, $h^{-1}$ | $T_{min}$, $^oC$ | $T_{opt}$, $^oC$ | $T_{max}$, $^oC$ |
|---|---|---|---|---|
| C-600-1, minimal (O'Donovan *et. al.* 1965a) | - | - | - | - |
| K-I-01, minimal (O'Donovan *et. al.* 1965a) | - | - | - | - |
| C-600-1, complex (O'Donovan *et. al.* 1965b) | 1.224 | 9.23 | 39.24 | 45.48 |
| K-II-27, complex (O'Donovan *et. al.* 1965b) | 1.224 | 6.78 | 40.16 | 44.96 |
| C-600-1, minimal (O'Donovan *et. al.* 1965b) | 0.604 | 7.35 | 40.17 | 44.46 |
| K-II-27, minimal (O'Donovan *et. al.* 1965b) | 0.565 | 16.65 | 40.89 | 44.42 |
| K-II-27 plus 10 μg $mL^{-1}$ of histidine, minimal (O'Donovan *et. al.* 1965b) | 0.622 | 6.41 | 40.56 | 44.37 |
| ML 30 replicate1, minimal (Ng 1969) | - | - | - | - |
| ML 30 replicate2, minimal (Ng 1969) | - | - | - | - |
| ML 30 G, minimal (Shehata and Marr 1975) | 1.063 | 3.47 | 37.67 | 41.19 |
| ML 30 G, complex (Shehata and Marr 1975) | - | - | - | - |
| B/r, complex (Herendeen *et. al.* 1979) | 1.751 | 7.37 | 39.97 | 48.30 |
| (Not defined), complex (Ratkowsky *et. al.* 1983) | 1.421 | 5.05 | 40.28 | 47.31 |
| SF, meat (Smith 1985) | - | - | - | - |
| O157:H7, complex (*pers. comm.* Buchanan 1992) | - | - | - | - |
| ML 30, minimal (Kovarova *et. al.* 1996) | 0.909 | 5.09 | 38.70 | 42.12 |
| M 23 and SB 1, complex (Ross *et. al.* 2003) | 1.853 | 4.95 | 41.69 | 47.96 |
| O157:H7, meat (Tamplin *et. al.* 2005) | 2.578 | 5.99 | 42.47 | 46.03 |
| **Overall mean:** | **1.256** | **7.12** | **40.16** | **45.14** |

- not presented because data for regression analyses inappropriate - *see* text.

**Table 4.3** Summary of the predicted value of maximum specific growth rate ($\mu_{max}$) for *E. coli* at 37 $^{O}$C for the Rosso *et. al.* (1993) model for 18 independent data sets.

| ***E. coli*, medium (source)** | $\mu_{max}$, $h^{-1}$ |
|---|---|
| C-600-1, minimal (O'Donovan *et. al.* 1965a) | - |
| K-I-01, minimal (O'Donovan *et. al.* 1965a) | - |
| C-600-1, complex (O'Donovan *et. al.* 1965b) | 1.177 |
| K-II-27, complex (O'Donovan *et. al.* 1965b) | 1.133 |
| C-600-1, minimal (O'Donovan *et. al.* 1965b) | 0.554 |
| K-II-27, minimal (O'Donovan *et. al.* 1965b) | 0.469 |
| K-II-27 plus 10 μg $mL^{-1}$ of histidine, minimal (O'Donovan *et. al.* 1965b) | 0.560 |
| ML 30 replicate1, minimal (Ng 1969) | - |
| ML 30 replicate2, minimal (Ng 1969) | - |
| ML 30 G, minimal (Shehata and Marr 1975) | 1.057 |
| ML 30 G, complex (Shehata and Marr 1975) | - |
| B/r, complex (Herendeen *et. al.* 1979) | 1.670 |
| (Not defined), complex (Ratkowsky *et. al.* 1983) | 1.338 |
| SF, meat (Smith 1985) | - |
| O157:H7, complex (*pers. comm.* Buchanan 1992) | - |
| ML 30, minimal (Kovarova *et. al.* 1996) | 0.878 |
| M 23 and SB 1, complex (Ross *et. al.* 2003) | 1.620 |
| O157:H7, meat (Tamplin *et. al.* 2005) | 2.112 |
| **Overall mean:** | **1.142** |

- not presented because data for regression analyses inappropriate - *see* text.

**Table 4.4** Summary of the *RiskNormal* (Mean, Standard deviation) simulations for the "accurate value" of maximum specific growth rate, $\mu_{max}$ ($h^{-1}$), of *E. coli*, at 37 $^{O}C$ for 18 independent data sets.

| ***E. coli*, medium (source)** | $\mu_{max}$ **(*RiskNormal*)[12], $h^{-1}$** | **Standard deviation** | **Total Uncertainty, %** |
|---|---|---|---|
| C-600-1, minimal (O'Donovan *et. al.* 1965a) | - | - | - |
| K-I-01, minimal (O'Donovan *et. al.* 1965a) | - | - | - |
| C-600-1, complex (O'Donovan *et. al.* 1965b) | 1.174 (1.044, 1.303) | 0.064 | 5.45 |
| K-II-27, complex (O'Donovan *et. al.* 1965b) | 1.129 (0.980, 1.298) | 0.079 | 6.99 |
| C-600-1, minimal (O'Donovan *et. al.* 1965b) | 0.554 (0.504, 0.604) | 0.025 | 4.51 |
| K-II-27, minimal (O'Donovan *et. al.* 1965b) | 0.469 (0.436, 0.502) | 0.016 | 3.41 |
| K-II-27 plus 10 μg $mL^{-1}$ of histidine, minimal (O'Donovan *et. al.* 1965b) | 0.559 (0.493, 0.624) | 0.032 | 5.72 |
| ML 30 replicate1, minimal (Ng 1969) | - | - | - |
| ML 30 replicate2, minimal (Ng 1969) | - | - | - |
| ML 30 G, minimal (Shehata and Marr 1975) | 1.054 (0.990, 1.107) | 0.029 | 2.75 |
| ML 30 G, complex (Shehata and Marr 1975) | - | - | - |
| B/r, complex (Herendeen *et. al.* 1979) | 1.669 (1.478, 1.859) | 0.095 | 5.69 |
| (Not defined), complex (Ratkowsky *et. al.* 1983) | 1.337 (1.163, 1.510) | 0.086 | 6.43 |
| SF, meat (Smith 1985) | - | - | - |
| O157:H7, complex (*pers. comm.* Buchanan 1992) | - | - | - |
| ML 30, minimal (Kovarova *et. al.* 1996) | 0.878 (0.841, 0.915) | 0.018 | 2.10 |
| M 23 and SB 1, complex (Ross *et. al.* 2003) | 1.651 (1.563, 1.738) | 0.043 | 2.60 |
| O157:H7, meat (Tamplin *et. al.* 2005) | 2.106 (1.713, 2.500) | 0.196 | 9.30 |

- not presented because data for regression analyses inappropriate - *see* text.

[12] The values of $\mu_{max}$ obtained from *RiskNormal* simulations are expressed as: expected value ($2.5^{th}$ percentile, $97.5^{th}$ percentile).

## 4.7 Summary and Conclusion

1. The Rosso predictive model for growth of *E. coli* was fitted to 18 appropriate independent data sets (n = 191) using non-linear regression analyses. Estimates for each of the four input parameters of the model, $\mu_{opt}$, $T_{min}$, $T_{opt}$ and $T_{max}$, from the regressions for each data set were used to determine a value for $\mu_{max}$. These values of $\mu_{max}$ ranged from 0.469 $h^{-1}$ to 2.112 $h^{-1}$ over a temperature range of 10 $^{O}C$ to 45 $^{O}C$ for growth of *E. coli* on a range of media that included: minimal media supplemented with glucose, complex media such as nutrient broths, and unblended meat. However, these values for $\mu_{max}$ do not take into account the importance of Uncertainty and Variability on each of the model parameters. Uncertainty is expressed as a standard deviation on the mean from the regression analyses and Variability is expressed by adding residual standard error to $\mu_{max}$.

2. To obtain a best estimate or "accurate value" of $\mu_{max}$ for growth of *E. coli*, Uncertainty and Variability were included for each of the data sets. The value obtained after the inclusion of Uncertainty and Variability is a more "accurate value" of $\mu_{max}$ than is obtained from the non-linear regression analyses (with substitution of the values of the four parameters in the Rosso model to obtain a value for $\mu_{max}$).

In the next chapter, a QRA of a Monod continuous fermenter using the "accurate value" of $\mu_{max}$ that includes Uncertainty and Variability is presented. Results of simulations of the QRA model are presented and discussed.

CHAPTER **FIVE**

# A REVISED QRA MODEL OF THE MONOD CONTINUOUS FERMENTER

Parts of this chapter are being prepared for publication as:

Davey, K. R., Patil, R. A. and Daughtry, B. J. 2006. A new quantitative risk assessment of a Monod continuous fermenter, *Transactions of the Institution of Chemical Engineers, Part C, Food and Bio products Processing* - in preparation.

## 5.1 Introduction

In this chapter, a revised QRA of the Monod process model for a continuous fermenter is presented.

With the revised QRA model a realistic simulation can be carried out to predict an "accurate value" of the number of actual practical failures that could occur in a continuous fermenter.

