THE EFFECT OF TEMPERATURE AND WATER ACTIVITY ON MICROBIAL GROWTH RATE AND FOOD SPOILAGE.

Robert Edward Chandler, B.Sc.(Hons.),

Being a thesis in fulfilment of the requirements for the degree of Doctor of Philosophy (Microbiology), at the University of Tasmania.

University of Tasmania,

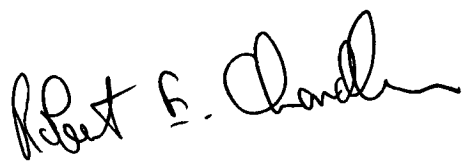
Hobart, Tasmania,

Australia,

January, 1988.

DECLARATION.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University, and to the best of my knowledge contains no copy or paraphrase of material previously published or written by any other person, except where due reference is made in the text of this thesis.



Robert Edward Chandler,
University of Tasmania,
Hobart, Tasmania,
Australia,
January, 1988.

ABSTRACT.

The Square Root Model [$\sqrt{r} = b(T - T_o)$] was used to describe the temperature dependence of bacterial growth rate under conditions where temperature was the only limiting factor. It was validated for predicting the growth of the bacteria responsible for the spoilage of pasteurised, homogenised milk and for the *in situ* spoilage of the milk, over the storage range 0 to 15°C.

A temperature function integrator, incorporating the Square Root Model and a T_o value of 263K was successfully used to monitor the temperature history of pasteurised, homogenised milk over a range of storage temperatures and to display the cumulated storage history at an arbitrary reference temperature (4°C).

The spoilage rate of pasteurised homogenised milk, with respect to temperature, was described accurately by a Square Root Equation, possessing a T_o value similar to that of the psychrotrophic pseudomonads responsible for the spoilage of the milk.

The Square Root Model described the temperature dependent variation in an induced bacterial lag phase, with the parameter, T_o, being similar to that of the exponentially growing cell.

The Square Root Model was shown to accurately predict bacterial growth under conditions where both temperature and water activity were limiting. Growth of the moderate halophile, *Staphylococcus xylosus* strain CM21/3, in media of different water activities, continued to be described by the Square Root Model, when either sodium chloride or glycerol was used as the humectant. The parameter T_o was constant, irrespective of water activity or the type of humectant used.

A decreasing linear relationship was demonstrated between growth rate and decreasing water activity, with the minimum water activity for growth being dependant upon humectant used. This enabled the derivation of a modified Square Root Model, which was capable of describing the effect of both temperature and water activity on bacterial growth rate.

The Square Root Model was validated for predicting the growth of the extreme halophiles, Halobacterium sp. strain HB9 and Halobacterium salinarium strain CM42/12, under conditions of varying water activity/salt concentration and temperature. The parameter T_o was constant irrespective of water activity. In addition, little change in growth rate, with change in water activity was noted.

TABLE OF CONTENTS.

LIST OF FIGURES.

LIST OF TABLES.

LIST OF COMMONLY USED ABBREVIATIONS.

$[CO_2]$	carbon dioxide concentration.
°C	degrees Celsius.
μ	temperature characteristic (=E).
A	collision factor.
a_w	water activity.
a_{wo}	theoretical a_w below which growth ceases.
ATP	adenosine triphosphate.
b	slope of the line predicted by the Square Root Model.
b_o	a constant in the general model relating growth rate to temperature and water activity
cfu	colony forming units.
E	apparent activation energy (=μ).
Eh	redox potential.
ERV	extract release volume.
GABA	gamma aminobutyric acid.
GT	generation time.
hr	hour.
K	Kelvin.
min	minute.
MHA	moderate halophile agar.
MHB	moderate halophile broth.
MHS	moderate halophile saline.
MPN	most probable number.
NA	nutrient agar.
OD	optical density.
PCA	plate count agar.
PCB	plate count broth.
pH	$-\log[H^+]$.
pO_2	oxygen partial pressure.
PsA	*Pseudomonas* agar.
Q_{10}	temperature coefficient.
r	growth rate.
RH	relative humidity.
saline	0.9% NaCl(w/v).
T_o	theoretical temperature at which growth rate is zero.
T_{min}	analagous to T_o.
TGI	temperature gradient incubator.

TTC	2,3,4-triphenyltetrazolium chloride.
TTFI	time/temperature function integrator(s).
TTM	time/temperature monitor.
XHA	extreme halophile agar.
XHB	extreme halophile broth.
XHS	extreme halophile saline.

PUBLICATIONS.

(1) Chandler, R.E. & McMeekin. T.A., (1985). Temperature function integration and the prediction of the shelf-life of milk. Aust. J. Dairy Technol. 40: 10-13.

(2) Chandler, R.E. & McMeekin, T.A., (1985). Temperature function integration and its relationship to the spoilage of pasteurized, homogenized milk. Aust. J. Dairy Technol. 40: 37-39, v.

(3) McMeekin, T.A., Chandler, R.E., Doe, P.E., Garland, C.D., Olley, J., Putro, S. & Ratkowsky, D.A., (1987). Model for combined effect of temperature, salt concentration/water activity on the growth rate of Staphylococcus xylosus. J. Appl. Bacteriol. 62: 543-550.

ACKNOWLEDGEMENTS.

I wish to acknowledge most sincerely my supervisor Dr. T.A. McMeekin, Reader in Agricultural Microbiology in the Faculty of Agricultural Science, University of Tasmania, for his constant encouragement, aid, guidance and criticism throughout this study.

Dr. J. Olley, CSIRO Division of Fisheries Research, Hobart, for her advice and lateral thinking ability.

Dr. D.A. Ratkowsky, CSIRO Division of Mathematics and Statistics, Hobart, for his assistance with mathematical modelling and statistics.

The technical and teaching staff of the Department of Agricultural Science at the University of Tasmania for their assistance.

The Australian Dairy Research Committee and the Australian Centre for International Agricultural Research, for without their funding, this study would never have been undertaken.

To my fiancee, Robyn Burrows, for her care, love and understanding throughout my studies.

1. LITERATURE REVIEW.

1.1: INTRODUCTION.

During storage, foods develop a characteristic spoilage association. As a result of microbial growth the quality of perishable stored food deteriorates to the point where overt spoilage is reached (McMeekin & Olley, 1986). Hence, in order to establish the effectiveness of a particular food process or storage regime on product shelf-life, it is necessary to understand and to be able to predict the responses of the microbial microbiota involved.

1.2: FACTORS AFFECTING THE GROWTH OF SPOILAGE MICROORGANISMS.

Four groups of factors are active in selecting which members of an initial microbiota will be responsible for the spoilage of a food:

(1) intrinsic factors, which are an expression of the physical properties, the chemical composition and some biological attributes of the food itself. These include water activity (a_w), pH, redox potential (Eh) and the availability of nutrients.

(2) modification of the initial microbiota, due to, processing the food, using irradiation, a heat treatment of variable lethality, a chemical treatment resulting in a change in the chemical composition of the food, or specifically bactericidal to certain strains of bacteria.

(3) extrinsic factors, such as, the temperature, relative humidity, or oxygen partial pressure (pO_2), of the environment in which the food is stored.

(4) implicit factors, such as, the comparative specific growth rates and antagonistic or synergistic interactions amongst the microorganisms selected by the the above three factors (Mossel, 1977).

It is often the case that the development of a spoilage

microbiota is not solely determined by any one factor or group of factors but is as a result of the interaction and combination of the above factors and/or groups. For example: changes in the a_w of a food may be directly related to the changes in relative humidity of the storage atmosphere; the inhibitory effect on bacterial growth of a specific pH food, depends upon the values of other parameters, such as, the type of acid used to establish a specified pH, temperature and pO_2; the Eh of a food is dependent on the chemical composition of the food and the pO_2 prevailing during storage (Mossel, 1977).

Of the many factors that influence the rate of change of microbial numbers in foods, temperature, a_w and pH have been considered the most important (Wodzinski & Frazier, 1960 and Roberts & Jarvis, 1983). Increased shelf-life of food products may be achieved by retarding bacterial growth by altering these factors to establish conditions which are not favourable for the growth of the relevant spoilage microorganisms.

In order to understand how a particular food product will deteriorate under specified storage conditions, it is necessary to understand how the microorganisms associated with that product will be affected by the storage environment.

1.3: THE EFFECT OF STORAGE TEMPERATURE ON MICROBIAL SELECTION.

Under conditions where water activity and pH are non-limiting, temperature is the major factor affecting the rate of microbial growth and hence the deterioration and spoilage of foods, such as milk, chicken, red meat, and fish (Pooni & Mead, 1984). These foods are generally of pH >5.5 and a_w >0.98. When stored aerobically at chill temperatures, they are spoiled by psychrotrophs, which are members of a physiological group of microorganisms, capable of

growing well at refrigeration temperatures having temperature optima of <35°C (Eddy, 1960 and Morita, 1975).

Pseudomonas, Alteromonas, Acinetobacter, Moraxella, lactobacilli and some members of the Enterobacteriaceae, constitute the psychrotrophic bacteria which are important in the chill store spoilage of fish (Shewan, 1962 and Lerke et al., 1965), chicken (Barnes & Impey, 1968 and Thomas & McMeekin, 1981), meat (Ingram & Dainty, 1971; Gill & Newton, 1977 and 1978) and milk (Sherman et al., 1941; Olson, 1967 and Cousin, 1982). Under aerobic conditions at temperatures close to 0°C, the pseudomonads are usually dominant, as they possess faster growth rates (Olsen & Jezeski, 1963; Gill & Newton, 1977 and Mol & Vincentie, 1981) and have a greater affinity for oxygen than their competitors (Gill & Newton, 1978). However, as the storage temperature increases, Acinetobacter, Moraxella and enteric species begin to become the dominant spoilage microorganisms present (Barnes & Thornley, 1966).

As storage temperatures approach 20°C, the spoilage association becomes predominantly mesophilic. High levels of carbon dioxide, oxygen limiting and microaerophilic conditions suppress pseudomonad growth and allow facultatively anaerobic bacteria such as Lactobacillus, Alteromonas and members of the family Enterobacteriaceae to become dominant (Gill & Newton, 1978 and Thomas, 1984).

Under some circumstances, spoilage may be a mesophilic process primarily due to poor temperature control during handling. Gill (1984) reported the initial temperatures of livers in abattoirs, to be between 30-39°C. When these temperatures were maintained for several hours and were followed by a period of 8-10 hours before a chill temperature of 5°C was achieved, the microbiota was composed predominantly of Escherichia coli and the shelf-life of the livers

was greatly reduced.

1.4: THE RELATIONSHIP BETWEEN TEMPERATURE AND MICROBIAL GROWTH RATE.

Attempts to formulate a relationship between microbial growth rate and temperature, have led to the development of several different mathematical models:

1.4a: The Reaction Rate Temperature Rule:

The reaction rate temperature rule may be used to give an indication of the alteration of biological reaction rates with respect to temperature. It yields the temperature coefficient Q_{10}, which is calculated from the quotient of the measured rates at experimental temperatures which differ by 10°C (Precht et al.,1973). However, Q_{10}'s have the inherent property of varying with temperature (Bělehrádek, 1926; Precht et al.,1973 and Olley & McMeekin, 1985) and hence are of limited use.

1.4b: The Arrhenius Equation:

The effect of temperature on the rate of microbial growth has been traditionally described by a modification of the Arrhenius equation. In this modified equation, the chemical reaction rate constant has been replaced by: the growth rate constant for bacterial growth (Ingraham, 1958; Baig & Hopton, 1969 and Reichardt & Morita, 1982); or the spoilage rate (Labuza, 1982). The modified Arrhenius equation is of the form:

$$k = A \exp^{(-\mu/RT)} \qquad (1)$$

where k = spoilage/growth rate at temperature T (K),

μ = temperature characteristic,

R = universal gas constant.

Figure 1.1 (reproduced from Ratkowsky et al.,1982) demonstrates the typical non-linear response of microbial growth rate with respect to temperature, when represented graphically as Arrhenius plots.

The significance and use of the Arrhenius equation will be discussed in detail in the sections which follow.

1.4c: The Schoolfield Model:

The non-linear Arrhenius relationship of Schoolfield et al.,(1981) was modified by Broughall et al.,(1983). The latter developed a two dimensional model capable of describing the combined effects of temperature and water activity, on bacterial growth kinetics.

The model proposed by Schoolfield et al.,(1981) (which is a reparameterisation of the model proposed by Sharpe & DeMichele, 1977) is of the form:

$$\frac{1}{K} = \frac{\rho(25)\,\frac{T}{298}\exp\frac{HA}{R}\left(\frac{1}{298}-\frac{1}{T}\right)}{1+\exp\frac{HL}{R}\left(\frac{1}{T_{\frac{1}{2}L}}-\frac{1}{T}\right)} \qquad (2)$$

where R is the universal gas constant, K is the kinetic parameter (either lag time or generation time), $\rho(25)$ is the inverse of the fitted kinetic parameter at 25°C, T the temperature in degrees absolute, HA is a constant describing the enthalpy of activation for microbial growth, HL is another constant describing the enthalpy of low temperature inactivation of growth and $T_{\frac{1}{2}L}$ describes the temperature inactivation of growth rate.

This model was extended by Broughall & Brown (1984) to include the influences of both a_w and pH on K, by replacing the constants in equation (2) with the following equations:

$$\ln\rho(25) = F1 + G1(X - XS) + H1(Y - YS) \qquad (3)$$

Figure 1.1: Arrhenius Plots of Five Bacteria and a Fungus.*

Data sets redrawn from Johnson et al.,(1974).

for: △ Bacillus circulans,

○ Lactobacillus delbruckii,

□ ,✱ Escherichia coli,

◇ Enterobacter aerogenes,

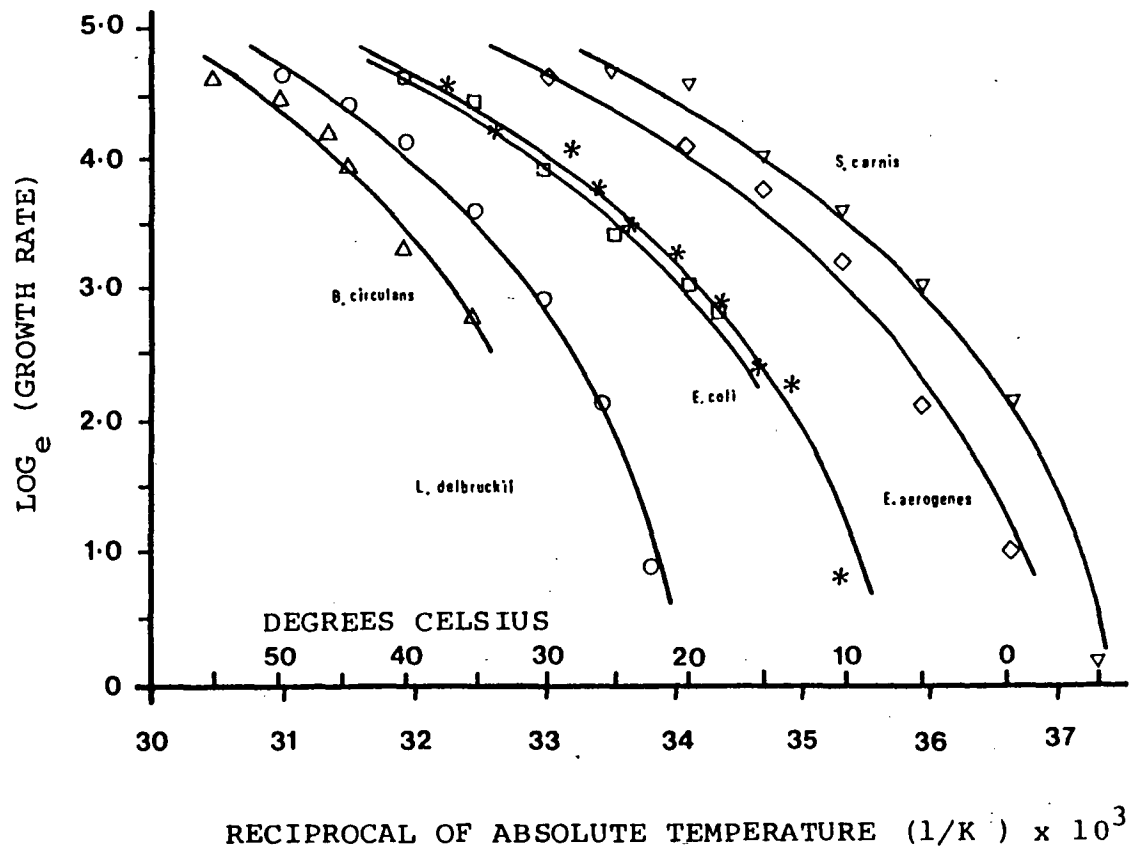
▽ Sporotrichum carnis.

The solid curves correspond to the equation:

$\sqrt{r} = b(T - T_o)$

* Reproduced from Ratkowsky et al.,(1982).

Figure 1.1: Arrhenius Plots of Five Bacteria and a Fungus.



$$HA = F2 + G2(X - XS) + H2(Y - YS) \quad (4)$$

$$HL = F3 + G3(X - XS) + H3(Y - YS) \quad (5)$$

$$T\tfrac{1}{2}L = F4 + G4(X - XS) + H4(Y - YS) \quad (6)$$

where F1-F4, G1-G4 and H1-H4 are constants fitted to give the best fit for the data, X and Y are the values for the a_w and pH variables respectively and XS and YS are the approximate midrange values for the variables X and Y.

Lowry & Ratkowsky (1983) have criticised the statistical properties of the Schoolfield model. They stated that it had an undesirably high degree of intrinsic and parameter-effects non-linearity, which lead to biases and non-normally distributed parameter estimators.

1.4d: The Square Root Models:

The "Square Root Model" was developed by Olley and Ratkowsky from the observation by Ohta and Hirahara (1977), that an empirical linear relationship existed between temperature and the square root of the rate of rigor nucleotide breakdown, in chill stored carp muscle. Olley (1983), stated that she had been interested in the effect of rigor mortis on spoilage and that upon noticing this relationship, had applied it to other spoilage data from the literature. It was found that experimental data over the temperature range 0-16°C, could be predicted accurately by the Square Root Model. Olley's observations led Ratkowsky et al.,(1982), to demonstrate that a relationship of the same form, could be used to accurately describe the effect of temperature on bacterial growth rate (Figure 1.2):

$$\sqrt{r} = b(T - T_o) \quad (7)$$

where r = the growth rate constant,

b = the slope of the regression line,

Figure 1.2: Square Root of Growth Rate Versus Temperature for Pseudomonas sp. Strain 16L16.*

Growth rates were measured as the reciprocal of the time to reach 25% turbidity.

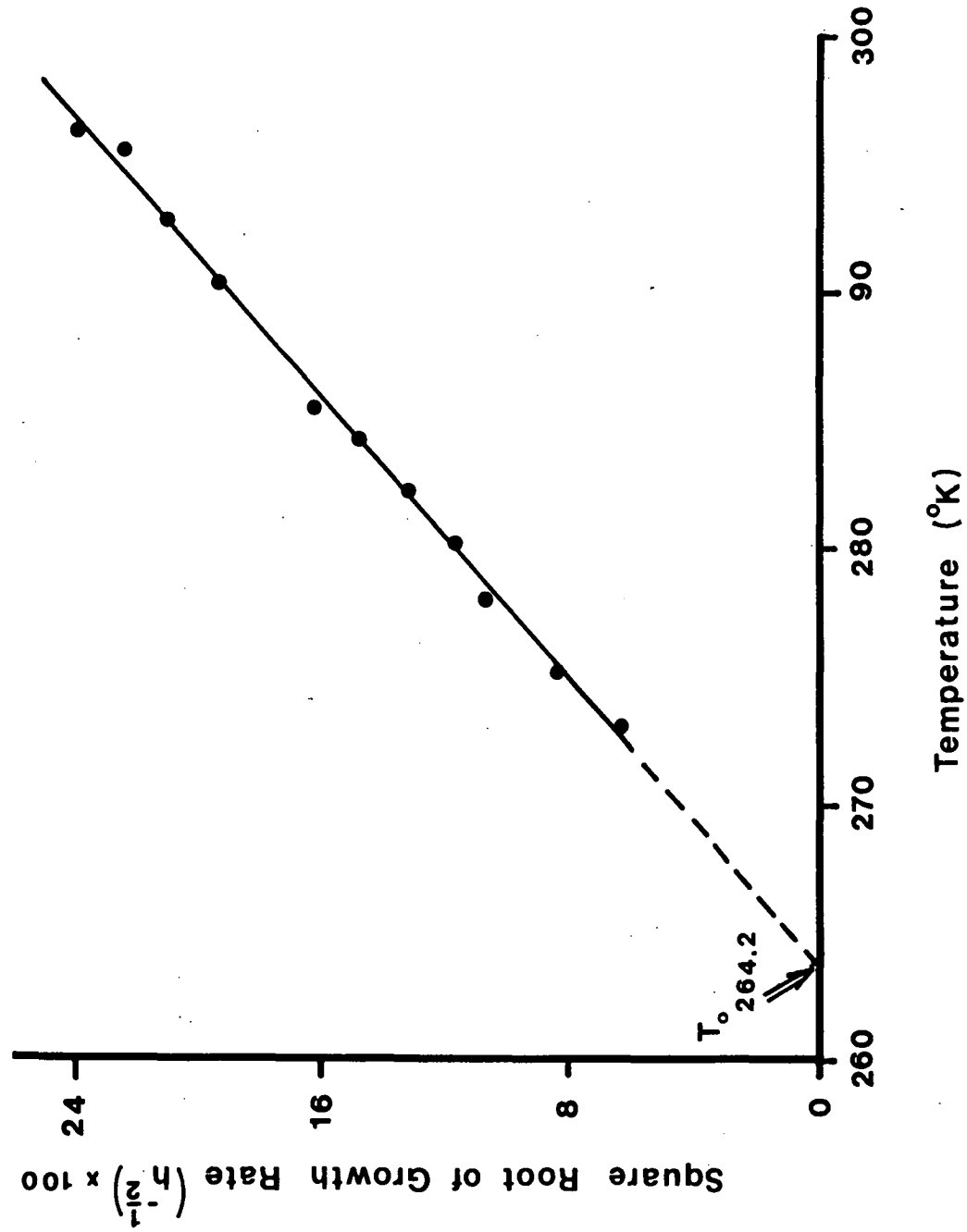
The fitted line predicted by the equation:

$\sqrt{r} = b(T - T_o)$

is extrapolated to the x-axis to yield a T_o value of 264.2K.

* Reproduced from Ratkowsky et al.,(1982).

Figure 1.2: Square Root of Growth Rate Versus Temperature for Pseudomonas sp. Strain 16L16.



T = the temperature in K,

T_o = a conceptual temperature of no metabolic significance.

It must be noted that this equation was limited to describing bacterial growth, only at temperatures below the optimum temperature for growth. Subsequently, Ratkowsky et al.,(1983) extended the Square Root Model to allow for the prediction of bacterial growth rates throughout the entire biokinetic temperature range (Figure 1.3):

$$\sqrt{r} = b(T - T_{min})[1 - \exp^{c(T - T_{max})}] \quad (8)$$

where T_{min} and T_{max} are respectively the minimum and maximum temperatures at which the rate of growth is zero (T_{min} is equivalent to T_o in equation (7)); b, as in equation (7), is the regression coefficient of the line formed at temperatures below the optimum temperature and c is a constant which enables the model to fit the data for temperatures greater than the optimum temperature.

Gill (1984) acknowledged the usefulness of the Square Root Model of Ratkowsky et al.,(1982) to describe the growth rate of E. coli, but stated that there existed a distinct change in slope above 30°C and a plateau between 40°C and 45°C. He terminated his simple three phase plot at the observed maximum and minimum temperatures for growth of 8°C and 45°C. He also applied the extended Square Root Model (equation (8)) to his data for E. coli but considered that it offered no obvious advantage over the simple Square Root Model, as at higher temperatures, it did not fit the experimental growth rate values well.

Ingraham et al.,(1983) indicated that the T_{min} value of E. coli (3.5°C) did not accurately predict the minimum temperature for growth of 8°C. This led them to suggest that the square root relationship

Figure 1.3: Square Root of Growth Rate Versus Temperature for Nine Bacterial Cultures.*

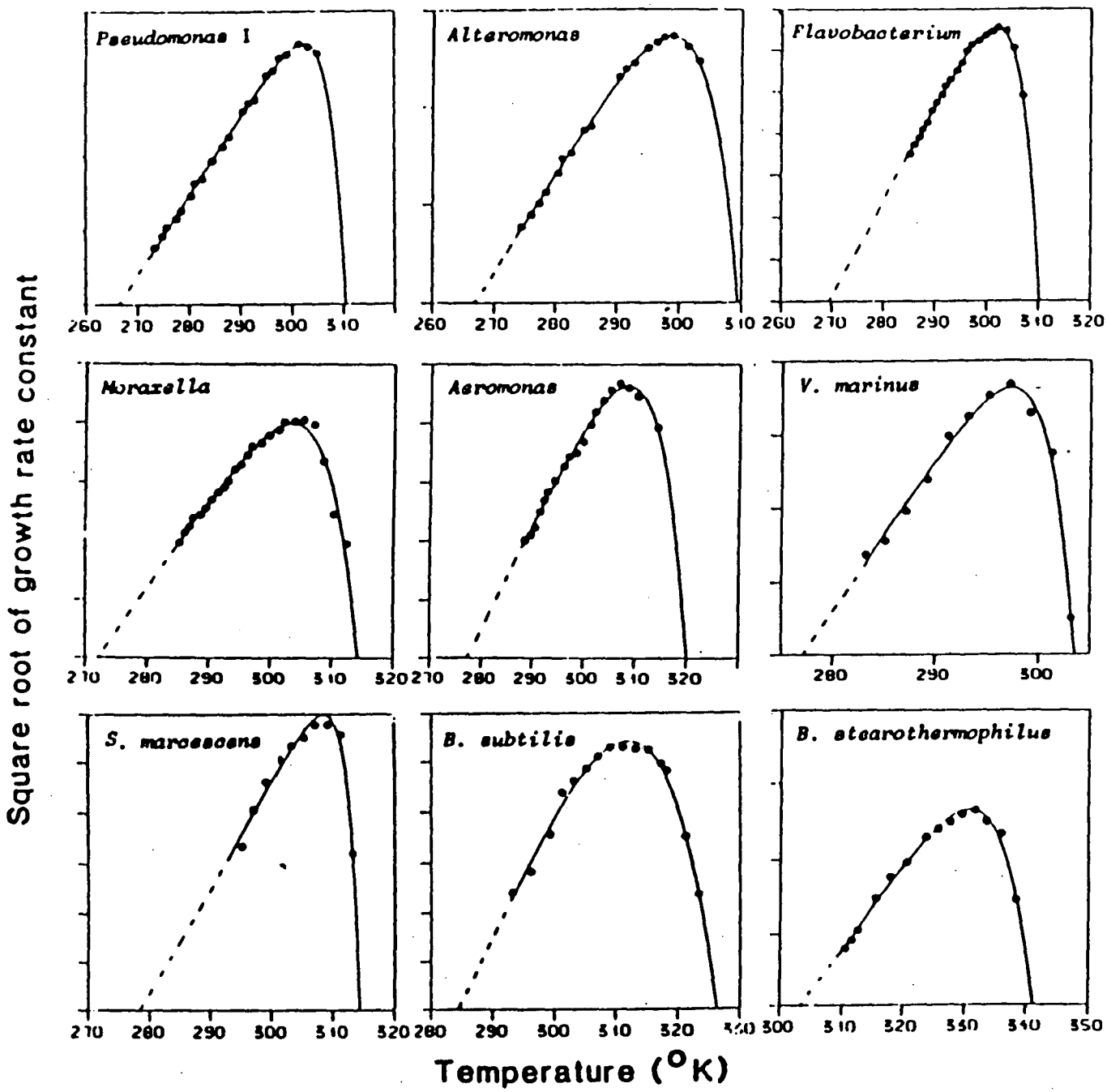
Data and fitted lines for the equation:

$$\sqrt{r} = b(T - T_{min})[1 - \exp^{c(T - T_{max})}]$$

for nine bacterial cultures.

* Reproduced from Ratkowsky et al.,(1983).

Figure 1.3: Square Root of Growth Rate Versus Temperature for Nine Bacterial Cultures.



was not valid in the extreme low temperature range. Inspection by McMeekin et al.,(1987) of the data of Ingraham et al.,(1983) indicated an almost perfect linearity between 13.5°C and 35°C. Shaw et al.,(1971) found no growth for E. coli at temperatures less than 7.8°C. McMeekin et al.,(1987) found the minimum temperature for growth also to be 7.8°C and reported no growth after 30 days incubation at 7.2°C. Similarly, cells incubated at 5°C for 5 months, failed to grow. Hence, it was concluded, that even though the minimum temperature for growth may occur several degrees above the extrapolated T_{min} value, the square root relationship remained valid to the point at which growth ceased.

Smith (1985) determined the effect of temperature on lag and generation times for E. coli. Analysis of his data by McMeekin et al.,(1987), indicated that both parameters obeyed the Square Root Model and yielded similar T_{min} values, respectively 274.9K and 275.8K.

McMeekin et al.,(1987) stated that both the Square Root Model and the Arrhenius equation were empirically based and that as yet no physiological explanation for the square root response of bacterial growth rate to temperature was available. However, they noted that Koch (1970) had shown bacterial growth rate to be related to the fourth root of the rate of ribosome synthesis. They stated that the physiological basis of the relationship was still awaiting detailed studies in the fields of microbial physiology and genetics, with particular reference to biosynthetic processes, which were likely to be the key to the control of growth rate. The degree of uncoupling of catabolic and anabolic processes as temperatures diverge from the optimum was also stated to warrant consideration.

1.4e: The Bělehrádek Equation:

Equation (7) has been shown by Ross (1986) to be a special case of the temperature function described by Bělehrádek (1935), which is given by the equation:

$$r = a(t - \alpha)^d \qquad (9)$$

where r = development rate, and a, d and α are constants to be fitted for the particular organism under study.

This equation has been used extensively for describing the temperature dependence of the rate of development or growth of a wide variety of aquatic animals (McLaren et al., 1969). The constant α is the so-called "biological zero", the theoretical temperature at which the development rate is zero, or the development time is infinite, and is equivalent to T_{min} in the Square Root Model. The exponent d varies from organism to organism but values around 2 for growth rate (or -2 for development time) are common (Cooley & Minns, 1978). When d = 2, equations (9) and (7) are equivalent, as the exponent 2 in equation (9) may be taken to the left-hand side of equation (7) as a square root.

1.5: THE MECHANISM OF ACTION OF TEMPERATURE ON MICROBIAL GROWTH.

1.5a: Low Temperature:

The effect of decreasing the incubation temperature of microorganisms from their optimum growth temperature, is to gradually slow down their growth rate, which eventually results in the cessation of growth. For many microorganisms, growth ceases at temperatures above the freezing point of the medium. As cold gradually slows down and finally prevents growth, there is a common effect of increasing both lag period and generation time. The pattern of response is virtually the same for both. The minimum growth temperature can be regarded as the point when either the lag period

or generation time becomes infinite (Ingram & Mackey, 1976). Campbell & Williams (1953) and Long & Williams (1959) demonstrated that the minimum temperature for the growth of thermophiles decreased with the addition of suitable nutrients.

The effect of altering optimal growth conditions by altering water activity or pH, is to increase the minimum temperature at which growth is observed. If the limitation of growth at low temperatures was due to specific effects such as changes in the permeability or fluidity of membranes, as is suggested by Farrell & Rose (1967 & 1968), then a reduction in water activity may cause these changes to occur at a higher temperature. However, as the same effect is observed for alterations caused by a change in pH, or sublethal damage caused by heat or radiation, then it is unlikely that all these factors could produce essentially the same response from the cell, by the mechanism suggested by Farrell & Rose (Ingram & Mackey, 1976).

Ingram & Mackey (1976) suggested that if the inactivation was due to a more general phenomenon, such as the failure of the cellular homeostatic mechanisms at low temperatures, then it was possible that, in the presence of inhibitors, the same temperature dependent imbalance may occur at higher temperatures. They stated that it would then be possible to make biochemical comparisons of the growth limitations of a psychrotroph and mesophile at the same temperature, by adjusting factors like pH and solute content of the medium.

1.5b: High Temperature:

At temperatures greater than the optimum temperature for growth, growth rate generally decreases rapidly to a minimum. Allwood & Russell (1970), in a review article on thermal injury in bacteria,

indicated that exposure to temperatures above the optimum may cause: damage to the cell wall and cytoplasmic membrane, and hence a loss in the selective permeability character of cells; alteration of metabolic and biosynthetic capabilities; alteration or loss of enzyme activity; degradation of ribosomal RNA; and damage to DNA.

Three general theories have been presented to explain the survival and growth of thermophiles at elevated temperatures. These have been developed around the following concepts: (i) stabilisation may be achieved through lipid interaction; (ii) heat-denatured components may be rapidly resynthesised; and (iii) thermophilic microorganisms may possess macromolecular complexes with an inherent heat stability (Singleton & Amelunxen, 1973).

(i) It has been noted that the heat stability of cells, and hence the upper limit for growth, is correlated with the melting point of their lipids. Bělehrádek (1935) developed the concept that the collapsing of structures in the protoplasm or plasma membrane, depended upon the melting of the cellular fat. Gaughran (1947) reported that the lipids of a stenothermophilic bacillus approached solidity as the minimum temperature for growth was approached. He suggested that the consistency of the fats may prevent active metabolism at low temperatures and hence fix the minimum temperature for growth, as below the solidification point, cells are not able to grow. The fact that the degree of saturation, length of chains and melting point of lipids increase with increasing growth temperature, is in agreement with both of the above views (Allwood & Russell, 1970).

(ii) In a review by Allen (1953), evidence is presented to support the hypothesis that growth at elevated temperatures is simply the result of rapid resynthesis of heat denatured cellular components. Further supporting evidence has been provided by Bubela &

Holdsworth (1966a,b) who noted that the rates of protein and nucleic acid synthesis and turnover in Bacillus stearothermophilus was much faster than those for E. coli.

(iii) Singleton & Amelunxen (1973) proposed three mechanisms to explain the survival of thermophiles in molecular terms: (a) thermophiles may contain factors which increase the stability of their components with respect to heat; (b) mesophiles may contain components which increase their lability with respect to heat; and (c) cellular components of thermophiles may have an inherent heat stability independent of exogenous factors.

Amelunxen & Lins (1968) compared the thermostability of eleven enzymes from a thermophilic and a mesophilic bacillus and found that with only two exceptions, the enzymes from the mesophile were more heat labile than the enzymes from the thermophile. They also demonstrated that the proteins from the thermophile showed greater resistance to coagulation by heat than those of the mesophile.

Singleton & Amelunxen (1973) concluded that most of the available evidence supported the hypothesis that thermophilic microorganisms synthesise macromolecular components which possess an intrinsic thermostability which is independent of any transferable stabilising factors. They stated that in all likelihood, the survival of a thermophile was the result of the interaction of several mechanisms, e.g., a more stable membrane, more rapid growth, some type of structural stabilising, with the primary contributing factor being the inherent heat stability of its cellular proteins.

1.6: THE EFFECT OF WATER ACTIVITY ON MICROBIAL GROWTH.

Water activity (a_w) may be defined in terms of: the ratio of the vapour pressure of a solution, to the vapour pressure of pure water at the same temperature; solute concentration; osmotic pressure;

water potential; and the equilibrium relative humidity (Troller, 1983).

Water activity is a measure of the portion of the total water in a system, which is readily available for chemical and biological reactions (Gailani & Fung, 1986). Hence, the a_w of an environment, has a dramatic effect on the microbiota present and its ability to proliferate (Table 1.1). At water activities of 1.00 to 0.98, bacteria dominate the spoilage microbiota of foods as they generally outgrow both the fungi and yeasts present. At water activities <0.95, most Gram negative rods cease to grow and their spoilage role is taken over by Gram positive cocci and lactobacilli. At water activities <0.88, growth of most bacteria and yeasts cease, with the exception of osmophilic yeasts in syrups and halophilic bacteria in strongly salted foods. In this range, moulds are usually dominant, with xerophilic species dominating at water activities <0.75 (Mossel, 1971).

It is often more useful to consider the water content (W) of a food, rather than its water activity. The "alarm water content" of a food is defined as the highest value at which microbial spoilage will not occur during the usual conditions and period of storage of the food (Mossel, 1977).

To interpret water activity data in terms of W, the relationship between the percentage water content and a_w, the water sorption isotherm, is required. This function varies between foods, as it is dependent upon the intensity by which water is bound to the components of the dry food. Lipids do not possess any significant water binding capacity and hence there is a greater proportion of free water molecules. Cellulose binds water weakly, whereas starch, sugars, salts and the water soluble extractives of animal tissues

Table 1.1: Water Activity, Water Content and the Microbial Spoilage of Some Foods*.

a_w range	Organisms inhibited by the lowest value of this range	Examples of foods with such a water activity
1.00-0.95	Gram negative rods; bacterial spores; some yeasts	Foods of c. 40% (w/w) sucrose or c. 7% (w/w) NaCl[a] e.g. cooked sausages; bread crumbs
0.95-0.91	Most cocci; lactobacilli; some moulds; vegetative cells of Bacillaceae	Foods of c. 55% (w/w) sucrose or c. 12% NaCl[a] e.g. dry ham; medium age cheese
0.91-0.87	Most yeasts	Foods of c. 65 %(w/w) sucrose (saturated) or foods of c. 15% NaCl[a] e.g. salami; "old" cheese
0.87-0.80	Most moulds; *Staphylococcus aureus*	Flour, rice, pulses, etc.; of 15-17% water; sweetened condensed milk (c. 0.83)
0.80-0.75	Most halophilic bacteria	Foods with 26% NaCl[a] (saturated); marzipan with 15-17% water; jam and marmalade
0.75-0.65	Xerophilic moulds	Rolled oats containing c. 10% water
0.65-0.60	Osmophilic yeasts	Dried fruits containing 15-20% water; toffees and caramels with c. 8% water
0.60-0.50	Area of water activity which will not allow any microbial proliferation	Noodles etc., of c. 12% water; spices of c. 10% water
0.30		Egg powder of c. 5% water Biscuits; rusks; bread crusts of c. 3-5% water
0.20		Milk powder of 2-3% water; dried vegetables of c. 5% water Humidity existing in deserts

[a] for foods containing more than traces of fat, solute contents are expressed as % of aqueous phase.

* Reproduced from Mossel (1971).

(Olley, 1980), bind water strongly. Mossel (1977) stated that as a water activity of approximately 0.70 gives reasonable protection against microbial spoilage, the alarm water content for different foods can be calculated from their respective water sorption isotherms.

When considering the growth of microorganisms in foods of lowered water activity, it is necessary to consider the method used to prepare the food. Labuza et al.,(1972) prepared intermediate moisture systems (water activity range 0.9 to 0.6) to similar water activities, using both adsorption and desorption methods. They found that moisture sorption hysteresis occurred (i.e., at any given moisture content, the water activity during adsorption is greater than the water activity during desorption) and that the minimum water activity for growth on the desorption branch was lower than that on the adsorption branch. As the desorption branch represents a higher moisture content than the adsorption branch at the same water activity, they concluded that not only water activity but also the amount of moisture contributes to controlling microbial growth.

Acott & Labuza (1975) found that hysteresis occurred above a water activity of 0.86 for an intermediate moisture pork system. When the system was adjusted to a water activity of 0.92 by desorption, it was found to contain 67.5% moisture. However, when prepared to the identical water activity by adsorption, it contained 55.9% moisture. A strain of Staphylococcus aureus inoculated into these systems grew in the presence of 67.5% moisture but not in 55.9% moisture.

1.7: THE EFFECT OF WATER ACTIVITY ON MICROBIAL GROWTH RATE.

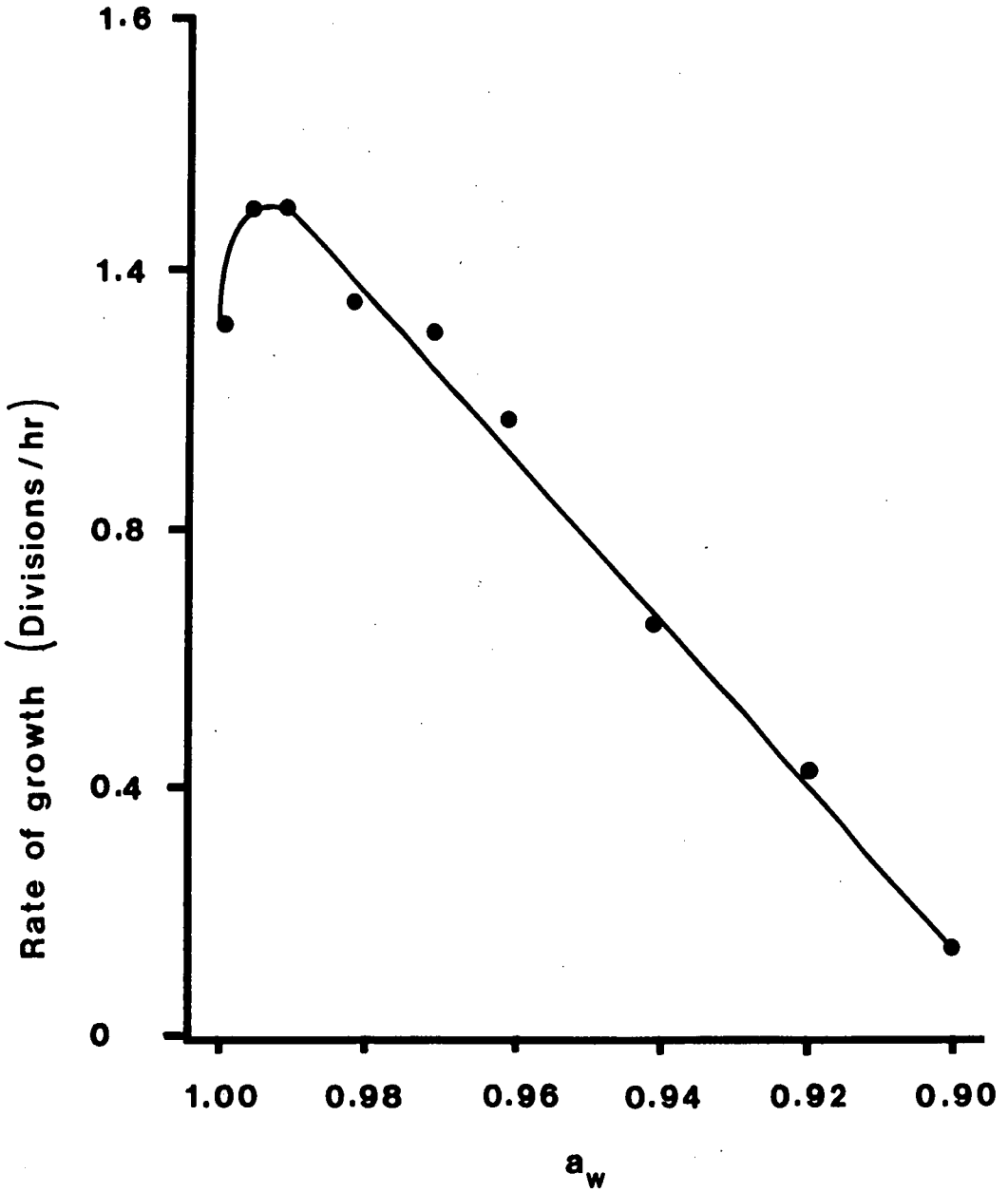
Growth rates for individual microorganisms alter with change in water activity. Figure 1.4 (reproduced from Scott, 1953) shows the

Figure 1.4: Relation Between Mean Growth Rate and Water Activity for 14 Strains of Staphylococcus aureus.*

S. aureus strains were grown in a basal medium of mineral salts, casamino acids, yeast extract and casitone, with a_w being adjusted using a mixture of NaCl, KCl and Na_2SO_4 in the ratio 5:3:2.

* Reproduced from Scott (1953).

Figure 1.4: Relation Between Mean Growth Rate and Water Activity for 14 Strains of Staphylococcus aureus.



typical relationship between a_w and bacterial growth rate for Staphylococcus aureus, i.e. as a_w decreases from 1.00, the growth rate increases to a maximum, and then decreases in a linear fashion. This linear decrease has been demonstrated for both bacteria (Christian & Scott, 1953 and Brownlie, 1966) and yeasts (Anand & Brown, 1968; Casolari et al., 1978 and Troller & Christian, 1978).

Christian & Scott (1953), Marshall & Scott (1958) and Anand & Brown (1968), have also presented graphs similar to Figure 1.4, with the data being interpreted as curves. However, examination of the graphs revealed them to be almost linear.

Scott (1957) concluded, that as water activity was reduced below an optimum level, there was an increase in the lag phase, a decrease in growth rate and a decrease in the amount of cell substance synthesised. He also noted that these effects were very similar to those produced by a reduction in growth temperature. At water activities lower than the minimum for growth, cells either remain dormant or die (Sperber, 1983).

When studying the influence of water activity on microbial growth rate and selection, changes in water activity result in different microbial growth profiles. The solute used to achieve the reduced water activity has a definite effect on the magnitude of growth achieved, the rate of growth and the limiting water activity at which growth would occur (Anand & Brown, 1968 and Strong et al., 1970). Materials such as, sugars, high molecular weight carbohydrates, proteins, salts and additives such as polyols, are used to alter the water activity of solutions (Strong et al., 1970; Marshall et al., 1971 and Notermans & Heulvelman, 1983). Marshall et al., (1971) found that when glycerol and NaCl were compared at similar levels of water activity, glycerol was more inhibitory than NaCl, to

relatively salt tolerant bacteria and less inhibitory than NaCl, to salt sensitive bacteria.

1.8: THE MECHANISM OF ACTION OF WATER ACTIVITY.

The lower limit for growth of microorganisms depends in part upon the abilities and characteristics of the various microorganisms to accumulate various solutes within their cells. Such "compatible" solutes are capable of replacing cellular water without impairing the normal functioning of the cell (Sperber, 1983). Different groups of microorganisms possess different mechanisms for handling the changes in water activity of their growth media.

Osmophilic yeasts (Brown, 1974) and fungi (Lewis & Smith, 1967) have been shown to accumulate intracellularly, polyalcohols such as glycerol, arabitol or mannitol, in order to counteract the osmotic imbalance which occurs when the water activity of the surrounding environment is lowered. Intracellular accumulation of polyols, may function in preventing the dehydration of the aerial mycelia of moulds.

Edgley & Brown (1978) found the limit of tolerance of various yeasts to water stress was not only due to the ability of the cells to accumulate compatible solutes but also to the amount of energy required to produce the solutes. They showed that Saccharomyces cerevisiae accumulated as much glycerol as Saccharomyces rouxii, when exposed to reduced water activity, but that it could not tolerate water activities as low as those tolerated by S. rouxii. The difference in tolerance, was attributed in part to the different methods by which glycerol was accumulated by the two species. In S. rouxii, the osmoregulatory mechanism was primarily conservative in biophysical (permeation/transportation), whereas S. cerevisiae, it was

primarily metabolic and hence required a greater energy input.

Generally, bacteria accumulate intracellularly an amino acid or related molecule, such as glutamate, proline or gamma-aminobutyric acid (GABA), when stressed osmotically (Measures, 1975 and Sperber, 1983). Notable exceptions to this, are members of the family Halobacteriaceae, which actively accumulate ionic potassium against a concentration gradient without any adverse intracellular effects (Tindall & Trüper, 1986).

Under normal conditions, Gram negative bacteria usually have a low total amino acid pool and low internal potassium ion concentration. When stressed osmotically, they generally accumulate glutamic acid, which is negatively charged at cellular pH and usually enters the cell as a potassium salt. When the glutamate concentration is high enough to reduce the intracellular water activity to around 0.95, the potassium ion concentration begins to inhibit enzymic processes and causes the cessation of growth (Measures, 1975 and Sperber, 1983). The minimum water activities for growth in NaCl for Pseudomonas aeruginosa and Vibrio parahaemolyticus were stated by Measures (1975) to be 0.970 and 0.950 respectively.