Results of simulations of the revised QRA model are presented and discussed, and a comparison made with the prediction of the QRA model of Chapter 3.

## 5.2 QRA model using the revised data

The revised QRA model of the Monod continuous fermenter uses the best estimate or "accurate value" of $\mu_{max}$ for growth of *E. coli*. The "accurate value" of $\mu_{max}$ was obtained by defining the *RiskNormal* distributions for each of the four Rosso model parameters, $\mu_{opt}$, $T_{min}$, $T_{opt}$ and $T_{max}$, and by adding the residual standard error (RSE) to $\mu_{max}$ that were obtained from the non-linear regression analyses (Table 4.4 of Chapter 4).

QRA simulations with the more "accurate value" of $\mu_{max}$ for each of the 18 data sets were carried out using *@Risk*™ software with 100,000 iterations.

## 5.3 Results and Discussion

Simulation results from the revised QRA model of the Monod continuous fermenter for *E. coli* growth for each of the 18 independent data sets are summarised in Tables 5.1 and 5.2.

Table 5.1 presents predictions for failure of the continuous fermenter from the revised QRA model. The table shows that the number of QRA predicted failures varies from 0.01 % to 5 % for *E. coli* grown in a minimal or complex medium, to 10.27 % in case of *E. coli* grown on meat. It can be seen from the table that there is an overall mean value for

predicted failure rate of 2.43 % for *E. coli* grown in a minimal or complex media, or, on meat.

A plot of % Failure *vs* % Total Uncertainty is presented in Figure 5.1. The values for % Failure were taken from Table 5.1 and for those of % Total Uncertainty were taken from Column 4 of Table 4.4. This plot highlights that the number of failures in the Monod continuous fermenter increases with an increase in the Total Uncertainty in the value of $\mu_{max}$ for *E. coli* growth.

Figure 5.2 presents a plot of % Failure *vs* % Total Uncertainty with the values of assumed Variability ranging from 1 % to 15 % about the means (*see* Table 3.3 of Chapter 3). It can be seen from the figure that the assumed values of % Variability from the Chapter 3 lie on the curve that is obtained by plotting the % Failure *vs* % Total Uncertainty. Therefore it can be concluded that these assumptions are reasonable.

Table 5.2 presents a comparison of predicted results for $\mu_{max}$ ($h^{-1}$) and failure (%) from the revised QRA model - with those from a QRA model (Chapter 3) of the Monod continuous fermenter with assumed Variability ranging from 1 % to 15 % about the means.

It can be seen from Table 5.2 that a failure rate of 2.43 % is predicted from the revised ("accurate value") QRA model over the 18 independent data sets collated from various published sources for growth of *E. coli*. This compares with 8.18 % from the initial QRA model of Chapter 3. That is, the number of predicted failures has been reduced by a factor of about 3 over the QRA model of Chapter 3 in the revised QRA model with an accurate estimate of $\mu_{max}$.

A close inspection of Table 5.2 shows that the mean value of $\mu_{max}$ is nearly the same for both QRA models: i.e. the revised and initial (Chapter 3) models. However, the number of failures predicted to actually occur in the Monod continuous fermenter is reduced by about 70 % (i.e. $\frac{8.18-2.43}{8.18}\times 100$) in the revised QRA model. This underscores the need for the best estimate, i.e. "accurate value" of $\mu_{max}$ for QRA modelling of the continuous fermenter and justifies the effort expended in determining its value.

**Table 5.1** Summary of the number of predicted failures (%) from a revised QRA model of the Monod continuous fermenter for each of 18 independent data sets.

| ***E. coli*, medium (source)** | **Failure, %** |
|---|---|
| C-600-1, minimal (O'Donovan *et. al.* 1965a) | - |
| K-I-01, minimal (O'Donovan *et. al.* 1965a) | - |
| C-600-1, complex (O'Donovan *et. al.* 1965b) | 1.87 |
| K-II-27, complex (O'Donovan *et. al.* 1965b) | 5 |
| C-600-1, minimal (O'Donovan *et. al.* 1965b) | 0.6 |
| K-II-27, minimal (O'Donovan *et. al.* 1965b) | 0.14 |
| K-II-27 plus 10 $\mu g\ mL^{-1}$ of histidine, minimal (O'Donovan *et. al.* 1965b) | 3.32 |
| ML 30 replicate1, minimal (Ng 1969) | - |
| ML 30 replicate2, minimal (Ng 1969) | - |
| ML 30 G, minimal (Shehata and Marr 1975) | < 0.1 |
| ML 30 G, complex (Shehata and Marr 1975) | - |
| B/r, complex (Herendeen *et. al.* 1979) | 2 |
| (Not defined), complex (Ratkowsky *et. al.* 1983) | 3.53 |
| SF, meat (Smith 1985) | - |
| O157:H7, complex (*pers. comm.* Buchanan 1992) | - |
| ML 30, minimal (Kovarova *et. al.* 1996) | < 0.01 |
| M 23 and SB 1, complex (Ross *et. al.* 2003) | < 0.01 |
| O157:H7, meat (Tamplin *et. al.* 2005) | 10.27 |
| **Overall mean:** | **2.43** |

- not presented because regression analyses inappropriate - *see* Chapter 4.

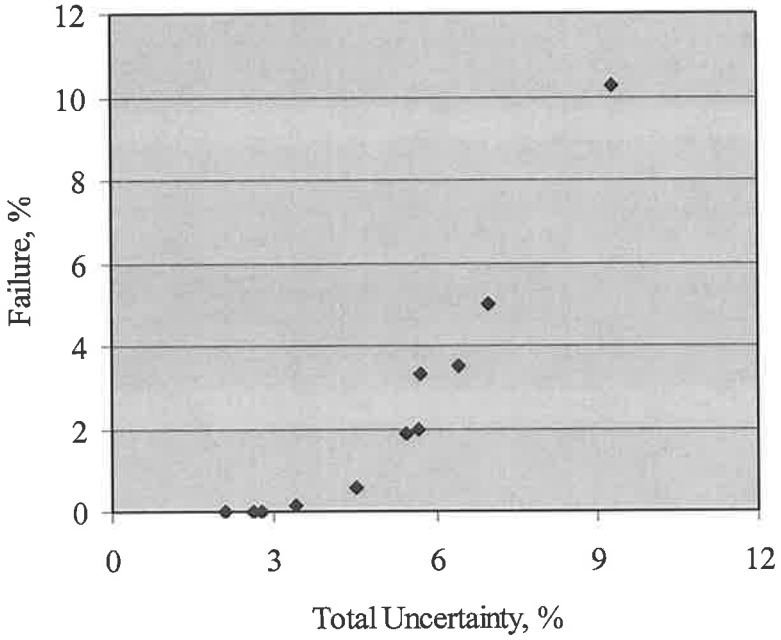



**Figure 5.1** Plot to highlight the relationship of % Failure with % Total Uncertainty in the revised QRA model for Monod.

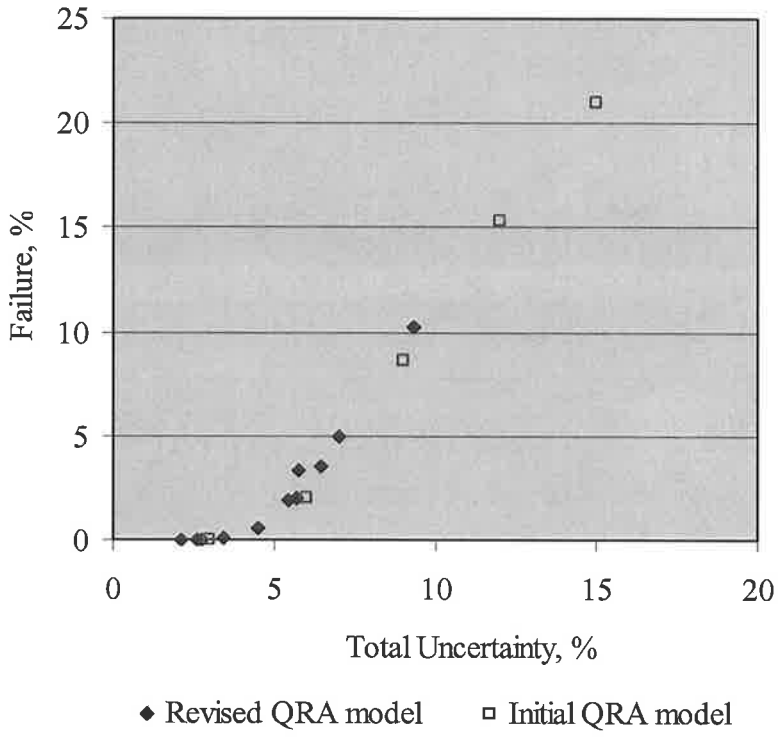



**Figure 5.2** Plot to highlight the relationship of % Failure with % Total Uncertainty in the revised QRA model and initial QRA model (Chapter 3) with the values of assumed Variability ranging from 1 % to 15 % about the means.

**Table 5.2** Comparison of the mean maximum specific growth rate ($\mu_{max}$) for *E. coli* and resulting predicted failures (%) from a revised QRA model of the Monod continuous fermenter - with those obtained from a QRA model (Chapter 3) of the continuous fermenter where $\mu_{max}$ has an assumed Variability ranging from 1 % to 15 % about the means.

| | $\mu_{max}$*, $h^{-1}$ | Failure*, % |
|---|---|---|
| Revised or "accurate value" QRA model | 1.14 | 2.43 |
| Initial QRA model from Chapter 3 | 1.00 | 8.18 |

* results expressed as mean values.