Measures (1975) reported that under normal conditions, Gram positive bacteria already have a large free amino acid pool (of which a large proportion is glutamate) and also a high potassium ion concentration. Therefore, they have evolved mechanisms to metabolically convert glutamate to form either proline, or both proline and GABA. As these molecules are not highly charged at neutral pH, they do not require the presence of a neutralising cation. This form of regulation is effective in lowering the intracellular water activity of Gram positive bacteria to levels which allow growth in media of around a_w 0.860. Measures (1975) found

the minimum water activities for growth of Bacillus subtilis and Staphylococcus aureus in NaCl, to be 0.909 and 0.860 respectively.

Decreased water activity is often combined with other microbiological control factors and used to inhibit the growth of spoilage microorganisms. Generally, as the minimum water activity for growth of a microorganism is approached, it becomes more sensitive to other inhibitors and inhibitory conditions that may be present in its environment. Such factors affecting growth include, pH, antioxidants, modified storage atmosphere, preservatives and temperature (Troller, 1986). Scott (1957) found that the minimum water activity for growth could change by 0.01 to 0.05 for a 10°C change in incubation temperature, with larger effects being observed at temperatures near the growth limits of the microorganism.

1.9: EVALUATION OF SPOILAGE.

Jay (1978) categorised the methods which have been used for the evaluation of spoilage in meat and meat products (Table 1.2). Ideally, the technique used should be rapid, reproducible and not require the use of elaborate or expensive equipment. In addition, the technique should give a measure of the degree of spoilage and not simply diagnose rank spoilage, which in any case, could be detected organoleptically. The last criterion places a severe constraint on most chemical and physical methods (McMeekin, 1982).

The majority of methods listed in Table 1.2 are diagnostic, rather than predictive in nature. They simply indicate whether or not spoilage has occurred and have little or no value for predicting shelf-life.

Most chemical methods which detect bacterial activity are reliant on end product or enzyme detection techniques and do not show

Table 1.2: Categories of Methods for the Evaluation of Spoilage*.

Chemical Methods:

(a) Measurement of H_2S production.
(b) Measurement of mercaptans produced.
(c) Determination of non-coagulable nitrogen.
(d) Determination of di- and trimethylamines.
(e) Determination of tyrosine complexes.
(f) Determination of indole and skatole.
(g) Determination of amino acids.
(h) Determination of volatile reducing substances.
(i) Determination of amino nitrogen.
(j) Determination of BOD.
(k) Determination of nitrate reduction.
(l) Measurement of total nitrogen.
(m) Measurement of catalase.
(n) Determination of creatinine content.
(o) Determination of dye reducing capacity.
(p) Measurement of hypoxanthine.

Physical Methods:

(a) Measurement of pH changes.
(b) Measurement of refractive index of muscle juices.
(c) Determination of alteration in electrical conductivity.
(d) Measurement of surface tension.
(e) Measurement of UV illumination (fluorescence).
(f) Determination of surface charges.
(g) Determination of cryoscopic properties.

Direct Bacteriological Methods:

(a) Determination of total aerobes.
(b) Determination of total anaerobes.
(c) Determination of ratio of total aerobes to anaerobes.
(e) Determination of any of the above at different temperatures.
(f) Determination of Gram negative endotoxins.

Physicochemical Methods:

(a) Determination of extract release volume.
(b) Determination of water holding capacity.
(c) Determination of viscosity.
(d) Determination of meat swelling capacity.

* Reproduced from Jay (1978).

a response until 10^7-10^8 cells g^{-1} or mL^{-1} are present. Generally by this time the product has at least reached the stage of incipient spoilage (Edwards et al.,1985).

Similarly, physical measurements of the degree of spoilage, tend to be diagnostic rather than predictive. However, rapid advances are occurring in the field of impedance microbiology. Present day equipment is now sensitive enough to detect the changes in electrical conductivity of test media, only hours after they have been inoculated. Impedance microbiology offers a realistic method of measuring growth automatically and has been adopted in the USA as an alternative to the standard plate count for the evaluation of dairy products (Brooks, 1986).

Brooks (1986) stated that the initial bacterial count is of limited value for predicting the shelf-life of perishable foods, mainly because the activity of the initial population is more important than its magnitude. The measured impedance change is a function of metabolic activity rather than bacterial numbers and is thus well suited for estimating shelf-life. Most impedance work has involved the prediction of the shelf-life of dairy products (Firstenberg-Eden & Tricarico, 1983; Bishop et al., 1984 and Griffiths & Phillips, 1984).

Direct bacteriological methods such as total and selective plating techniques, are also of little value as they require a 48 to 72 hour incubation before colony forming units can be enumerated.

The physicochemical technique which is most widely used is the determination of the extract release volume (ERV) of the food. Jay (1966) found that meat of good organoleptic quality released a large volume of extract, which decreased with the onset of spoilage. He stated that an ERV of approximately 25-30, corresponded to a log bacterial number of $8.4g^{-1}$ and that it could be used predictively to

discriminate between acceptable and spoiled meat. However, Ingram & Dainty (1971) claimed that the predictive value of the test was little better than other methods, as the cut off level was simply related to bacterial numbers and the organoleptic characteristics associated with incipient spoilage.

The sensory system of Branch & Vail (1985), is a method developed for evaluating the deterioration of fish, which encompasses the effects of bacterial, chemical, physical and enzymic deteriorative processes on the fish. The system provides a linear relationship between a demerit score and the duration of storage of the fish. The score is derived from the allocation of demerit points to salient features commonly used to judge stored fish. Once the pattern of spoilage for a particular species has been established, the demerit point score can be related directly to a suitable reference, such as days in ice and the remaining shelf-life or time elapsed post mortem can be calculated.

1.10: SPOILAGE PREDICTION BASED ON PRODUCT TEMPERATURE HISTORY.

High moisture content foods (water activity >0.90) are susceptible to rapid spoilage, i.e. have a limited shelf-life, unless stored at suitably controlled low temperatures. In order to determine the shelf-life of such foods, it is normal practice to conduct storage trials under controlled conditions using constant temperatures. However, the commercial situation is quite different. The product may be subjected to wide temperature variation during separate stages of collection, transport, processing, storage, distribution and retail sale. Thus, in practice, shelf-life is determined by the cumulative effects of fluctuating temperature throughout the product's handling history (Pooni & Mead, 1984).

Research workers have attempted to predict the shelf-life of aerobically stored products, by utilising the products' temperature histories (Spencer & Baines, 1964; Olley & Ratkowsky, 1973a,b and Ronsivalli & Charm, 1975). Such attempts were based on the fact that the storage temperature of the product was considered to be the cardinal factor controlling the rate of development of the microbiota and hence the product's rate of spoilage. This led to the development of the following predictive relationships:

1.10a: The Spencer and Baines Equation:

This equation was proposed by Spencer & Baines (1964) as a means to predict the shelf-life of fish. It assumes a linear relationship between the rate of spoilage of the food at 0°C and its storage temperature (Figure 1.5a):

$$k_T = k_0(1 + cT) \qquad (10)$$

where k_T = spoilage rate at temperature T (°C),

k_0 = spoilage rate at 0°C,

c = constant for linear response.

This equation was considered valid over the temperature range -1°C to 25°C. However, upon reviewing the literature for the spoilage of flesh foods, Olley & Ratkowsky (1973a) found that the spoilage rate coefficient c, had a value of 0.24 at temperatures up to 6°C but above this temperature, c increased and became more variable. Nevertheless, it was shown that the model was valid over the restricted temperature range of 0°C to 8°C and has been used by Ronsivalli & Charm (1975) who produced a simple slide rule calculator enabling fishermen to predict the shelf-life of fish stored within the above temperature range.

Figure 1.5: Mathematical Relationships Between Temperature and Spoilage Constants.*

(a): mean relative spoilage rates. Each plotted against mean maximum temperature.

O Olley/Ratkowsky Relative Rate Curve.

● Spencer & Baines Linear Equation.

(b): mean apparent activation energy.

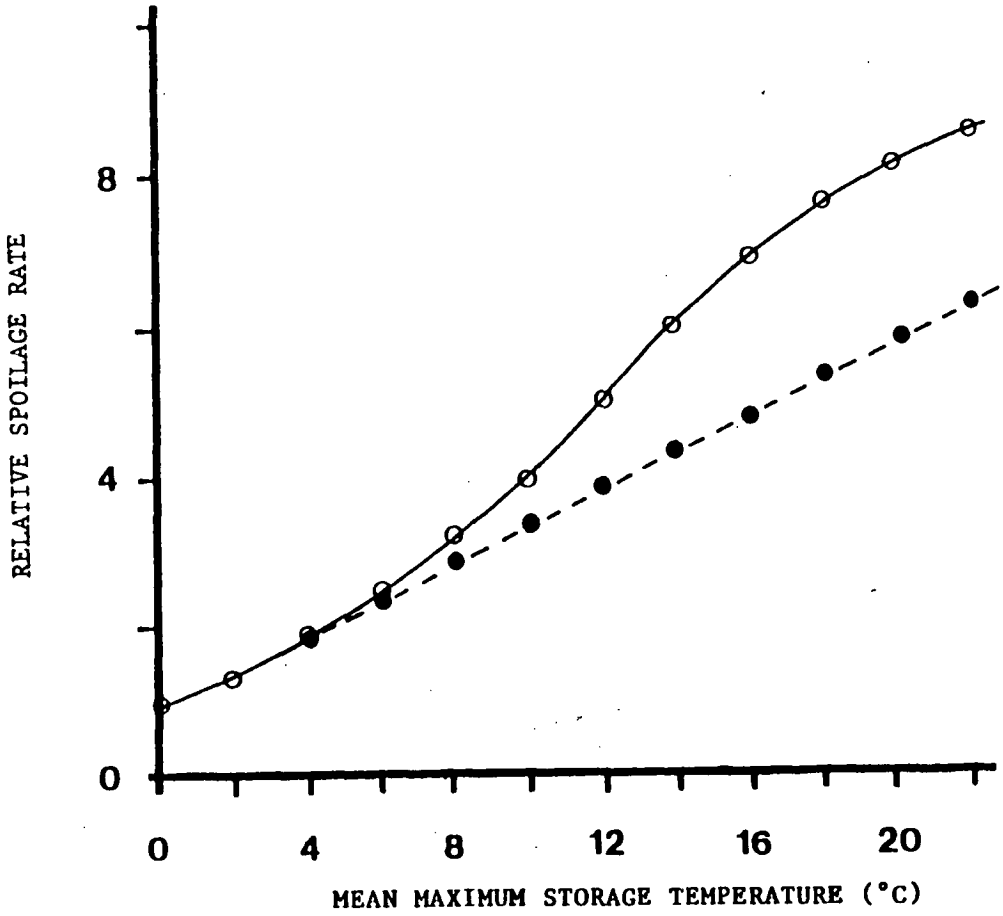
(c): mean spoilage coefficient of Spencer & Baines.

In (b) and (c), vertical lines represent standard deviations.

* Reproduced from Olley & Ratkowsky (1973a).
Thomas (1984).

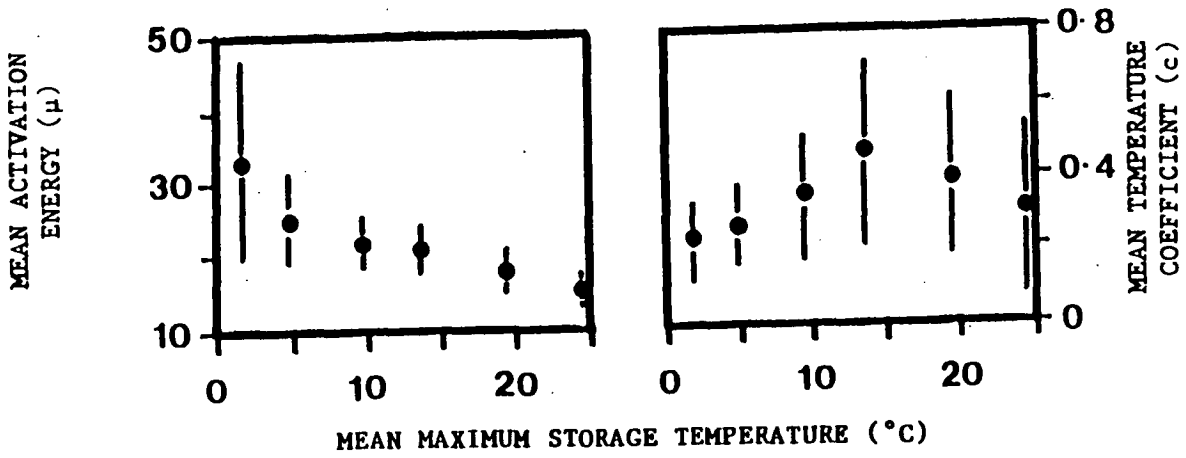
Figure 1.5: Mathematical Relationships Between Temperature and Spoilage Constants.

a



b

c



1.10b: The Olley and Ratkowsky Relative Rate Curve:

Olley & Ratkowsky (1973a,b) derived a general spoilage model between 0°C and 25°C for proteinaceous food products relative to their spoilage rate at 0°C. The Olley/Ratkowsky Relative Rate Curve was derived from 70 spoilage data sets obtained from the literature (Figure 1.5a).

In an attempt to decide whether the Spencer & Baines Model or the Arrhenius equation could accurately describe the Olley/Ratkowsky Curve, estimates of both the activation energy (μ) and c were obtained for specified temperature ranges using a computer. Olley & Ratkowsky's analyses of these data points, showed that the activation energy decreased with temperature as expected but that it had a lower standard deviation at higher temperatures, than at lower temperatures (Figure 1.5b). The constant for linear response (c) was found to vary from 0.24 at 1.8°C, to 0.46 at 13.5°C (Figure 1.5c).

Olley & Ratkowsky (1973a) concluded that the model which best fitted the experimental data, was the Arrhenius equation. However, it was accurate only over the limited temperature range of 3°C to 15°C, where the activation energy was found to be relatively constant.

Daud (1978) and Daud et al.,(1978) showed the rate of spoilage of chicken tissue, the development of spoilage bacteria and the utilisation of amino acids by spoilage bacteria, to be a function of temperature, and that these were more accurately described by the Olley/Ratkowsky Curve, than by the Spencer & Baines Equation. However, at temperatures greater than 16°C the poultry spoilage curve continued to rise and did not show the plateau effect found by the Olley/Ratkowsky Curve, at a relative rate of approximately 9.

1.11: APPLICATIONS OF THE SQUARE ROOT MODEL AND THE ARRHENIUS EQUATION.

Both the Arrhenius equation and the Square Root Model have been used as a basis for modelling spoilage prediction (Olley & Ratkowsky, 1973a,b; Pooni & Mead, 1984 and Gill & Harrison, 1985). The application of the Arrhenius equation by Olley & Ratkowsky has been discussed in Section 1.10b. The application of the Square Root Model, in particular the use of T_{min} (T_o) values as a basis for producing the relative rate curves used in temperature function integration, will be discussed in the sections that follow.

Workers such as Ingraham (1958) and Baig & Hopton (1969) have categorised various groups of bacteria (psychrophiles, psychrotrophs, mesophiles and thermophiles) using the temperature characteristics obtained from Arrhenius plots. Ingraham (1958) suggested that psychrotrophs should have temperature characteristics lower than those of mesophiles. Mohr & Krawiec (1980) suggested that psychrophiles and psychrotrophs were best described by Arrhenius plots which yielded only one distinct temperature characteristic, whereas mesophiles were best described by plots which yielded two temperature characteristics. Reichardt & Morita (1982) criticised the work of Mohr & Krawiec (1980) and stated that they had found disparate Arrhenius profiles for psychrotrophic bacteria.

These concepts have been refuted by Shaw (1967), Hanus & Morita (1968), Herbert & Bhakoo (1979) and Stannard *et al.*,(1985), who found no significant correlation between the temperature characteristics of the different groups. Hanus & Morita (1968) demonstrated several closely related vibrios, including a psychrophile, psychrotroph and mesophile, to have temperature characteristics of 16200, 16400, and 14400 cal mol^{-1} respectively. Epstein & Grossowicz (1969) found that

different temperature characteristics were obtained when a thermophilic bacillus was grown in either a minimal (13500 cal mol^{-1}) or rich medium (15000 cal mol^{-1}). Baker (1974) compared the temperature characteristics of both psychrophiles and mesophiles from the literature and his own work and concluded that there was no apparent correlation between the presence or absence of psychrophily and the value of the temperature characteristic. Reports such as these, further complicate the notion that it is possible to categorise bacteria by their temperature characteristics, instead of by using their temperature growth ranges.

Ratkowsky et al.,(1982) proposed that the temperature parameter T_o, which is derived from the Square Root Model, may be useful to categorise various bacteria. T_o has two advantages over the temperature characteristic (μ), in that it does not alter with temperature and that it appears to be constant for any bacterium grown under various nutritional conditions. They suggested that psychrophiles possessed T_o values around 248K, psychrotrophs had values in the range 260K to 269K, mesophiles had values in the range 270K to 280K and that thermophiles had values in the range 290K to 296K. Examples of bacteria fitting into the above groups, were summarised by Olley (1983) (Table 1.3).

Ratkowsky et al.,(1983) stated that they had found two anomalies to the above grouping method. Vibrio marinus and Vibrio psychroerythrus were found to have T_{min} (T_o) values of 276K, which would class them as mesophiles, however, their optimum and maximum temperatures for growth indicate that they are psychrophilic in nature.

Comparative studies by Stannard et al.,(1985) have shown that the Square Root relationship fits bacterial temperature/growth data better than the Arrhenius relationship. They also suggested that T_o

Table 1.3: T_o Values for Various Bacteria*.

Culture	T_o(K)
PSYCHROPHILE:	
Pseudomonas sp. L12	248
PSYCHROTROPH:	
Pseudomonas sp. GpIV	263
Pseudomonas fluorescens	263.5
Pseudomonas sp. Gp. I	264
MESOPHILES:	
Proteus morganii	272
Escherichia coli	275
Pseudomonas aeruginosa	276
Acinetobacter sp.	277
THERMOPHILES:	
Lactobacillus delbrueckii	290
Bacillus circulans	296

* Reproduced from Olley (1983).

values may be useful in the classification of microorganisms as psychrotrophs, mesophiles or thermophiles.

1.12: RECONCILING THE SQUARE ROOT MODEL AND THE ARRHENIUS EQUATION.

McMeekin et al.,(1987) stated that the non-applicability of the Arrhenius law to modelling the temperature dependence of microbial growth, could be viewed as a result of the temperature characteristic decreasing with increasing temperature. Bacterial growth involves the interaction of a highly complex series of reactions comprising both catabolic and anabolic processes and it is therefore not surprising that Arrhenius plots of bacterial growth rate deviate markedly from linearity. This is evident in Figure 1.1, (reproduced from Ratkowsky et al.,1982) which shows the non-linearity of the Arrhenius plots of six microorganisms.

McMeekin et al.,(1987) derived the following equation to demonstrate how the temperature characteristic/activation energy, (μ/E) changes with temperature and can be related to the Square Root Model:

$$E = 2RT^2/(T - T_{min}) \qquad (11)$$

For a given organism, the rate of change of E is greater for low values of $T-T_{min}$ than for higher values. A high activation energy means a high energy barrier to reaction and hence the rate of bacterial growth will be low for organisms that are only 10°C or so above their T_{min} values. As the values of $T-T_{min}$ increase, the energy barrier decreases, allowing an increase in growth rate (McMeekin et al.,1987).

1.13: SPOILAGE PREDICTION BASED ON RELATIVE RATES OF MICROBIAL GROWTH.

Temperature is the major factor controlling the rate of growth

of the spoilage microorganisms responsible for the deterioration of proteinaceous foods such as milk, poultry, red meat and fish, stored at chill temperatures. Other factors such as nutrient status and water activity are non-limiting and no microbial interactions occur until maximum cell densities are reached (Gill & Newton, 1977). Hence a knowledge of the effect of temperature on the rate of growth of the spoilage microbiota may be used to monitor the time/temperature history of the product.

When a T_{min} of 263K (based on the T_{min} value for a typical psychrotroph) is used, the relative rate of growth at 0°C can be calculated from equation (7), after the following algebraic manipulation:

$$r = (0.1T + 1)^2 \qquad (12)$$

where r and T, are the rate relative to that at 0°C and temperature in degrees Celsius respectively.

When the relative rate of spoilage curve is computed, the values derived from the Square Root Model are practically identical to those predicted by the Olley/Ratkowsky curve, up to approximately 15°C, and provides an excellent fit for spoilage data (Table 1.4) (Thomas, 1984).

1.14: TEMPERATURE FUNCTION INTEGRATION.

Temperature function integration provides an alternative to traditional bacteriological and biochemical approaches to estimating product deterioration. Many methods have been used to determine the bacteriological quality of foods (Table 1.2) and there is considerable disagreement as to which may provide reliable estimates of shelf-life. Temperature function integration is based on the observation that temperature is generally the major factor controlling the rate of microbial growth and hence the rate of

Table 1.4: Comparison of Relative Rates of Spoilage as Predicted by the Olley/Ratkowsky Curve, the Square Root Model and Experimental Data*.

Temperature (°C)	O/R Curve	Square Root Model (T_{min} = 263K)	Poultry Data**
0	1.00	1.00	1.00
2	1.40	1.44	1.40
4	1.90	1.96	1.47
6	2.50	2.56	2.17
8	3.20	3.24	2.55
10	4.00	4.00	3.23
12	5.00	4.84	4.58
14	6.00	5.76	5.77
16	6.90	6.76	6.15
18	7.60	7.84	7.55
20	8.10	9.00	9.42
22	8.50	10.24	-

* Reproduced from Thomas (1984).

** Reproduced from Daud et al.,(1978).

spoilage.

To be effective, temperature function integration requires a knowledge of the effect of temperature on the rate of microbial growth, i.e. relative rate data provided by the Square Root Model, and a device, capable of continually monitoring the time/temperature history of the product. Such a device, called a temperature function integrator, has been described by Nixon (1971) and Owen & Nesbitt (1984). In this device the sensor (a thermistor) is attached to the circuitry (programmed with a relative rate formula such as equation (12)) and a display unit. The circuitry converts impulses received from the sensor, sums the temperature history and displays the integrated information as an equivalent number of days at an arbitrarily programmed reference temperature.

Temperature function integration has two advantages over traditional methods of monitoring shelf-life and product deterioration:

(i) it provides a means of continually monitoring the deterioration of a product, and

(ii) it takes account of temperature fluctuations and integrates these to provide a reading for an equivalent number of days at a specified reference temperature, which then allows prediction of the remaining shelf-life of the product.

Devices such as temperature indicators, of the "go/no go" variety and time temperature integrators, which are based on processes such as enzyme reactions, have also been developed to monitor the temperature history of products. They operate on the principle that a temperature dependent reaction occurs at a similar rate to the temperature related spoilage of the product (Olley, 1978). Olley & Lisac (1985) studied time/temperature monitors (TTM) which were capable of keeping track of both accumulated time and

temperature. It was found that for a TTM to be useful, it had to be capable of simulating the growth of common psychrotrophic bacteria. Only one of the monitors manufactured by i-point (Malmo, Sweden), type 3270, was found to be comparable with a psychrotrophic relative rate curve and hence suitable to monitor chill stored products.

The mode of action of temperature function integrators is superior to other temperature integrators, as they operate by mimicking the temperature dependent growth patterns of the significant spoilage bacteria.

Temperature function integration was originally developed to describe the chill store spoilage of fish using the Olley/Ratkowsky curve and has been reviewed by Olley (1983). It soon became apparent that it had a more widespread use and that it could be applied to the spoilage of other foods, viz., poultry (Pooni & Mead, 1984) and offal (Gill & Harrison, 1985), with the Square Root Model replacing the Olley/Ratkowsky curve, as the basis for relative rate prediction.

The psychrotrophic relative rate curve is accurate at temperatures up to approximately 15°C, with discrepancies being noted at elevated temperatures, due to the increased influence of the mesophilic microbiota (Pooni & Mead, 1984). McMeekin & Olley (1986) suggested that prolonged storage at elevated temperatures would lead to such rapid spoilage that prediction would hardly be necessary. However, if transient excursions into the upper temperature region were considered to have a significant effect on spoilage rate, then two relative rate curves may be incorporated into an integrator which switches from a psychrotrophic, to a mesophilic mode, at approximately 15°C.

Smith (1987) found that he could predict the rate of multiplication of coliform microorganisms in blended mutton tissue by

using a mesophilic relative rate curve. Given the temperature history of the product, he correctly predicted the increase in the number of bacteria during incubation.

1.15: SHELF-LIFE PREDICTION.

In order to predict shelf-life, it is necessary to estimate the keeping quality of the product. This can be done either by incubation trials under specified storage conditions or by calculation from a knowledge of the microbial ecology of the product (McMeekin & Thomas, 1980). In the latter case, it is necessary to obtain an estimate of the initial number of psychrotrophs present, as the actual time required to reach a specified spoilage level will be dependent on the initial number of bacteria capable of growth at a particular storage temperature.

Table 1.5 is reproduced from Thomas (1984) and demonstrates how the shelf-life of chicken under specified conditions (spoilage occurring at a level of 10^8 cells cm^{-2} and a generation time of 8 hours at 5°C) is affected by both storage temperature and the initial number of psychrotrophs. It shows the advantages of keeping the product at as low a temperature as possible and the importance of minimising the initial psychrotrophic load of the product.

Mead (1985) reported the shelf-life of poultry to be inversely proportional to the number of pseudomonads initially present on the product. This observation has been supported by Knabel et al.,(1987), who have shown the shelf-life of poultry to be negatively correlated ($r = -0.86$) to initial numbers of fluorescent pseudomonads prior to storage at 5°C.

McMeekin & Thomas (1980) suggested that temperature function integrators, offered the possibility of monitoring the complete

Table 1.5: The Effect of Temperature and Initial Number of Psychrotrophic Bacteria on the Shelf-life of Poultry Carcasses*.

Storage Temperature (°C)	Relative Rate of Spoilage	Initial Number of Psychrotrophs 500	1000	10000
0	1.00	[a] 9.42	8.92	7.23
2	1.44	6.54	6.19	5.02
4	1.96	4.81	4.55	3.69
6	2.56	3.68	3.48	2.82
8	3.24	2.91	2.75	2.23
10	4.00	2.36	2.33	1.81

Each shelf-life is calculated assuming a spoilage level of 10^8 cells cm^{-2} and a generation time of 8 hours at 5°C.

* Reproduced from Thomas (1984).

[a] Predicted shelf-life in days.

temperature history of aerobically stored poultry throughout the entire processing, transport and retailing sequence and that they could be used to estimate the remaining shelf-life of a product or identify weak links in the cold chain. McMeekin (1982) reported that an integrator had been used successfully in a poultry processing plant and that it allowed the processor to estimate remaining shelf-life during transport and retail storage. Campbell-Platt (1985) and Pooni & Mead (1984) have also noted the usefulness of temperature function integration as a management and control tool to monitor the actual temperature conditions in the food distribution chain. Pooni & Mead (1984) stated that the use of temperature function integrators would also be of educational value to plant operators and shop personnel, by increasing their awareness of the importance of temperature control.

Gill & Harrison (1985) found that the in-plant sampling for the direct estimation of microbial proliferation on offals was inadequate. They fitted the Square Root Model to generation time data for E. coli and calculated the number of generations which should proliferate during specified offal cooling regimes. The hygienic efficiency of any offal cooling process could then be quantified by a Bacterial Growth Number, which was equivalent to the number of bacterial generations occurring during cooling. This was thought to be a good basis for routine process evaluation and provided the cooling regime yielded a sufficiently low Bacterial Growth Number, the process could be considered satisfactory and would lead to a simplification of process supervision.

1.16: CONCLUSION.

Farber (1986), stated that the information provided by mathematical models, can be used advantageously in many situations: to predict the relative microbial stability of a new product, which may differ only slightly from an older established product; to aid in the assessment of the direct and interactive effects on microbial growth of a novel combination of food preservatives in a food product; or to assess the safety of foods which are subjected to various degrees of temperature abuse as they pass through wholesale, retail and domestic outlets.

The Square Root Model, which is a specific form of the Belehradek equation, may be criticised as being a parabola, based upon no firm theoretical understanding. However, an empirically based general equation, which is successful in predicting bacterial growth and spoilage rates, is more beneficial to workers than an experimentally derived model, which is either not general or may lead to inaccurate predictions.

The aims of this study are: to validate the application of the Square Root Model, as a method of monitoring and predicting the growth of microorganisms in, and the shelf-life of, pasteurised, homogenised milk; and to validate the applicability of the Square Root Model to predict the growth of moderate and extreme halophiles under conditions of varying water activity and temperature.

2. MATERIALS AND METHODS.

2.1: THE EFFECT OF TEMPERATURE ON MILK SPOILAGE.

All pasteurised, homogenised milk samples were obtained directly from the processing lines of Tasmaid foods immediately prior to use in experiments.

2.1a: The Relationship Between Carbon Dioxide Concentration and the Spoilage of Pasteurised, Homogenised Milk.

(i) Five pairs of L shaped, glass tubes (L-tubes) (Toyo Kagaku Sangyo Co. Ltd., Tokyo Japan), each containing 15mL of commercial, pasteurised, homogenised milk were stored horizontally in a temperature gradient incubator (TGI) (Toyo Kagaku Sangyo Co. Ltd., Tokyo Japan) at 15.1, 9.9, 7.1, 4.2 and 2.0°C. One tube at each temperature was capped with a rubber suba-seal and the other with a loose aluminium cap. After the onset of organoleptic spoilage, the CO_2 concentration ($[CO_2]$) in the head space of each tube was analysed in duplicate, using a Perkin-Elmer Sigma 3B Gas Chromatograph, fitted with a SA 80-100 molecular sieve and calibrated using a CIG CO_2 gas mixture (Lab. Ref. No. 35876). Organoleptic spoilage was assessed by the presence or absence of off odours in the aluminium capped tubes.

To determine the number of microbial colony forming units (cfu) initially present and at each sample time, until organoleptic spoilage had been attained, duplicate 10-fold serial dilutions of the milk were made using saline (Appendix 6.1a) and 0.1mL aliquots were spread onto Plate Count Agar (PCA) (Appendix 6.1b) and 1.0mL aliquots were analysed using the PCA pour plate method (Anonymous, 1973).

(ii) Twenty four 600mL cartons of pasteurised, homogenised milk were divided arbitrarily into 4 groups and stored at 10.5, 7.2, 4.4 and 1.8°C. The head space $[CO_2]$ and the number of microbial cfu present in the milk were determined as in Section 2.1a(i), at regular

intervals during storage. In addition, 0.1mL aliquots were spread onto Pseudomonas Agar (PsA) (Appendix 6.1c) to enumerate the number of pseudomonads present.

(iii) Fifteen millilitres of pasteurised, homogenised milk were dispensed into 16 pairs of L-tubes, sealed as in Section 2.1a(i) and stored in a TGI over the temperature range 0-25°C. Carbon dioxide concentrations for the suba-sealed L-tubes and the number of microbial cfu for all the L-tubes were determined as in Section 2.1a(i) at regular time intervals.

2.1b: The Relationship Between Storage Temperature and the Spoilage of Pasteurised, Homogenised Milk.

(i) The spoilage rate at each temperature in Section 2.1a(iii), Section 2.1b(ii), Section 2.1b(iii), Section 2.1b(iv) and Section 2.1b(v), was determined by calculating the reciprocal of the time required for microbial numbers to reach log(7.5) cfu mL^{-1}. This spoilage level corresponds to the detection of organoleptic changes in most dairy products (Griffiths et al.,1984). Spoilage rate data were fitted to the Square Root Model of Ratkowsky et al.,(1982).

(ii) Fifteen millilitres of pasteurised, homogenised milk were dispensed into 16 L-tubes, sealed with loose aluminium caps and stored in a TGI over the temperature range 0-25°C. Microbial numbers and spoilage rates were determined and treated as in Section 2.1b(i).

(iii) Twenty L-tubes, each containing 15mL of pasteurised, homogenised milk, were stored as in Section 2.1b(ii). Microbial numbers and spoilage rates were determined and treated as in

Section 2.1b(i). In addition, 0.1mL aliquots were taken from the serial dilutions of the fresh milk and milk stored at 23.4, 15.4, 10.1, 6.6 and 4.2°C and spread onto PCA.

(iv) Eighteen L-tubes, each containing 15mL of pasteurised, homogenised milk, were treated, as in Section 2.1b(ii).

(v) Twenty one L-tubes, each containing 15mL of pasteurised, homogenised milk, were treated as in Section 2.1b(ii). The milk was sampled as in Section 2.1b(iii), with the 0.1mL aliquots being taken from the fresh milk, and milk stored at 25.0, 19.7, 15.6, 11.5 and 6.3°C.

Least squares linear regression was used to fit predicted lines to the experimental data obtained in Section 2.1b(i), Section 2.1b(ii), Section 2.1b(iii), Section 2.1b(iv) and Section 2.1b(v).

2.1c: The Effect of Storage Temperature on the Microbiota of Pasteurised, Homogenised Milk.

2.1c(i): Analyses of Initial and Spoilage Microbiota.

Individual colonies were picked randomly from the spread plates obtained from the experiments in Section 2.1b(iii) and Section 2.1b(v). Where possible, every cfu from the PCA spread plates used to enumerate microbial numbers, was isolated and purified by streaking onto to PCA. Each isolate was then transferred to slopes of PCA and after incubation, stored at 4°C.

Cultures of the isolates were grown on slopes of PCA at 25°C for between 48 and 96 hours prior to assessment of the characteristics shown in Table 2.1 and identification using a modification of the scheme of Shewan *et al.*,(1960) (Figure 2.1a,b).

Table 2.1: Tests Used To Characterise Bacterial Isolates.

	Test	Method/Medium
(a)	Non-diffusible pigment	Visually on PCA
(b)	Cell morphology	Light microscopy
(c)	Gram reaction	Appendix 6.1d
(d)	Mode of glucose utilisation	Appendix 6.1e
(e)	Motility	Phase contrast microscopy
(f)	Flagella arrangement	Appendix 6.1f
(g)	Cytochrome oxidase	Appendix 6.1g
(h)	Catalase	Appendix 6.1h
(i)	Psychrotrophic growth	Appendix 6.1i
(j)	Fluorescent pigment production	Appendix 6.1j

Figure 2.1a: Diagnostic Key for the Identification of Certain Gram-Negative Isolates (Modified from Shewan et al., 1960).

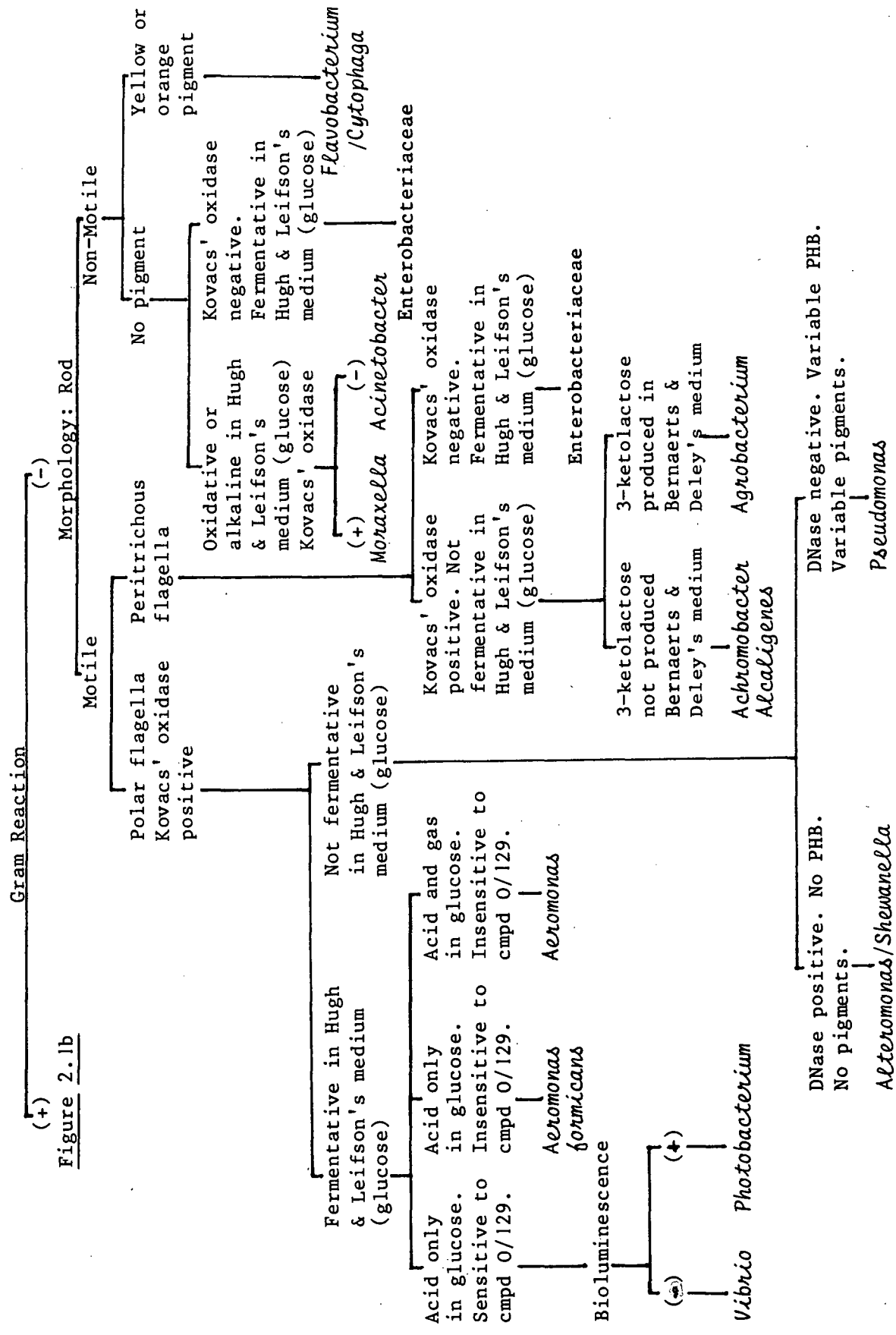
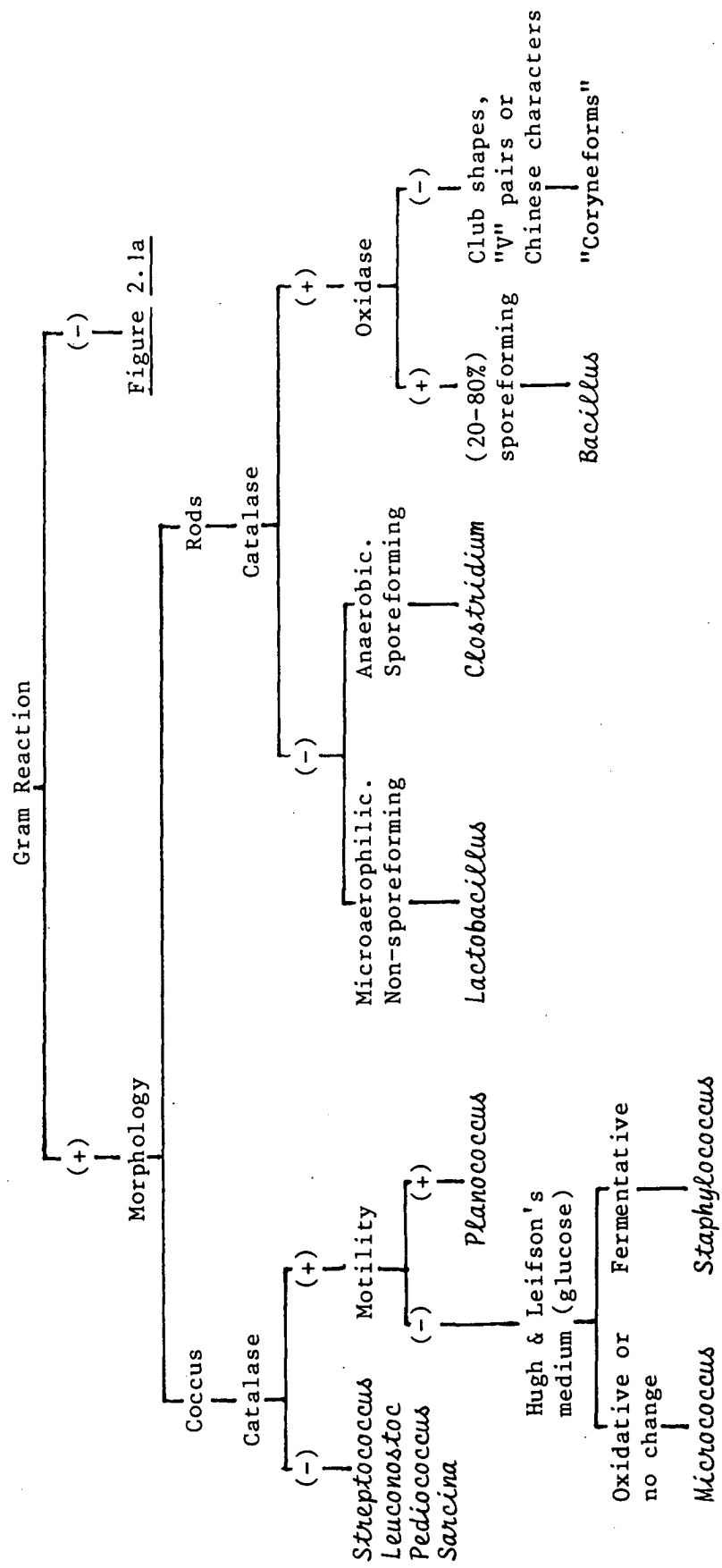


Figure 2.1b: Diagnostic Key for the Identification of Certain Gram-Positive Isolates (Modified from Shewan et al., 1960).



2.1c(ii): Changes in the Microbiota During Storage.

Three 1L cartons of pasteurised, homogenised milk were combined and then aseptically dispensed into three 500mL sterile flasks, prior to storage at 14.6, 10.0 and 4.1°C, until organoleptic spoilage had been reached. Estimates of microbial and pseudomonad cfu, present prior to and during storage, were obtained by preparing a dilution series of the milk in saline and spreading duplicate 0.1mL aliquots onto both PCA and PsA.

2.1c(iii): Development of a Most Probable Number Technique to Detect Pseudomonads.

A most probable number (MPN) medium, based on the ingredients in PsA and utilising its C-F-C selective supplement (Appendix 6.1k), was tested for its ability to enumerate the low numbers of pseudomonads present in freshly pasteurised, homogenised milk and during its subsequent storage at approximately 5°C.

The initial pseudomonad contamination of four 2L cartons of pasteurised, homogenised milk was determined. Each was tested using a 5 tube, 3 dilution series, MPN technique (Anonymous, 1982). To determine the specificity of the MPN medium, samples from both the positive and negative tubes obtained from the first carton of milk, were streaked onto PsA and PCA. The colonies which grew on the PsA plates were tested for the presence of cytochrome oxidase. Microbial and pseudomonad cfu, were determined by spreading duplicate 0.1mL aliquots of 10-fold serial dilutions of each milk sample, onto both PCA and PsA. Each carton was tested initially and then daily for 3 or 4 days, using MPN, PsA and PCA. During each experiment, a control containing pasteurised, homogenised milk, sterilised by autoclaving for 30 minutes at 34.5kPa, was also inoculated into the MPN medium.

2.1d: Determination of the Shelf-Life of Pasteurised, Homogenised Milk by Time/Temperature Function Integration (TTFI).

2.1d(i): Correlation of TTFI Readings with Microbial Numbers.

One litre cartons of pasteurised, homogenised milk were stored at either 6, 10, 12 or 15°C. Each was sampled at least daily, for either microbial, and/or pseudomonad cfu, by spreading duplicate 0.1mL aliquots from the milk or from 10-fold serial dilutions in saline, onto plates of PCA or PsA.

At the commencement of storage of each sample, the thermistor of a time-temperature function integrator (TTFI) (Don Whitley Scientific Ltd., West Yorkshire, Model WF1) was placed into the milk and whenever it was sampled, the reading on the TTFI was noted. The TTFI was set to operate using a T_o value of 263K and to display elapsed storage time in equivalent days at 4°C (Owen & Nesbitt, 1984). Least squares linear regression was used to fit predicted lines to experimental data.

2.1d(ii): Correlation of TTFI Readings with Organoleptic Evaluation of Spoilage at Elevated Temperatures.

Three 1L cartons of pasteurised, homogenised milk were stored at 10°C, four 1L cartons at 12°C, and five 1L cartons were stored at 15°C, until organoleptic spoilage was attained. Microbial and pseudomonad cfu were determined for each milk sample, as in Section 2.1c(iii). From each of the initial and spoilage PCA plates used for enumeration, between 15 and 30 isolates were inoculated into individual test tubes containing PCB and incubated for 48 hours at 25°C. Each isolate was then tested for its ability to grow on PsA and as a psychrotroph. Positive isolates were then tested for the presence of cytochrome oxidase and motility, to enable a presumptive pseudomonad identification. TTFI readings were obtained as in Section 2.1d(i).

2.2: THE EFFECT OF TEMPERATURE ON MICROBIAL GROWTH RATE.

All bacterial strains were maintained on agar slopes and stored at 4°C prior to inoculation into broths for use in the growth studies reported in the following sections.

2.2a: Calibration of a Nephelometer for Pseudomonas sp. Strain E5.2.

Pseudomonas sp. strain E5.2 was isolated from spoiled, pasteurised, homogenised milk which had been stored at 4.2°C and the culture identified by the method outlined in Section 2.1c(i).

An exponential phase culture of Pseudomonas sp. strain E5.2 was obtained after incubation in Plate Count Broth (PCB) (Appendix 6.11) on a rotary shaker (100rpm and 25°C) for 14.5 hours. A 10^{-2} dilution of the culture was made using PCB, prior to 1mL being inoculated into an L-tube containing 14mL of PCB, equilibrated at 25°C. Growth was monitored by measuring the increase in optical density (OD) of the culture with a nephelometer (EEL Unigalvo).

When the OD of the culture exceeded full scale deflection on the nephelometer, doubling dilutions were made using PCB. At each measurable OD, values were correlated to the number of bacterial cfu by removal of 1mL of culture and spreading duplicate 0.1mL aliquots of the subsequent 10^{-1} serial dilutions in saline, onto PCA. The generation time at 25°C was determined. Predicted lines were fitted to experimental data using least squares linear regression.

2.2b: Determination of Temperature/Growth Profiles for Two Bacteria, Isolated from Spoiled, Pasteurised, Homogenised Milk.

Pseudomonas sp. strain 12.48 and Enterobacter agglomerans, strain 11.33 (the latter identified using API 20E strips, API System, France), were isolated in Section 3.1c(i).

The temperature/growth profiles of the bacteria were studied

using a TGI over the temperature range 0-40°C at approximately 1°C intervals. In each case, the growth medium (PCB) was inoculated with 1.0mL of an exponentially growing culture, which had been incubated for 18 hours on a rotary shaker (100rpm and 25°C).

Growth at each temperature was monitored by measuring the change in OD of the broth culture with a nephelometer. The growth rate was calculated at each temperature, as the reciprocal of the time required for a change in OD of 0.3 (OD data). The generation time at each temperature was calculated as the time required for a doubling in OD (GT data), with the growth rate being the reciprocal of the GT.

Experimental data were interpreted using the Square Root Model of Ratkowsky *et al.*,(1982). Predicted lines were fitted to experimental data using least squares linear regression.

2.2c: The Effect of Temperature on the Duration of the Lag Phase in Stationary Phase Cells of *Pseudomonas* sp. Strain E5.2.

(i) *Pseudomonas* sp. strain E5.2 was grown in PCB on a rotary shaker (100rpm and 25°C) for 72 hours. Growth of the culture was monitored by making a 10^{-2} dilution of the culture in PCB and determining its OD by nephelometry. This process was repeated at regular time intervals until continued incubation resulted in no further increase in OD (*i.e.*, the bacteria had reached stationary phase).

(ii) *Pseudomonas* sp. strain E5.2 was grown to stationary phase in PCB, on a rotary shaker (100rpm and 25°C). Two TGI experiments were conducted over the temperature range 0-50°C, using cultures incubated for 63 and 67 hours. A 1mL inoculum of these cultures resulted in an initial OD of between 0.1 and 0.15 for both experiments. OD and GT data were calculated at each incubation temperature by the method

described in Section 2.2b. The lag time at each temperature was calculated from this data and the results obtained in Section 3.2a. A sample calculation is given in Appendix 6.2c.

(iii) Pseudomonas sp. strain E5.2 was grown to stationary phase in PCB, on a rotary shaker (100rpm and 25°C). Three TGI experiments were conducted over the temperature range 5-40°C, using cultures incubated for 92, 95 and 93 hours. A 1mL inoculum of these cultures resulted in an initial OD of approximately 0.1 for each experiment. The duration of the lag phase at each temperature was estimated directly, as the time required to observe a detectable (0.02) change in OD.

Data derived from the experiments conducted in Section 2.2c(ii) and Section 2.2c(iii), were interpreted using the Square Root Model of Ratkowsky et al.,(1982). Predicted lines were fitted to experimental data using least squares linear regression.

2.3: THE EFFECT OF TEMPERATURE AND WATER ACTIVITY ON MICROBIAL GROWTH RATE.

2.3a: Growth of the Moderate Halophile Staphylococcus xylosus Strain CM21/3.

2.3a(i): Calibration of Nephelometer.

An exponential phase culture of Staphylococcus xylosus strain CM21/3, isolated from salted, dried chub mackerel, was obtained by growing the bacterium in moderate halophile broth (MHB) (Appendix 6.1m). Incubation was for 11 hours on a rotary shaker (100rpm and 25°C). One millilitre of the culture was inoculated into 14mL of MHB, in an L-tube equilibrated to 35.3°C. Once the culture in the L-tube had obtained full scale deflection on the nephelometer, doubling dilutions were made using MHB and the corresponding OD values were determined. This process was repeated using MHB which had been adjusted to contain 3.5 molal NaCl or 4.5 molal glycerol.

Staphylococcus xylosus strain CM21/3 was inoculated into three flasks containing 100 mL of MHB, two of which were adjusted to contain 3.5 molal NaCl and 4.5 molal glycerol and incubated on a rotary shaker at 25°C. The cell dimensions of bacteria from each culture were measured using an eyepiece micrometer (Olympus, Tokyo) calibrated with an objective micrometer (Olympus, Tokyo). When the OD of each culture reached 0.3, bacterial numbers were determined as in Section 2.2a, with cultures plated onto moderate halophile agar (MHA) (Appendix 6.1n) instead of PCA.

2.3a(ii): The Effect of Temperature and Sodium Chloride Concentration on Growth Rate.

The effect of temperature and NaCl concentration on the growth rate of Staphylococcus xylosus strain CM21/3, over the NaCl range 0.0-4.5 molal, was examined using a TGI over the temperature range

5-30°C, at approximately 1°C intervals. The water activity of the basal growth medium (MHB) was adjusted by the addition of NaCl (Table 2.2).

The resultant broths were inoculated with CM21/3 and grown at 35°C for 18-42 hours (dependent upon a_w), on a rotary shaker at 100rpm. One millilitre of each exponentially growing culture was inoculated into L-tubes which had been heat sterilised for 90 minutes at 170°C and aseptically filled with sterile medium and placed in the TGI to equilibrate. Growth at each temperature was determined as in Section 2.2b. OD and GT data were interpreted using the Square Root Model (Ratkowsky et al.,1982).

Predicted lines were fitted using least squares linear regression. Non-linear least squares regression (Kennedy & Gentle, 1980) was used to verify the Square Root Model as a specific example of the Bělehrádek equation. Convergence of OD and GT data was conducted according to the method described by Ratkowsky (1983).

2.3a(iii): The Effect of Temperature and Glycerol Concentration on Growth Rate.

The effect of temperature and glycerol concentration on the growth rate of Staphylococcus xylosus strain CM21/3, over the glycerol range 1.0-6.0 molal, was examined as in Section 2.3a(ii). The water activity of the MHB was adjusted with glycerol (Table 2.3) instead of NaCl. OD and GT data were analysed as in Section 2.3a(ii). The water activity of the 3.0 molal culture grown at 35°C, was measured after it had grown sufficiently to result in a full scale deflection of the nephelometer.

2.3a(iv): Determination of the Minimum Water Activity for Growth.

The effect of either NaCl or glycerol concentration, on the

Table 2.2: Water Activities of Moderate Halophile Broths Containing Different Sodium Chloride Concentrations.

Molality	NaCl	Water Activity*	pH
0.0	0.0	0.996	7.01
0.7	35.1	0.976	7.00
1.4	81.8	0.949	7.00
2.0	116.9	0.928	7.00
2.5	146.1	0.909	7.00
3.0	175.4	0.889	7.06
3.5	204.6	0.869	7.00
3.75	219.2	0.858	7.03
4.0	233.8	0.848	7.01
4.5	263.0	0.826	7.02

* Values calculated (Appendix 6.2a).

NaCl values in grams per Kg water.

Table 2.3: Water Activities of Moderate Halophile Broths Containing Different Glycerol Concentrations.

Molality	Glycerol	Water Activity*	pH
0.0	0.0	0.996	7.01
1.0	92.1	0.975	7.00
2.0	184.2	0.958	7.02
3.0	276.3	0.943	7.00
4.5	414.4	0.919	7.00
6.0	552.5	0.896	7.01

* Values determined using a Sina-Scope (Sina, Zurich, Switzerland).

Glycerol values in grams per Kg water.

growth of Staphylococcus xylosus strain CM21/3 at 38°C, was studied. The water activity of flasks containing 50mL of MHB, was altered by either the addition of NaCl (3.5-6.0 molal) (Table 2.4) or glycerol (4.0-6.5 molal) (Table 2.4). The resultant broths were inoculated with CM21/3 and incubated for up to 35 days on a rotary shaker (100rpm) at 38°C. A turbid broth indicated growth.

2.3a(v): Determination of the Effect of Temperature on the Minimum Water Activity for Growth.

The combined effects of sodium chloride and temperature on the growth of Staphylococcus xylosus strain CM21/3 were studied over the NaCl range 0.0-4.5 molal (Table 2.5) and at 40.1, 35.1, 30.0, 25.2, 20.0, 15.1, and 9.1°C. Flasks containing 20mL of MHB and the specified NaCl concentrations, were incubated for up to 7 days, at each specified temperature. A turbid broth indicated growth.

2.3b: GROWTH OF EXTREME HALOPHILES, HALOBACTERIUM SP. STRAIN HB9 AND HALOBACTERIUM SALINARIUM STRAIN CM42/12.

2.3b(i): Calibration of Nephelometer for Halobacterium sp. Strain HB9.

An exponential phase culture of Halobacterium sp. strain HB9, isolated from salted, dried chub mackerel, was obtained by growing the bacterium in extreme halophile broth (XHB) (Appendix 6.1o), which had been adjusted to contain 6.0 molal NaCl. Incubation was for 72 hours on a rotary shaker (100rpm and 35°C). One millilitre of the culture was inoculated into 14mL of XHB, in an L-tube equilibrated to 35.0°C. Once the culture in the L-tube had obtained full scale deflection on the nephelometer, doubling dilutions were made using XHB and the corresponding OD values determined. This process was

Table 2.4: Water Activities of Moderate Halophile Broths Containing Different Sodium Chloride and Glycerol Concentrations.

Molality	NaCl	Water Activity*	pH
3.5	204.6	0.869	7.08
4.0	233.8	0.848	7.04
4.5	263.0	0.826	6.99
5.0	292.2	0.803	7.06
5.5	321.4	0.780	7.01
6.0	350.6	0.757	7.08
Molality	**Glycerol**	**Water Activity****	**pH**
4.0	368.4	0.915	7.02
4.5	414.4	0.908	7.01
5.0	460.5	0.900	7.02
5.5	506.6	0.888	7.00
6.0	552.5	0.883	7.01
6.5	598.7	0.872	7.00

* Values calculated (Appendix 6.2a).

** Values determined using a Sina-Scope (Sina, Zurich, Switzerland).

NaCl and glycerol values in grams per Kg water.

Table 2.5: Water Activities of Moderate Halophile Broths Containing Different Sodium Chloride Concentrations.

Molality	NaCl	Water Activity*	pH
0.0	0.0	0.992	7.02
0.7	40.0	0.970	7.01
1.4	80.0	0.945	7.00
2.1	120.0	0.921	7.02
2.7	160.0	0.890	7.00
3.4	200.0	0.871	7.01
3.8	220.0	0.860	7.00
4.1	240.0	0.841	6.99
4.5	260.0	0.826	7.01

* Values determined using a Sina-Scope (Sina, Zurich, Switzerland).

NaCl values in grams per Kg water.

repeated using XHB adjusted to contain 3.5 molal NaCl.

Halobacterium sp. strain HB9 was inoculated into two flasks containing 100mL of XHB adjusted to contain 6.0 and 3.5 molal NaCl and incubated on a rotary shaker at 35°C. Bacterial numbers and cell dimensions were determined as in Section 2.3a(i). Dilution of both cultures used extreme halophile saline (XHS) (Appendix 6.1p) with aliquots plated onto extreme halophile agar (XHA) (Appendix 6.1q).

2.3b(ii): The Effect of Temperature and Sodium Chloride Concentration on the Growth Rate of Halobacterium sp. Strain HB9.

The effect of temperature and NaCl concentration on the growth rate of Halobacterium sp. strain HB9, within the temperature range 15-50°C and the NaCl range 2.75-6.0 molal, was examined according to the protocol described in Section 2.3a(ii). NaCl was used to adjust the water activity of the basal growth medium (XHB) (Table 2.6). Growth at each temperature was determined as in Section 2.2b. OD and GT data were analysed by the methods described in Section 2.3a(ii).

2.3b(iii): The Effect of Temperature and Sodium Chloride Concentration on the Growth Rate of Halobacterium salinarium Strain CM42/12.

The effect of temperature and NaCl concentration on the growth rate of Halobacterium salinarium strain CM42/12, within the temperature range 15-50°C and the NaCl range 3.0-6.0 molal, was examined according to the protocol described in Section 2.3a(ii). NaCl was used to adjust the water activity of the basal growth medium (XHB) (Table 2.7). Growth at each temperature was determined as in Section 2.2b. OD and GT data were analysed by the methods described in Section 2.3a(ii).

Table 2.6: Water Activities of Extreme Halophile Broths Containing Different Sodium Chloride Concentrations, Used for Growth of *Halobacterium* sp. Strain HB9.

Molality	NaCl	Water Activity*	pH
2.75	160.7	0.896	6.76
3.0	175.3	0.892	6.92
3.25	189.9	0.878	6.94
3.5	204.5	0.865	6.92
4.0	233.8	0.846	7.15
4.5	263.0	0.826	7.10
5.0	292.3	0.806	7.15
5.0	292.3	0.801	6.95
5.5	321.4	0.780	7.02
6.0	350.6	0.748	7.06

* Values determined using a Sina-Scope (Sina, Zurich, Switzerland).

NaCl values in grams per Kg water.

Table 2.7: Water Activities of Extreme Halophile Broths Containing Different Sodium Chloride Concentrations, Used for Growth of Halobacterium salinarium Strain CM42/12.

Molality	NaCl	Water Activity*	pH
3.0	175.3	0.888	7.01
3.5	204.5	0.870	7.05
4.0	233.8	0.846	7.01
4.5	263.0	0.822	6.95
5.0	292.3	0.790	6.95
5.5	321.4	0.776	6.98
6.0	350.6	0.748	7.00

* Values calculated (Appendix 6.2a).

NaCl values in grams per Kg water.

3. RESULTS AND DISCUSSION.

3.1: THE EFFECT OF TEMPERATURE ON MILK SPOILAGE.

It has been shown that incubation temperature and duration of storage, directly influence the keeping quality and hence the shelf-life of milk (Barnard, 1972; Hankin et al.,1977 & Janzen et al.,1981). The organoleptic quality of the milk is directly related to the level of microorganisms present in the milk, with spoilage often occurring at microbial levels of approximately log(7.5) cfu mL^{-1} (Griffiths et al.,1984).

The general aim of the experiments in this section was to determine the response to temperature of the post pasteurisation microbiota of homogenised milk. This response was assessed in three ways. The first involved the measurement of a product of microbial metabolism, viz., carbon dioxide concentration. The second involved monitoring changes in the microbiota during storage of the milk, by determining microbial numbers. The third involved organoleptic assessment of the milk in order to determine spoilage.

The first two methods described, represent general methods of assessing the microbial quality of food products. However, metabolic end products tend to accumulate to significant levels when microbial levels reach 10^{7-8} cfu mL^{-1} or g^{-1} (Edwards et al.,1985) and are hence of little predictive value. Similarly, determination of microbial numbers, gives a retrospective result, which is often available only after the product has reached the end of its shelf-life and hence the result is of no predictive value.

Determination of the organoleptic quality of some products may have predictive value when assessed by a trained taste panel but often provides only an end point measurement indicative of the onset of spoilage. A significant advance in the predictive value of any spoilage indicator would be provided if the relationship between spoilage rate and storage conditions was known. By interpolation,

this could be used to assess elapsed and remaining shelf-life, before the degree of spoilage is determined using currently available techniques. In this study, time/temperature function integration (TTFI) has been used as the basis for such a process.

3.1a: The Relationship Between Carbon Dioxide Concentration and the Spoilage of Pasteurised, Homogenised Milk.

In addition to determining the response to temperature of the post-pasteurisation microbiota of milk, the aim of (i), (ii) and (iii), was to utilise headspace carbon dioxide concentration ($[CO_2]$), as a rapid method of monitoring any increase in microbial levels.

(i) The five pairs of L-tubes containing pasteurised, homogenised milk, were sampled at organoleptic spoilage, to determine microbial numbers on PCA, using a 1.0mL pour plate and a 0.1mL spread plate method. A comparison of the $[CO_2]$ produced in the closed and open tubes at spoilage also was made (Table 3.1).

The time taken for organoleptic spoilage to occur, increased with decreasing temperature and microbial numbers were similar at each temperature. No difference between the two methods of determining microbial numbers was observed, as both spread and pour plate techniques yielded similar results. Discrepancies between the two methods were due to errors inherent in the viable count technique. The Standards Association of Australia recommends the use of the pour plate technique for the enumeration of microorganisms in milk (Anonymous, 1973), however, it is more practical to isolate colonies for identification from the surface of an agar spread plate, than from within an agar pour plate. Hence, both methods were used at various times in this study.

The sealed and unsealed tubes also yielded similar microbial

Table 3.1: Microbial Numbers and Carbon Dioxide Concentrations Associated with Spoiled, Pasteurised, Homogenised Milk, Stored at Different Temperatures.

Temperature (°C)		Time (hr)	PCA 0.1mL	PCA 1.0mL	$\%CO_2$
15.1	unsealed	48	>9.00	>8.70	0.81
	sealed		8.46	8.70	10.44
9.9	unsealed	74	8.69	8.92	0.92
	sealed		8.28	8.61	7.70
7.1	unsealed	121	8.81	>9.00	0.52
	sealed		8.72	>9.00	11.25
4.2	unsealed	192	8.77	8.43	0.54
	sealed		7.78	7.54	6.73
2.0	unsealed	241	8.83	8.92	0.66
	sealed		8.63	8.86	9.65
Initial milk			3.94		

PCA 0.1mL: log of microbial numbers (cfu mL^{-1}) on 0.1mL PCA spread plates.

PCA 1.0mL: log of microbial numbers (cfu mL^{-1}) in 1.0mL PCA pour plates.

All figures are the mean of results from duplicate samples.

numbers within the limits of accuracy of the plate count method, with the exception of the L-tubes stored at 4.2°C. In general, the quantity of milk used and horizontal storage of the L-tubes, provided enough air and a large enough surface area for sufficient diffusion of oxygen into the milk, to allow unrestricted aerobic microbial growth for the duration of the experiment.

There was a large increase in $[CO_2]$ in the sealed L-tubes, at all storage temperatures, caused by the metabolic activity of the spoilage microbiota. It was concluded that it may be possible to use the change in $[CO_2]$ as a method of monitoring the development of the spoilage microbiota. Obviously, such a method would be valid only in a closed system as the $[CO_2]$ of the open L-tubes did not change.

(ii) The effect of incubation time and temperature, on the development of microbial and pseudomonad numbers in relation to $[CO_2]$ were monitored. Even though microbial numbers, as determined by PCA spread and pour plates and PsA spread plates, increased at each storage temperature with time, there were no concomitant increases in the $[CO_2]$ within the head spaces of the milk cartons (Table 3.2).

There are two possible explanations. The first, is that any CO_2 produced, leaked from the cartons, and the second, is that high levels of CO_2 are associated with microbial numbers similar to those found at organoleptic spoilage. The second is favoured, as the final $[CO_2]$ measured at 4.4 and 1.8°C, were slightly higher than their preceding $[CO_2]$ and would be expected to increase as spoilage progressed.

(iii) To determine the relationship between microbial numbers and $[CO_2]$, pasteurised, homogenised milk was stored at different temperatures until organoleptic spoilage was reached. Both microbial

Table 3.2: Changes in the Microbial Numbers and Carbon Dioxide Concentrations of Cartoned, Pasteurised, Homogenised Milk, During Storage at Different Temperatures.

Temp. (°C)	Time (hr)	PCA 0.1mL	PCA 1.0mL	PsA 0.1mL	$\%CO_2$
10.5	21.3	<2.00	Cont.	<2.00	0.35
	33.3	<2.00	Cont.	2.30	0.36
	45.3	<2.00	3.00	3.28	0.44
	57.5	4.08	3.16	2.30	0.42
	69.8	4.48	3.99	3.48	0.51
	94.5	5.98	5.42	6.19	0.30
7.2	23.3	<2.00	2.68	<1.00	0.41
	47.2	<2.00	4.05	<1.00	0.37
	71.8	2.30	3.70	1.00	0.52
	96.3	2.00	3.00	1.74	0.39
	120.8	3.85	3.18	4.08	0.45
	169.8	6.57	5.48	6.83	0.34
4.4	50.0	4.00	4.03	<1.00	0.33
	95.5	<2.00	<2.00	<2.00	0.41
	143.4	3.90	3.99	2.48	0.51
	192.0	4.19	3.75	4.42	0.31
	289.2	6.83	6.41	7.03	0.44
	339.3	7.97	7.72	7.88	1.25
1.8	121.5	<2.00	<2.00	<2.00	0.49
	190.8	3.87	3.94	<2.00	0.29
	313.0	5.12	3.72	5.38	0.41
	363.3	6.32	5.36	6.38	0.35
	456.3	7.65	7.03	7.48	1.12

PCA 0.1mL: log of cfu mL^{-1} on 0.1mL PCA spread plates.

PCA 1.0mL: log of cfu mL^{-1} in 1.0mL PCA pour plates.

PsA 0.1mL: log of cfu mL^{-1} on 0.1mL PsA spread plates.

Cont.: plates contaminated.

numbers and associated [CO_2] were monitored. The [CO_2] in the head space of the L-tubes associated with any given number of microorganisms, irrespective of temperature, were similar. However, no linear relationship between [CO_2] and microbial numbers was evident. An increase in [CO_2] was detected only when microbial levels exceeded approximately 10^8 cfu mL^{-1} (Figure 3.1).

This result was similar to those for other end product and enzyme detection techniques, which tend only to be of use when microbial numbers are at least 10^{7-8} cfu mL^{-1}. Edwards et al.,(1985) reported that the significant increases in diamine levels associated with the onset of spoilage of stored beef, occurred at microbial levels of approximately 10^7 cfu g^{-1}.

For this reason, rapid methods for determining microbial numbers, such as the $^{14}CO_2$ radiometric method (Waters, 1972) and the impedimetric method (Firstenberg-Eden & Tricarico, 1983), rely on the incubation of the food sample to achieve microbial levels of approximately 10^{7-8} cfu mL^{-1} before obtaining a detectable response.

3.1b: The Relationship Between Storage Temperature and the Spoilage of Pasteurised, Homogenised Milk.

Pooni & Mead (1984) have used the Square Root Model of Ratkowsky et al.,(1982), to demonstrate the existence of a linear relationship between storage temperature and the rate of spoilage of poultry. The aim of this section was to examine the ability of the Square Root Model to describe accurately the relationship between temperature and the rate of spoilage of pasteurised, homogenised milk.

A plot of the square root of the spoilage rate of pasteurised, homogenised milk versus temperature obtained from spoilage experiment 2.1b(i), was linear (Figure 3.2a). Similar results were obtained from

Figure 3.1: Plot of Carbon Dioxide Concentrations Versus Microbial Numbers.

A plot of the carbon dioxide concentration in the head space of sealed L-tubes containing pasteurised, homogenised milk versus the microbial numbers present in the milk (cfu mL^{-1}).

For data refer to Appendix 6.3.

Figure 3.1: Plot of Carbon Dioxide Concentrations Versus Microbial Numbers.

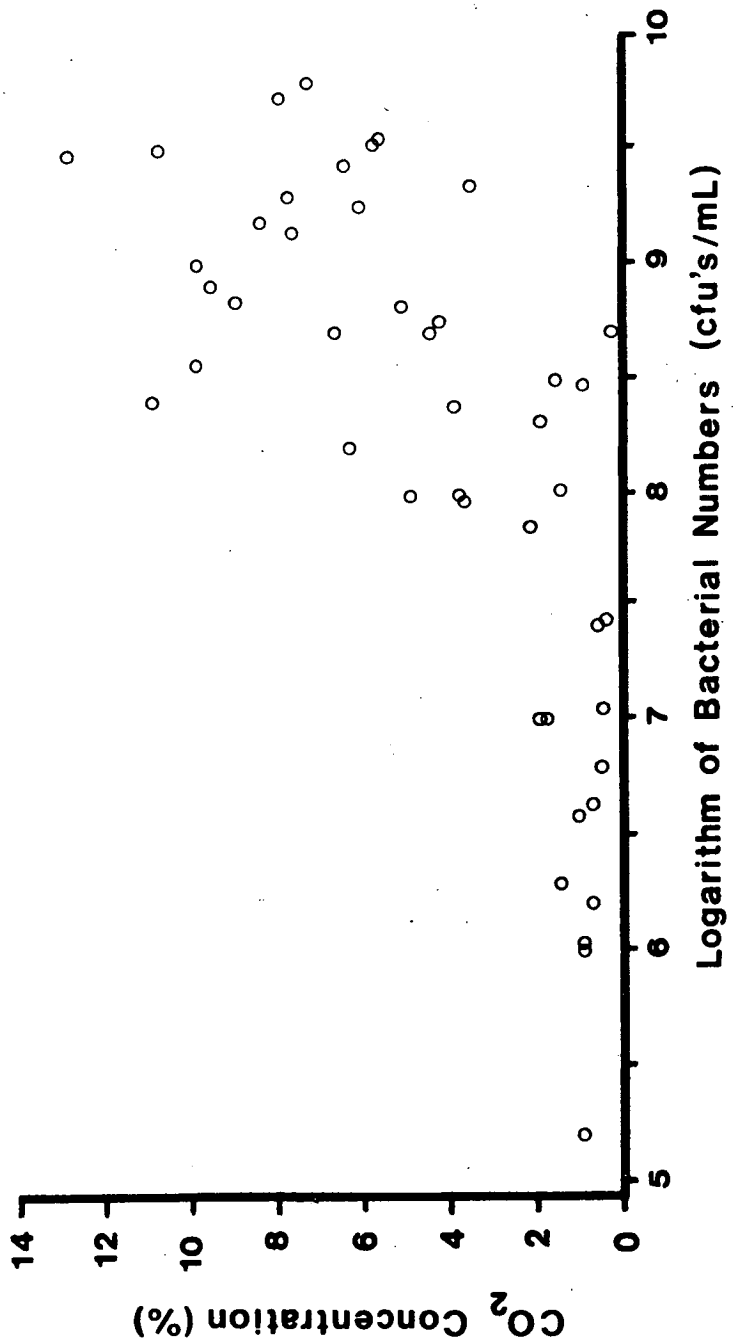


Figure 3.2: Square Root Plots of the Effect of Temperature on the Spoilage of Pasteurised, Homogenised Milk.

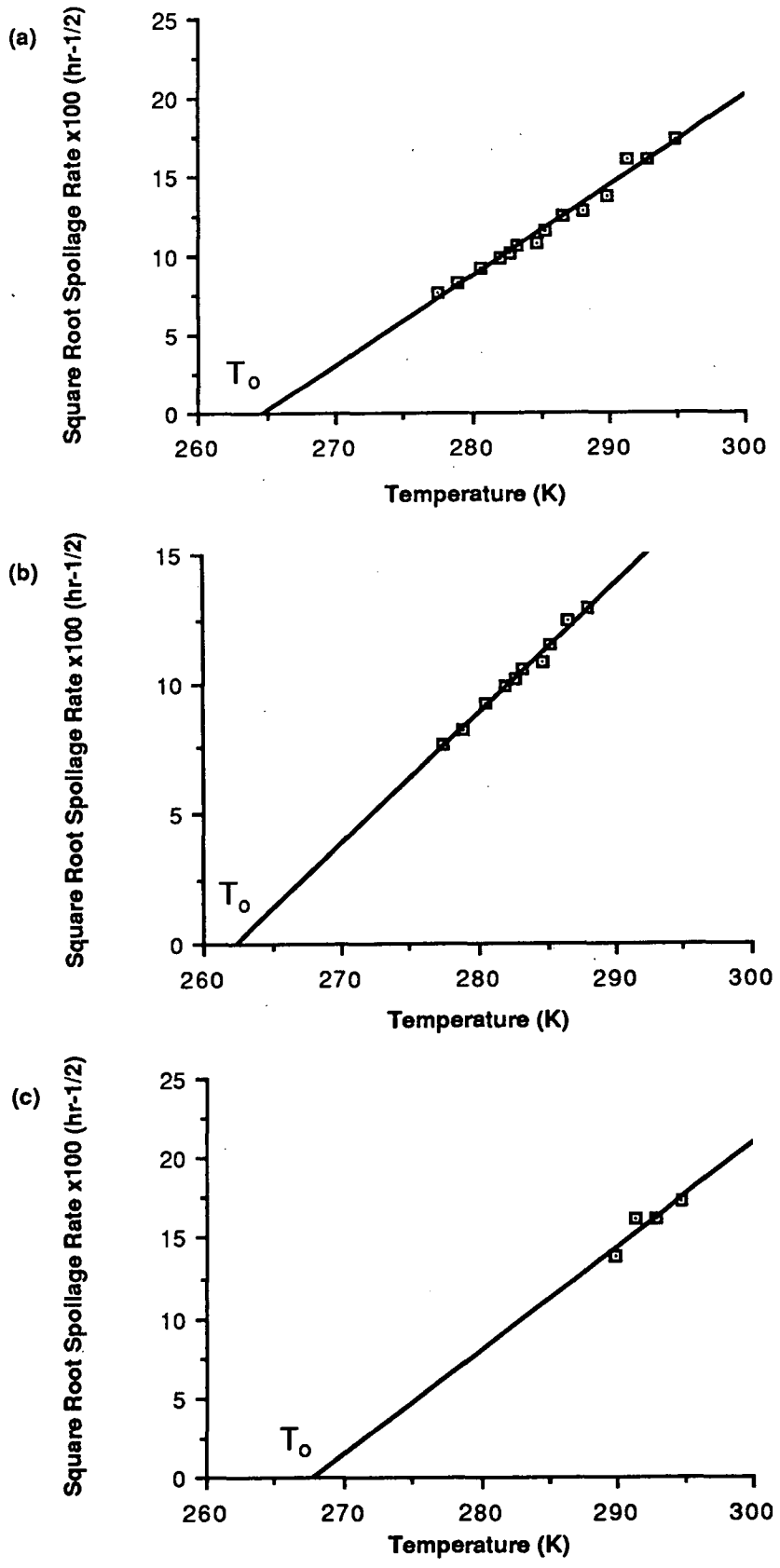
(a): the Square Root plot contains data points for all temperatures from the milk spoilage experiment conducted in 2.1b(i).

(b): the Square Root plot of data points for temperatures <15°C from 2.1b(i).

(c): the Square Root plot of data points for temperatures >15°C from 2.1b(i).

In each graph, the solid line represents the least squares fit of the experimental data. For experimental and predicted values, refer to Appendix 6.4a.

Figure 3.2: Square Root Plots of the Effect of Temperature on the Spoilage of Pasteurised, Homogenised Milk.



the spoilage experiments conducted in 2.1b(ii), 2.1b(iii), 2.1b(iv) and 2.1b(v) (Figures 3.3a, 3.4a, 3.5a and 3.6a, respectively). The mean T_o (T_{min}) value for the data over the entire temperature range studied was 267.8K (Table 3.3).

Even though all the correlation coefficients were greater than 0.987, each spoilage experiment was analysed further, over two temperature ranges, i.e., above and below 15°C. The basis for this was the observation that psychrotrophic pseudomonads were responsible for spoilage below 15°C, and that above this temperature, there was an increasing proportion of mesophilic microorganisms present in the spoilage microbiota [Section 3.1c(i)].

Figures 3.2b, 3.3b, 3.4b, 3.5b and 3.6b, contain the square root plots of the spoilage data at temperatures <15°C. The mean T_o value was 265.4K. This falls within the T_o range for psychrotrophic microorganisms (Olley, 1983). The correlation coefficients were similar to those calculated for each spoilage experiment over their entire temperature range.

Figures 3.2c, 3.3c, 3.4c, 3.5c and 3.6c, contain the square root plots of the spoilage data at temperatures above 15°C. The mean T_o value of 271.2K for temperatures >15°C, was in the lower portion of the T_o range for mesophilic microorganisms (Olley, 1983). This was probably due to the continuing influence of psychrotrophs in the spoilage microbiota above 15°C, which decreased with further increase in temperature. The correlation coefficients for this data were also similar to those calculated for each individual experiment over their entire temperature range.

The change in microbiota at approximately 15°C, demonstrated by the analyses reported in Section 3.1c(i), was confirmed by the change in temperature response of the spoilage microorganisms in pasteurised, homogenised milk at approximately 15°C. At temperatures

Figure 3.3: Square Root Plots of the Effect of Temperature on the Spoilage of Pasteurised, Homogenised Milk.

(a): the Square Root plot contains data points for all temperatures from the milk spoilage experiment conducted in 2.1b(ii).

(b): the Square Root plot of data points for temperatures <15°C from 2.1b(ii).

(c): the Square Root plot of data points for temperatures >15°C from 2.1b(ii).

In each graph, the solid line represents the least squares fit of the experimental data. For experimental and predicted values, refer to Appendix 6.4b.

Figure 3.3: Square Root Plots of the Effect of Temperature on the Spoilage of Pasteurised, Homogenised Milk.

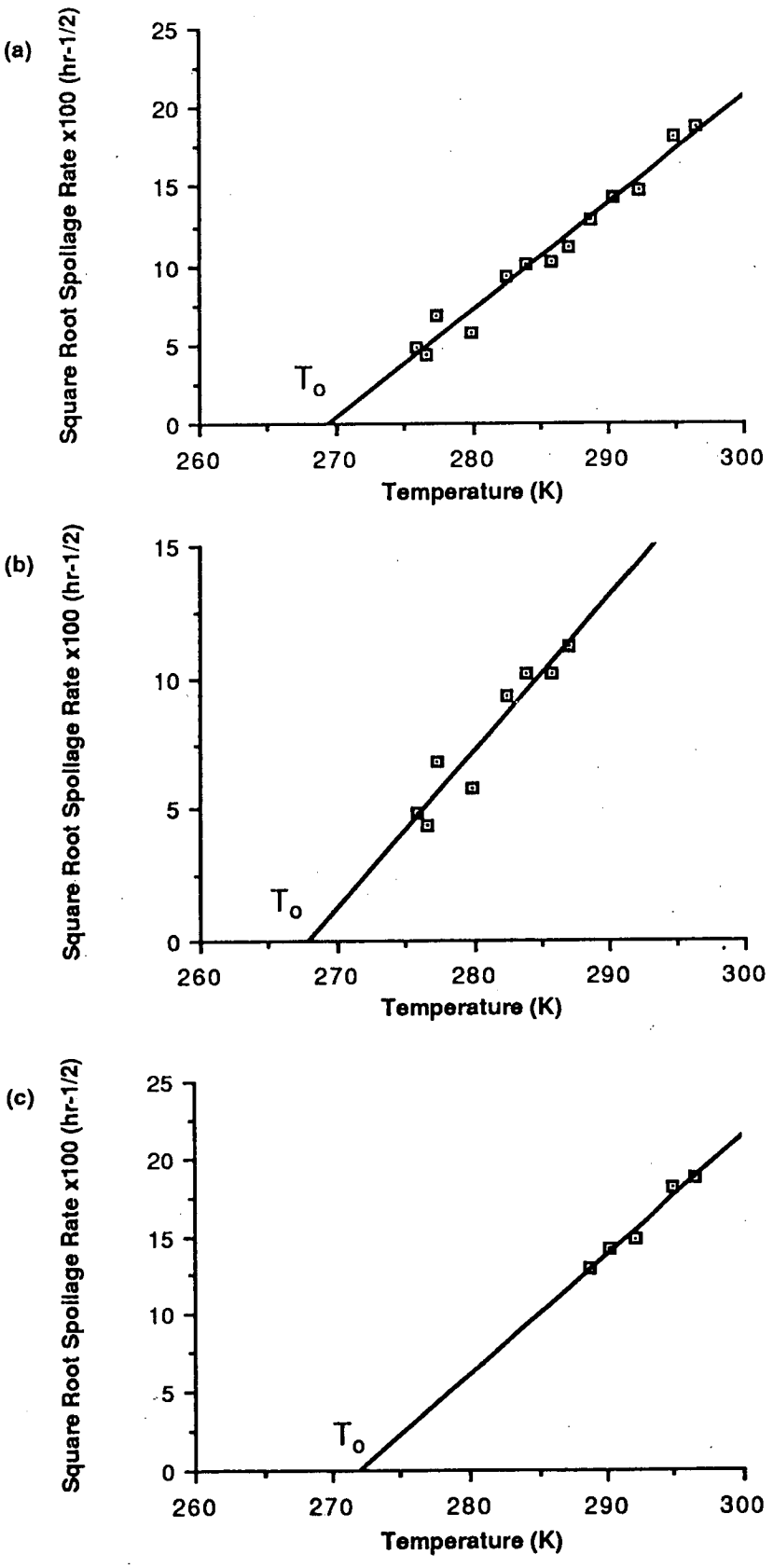


Figure 3.4: Square Root Plots of the Effect of Temperature on the Spoilage of Pasteurised, Homogenised Milk.

(a): the Square Root plot contains data points for all temperatures from the milk spoilage experiment conducted in 2.1b(iii).

(b): the Square Root plot of data points for temperatures <15°C from 2.1b(iii).

(c): the Square Root plot of data points for temperatures >15°C from 2.1b(iii).

In each graph, the solid line represents the least squares fit of the experimental data. For experimental and predicted values, refer to Appendix 6.4c.

Figure 3.4: Square Root Plots of the Effect of Temperature on the Spoilage of Pasteurised, Homogenised Milk.

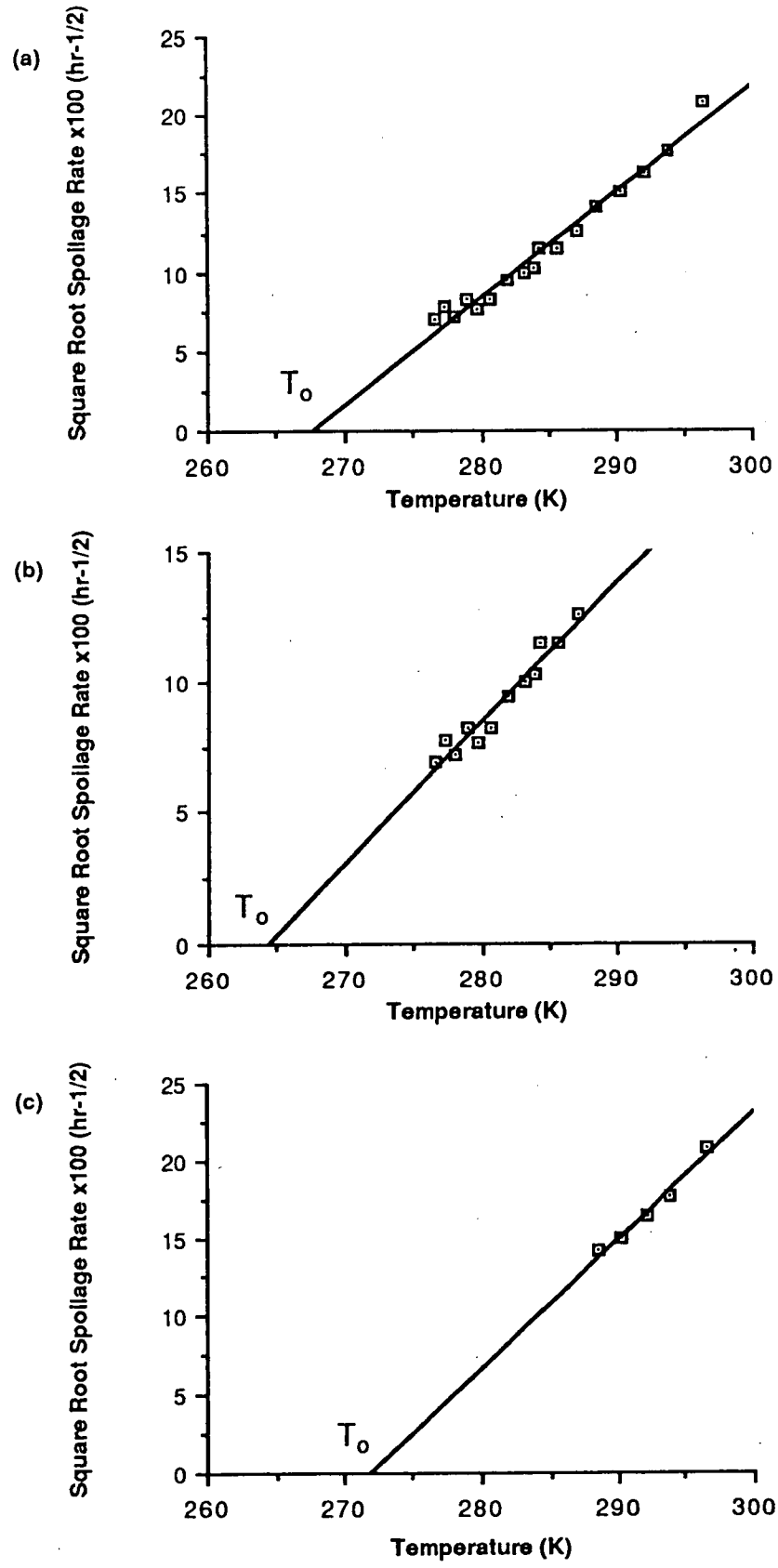


Figure 3.5: Square Root Plots of the Effect of Temperature on the Spoilage of Pasteurised, Homogenised Milk.

(a): the Square Root plot contains data points for all temperatures from the milk spoilage experiment conducted in 2.1b(iv).

(b): the Square Root plot of data points for temperatures <15°C from 2.1b(iv).

(c): the Square Root plot of data points for temperatures >15°C from 2.1b(iv).

In each graph, the solid line represents the least squares fit of the experimental data. For experimental and predicted values, refer to Appendix 6.4d.

Figure 3.5: Square Root Plots of the Effect of Temperature on the Spoilage of Pasteurised, Homogenised Milk.

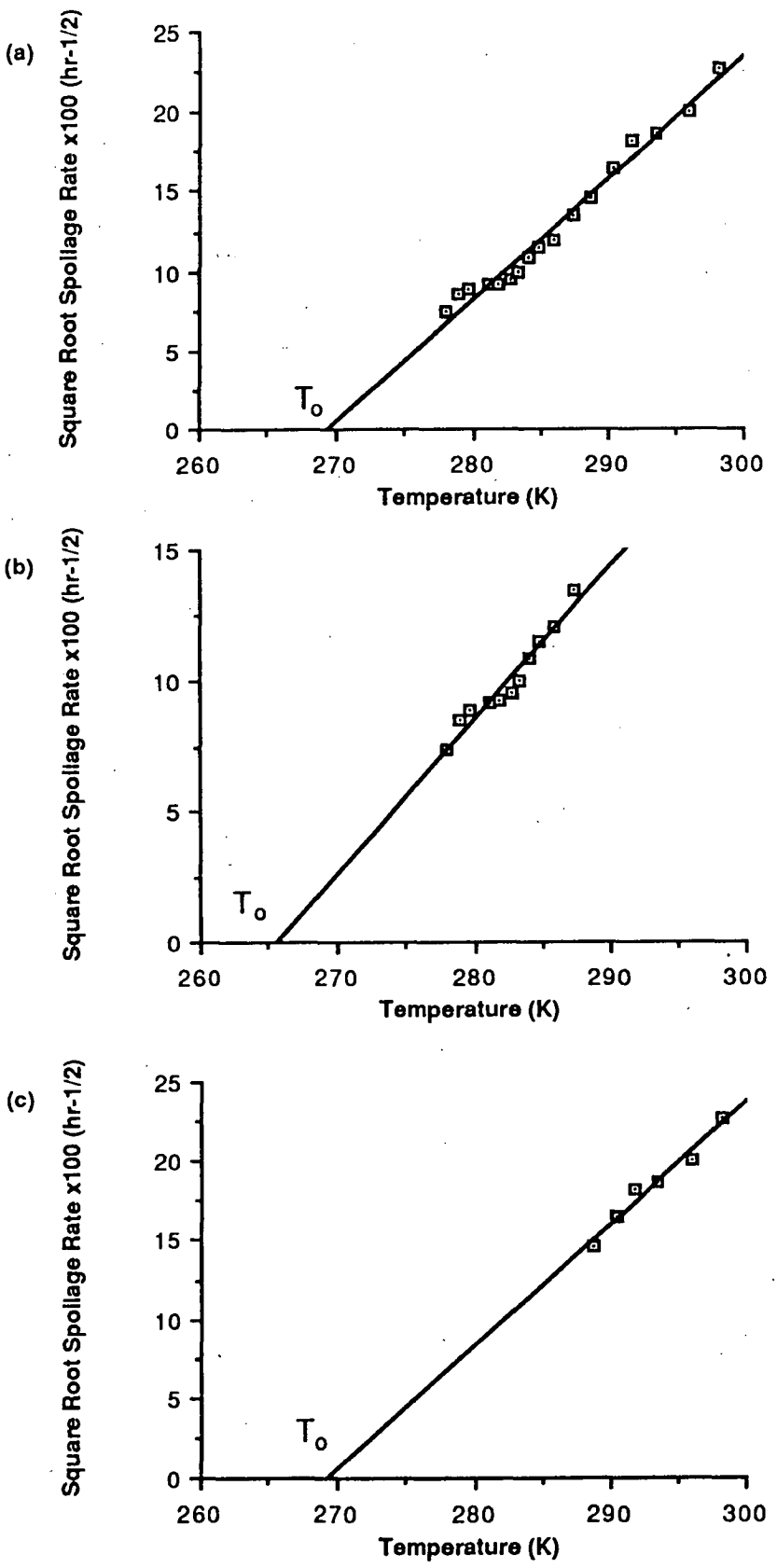


Figure 3.6: Square Root Plots of the Effect of Temperature on the Spoilage of Pasteurised, Homogenised Milk.

(a): the Square Root plot contains data points for all temperatures from the milk spoilage experiment conducted in 2.1b(v).

(b): the Square Root plot of data points for temperatures <15°C from 2.1b(v).

(c): the Square Root plot of data points for temperatures >15°C from 2.1b(v).

In each graph, the solid line represents the least squares fit of the experimental data. For experimental and predicted values, refer to Appendix 6.4e.

Figure 3.6: Square Root Plots of the Effect of Temperature on the Spoilage of Pasteurised, Homogenised Milk.

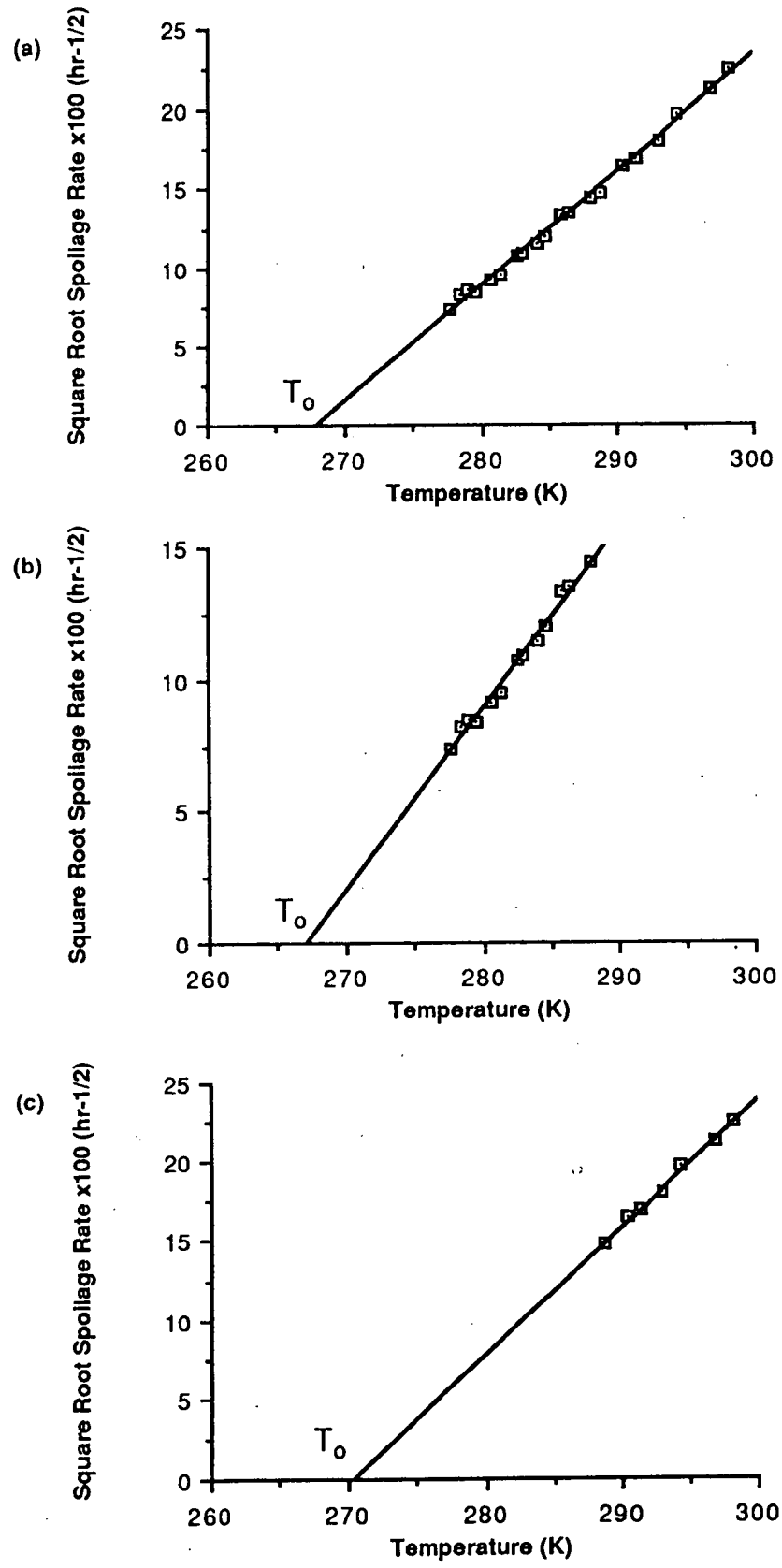


Table 3.3: T_o Values and Correlation Coefficients Predicted by the Square Root Model for the Spoilage of Pasteurised, Homogenised Milk.

Spoilage Experiment	T all		T<15°C		T>15°C	
	T_o	r	T_o	r	T_o	r
2.1b(i)	264.7	0.991	262.3	0.995	267.7	0.913
2.1b(ii)	269.4	0.987	267.9	0.951	271.9	0.987
2.1b(iii)	267.6	0.988	264.3	0.972	271.8	0.988
2.1b(iv)	269.3	0.991	265.5	0.972	269.2	0.981
2.1b(v)	267.9	0.998	267.0	0.994	270.3	0.997
Mean	267.8		265.4		271.2	

T all, T<15°C, T>15°C: temperature ranges of the data for analysis by least squares regression.

All T_o values (K).

r: correlation coefficient.

<15°C the spoilage of pasteurised, homogenised milk was found to be a psychrotrophic process which was best described using a T_o value similar to those of the psychrotrophs responsible for spoilage. Similarly, at temperatures >15°C, spoilage was a mesophilic process and the T_o value obtained was similar to those indicative of mesophilic microorganisms.