## 5.4 Summary and Conclusions

1. A revised QRA model of a Monod continuous fermenter for *E. coli* growth with an "accurate value" of the maximum specific growth rate ($\mu_{max}$) predicted an overall mean of 2.43 % of actual practical fermentations will fail. The number of predicted failures ranged about this mean value from 0.01 % to 10.27 %. The "accurate value" of $\mu_{max}$ was determined from non-linear regression analyses of 18 independent data sets collated from various published sources

2. This number of predicted failures is about one-third that predicted from an initial QRA model of a continuous fermenter based on estimated values of Variability in the mean value of $\mu_{max}$.

3. Small changes in the value of $\mu_{max}$ have a highly significant effect on predicted failure rates of growth of *E. coli* in a Monod continuous fermenter. The effort expended in determining the best estimate of $\mu_{max}$ is justified by more reliable prediction of failure of the continuous fermenter.

In the following chapter the conclusions that can be reached from this research are presented.

CHAPTER SIX

# CONCLUSIONS

From this research the following can be concluded:

1. A novel Quantitative Risk Assessment (QRA) model of a Monod continuous fermenter can be carried out. Failure of the fermenter is defined as washout.

2. Predictions from the novel QRA model of the Monod continuous fermenter for growth of *Escherichia coli* at the dilution rate for maximum productivity highlighted that the maximum specific growth rate ($\mu_{max}$) was the input parameter that most influenced de-stabilising of an otherwise well-operated plant that led to failure. A comparison of simulation results from the QRA model with a traditional Single Value Assessment, underscored the need for a more "accurate value" of $\mu_{max}$ to accurately predict the practical failures that could occur in the Monod continuous fermenter.

3. An "accurate value" of the natural Variability in the value of $\mu_{max}$ for *E. coli* can be obtained from collation and analyses of published (18) independent growth data sets over a range of growth temperature from 10 $^{O}$C to 45 $^{O}$C and wide range of growth media (minimal, complex, and blended meat).

4. Simulations with the revised QRA model of the Monod continuous fermenter at the dilution rate for maximum productivity with an "accurate value" of $\mu_{max}$ for *E. coli* growth predicted that 2.43 % of actual practical failures can occur. The rate of fermentation failures was reduced from 8.18 % to 2.43 % or by about 70 % in the revised QRA model. That is, the number of predicted failures was reduced by a factor of about 3 over the initial QRA model of the continuous fermenter for growth of *E. coli*. This finding underscores that effort should be expended in determining the best estimate of $\mu_{max}$ for reliable predictions.

5. The notion of global food process can be glimpsed through the successful application of this novel QRA modelling approach to a continuous fermenter and earlier a UHT plant (Cerf and Davey 2001), and could, in principle, be applied to a range of single or connected unit operations.

## 6.1 Recommendations for further research

The novel application of QRA modelling to a continuous fermenter, in principle, can be applied to a wide range of a standard 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 (K R Davey *pers. comm.*). 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. The success of this research strongly supports this. The benefits of using QRA will assist in evaluating the risk involved in any unit operation together with the Uncertainty and Variability in the model input parameters.

# APPENDICES A - E

## APPENDIX A - A definition of some important terms used in this research

| | |
|---|---|
| Chance | *see* Variability |
| Chemostat | *see* CSTR |
| CSTR | Continuous Stirred Tank Reactor - defined as a *Chemostat* in general microbiology or as a CSTF in biochemical engineering literature (*see* Lee 1992) |
| Fact | *see* Uncertainty |
| Failure | *see* Washout |
| Fermentation | An enzymatically controlled transformation of an organic compound usually carried out in a *CSTR* |
| Friday 13$^{th}$ Syndrome | Events defined by where just about all the bad in everything seems to combine to make a failure of plans and opportunities despite all good design and operation (defined by Cerf and Davey 2003) |
| Generation time | Time required to double the population for a micro organism |
| Global food process | A food process in which there are two or more process *unit operations* interconnected (defined by Cerf and Davey 2003) |
| Inactivation | Rendering of the inability of *viable* cells to reproduce |
| Lag time | Phase in a microbial growth curve in which there is no significant increase in cell numbers |

| | |
|---|---|
| Mean | The value of a random variable for which the weighted *probability* mass for all values less than the mean is equal to the weighted probability mass for all values greater than the mean. The mean can be regarded as the center of gravity of a probability density function. Also, the mean is the first moment of the distribution with respect to the origin |
| Median | It is a point such that exactly half of the probability is associated with values less than the median and half of the probability is associated with values greater than the median. It is also regarded as the mid-point or $50^{th}$ percentile of the distribution |
| Monte Carlo Assessment (MCA) | MCA involves the random sampling of each *probability distribution* within a parameter to produce hundred's or even thousand's of iterations. Each probability distribution is sampled in a manner that reproduces the shape of the distribution. The distribution of the values calculated for the parameter outcome therefore reflects the probability of the values that could occur practically in plant operation. This methodology is referred to as Monte Carlo Assessment (MCA) (Cerf and Davey 2001) |
| Predictive (microbial) Modelling, Predictive Microbiology, Microbiological Process Modelling | A description of the microbial response to a particular environmental condition, using models as a basis on which predictions are made. A term widely used by microbiologists. The models use experimental data, and equations to produce a prediction. A prediction should be used as a guide to the response of a micro organism to a particular set of conditions. Predictive microbiology is too broad a title. For chemical |

| | |
|---|---|
| | engineers, biochemical engineers a more accurate descriptor is: microbiological process modelling (*see* Davey 1993) |
| Probability | A numerical measure of the likelihood of a particular outcome of a *stochastic* process |
| Probability distribution | A distribution of probable values a parameter may take, with the likelihood that the parameter will take a unique value |
| Quantile | The fraction (or percent) of points lying below the given value i.e. 0.5 (or 50 %) quantile is the point (median) at which 50 % percent of the data fall below and 50 % fall above that value |
| Single Value Assessment (SVA) | Assessment of a desired model output using a single value input (defined by Cerf and Davey 2001) |
| Standard deviation | It is the square root of variance. It is used to estimate probability bands for many standard probability distributions |
| Sterilisation | *Inactivation* of all living matter. However, a working definition is a given reduction in the number of *viable* micro organisms |
| Stochastic process | A system of countable events to a well defined random process |
| Strain | A group of micro organisms of the same species, having distinctive characteristics but not usually considered a separate breed or variety |

| | |
|---|---|
| Uncertainty | A lack of knowledge, or level of ignorance, about the parameters that characterise the physical system. It is also referred to as a *Fact*. Uncertainty is sometimes reducible through further measurement or careful study, or through consulting more experts (Vose 2000) |
| Unit operation | The operation in which chemical as well as physical changes take place e.g. *fermentation*, *sterilisation*, drying, mixing, etc |
| Variability | The effect of *chance* on an outcome. It is a function of the system. Variability is not reducible through further study or careful measurement. It can be reduced through changing the physical system (Vose 2000) |
| Viable | Living or active in terms of microbiology |
| Washout | A condition of loss of all viable cells from the fermenter |

## APPENDIX B - Collated growth data for *Escherichia coli* from various published sources

Data collated in this appendix for maximum specific growth rate ($\mu_{max}$) for *Escherichia coli* were either: taken from tabulated data directly from the published sources (e.g. Table B.18), taken from tabulated data expressed as generation time ($t_G$) and converted to give $\mu_{max}$ (e.g. Table B.14 and B.17), or taken from graphical data and converted to give $\mu_{max}$ by linear interpolation of suitably enlarged diagrams (e.g. Table B.1-B.13, B.15 and B.16).

All data sets were converted to give consistent units of $\mu_{max}$ of $h^{-1}$. Each strain of *E. coli* is identified except the data of Ratkowsky *et. al.* (1983), (*see* Table B.13).