Once the T_o value for a spoilage process has been determined, the Square Root Model may be used to predict spoilage rates relative to a specified temperature. Pooni & Mead (1984) used the Square Root Model and a T_o value of 263K to predict the spoilage of poultry at temperatures <15°C by psychrotrophic pseudomonads. Gill & Harrison (1985) used a T_o value of approximately 277K for their work on predicting the growth of Escherichia coli on sheep offal. Smith (1985, 1987) used a T_o value of 276.5K for his work on predicting the growth of coliform microorganisms and the growth of Salmonella typhimurium and E. coli, in minced mutton tissue.

The mean T_o value obtained for the spoilage of pasteurised, homogenised milk at temperatures <15°C was approximately 265K. Therefore, a relative rate curve based on this value would be more appropriate than the currently used 263K curve. Table 3.4 contains the relative rates predicted by the two curves. At low temperatures, the comparative relative rates are similar. However, with increasing temperature, the 265K curve predicts spoilage rates which are faster than those predicted by the 263K curve.

Information concerning relative spoilage rates derived from the Square Root Model and a specified T_o value, is used to program time/temperature function integrators (TTFI) (Owen & Nesbitt, 1984). These devices are capable of monitoring the temperature related

Table 3.4: Comparison Between the 263K and 265K Curves Predicted by the Square Root Model.

Temperature (°C)	Relative Rate 263K	Relative Rate 265K
0	1.00	1.00
2	1.44	1.56
4	1.96	2.25
6	2.56	3.06
8	3.24	4.00
10	4.00	5.06
12	4.84	6.25
14	5.76	7.56
15	6.25	8.27

All rates are relative to 0°C (273K).

deterioration of a food product. TTFI are currently based on a 263K relative rate curve and are easily modified to operate on single or multiple relative rate curves. In the case of pasteurised, homogenised milk it appears that a TTFI with a two curve system set to operate on a 265K curve below 15°C and a 271K curve above 15°C, would be more appropriate. However, in a practical situation, a single 265K curve may suffice, as extended periods of storage at temperatures above 15°C would lead to the rapid deterioration of the milk.

3.1c: The Effect of Storage Temperature on the Microbiota of Pasteurised, Homogenised Milk.

3.1c(i): Analyses of Initial and Spoilage Microbiota.

Characterisation of the psychrotrophic nature of isolates from the initial microbiota of pasteurised, homogenised milk (Analysis 1 and 2 respectively in Table 3.5), showed that none of the isolates was capable of growth at 4°C and hence could not contribute to the spoilage microbiota at refrigeration temperatures. This is not unusual, as the majority of cases reported in the literature, describe the causative microorganisms as belonging to the genus Pseudomonas (Sherman et al., 1941; Boyd et al., 1953; Alexander & Higginbottom, 1953 and Maxcy, 1967). These bacteria are generally psychrotrophic, post-pasteurisation contaminants, initially present in very low numbers (Maxcy, 1967; Olson, 1967 and Cousin, 1982).

It can be seen from Analysis 1, that pseudomonads constituted 100% of the microbiota after spoilage at 4.2, 6.6 and 10.1°C. At 15.4°C, mesophilic Gram-positive microorganisms were present at spoilage and constituted 100% of the microbiota identified at 23.4°C.

Analysis 2 yielded similar results, with pseudomonads constituting 100% of the microbiota at 6.3°C, 70% at 11.5°C and 60%

Table 3.5: Analyses of the Initial and Spoilage Microbiota of Pasteurised, Homogenised Milk Stored at Different Temperatures.

	Bacterial Groups (%)							
	Ps.	Ent.	Ac.	Bac.	G+ve.	N.G.	%Psy.	Tot.
Analysis 1								
Initial	0	0	0	0	98	5	0	41
23.4°C	0	0	0	13	56	28	3	32
15.4°C	79	0	0	6	16	0	78	32
10.1°C	100	0	0	0	0	0	100	24
6.6°C	100	0	0	0	0	0	100	21
4.2°C	100	0	0	0	0	0	100	34
Analysis 2								
Initial	0	0	2	2	96	0	0	48
25.0°C	0	0	85	15	0	0	0	40
19.7°C	60	25	10	0	4	0	88	48
15.6°C	60	29	10	0	0	0	83	48
11.5°C	70	20	10	0	0	0	90	40
6.3°C	100	0	0	0	0	0	100	48

Ps. = Pseudomonas spp.; Ent. = Enterobacteriaceae spp.; Ac. = Acinetobacter spp.; Bac. = Bacillus spp.; G+ve. = Other Gram positives include lactobacilli, micrococci and coryneforms; N.G. = No growth after initial isolation; %Psy. = % capable of psychrotrophic growth; Tot. = Total number of isolates.

The spoilage microbiota at each temperature correspond to percentages present at Log(7.5) cfu mL^{-1} of milk.

For identification of the microflora, refer to Appendix 6.5.

at 15.6°C. The remaining bacteria at these temperatures were identified as species of Acinetobacter and Enterobacteriaceae. None of the Acinetobacter was found to be psychrotrophic, whereas, all but one of the Enterobacteriaceae were capable of psychrotrophic growth at 4°C.

The dominance of the pseudomonads over the Enterobacteriaceae at low temperatures, even though the latter were capable of growth at 4°C and above, was evident when generation times for representatives from the two groups were compared. Generation times for Pseudomonas sp. strain 12.48 and Enterobacter agglomerans strain 11.33 at 4°C, were 5.6 and 10.5 hours, respectively; at 8°C, 2.7 and 4.0 hours; at 12°C, 1.6 and 2.1 hours; and at 16°C, 1.1 and 1.3 hours (results from Section 3.2b). Hence, it was not surprising that strains of Enterobacteriaceae were present in the spoilage microbiota of pasteurised, homogenised milk at storage temperatures of approximately 12°C and above. It can be calculated from the generation times at 12°C, that during the period of time required for a four fold increase in Pseudomonas numbers, there would be a three fold increase in Enterobacter numbers.

Psychrotrophic, thermoduric bacilli have also been reported to be responsible for the spoilage of pasteurised milk (Grosskopf & Harper, 1969; Shehata et al., 1971; Credit et al., 1972 and Coghill, 1982). However, these bacteria only grow very slowly at refrigeration temperatures: 20.9-35.6 hours per generation at 4.5°C (Tinuoye & Harmon, 1975); and 22 hours at 4°C (Langeveld & Cuperus, 1980). Comparison of these generation times with those presented above, showed the psychrotrophic pseudomonads to be capable of significantly faster growth. The calculated generation times agree well with those from the literature: 5 hours at 5°C (Frazier & Westhoff, 1978; and

4.2-6.7 hours at 4°C (Olsen & Jezeski, 1963). In addition, Mol & Vincentie (1981), compared the generation times of thermoduric psychrotrophs at 7°C with those of pseudomonads and found times of 24-30 hours and 4 hours respectively. Hence, the psychrotrophic pseudomonads will rapidly outgrow thermoduric bacilli at cool store temperatures, even if present initially in low numbers.

3.1c(ii): Changes in the Microbiota During Storage.

The dominance of the pseudomonads during and at completion of storage at refrigeration temperatures, is evident in Table 3.6, in which details are presented of the pseudomonad and microbial numbers present in pasteurised, homogenised milk samples incubated at 4.1, 10.0 and 14.6°C. Although the pseudomonads were present initially in numbers of $<10mL^{-1}$, they rapidly became the dominant constituent of the microbiota present at each incubation temperature and hence at spoilage. At 14.6°C the pseudomonad count was similar to the microbial count after 32 hr. At 10.0°C, the counts were effectively the same after 47 hr and at 4.1°C, the counts were similar after 68 hr.

At each storage temperature the initial microbial count on PCA (equivalent to the initial thermoduric count), did not alter during incubation, until the pseudomonad count on PsA reached a similar level. From this time, microbial numbers on PCA and PsA, increased at similar rates.

Maxcy (1967), demonstrated similar results for freshly pasteurised milk, which had been stored at 5°C for intervals up to 14 days and then incubated at 32°C. Post-pasteurisation contaminants were monitored using a selective medium based on alkyl aryl sulfonate and were compared to the Standard Plate Count. The two methods of enumeration showed similar patterns of growth, with the counts from

Table 3.6: Microbial and Pseudomonad Numbers During Storage of Pasteurised, Homogenised Milk.

Temp.(°C).		Incubation Period (hr) 0	18	32	47	68	100	124	146	168
14.6	PCA	3.16	3.05	4.07	5.64	7.94	-	-	-	-
	PsA	<1	0.78	4.31	>5	7.82	-	-	-	-
10.0	PCA	3.13	3.11	-	3.79	5.63	7.59	7.70	-	-
	PsA	<1	<1	-	3.93	>5	7.37	7.98	-	-
4.1	PCA	3.09	3.12	-	2.85	3.09	3.85	5.08	6.19	7.10
	PsA	<1	<1	-	1.00	3.15	3.89	5.17	6.30	7.20

Incubation period of 0 hours = initial count.

PCA: log of microbial count (cfu mL^{-1}) on 0.1mL PCA spread plates.

PsA: log of pseudomonad count (cfu mL^{-1}) on 0.1mL PsA spread plates.

the selective medium starting at approximately 3% of the total count at the beginning of the incubation period and accounting for an increasing percentage as the test period progressed.

The data presented in Table 3.6 demonstrates the usefulness PsA as selective medium for the enumeration of pseudomonads. It provided a method of monitoring the increase in pseudomonad numbers whilst they were being masked by larger numbers of thermoduric microorganisms. It also provided a useful method for obtaining a presumptive psychrotrophic pseudomonad count. Each of the 143 presumptively identified pseudomonads from Section 3.1d(ii) which were capable of growth on PsA, were also capable of psychrotrophic growth at 4°C.

Phillips & Griffiths (1986), concluded that even though PsA was inhibitory to the growth of Gram-positive bacteria, it was not solely selective for pseudomonad growth. Results obtained within this study by testing colonies capable of growth on the medium, for the presence of cytochrome oxidase and motility, have shown PsA to be reasonably selective for the growth of pseudomonads [Section 3.1d(ii)].

3.1c(iii): Development of a Most Probable Number Technique to Detect Pseudomonads.

Pseudomonads generally occur in milk as post pasteurisation contaminants in very low numbers (Olson, 1967 and Cousin, 1982), often at levels which cannot be enumerated by normal plating techniques. Pour and spread plate methods, which use 1 and 0.1mL volumes, enable the enumeration of microbial numbers greater than 1 and 10 cfu mL^{-1} respectively. Maxcy & Wallen (1983) stated that the number of bacteria of significance in a freshly pasteurised, packaged sample of milk, is so low that the variation in a standard plate

count far exceeded the number of bacteria of real interest. The use of 1mL pour plates presents the additional problem of the opacity of the milk, making direct enumeration extremely difficult.

The most probable number method is often used in situations were normal plate count determinations are of limited or no use. The method is suited to counting microbial groups that fail to form colonies on agar or particular groups in mixed cultures, where some selective medium or technique is available. For example, the presumptive coliform count in water analysis (Anonymous, 1982). Hence, the MPN technique would be useful to enumerate the low numbers of pseudomonads present in freshly pasteurised, homogenised milk.

An MPN medium selective for the growth of pseudomonads was developed from the components of Oxoid Pseudomonas Agar Base and the C-F-C antibiotic supplement. 2,3,4-triphenyltetrazolium chloride (TTC) was added as an indicator of microbial metabolic activity.

When samples from the positive (pink) tubes were streaked onto PsA and PCA, it was found that the resultant colonies grew equally well on PCA and PsA, with the colonies present on PsA, also being cytochrome oxidase positive. When samples from the negative (white) tubes were streaked onto both media, no growth was obtained on the PsA plates. In addition, no colour change was observed when sterile milk was incubated with the MPN medium. Therefore, it was possible to conclude that the MPN medium developed was specific for selecting and enriching pseudomonads from pasteurised, homogenised milk.

The correlation between the MPN and spread plate results for the development of both microbial and pseudomonad numbers in pasteurised, homogenised milk, incubated at approximately 5°C, is presented in Table 3.7. MPN results were recorded after 24, 48 and 72 hours

Table 3.7: The Enumeration of the Microbiota of Pasteurised, Homogenised Milk, Using Most Probable Number and Spread Plate Techniques.

No.	S.T.	TTFI	MPN24	MPN48	MPN72	PCA	PsA
1	0	0.00	0.23	0.49	1.1	1685	<10
	24	1.23	0.23	0.49	1.1	1655	<10
	48	2.47	0.23	3.50	>16.0	940	35
	72	3.72	2.40	>16.0	>16.0	7850	8000
2	0	0.00	0.46	1.58	1.58	2725	<10
	23	1.20	0.46	4.8	7.0	2550	10
	47	2.40	0.46	4.8	7.0	2600	120
	72	3.67	-	46.0	98.0	1300	14 10
3	0	0.00	0.46	0.46	0.66	3305	<10
	23	1.17	0.46	0.98	0.98	3800	<10
	47	2.43	0.46	4.8	4.8	3960	20
	71	3.70	15.8	26.0	108.0	3550	4 10
4	0	0.00	0.46	7.0	7.0	2360	10
	23	1.15	0.46	4.8	18.4	3480	<10
	48	2.23	0.46	4.8	10.8	3220	15

Each carton of pasteurised, homogenised milk was incubated at approximately 5°C.

No.: carton number.

S.T.: storage time (hours).

TTFI: time/temperature function integrator reading in days.

MPN24: MPN of pseudomonads mL^{-1} after 24 hours incubation.

MPN48: MPN of pseudomonads mL^{-1} after 48 hours incubation.

MPN72: MPN of pseudomonads mL^{-1} after 72 hours incubation.

PsA: pseudomonad numbers (cfu mL^{-1}) on PsA.

PCA: microbial numbers (cfu mL^{-1}) on PCA.

incubation at 25°C, to determine the most suitable incubation period. The results suggested that at least 72 hours incubation was required for the maximum number of positive tubes to be recorded.

As has been previously noted, microbial numbers on PCA, did not increase unless the pseudomonad numbers were at a similar level. The MPN 72 hour results showed the MPN method to predict very small numbers of pseudomonads at times when the PsA plates showed no bacterial growth, i.e., a count of <10 cfu mL^{-1}. However, when pseudomonad levels were high enough for enumeration on PsA, the MPN method predicted numbers which were less than those found on PsA.

In general, the MPN technique predicted pseudomonad numbers which were less than those determined from PsA spread plates. In addition, the broth system used, was a good enrichment medium for the detection of the post-pasteurisation contamination of milk by pseudomonads.

3.1d: Determination of the Shelf-Life of Pasteurised, Homogenised Milk by Time/Temperature Function Integration (TTFI).

3.1d(i): Correlation of TTFI Readings with Microbial Numbers.

The relationship between the change in microbial numbers and TTFI readings is presented graphically in Figure 3.7a and Figure 3.7b. Analysis of the data by least squares linear regression yielded a correlation coefficient of 0.93. Even though this value is close to 1.00, it is evident that there is a plateau in the region 0 to 5 days. As was noted in Section 3.1c(ii), this was due to the presence of mesophilic, thermoduric microorganisms, which did not increase in numbers during storage at low temperature.

The progressive increase in pseudomonad numbers with storage time, especially during the first 5 days of storage, is displayed in Figure 3.7b. The regression analysis resulted in a better fit than

Figure 3.7: Plots of Microbial and Pseudomonad Numbers Versus Time/Temperature Function Integrator Readings.

(a): A plot of the logarithm of microbial numbers (cfu mL^{-1}) present at different time/temperature function integrator readings (TTFI) during the incubation of pasteurised, homogenised milk at 6, 10, 12 or 15°C.

The solid line is the least squares regression line and is described by the equation:

$y = 1.6023 + 0.5825x$

Correlation coefficient = 0.93

(b): A plot of the logarithm of pseudomonad numbers (cfu mL^{-1}) present at different time/temperature function integrator readings (TTFI) during the incubation of pasteurised, homogenised milk at 6, 10, 12 or 15°C.

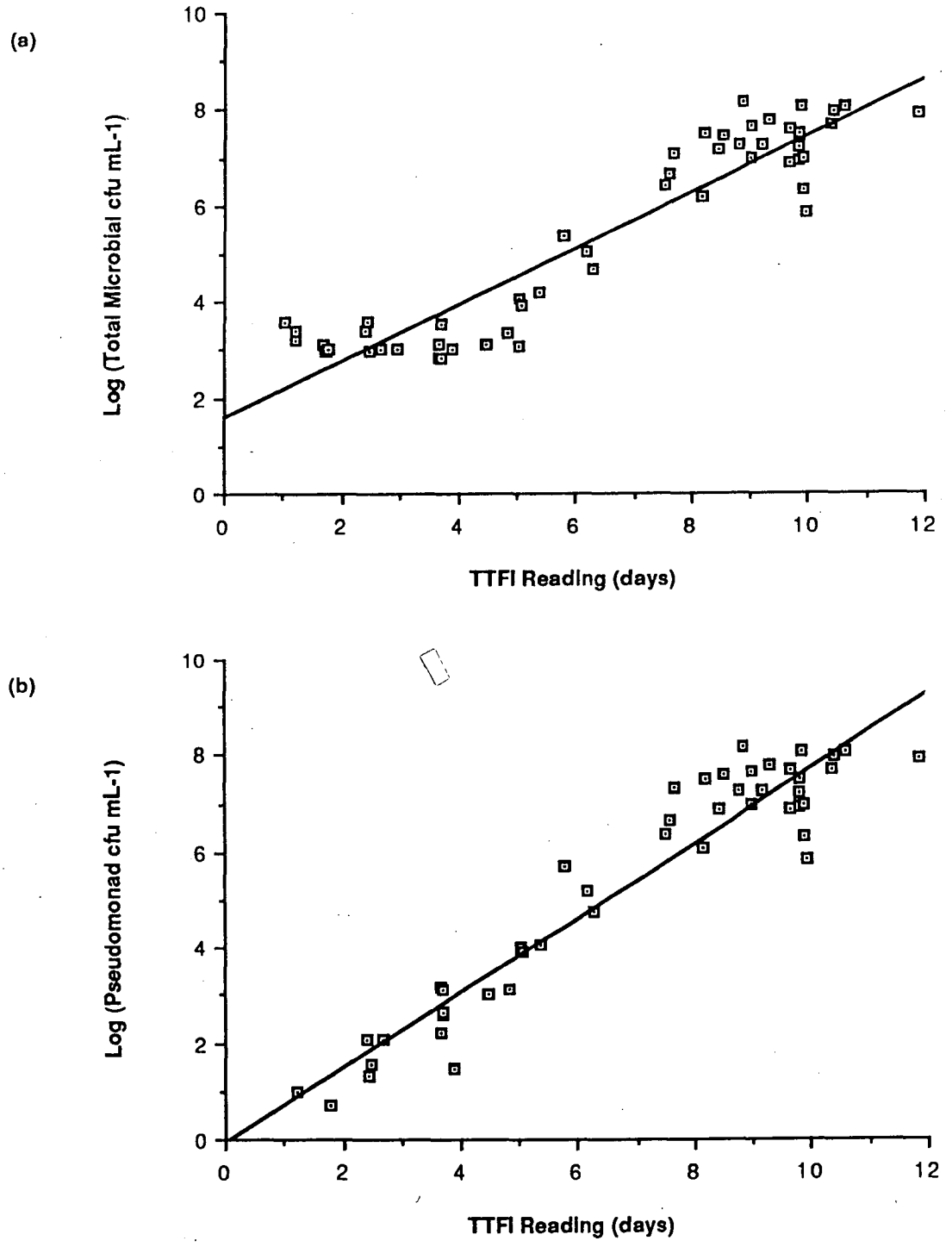
The solid line is the least squares regression line and is described by the equation:

$y = -0.0526 + 0.7755x$

Correlation coefficient = 0.96

For data refer to Appendix 6.6.

Figure 3.7: Plots of Microbial and Pseudomonad Numbers Versus Time/Temperature Function Integrator Readings.



the data for Figure 3.7a, having a correlation coefficient of 0.96. This better fit was due to the pseudomonads being present initially in only very small numbers, often $<1 mL^{-1}$, as compared to 10^{3-4} mL^{-1} thermoduric microorganisms present after pasteurisation. The increase in pseudomonad numbers during storage over the 0 to 5 days region was determined on PsA, which did not enumerate the thermoduric, Gram-positive microorganisms present.

3.1d(ii): Correlation of TTFI Readings with Organoleptic Evaluation of Spoilage at Elevated Temperatures.

The microbiological quality of pasteurised milk which had been stored at elevated temperatures until organoleptically spoiled (in all but one case), is summarised in Table 3.8. The temperature history of each milk carton was monitored using a TTFI and displayed as an equivalent time incubated at 4°C. The actual duration of incubation at each temperature is calculated easily using the Square Root Model and a T_o value of 263K (Appendix 6.2b). The T_o value of 263K and not 265K was applied in this case, as the TTFI used was programmed to operate on a 263K relative rate curve.

Both microbial and pseudomonad counts were estimated initially and at spoilage. Even though there were very few pseudomonads initially present, they constituted the entire spoilage microbiota at 10°C and were the major group present at 12°C. The exception was number 6, which was not spoiled when finally sampled. It is likely that the pseudomonad numbers would have represented a greater proportion of the microorganisms present had this sample been allowed to spoil. At 15°C the pseudomonads were only a minor proportion of the spoilage microbiota, although actual numbers recovered on PsA were $>10^{8}$ mL^{-1} in each of the 5 samples stored at this temperature.

Table 3.8: Microbial and Pseudomonad Numbers in Pasteurised, Homogenised Milk, Before and After Storage at 10, 12 and 15°C.

		Before Storage					After Storage				
No.	T.	PsA	PCA	Ps	pPs	TTFI	PsA	PCA	Ps	pPs	Org
1	15	<1.00	3.64	0	0	9.67	8.06	9.09	9	7	0
2	15	<1.00	3.66	0	0	8.80	8.24	9.35	8	9	0
3	15	<1.00	3.67	0	0	8.68	8.40	9.03	23	11	0
4	15	<1.00	3.19	0	0	8.72	8.34	9.16	15	4	0
5	15	<1.00	3.45	0	0	8.63	8.39	9.12	19	4	0
6	12	<1.00	3.43	0	0	7.22	6.00	7.49	3	0	N
7	12	<1.00	3.60	0	0	9.30	7.20	7.57	43	96	0
8	12	<1.00	3.63	0	0	9.35	7.92	7.74	100	100	0
9	12	1.40	3.63	0	0	9.67	8.38	8.10	100	100	0
10	10	<1.00	2.90	0	0	9.68	7.71	7.59	100	100	0
11	10	<1.00	3.03	0	0	9.87	8.07	8.09	97	100	0
12	10	<1.00	3.00	0	0	9.83	7.13	7.28	71	100	0

No.: carton number.

T: temperature (°C).

TTFI: time/temperature function integrator reading in days at 4°C.

PsA: log of pseudomonad numbers (cfu mL^{-1}) on PsA.

PCA: log of microbial numbers (cfu mL^{-1}) on PCA.

Ps: percentage pseudomonads calculated from PsA and PCA cfu.

pPs: percentage presumptive pseudomonads identified from PCA plates.

Org: organoleptic assessment, whether (O)ff or (N)ot Off.

In order to determine the selectivity of PsA, isolates capable of growth on the medium from each accelerated shelf-life experiment, were tentatively identified as pseudomonads, if they were capable of psychrotrophic growth, were cytochrome oxidase positive and motile. Of the 188 isolates capable of growth on PsA, 143 were positive for each characteristic and presumptively identified as pseudomonads (76%). Hence, the medium was reasonably selective for the isolation and enumeration of pseudomonads from pasteurised, homogenised milk.

3.1d(iii): Potential Application of TTFI in Quality Assurance and Shelf-life Prediction.

Mead (1985) stated that TTFI has considerable potential in the area of shelf-life control, as it is capable of detecting and analysing product temperature fluctuations and abuse during processing and subsequent storage. The ideal test for shelf-life, should be accurate, provide results in the shortest time possible and be simple and economical to perform (Bishop & White, 1986).

Results presented in Section 3.1d(i) and Section 3.1d(ii) suggest that TTFI may satisfy most of these criteria. It is generally accepted that microbial growth is responsible for the onset of spoilage of milk and other dairy products and that a level of log(7.5) microorganisms mL^{-1} represents the end of shelf-life (Griffiths et al.,1984). The spoilage microorganisms produce biochemical changes in the substrate and the amount of substrate utilised and product formed, is proportional to the number of cells present (Pirt, 1975 and Tan & Gill, 1981). In turn, the products formed are recognised organoleptically as spoilage changes.

Hence, the end point of shelf-life may be determined by storage trials in which the time to reach a specified level of either microbial numbers, metabolic product, or sensory deterioration, is

estimated. The rates at which the spoilage criteria change, are related to the environmental parameters acting on them. In this section temperature is the only parameter which varies. The Square Root Model provides an unifying relationship, which may be used with any of the three types of measurements outlined above.

In Section 3.1a, it was demonstrated that measurement of the metabolic product CO_2, had little predictive value, as a high $[CO_2]$ was only associated with a microbial level which resulted in organoleptic spoilage of the milk. When the microbial level was low, no change in $[CO_2]$ was measured. Therefore, in Section 3.1c and Section 3.1d, only microbial numbers and TTFI readings were examined, with the latter being correlated to microbial numbers. Sensory deterioration was used to signify the onset of organoleptic spoilage.

The information in Figure 3.7b indicates that TTFI readings correlate well with the number of spoilage microorganisms (pseudomonads) present in pasteurised, homogenised milk. Hence, the TTFI can be used directly to predict elapsed and remaining shelf-life of the product.

It is obvious that the time to reach a specified level of microorganisms and hence product shelf-life, will depend upon the initial level of contamination of the product (McMeekin & Thomas, 1980; Pooni & Mead, 1984 and Knabel et al., 1987). Maxcy & Wallen (1983) noted that it was the variability in the initial levels of contamination of pasteurised milk, which caused differences in observed product shelf-life.

Mead (1985) stated that accurate shelf-life prediction was dependent upon a knowledge of the initial level of product contamination with spoilage bacteria and that it was necessary for a consistently good standard of plant hygiene to be maintained, in

order to avoid undue variation in the expected shelf-life of the product.

For the practical application of TTFI to shelf-life prediction, a maximum initial level of contamination must be specified. This would be set by a value which was obtainable by good manufacturing practice. For example, Table 3.8 indicates that 11 of the 12 samples had an initial level of $<10mL^{-1}$. By specifying $10mL^{-1}$ as a maximum initial value, the number of generations required to reach a specified spoilage level is known and from storage trials, the average generation time of the population and hence the predicted shelf-life at a reference temperature can be calculated.

Correlation of this information with the TTFI readings shown in Figure 3.7b, provides a method of estimating the shelf-life of future milk samples directly from a TTFI reading.

A major problem associated with determining the shelf-life of pasteurised, homogenised milk at the recommended storage temperature of 4°C, by the estimation of microbial numbers, is that the result obtained is useful only in retrospect. To satisfy the criterion of Bishop & White (1986) that results should be obtained in the shortest possible time, sensory evaluation may be substituted for the estimation of microbial numbers. In addition, the rate of deterioration can be increased by storage of the milk samples at an elevated temperature.

It can be seen from Table 3.8 that the most suitable temperature was 12°C, as this was the highest temperature at which the dominant spoilage microbiota at 4°C (pseudomonads), remained dominant. The observed shelf-life at 12°C can be linked directly to the predicted shelf-life at 4°C by the application of the relative rate calculated from the Square Root Model with a T_o value of 263K (Table 3.9). In

Table 3.9: Relative Rates Predicted by the Square Root Model Using a T_o Value of 263K.

Temperature (°C)	Relative Rate 263K
4	1.00
6	1.31
8	1.65
10	2.04
12	2.47
14	2.94
15	3.19

All rates are relative to 4°C (277K).

this case the relative rate factor is 2.47.

TTFI and accelerated spoilage at 12°C could be used to assess the validity of the established shelf-life date for a particular milk product. Provided the product is still organoleptically acceptable at its use-by date, it can be assumed that adequate hygiene practices are active in the processing plant. By constantly monitoring the product under the same conditions, deviations in the overall hygiene of the plant, can be assessed by the detection of a product of poor keeping quality. The actual initial number of pseudomonads present is not important, provided it remains below the specified maximum initial level obtainable by good manufacturing practice. If initial microbial numbers rise above this level, organoleptic spoilage will occur prior to the specified use-by date.

It can be seen from Table 3.8, that storage of milk until a TTFI reading of 8 to 9 days equivalent at 4°C, resulted in an organoleptically unacceptable product. An incubation period of 3.6 days at 12°C was equivalent to 9 days of storage at 4°C. This method for determining shelf-life, can be compared with most accelerated shelf-life techniques. Generally they utilise a preincubation step, for example 25 hours at 21°C (Phillips et al., 1984) or 14 hours at 21°C (Bishop & White, 1985), in a medium containing selective agents, which is then followed by plating onto a non-selective medium and enumeration after incubation for 25 hours at 21°C.

Both the above methods are shorter than the tested accelerated technique (approximately half the time). However, both involve precise preincubation periods, followed by microbial enumeration by plating. The latter could be easily replaced by impedimetric detection techniques (Bishop et al., 1984), which then makes it necessary to relate the detection time obtained, to a standard curve to determine the potential shelf-life of the product. The accelerated

method utilising the TTFI, gives a direct reading of potential shelf-life and only requires organoleptic analysis of the milk to determine its level of acceptability.

The use of TTFI in an accelerated shelf-life test system is not limited to pasteurised, homogenised milk. It can be used in any system where spoilage occurs as a result of the activity of a dominant microbiota. Such a process occurs when UHT milk is spoiled by Bacillus stearothermophilus. By calibrating a TTFI to operate with the T_o value characteristic of B. stearothermophilus and by incubating the milk at a temperature close to its optimum for growth, the milk can be assessed organoleptically (for gelation) at its equivalent use-by date.

Hence, it has been shown that the spoilage of pasteurised, homogenised milk at refrigeration temperatures, is a process which can be described by the Square Root Model of Ratkowsky et al.,(1982). Utilisation of this information by the process of TTFI allows for a simple method of monitoring and predicting the shelf-life of the milk.

3.2: THE EFFECT OF TEMPERATURE ON MICROBIAL GROWTH RATE.

Temperature is the major factor affecting the rate of microbial growth under conditions where water activity and pH are non-limiting. Pseudomonas, Alteromonas, Acinetobacter, Moraxella, lactobacilli and some members of the Enterobacteriaceae, constitute the psychrotrophic bacteria which are important in the chill store spoilage of milk (Sherman et al.,1941; Olson, 1967 and Cousin, 1982).

Attempts to formulate a relationship between microbial growth rate and temperature, have led to the development of several different mathematical models, of which the Square Root Models of Ratkowsky et al.,(1982, 1983) appear to provide the best fit to experimental growth rate data (Langeveld, 1983 and Stannard et al.,1985). They have been shown to describe accurately the effect of temperature on the growth of bacteria of dairy origin. The data of Greene & Jezeski (1954) and Olsen & Jezeski (1963), obtained from bacterial dairy isolates cultured in laboratory media have been shown to fit the Square Root Model (Ratkowsky et al.,1982).

Smith (1985) demonstrated the Square Root Model to predict accurately the effect of temperature on lag and generation times for Escherichia coli. Analysis of his data by McMeekin et al.,(1987), indicated that both parameters obeyed the Square Root Model and yielded similar T_{min} (T_o) values.

The aims of this section were: to verify the ability of the Square Root Model to describe accurately the effect of temperature on the growth rate of bacteria responsible for the spoilage of pasteurised, homogenised milk at refrigeration temperatures and to determine if their T_o values correlated with those which describe the actual rate of spoilage of the milk; and also to determine the effect of temperature on the duration of the lag phase of a pseudomonad and

compare its response to that of the exponentially growing culture.

3.2a: Calibration of a Nephelometer for Pseudomonas sp. Strain E5.2.

Prior to the use of the nephelometer for the measurement of bacterial growth, it was necessary to calibrate the instrument for a bacterium representative of those being studied. Figure 3.8a contains the calibration curve for Pseudomonas sp. strain E5.2, a typical Gram negative rod which verified the assumption that a doubling in optical density (OD), approximated a doubling in microbial numbers, i.e., an increase by 1.0 generation. From the initial inoculum level of an OD of 0.1, a change in OD of 0.3, to an OD of 0.4, corresponded to an increase in microbial numbers by 2.1 generations.

It was possible to calculate the generation time (GT) for Pseudomonas sp. strain E5.2 from Figure 3.8b. The assumption that doubling OD corresponded to a doubling in microbial numbers, resulted in calculation of a GT of 25.1 min at 25°C. Even though other workers have reported slower GT of approximately 54 min (Reichelt & Baumann, 1974) and 66 min (Nelson & Parkinson, 1978) for pseudomonads at this temperature, separate experiments in Section 3.2c confirmed the generation time of Pseudomonas sp. strain E5.2 to be 31.3 and 29.6 minutes at 25°C.

3.2b: Determination of Temperature/Growth Profiles for Two Bacteria, Isolated from Spoiled, Pasteurised, Homogenised Milk.

For the two bacteria involved in growth studies, OD data were derived from the time required for a change in OD by 0.3. This figure was chosen as it was situated in the mid to late exponential phase. GT data were based on the assumption that a doubling in OD corresponded to a doubling in microbial numbers. As both forms of data involve the interpretation of bacterial growth rates, the T_o

Figure 3.8: Nephelometer Calibration Curves for Pseudomonas sp. Strain E5.2.

(a): A plot of the number of cfu mL^{-1} of Pseudomonas sp. strain E5.2 at different optical density values.

The solid line was produced by least squares linear regression line and is described by the equation: y = .00581 + 2.09E-09x

Correlation coefficient = 0.995

A doubling in optical density approximated to a doubling in microbial numbers.

The change in OD of 0.3 from 0.1 to 0.4, corresponded to an increase in microbial numbers by 2.1 generations.

For data refer to Appendix 6.7a.

(b): A plot of the increase in the logarithm of the optical density of a culture of Pseudomonas sp. strain E5.2, incubated at 25°C, with respect to time.

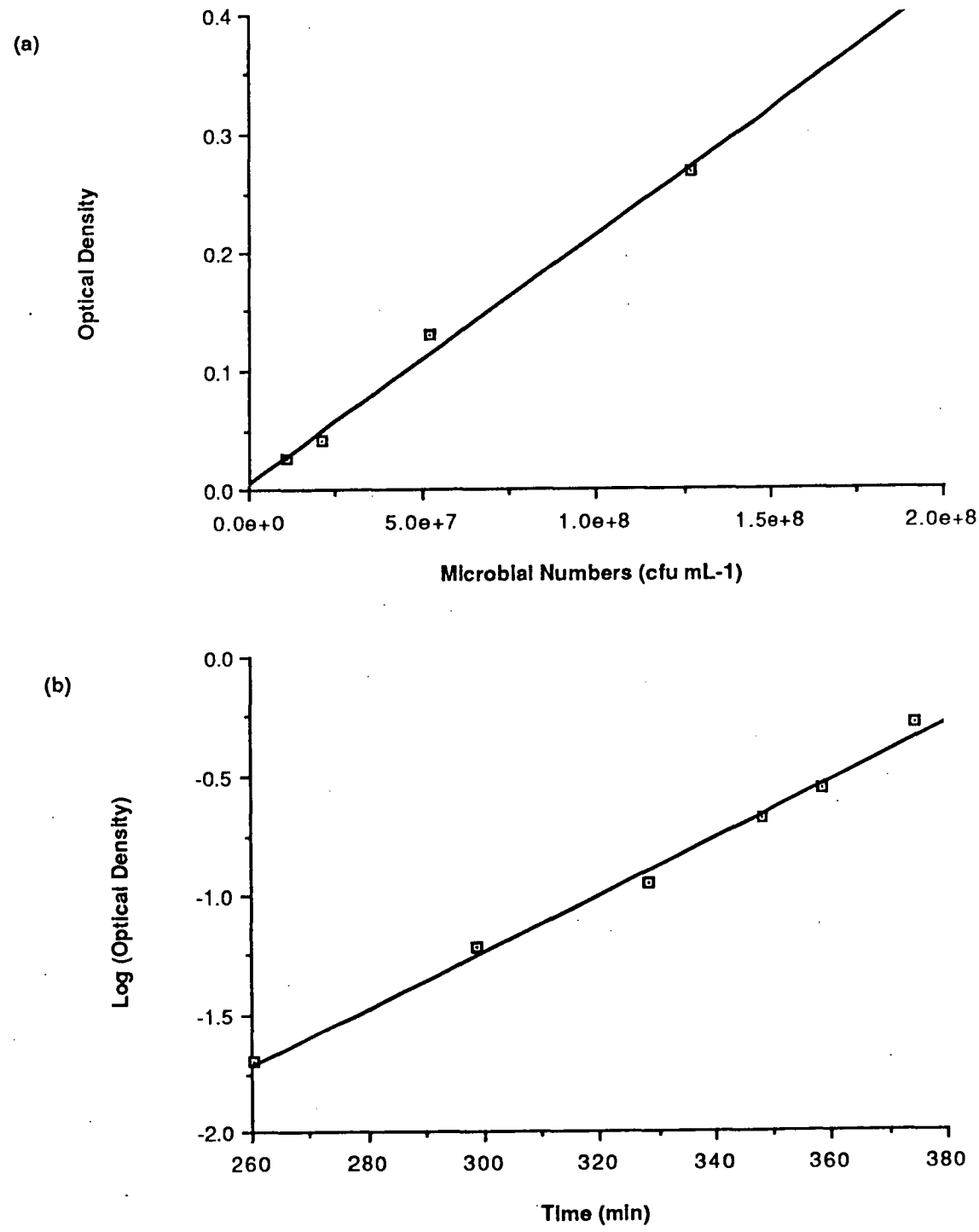
The solid line was produced by least squares linear regression and is described by the equation: y = -4.8219 + 0.012x

Correlation coefficient = 0.999

The time required for a doubling in optical density was 25.1 minutes.

For data refer to Appendix 6.7b.

Figure 3.8: Nephelometer Calibration Curves for Pseudomonas sp. Strain E5.2.



values generated from the Square Root Model for each bacterium should be the same.

(i) The temperature/growth profiles of two bacteria isolated from the spoilage microbiota of pasteurised, homogenised milk in Section 3.1c(i) were studied to determine their temperature characteristics, which may in turn explain their presence in large numbers in the spoilage microbiota at different storage temperatures. Enterobacter agglomerans strain 11.33 was isolated at 11.5°C and Pseudomonas sp. strain 12.48 was isolated at 6.3°C.

The Square Root plots for the two bacteria (Figure 3.9), were based on results generated from both OD and GT data. Figure 3.9a and Figure 3.9b indicated T_o values for E. agglomerans strain 11.33 of 270.4K and 270.7K, for OD and GT data respectively. These T_o values were at the lower end of the mesophilic range or upper end of the psychrotrophic range (Olley, 1983). These results were expected, as this strain and other members of the Enterobacteriaceae were capable of successfully competing against the psychrotrophic pseudomonads at temperatures of approximately 12°C and above.

Figure 3.9c and Figure 3.9d indicated T_o values for Pseudomonas sp. strain 12.48 of 268.7K and 268.0K, for OD and GT data respectively. The two T_o values tended toward the upper end of the psychrotrophic range. Many pseudomonads have T_o values of approximately 263-264K (Olley, 1983) and the higher T_o value possessed by this strain provided further explanation for its competitiveness against members of the Enterobacteriaceae in the spoilage microbiota at 11.5°C in Section 3.1c(i).

The mean T_o value for Pseudomonas sp. strain 12.48 of 268.4K was similar to the mean T_o value observed for the spoilage of

Figure 3.9: Square Root Plots of the Effect of Temperature on the Growth Rates of Enterobacter agglomerans Strain 11.33 and Pseudomonas sp. Strain 12.48.

(a): Square root of growth rate versus temperature for Enterobacter agglomerans strain 11.33 (OD data).
Equation to line: $\sqrt{r} = -0.71110 + 0.00263T$
Correlation coefficient = 0.998; T_o = 270.4K.

(b): Square root of growth rate versus temperature for Enterobacter agglomerans strain 11.33 (GT data).
Equation to line: $\sqrt{r} = -1.67304 + 0.00618T$
Correlation coefficient = 0.995; T_o = 270.7K.

For experimental and predicted values refer to Appendix 6.8a.

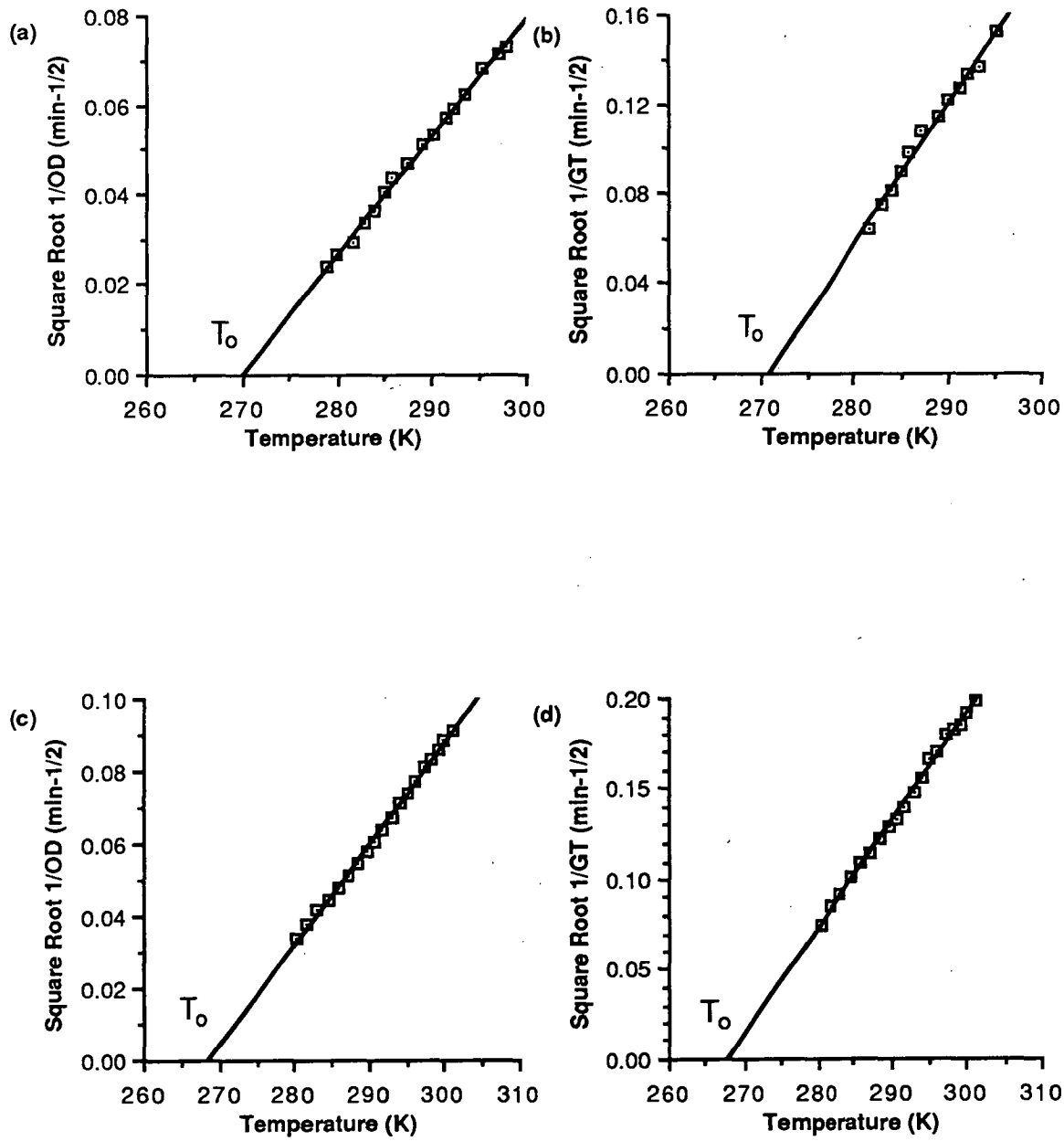
(c): Square root of growth rate versus temperature for Pseudomonas sp. strain 12.48 (OD data).
Equation to line: $\sqrt{r} = -0.74430 + 0.00277T$
Correlation coefficient = 0.999; T_o = 268.7K

(d): Square root of growth rate versus temperature for Pseudomonas sp. strain 12.48 (GT data).
Equation to line: $\sqrt{r} = -1.59196 + 0.00594T$
Correlation coefficient = 0.998; T_o = 268.0K.

For experimental and predicted values refer to Appendix 6.8b.

All equations produced by least squares linear regression of experimental data.

Figure 3.9: Square Root Plots of the Effect of Temperature on the Growth Rates of Enterobacter agglomerans Strain 11.33 and Pseudomonas sp. Strain 12.48.



pasteurised, homogenised milk of 267.8K (Section 3.1b). This supported the assertion that spoilage of the milk was a process which responded to temperature in a manner similar to that of representatives of the actual spoilage microbiota.

The T_o values for the two bacteria provided information on their temperature/growth characteristics. The lower the T_o value, the more adapted the bacterium is to growth at low temperature. Hence, it would be expected that the pseudomonad should grow better at low temperatures than the enterobacter. This assertion was verified when generation times for the two bacteria at any specified temperature were compared. It was demonstrated in Section 3.1c(i) that Pseudomonas sp. strain 12.48 would grow faster than E. agglomerans strain 11.33 at temperatures less than 16°C and that this was the reason for the dominance of pseudomonads in the spoilage microbiota of chill stored pasteurised, homogenised milk.

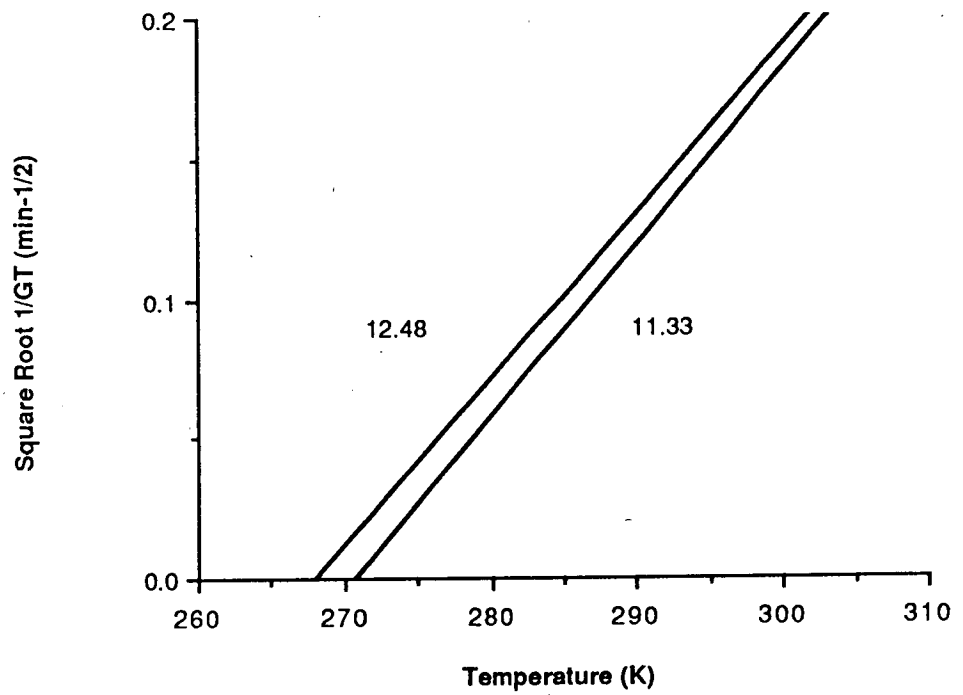
This was apparent when the two Square Root plots generated from GT data were overlayed (Figure 3.10). It was evident that the T_o value of a bacterium was not the only important factor to be considered when growth rates were compared. The slope of the line was also important, as it was a measure of the rate of increase in growth rate with increase in temperature. It can be seen from Figure 3.10, that the temperature/growth profiles of the two bacteria were similar except for their predicted T_o values and that Pseudomonas sp. strain 12.48 should outgrow E. agglomerans strain 11.33 at any specified incubation temperature, as the profiles do not intersect within their common temperature growth range.

It is of interest to note that the data derived from OD and GT readings yielded similar T_o values. It was necessary to calculate

Figure 3.10: Comparison of the Square Root Plots of the Effect of Temperature on the Growth Rates of Enterobacter agglomerans Strain 11.33 and Pseudomonas sp. Strain 12.48.