Table B.1 *E. coli* C-600-1 growth in glucose-minimal medium over a range of temperature $15 \leq T \leq 37\ ^{o}C$ (O'Donovan *et. al.* 1965a).

| $T$, $^{o}C$ | $\mu_{max}$, $h^{-1}$ |
|---|---|
| 15 | 0.049 |
| 20 | 0.102 |
| 22.5 | 0.160 |
| 25 | 0.199 |
| 27.5 | 0.244 |
| 30 | 0.315 |
| 35 | 0.465 |
| 37 | 0.572 |

Table B.2 *E. coli* K-I-01 growth in glucose-minimal medium over a range of temperature $20 \leq T \leq 37\ ^{o}C$ (O'Donovan *et. al.* 1965a).

| $T$, $^{o}C$ | $\mu_{max}$, $h^{-1}$ |
|---|---|
| 20 | 0.028 |
| 22.5 | 0.056 |
| 25 | 0.129 |
| 27.5 | 0.178 |
| 30 | 0.269 |
| 35 | 0.444 |
| 37 | 0.482 |

Table B.3 *E. coli* C-600-1 growth in nutrient broth over a range of temperature $9.5 \leq T \leq 44\ ^{O}C$ (O'Donovan *et. al.* 1965b).

| $T$, $^{O}C$ | $\mu_{max}$, $h^{-1}$ |
|---|---|
| 9.5 | 0.014 |
| 15 | 0.081 |
| 20 | 0.258 |
| 22.5 | 0.388 |
| 26 | 0.419 |
| 30 | 0.799 |
| 37 | 1.202 |
| 39 | 1.235 |
| 42 | 1.071 |
| 44 | 0.712 |

Table B.4 *E. coli* K-II-27 growth in nutrient broth over a range of temperature $10 \leq T \leq 44\ ^{O}C$ (O'Donovan *et. al.* 1965b).

| $T$, $^{O}C$ | $\mu_{max}$, $h^{-1}$ |
|---|---|
| 10 | 0.014 |
| 15 | 0.078 |
| 20 | 0.258 |
| 22.5 | 0.410 |
| 26 | 0.419 |
| 30 | 0.799 |
| 37 | 1.071 |
| 39 | 1.261 |
| 42 | 1.146 |
| 44 | 0.712 |

Table B.5 *E. coli* C-600-1 growth in glucose-minimal medium over a range of temperature $15 \leq T \leq 37$ $^{O}$C (O'Donovan *et. al.* 1965b).

| $T$, $^{O}$C | $\mu_{max}$, $h^{-1}$ |
|---|---|
| 11.8 | 0.015 |
| 15 | 0.041 |
| 20 | 0.120 |
| 26 | 0.245 |
| 30 | 0.335 |
| 37 | 0.589 |
| 39 | 0.568 |
| 42 | 0.568 |
| 44 | 0.240 |

Table B.6 *E. coli* K-II-27 growth in glucose-minimal medium over a range of temperature $20 \leq T \leq 44$ $^{O}$C (O'Donovan *et. al.* 1965b).

| $T$, $^{O}$C | $\mu_{max}$, $h^{-1}$ |
|---|---|
| 20 | 0.014 |
| 22.5 | 0.055 |
| 26 | 0.117 |
| 30 | 0.197 |
| 37 | 0.478 |
| 39 | 0.535 |
| 42 | 0.542 |
| 44 | 0.228 |

Table B.7 *E. coli* K-11-27 plus 10 μg $mL^{-1}$ histidine growth in glucose-minimal medium over a range of temperature $11.8 \leq T \leq 44\ ^{O}C$ (O'Donovan *et. al.* 1965b).

| $T$, $^{O}C$ | $\mu_{max}$, $h^{-1}$ |
|---|---|
| 11.8 | 0.015 |
| 15 | 0.043 |
| 20 | 0.128 |
| 26 | 0.257 |
| 30 | 0.335 |
| 37 | 0.602 |
| 39 | 0.568 |
| 42 | 0.602 |
| 44 | 0.245 |

Table B.8 *E. coli* ML 30 replicate 1 growth data in a glucose-minimal medium over a range of temperature $9.29 \leq T \leq 34.96\ ^{O}C$ (Ng 1969).

| $T$, $^{O}C$ | $\mu_{max}$, $h^{-1}$ |
|---|---|
| 9.29 | 0.042 |
| 15.07 | 0.141 |
| 17.07 | 0.183 |
| 20.25 | 0.272 |
| 25.21 | 0.485 |
| 30.17 | 0.678 |
| 34.96 | 0.862 |

Table B.9 *E. coli* ML 30 replicate 2 growth data in a glucose-minimal medium over a range of temperature $9.29 \leq T \leq 34.96$ $^{O}$C (Ng 1969).

| $T$, $^{O}$C | $\mu_{max}$, $h^{-1}$ |
|---|---|
| 9.29 | 0.037 |
| 12.68 | 0.086 |
| 15.07 | 0.148 |
| 20.34 | 0.290 |
| 25.21 | 0.427 |
| 30.37 | 0.625 |
| 34.96 | 0.809 |

Table B.10 *E. coli* ML 30 G growth data in a minimal medium over a range of temperature $11 \leq T \leq 40$ $^{O}$C (Shehata and Marr 1975).

| $T$, $^{O}$C | $\mu_{max}$, $h^{-1}$ |
|---|---|
| 11 | 0.058 |
| 15 | 0.154 |
| 20 | 0.306 |
| 25 | 0.485 |
| 30 | 0.756 |
| 35 | 0.99 |
| 40 | 0.866 |

Table B.11 *E. coli* ML 30 G growth data in a complex medium over a range of temperature $14 \leq T \leq 40$ $^{O}$C (Shehata and Marr 1975).

| $T$, $^{O}$C | $\mu_{max}$, h$^{-1}$ |
|---|---|
| 14 | 0.194 |
| 20 | 0.507 |
| 25 | 0.835 |
| 30 | 1.26 |
| 35 | 1.8 |
| 40 | 2.08 |

Table B.12 *E. coli* B/r growth data in a complex medium over a range of temperature $13.5 \leq T \leq 48$ $^{O}$C (Herendeen *et. al.* 1979).

| $T$, $^{O}$C | $\mu_{max}$, h$^{-1}$ |
|---|---|
| 13.5 | 0.149 |
| 15 | 0.195 |
| 17 | 0.298 |
| 19 | 0.367 |
| 21 | 0.518 |
| 23 | 0.587 |
| 28 | 0.875 |
| 30 | 1.160 |
| 33 | 1.298 |
| 36 | 1.546 |
| 37 | 1.776 |
| 39 | 1.866 |
| 42 | 1.808 |
| 45 | 1.197 |
| 46 | 0.992 |
| 47 | 0.690 |
| 48 | 0.238 |

Table B.13 *E. coli* (strain not defined) growth data over a range of temperature $21 \leq T \leq 47\ ^{o}C$ (Ratkowsky *et. al.* 1983).

| $T$, $^{o}C$ | $\mu_{max}$, $h^{-1}$ |
|---|---|
| 21 | 0.25 |
| 23 | 0.56 |
| 25 | 0.61 |
| 27 | 0.79 |
| 29 | 0.94 |
| 31 | 1.04 |
| 33 | 1.16 |
| 35 | 1.23 |
| 37 | 1.36 |
| 39 | 1.32 |
| 41 | 1.36 |
| 43 | 1.34 |
| 45 | 0.96 |
| 46 | 0.83 |
| 47 | 0.16 |

Table B.14 *E. coli* SF growth data on meat over a range of temperature $8.2 \leq T \leq 40\ ^{o}C$ (Smith 1985).

| $T$, $^{o}C$ | $\mu_{max}$, $h^{-1}$ |
|---|---|
| 8.2 | 0.04 |
| 10 | 0.10 |
| 15 | 0.27 |
| 20 | 0.50 |
| 25 | 0.89 |
| 30 | 1.33 |
| 35 | 1.87 |
| 40 | 2.31 |

Table B.15 *E. coli* O157:H7 growth data in a complex medium over a range of temperature $11 \leq T \leq 40$ $^{O}$C (*pers. comm.* Buchanan 1992).

| $T$, $^{O}$C | $\mu_{max}$, h$^{-1}$ |
|---|---|
| 10 | 0.112 |
| 10 | 0.108 |
| 10 | 0.115 |
| 12 | 0.163 |
| 19 | 0.584 |
| 19 | 0.561 |
| 19 | 0.561 |
| 28 | 1.254 |
| 28 | 1.303 |
| 28 | 1.227 |
| 37 | 2.288 |
| 37 | 2.309 |
| 37 | 2.314 |
| 42 | 2.387 |
| 42 | 2.507 |
| 42 | 2.323 |

Table B.16 *E. coli* ML 30 growth data in a glucose-minimal medium over a range of temperature $10.6 \leq T \leq 39.83$ $^{O}$C (Kovarova *et. al.* 1996).

| $T$, $^{O}$C | $\mu_{max}$, h$^{-1}$ |
|---|---|
| 10.06 | 0.021 |
| 20.10 | 0.211 |
| 25.93 | 0.445 |
| 28.54 | 0.529 |
| 30.15 | 0.578 |
| 31.43 | 0.639 |
| 32.72 | 0.690 |
| 34.83 | 0.789 |
| 37.58 | 0.912 |
| 38.54 | 0.924 |
| 39.83 | 0.868 |
| 42.12 | 0 |

Table B.17 *E. coli* M 23 and SB 1 growth data in a complex medium over a range of temperature $7.63 \leq T \leq 47.43$ $^{O}$C (Ross *et. al.* 2003).

| $T$, $^{O}$C | $\mu_{max}$, $h^{-1}$ |
|---|---|
| 7.63 | 0.011 |
| 10.30 | 0.036 |
| 12.03 | 0.074 |
| 13.20 | 0.105 |
| 14.48 | 0.143 |
| 16.03 | 0.200 |
| 17.38 | 0.256 |
| 20.18 | 0.415 |
| 21.50 | 0.471 |
| 22.68 | 0.587 |
| 24.05 | 0.693 |
| 25.00 | 0.778 |
| 25.00 | 0.745 |
| 25.43 | 0.796 |
| 26.70 | 0.855 |
| 27.90 | 1.066 |
| 29.30 | 1.034 |
| 30.60 | 1.195 |
| 32.08 | 1.307 |
| 33.60 | 1.386 |
| 34.98 | 1.474 |
| 36.70 | 1.611 |
| 38.03 | 1.650 |
| 40.08 | 1.824 |
| 41.85 | 1.777 |
| 43.63 | 1.824 |
| 45.55 | 1.506 |
| 47.43 | 0.521 |

Table B.18 *E. coli* O157:H7 growth data on meat over a range of temperature $6 \leq T \leq 45$ $^{O}$C (Tamplin *et. al.* 2005).

| $T$, $^{O}$C | $\mu_{max}$, $h^{-1}$ |
|---|---|
| 6 | 0.003 |
| 8 | 0.022 |
| 10 | 0.107 |
| 10.5 | 0.068 |
| 11 | 0.109 |
| 12.5 | 0.158 |
| 15 | 0.245 |
| 17 | 0.254 |
| 18 | 0.404 |
| 20 | 0.471 |
| 25 | 0.878 |
| 30 | 1.07 |
| 35 | 1.79 |
| 40 | 2.45 |
| 42 | 2.93 |
| 44 | 2.16 |
| 45 | 2.07 |

## APPENDIX C – R software for non-linear regression analysis of fit of Rosso model to data for growth of *E. coli*

### C.1 R program for published *E. coli* data sets

Before starting the program, save the *E. coli* growth data on the C:// (as filename.dat).

```
filename <- read.table ("C:\\ filename.dat", header=T)
attach(filename)
filename
rosso.func <- function(temp,mu.opt,tmin,topt,tmax){mu.opt*(temp-
tmax)*(temp-tmin)^2/
((topt-tmin)*((topt-tmin)*(temp-topt)-(topt-tmax)*(topt+tmin-
2*temp)))}
filename.func <- function(temp,b,tmin,c,tmax){b*(temp-tmin)*(1-
exp(c*(temp-tmax)))}

# Rosso model

filename.nls1 <- nls(mu.max ~ rosso.func (Temp, mu.opt, tmin,
topt, tmax),data= filename,start=list(mu.opt=2, tmin=5, topt=41,
tmax=49), trace=T)

filename.nls1

summary(filename.nls1)

shapiro.test(resid(filename.nls1))

vcov(filename.nls1)

plot(Temp,mu.max, xlim=c(coef(filename.nls1)[2],
coef(filename.nls1)[4]))
x <- seq(coef(filename.nls1)[2], coef(filename.nls1)[4], len=101)
lines(x, rosso.func(x, coef(filename.nls1)[1],
coef(filename.nls1)[2], coef(filename.nls1)[3],
coef(filename.nls1)[4]))
mean(resid(filename.nls1))
sd(resid(filename.nls1))
hist(resid(filename.nls1), breaks=11)
plot(fitted(filename.nls1), resid(filename.nls1))
abline(h=0)
qqnorm(resid(filename.nls1))
qqline(resid(filename.nls1))

par(mfrow=c(2,2))
plot(Temp,mu.max, xlim=c(coef(filename.nls1)[2],
coef(filename.nls1)[4]))
x <- seq(coef(filename.nls1)[2], coef(filename.nls1)[4], len=101)
lines(x, rosso.func(x, coef(filename.nls1)[1],
coef(filename.nls1)[2], coef(filename.nls1)[3],
coef(filename.nls1)[4]))
```

```
hist(resid(filename.nls1), breaks=11)
plot(fitted(filename.nls1), resid(filename.nls1))
abline(h=0)
qqnorm(resid(filename.nls1))
qqline(resid(filename.nls1))
```

## C.2 R program output for *E. coli* M 23 and SB1 growth data

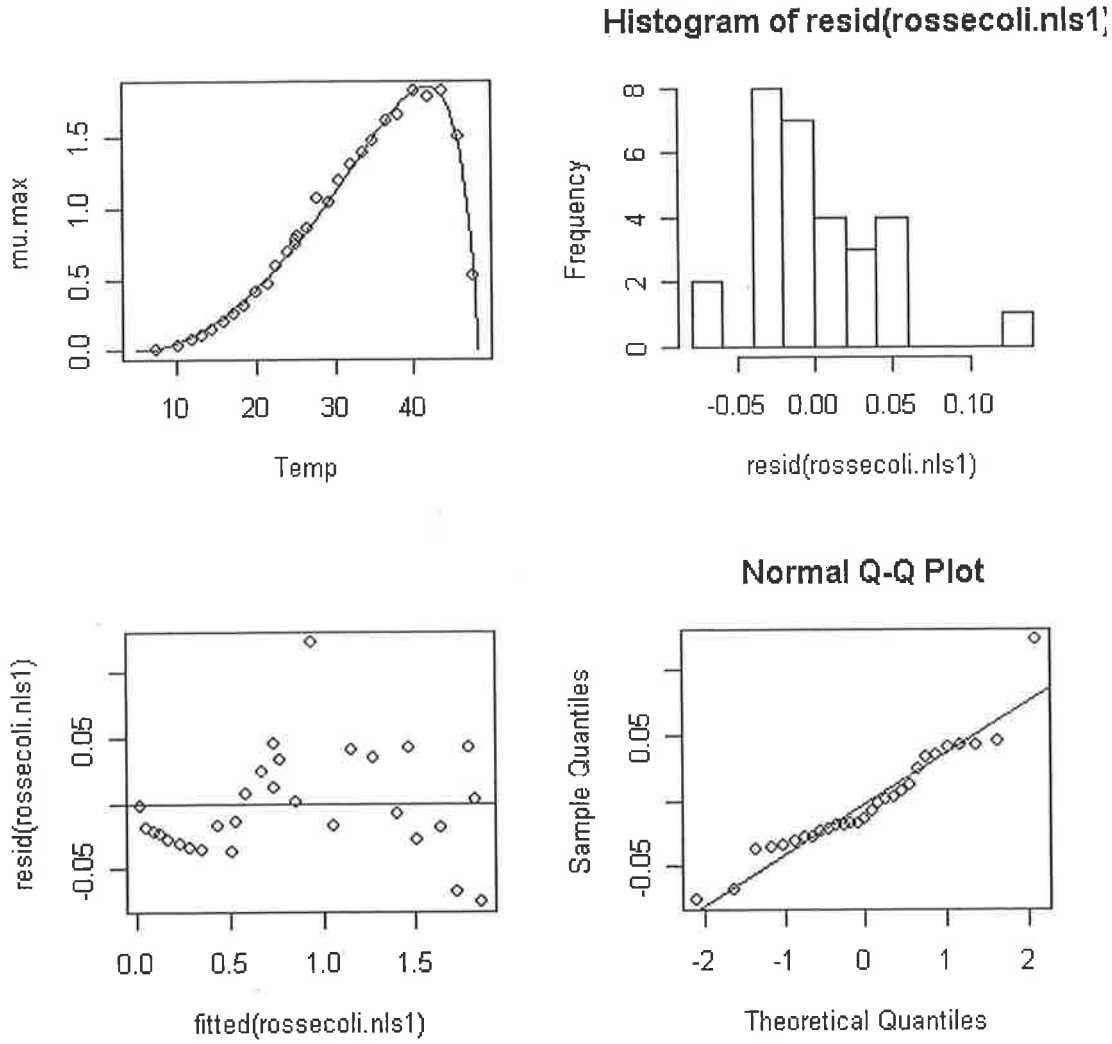
```
> rossecoli <- read.table ("C:\\rossecoli.dat", header=T)
> attach(rossecoli)
> rossecoli

    Temp     mu.max
1   7.63 0.0114
2  10.30 0.0362
3  12.03 0.0744
4  13.20 0.1058
5  14.48 0.1438
6  16.03 0.2003
7  17.38 0.2567
8  18.53 0.3108
9  20.18 0.4150
10 21.50 0.4715
11 22.68 0.5874
12 24.05 0.6931
13 25.00 0.7788
14 25.00 0.7453
15 25.43 0.7967
16 26.70 0.8557
17 27.90 1.0663
18 29.30 1.0345
19 30.60 1.1950
20 32.08 1.3078
21 33.60 1.3862
22 34.98 1.4747
23 36.70 1.6119
24 38.03 1.6503
25 40.08 1.8240
26 41.85 1.7773
27 43.63 1.8240
28 45.55 1.5068
29 47.43 0.5211
> rosso.func <- function(temp,mu.opt,tmin,topt,tmax){mu.opt*(temp-
tmax)*(temp-tmin)^2/ ((topt-tmin)*((topt-tmin)*(temp-topt)-(topt-
tmax)*(topt+tmin-2*temp)))}
> ratkowsky.func <- function(temp,b,tmin,c,tmax){b*(temp-tmin)*(1-
exp(c*(temp-tmax)))}
> # Rosso model
```

```
> rossecoli.nls1 <- nls(mu.max ~ rosso.func (Temp, mu.opt, tmin,
topt, tmax),data= rossecoli,start=list(mu.opt=2, tmin=5, topt=41,
tmax=49), trace=T)
0.7840829 :   2  5 41 49
0.1114702 :   1.852255  4.885935 41.536812 47.685596
0.04495153 :   1.853627  4.899539 41.704921 47.932771
0.04432389 :   1.853268  4.945065 41.698292 47.967508
0.04432206 :   1.853091  4.953393 41.694421 47.969654
0.04432204 :   1.853071  4.954204 41.693983 47.969824
0.04432204 :   1.853069  4.954282 41.693941 47.969840
> rossecoli.nls1

Nonlinear regression model
  model:  mu.max ~ rosso.func(Temp, mu.opt, tmin, topt, tmax)
   data:  rossecoli
   mu.opt       tmin       topt       tmax
 1.853069  4.954282 41.693941 47.969840
 residual sum-of-squares:  0.04432204
> summary(rossecoli.nls1)
Formula: mu.max ~ rosso.func(Temp, mu.opt, tmin, topt, tmax)
Parameters:
       Estimate Std. Error t value Pr(>|t|)
mu.opt   1.8531     0.0179 103.515  < 2e-16 ***
tmin     4.9543     0.5565   8.903 3.17e-09 ***
topt    41.6939     0.1708 244.064  < 2e-16 ***
tmax    47.9698     0.0719 667.144  < 2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Residual standard error: 0.04211 on 25 degrees of freedom
Correlation of Parameter Estimates:
      mu.opt    tmin    topt
tmin  0.1832
topt  0.3462 -0.5838
tmax -0.3303  0.3823 -0.5545
> shapiro.test(resid(rossecoli.nls1))
        Shapiro-Wilk normality test
data:  resid(rossecoli.nls1)
W = 0.9276, p-value = 0.04767
> vcov(rossecoli.nls1)
              mu.opt         tmin         topt          tmax
mu.opt  0.0003204623  0.001825372  0.001058752 -0.0004252107
tmin    0.0018253725  0.309674769 -0.055501388  0.0152983342
topt    0.0010587519 -0.055501388  0.029183667 -0.0068111900
tmax   -0.0004252107  0.015298334 -0.006811190  0.0051700744
> plot(Temp,mu.max, xlim=c(coef(rossecoli.nls1)[2],
coef(rossecoli.nls1)[4]))
> x <- seq(coef(rossecoli.nls1)[2], coef(rossecoli.nls1)[4],
len=101)
> lines(x, rosso.func(x, coef(rossecoli.nls1)[1],
coef(rossecoli.nls1)[2], coef(rossecoli.nls1)[3],
coef(rossecoli.nls1)[4]))
> mean(resid(rossecoli.nls1))
[1] -0.002642282
> sd(resid(rossecoli.nls1))
[1] 0.03969508
> hist(resid(rossecoli.nls1), breaks=11)
```

```
> plot(fitted(rossecoli.nls1), resid(rossecoli.nls1))
> abline(h=0)
> qqnorm(resid(rossecoli.nls1))
> qqline(resid(rossecoli.nls1))
> par(mfrow=c(2,2))
> plot(Temp,mu.max, xlim=c(coef(rossecoli.nls1)[2],
coef(rossecoli.nls1)[4]))
> x <- seq(coef(rossecoli.nls1)[2], coef(rossecoli.nls1)[4],
len=101)
> lines(x, rosso.func(x, coef(rossecoli.nls1)[1],
coef(rossecoli.nls1)[2], coef(rossecoli.nls1)[3],
coef(rossecoli.nls1)[4]))
> hist(resid(rossecoli.nls1), breaks=11)
> plot(fitted(rossecoli.nls1), resid(rossecoli.nls1))
> abline(h=0)
> qqnorm(resid(rossecoli.nls1))
> qqline(resid(rossecoli.nls1))
```