An overlay plot of the curves predicted by the Square Root Model from Figure 3.9(b) and Figure 3.9(d), directly comparing the square root of growth rate of Enterobacter agglomerans strain 11.33 and Pseudomonas sp. strain 12.48 at different temperatures.

Figure 3.10: Comparison of the Square Root Plots of the Effect of Temperature on the Growth Rates of Enterobacter agglomerans Strain 11.33 and Pseudomonas sp. Strain 12.48.



both sets of data as they have different uses. When OD data was plotted using the Square Root Model, the data points resulted in a line which had considerably less scatter than GT generated data and was therefore more accurate in predicting the T_0 value of the bacterium. This was evident, as the correlation coefficients for predicted lines derived from OD data were generally higher than those derived from GT data.

GT data was more scattered than OD data, as the method of obtaining the data points was reliant on a line of best fit through the actual growth data at each temperature, which was less precise than obtaining a time to reach a specified OD. However, calculation of GT data was useful, as it enabled the direct comparison of data derived from different experiments under specified conditions. When factors such as, initial inoculum level, water activity or pH, differ from one experiment to the next, GT data and not OD data must be used, before any meaningful comparison between the experiments can be made.

3.2c: The Effect of Temperature on the Duration of the Lag Phase in Stationary Phase Cells of Pseudomonas sp. Strain E5.2.

The results presented below, describe the effect of temperature on the duration of the lag phase present in stationary phase cells of Pseudomonas sp. strain E5.2. The Square Root Model was used to describe the temperature dependent variation in lag phase duration and was compared to values obtained from Square Root plots generated from OD and GT data.

(i) The period of incubation at 25°C required to induce stationary phase in Pseudomonas sp. strain E5.2 was determined. The absorbance changes of 10^{-2} dilutions of the original culture, with corresponding

incubation time, are shown in Table 3.10. After approximately 50 hours, continued incubation resulted in no further increase in OD and the original culture was deemed to have entered stationary phase.

Hence, any culture of Pseudomonas sp. strain E5.2 incubated for greater than 50 hours under similar conditions, will result in the production of stationary phase cells. These cells will exhibit a lag phase prior to exponential growth, when inoculated into fresh growth media.

(ii) Two TGI experiments were conducted on Pseudomonas sp. strain E5.2. Measurement of variation in time to reach a specified growth level (change in OD = 0.3), the length of the generation time and the duration of the lag phase at different temperatures, showed that each of these three growth parameters, was readily described by the Square Root Model (Figure 3.11a, Figure 3.11b and Figure 3.11c).

Similar results were obtained for the other TGI experiment (Figure 3.12a, Figure 3.12b and, Figure 3.12c). As the inocula for both experiments were greater than the minimum for detection by the nephelometer, it was possible to monitor bacterial growth immediately after completion of the lag phase.

The T_o values for each of the three parameters used to assess growth were determined from the Square Root plots in Figure 3.11 and Figure 3.12. These are summarised in Table 3.11. As was expected from the results in Section 3.2b(ii), the comparison between the T_o values generated from OD and GT data, yielded similar figures in both TGI experiments, with mean values of 267.9K and 267.1K. The T_o values generated from the lag phase data, were similar to the OD and GT values, having a mean value of 268.5K.

Table 3.10: Determination of Time to Achieve Stationary Phase for *Pseudomonas* sp. Strain E5.2 at 25°C.

Time (hours)	Optical Density
15.9	0.104
17.6	0.143
19.4	0.201
20.7	0.215
24.5	0.302
27.1	0.386
30.5	0.538
39.8	0.714
42.0	1.244
44.4	0.991
46.4	1.357
63.8	1.658
66.5	1.638
68.1	1.444

All optical density measurements were determined using 10^{-2} dilutions of the incubated culture.

Figure 3.11: Square Root Plots of the Effect of Temperature on the Growth Rate and Lag Phase of Pseudomonas sp. Strain E5.2.

(a): The square root of growth rate (OD data) versus temperature.

Equation to line: $\sqrt{r} = -0.667641 + 0.0025T$

Correlation coefficient = 0.998; T_o = 267.1K.

(b): The square root of growth rate (GT data) versus temperature.

Equation to line: $\sqrt{r} = -1.5224 + 0.00571T$

Correlation coefficient = 0.997; T_o = 266.6K.

(c): The square root of rate (1/Lag) versus temperature.

Equation to line: $\sqrt{r} = -0.86292 + 0.00323T$

Correlation coefficient = 0.996; T_o = 267.2K

For experimental and predicted values refer to Appendix 6.9a.

All equations produced by least squares linear regression of experimental data.

Figure 3.11: Square Root Plots of the Effect of Temperature on the Growth Rate and Lag Phase of Pseudomonas sp. Strain E5.2.

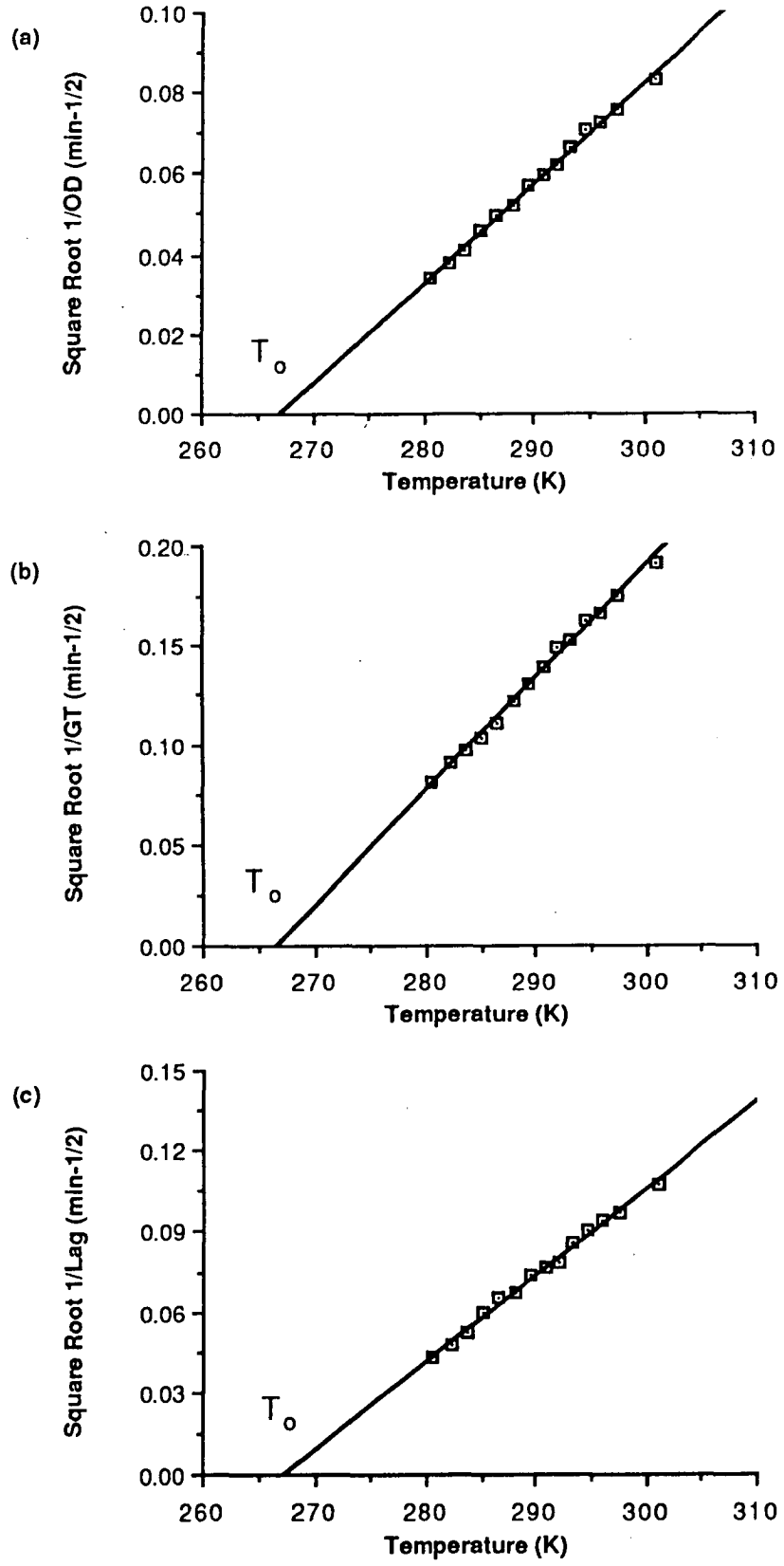


Figure 3.12: Square Root Plots of the Effect of Temperature on the Growth Rate and Lag Phase of Pseudomonas sp. Strain E5.2.

(a): The square root of growth rate (OD data) versus temperature.

Equation to line: $\sqrt{r} = -0.698483 + 0.0026T$

Correlation coefficient = 0.998; T_o = 268.7K.

(b): The square root of growth rate (GT data) versus temperature.

Equation to line: $\sqrt{r} = -1.57346 + 0.00588T$

Correlation coefficient = 0.995; T_o = 267.6K.

(c): The square root of rate (1/Lag) versus temperature.

Equation to line: $\sqrt{r} = -0.90610 + 0.00336T$

Correlation coefficient = 0.996; T_o = 269.7K

Refer to Appendix 6.9b for experimental and predicted values.

All equations produced by least squares linear regression of experimental data.

Figure 3.12: Square Root Plots of the Effect of Temperature on the Growth Rate and Lag Phase of Pseudomonas sp. Strain E5.2.

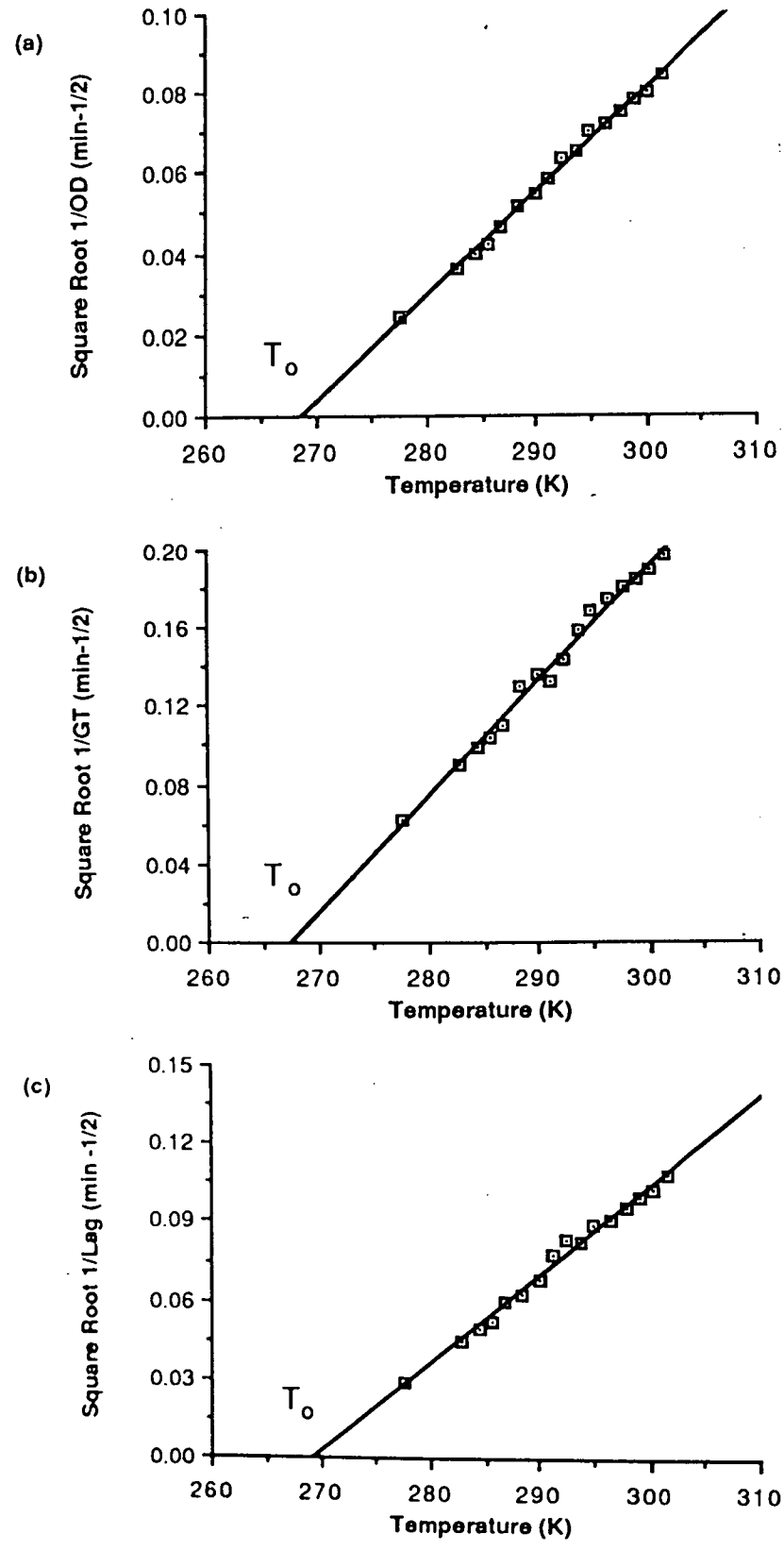


Table 3.11: T_o Values for *Pseudomonas* sp. Strain E5.2, Calculated from Optical Density, Generation Time and Lag Phase Data.

Xpt#	OD	GT	Lag	Mean
1	267.1	266.6	267.2	267.0
2	268.7	267.6	269.7	268.7
Mean	267.9	267.1	268.5	

Mean of all T_o values = 267.8K.

Standard deviation = +/- 1.2K.

Xpt#: experiment number.

OD : optical density data.

GT : generation time data.

Lag : lag phase data.

All temperatures in Kelvin.

Gibson et al.,(1987), recognised that the time to reach maximum growth rate, was an appropriate parameter for modelling bacterial growth, as it took into account both lag time and growth rate. Measurement of the time to reach a specified change in OD, is a similar parameter, which incorporates both lag phase and GT data into one reading.

It is apparent from Figure 3.11 and Figure 3.12, that the effect of temperature on growth rate as measured by OD or GT, was the same as the effect of temperature on lag phase, with T_o values being similar and only the slopes of the lines varying.

The mean T_o value for lag phase of 268.7K, was similar to the T_o value for Pseudomonas sp. strain E5.2 obtained in Section 3.2b(ii) of 268.6K. The latter value was obtained from a TGI experiment which used an exponentially growing inoculum which did not exhibit a lag phase. Hence, the relative effects of a changing temperature regime on a lag phase culture of Pseudomonas sp. strain E5.2, was the same as for the exponentially growing culture and can be described by the Square Root Model with the same T_o value.

Smith (1985, 1987) demonstrated the ability of the Square Root Model to fit data describing the effect of temperature on lag phase length and generation times for coliform bacteria in blended mutton. The calculated T_o values for lag phase and GT data were 276.2K and 276.7K, respectively. The subsequent information predicted by the Square Root Model for the lag phase and GT data was successfully used to predict the growth of the coliform bacteria from the time/temperature history of the mutton.

(iii) Section 3.2c(ii) demonstrated the presence of a lag phase in

cultures of Pseudomonas sp. strain E5.2. The duration of the lag phase at each incubation temperature was the result of a calculation using GT and OD data (Appendix 6.2c) and as such, an indirect measurement. In this section, lag phase duration was measured as the elapsed time from inoculation to the onset of exponential growth (signified by a 0.02 change in OD). This method was used as it was a direct measurement of lag phase duration.

Figure 3.13 contains the Square Root plots for the three TGI experiments. In each case the Square Root Model fitted the experimental data well. T_o values of 270.0, 268.9 and 268.6K were predicted. The mean T_o value of 269.2K obtained from direct measurement of lag phase duration was similar to the T_o value obtained by indirect calculation in Section 3.2c(ii) of 268.7K. It was also similar to the T_o value obtained for the exponentially growing culture of 268.6K [Section 3.2b(ii)].

Hence, it has been shown that the effect of temperature on the duration of the lag phase was accurately described by the Square Root Model, with lag phase and exponential growth data yielding similar T_o values. The use of the growth parameter, time for a specified change in OD, incorporated both lag and exponential growth data and yielded a T_o value similar to the individual values obtained for lag and exponential growth data.

Figure 3.13: Square Root Plots of the Effect of Temperature on the Lag Phase of Pseudomonas sp. Strain E5.2.

(a): The square root of rate (1/Lag) versus temperature.

Equation to line: $\sqrt{r} = -1.0909 + 0.00404T$

Correlation coefficient = 0.998; T_o = 270.0K.

For experimental and predicted values refer to Appendix 6.10a.

(b): The square root of rate (1/Lag) versus temperature.

Equation to line: $\sqrt{r} = -0.884718 + 0.00329T$

Correlation coefficient = 0.997; T_o = 268.9K.

For experimemtal and predicted values refer to Appendix 6.10b.

(c): The square root of rate (1/Lag) versus temperature.

Equation to line: $\sqrt{r} = -0.682223 + 0.00254T$

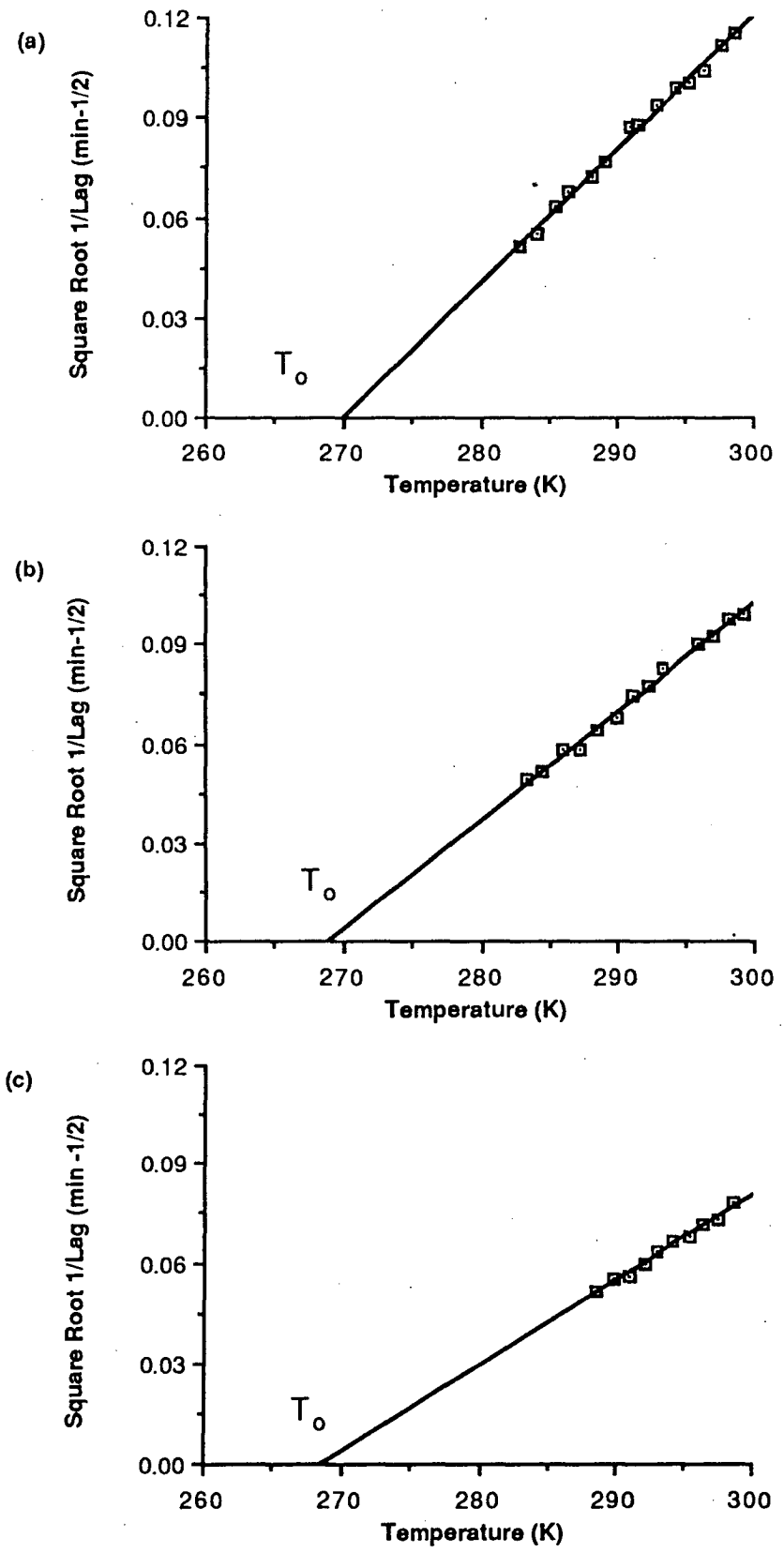
Correlation coefficient = 0.994; T_o = 268.6K

For experimental and predicted values refer to Appendix 6.10c.

In all graphs, Lag = time for a 0.02 change in OD.

All equations produced by least squares linear regression of experimental data.

Figure 3.13: Square Root Plots of the Effect of Temperature on the Lag Phase of Pseudomonas sp. Strain E5.2.



3.3: THE EFFECT OF TEMPERATURE AND WATER ACTIVITY ON MICROBIAL GROWTH RATE.

It was shown in Section 3.1 and Section 3.2, that the Square Root Model was capable of accurately describing the spoilage of pasteurised, homogenised milk and the growth of relevant spoilage microorganisms under conditions where temperature was the only limiting factor. Water activity is another factor which is capable of influencing microbial growth and hence the deterioration of foods. It may be used alone or in combination with other factors such as temperature or pH.

Every microorganism is capable of growth over a range of water activities. It was reported in Table 1.1 that various microbial groups grew better than others under specified conditions. The bacterial groups which are adapted for growth at low water activities (0.9-0.75) are broadly categorised as moderate (no specific requirement for salt) and extreme (specific requirement for salt) halophiles.

The general aim of this section was to quantify the combined effects of temperature and water activity on bacterial isolates from these two microbial groups and to modify the Square Root Model of Ratkowsky *et al.*,(1982), to incorporate a water activity parameter.

3.3a: *Growth of the Moderate Halophile Staphylococcus xylosus Strain CM21/3*.

3.3a(i): Calibration of Nephelometer.

It was demonstrated in Section 3.2a that doubling dilutions of a broth culture of *Pseudomonas* sp. strain E5.2 halved the observed OD with each successive dilution. This method was used to calibrate the nephelometer for growth of *Staphylococcus xylosus* strain CM21/3 in MHB at a water activity of 0.996 (Figure 3.14a). It was also

Figure 3.14: Nephelometer Calibration Curves for *Staphylococcus xylosus* Strain CM21/3.

(a): A plot of the OD of doubling dilutions of a culture incubated at 25°C in MHB.

Equation to line: $y = 0.01025 + 0.59858x$

Correlation coefficient = 0.999

For data refer to Appendix 6.11a.

(b): A plot of the OD of doubling dilutions of a culture incubated at 25°C in MHB containing 3.5 molal NaCl.

Equation to line: $y = 0.01167 + 0.68370x$

Correlation coefficient = 0.999

For data refer to Appendix 6.11b.

(c): A plot of the OD of doubling dilutions of a culture incubated at 25°C in MHB containing 4.5 molal glycerol.

Equation to line: $y = -0.00230 + 0.52007x$

Correlation coefficient = 0.999

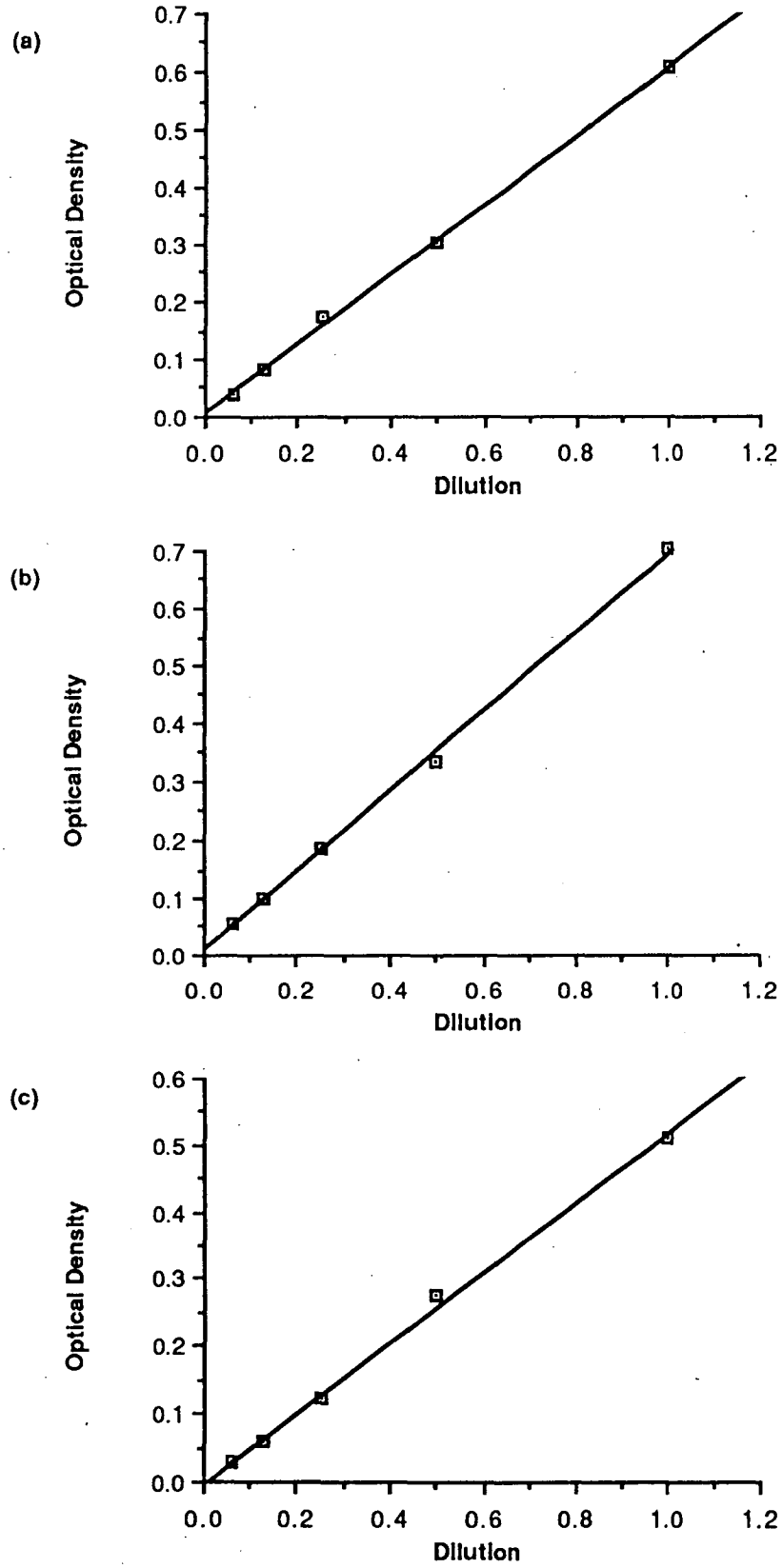
For data refer to Appendix 6.11c.

In each graph, the lowest dilution was arbitrarily valued at 1.

All equations produced by least squares linear regression.

For each dilution series, a doubling in OD approximated to a doubling in microbial numbers.

Figure 3.14: Nephelometer Calibration Curves for Staphylococcus xylosus Strain CM21/3.



calibrated under conditions where water activity markedly affected bacterial growth rate: at a water activity of 0.869 (MHB + 3.5 molal NaCl) (Figure 3.14b); and a water activity of 0.919 (MHB + 4.5 molal glycerol) (Figure 3.14c). The assertion that doubling optical density corresponded to doubling microbial numbers, was found to be valid for Staphylococcus xylosus strain CM21/3 in each of the growth media.

The effect of changing the NaCl and glycerol concentrations and hence the water activities of MHB on the cellular dimensions of Staphylococcus xylosus strain CM21/3 were examined after growth to an OD of 0.3 in MHB and MHB containing 3.5 molal NaCl (a_w = 0.869) and 4.5 molal glycerol (a_w = 0.919). The bacterial cells which grew in MHB had a mean diameter of 0.87 +/- 0.09μ. The cells which grew in MHB + 3.5 molal NaCl had a mean diameter of 0.91 +/- 0.13μ. The cells which grew in MHB + 4.5 molal glycerol had a mean diameter of 0.93 +/- 0.09μ (Appendix 6.12).

The dimensions of Staphylococcus xylosus strain CM21/3 did not alter significantly when it was grown in media of differing water activities. The water activities tested were close to the limits for bacterial growth in media containing NaCl and glycerol. It was concluded that cell dimensions would remain constant when grown over the water activity range 0.996-0.869 in MHB + NaCl. A similar conclusion was made for growth over the water activity range 0.996-0.919 for MHB + glycerol. In addition, there was no observable difference between the dimensions of cells which were grown in MHB containing either NaCl or glycerol as the humectant.

The number of cfu mL^{-1} which corresponded to an OD of 0.3 in MHB, MHB + 3.5 molal NaCl and MHB + 4.5 molal glycerol were 1.02×10^8, 1.14×10^8 and 2.05×10^8 cfu mL^{-1} respectively. As the

three values were similar, OD levels over the range of water activities in each type of media should correspond to similar microbial levels.

3.3a(ii): The Effect of Temperature and Sodium Chloride Concentration on Growth Rate.

In the experiments conducted by Ratkowsky et al.,(1982) and in Section 3.2, conditions were chosen to allow unrestricted growth at a rate characteristic of each incubation temperature. Experiments in Section 3.3a(ii) tested the general applicability of the Square Root Model to describe microbial growth, in the presence of two growth limiting factors (temperature and water activity adjusted by elevated NaCl concentrations). Four responses to these factors were possible: the Bělehrádek exponent (d) may have changed with decreased water activity; the Square Root Model may not have been adequate in describing growth with the addition of a water activity constraint, i.e., the response would be non-linear; the Square Root Model may be accurate, with the predicted T_o values not being constant; or the Square Root Model may be accurate, with the predicted T_o values being constant.

Ten TGI experiments were conducted to test the validity of the Square Root Model under conditions where NaCl and water activity were limiting. OD and GT data were calculated from the growth of Staphylococcus xylosus strain CM21/3 in MHB containing NaCl: 0.0 (Figure 3.15a and Figure 3.15b); 0.7 (Figure 3.15c and Figure 3.15d); 1.4 (Figure 3.16a and Figure 3.16b); 2.0 (Figure 3.16c and Figure 3.16d); 2.5 (Figure 3.17a and Figure 3.17b); 3.0 (Figure 3.17c and Figure 3.17d); 3.5 (Figure 3.18a and Figure 3.18b); 3.75 (Figure 3.18c and Figure 3.18d); 4.0 (Figure 3.19a and Figure 3.19b);

Figure 3.15: Square Root Plots of the Effect of Temperature and Sodium Chloride Concentration on the Growth Rate of Staphylococcus xylosus Strain CM21/3.

(a): Square root of growth rate versus temperature at NaCl = 0.0 molal, a_w = 0.996 (OD data).
Equation to line: $\sqrt{r}$ = -0.82836 + 0.00299T
Correlation coefficient = 0.999; T_o = 277.0K.

(b): Square root of growth rate versus temperature at NaCl = 0.0 molal, a_w = 0.996 (GT data).
Equation to line: $\sqrt{r}$ = -2.03895 + 0.00737T
Correlation coefficient = 0.998; T_o = 276.7K.

For experimental and predicted values refer to Appendix 6.13a.

(c): Square root of growth rate versus temperature at NaCl = 0.7 molal, a_w = 0.976 (OD data).
Equation to line: $\sqrt{r}$ = -0.84796 + 0.00307T
Correlation coefficient = 0.999; T_o = 276.2K.

(d): Square root of growth rate versus temperature at NaCl = 0.7 molal, a_w = 0.976 (GT data).
Equation to line: $\sqrt{r}$ = -2.12521 + 0.00769T
Correlation coefficient = 0.995; T_o = 276.4K.

For experimental and predicted values refer to Appendix 6.13b.

All equations produced by least squares linear regression of experimental data.

Figure 3.15: Square Root Plots of the Effect of Temperature and Sodium Chloride Concentration on the Growth Rate of Staphylococcus xylosus Strain CM21/3.

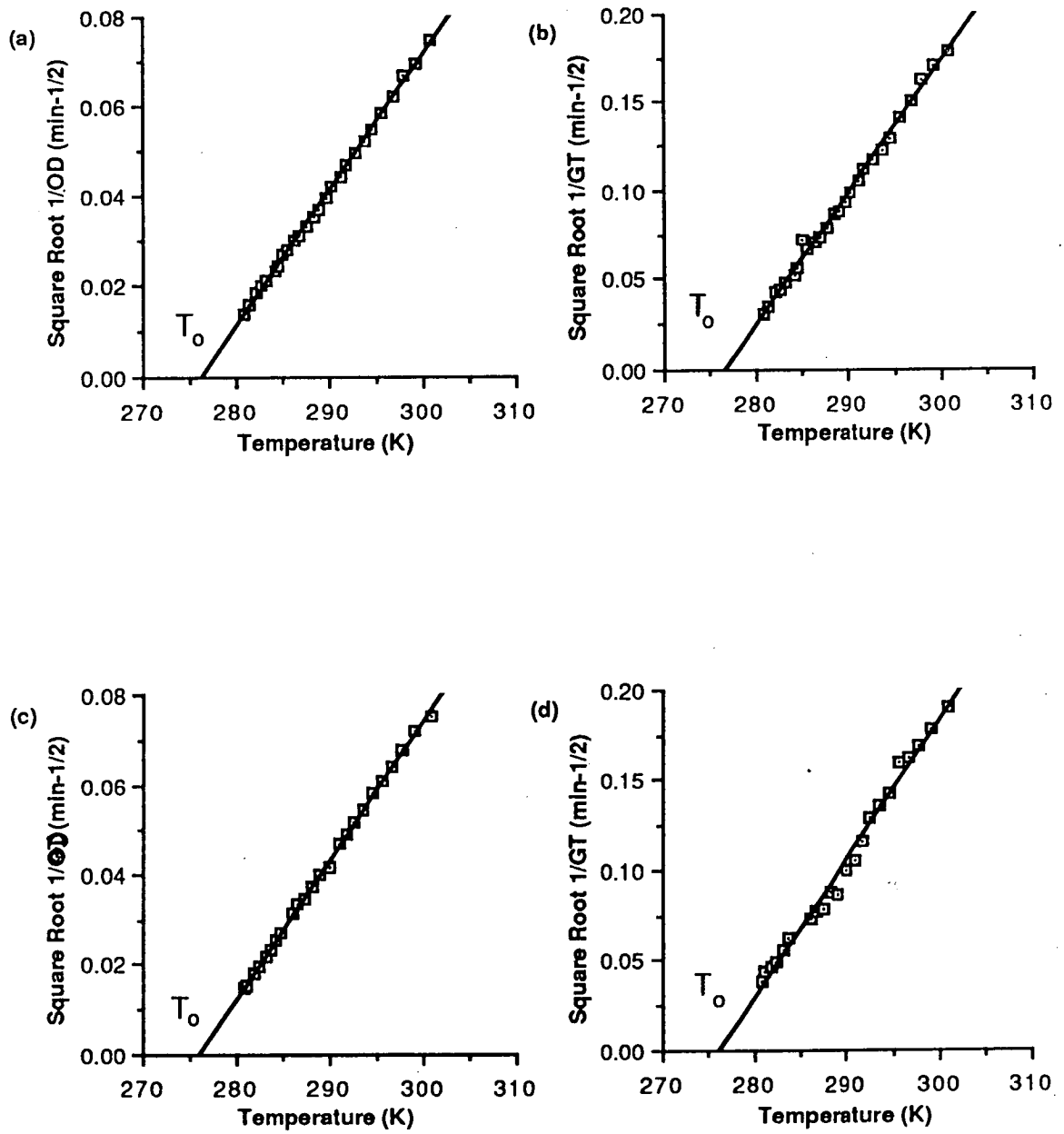


Figure 3.16: Square Root Plots of the Effect of Temperature and Sodium Chloride Concentration on the Growth Rate of Staphylococcus xylosus Strain CM21/3.

(a): Square root of growth rate versus temperature at NaCl = 1.4 molal, a_w = 0.949 (OD data).
Equation to line: $\sqrt{r}$ = -0.82910 + 0.00300T
Correlation coefficient = 0.999; T_o = 276.4K.

(b): Square root of growth rate versus temperature at NaCl = 1.4 molal, a_w = 0.949 (GT data).
Equation to line: $\sqrt{r}$ = -1.78703 + 0.00649T
Correlation coefficient = 0.999; T_o = 275.4K.

For experimental and predicted values refer to Appendix 6.13c.

(c): Square root of growth rate versus temperature at NaCl = 2.0 molal, a_w = 0.928 (OD data).
Equation to line: $\sqrt{r}$ = -0.59566 + 0.00216T
Correlation coefficient = 0.999; T_o = 275.8K.

(d): Square root of growth rate versus temperature at NaCl = 2.0 molal, a_w = 0.928 (GT data).
Equation to line: $\sqrt{r}$ = -1.75740 + 0.00638T
Correlation coefficient = 0.991; T_o = 275.5K.

For experimental and predicted values refer to Appendix 6.13d.

All equations produced by least squares linear regression of experimental data.

Figure 3.16: Square Root Plots of the Effect of Temperature and Sodium Chloride Concentration on the Growth Rate of Staphylococcus xylosus Strain CM21/3.

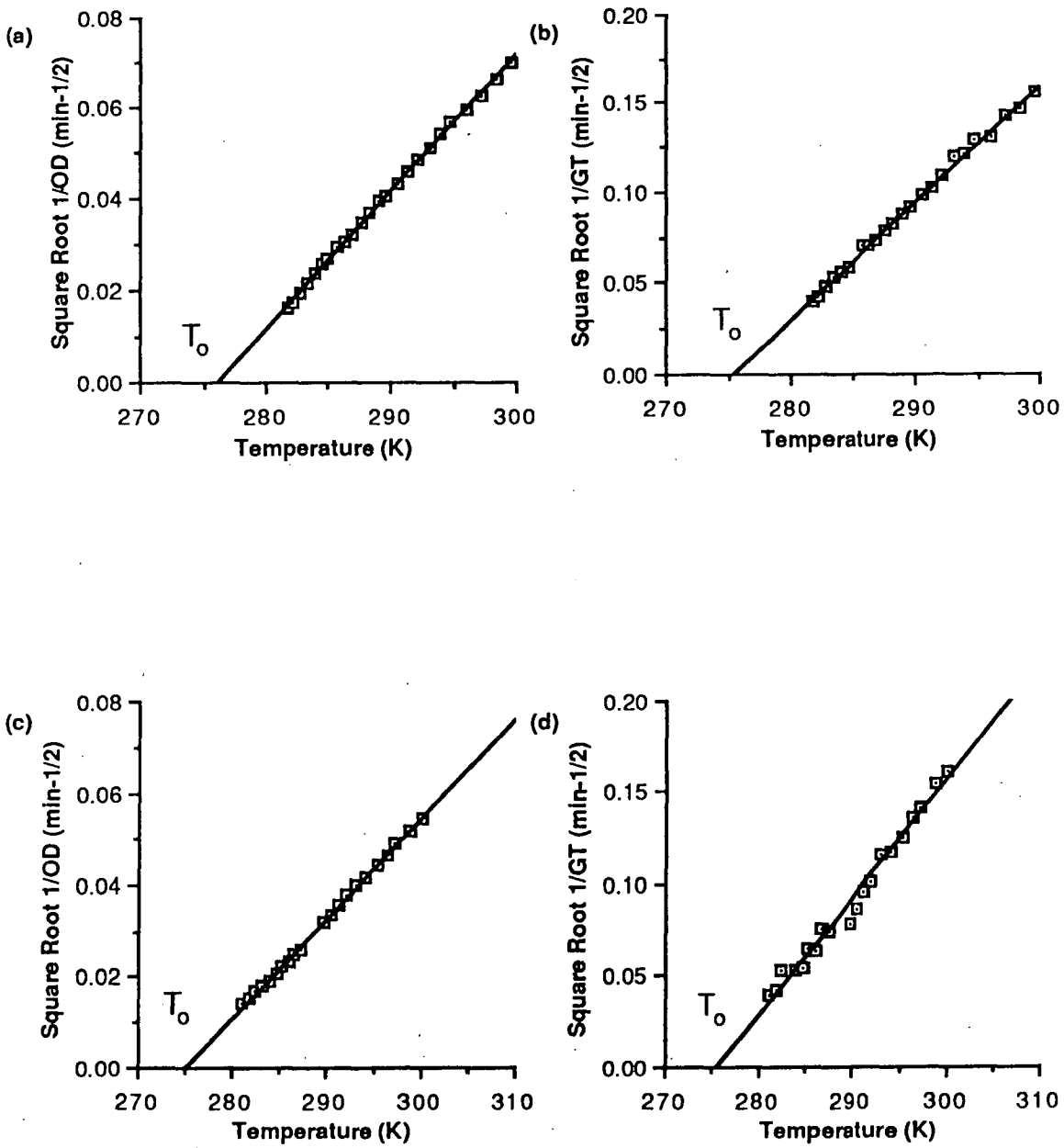


Figure 3.17: Square Root Plots of the Effect of Temperature and Sodium Chloride Concentration on the Growth Rate of Staphylococcus xylosus Strain CM21/3.

(a): Square root of growth rate versus temperature at NaCl = 2.5 molal, a_w = 0.909 (OD data).
Equation to line: $\sqrt{r}$ = -0.61431 + 0.00222T
Correlation coefficient = 0.999; T_o = 276.7K.

(b): Square root of growth rate versus temperature at NaCl = 2.5 molal, a_w = 0.909 (GT data).
Equation to line: $\sqrt{r}$ = -1.47023 + 0.00532T
Correlation coefficient = 0.991; T_o = 276.4K.

For experimental and predicted values refer to Appendix 6.13e.

(c): Square root of growth rate versus temperature at NaCl = 3.0 molal, a_w = 0.889 (OD data).
Equation to line: $\sqrt{r}$ = -0.56681 + 0.00206T
Correlation coefficient = 0.999; T_o = 275.2K.

(d): Square root of growth rate versus temperature at NaCl = 3.0 molal, a_w = 0.889 (GT data).
Equation to line: $\sqrt{r}$ = -1.18961 + 0.00432T
Correlation coefficient = 0.976; T_o = 275.4K.

For experimental and predicted values refer to Appendix 6.13f.

All equations produced by least squares linear regression of experimental data.

Figure 3.17: Square Root Plots of the Effect of Temperature and Sodium Chloride Concentrations on the Growth Rate of Staphylococcus xylosus Strain CM21/3.

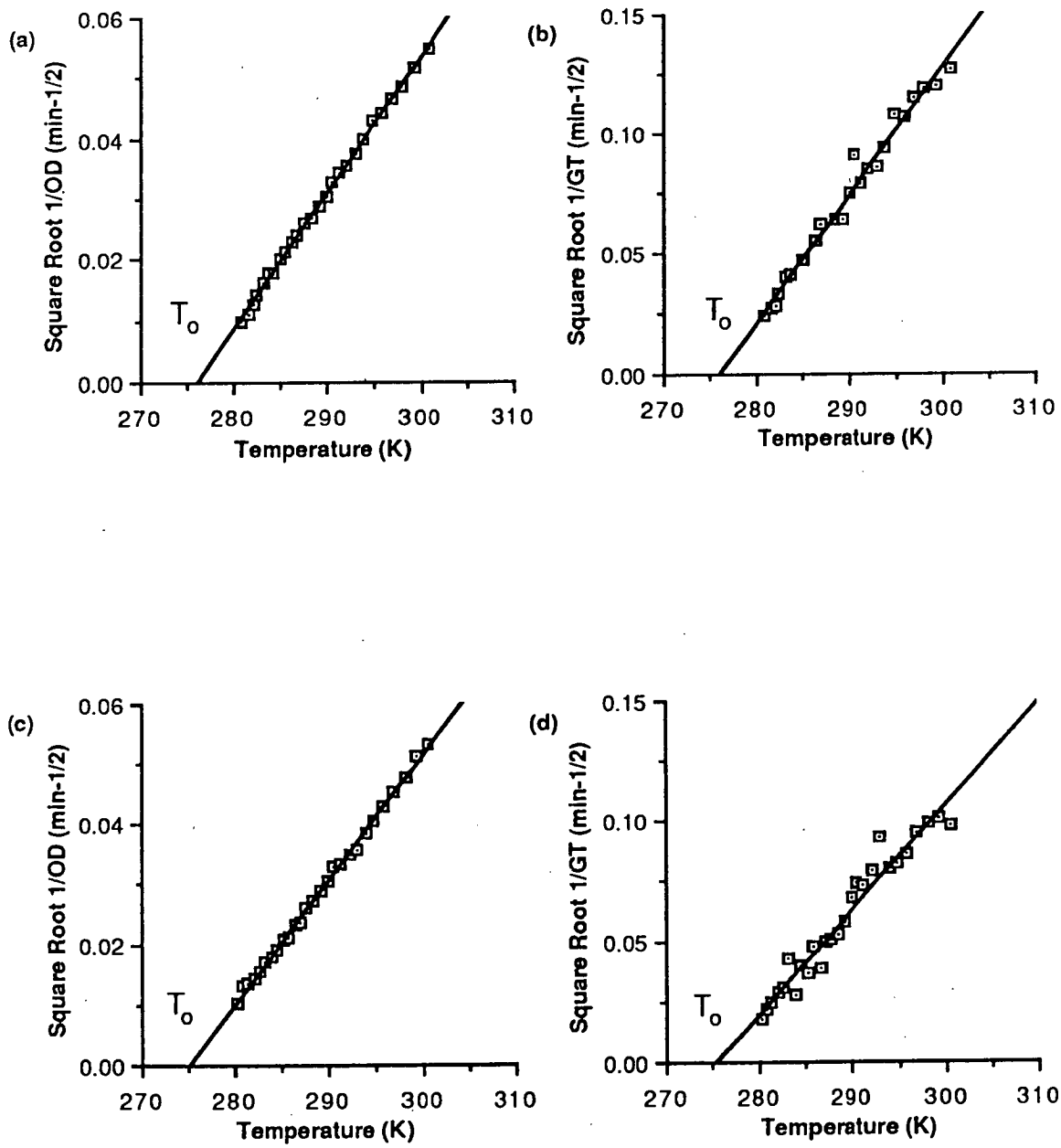


Figure 3.18: Square Root Plots of the Effect of Temperature and Sodium Chloride Concentrations on the Growth Rate of Staphylococcus xylosus Strain CM21/3.

(a): Square root of growth rate versus temperature at NaCl = 3.5 molal, a_w = 0.869 (OD data).
Equation to line: $\sqrt{r}$ = -0.38116 + 0.00138T
Correlation coefficient = 0.993; T_o = 276.2K.

(b): Square root of growth rate versus temperature at NaCl = 3.5 molal, a_w = 0.869 (GT data).
Equation to line: $\sqrt{r}$ = -0.89504 + 0.00325T
Correlation coefficient = 0.917; T_o = 275.4K.

For experimental and predicted values refer to Appendix 6.13g.

(c): Square root of growth rate versus temperature at NaCl = 3.75 molal, a_w = 0.858 (OD data).
Equation to line: $\sqrt{r}$ = -0.22898 + 0.00828T
Correlation coefficient = 0.989; T_o = 276.6K.

(d): Square root of growth rate versus temperature at NaCl = 3.75 molal, a_w = 0.858 (GT data).
Equation to line: $\sqrt{r}$ = -0.87307 + 0.00313T
Correlation coefficient = 0.896; T_o = 278.9K.

For experimental and predicted values refer to Appendix 6.13h.

All equations produced by least squares linear regression of experimental data.

Figure 3.18: Square Root Plots of the Effect of Temperature and Sodium Chloride Concentration on the Growth Rate of Staphylococcus xylosus Strain CM21/3.