## APPENDIX D – Output estimates of non-linear regression fit of the Rosso model to *E. coli* growth data

Table D.1 Initial parameter estimates of the Rosso model.

| *E. coli*, medium (source) | $\mu_{opt}$, $h^{-1}$ | $T_{min}$, °C | $T_{opt}$, °C | $T_{max}$, °C |
|---|---|---|---|---|
| C-600-1, minimal (O'Donovan *et. al.* 1965a) | - | - | - | - |
| K-I-01, minimal (O'Donovan *et. al.* 1965a) | - | - | - | - |
| C-600-1, complex (O'Donovan *et. al.* 1965b) | 1.23 | 9 | 39 | 45 |
| K-II-27, complex (O'Donovan *et. al.* 1965b) | 1.26 | 9 | 39 | 45 |
| C-600-1, minimal (O'Donovan *et. al.* 1965b) | 0.5 | 10 | 40 | 45 |
| K-II-27, minimal (O'Donovan *et. al.* 1965b) | 0.54 | 19 | 42 | 45 |
| K-II-27 plus 10 μg $mL^{-1}$ of histidine, minimal (O'Donovan *et. al.* 1965b) | 0.6 | 11 | 37 | 45 |
| ML 30 replicate1, minimal (Ng 1969) | - | - | - | - |
| ML 30 replicate2, minimal (Ng 1969) | - | - | - | - |
| ML 30 G, minimal (Shehata and Marr 1975) | 1.2 | 10 | 37 | 42 |
| ML 30 G, complex (Shehata and Marr 1975) | - | - | - | - |
| B/r, complex (Herendeen *et. al.* 1979) | 2 | 10 | 39 | 50 |
| (Not defined), complex (Ratkowsky *et. al.* 1983) | 2 | 5 | 41 | 49 |
| SF, meat (Smith 1985) | - | - | - | - |
| O157:H7, complex (*pers. comm.* Buchanan 1992) | - | - | - | - |
| ML 30, minimal (Kovarova *et. al.* 1996) | 0.92 | 10 | 38 | 43 |
| M 23 and SB 1, complex (Ross *et. al.* 2003) | 2 | 5 | 41 | 49 |
| O157:H7, meat (Tamplin *et. al.* 2005) | 2.9 | 5 | 42 | 46 |

- not presented because data for regression analyses inappropriate - *see* Chapter 4.

Table D.2 Mean and Standard Deviation of the parameter estimates.

| *E. coli*, medium (source) | Parameters | Mean | Standard Deviation |
|---|---|---|---|
| C-600-1, minimal (O'Donovan *et. al.* 1965a) | $\mu_{opt}$, $h^{-1}$ | - | - |
| | $T_{min}$, $^{o}C$ | - | - |
| | $T_{opt}$, $^{o}C$ | - | - |
| | $T_{max}$, $^{o}C$ | - | - |
| K-I-01, minimal (O'Donovan *et. al.* 1965a) | $\mu_{opt}$, $h^{-1}$ | - | - |
| | $T_{min}$, $^{o}C$ | - | - |
| | $T_{opt}$, $^{o}C$ | - | - |
| | $T_{max}$, $^{o}C$ | - | - |
| C-600-1, complex (O'Donovan *et. al.* 1965b) | $\mu_{opt}$, $h^{-1}$ | 1.2242 | 0.034 |
| | $T_{min}$, $^{o}C$ | 9.23 | 1.8293 |
| | $T_{opt}$, $^{o}C$ | 39.24 | 0.5416 |
| | $T_{max}$, $^{o}C$ | 45.48 | 0.4756 |
| K-II-27, complex (O'Donovan *et. al.* 1965b) | $\mu_{opt}$, $h^{-1}$ | 1.2249 | 0.0407 |
| | $T_{min}$, $^{o}C$ | 6.78 | 2.1783 |
| | $T_{opt}$, $^{o}C$ | 40.16 | 0.6200 |
| | $T_{max}$, $^{o}C$ | 44.96 | 0.4093 |
| C-600-1, minimal (O'Donovan *et. al.* 1965b) | $\mu_{opt}$, $h^{-1}$ | 0.6043 | 0.0132 |
| | $T_{min}$, $^{o}C$ | 7.35 | 1.4690 |
| | $T_{opt}$, $^{o}C$ | 40.17 | 0.3756 |
| | $T_{max}$, $^{o}C$ | 44.69 | 0.1223 |
| K-II-27, minimal (O'Donovan *et. al.* 1965b) | $\mu_{opt}$, $h^{-1}$ | 0.5651 | 0.0095 |
| | $T_{min}$, $^{o}C$ | 16.65 | 0.7582 |
| | $T_{opt}$, $^{o}C$ | 40.89 | 0.2265 |
| | $T_{max}$, $^{o}C$ | 44.42 | 0.0789 |
| K-II-27 plus 10 μg $mL^{-1}$ of histidine , minimal (O'Donovan *et. al.* 1965b) | $\mu_{opt}$, $h^{-1}$ | 0.6228 | 0.0178 |
| | $T_{min}$, $^{o}C$ | 6.41 | 1.9304 |
| | $T_{opt}$, $^{o}C$ | 40.56 | 0.4997 |
| | $T_{max}$, $^{o}C$ | 44.37 | 0.1366 |
| ML 30 replicate1, minimal (Ng 1969) | $\mu_{opt}$, $h^{-1}$ | - | - |
| | $T_{min}$, $^{o}C$ | - | - |
| | $T_{opt}$, $^{o}C$ | - | - |
| | $T_{max}$, $^{o}C$ | - | - |

- not presented because data for regression analyses inappropriate - *see* Chapter 4.