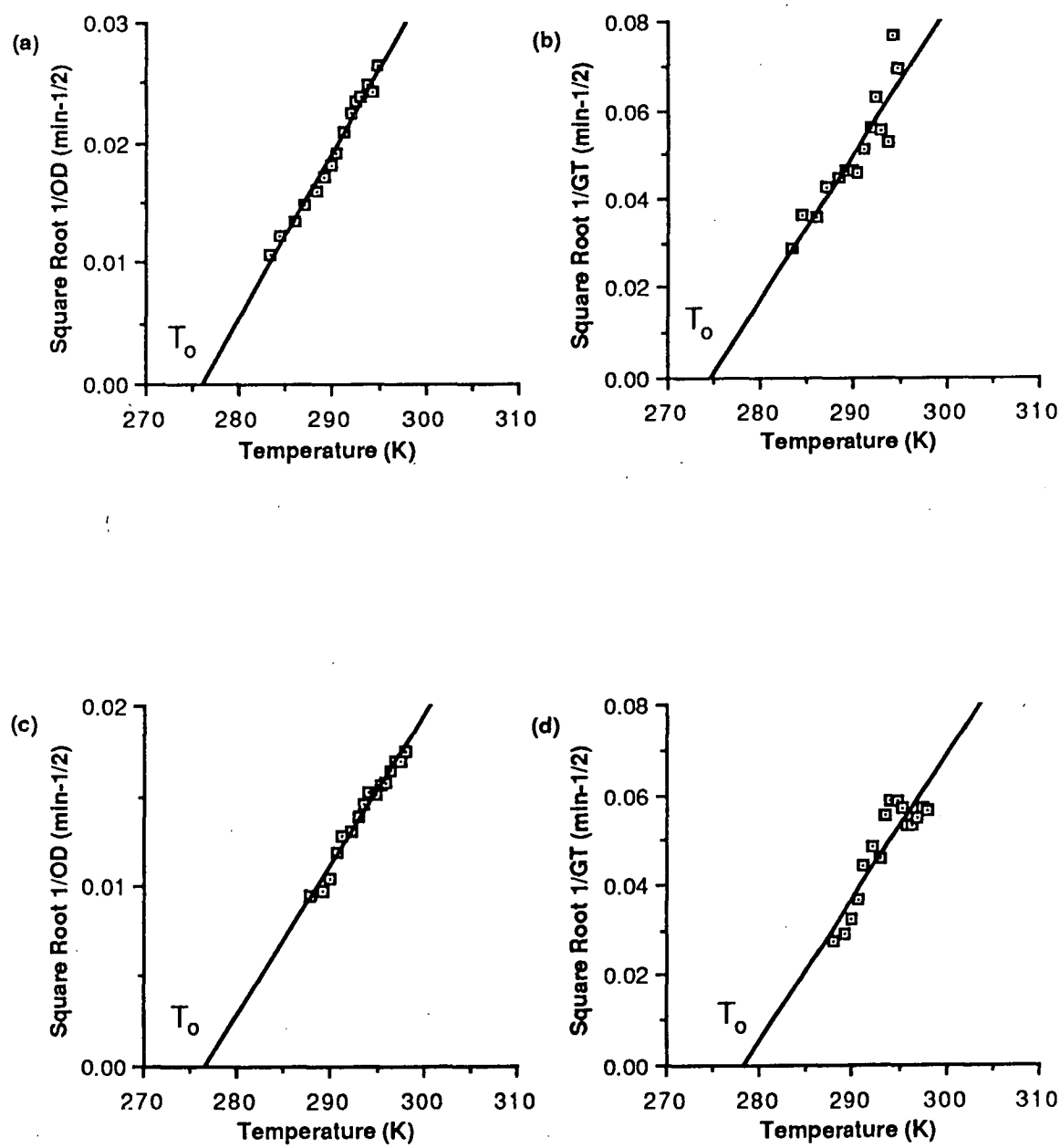


Figure 3.19: Square Root Plots of the Effect of Temperature and Sodium Chloride Concentration on the Growth Rate of Staphylococcus xylosus Strain CM21/3.

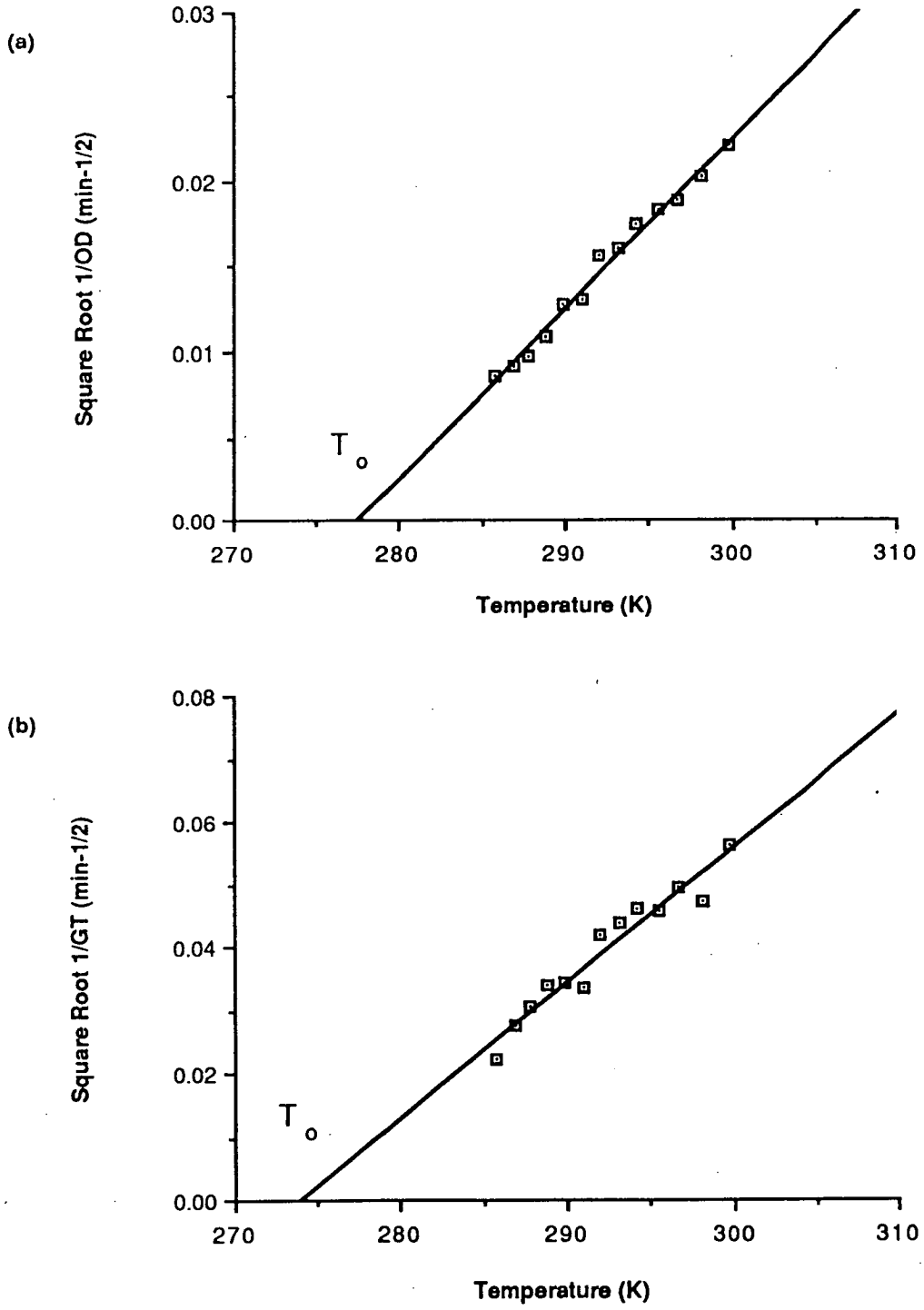
(a): Square root of growth rate versus temperature at NaCl = 4.0 molal, a_w = 0.848 (OD data).
Equation to line: $\sqrt{r} = -0.276246 + 0.000995T$
Correlation coefficient = 0.994; T_o = 277.6K.

(b): Square root of growth rate versus temperature at NaCl = 4.0 molal, a_w = 0.848 (GT data).
Equation to line: $\sqrt{r} = -0.58562 + 0.00213T$
Correlation coefficient = 0.970; T_o = 274.9K.

For experimental and predicted values refer to Appendix 6.13i.

Both equations produced by least squares linear regression of experimental data.

Figure 3.19: Square Root Plots of the Effect of Temperature and Sodium Chloride Concentration on the Growth Rate of Staphylococcus xylosus Strain CM21/3.



and 4.5 molal (a_w: 0.996; 0.976; 0.949; 0.928; 0.909; 0.889; 0.869; 0.858; 0.848; and 0.826). The 4.5 molal culture initially grew poorly at 35°C and upon inoculation for TGI studies, failed to grow after 7 days incubation over the temperature range 5-28°C.

From the results presented in Figure 3.15, Figure 3.16, Figure 3.17, Figure 3.18 and Figure 3.19, it was demonstrated that the Square Root Model accurately described the growth of Staphylococcus xylosus strain CM21/3 under conditions where NaCl and water activity were limiting.

As was noted in Section 1.4e, the Square Root Model of Ratkowsky et al.,(1982) was a specific case of the temperature function described by Bělehrádek (1935). To test the validity of the use of the exponent d = 2 [Equation (9)] and the hypothesis that T_o or α was constant, the OD data generated from the growth of Staphylococcus xylosus strain CM21/3 over the sodium chloride range 0.0-4.0 was analysed. Non-linear least squares regression (Kennedy & Gentle, 1980) was used to estimate 11 coefficients (i.e., α, d, and nine values of a). The exponent d was estimated to be 1.97 +/- 0.04. Since this was within one standard error from 2, the use of the Square Root Model to describe the effect of temperature on the growth rate of Staphylococcus xylosus strain CM21/3 over a wide range of NaCl/water activity values, was valid.

The T_o values calculated from OD and GT data at each water activity are summarised in Table 3.12. It can be seen that the T_o values were relatively constant and that there was no consistent trend or change in T_o value with decreasing water activity. The mean T_o values for OD and GT data were similar and were calculated to be 276.4 +/- 0.4K and 276.1 +/- 1.2K respectively.

Table 3.12: T_o Values for *Staphylococcus xylosus* Strain CM21/3 at Different Sodium Chloride/Water Activity Levels, Derived from the Square Root Model Using OD and GT Data.

NaCl (molal)	Water Activity	T_o(K) OD data	GT data
0.0	0.996	277.0	276.7
0.7	0.976	276.2	276.4
1.4	0.949	276.4	275.4
2.0	0.928	275.8	275.5
2.5	0.909	276.7	276.4
3.0	0.889	275.2	275.4
3.5	0.869	276.2	275.4
3.75	0.858	276.6	278.9
4.0	0.848	277.6	274.9

Mean T_o value for OD data = 276.4 +/- 0.4K.

Mean T_o value for GT data = 276.1 +/- 1.2K.

When both OD and GT data were forced to converge to a common T_o value, the T_o value for OD data was estimated to be 275.7 +/- 0.1K and for GT data, it was estimated to be 275.9 +/- 0.2K. These values were similar to the mean T_o values described in Table 3.12. These results support the notion of Ratkowsky et al.,(1982), that the parameter T_o was an intrinsic property of the microorganism concerned and that it was not influenced by different temperature and water activity regimes.

A clear distinction must be made between T_o, which is a notional temperature where growth rate is zero and the observed minimum temperature for growth. No attempt was made in these experiments to define accurately the minimum temperature at which growth could be observed at each water activity. However, the minimum temperature at which growth was observed, generally increased with decreased water activity (Figure 3.20). This trend was consistent with reports from the literature (Lotter & Leistner, 1978; Lee et al.,1981 and Notermans & Heuvelman, 1983).

At a_w = 0.996, 27 values were determined over the temperature range 6.7-29.2°C, whilst at a_w = 0.848, only 13 values were determined from 12.6-28.5°C. At a_w = 0.828, slight and inconsistent growth was observed at approximately 35°C. However, cells from this culture used to inoculate tubes of the same medium held at temperatures from 5-28°C, failed to grow within 7 days.

Ingraham et al.,(1983) noted the discrepancy between T_o (3.5°C) and the observed minimum temperature for growth (8°C) for Escherichia coli and suggested that in the extreme low range the Square Root Model may not be valid. However, despite the increasing distance between T_o and the observed minimum temperature for growth, of Staphylococcus xylosus strain CM21/3, with decreasing water activity,

Figure 3.20: Square Root Plots of the Effect of Temperature and Sodium Chloride Concentration/Water Activity on the Growth Rate of Staphylococcus xylosus Strain CM21/3.

Square Root plots of the predicted lines for GT data for Staphylococcus xylosus strain CM21/3 at nine different sodium chloride/water activity levels. Each line was forced to converge statistically at the common T_o value of 275.9K.

Table 3.13 reports the predicted value of b for each line.

No growth occurred over the temperature range 278.2-301.2K at a_w = 0.826

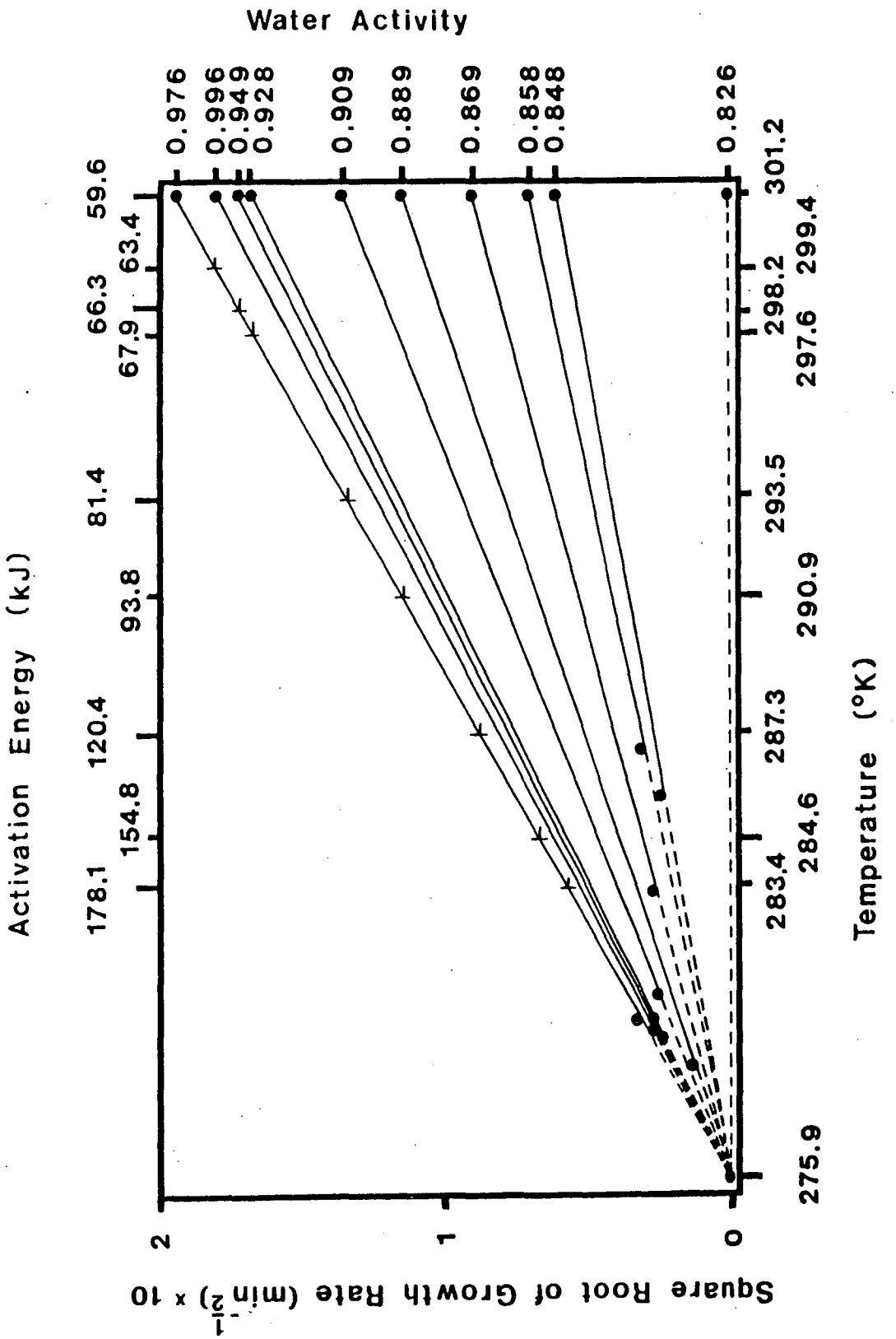
Apparent activation energies (kJ mol^{-1}) (E) were calculated from the differential form of the Arrhenius equation:

$$d \ln r/dT = E/RT^2 \qquad (13)$$

For predicted values see Appendix 6.14.

The minimum temperature at which growth was measured for each a_w is indicated by ●.

Figure 3.20: Square Root Plots of the Effect of Temperature and Sodium Chloride Concentration/Water Activity on the Growth Rate of Staphylococcus xylosus Strain CM21/3.



the Square Root Model accurately predicted the growth response at temperatures above the minimum observed temperature for growth at each water activity (Figure 3.15, Figure 3.16, Figure 3.17, Figure 3.18, Figure 3.19 and Figure 3.20). This was also shown to be the case for *E. coli*, which obeyed the Square Root Model at low temperatures until growth ceased (McMeekin *et al.*, 1987).

A direct comparison of growth rates at different water activities was made by comparing GT data (Figure 3.20). Growth was slower at a_w = 0.996 than at a_w = 0.976 and growth rate declined sequentially at lower water activity values. Generation times at 301.2K (28°C) and values for the constant b (slope of line predicted by the Square Root Model) are listed in Table 3.13. Generation times varied from approximately 27 min at a_w = 0.976 to 275 min at a_w = 0.848.

3.3a(iii): Further Reconciliation of the Square Root Model and the Arrhenius Equation.

As was stated in Section 1.12, the value of the temperature characteristic/activation energy predicted by the Arrhenius equation was a function of temperature and was related to the Square Root Model by Equation (11). Figure 3.20 showed that the effect of changing water activity was to alter the slope b in the Square Root Model, but not to alter T_o. Hence, according to Equation (11), the activation energy (E) is constant at any given temperature.

The Arrhenius equation is often written in the integrated form obtained by assuming a constant activation energy:

$$r = A_T \exp^{(-E_T/RT)} \qquad (14)$$

where A_T is the collision factor and E_T the activation energy. (The subscript T emphasises the fact that these

Table 3.13: b Values Predicted by the Square Root Model for *Staphylococcus xylosus* Strain CM21/3, at Different Sodium Chloride/Water Activity Levels.

Water Activity	Predicted b Value	Generation Time at 301.2K
0.996	0.0070715	31.1
0.976	0.0075929	27.1
0.949	0.0067114	34.7
0.928	0.0065725	36.2
0.909	0.0053095	55.2
0.889	0.0044727	78.2
0.869	0.0035338	125.0
0.858	0.0027498	207.0
0.848	0.0023851	275.0

Predicted b values were derived from lines predicted by the Square Root Model for GT data forced to converge statistically at their common T_o value of 275.9K.

All times in minutes.

parameters are temperature dependent).

Thus with E_T constant at a particular temperature, the reduced growth rate caused by lowered water activity (i.e., by increased NaCl concentration) was due to a reduced value of the collision factor A_T. The Square Root Model and the Arrhenius equation can thus be viewed as being totally reconciled. The advantage of the Square Root Model for practical use, is the fact that T_o and b do not change with temperature, whereas E_T and A_T do. The same reconciliation can be carried out between the Arrhenius and Bělehrádek equations. Provided the exponent d is constant, a substitutes for A_T and α substitutes for E_T.

3.3a(iv): Derivation of a Mathematical Model Relating Growth Rate to Temperature and Water Activity.

Figure 3.21 contains the plot of b^2 versus water activity, from which it is seen that for the a_w range 0.976-0.848, the relationship is approximately linear. As b alone in the Square Root Model changes with water activity, growth as a function of temperature (between the minimum and optimum values may be modelled by replacing b by its relationship with water activity. From the Square Root Model, b^2 is proportional to the growth rate r. Hence, the linear relationship between b^2 and salt concentration/water activity is equivalent to a linear relationship between r and a_w. This is in agreement with the observations of Scott (1953) and Troller & Christian (1978), who noted that below the optimum water activity, growth often declined linearly.

Extrapolation of the fitted straight line in Figure 3.21, predicted a minimum a_w of 0.838 (corresponding to a zero growth rate in MHB containing NaCl). When the equation for this line was substituted for b in the Square Root Model, the expression:

Figure 3.21: A Plot of the Value of b^2 Associated with Different Sodium Chloride / Water Activity Levels for Staphylococcus xylosus Strain CM21/3.

Predicted values of b derived from the Square Root Model (at a_w values from 0.976-0.848) were plotted as b^2 against their corresponding water activities, for GT data forced to converge statistically at their common T_o value of 275.9K.

Equation to line:

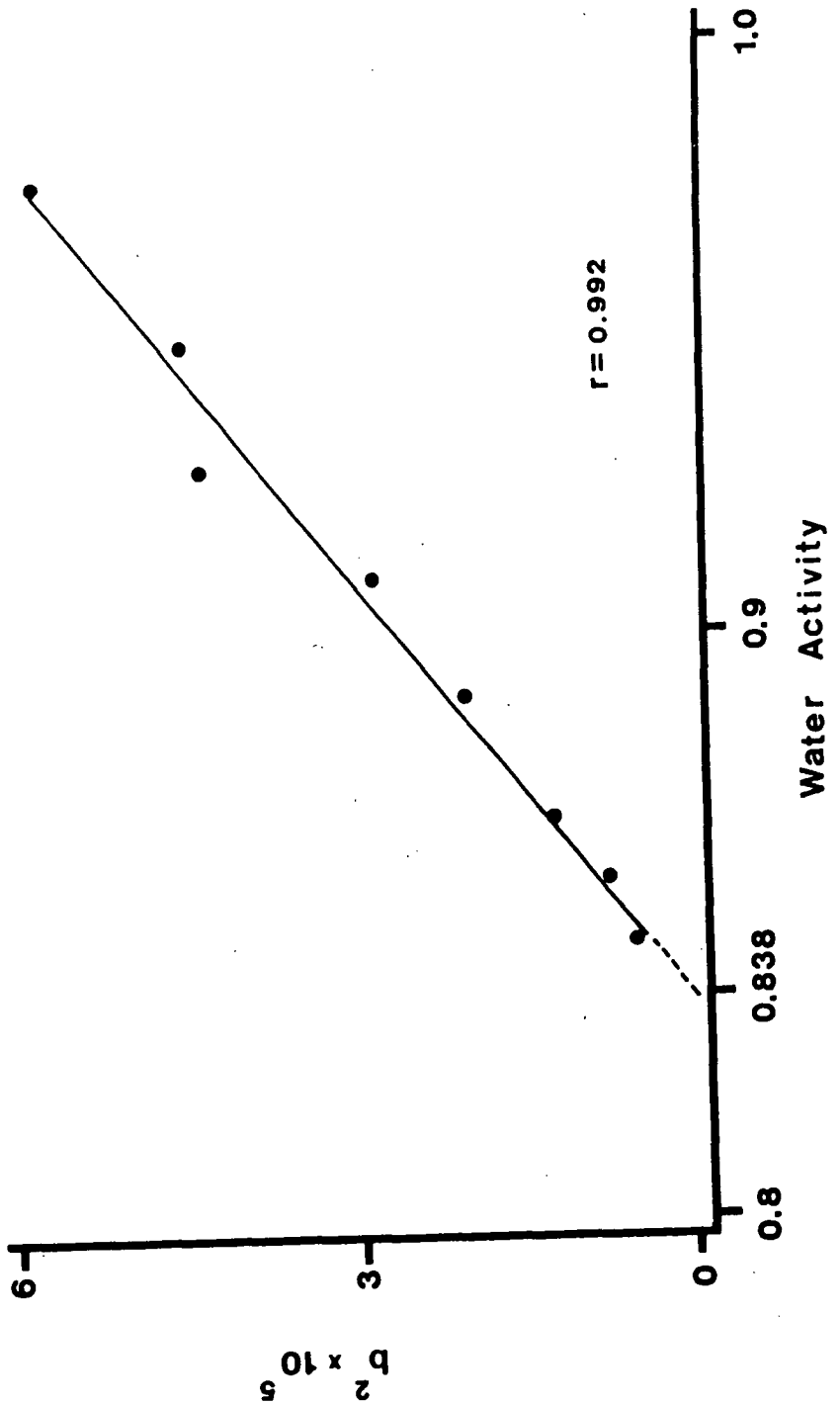
$$b^2 = -3.53365 \times 10^{-4} + 4.216376 \times 10^{-4}(a_w)$$

Correlation coefficient = 0.992

Predicted minimum a_w for growth = 0.838

Table 3.13 reports the predicted value of b at each water activity.

Figure 3.21: A Plot of the Value of b^2 Associated with Different Sodium Chloride / Water Activity Levels for Staphylococcus xylosus Strain CM21/3.



$$\sqrt{r} = 0.0205 \sqrt{(a_w - 0.838)}(T - 275.9) \quad (15)$$

was obtained. Equation (15) may be used for predictive purposes at a_w levels from 0.848, to the optimum at 0.976 and at temperatures from the minimum observed temperature for growth, to 301.2K (28°C). The mean absolute deviation between predicted and experimental values of r was 15.4% (Appendix 6.13).

Equation (15) is a specific form of the more general equation:

$$\sqrt{r} = b_o \sqrt{(a_w - a_{wo})}(T - T_o) \quad (16)$$

where b_o is a constant and a_{wo} is a theoretical water activity, below which growth does not occur.

Equation (16) describes the growth of any microorganism showing a response to temperature and water activity similar to that of Staphylococcus xylosus. To determine the parameters T_o and a_{wo}, it is necessary to conduct two simple experiments. The first is to grow the microorganism over a range of temperatures at a single water activity and to determine T_o by using the Square Root Model. The second is to grow the microorganism over a range of water activities at a specified temperature and to determine the a_{wo} value from a plot of r versus water activity. It is not necessary to determine a value for b_o, as when the equation is used to predict relative growth rates for any combination of temperature and water activity, the constant will cancel during subsequent calculations.

3.3a(v): The Effect of Temperature and Glycerol Concentration on the Growth Rate.

In Section 3.3a(iii), water activity was controlled by the addition of NaCl to the basal growth medium (MHB). It was recognised under those conditions that growth was also influenced by a specific solute effect. In order to determine whether or not the response to temperature and water activity displayed in Section 3.3a(iii) was due

to the presence of NaCl, a similar series of experiments was conducted, with glycerol replacing NaCl as the humectant.

Four TGI experiments were conducted to test the validity of the Square Root Model under conditions where glycerol and water activity were limiting. OD and GT data were calculated from the growth of Staphylococcus xylosus strain CM21/3 in MHB containing glycerol: 1.0 (Figure 3.22a and Figure 3.22b); 2.0 (Figure 3.22c and Figure 3.22d); 3.0 (Figure 3.23a and Figure 3.23b); and 4.5 molal (Figure 3.23c and Figure 3.23d) (a_w: 0.975; 0.958; 0.943; and 0.919). Poor growth was observed in 6.0 molal (a_w = 0.896 glycerol after incubation at 35°C for 5 days. The growth was not sufficient for use as an inoculum for a TGI experiment.

To ensure the water activity of MHB + glycerol did not change during growth, the water activity of the 3.0 molal system was determined before and after growth of Staphylococcus xylosus strain CM21/3 at 35°C. The a_w prior to growth was 0.943 and the a_w after growth was 0.945. Therefore, there was little or no decrease in glycerol concentration over the duration of the growth studies.

From the results presented in Figure 3.22 and Figure 3.23, it was demonstrated that the Square Root Model also accurately described the growth of Staphylococcus xylosus strain CM21/3 under conditions where glycerol and water activity were limiting.

The T_o values calculated from OD and GT data at each water activity are summarised in Table 3.14. It can be seen that the T_o values were relatively constant and that there was no consistent trend or change in T_o value with decreasing water activity. The mean

Figure 3.22: Square Root Plots of the Effect of Temperature and Glycerol Concentration on the Growth Rate of Staphylococcus xylosus Strain CM21/3.

(a): Square root of growth rate versus temperature at glycerol = 1.0 molal, a_w = 0.975 (OD data).
Equation to line: $\sqrt{r} = -0.84587 + 0.00305T$
Correlation coefficient = 0.999; T_o = 277.3K.

(b): Square root of growth rate versus temperature at glycerol = 1.0 molal, a_w = 0.975 (GT data).
Equation to line: $\sqrt{r} = -2.59010 + 0.00930T$
Correlation coefficient = 0.974; T_o = 278.5K.

For experimental and predicted values refer to Appendix 6.15a.

(c): Square root of growth rate versus temperature at glycerol = 2.0 molal, a_w = 0.958 (OD data).
Equation to line: $\sqrt{r} = -0.53487 + 0.00195T$
Correlation coefficient = 0.999; T_o = 274.3K.

(d): Square root of growth rate versus temperature at glycerol = 2.0 molal, a_w = 0.958 (GT data).
Equation to line: $\sqrt{r} = -1.92758 + 0.00696T$
Correlation coefficient = 0.982; T_o = 277.0K.

For experimental and predicted values refer to Appendix 6.15b.

All equations produced by least squares linear regression of experimental data.

Figure 3.22: Square Root Plots of the Effect of Temperature and Glycerol Concentration on the Growth Rate of Staphylococcus xylosus Strain CM21/3.

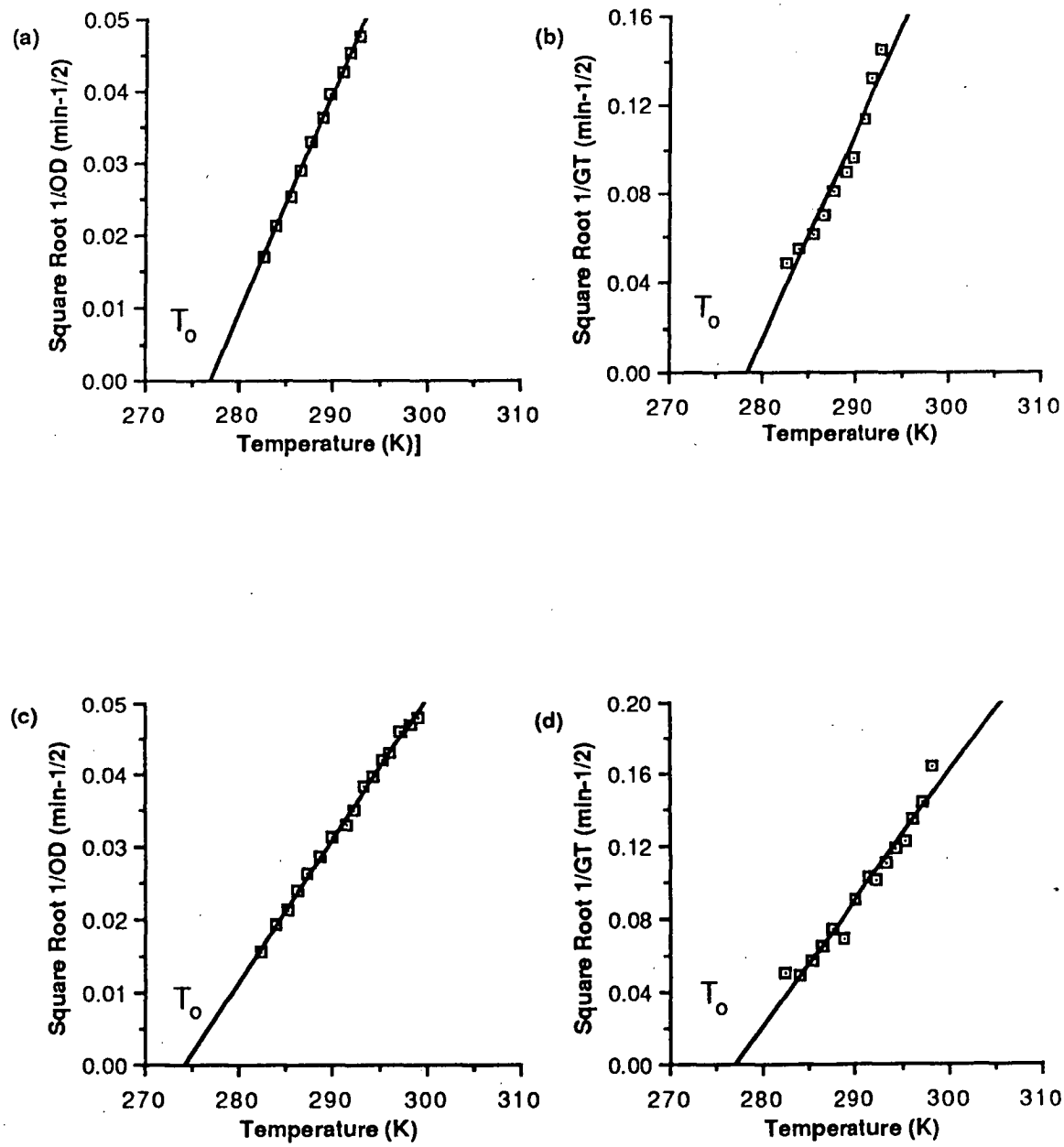


Figure 3.23: Square Root Plots of the Effect of Temperature and Glycerol Concentration on the Growth Rate of Staphylococcus xylosus Strain CM21/3.

(a): Square root of growth rate versus temperature at glycerol = 3.0 molal, a_w = 0.943 (OD data).
Equation to line: $\sqrt{r}$ = -0.51922 + 0.00189T
Correlation coefficient = 0.998; T_o = 274.7K.

(b): Square root of growth rate versus temperature at glycerol = 3.0 molal, a_w = 0.943 (GT data).
Equation to line: $\sqrt{r}$ = -1.45037 + 0.00527T
Correlation coefficient = 0.994; T_o = 275.2K.

For experimental and predicted values refer to Appendix 6.15c.

(c): Square root of growth rate versus temperature at glycerol = 4.5 molal, a_w = 0.919 (OD data).
Equation to line: $\sqrt{r}$ = -0.28978 + 0.00105T
Correlation coefficient = 0.998; T_o = 276.0K.

(d): Square root of growth rate versus temperature at glycerol = 4.5 molal, a_w = 0.919 (GT data).
Equation to line: $\sqrt{r}$ = -0.85961 + 0.00311T
Correlation coefficient = 0.997; T_o = 276.4K.

For experimental and predicted values refer to Appendix 6.15d.

All equations produced by least squares linear regression of experimental data.

Figure 3.23: Square Root Plots of the Effect of Temperature and Glycerol Concentration on the Growth Rate of Staphylococcus xylosus Strain CM21/3.

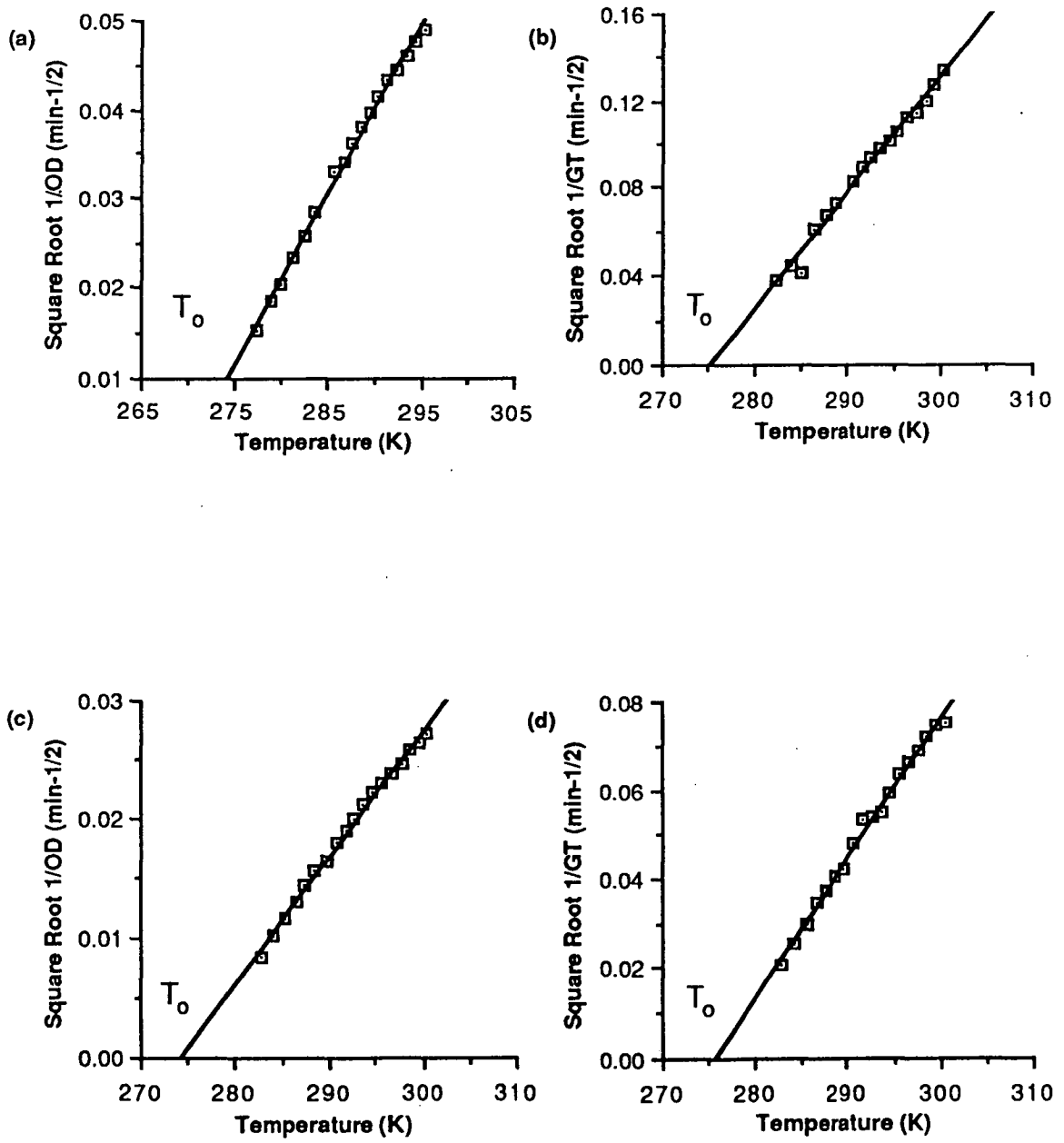


Table 3.14: T_o Values for *Staphylococcus xylosus* Strain CM21/3 at Different Glycerol/Water Activity Levels, Derived from the Square Root Model Using OD and GT Data.

Glycerol (molal)	Water Activity	T_o(K) OD data	GT data
0.0*	0.996	277.0	276.7
1.0	0.975	277.3	278.5
2.0	0.958	274.3	277.0
3.0	0.943	274.7	275.2
4.5	0.919	276.0	276.4

Mean T_o value for OD data = 275.9 +/- 1.3K.

Mean T_o value for GT data = 276.8 +/- 1.2K.

* data for this value was presented in Table 3.12 (Figure 3.15a and Figure 3.15b).

T_o values for OD and GT data were similar and calculated to be 275.9 +/- 1.3K and 276.8 +/- 1.2K respectively. These values are slightly higher and lower than the corresponding values observed for growth in MHB + NaCl.

Both OD and GT data were forced to converge to a common T_o value. For OD data the T_o value was estimated to be 275.6 +/- 1.3K and for GT data it was estimated to be 276.6 +/- 2.3K. These values were similar to the converged T_o values obtained from OD and GT data, for growth in MHB + NaCl. These results further support the assertion that the T_o value of a microorganism is an intrinsic property and remains fixed irrespective of the water activity of the growth medium or the humectant used.

A direct comparison of the growth rates of Staphylococcus xylosus strain CM21/3 at different water activities was made by comparing GT data (Figure 3.24). The growth response in MHB containing different concentrations of glycerol was the same as the response for MHB containing different concentrations of NaCl. Growth was slower at a_w = 0.996 than at a_w = 0.975 and growth declined sequentially at lower water activity values. Generation times varied from approximately 26 min at a_w =0.975 to 154 min at a_w = 0.919.

Similarly a plot of b^2 (b is the slope of the line predicted by the Square Root Model) versus water activity (Figure 3.25), resulted in an approximately linear relationship. Extrapolation of the fitted straight line predicted a minimum a_w for growth in MHB containing glycerol, of 0.908.

It was possible to derive an equation similar to Equation (15):

$$\sqrt{r} = 0.0308 \sqrt{(a_w - 0.908)}(T - 276.6) \qquad (17)$$

Equation (17) may be used for predictive purposes at a_w levels

Figure 3.24: Square Root Plots of the Effect of Temperature and Glycerol Concentration/Water Activity on the Growth Rate of Staphylococcus xylosus Strain CM21/3.

Square Root plots of the predicted lines for GT data for Staphylococcus xylosus strain CM21/3 at five different glycerol/water activity levels. Each line was forced to converge statistically at the common T_o value of 276.6K.

The predicted value of b for each line was:

$a_w = 0.996$, b = 0.0073981

$a_w = 0.975$, b = 0.0079973

$a_w = 0.958$, b = 0.0068597

$a_w = 0.943$, b = 0.0056992

$a_w = 0.919$, b = 0.0032794

No growth occurred over the temperature range 278.2-301.2K at $a_w = 0.896$

Figure 3.24: Square Root Plots of the Effect of Temperature and Glycerol Concentration/Water Activity on the Growth Rate of Staphylococcus xylosus Strain CM21/3.

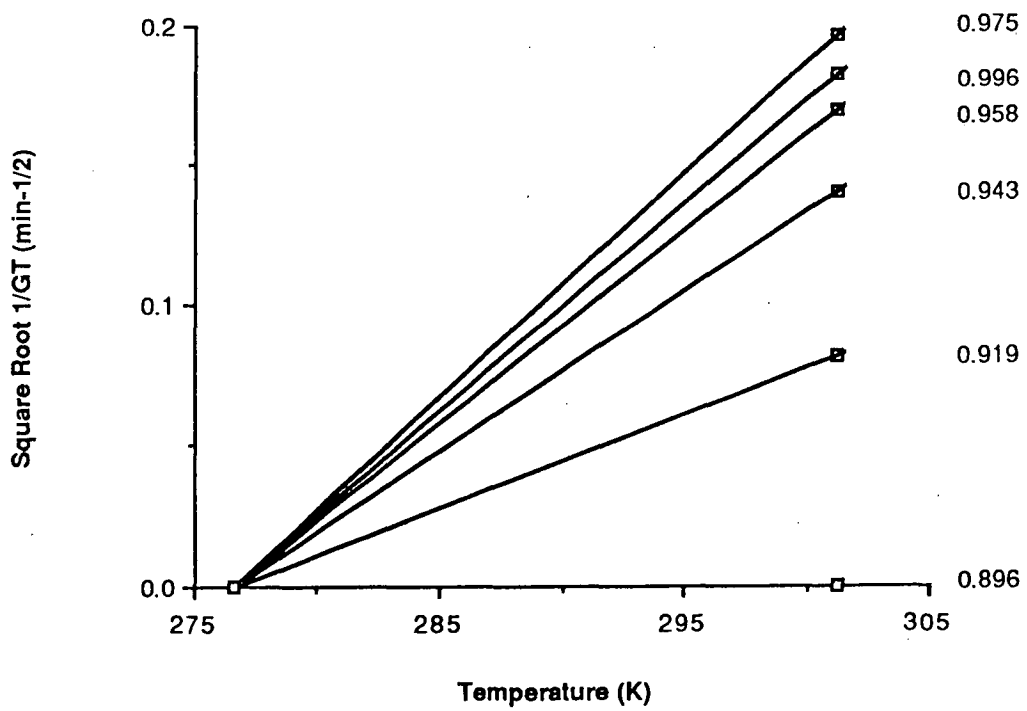


Figure 3.25: A Plot of the Value of b^2 Associated with Different Glycerol / Water Activity Levels for Staphylococcus xylosus Strain CM21/3.

Predicted values of b derived from the Square Root Model (at a_w values from 0.975-0.919) (Figure 3.24) were plotted as b^2 against their corresponding water activities, for GT data forced to converge statistically at their common T_o value of 276.6K.

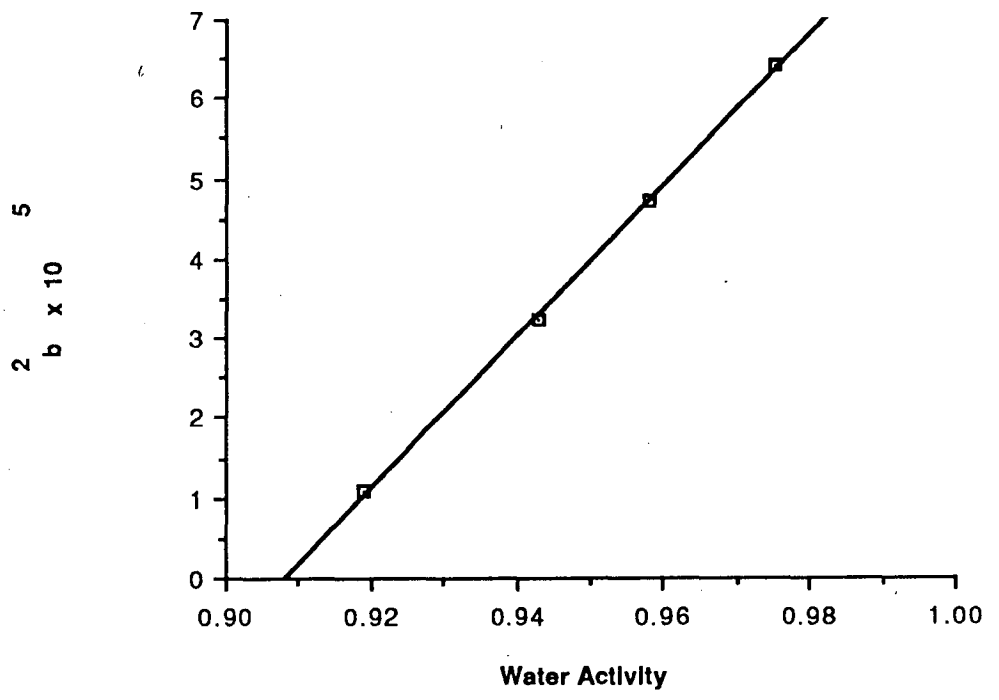
Equation to line:

$$b^2 = -8.62364 \times 10^{-4} + 9.495935 \times 10^{-4}(a_w)$$

Correlation coefficient = 0.999

Predicted minimum a_w for growth = 0.908

Figure 3.25: A Plot of the Value of b^2 Associated with Different Glycerol / Water Activity Levels for Staphylococcus xylosus Strain CM21/3.



from 0.919, to the optimum at 0.975 and at temperatures from the minimum observed temperature for growth, to 301.2K (28°C). The mean absolute deviation between predicted and experimental values of r was 10.5% (Appendix 6.15).

Hence, it has been shown that Staphylococcus xylosus strain CM21/3, exhibited a similar response to water activity and temperature, independent of the type of humectant used. The major difference between the two broth systems was the fact that glycerol was more inhibitory to growth than NaCl at similar water activity levels. Marshall et al.,(1971) reported a similar result for salt tolerant bacteria.

3.3a(vi): Determination of the Minimum Water Activity for Growth.

In an attempt to establish the minimum water activities for growth in MHB containing NaCl or glycerol, Staphylococcus xylosus strain CM21/3 incubated at water activities above and below the a_{wo} values predicted by Equation (15) and Equation (17). However, it must be noted that both equations were based upon data derived from within the temperature range 5-30°C and hence were not strictly valid for predicting growth at the optimum temperature of 38°C.

For both NaCl and glycerol, the results presented in Table 3.15, indicated that slight growth occurred at water activities just below the values predicted by the Equation (15) and Equation (17). Very slightly turbid cultures were found after incubation in MHB + NaCl at a_w = 0.826 and MHB + glycerol at a_w 0.900 at 38°C, with no growth observed at a_w = 0.803 and 0.888. The predicted a_{wo} values for the two broth systems were 0.838 and 0.908, respectively. Hence, even at 38°C, the extended Square Root Model predicted minimum water activities for growth which were close to experimentally derived

Table 3.15: Determination of the Minimum Water Activity for Growth of Staphylococcus xylosus Strain CM21/3 at Different Sodium Chloride and Glycerol Concentrations.