| | | | |
|---|---|---|---|
| ML 30 replicate2, minimal (Ng 1969) | $\mu_{opt}$, $h^{-1}$ | - | - |
| | $T_{min}$, $^{o}C$ | - | - |
| | $T_{opt}$, $^{o}C$ | - | - |
| | $T_{max}$, $^{o}C$ | - | - |
| ML 30 G, minimal (Shehata and Marr 1975) | $\mu_{opt}$, $h^{-1}$ | 1.0638 | 0.0347 |
| | $T_{min}$, $^{o}C$ | 3.47 | 0.7927 |
| | $T_{opt}$, $^{o}C$ | 37.67 | 0.3461 |
| | $T_{max}$, $^{o}C$ | 41.19 | 0.5504 |
| ML 30 G, complex (Shehata and Marr 1975) | $\mu_{opt}$, $h^{-1}$ | - | - |
| | $T_{min}$, $^{o}C$ | - | - |
| | $T_{opt}$, $^{o}C$ | - | - |
| | $T_{max}$, $^{o}C$ | - | - |
| B/r, complex (Herendeen *et. al.* 1979) | $\mu_{opt}$, $h^{-1}$ | 1.7513 | 0.0398 |
| | $T_{min}$, $^{o}C$ | 7.37 | 1.3523 |
| | $T_{opt}$, $^{o}C$ | 39.7 | 0.3756 |
| | $T_{max}$, $^{o}C$ | 48.30 | 0.1595 |
| (Not defined), complex (Ratkowsky *et. al.* 1983) | $\mu_{opt}$, $h^{-1}$ | 1.4212 | 0.0362 |
| | $T_{min}$, $^{o}C$ | 5.05 | 2.3820 |
| | $T_{opt}$, $^{o}C$ | 40.28 | 0.4121 |
| | $T_{max}$, $^{o}C$ | 47.31 | 0.1544 |
| SF, meat (Smith 1985) | $\mu_{opt}$, $h^{-1}$ | - | - |
| | $T_{min}$, $^{o}C$ | - | - |
| | $T_{opt}$, $^{o}C$ | - | - |
| | $T_{max}$, $^{o}C$ | - | - |
| O157:H7, complex (*pers. comm.* Buchanan 1992) | $\mu_{opt}$, $h^{-1}$ | - | - |
| | $T_{min}$, $^{o}C$ | - | - |
| | $T_{opt}$, $^{o}C$ | - | - |
| | $T_{max}$, $^{o}C$ | - | - |
| ML 30, minimal (Kovarova *et. al.* 1996) | $\mu_{opt}$, $h^{-1}$ | 0.9093 | 0.0089 |
| | $T_{min}$, $^{o}C$ | 5.09 | 0.9472 |
| | $T_{opt}$, $^{o}C$ | 38.70 | 0.1919 |
| | $T_{max}$, $^{o}C$ | 42.12 | 0.0106 |
| M 23 and SB 1, complex (Ross *et. al.* 2003) | $\mu_{opt}$, $h^{-1}$ | 1.8531 | 0.0179 |
| | $T_{min}$, $^{o}C$ | 4.95 | 0.5565 |
| | $T_{opt}$, $^{o}C$ | 41.69 | 0.1708 |
| | $T_{max}$, $^{o}C$ | 47.96 | 0.0719 |

- not presented because data for regression analyses inappropriate - *see* Chapter 4.

| | | | |
|---|---|---|---|
| O157:H7, meat (Tamplin *et. al.* 2005) | $\mu_{opt}$, $h^{-1}$ | 2.5786 | 0.0990 |
| | $T_{min}$, $^{o}C$ | 5.99 | 0.0602 |
| | $T_{opt}$, $^{o}C$ | 42.47 | 0.6059 |
| | $T_{max}$, $^{o}C$ | 46.03 | 0.6963 |

Table D.3 Residual Sum-of-Squares (RSS) and Residual Standard Error (RSE).

| *E. coli*, medium (source) | Residual Sum-of-Squares (RSS) | Residual Standard Error (RSE) |
|---|---|---|
| C-600-1, minimal (O'Donovan *et. al.* 1965a) | - | - |
| K-I-01, minimal (O'Donovan *et. al.* 1965a) | - | - |
| C-600-1, complex (O'Donovan *et. al.* 1965b) | 0.0182 | 0.0551 |
| K-II-27, complex (O'Donovan *et. al.* 1965b) | 0.0243 | 0.0636 |
| C-600-1, minimal (O'Donovan *et. al.* 1965b) | 0.0022 | 0.0213 |
| K-II-27, minimal (O'Donovan *et. al.* 1965b) | 0.0007 | 0.0137 |
| K-II-27 plus 10 $\mu g\ mL^{-1}$ of histidine, minimal (O'Donovan *et. al.* 1965b) | 0.0039 | 0.0279 |
| ML 30 replicate1, minimal (Ng 1969) | - | - |
| ML 30 replicate2, minimal (Ng 1969) | - | - |
| ML 30 G, minimal (Shehata and Marr 1975) | 0.0005 | 0.0133 |
| ML 30 G, complex (Shehata and Marr 1975) | - | - |
| B/r, complex (Herendeen *et. al.* 1979) | 0.1023 | 0.0887 |
| (Not defined), complex (Ratkowsky *et. al.* 1983) | 0.0712 | 0.0805 |
| SF, meat (Smith 1985) | - | - |
| O157:H7, complex (*pers. comm.* Buchanan 1992) | - | - |
| ML 30, minimal (Kovarova *et. al.* 1996) | 0.0022 | 0.0168 |
| M 23 and SB 1, complex (Ross *et. al.* 2003) | 0.0443 | 0.0421 |
| O157:H7, meat (Tamplin *et. al.* 2005) | 0.3040 | 0.1529 |

- not presented because data for regression analyses inappropriate - *see* Chapter 4.

Table D.4 Correlation of Parameter Estimates for: *E. coli*, medium (source).

Table D.4.1 Correlation of Parameter Estimates for C-600-1, complex (O'Donovan *et. al.* 1965b).

| Correlations | $\mu_{opt}$, $h^{-1}$ | $T_{min}$, $^{o}C$ | $T_{opt}$, $^{o}C$ | $T_{max}$, $^{o}C$ |
|---|---|---|---|---|
| $\mu_{opt}$, $h^{-1}$ | 1 | | | |
| $T_{min}$, $^{o}C$ | 0.1183 | 1 | | |
| $T_{opt}$, $^{o}C$ | 0.1724 | -0.6905 | 1 | |
| $T_{max}$, $^{o}C$ | -0.3623 | 0.6129 | -0.7865 | 1 |

Table D.4.2 Correlation of Parameter Estimates for K-II-27, complex (O'Donovan *et. al.* 1965b).

| Correlations | $\mu_{opt}$, $h^{-1}$ | $T_{min}$, $^{o}C$ | $T_{opt}$, $^{o}C$ | $T_{max}$, $^{o}C$ |
|---|---|---|---|---|
| $\mu_{opt}$, $h^{-1}$ | 1 | | | |
| $T_{min}$, $^{o}C$ | 0.0937 | 1 | | |
| $T_{opt}$, $^{o}C$ | 0.2904 | -0.6352 | 1 | |
| $T_{max}$, $^{o}C$ | -0.4158 | 0.5829 | -0.8406 | 1 |

Table D.4.3 Correlation of Parameter Estimates for C-600-1, minimal (O'Donovan *et. al.* 1965b).

| Correlations | $\mu_{opt}$, $h^{-1}$ | $T_{min}$, $^{o}C$ | $T_{opt}$, $^{o}C$ | $T_{max}$, $^{o}C$ |
|---|---|---|---|---|
| $\mu_{opt}$, $h^{-1}$ | 1 | | | |
| $T_{min}$, $^{o}C$ | 0.2145 | 1 | | |
| $T_{opt}$, $^{o}C$ | 0.2001 | -0.5186 | 1 | |
| $T_{max}$, $^{o}C$ | -0.2790 | 0.4882 | -0.8099 | 1 |

Table D.4.4 Correlation of Parameter Estimates for K-II-27, minimal (O'Donovan *et. al.* 1965b).

| Correlations | $\mu_{opt}$, $h^{-1}$ | $T_{min}$, $^{o}C$ | $T_{opt}$, $^{o}C$ | $T_{max}$, $^{o}C$ |
|---|---|---|---|---|
| $\mu_{opt}$, $h^{-1}$ | 1 | | | |
| $T_{min}$, $^{o}C$ | 0.1183 | 1 | | |
| $T_{opt}$, $^{o}C$ | 0.3321 | -0.4957 | 1 | |
| $T_{max}$, $^{o}C$ | -0.3941 | 0.4781 | -0.8283 | 1 |

Table D.4.5 Correlation of Parameter Estimates for K-II-27 plus 10 μg $mL^{-1}$ of histidine, minimal (O'Donovan *et. al.* 1965b).

| Correlations | $\mu_{opt}$, $h^{-1}$ | $T_{min}$, °C | $T_{opt}$, °C | $T_{max}$, °C |
|---|---|---|---|---|
| $\mu_{opt}$, $h^{-1}$ | 1 | | | |
| $T_{min}$, °C | 0.1776 | 1 | | |
| $T_{opt}$, °C | 0.2778 | -0.5172 | 1 | |
| $T_{max}$, °C | -0.3312 | 0.4990 | -0.8477 | 1 |

Table D.4.6 Correlation of Parameter Estimates for ML 30 G, minimal (Shehata and Marr 1975).

| Correlations | $\mu_{opt}$, $h^{-1}$ | $T_{min}$, °C | $T_{opt}$, °C | $T_{max}$, °C |
|---|---|---|---|---|
| $\mu_{opt}$, $h^{-1}$ | 1 | | | |
| $T_{min}$, °C | -0.6005 | 1 | | |
| $T_{opt}$, °C | 0.9168 | -0.7777 | 1 | |
| $T_{max}$, °C | -0.9540 | 0.7430 | -0.9702 | 1 |

Table D.4.7 Correlation of Parameter Estimates for B/r, complex (Herendeen *et. al.* 1979).

| Correlations | $\mu_{opt}$, $h^{-1}$ | $T_{min}$, °C | $T_{opt}$, °C | $T_{max}$, °C |
|---|---|---|---|---|
| $\mu_{opt}$, $h^{-1}$ | 1 | | | |
| $T_{min}$, °C | 0.3003 | 1 | | |
| $T_{opt}$, °C | 0.1176 | -0.4436 | 1 | |
| $T_{max}$, °C | -0.2489 | 0.3000 | -0.5194 | 1 |

Table D.4.8 Correlation of Parameter Estimates for (Not defined), complex (Ratkowsky *et. al.* 1983).

| Correlations | $\mu_{opt}$, $h^{-1}$ | $T_{min}$, °C | $T_{opt}$, °C | $T_{max}$, °C |
|---|---|---|---|---|
| $\mu_{opt}$, $h^{-1}$ | 1 | | | |
| $T_{min}$, °C | 0.4462 | 1 | | |
| $T_{opt}$, °C | 0.0185 | -0.5339 | 1 | |
| $T_{max}$, °C | -0.1317 | 0.3795 | -0.5557 | 1 |

Table D.4.9 Correlation of Parameter Estimates for ML 30, minimal (Kovarova *et. al.* 1996).

| Correlations | $\mu_{opt}$, $h^{-1}$ | $T_{min}$, $^{o}C$ | $T_{opt}$, $^{o}C$ | $T_{max}$, $^{o}C$ |
|---|---|---|---|---|
| $\mu_{opt}$, $h^{-1}$ | 1 | | | |
| $T_{min}$, $^{o}C$ | 0.1504 | 1 | | |
| $T_{opt}$, $^{o}C$ | 0.3746 | -0.6330 | 1 | |
| $T_{max}$, $^{o}C$ | -0.0085 | 0.0071 | 0.0076 | 1 |

Table D.4.10 Correlation of Parameter Estimates for M 23 and SB 1, complex (Ross *et. al.* 2003).

| Correlations | $\mu_{opt}$, $h^{-1}$ | $T_{min}$, $^{o}C$ | $T_{opt}$, $^{o}C$ | $T_{max}$, $^{o}C$ |
|---|---|---|---|---|
| $\mu_{opt}$, $h^{-1}$ | 1 | | | |
| $T_{min}$, $^{o}C$ | 0.1832 | 1 | | |
| $T_{opt}$, $^{o}C$ | 0.3462 | -0.5838 | 1 | |
| $T_{max}$, $^{o}C$ | -0.3303 | 0.3823 | -0.5545 | 1 |

Table D.4.11 Correlation of Parameter Estimates for O157:H7, meat (Tamplin *et. al.* 2005).

| Correlations | $\mu_{opt}$, $h^{-1}$ | $T_{min}$, $^{o}C$ | $T_{opt}$, $^{o}C$ | $T_{max}$, $^{o}C$ |
|---|---|---|---|---|
| $\mu_{opt}$, $h^{-1}$ | 1 | | | |
| $T_{min}$, $^{o}C$ | 0.005 | 1 | | |
| $T_{opt}$, $^{o}C$ | 0.3882 | -0.5575 | 1 | |
| $T_{max}$, $^{o}C$ | -0.5821 | 0.4897 | -0.8155 | 1 |

## APPENDIX E – Referred publications from this research

Patil, R. A., Davey, K. R. and Daughtry, B. J. 2005. A new quantitative risk assessment of a fermenter for Friday 13th Syndrome, In: *Proc. 32nd Australasian Chemical Engineering Conference* (*Smart Solutions - Doing More with Less*), CHEMECA 2005, *Brisbane*, Queensland, Australia, September 25-29, paper 79 (ISBN 1864998326).

Davey, K. R., Patil, R. A. and Daughtry, B. J. 2006. A new quantitative risk assessment of a Monod continuous fermenter, *Transactions of the Institution of Chemical Engineers, Part C, Food and Bio products Processing* - in preparation.

Patil, R. A., Davey, K. R. and Daughtry, B. J. 2006. Assessment of cardinal - temperature predictive model for growth of *Escherichia coli* in a Monod continuous fermenter, *Food Research International* - in preparation.

Patil, R. A., Davey, K. R. & Daughtry, B. J. (2005, September). A new quantitative risk assessment of a fermenter for Friday 13th Syndrome. In *33rd Australasian Chemical Engineering Conference, CHEMECA 2005*. (p. 56). Brisbane, Australia.

## NOMENCLATURE

| Symbol | Definition | Unit |
|---|---|---|
| $a$ , $b$ , $c$ | mathematical parameters (Chapter 2) | dimensionless |
| $a_w$ | water activity (Chapter 2) | dimensionless |
| $A$ | asymptote [= $\ln(N_\infty / N_0)$ ] i.e. the maximal value reached (Chapter 2) | dimensionless |
| $b_1$ , $b_2$ , $b_3$ | Ratkowsky parameters (Chapter 2) | $^oC^{-1}\ h^{-0.5}$ |
| $c_2$ , $c_3$ | Ratkowsky parameters (Chapter 2) | $^oC^{-1}$ |
| $D$ | dilution rate (Chapter 3) | $h^{-1}$ |
| $D_{max}$ | maximum dilution rate (Chapter 3) | $h^{-1}$ |
| $D_{max\,output}$ | dilution rate at maximum output (Chapter 3) | $h^{-1}$ |
| $E$ | activation energy (Chapter 2) | J $mole^{-1}$ |
| $F$ | feed (Chapter 3) | $m^3\ h^{-1}$ |
| $H$ | enthalpy of activation (Chapter 2) | J $mole^{-1}$ |
| $k$ | rate constant (Chapter 2) | $s^{-1}\ (mole\ m^{-3})^{-1}$ |
| $k_0$, $k_1$, $k_2$ | collision factors or frequency factors (Chapter 2) | $s^{-1}\ (mole\ m^{-3})^{-1}$ |
| $K$ , $L$ , $M$ | constants (Chapter 2) | dimensionless |
| $K_s$ | limiting nutrient concentration at $\mu = \frac{\mu_{max}}{2}$ (Chapter 2, 3) | dimensionless |
| $N$ | number of micro organisms (Chapter 2) | cfu $mL^{-1}$ |
| $N_0$ | initial population density (Chapter 2) | cfu $mL^{-1}$ |
| $N_\infty$ | maximum population density (Chapter 2) | cfu $mL^{-1}$ |
| $R$ | universal gas constant (Chapter 2) | J $mole^{-1}\ K^{-1}$ |
| $s$ | concentration of the substrate (Chapter 3) | kg $m^{-3}$ |
| $s_f$ | concentration of the substrate in feed (Chapter 3) | kg $m^{-3}$ |

| | | |
|---|---|---|
| $t$ | time | s |
| $T$ | temperature (Chapter 2, 4) | $^{o}C$ |
| $T_h$ | temperature at which the enzyme is 50% inactivated (Chapter 2) | $^{o}C$ |
| $T_l$ | temperature at which the enzyme is 50 % inactivated (Chapter 2) | $^{o}C$ |
| $T_{max}$ | temperature above which no growth occurs (Chapter 2, 4, 5) | $^{o}C$ |
| $T_{min}$ | temperature below which growth is no longer observed (Chapter 2, 4, 5) | $^{o}C$ |
| $T_{opt}$ | optimum temperature (Chapter 2, 4, 5) | $^{o}C$ |
| $\upsilon$ | shape parameter (Chapter 2) | dimensionless |
| $V$ | reactor volume (Chapter 3) | $m^3$ |
| $x$ | concentration of cells (Chapter 3) | $kg\ m^{-3}$ |
| $(xD)_{max\ output}$ | productivity of the continuous fermenter at maximum output dilution rate (Chapter 3) | $g\ L^{-1}\ h^{-1}$ |
| $y$ | $[=\ln(N/N_0)]$ is the population density (Chapter 2) | dimensionless |
| $Y_{x/s}$ | yield factor (Chapter 3) | dimensionless |

**Greek letters**

| | | |
|---|---|---|
| $\lambda$ | lag time (Chapter 2) | h |
| $\mu$ | specific growth rate (Chapter 2, 3) | $h^{-1}$ |
| $\mu_{max}$ | maximum specific growth rate (Chapter 2, 3, 4, 5) | $h^{-1}$ |
| $\mu_{opt}$ | optimum specific growth rate (Chapter 2, 4, 5) | $h^{-1}$ |
| $\omega$ | $= \mu/\mu_{max}$ (Chapter 2) | dimensionless |

**Subscripts**

| | |
|---|---|
| *f* | input or feed (Chapter 2, 3) |
| *h* | high (Chapter 2) |
| *i* | initial condition (Chapter 2, 3) |
| *l* | low (Chapter 2) |
| *max* | maximum (Chapter 2, 3, 4, 5) |
| *min* | minimum (Chapter 2, 4, 5) |
| *o* | output (Chapter 3) |
| *opt* | optimum (Chapter 2, 4, 5) |
| *p* | product (Chapter 2) |
| *s* | substrate (Chapter 2) |
| *x* | cells (Chapter 2) |

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