Sodium Chloride	Water Activity	Days incubation at 38°C 1	2	3	5	10	20	35
3.5	0.869	+	++	++	+++	/	/	/
4.0	0.848	+	+	++	++	+++	/	/
4.5	0.826	-	-	-	+	+	+	+
5.0	0.803	-	-	-	-	-	-	-
5.5	0.780	-	-	-	-	-	-	-
6.0	0.757	-	-	-	-	-	-	-
Glycerol								
4.0	0.915	++	++	++	+++	/	/	/
4.5	0.908	+	++	++	++	+++	/	/
5.0	0.900	-	-	-	+	+	+	+
5.5	0.888	-	-	-	-	-	-	-
6.0	0.883	-	-	-	-	-	-	-
6.5	0.872	-	-	-	-	-	-	-

Sodium chloride and glycerol values expressed as molalities.

\+ : culture just turbid.

++ : culture turbid.

+++: culture very turbid.

/ : culture no longer monitored.

\- : culture not turbid.

values.

3.3a(vii): Determination of the Effect of Temperature on the Minimum Water Activity for Growth.

It was previously noted in Section 3.3a(ii) that the minimum water activity for growth appeared to increase with decreasing temperature and that this observation was consistent with reports from the literature (Lee et al.,1981). The results reported in this section support this assertion further.

The effect of temperature on the minimum water activity for growth of Staphylococcus xylosus strain CM21/3 is described in Table 3.16. It can be seen that the most rapid growth and growth to the lowest water activity occurred at 35.1°C (a temperature close to the optimum temperature for growth of approximately 38°C). At higher or lower temperatures, the growth rate decreased and the minimum water activity for growth increased.

The minimum temperature and/or water activity at which growth occurs, are difficult parameters to measure accurately. Growth rates decrease, with both decrease in temperature and water activity, until a point is reached, where it is difficult to determine, whether or not growth has ceased. In this experiment the incubation period was 7 days and it is therefore difficult to assess the effect of continued incubation (e.g., 7 weeks) on growth at lower temperature and/or water activity regimes. However, it is probable that continued incubation would have resulted in growth at a_w values of 0.841 and 0.826, at least at temperatures close to the optimum (Table 3.15).

3.3a(viii): Conclusion.

The results presented in Section 3.3a have demonstrated that the Square Root Model accurately predicts the effect of temperature and

Table 3.16: Determination of the Effect of Temperature on the Minimum Water Activity for Growth of Staphylococcus xylosus Strain CM21/3 at Different Sodium Chloride Concentrations.

Sodium Chloride	Water Activity	Temperature °C 40.1	35.1	30.0	25.2	20.0	15.1	9.1
0.0	0.992	1	1	1	1	1	1	1
0.7	0.970	1	1	1	1	1	1	1
1.4	0.945	1	1	1	1	1	1	2
2.1	0.921	2	1	1	1	1	2	3
2.7	0.890	3	2	2	2	2	3	4
3.4	0.871	-	3	3	4	4	5	6
3.8	0.860	-	4	5	6	7	-	-
4.1	0.841	-	-	-	-	-	-	-
4.5	0.826	-	-	-	-	-	-	-

Sodium chloride values expressed as molalities.

The figures 1-7 indicate the number of days required for positive growth for any particular combination of temperature and water activity.

A "-" indicates growth no growth occurred after 7 days incubation.

water activity on the growth rate of Staphylococcus xylosus strain CM21/3. The constancy of the theoretical T_o value provided a significant advantage in the development of a combined temperature/water activity model [Equation (16)]. As only the constant b in the Square Root Model varied with water activity, it was possible to simply extend the Square Root Model by introducing a term to describe the variation of b with water activity.

3.3b: Growth of Extreme Halophiles, Halobacterium sp. Strain HB9 and Halobacterium salinarium Strain CM42/12.

It has been shown in the preceding Sections that the Square Root Model of Ratkowsky et al.,(1982) was accurate in describing the growth of bacteria from different physiological groups i.e., NaCl sensitive and NaCl tolerant. Experiments in Section 3.3b(ii) and Section 3.3b(iii), tested the general applicability of the Square Root Model to describe the growth of two Halobacterium strains which have an obligate requirement for NaCl. They also tested the constancy of the predicted T_o value at different NaCl/water activity levels. An attempt was made to model the variation in response to temperature, at each of the different water activities tested.

3.3b(i): Calibration of Nephelometer.

It was demonstrated in Section 3.2a that doubling dilutions of a broth culture of Pseudomonas sp. strain E5.2 halved the observed OD with each successive dilution. This method was used to calibrate the nephelometer for Halobacterium sp. strain HB9 and Halobacterium salinarium strain CM42/12. Calibrations were conducted in XHB containing NaCl at a level close to the minimum level required for growth (3.5 molal, a_w = 0.865) (Figure 3.26a and Figure 3.26b) and at a level close to saturation of the XHB (6.0 molal, a_w = 0.748)

Figure 3.26: Nephelometer Calibration Curves for Halobacterium sp. Strain HB9 and Halobacterium salinarium Strain CM42/12.

(a): A plot of the OD of doubling dilutions of Halobacterium sp. strain HB9 in XHB + 3.5 molal NaCl.
Equation to line: y = 0.00013 + 0.420522x
Correlation coefficient = 0.997
For data refer to Appendix 6.16a.

(b): A plot of the OD of doubling dilutions of Halobacterium salinarium strain CM42/12 in XHB + 3.5 molal NaCl.
Equation to line: y = -0.0020 + 0.386226x
Correlation coefficient = 0.998
For data refer to Appendix 6.16b.

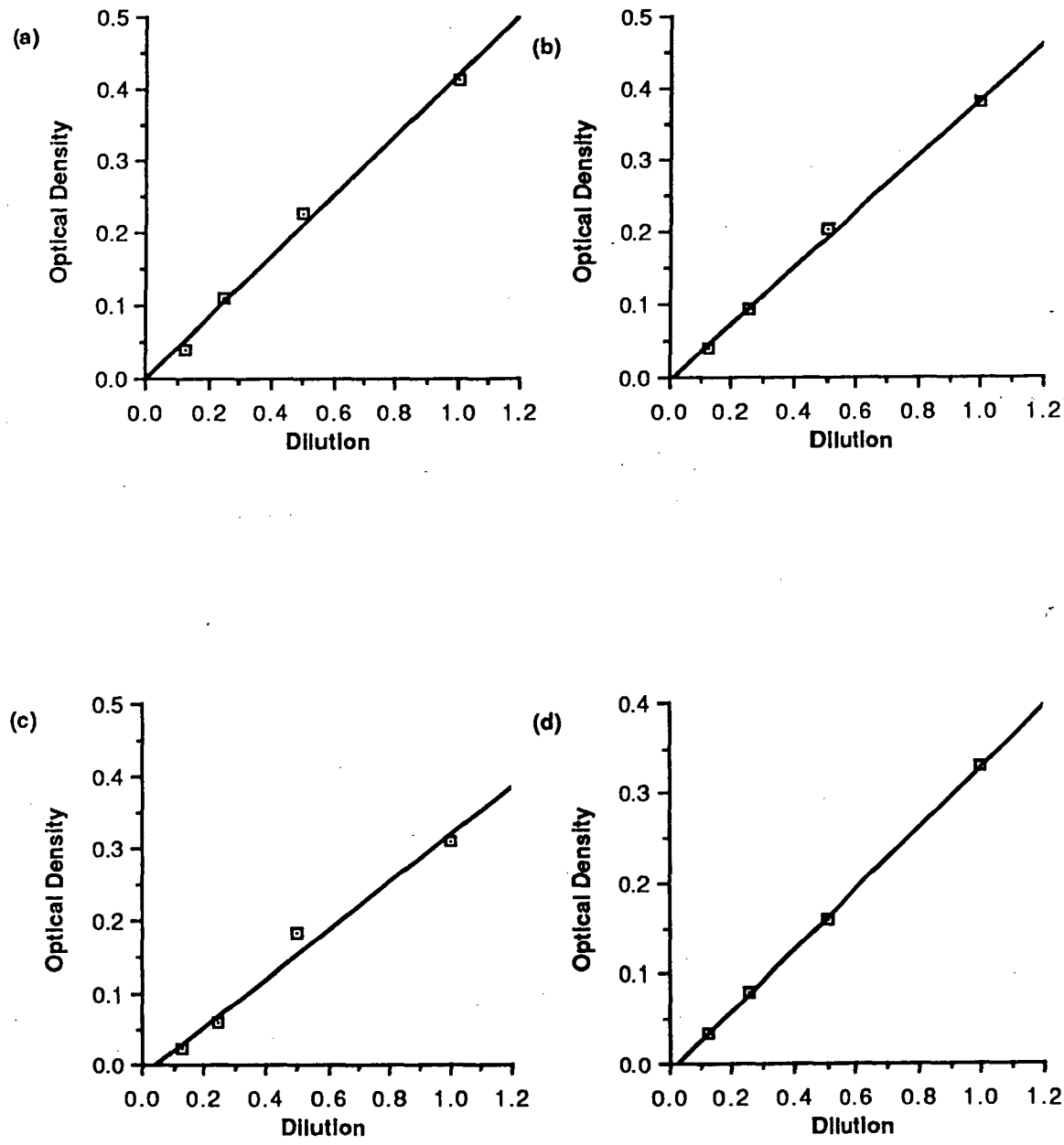
(c): A plot of the OD of doubling dilutions of Halobacterium sp. strain HB9 in XHB + 6.0 molal NaCl.
Equation to line: y = -0.012391 + 0.332035x
Correlation coefficient = 0.987
For data refer to Appendix 6.16c.

(d): A plot of the OD of doubling dilutions of Halobacterium salinarium strain CM42/12 in XHB + 6.0 molal NaCl.
Equation to line: y = -0.0067 + 0.337670x
Correlation coefficient = 0.993
For data refer to Appendix 6.16d.

In each graph, the lowest dilution was arbitrarily valued at 1.
All equations produced by least squares linear regression.

For each dilution series, a doubling in OD approximated to a doubling in microbial numbers.

Figure 3.26: Nephelometer Calibration Curves for Halobacterium sp. Strain HB9 and Halobacterium salinarium Strain CM42/12.



(Figure 3.26c and Figure 3.26d). The assertion that doubling optical density corresponded to doubling microbial numbers, was found to be valid for both Halobacterium strains at both NaCl/water activity levels.

The effect of changing the NaCl concentration and hence the water activity of the XHB on the cellular dimensions of both Halobacterium strains was examined after growth to an OD of 0.3 in XHB + 3.5 molal NaCl (a_w = 0.865) and XHB + 6.0 molal NaCl (a_w = 0.748). At 6.0 molal NaCl, cells of Halobacterium sp. strain HB9 had a mean length of 3.46 +/- 0.59μ and a mean width of 0.64 +/- 0.08μ. At the same NaCl concentration, the cellular dimensions of Halobacterium salinarium strain CM42/12 were similar, with a mean length of 3.29 +/- 0.57μ and mean width of 0.59 +/- 0.11μ. Similar results were observed for both bacteria after growth in XHB + 3.5 molal NaCl, with mean lengths of 2.74 +/- 0.38μ and 2.82 +/- 0.52μ, and mean widths of 0.72 +/- 0.10μ and 0.69 +/- 0.12μ, respectively (Appendix 6.17).

Comparison of cellular dimensions of the two Halobacterium strains at the two NaCl concentrations, showed that at 3.5 molal NaCl, the cells were generally slightly shorter and wider. This observation is consistent with the assertion that the cells are capable of adjusting their intracellular solute concentrations to counterbalance the changing osmotic strength of the surrounding medium. However, when the NaCl concentration of the surrounding medium decreases below approximately 3.0 molal NaCl, the cells are no longer capable of counterbalancing the influx of water and start to swell, ultimately resulting in lysis (Tindall & Trüper, 1986).

Hence, over the water activity ranges being studied, the cell size for both strains should remain relatively constant. This was

confirmed when the actual cell volumes were calculated from the mean cell lengths and widths (Appendix 6.2d). It was found that the cell volumes did not alter greatly with change in water activity/NaCl concentration. At 3.5 molal NaCl, the volumes of Halobacterium sp. strain HB9 and Halobacterium salinarium strain CM42/12 were $1.417\mu^3$ and $1.145\mu^3$ and at 6.0 molal NaCl, the volumes were $1.420\mu^3$ and $1.343\mu^3$, respectively.

The observed slight change in cell size did not alter the number of cfu mL^{-1} which corresponded to an OD of 0.3. At 3.5 molal NaCl, it corresponded to 7.94×10^7 cfu mL^{-1} for Halobacterium sp. strain HB9 and 7.48×10^7 cfu mL^{-1} for Halobacterium salinarium strain CM42/12. At 6.0 molal it corresponded to 7.11×10^7 and 7.79×10^7 cfu mL^{-1}, respectively. As the OD levels in the two media for both Halobacterium strains were similar, OD levels over the range of water activities in XHB, should correspond to similar microbial levels.

3.3b(ii): The Effect of Temperature and Sodium Chloride Concentration on the Growth Rate of Halobacterium sp. Strain HB9.

Ten TGI experiments were conducted to test the validity of the Square Root Model under conditions of varying NaCl/water activity. OD and GT data were calculated from the growth of Halobacterium sp. strain HB9 in XHB containing NaCl: 3.0 (Figure 3.27a and Figure 3.27b); 3.25 (Figure 3.27c and Figure 3.27d); 3.5 (Figure 3.28a and Figure 3.28b); 4.0 (Figure 3.28c and Figure 3.28d); 4.5 (Figure 3.29a and Figure 3.29b); 5.0 (Figure 3.29c and Figure 3.29d); 5.0 (Figure 3.30a and Figure 3.30b); and 5.5 molal (Figure 3.30c and Figure 3.30d) (a_w: 0.892; 0.878; 0.865; 0.846; 0.826; 0.806; 0.801; and 0.780). Only OD data were determined for growth in XHB + 6.0 molal NaCl (Figure 3.31) (a_w = 0.748) as the GT

Figure 3.27: Square Root Plots of the Effect of Temperature and Sodium Chloride Concentration on the Growth Rate of Halobacterium sp. Strain HB9.

(a): Square root of growth rate versus temperature at NaCl = 3.0 molal, a_w = 0.892 (OD data).
Equation to line: $\sqrt{r}$ = -0.224426 + 0.000785T
Correlation coefficient = 0.999; T_o = 285.9K.

(b): Square root of growth rate versus temperature at NaCl = 3.0 molal, a_w = 0.892 (GT data).
Equation to line: $\sqrt{r}$ = -0.704489 + 0.00245T
Correlation coefficient = 0.993; T_o = 287.6K.

For experimental and predicted values refer to Appendix 6.18a.

(c): Square root of growth rate versus temperature at NaCl = 3.25 molal, a_w = 0.878 (OD data).
Equation to line: $\sqrt{r}$ = -0.286040 + 0.00100T
Correlation coefficient = 0.999; T_o = 286.0K.

(d): Square root of growth rate versus temperature at NaCl = 3.25 molal, a_w = 0.878 (GT data).
Equation to line: $\sqrt{r}$ = -0.799730 + 0.00280T
Correlation coefficient = 0.990; T_o = 285.6K.

For experimental and predicted values refer to Appendix 6.18b.

All equations produced by least squares linear regression of experimental data.

Figure 3.27: Square Root Plots of the Effect of Temperature and Sodium Chloride Concentration on the Growth Rate of Halobacterium sp. Strain HB9.

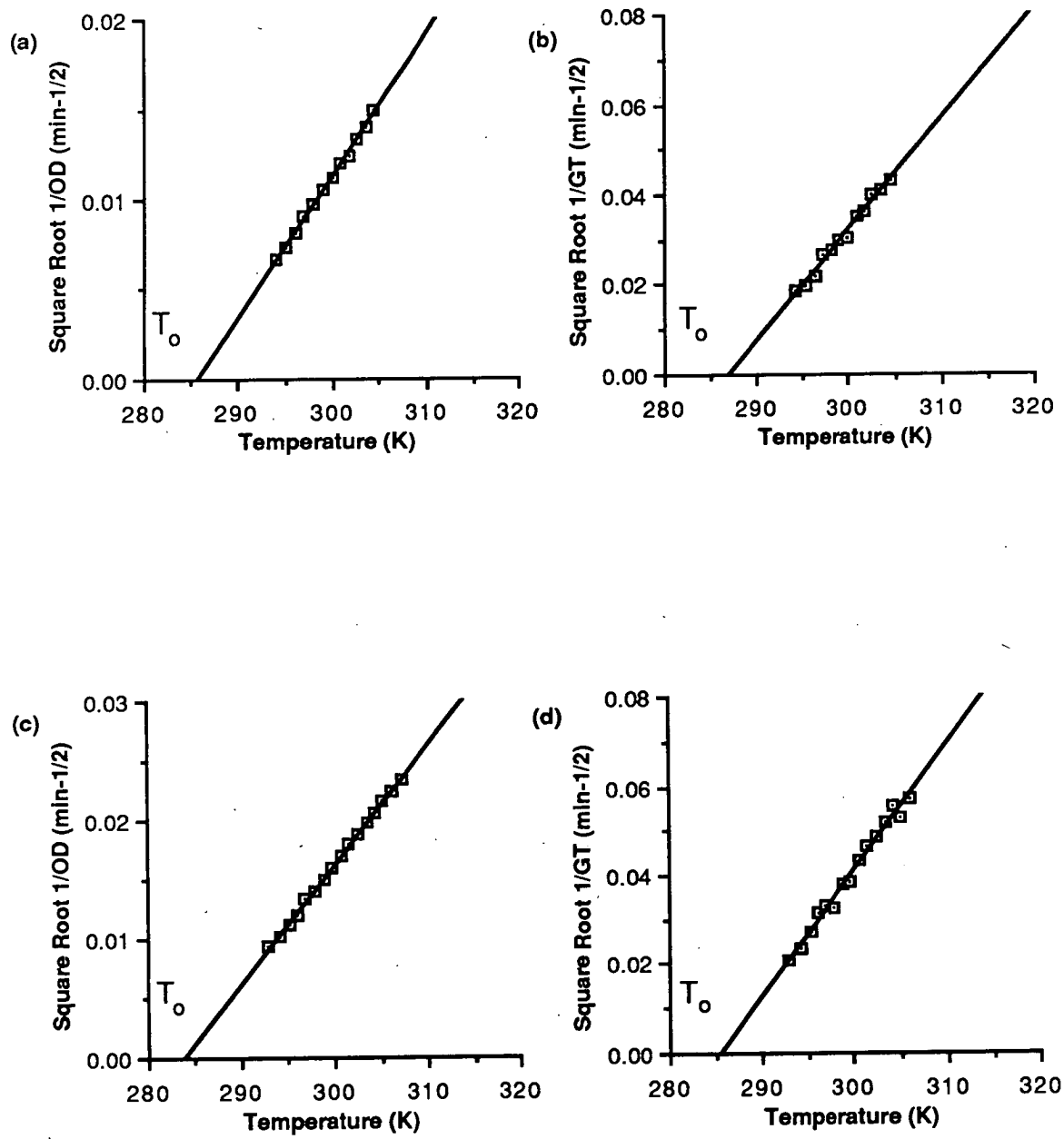


Figure 3.28: Square Root Plots of the Effect of Temperature and Sodium Chloride Concentration on the Growth Rate of Halobacterium sp. Strain HB9.

(a): Square root of growth rate versus temperature at NaCl = 3.5 molal, a_w = 0.865 (OD data).
Equation to line: $\sqrt{r}$ = -0.231884 + 0.000822T
Correlation coefficient = 0.999; T_o = 282.1K.

(b): Square root of growth rate versus temperature at NaCl = 3.5 molal, a_w = 0.865 (GT data).
Equation to line: $\sqrt{r}$ = -0.738138 + 0.00260T
Correlation coefficient = 0.994; T_o = 283.9K.

For experimental and predicted values refer to Appendix 6.18c.

(c): Square root of growth rate versus temperature at NaCl = 4.0 molal, a_w = 0.846 (OD data).
Equation to line: $\sqrt{r}$ = -0.218547 + 0.000774T
Correlation coefficient = 0.997; T_o = 282.4K.

(d): Square root of growth rate versus temperature at NaCl = 4.0 molal, a_w = 0.846 (GT data).
Equation to line: $\sqrt{r}$ = -0.678700 + 0.00241T
Correlation coefficient = 0.986; T_o = 281.6K.

For experimental and predicted values refer to Appendix 6.18d.

All equations produced by least squares linear regression of experimental data.

Figure 3.28: Square Root Plots of the Effect of Temperature and Sodium Chloride Concentration on the Growth Rate of Halobacterium sp. Strain HB9.

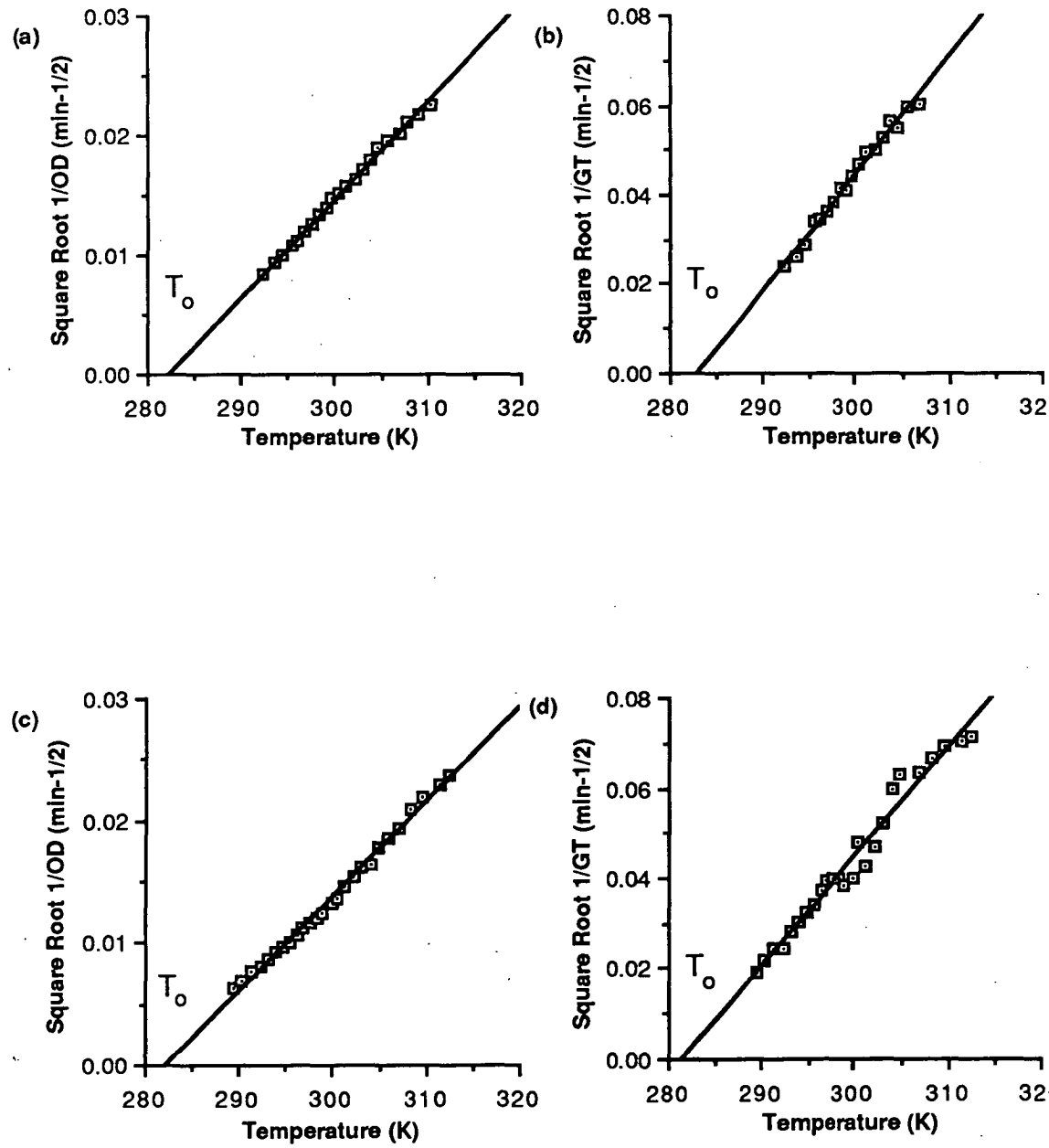


Figure 3.29: Square Root Plots of the Effect of Temperature and Sodium Chloride Concentration on the Growth Rate of Halobacterium sp. Strain HB9.

(a): Square root of growth rate versus temperature at NaCl = 4.5 molal, a_w = 0.826 (OD data).
Equation to line: $\sqrt{r}$ = -0.235037 + 0.000829T
Correlation coefficient = 0.995; T_o = 283.5K.

(b): Square root of growth rate versus temperature at NaCl = 4.5 molal, a_w = 0.826 (GT data).
Equation to line: $\sqrt{r}$ = -0.770156 + 0.00271T
Correlation coefficient = 0.986; T_o = 284.2K.

For experimental and predicted values refer to Appendix 6.18e.

(c): Square root of growth rate versus temperature at NaCl = 5.0 molal, a_w = 0.806 (OD data).
Equation to line: $\sqrt{r}$ = -0.259048 + 0.000919T
Correlation coefficient = 0.997; T_o = 281.9K.

(d): Square root of growth rate versus temperature at NaCl = 5.0 molal, a_w = 0.806 (GT data).
Equation to line: $\sqrt{r}$ = -0.752037 + 0.00266T
Correlation coefficient = 0.989; T_o = 282.7K.

For experimental and predicted values refer to Appendix 6.18f.

All equations produced by least squares linear regression of experimental data.

Figure 3.29: Square Root Plots of the Effect of Temperature and Sodium Chloride Concentration on the Growth Rate of *Halobacterium* sp. Strain HB9.

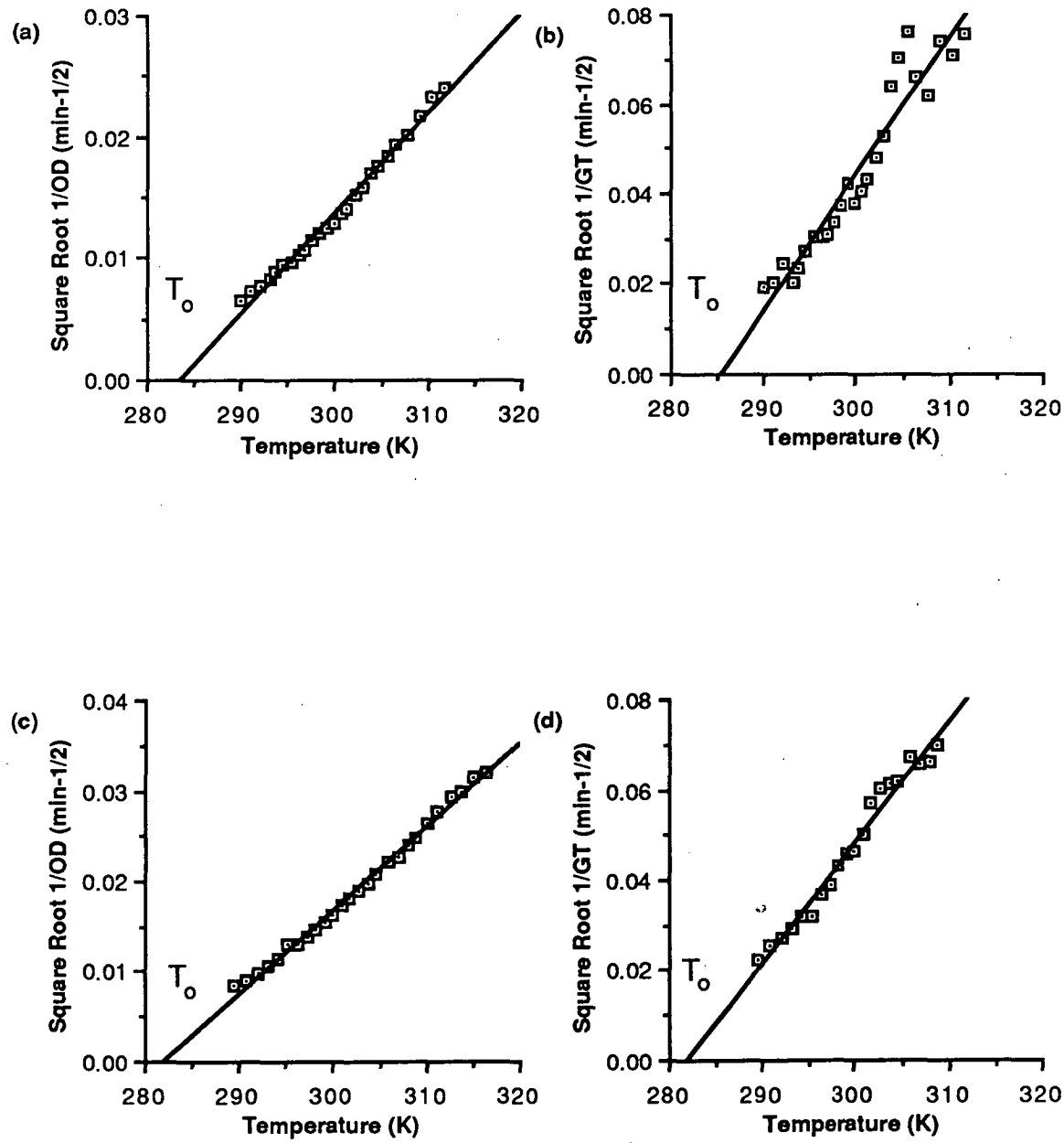


Figure 3.30: Square Root Plots of the Effect of Temperature and Sodium Chloride Concentration on the Growth Rate of Halobacterium sp. Strain HB9.

(a): Square root of growth rate versus temperature at NaCl = 5.0 molal, a_w = 0.801 (OD data).
Equation to line: $\sqrt{r}$ = -0.292600 + 0.00104T
Correlation coefficient = 0.997; T_o = 281.4K.

(b): Square root of growth rate versus temperature at NaCl = 5.0 molal, a_w = 0.801 (GT data).
Equation to line: $\sqrt{r}$ = -0.660758 + 0.00237T
Correlation coefficient = 0.990; T_o = 278.8K.

For experimental and predicted values refer to Appendix 6.18g.

(c): Square root of growth rate versus temperature at NaCl = 5.5 molal, a_w = 0.780 (OD data).
Equation to line: $\sqrt{r}$ = -0.258043 + 0.000920T
Correlation coefficient = 0.987; T_o = 280.5K.

(d): Square root of growth rate versus temperature at NaCl = 5.5 molal, a_w = 0.780 (GT data).
Equation to line: $\sqrt{r}$ = -0.686725 + 0.00243T
Correlation coefficient = 0.984; T_o = 282.6K.

For experimental and predicted values refer to Appendix 6.18h.

All equations produced by least squares linear regression of experimental data.

Figure 3.30: Square Root Plots of the Effect of Temperature and Sodium Chloride Concentration on the Growth Rate of Halobacterium sp. Strain HB9.

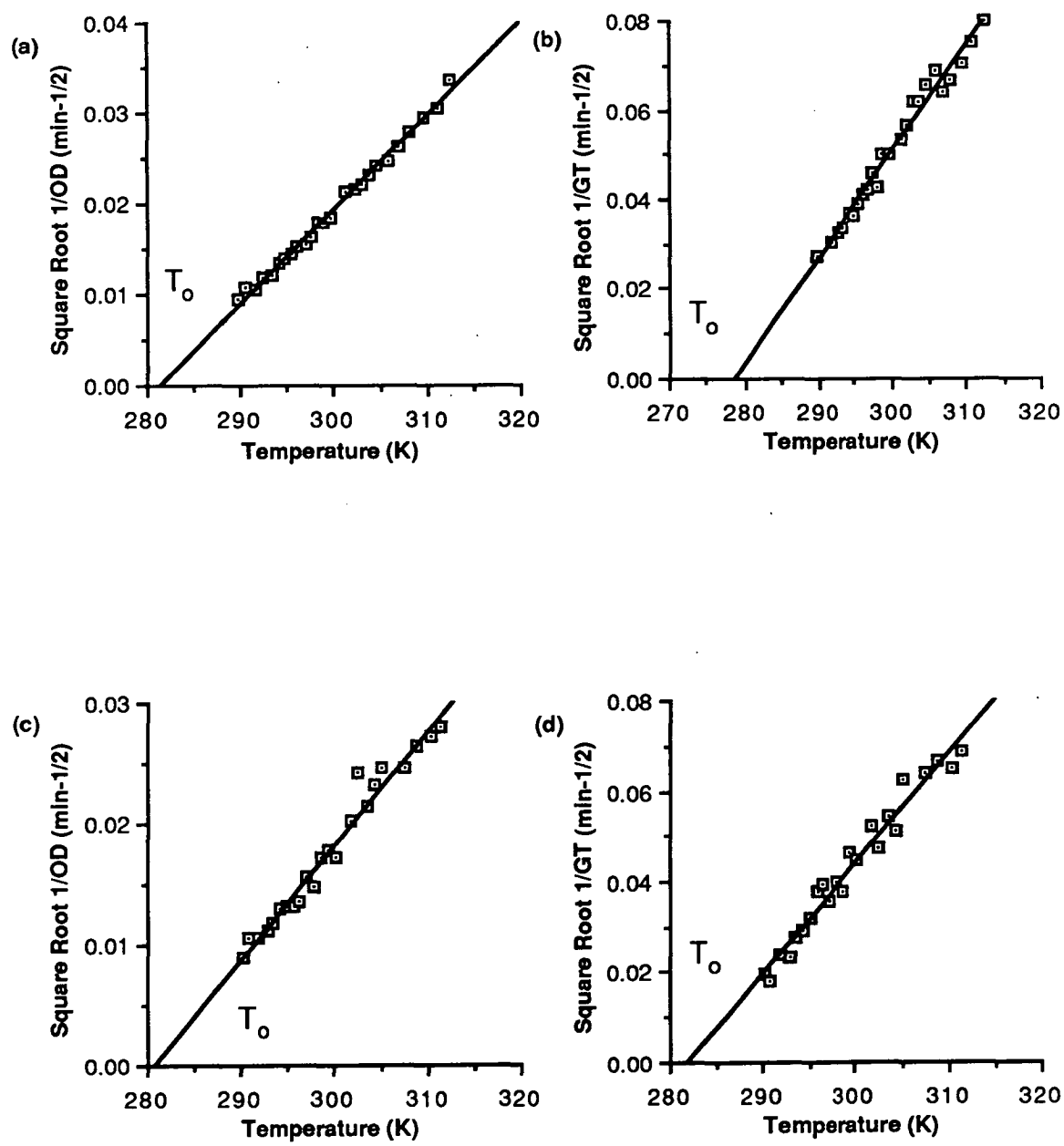


Figure 3.31: Square Root Plot of the Effect of Temperature and Sodium Chloride Concentration on the Growth Rate of Halobacterium sp. Strain HB9.

Square root of growth rate versus temperature at NaCl = 6.0 molal, a_w = 0.748 (OD data).

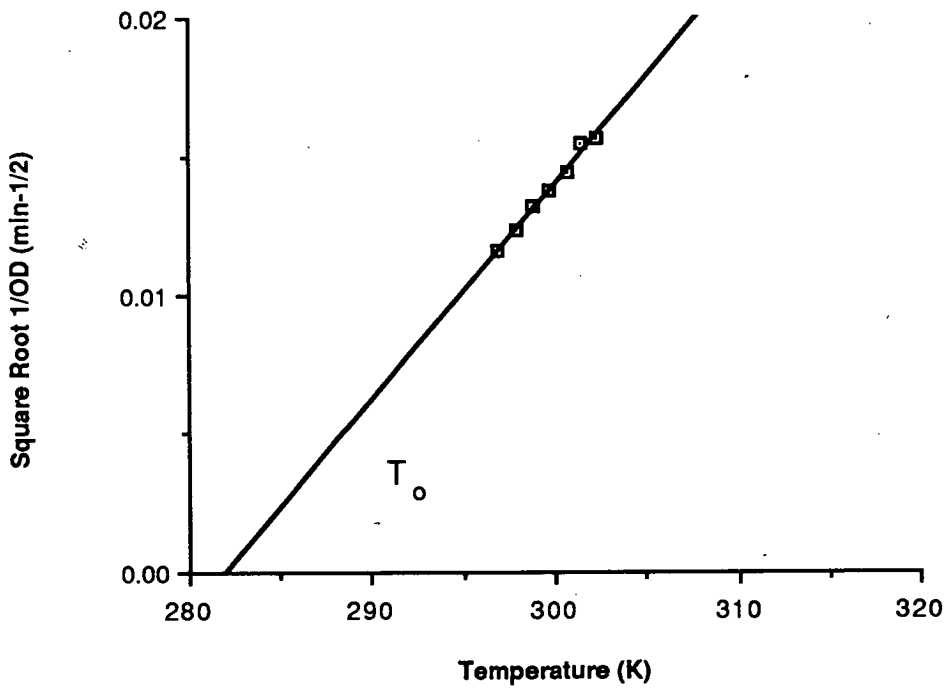
Equation to line: $\sqrt{r} = -0.216538 + 0.000768T$

Correlation coefficient = 0.993; T_o = 282.0.

For experimental and predicted values refer to Appendix 6.18i.

Equations produced by least squares linear regression of experimental data.

Figure 3.31: Square Root Plot of the Effect of Temperature and Sodium Chloride Concentration on the Growth Rate of Halobacterium sp. Strain HB9.



data gave inconsistent results.

Limited growth was observed over the temperature range 38-45°C, in XHB + 2.75 molal NaCl (a_w = 0.896), however insufficient data was obtained for analysis using the Square Root Model. The narrow growth range at this NaCl concentration implied the bacterium was close to its limit for growth. This was confirmed by direct microscopic observation of the cells of Halobacterium sp. strain HB9 grown in XHB containing 2.75 molal and 3.25 molal NaCl. The cells which were grown in 2.75 molal NaCl appeared either as very swollen, non-motile rods or granular like particles. The cells which were grown at 3.25 molal NaCl retained their motility and long rod morphology. Hence, at NaCl levels lower than 2.75 molal it would be expected that Halobacterium sp. strain HB9, would not grow due to lysis of the cells.

The T_o values calculated from OD and GT data at each water activity/NaCl concentration are summarised in Table 3.17. It can be seen that the T_o values were relatively constant and that there was no consistent trend or change in T_o value with change in water activity/NaCl concentration. The mean T_o values for OD and GT data were similar and were calculated to be 282.9 +/- 1.9K and 283.4 +/- 2.6K.

When both OD and GT data were forced to converge to a common T_o value, the T_o value for OD data was estimated to be 281.8 +/- 1.7K and for GT data, it was estimated to be 281.8 +/- 2.9K. These values were similar to the mean T_o values described in Table 3.17. These results further support the notion of Ratkowsky et al.,(1982) that the parameter T_o was an intrinsic property of the microorganism concerned and that it was not influenced by different temperature and water activity/NaCl regimes.

Table 3.17: T_o Values for Halobacterium sp. Strain HB9 at Different Sodium Chloride/Water Activity Levels, Derived from the Square Root Model Using OD and GT Data.

NaCl (molal)	Water Activity	T_o(K) OD data	GT data
3.0	0.892	285.9	287.6
3.25	0.872	286.0	285.6
3.5	0.865	282.1	283.9
4.0	0.846	282.4	281.6
4.5	0.826	283.5	284.2
5.0	0.806	281.9	282.7
5.0	0.801	281.4	278.8
5.5	0.780	280.5	282.6
6.0	0.748	282.0	

Mean T_o value for OD data = 282.9 +/- 1.9K.

Mean T_o value for GT data = 283.4 +/- 2.6K.

It was noted in Section 3.3a(ii) that associated with the decrease in water activity or increase in NaCl concentration of the growth medium, there was a general increase in the minimum observed temperature for growth of the moderate halophile, Staphylococcus xylosus strain CM21/3, of approximately 8°C (mean minimum temperature for growth of 282.7 +/- 2.8K). Even though precise minimum temperatures for growth were not determined, it appeared that this observation was not applicable for the growth of the extreme halophile, Halobacterium sp. strain HB9, at different water activities.

The minimum observed temperature for growth at each water activity/NaCl concentration is reported in Table 3.18, from which it can be seen that the minimum temperature for growth was relatively constant, (except at 3.0 molal) and had a mean value of 291.0 +/- 1.8K. At 3.0 molal, cells were stressed due to the lack of NaCl in the medium and when this value was excluded, the range of observed minimum growth temperatures was reduced to approximately 3.5°C and the standard error of the mean, decreased to 290.6 +/- 1.4K. Hence, it was only under the conditions of stress at 3.0 molal NaCl, that the water activity/NaCl level of the growth medium appeared to play a significant role in altering the minimum observed temperature for growth.

The assertion that changing the water activity/NaCl concentration of the growth medium at any specified temperature, had little effect on the growth rate of Halobacterium sp. strain HB9, was supported by the observation that there was little variation between the growth rates at different NaCl concentrations (Figure 3.32). Generation times at 301.2K (28°C) and values for the constant b

Table 3.18: Minimum Observed Temperatures for Growth of *Halobacterium* sp. Strain HB9 at Different Sodium Chloride/Water Activity Levels.

NaCl (molal)	Water Activity	Minimum T
3.0	0.892	294.1
3.25	0.872	292.9
3.5	0.865	292.3
4.0	0.846	289.4
4.5	0.826	290.1
5.0	0.806	289.5
5.0	0.801	289.7
5.5	0.780	290.1

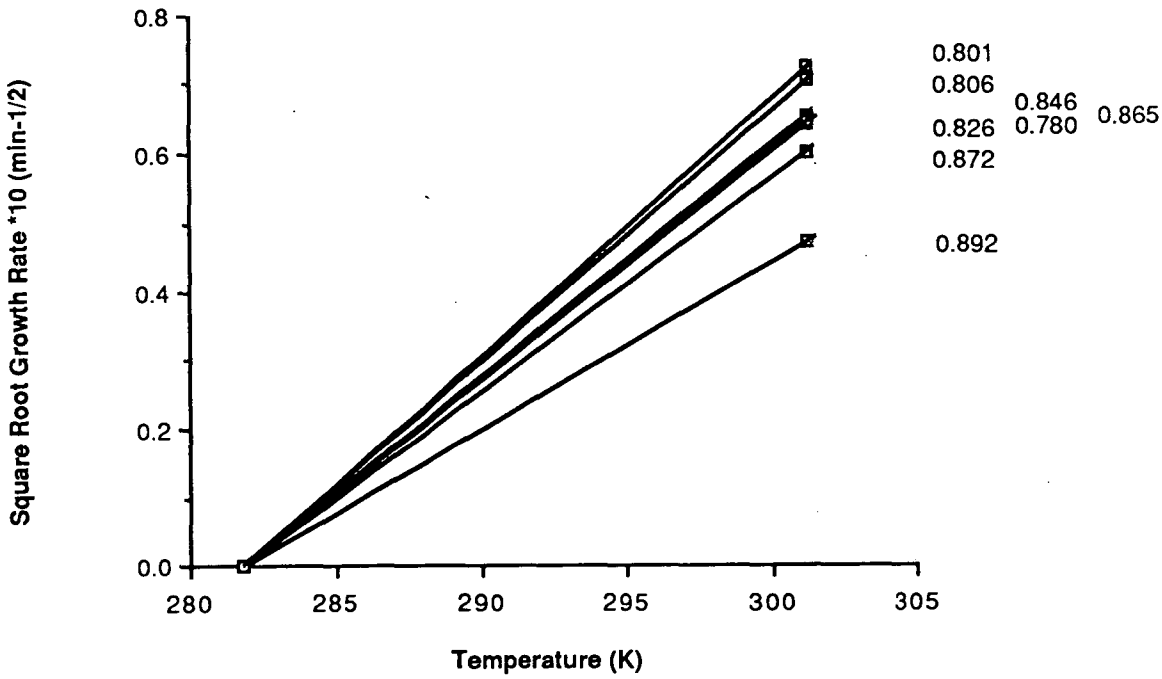
T: observed temperature for growth.

Figure 3.32: Square Root Plots of the Effect of Temperature and Sodium Chloride Concentration/Water Activity on the Growth Rate of Halobacterium sp. Strain HB9.

Square Root plots of the predicted lines for GT data for Halobacterium sp. strain HB9 at eight different sodium chloride/water activity levels. Each line was forced to converge statistically at the common T_o value of 281.8K.

Table 3.19 reports the predicted value of b for each line.

Figure 3.32: Square Root Plots of the Effect of Temperature and Sodium Chloride Concentration/Water Activity on the Growth Rate of Halobacterium sp. Strain HB9.



(slope of line predicted by the Square Root Model) are listed in Table 3.19.

Generation times varied from approximately 191 min at a_w = 0.801 to 451 min at a_w = 0.892, at 301.2K. The mean GT was calculated to be 259.5 +/- 81.8 min. When the GT at a_w 0.892 was excluded, the standard error of the mean decreased to 232.1 +/- 28.4 min. This was far less than the standard error of the mean for Staphylococcus xylosus strain CM21/3 of 96.6 +/- 89.1 min, which showed a definite correlation between change in water activity and growth rate.

3.3b(iii): The Effect of Temperature and Sodium Chloride Concentration on the Growth Rate of Halobacterium salinarium Strain CM42/12.

Seven TGI experiments were conducted to test the validity of the Square Root Model under conditions of varying NaCl/water activity. OD and GT and were calculated from the growth of Halobacterium salinarium sp. strain CM42/12 in XHB containing NaCl: 3.5 (Figure 3.33a and Figure 3.33b); 4.0 (Figure 3.33c and Figure 3.33d); 4.5 (Figure 3.34a and Figure 3.34b); 5.0 (Figure 3.34c and Figure 3.34d); and 5.5 (Figure 3.35a and Figure 3.35b) (a_w: 0.870; 0.846; 0.822; 0.790; and 0.776).

Only OD data was determined for growth in XHB + 6.0 molal NaCl (Figure 3.35c) (a_w = 0.748) as the GT data gave inconsistent results. This observation was the same as for Halobacterium sp. strain HB9 at the same NaCl level and implied that the observed variation in GT data was probably as a result of an external factor such as precipitation of salts in the growth medium and not an actual response of the bacterium.

Erratic and inconsistent growth was observed in XHB + 3.0 molal NaCl (a_w = 0.88), over the temperature range 20.8-37.6°C. This

Table 3.19: b Values Predicted by the Square Root Model for *Halobacterium* sp. Strain HB9 at Different Sodium Chloride/Water Activity Levels.

Water Activity	Predicted b Value	Generation Time at 301.2K
0.892	0.0017860	451.2
0.872	0.0022780	277.3
0.865	0.0024682	236.2
0.846	0.0024768	234.6
0.826	0.0024389	242.0
0.806	0.0026710	201.7
0.801	0.0027428	191.3
0.780	0.0024420	241.3

Predicted b values were derived from lines predicted by the Square Root Model for GT data forced to converge statistically at their common T_o value of 281.8K.

All times in minutes.

Figure 3.33: Square Root Plots of the Effect of Temperature and Sodium Chloride Concentration on the Growth Rate of Halobacterium salinarium Strain CM42/12.

(a): Square root of growth rate versus temperature at NaCl = 3.5 molal, a_w = 0.870 (OD data).
Equation to line: $\sqrt{r}$ = -0.191880 + 0.000687T
Correlation coefficient = 0.992; T_o = 279.3K.

(b): Square root of growth rate versus temperature at NaCl = 3.5 molal, a_w = 0.870 (GT data).
Equation to line: $\sqrt{r}$ = -0.607251 + 0.00215T
Correlation coefficient = 0.993; T_o = 282.4K.

For experimental and predicted values refer to Appendix 6.19a.

(c): Square root of growth rate versus temperature at NaCl = 4.0 molal, a_w = 0.846 (OD data).
Equation to line: $\sqrt{r}$ = -0.245387 + 0.000866T
Correlation coefficient = 0.998; T_o = 283.4K.

(d): Square root of growth rate versus temperature at NaCl = 4.0 molal, a_w = 0.846 (GT data).
Equation to line: $\sqrt{r}$ = -0.608006 + 0.00215T
Correlation coefficient = 0.969; T_o = 282.8K.

For experimental and predicted values refer to Appendix 6.19b.

All equations produced by least squares linear regression of experimental data.

Figure 3.33: Square Root Plots of the Effect of Temperature and Sodium Chloride Concentration on the Growth Rate of Halobacterium salinarium Strain CM42/12.

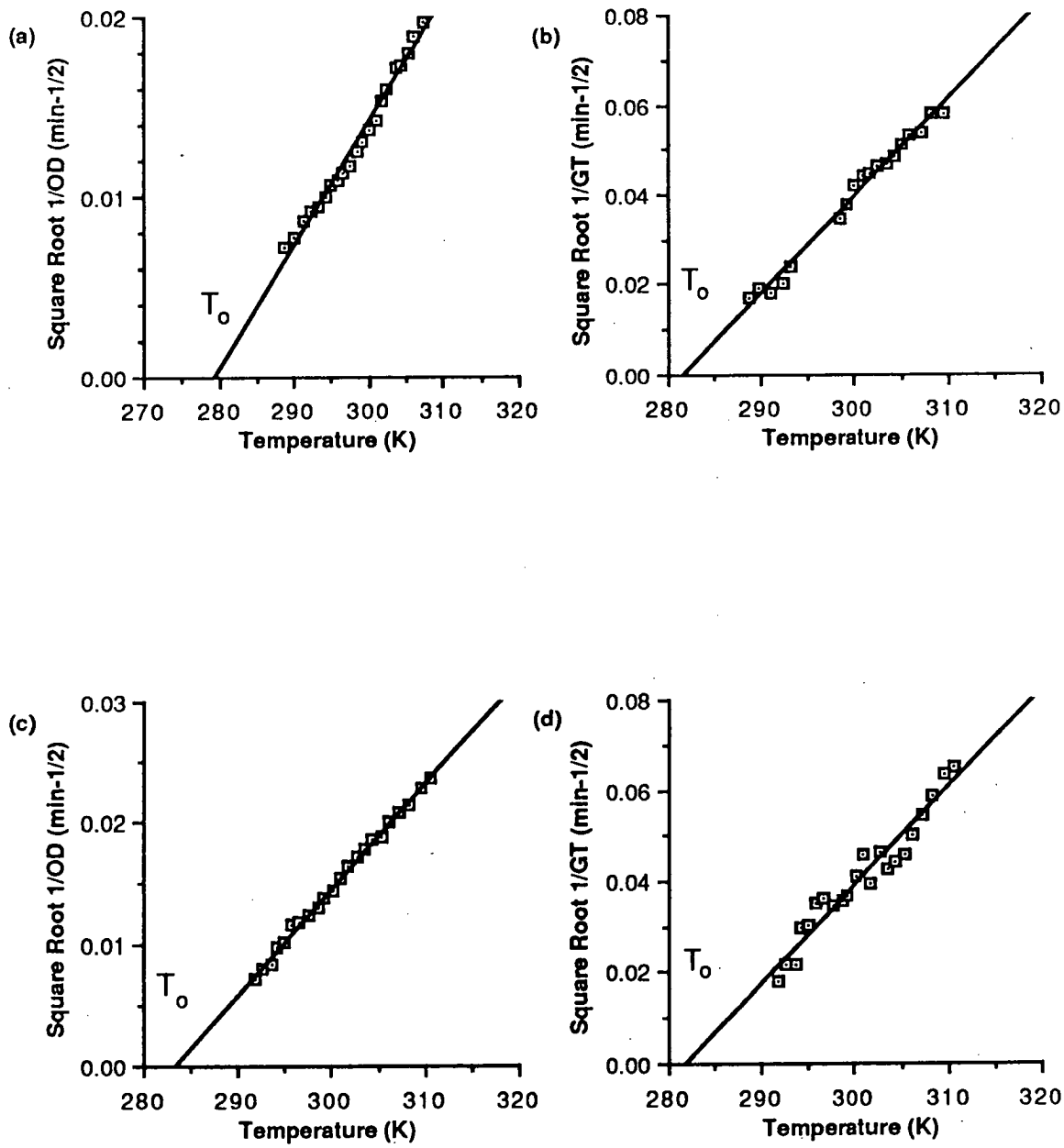


Figure 3.34: Square Root Plots of the Effect of Temperature and Sodium Chloride Concentration on the Growth Rate of Halobacterium salinarium Strain CM42/12.

(a): Square root of growth rate versus temperature at NaCl = 4.5 molal, a_w = 0.822 (OD data).
Equation to line: $\sqrt{r}$ = -0.230604 + 0.000818T
Correlation coefficient = 0.997; T_o = 281.9K.

(b): Square root of growth rate versus temperature at NaCl = 4.5 molal, a_w = 0.822 (GT data).
Equation to line: $\sqrt{r}$ = -0.642467 + 0.00226T
Correlation coefficient = 0.977; T_o = 284.3K.

For experimental and predicted values refer to Appendix 6.19c.

(c): Square root of growth rate versus temperature at NaCl = 5.0 molal, a_w = 0.790 (OD data).
Equation to line: $\sqrt{r}$ = -0.221726 + 0.000789T
Correlation coefficient = 0.998; T_o = 281.0K.

(d): Square root of growth rate versus temperature at NaCl = 5.0 molal, a_w = 0.790 (GT data).
Equation to line: $\sqrt{r}$ = -0.580052 + 0.00205T
Correlation coefficient = 0.959; T_o = 283.0K.

For experimental and predicted values refer to Appendix 6.19d.

All equations produced by least squares linear regression of experimental data.

Figure 3.34: Square Root Plots of the Effect of Temperature and Sodium Chloride Concentration on the Growth Rate of Halobacterium salinarium Strain CM42/12.

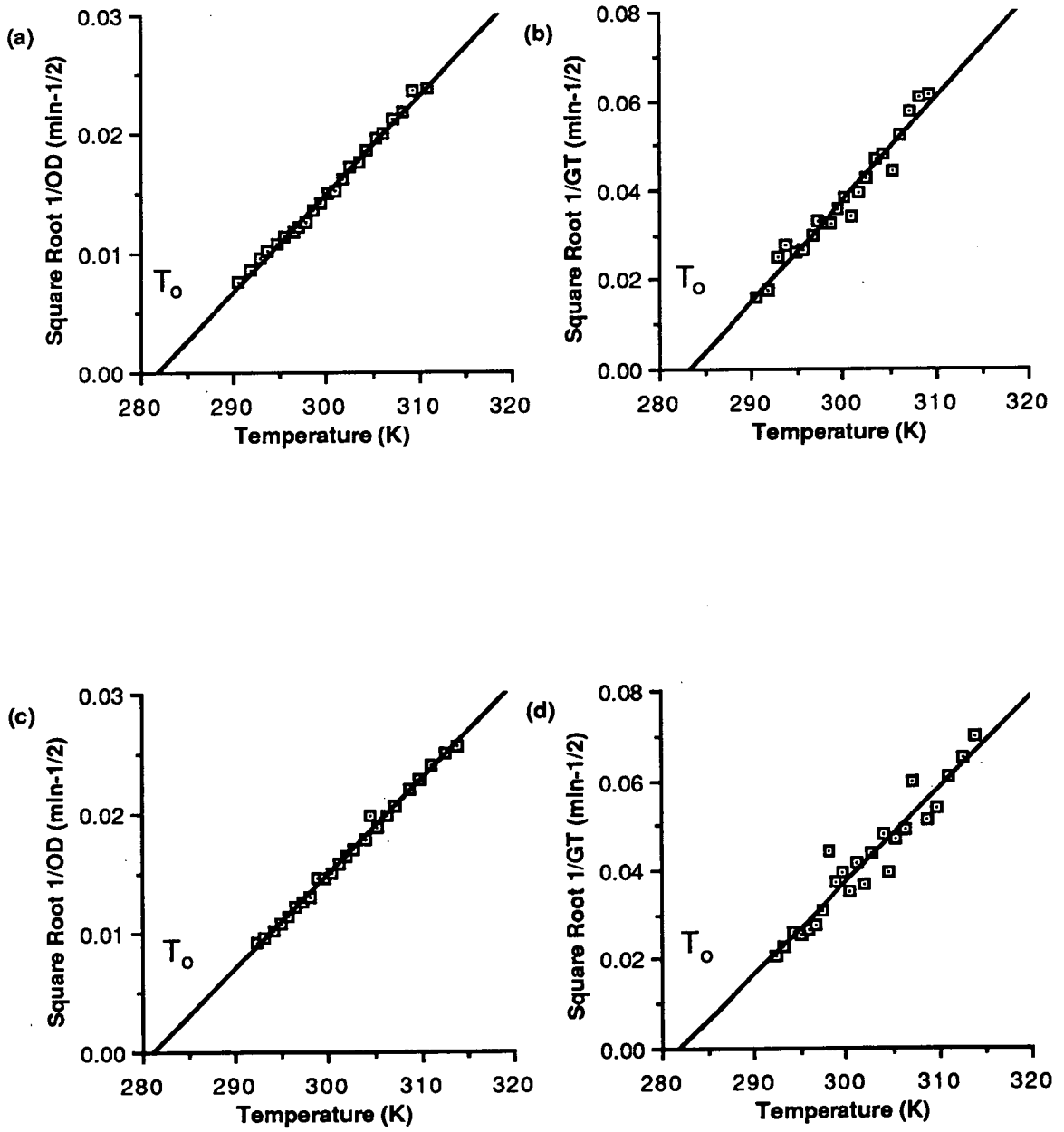


Figure 3.35: Square Root Plots of the Effect of Temperature and Sodium Chloride Concentration on the Growth Rate of Halobacterium salinarium Strain CM42/12.

(a): Square root of growth rate versus temperature at NaCl = 5.5 molal, a_w = 0.776 (OD data).
Equation to line: $\sqrt{r}$ = -0.225494 + 0.000798T
Correlation coefficient = 0.997; T_o = 282.6K.

(b): Square root of growth rate versus temperature at NaCl = 5.5 molal, a_w = 0.776 (GT data).
Equation to line: $\sqrt{r}$ = -0.567425 + 0.00201T
Correlation coefficient = 0.961; T_o = 282.3K.

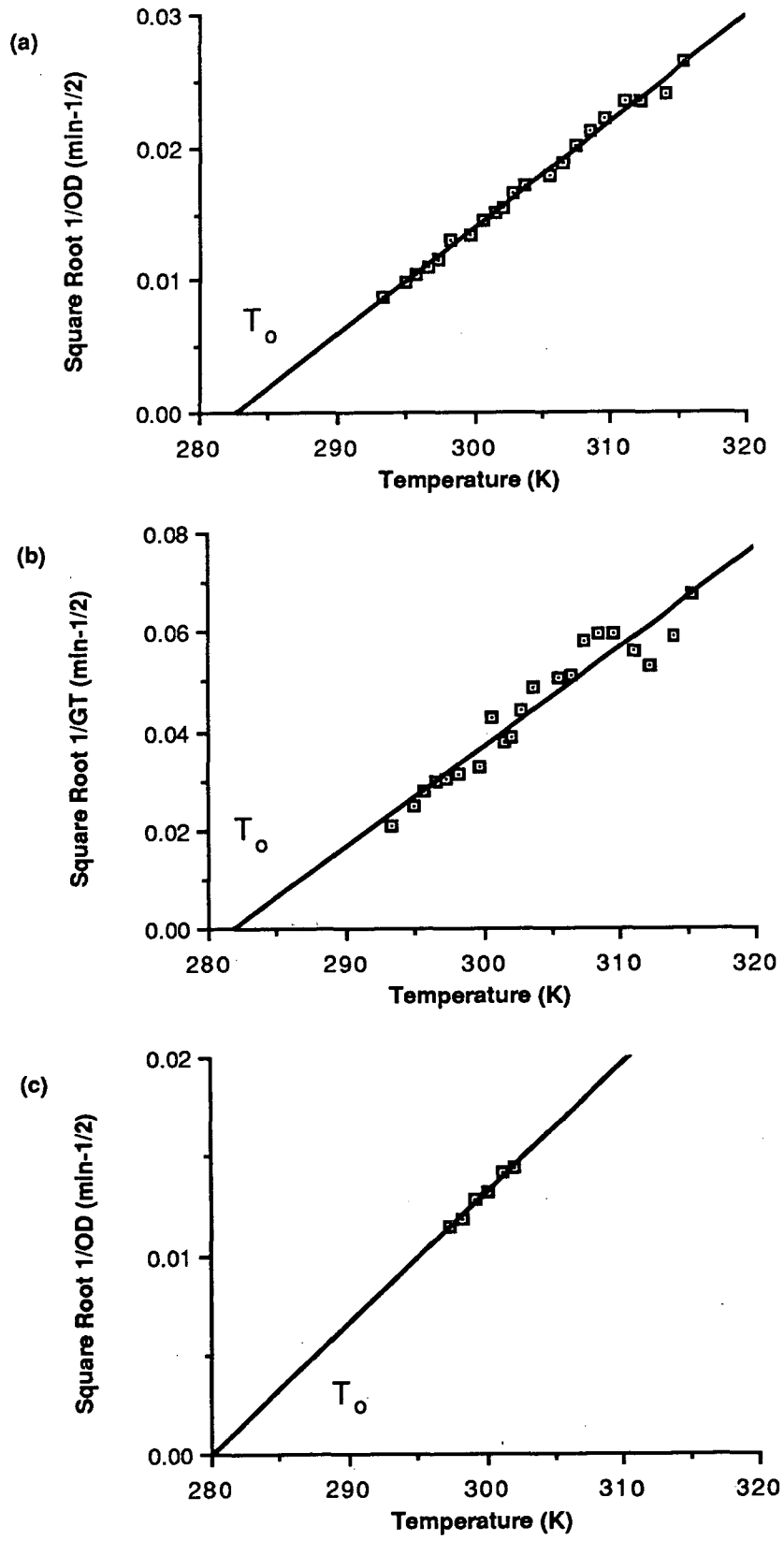
For experimental and predicted values refer to Appendix 6.19e.

(c): Square root of growth rate versus temperature at NaCl = 6.0 molal, a_w = 0.748 (OD data).
Equation to line: $\sqrt{r}$ = -0.184806 + 0.000659T
Correlation coefficient = 0.991; T_o = 280.4K.

For experimental and predicted values refer to Appendix 6.19f.

All equations produced by least squares linear regression of experimental data.

Figure 3.35: Square Root Plots of the Effect of Temperature and Sodium Chloride Concentration on the Growth Rate of Halobacterium salinarium Strain CM42/12.



implied that the bacterium was not an exponentially growing culture and cells were rapidly dying as well as growing. It would be expected that at NaCl levels slightly below 3.0 molal, that growth of Halobacterium salinarium strain CM42/12, would not occur.

The T_o values calculated from OD and GT data at each water activity/NaCl concentration are summarised in Table 3.20. It can be seen that the T_o values were relatively constant and that there was no consistent trend or change in T_o value with decreasing water activity. The mean T_o values for OD and GT data were similar and were calculated to be 281.4 +/- 1.5K and 283.0 +/- 0.8K.

When both OD and GT data were forced to converge to a common T_o value, the T_o value for OD data was estimated to be 282.8 +/- 1.9K and for GT data, it was estimated to be 282.0 +/- 0.5K. These values were similar to the mean T_o values reported in Table 3.20. These results further support the notion of Ratkowsky et al.,(1982) that the parameter T_o was an intrinsic property of the microorganism concerned and that it was not influenced by different temperature and water activity/NaCl regimes.

As was observed in Section 3.3b(ii), a decrease in the water activity or increase in NaCl concentration of the growth medium, gave rise to a more narrow range for the observed minimum temperature for growth of the extreme halophile Halobacterium salinarium strain CM42/12 (approximately 5°C) (Table 3.21), than for the moderate halophile Staphylococcus xylosus strain CM21/3. This was supported by the mean observed minimum temperature for growth, which was calculated to be 291.3 +/- 1.8K and allowed similar conclusions to be made to those obtained for Halobacterium sp. strain HB9.

Table 3.20: T_o Values for Halobacterium salinarium Strain CM42/12 at Different Sodium Chloride/Water Activity Levels, Derived from the Square Root Model Using OD and GT Data.

NaCl (molal)	Water Activity	T_o(K) OD data	GT data
3.5	0.870	279.3	282.4
4.0	0.846	283.4	282.8
4.5	0.822	281.9	284.3
5.0	0.790	281.0	283.0
5.5	0.776	282.6	282.3
6.0	0.748	280.4	

Mean T_o value for OD data = 281.4 +/- 1.5K.

Mean T_o value for GT data = 283.0 +/- 0.8K.

Table 3.21: Minimum Observed Temperatures for Growth of *Halobacterium salinarium* Strain CM42/12 at Different Sodium Chloride/Water Activity Levels.

NaCl (molal)	Water Activity	Minimum T
3.5	0.870	288.7
4.0	0.846	291.7
4.5	0.822	290.5
5.0	0.790	292.2
5.5	0.776	293.4

T: observed temperature for growth.

The assertion that changing the water activity/NaCl concentration of the growth medium had little effect on the growth rate of Halobacterium salinarium strain CM42/12 was supported by the observation that there was little variation between the growth rates at different NaCl concentrations (Figure 3.36). Generation times at 301.2K (28°C) and values for the constant b (slope of line predicted by the Square Root Model), are listed in Table 3.22.

Generation times varied from approximately 300 min at a_w = 0.870 to 352 min at a_w = 0.776, at 301.2K. The mean GT was calculated to be 323.6 +/- 21.9 min. The standard error of the mean for Halobacterium salinarium strain CM42/12 was far less than the standard error from the mean for Staphylococcus xylosus strain CM21/3 of 96.6 +/- 89.1 min.

The mechanism by which temperature and water activity interact to determine the minimum temperature for growth of a microorganism is a matter of speculation. Theron et al.,(1987) have reported that the adenosine triphosphate (ATP) levels of mesophilic bacteria were considerably lower at temperatures close to or less than their observed minimum temperature for growth (4°C), than at 30°C. A psychrotrophic bacterium studied under the same conditions had a similar ATP content at both temperatures. It is therefore possible to postulate that the ATP level in the cell is the major factor determining the minimum observed temperature for growth.

It is possible to speculate that a similar process is involved with the effect of water activity on microbial growth rate at a specified temperature. An analogy can be made between the mesophiles and the moderate halophile, Staphylococcus xylosus strain CM21/3 and between the psychrotroph and the extreme halophiles, Halobacterium sp. strain HB9 and Halobacterium salinarium strain CM42/12. This

Figure 3.36: Square Root Plots of the Effect of Temperature and Sodium Chloride Concentration/Water Activity on the Growth Rate of Halobacterium salinarium Strain CM42/12.

Square Root plots of the predicted lines for GT data for Halobacterium salinarium strain CM42/12 at five different sodium chloride/water activity levels. Each line was forced to converge statistically at the common T_o value of 282.0K.

Table 3.22 reports the predicted value of b for each line.

Figure 3.36: Square Root Plots of the Effect of Temperature and Sodium Chloride Concentration/Water Activity on the Growth Rate of Halobacterium salinarium Strain CM42/12.

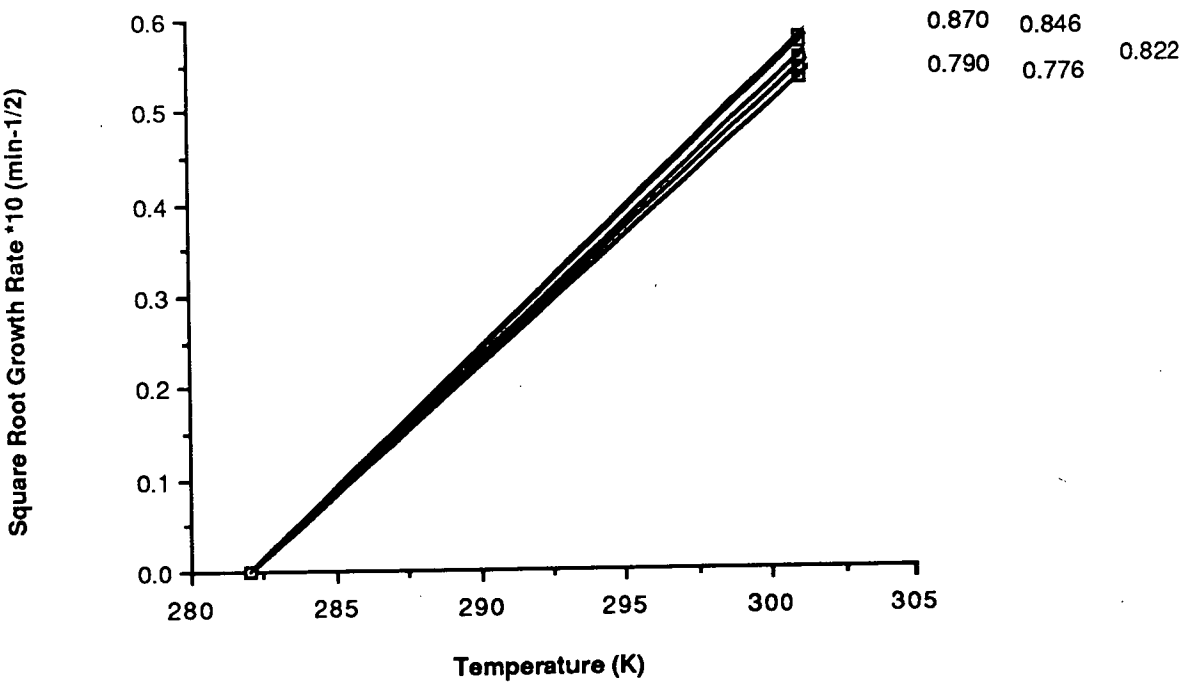


Table 3.22: b Values Predicted by the Square Root Model for *Halobacterium salinarium* Strain CM42/12 at Different Sodium Chloride/Water Activity Levels.

Water Activity	Predicted b Value	Generation Time at 301.2K
0.870	0.0022065	300.1
0.846	0.0021895	304.8
0.822	0.0021250	323.6
0.790	0.0020821	337.1
0.776	0.0020363	352.4

Predicted b values were derived from lines predicted by the Square Root Model for GT data forced to converge statistically at their common T_o value of 282.0K.

All times in minutes.

would imply that the mode of action of the effect of NaCl concentration, involves disruption of the energy balance of the cell. This is not surprising, as the moderate halophile is adapted to cope with NaCl levels up to a maximum value, whereas, the extreme halophiles are reliant on the presence of NaCl, and unless it is present at high enough levels, growth is not possible. The molecular biology of the halophiles is adapted to the presence of NaCl and hence any increase in concentration over the amount required for growth, has little or no effect on growth rate.

3.3b(iv): Conclusion.

It has been demonstrated in Section 3.3b(ii) and Section 3.3b(iii) that the Square Root Model accurately described the growth rate response to temperature, of the two extreme halophiles Halobacterium sp. strain HB9 and Halobacterium salinarium strain CM42/12. In this aspect they are similar to the salt sensitive and salt tolerant groups of microorganisms (for example, Pseudomonas sp. strain E5.2 and Staphylococcus xylosus strain CM21/3). However, they are very dissimilar to the latter bacterium in their response to changing water activity regimes. Under conditions which caused Staphylococcus xylosus strain CM21/3 to show a marked decrease in growth rate, with decrease in water activity, both the Halobacterium strains showed little change. Growth rate was markedly affected only when the NaCl concentration of the growth medium was decreased to near the level at which cell death and lysis occurred.

4: CONCLUSIONS AND PROJECTIONS.

Discussion of individual experimental sections has been presented with the results and where possible related to the existing body of knowledge. Rather than providing a repetitious general discussion, an attempt is made in this Section to present succinct conclusions, arising from the work described, together with suggestions for further work.

Conclusions:

(1): Microbial end product determination (measurement of $[CO_2]$) did not provide a useful means to predict the shelf-life of pasteurised, homogenised milk.

(2): The effect of temperature on the rate of spoilage of pasteurised, homogenised milk was described by the Square Root Model of Ratkowsky et al.,(1982).

(3): At temperatures <15°C, spoilage was a predominantly psychrotrophic process (T_o approximately 265K) but at temperatures >15°C, it was a mesophilic process (T_o approximately 271K). Pseudomonads dominated at <15°C and a mixed flora was present at >15°C.

(4): A good correlation was established between TTFI readings and pseudomonad numbers in chill stored, pasteurised, homogenised milk. TTFI may be used to monitor spoilage and to predict remaining shelf-life. An accelerated shelf-life test at 12°C was proposed, with results being related to the spoilage rate at the recommended storage temperature.

(5): Temperature/growth profiles of a psychrotroph (Pseudomonas sp. strain 12.48) and a mesophile (Enterobacter agglomerans strain 11.33) isolated from spoiled pasteurised, homogenised milk were

consistent with their roles in spoilage. Due to a lower T_o value and a higher b value, it was shown that the pseudomonad should outgrow the enterobacter over the temperature range 4-16°C.

(6): The effect of temperature on the lag phase of Pseudomonas sp. strain E5.2 was described by the Square Root Model, with a T_o value similar to that observed for the exponentially growing cells. Incorporation of lag and logarithmic phase data, did not affect the fit of the Square Root Model or the predicted T_o value.

(7): The Square Root Model was validated under conditions where both temperature and water activity limited microbial growth, using halotolerant (Staphylococcus xylosus strain CM21/3) and halophilic (Halobacterium sp. strain HB9 and Halobacterium salinarium strain CM42/12) bacteria.

(8): For Staphylococcus xylosus strain CM21/3, the Square Root Model was validated when either NaCl or glycerol was used as the humectant. The T_o value obtained was the same for both humectants.

(9): The growth rate of Staphylococcus xylosus strain CM21/3 decreased linearly with decreasing water activity. This allowed derivation of an extended Square Root Model incorporating a water activity term of the form: $\sqrt{r} = b_o\sqrt{(a_w - a_{wo})}(T - T_o)$.

(10): The Square Root Model was shown to be a specific example of the Bělehrádek equation, with exponent = 2.

(11): The Square Root Model and the Arrhenius equation were shown to be similar. The advantage of the Square Root Model is that the constants (T_o and b) are constant, whereas the "constants" in the Arrhenius equation (E and A) vary with temperature.

(12): The minimum temperature for growth of the halotolerant

Staphylococcus xylosus strain CM21/3, increased with decreased water activity, whereas, the minimum temperatures for growth of the halophiles, Halobacterium sp. strain HB9 and Halobacterium salinarium strain CM42/12, were affected to a lesser degree by decreased water activity.

Projections:

The work described in this thesis has confirmed the contentions of Ratkowsky et al.,(1982), that the Square Root Model may be used to describe the effect of temperature on microbial growth rate and of McMeekin and Olley (1986) that the Square Root Model may be used as a basis to predict the shelf-life of food in storage.

This thesis has advanced the knowledge in specifically applying the Square Root Model to the spoilage of pasteurised, homogenised milk, in demonstrating the temperature dependence of the lag phase of microbial growth and in validating and extending the Square Root Model to incorporate a water activity term.

Nevertheless, there are several areas of investigation that remain to be pursued from this point.

(1): Will the Square Root Model be valid when other factors, such as pH, are also limiting? The combined effects of temperature, water activity and pH have been considered important when considering the rate of change of microbial numbers in foods (Roberts & Jarvis, 1983).

(2): Much of the work described in this thesis is based upon pure culture systems. Further experiments involving the use of binary or mixed systems, to examine the possible effect of microbial interactions, are required. Such experiments might involve a study of the interactions between psychrotrophic and

mesophilic bacteria in chill stored products subject to temperature abuse, halotolerant and halophilic bacteria in products with reduced water activity and lactic acid bacteria with spoilage microorganisms in modified atmosphere stored products.

(3): The Square Root Model is still an empirical model and at this time, the physiological basis of the response of bacterial growth rate to temperature, remains unknown. Detailed study is required in the fields of microbial physiology and genetics, and particularly in biosynthetic processes, which are likely to be the key to the control of growth rate. The degree of uncoupling of catabolic and anabolic processes as temperatures diverge from the optimum also warrants consideration (McMeekin et al., 1987).

(4): It would also be of interest to consider further the influence of two or more restrictive factors on microbial growth rate and the cessation of microbial growth due to different combinations of these factors. The response described in this thesis suggests that temperature and water activity act independently, to the point at which growth ceases. This may be achieved by various combinations of the two factors. Theron et al.,(1987) reported that the minimum observed temperature for growth was associated with decreased ATP levels. If reduced water activity results in a similar reduction to a critical level of ATP or of the adenylate energy charge ratio, this may provide a reason for the cessation of growth at various combinations of temperature and water activity.

5. BIBLIOGRAPHY.

Acott, K.M. & Labuza, T.P., (1975). Microbial growth response to water sorption preparation. J. Food Technol. 10: 603-611.

Alexander, H. & Higginbottom, C. (1953). Bacteriological studies on pasteurized milk. J. Dairy Res. 20: 156-176.

Allen, M.B., (1953). The thermophilic aerobic sporeforming bacteria. Bacteriol. Rev. 17: 125-173.

Allwood, M.C. & Russell, A.D., (1970). Mechanisms of thermal injury in nonsporulating bacteria. Adv. Appl. Microbiol. 12: 89-119.

Amelunxen, R. & Lins, M., (1968). Comparative thermostability of enzymes from Bacillus stearothermophilus and Bacillus cereus. Arch. Biochem. Biophys. 125: 765-769.

Anand, J.C. & Brown, A.D., (1968). Growth rate patterns of the so-called osmophilic and non-osmophilic yeasts in solutions of polyethylene glycol. J. Gen. Microbiol. 52: 205-212.

Anonymous, (1973). Methods of Microbiological Examination of Dairy Products and for Dairy Purposes. Standards Association of Australia, 1095, Part 2, Section 1.

Anonymous, (1982). Manual of Laboratory Techniques in Microbiology. Faculty of Agricultural Science, University of Tasmania. Pp.: 36-38.

Baig, I.A. & Hopton, J.W., (1969). Psychrophilic properties and the temperature characteristics of growth of bacteria. J. Bacteriol. 100: 552-553.

Baird-Parker, A.C., (1976). Methods for classifying staphylococci and micrococci. In: Identification Methods for Microbiologists. Gibbs, B.M. & Skinner, F.A., (Eds.). Academic Press, London. Pp.: 59-64.

Baker, J.H., (1974). The use of a temperature-gradient incubator to investigate the temperature characteristics of some bacteria from Antarctic peat. Br. Antarct. Surv. Bull. 39: 49-59.

Barnard, S.E. (1972). Importance of shelf life for consumers of milk. J. Dairy Sci. 55: 134-136.

Barnes, E.M. & Impey, C.S., (1968). Psychrophilic spoilage bacteria of poultry. J. Appl. Bacteriol. 31: 97-107.

Barnes, E.M. & Thornley, M.J., (1966). The spoilage flora of eviscerated chickens stored at different temperatures. J. Food Technol. 1: 113-119.

Bělehrádek, J., (1926). Influence of temperature on biological processes. Nature. 118: 117-118.

Bělehrádek, J., (1935). Temperature and Living Matter. Protoplasma Monogr. No.8, Borntraeger, Berlin. 277 pp.

Bishop, J.R. & White, C.H. (1985). Estimation of potential shelf-life of pasteurized fluid milk utilizing bacterial numbers and metabolites. J. Food Protect. 48: 663-667.

Bishop, J.R. & White, C.H., (1986). Assessment of dairy product quality and potential shelf-life - a review. J. Food Prot. 49: 739-753.

Bishop, J.R., White, C.H. & Firstenberg-Eden, R., (1984). Rapid impedimetric method for determining the potential shelf-life of pasteurized whole milk. J. Food Prot. 47: 471-475.

Boyd, J.C., Smith, C.K. & Trout, G.M. (1953). The role of psychrophilic bacteria in the keeping quality of commercially pasteurized and homogenized milk. J. Dairy Sci. 36: 571.

Branch, A.C. & Vail, A.M.A., (1985). Bringing fish inspection into the computer age. Food Technol. Aust. 37: 352-355.

Brooks, J.D., (1986). Impedance microbiology. Food Technol. Aus. 38: 338-340.

Broughall, J.M., Anslow, P.A. & Kilsby, D.C., (1983). Hazard analysis applied to microbiological growth in foods: development of mathematical models describing the effect of water activity. J. Appl. Bacteriol. 55: 101-110.

Broughall, J.M. & Brown, C., (1984). Hazard analysis applied to microbiological growth in foods: development and application of three-dimensional models to predict bacterial growth. Food Microbiol. 1: 13-22.

Brown, A.D., (1974). Microbial water relations: features of the intracellular composition of sugar-tolerant yeasts. J. Bacteriol. 118: 769-777.

Brownlie, L.E., (1966). Effect of some environmental factors on psychrophilic microbacteria. J. Appl. Bacteriol. 29: 447-454.

Bubela, B. & Holdsworth, E.S., (1966a). Amino acid uptake, protein and nucleic acid synthesis and turnover in Bacillus stearothermophilus. Biochim. Biophys. Acta. 123: 364-375.

Bubela, B. & Holdsworth, E.S., (1966b). Protein synthesis in Bacillus stearothermophilus. Biochim. Biophys. Acta. 123: 376-389.

Campbell, L.L.Jr. & Williams, O.B., (1953). The effect of temperature on the nutritional requirements of facultative and obligate thermophilic bacteria. J. Bacteriol. 65: 141-145.

Campbell-Platt, G., (1985). Microbiological spoilage of chilled foods. Proceedings IFST (UK). 18: 2-6.

Casolari, A., Spotti, E. & Castelvetri, F., (1978). Water activity and microbial growth rate. In: Food Microbiology and Technology. Jarvis, B., Christian, J.H.B., & Michener, A.D., (Eds.). Medicina Viva, Parma Italy. Pp: 19-34.

Christian, J.H.B. & Scott, W.J., (1953). Water relations of salmonellae at 30°C. Aust. J. Biol. Sci. 6: 565-573.

Coghill, D., (1982). Studies on thermoduric psychrotrophic bacteria in south east Queensland dairy products. Aust. J. Dairy Technol. 37: 147-148.

Cooley, J.M. & Minns, C.K., (1978). Prediction of egg development times of freshwater copepods. J. Fish Res. Board Can. 35: 1322-1329.

Cousin, M.A., (1982). Presence and activity of psychrotrophic microorganisms in milk and dairy products: a review. J. Food Prot. 45: 172-207.

Credit, C., Hedeman, R., Heywood, P. & Westhoff, D., (1972). Identification of bacteria isolated from pasteurized milk following refrigerated storage. J. Milk Food Technol. 35: 708-709.

Daud, H.B., (1978). Poultry spoilage, growth and metabolism of the associated bacteria and the effect of storage temperature on model systems. B.Sc. Honours Thesis, University of Tasmania.

Daud, H.B., McMeekin, T.A. & Olley, J., (1978). Temperature function integration and the development and metabolism of poultry spoilage bacteria. Appl. Environ. Microbiol. 36: 650-654.

Eddy, B.P., (1960). The use and the meaning of the word psychrophilic. J. Appl. Bacteriol. 23: 189-190.

Edgley, M. & Brown, A.D., (1978). Response of xerotolerant and non-tolerant yeasts to water stress. J. Gen. Microbiol. 104: 343-345.

Edwards, R.A., Dainty, R.H. & Hibbard, C.M., (1985). The relationship of bacterial numbers and types to diamine concentration in fresh and aerobically stored beef, pork, and lamb. J. Appl. Bacteriol. 58: 13-20.

Epstein, I. & Grossowicz, N., (1969). Prototrophic thermophilic bacillus: isolation, properties, and kinetics of growth. J. Bacteriol. 99: 414-417.

Farber, J.M., (1986). Predictive modeling of food deterioration and safety. In: Foodborne Microorganisms and Their Toxins: Developing Methodology. Peirson, M.D. & Stern, N.J., (Eds.). Marcel Dekker Inc., New York. Pp: 57-90.

Farrel, J. & Rose, A.H., (1967). Temperature effects on microorganisms. Ann. Rev. Microbiol. 21: 101-120.

Farrel, J. & Rose, A.H., (1968). Cold shock in a mesophile and a psychrophilic pseudomonad. J. Gen. Microbiol. 50: 429-439.

Firstenberg-Eden, R. & Tricarico, M.K., (1983). Impedimetric determination of total, mesophilic and psychrotrophic counts in raw milk. J. Food Sci. 48: 1750-1754.

Frazier, W.C. & Westhoff, D.C. (1978). Preservation by use of low temperatures. In: Food Microbiology. 3rd Ed. McGraw-Hill, New York. Pp: 130-142.

Gailani, M.B. & Fung, D.Y.C., (1986). Critical review of water activities and microbiology of drying of meats. CRC Critical Reviews in Food Science and Nutrition. 25: 159-183.

Gaughran, E.R.L., (1947). The saturation of bacterial lipids as a function of temperature. J. Bacteriol. 53: 506.

Gibson, A.M.; Bratchell, N. & Roberts, T.A., (1987). The effect of sodium chloride and temperature on the rate and extent of growth of Clostridium botulinum type A in pasteurized pork slurry. J. Appl. Bacteriol. 62: 479-490.

Gill, C.O., (1984). Prevention of early spoilage of livers. Proceedings 30th. European Meeting of Meat Research Workers. Bristol. Pp: 240-241.

Gill, C.O. & Harrison, J.C.L., (1985). Evaluation of the hygienic efficiency of offal cooling procedures. Food Microbiol. 2: 63-69.

Gill, C.O. & Newton, K.G., (1977). The development of aerobic spoilage flora on meat stored at chill temperatures. J. Appl. Bacteriol. 43: 189-195.

Gill, C.O. & Newton, K.G., (1978). The ecology of bacterial spoilage of fresh meat at chill temperatures. Meat Science. 2: 207-217.

Greene, V.W. & Jezeski, J.J., (1954). Influence of temperature on the development of several psychrophilic bacteria of dairy origin. Appl. Microbiol. 2: 110-117.

Griffiths, M.W. & Phillips, J.D., (1984). Detection of post - pasteurization contamination of cream by impedimetric methods. J. Appl. Bacteriol. 57: 107-114.

Griffiths, M.W.; Phillips, J.D. & Muir, D.D., (1984). Methods for rapid detection of post-pasteurization contamination in cream. J. Soc. Dairy Technol. 37: 22-26.

Grosskopf, J.S. & Harper, W.J., (1969). Role of psychrophilic sporeformers in long life milk. J. Dairy Sci. 52: 897.

Hankin, L., Dillman, W.F. & Stephens, G.R. (1977). Keeping quality of pasteurized milk for retail sale related to code date, storage temperature, and microbial counts. J. Food Prot. 40: 848-853.

Hanus, F.J. & Morita, R.Y., (1968). Significance of the temperature characteristic of growth. J. Bacteriol. 95: 736-737.

Herbert, R.A. & Bhakoo, M., (1979). Microbial growth at low temperatures. In: Cold Tolerant Microbes in Spoilage and the Environment. Russell, A.D. & Fuller, R. (Eds.). Academic Press, London. Pp: 1-14.

Hugh, R. & Leifson, E., (1953). The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various Gram-negative bacteria. J. Bacteriol. 66: 24-26.

Ingraham, J.L., (1958). Growth of psychrophilic bacteria. J. Bacteriol. 76: 75-80.

Ingraham, J.L., Maaløe, O. & Neidhart, F.C., (1983). Growth of the Bacterial Cell. Sinauer Associates, Inc., Sunderland Massachusetts. P: 253.

Ingram, M. & Dainty, R.H., (1971). Changes caused by microbes in spoilage of meats. J. Appl. Bacteriol. 34: 21-39.

Ingram, M. & Mackey, B.M., (1976). Inactivation by cold. In: Inhibition and Inactivation of Vegetative Microbes. Skinner, F.A., & Hugo, W.B., (Eds.). Academic Press, London. Pp: 111-151.

Janzen, J.J., Bodine, A.B. & Bishop, J.R. (1981). Effects of package temperature and days of storage on the flavor score of processed milk. J. Food Prot. 44: 455-458.

Jay, J.M., (1966). Response of the extract-release volume and water-holding capacity phenomena to microbiologically spoiled beef and aged beef. Appl. Microbiol. 14: 492-496.

Jay, J.M., (1978). In: Modern Food Microbiology. 2nd. Ed. D. Van Nostrond Co., New York.

Johnson, F.H., Eyring, H. & Stover, B.J., (1974). The Theory of Rate Processes in Biology and Medicine. J. Wiley & Sons, New York.

Kennedy, W.J. & Gentle, J.E., (1980). Statistical Computing. Marcel Dekker Inc., New York.

King, E.O., Ward, M.K. & Raney, D.E., (1954). Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med. 44: 301-307.

Knabel, S.J., Walker, H.W. & Kraft, A.A., (1987). Enumeration of fluorescent pseudomonads on poultry by using the hydrophobic-grid membrane filter method. J. Food Sci. 52: 837-841, 845.

Koch, A.L., (1970). Overall controls on the biosynthesis of ribosomes in growing bacteria. J. Theor. Biol. 28: 203-231.

Kovacs, N., (1956). Identification of Pseudomonas pyocyanea by the oxidase reaction. Nature. 178: 703.

Labuza, T.P., (1982). Shelf Life Dating of Foods. Food & Nutrition Press, Westport.

Labuza, T.P., Cassil, S. & Sinskey, A.J., (1972). Stability of intermediate moisture foods. II. Microbiology. J. Food Sci. 37: 160-162.

Langeveld, L.P.M., (1983). Bacteria and the keeping quality of pasteurised milk. Neth. Milk Dairy J. 37: 244-245.

Langeveld, L.P.M. & Cuperus, F., (1980). The relation between temperature and growth rate in pasteurized milk of different types of bacteria which are important to the deterioration of that milk. Neth. Milk Dairy J. 34: 106-125.

Lee, R.Y., Silverman, G.J. & Munsey, D.T., (1981). Growth and enterotoxin A production by Staphylococcus aureus in precooked bacon in the intermediate moisture range. J. Food Sci. 46: 1687-1692, 1700.

Lerke, P., Adams, R. & Farber, L., (1965). Bacteriology of spoilage of fish muscle. III. Characterization of spoilers. Appl. Microbiol. 13: 625-630.

Lewis, D.H. & Smith, D.C., (1967). Sugar alcohols (polyols) in fungi and green plants. I. Distribution, physiology and metabolism. New Phytol. 66: 143-184.

Long, S.K. & Williams, O.B., (1959). Growth of obligate thermophiles at 37°C as a function of the cultural conditions employed. J. Bacteriol. 77: 545-547.

Lotter, L.P. & Leistner, L., (1978). Minimal water activity for enterotoxin A production and growth of Staphylococcus aureus. Appl. Environ. Microbiol. 36: 377-380.

Lowry, R.K. & Ratkowsky, D.A., (1983). A note on models for poikilotherm development. J. Theor. Biol. 105: 453-459.

Marshall, B.J., Ohye, D.F. & Christian, J.H.B., (1971). Tolerance of bacteria to high concentrations of NaCl and glycerol in the growth medium. Appl. Microbiol. 21: 363-364.

Marshall, B.J. & Scott, W.J., (1958). The water relations of Vibrio metchnikovi at 30°C. Aust. J. Biol. Sci. 11: 171-176.

Marshall, K.C., (1964). Unpublished manual of laboratory techniques, Faculty of Agricultural Science, University of Tasmania.

Maxcy, R.B. (1967). Nature and growth response of the microflora of pasteurized, packaged milk. J. Milk Food Technol. 30: 213-218.

Maxcy, R.B. & Wallen, S.E., (1983). Heterogeneity of samples as a problem in shelf-life prediction. J. Food Prot. 46: 542-544.

McLaren, I.A., Corkett, C.J. & Zillioux, E.J., (1969). Temperature adaptations of copepod eggs from the Arctic to the Tropics. Biol. Bull. 137: 486-493.

McMeekin, T.A., (1982). Microbial spoilage of meats. In: Developments in Food Microbiology-1. Davies, R., (Ed.). Applied Science, London. Pp: 1-40.

McMeekin, T.A. & Olley, J., (1986). Predictive microbiology. Food Technol. Aus. 38: 331-334.

McMeekin, T.A., Olley, J. & Ratkowsky, D.A., (1987). Temperature effects on bacterial growth rates. CRC. In: Mathematical Models in Microbiology. Bazin, M. & Prosser, J., (Eds.). CRC Press Inc., Boca Raton.

McMeekin, T.A. & Thomas, C.J., (1980). Microbiological problems associated with refrigerated poultry. CSIRO Food Research Quarterly. 40: 141-149.

Mead, G.C. (1985). Predict shelf-life of poultry meat. Poultry. 2: 22-23.

Mead, G.C. & Adams, B.W., (1977). A selective medium for the rapid isolation of pseudomonads associated with poultry meat spoilage. Br. Poult. Sci. 18: 661-670.

Measures, J.C., (1975). Role of amino acids in osmoregulation of non-halophilic bacteria. Nature (London). 257: 398-400.

Mohr, P.W. & Krawiec, S., (1980). Temperature characteristics and Arrhenius plots for nominal psychrophiles, mesophiles and thermophiles. J. Gen. Microbiol. 121: 311-317.

Mol, H. & Vincentie, H.M., (1981). Psychrotrophic microorganisms in milk and milk products in the Netherlands. In: Psychrotrophic Microorganisms in Spoilage and Pathogenicity. Roberts, T.A., Hobbs, G., Christian, J.H.B. & Skovgaard, N. (Eds.), Academic Press, London. Pp: 97-108.

Morita, R.Y., (1975). Psychrophilic bacteria. Bacteriol. Reviews. 39: 144-167.

Mossel, D.A.A., (1971). Physiological and metabolic attributes of microbial groups associated with foods. J. Appl. Bacteriol. 34: 95-118.

Mossel, D.A.A., (1977). Microbiology of Foods. Occurrence, Prevention and Monitoring of Hazards and Deterioration. 2nd Ed. University of Utrecht, Netherlands.

Nelson, L.M. & Parkinson, D., (1978). Growth characteristics of three bacterial isolates from arctic soil. Can. J. Microbiol. 24: 909-914.

Nixon, P.A., (1971). Temperature integration as a means of assessing storage conditions. In: Report on Quality in Fish Products. Wellington, New Zealand Fishing Industry Board. Seminar No. 3: 34-44.

Notermans, S. & Heulvelman, C.J., (1983). Combined effect of water activity, pH and sub-optimal temperature on growth and enterotoxin production of Staphylococcus aureus. J. Food Sci. 48: 1832-1835, 1840.

Ohta, F. & Hirahara, T., (1977). Rate of degredation of nucleotides in cooling-stored carp muscle. Mem. Fac. Fish. Kagoshima Univ. 26: 97-102.

Olley, J., (1978). Current status of the theory of the application of temperature indicators, temperature integrators, and temperature function integrators to the food spoilage chain. Int. J. Refrig. 1: 81-86.

Olley, J.N., (1980). Structure and proteins of fish and shellfish. Part 1. In: Advances in Fish Science and Technology. Connell, J.J., (Ed.). Fishing News Books Ltd., Farnham, Surrey. 65-77.

Olley, J., (1983). Temperature effects on histamine producing bacteria. In: Properties and Processing of Marine Foods. National Taiwan College of Marine Science and Technology Marine Food Science Series. Pp: 14-35.

Olley, J. & Lisac, H., (1985). Time/temperature monitors. Infofish Marketing Digest. 3: 45-47.

Olley, J. & McMeekin, T.A., (1985). Use of temperature function integrators. In: Histamine in marine products: production by bacteria, measurement and prediction of formation. Pan, B.S. & James, D. (Eds.). FAO Fish Tech. Pap. 252: 24-29.

Olley, J. & Ratkowsky, D.A., (1973a). Temperature function integration and its importance in the storage and distribution of flesh foods above the freezing point. Food Technol. Aust. 25: 66-73.

Olley, J. & Ratkowsky, D.A., (1973b). The role of temperature function integration in the monitoring of fish spoilage. Food Technol. N.Z. 8: 13-17.

Olsen, R.H. & Jezeski, J.J., (1963). Some effects of carbon source, aeration, and temperature on growth of a psychrophilic strain of Pseudomonas fluorescens. J. Bacteriol. 86: 429-433.

Olson, H.C., (1967). Relationship between bacterial counts and shelf life of milk. J. Dairy Sci. 50: 607.

Owen, D. & Nesbitt, M., (1984). A versatile time temperature function integrator. Laboratory Practice. 33: 70-75.

Phillips, J.D. & Griffiths, M.W., (1986). Estimation of Gram-negative bacteria in milk: a comparison of inhibitor systems for preventing Gram-positive bacterial growth. J. Appl. Bacteriol. 60: 491-500.

Phillips, J.D., Griffiths, M.W. & Muir, D.D. (1984). Preincubation test to rapidly identify post-pasteurization contamination in milk and single cream. J. Food Prot. 47: 391-393.

Pirt, S.J., (1975). In: Principles of Microbiology and Cell Cultivation. Blackwell, Oxford.

Pooni, G.S. & Mead, G.C., (1984). Prospective use of temperature function integration for predicting the shelf-life of non-frozen poultry-meat products. Food Microbiol. 1: 67-78.

Precht, H., Laudien, H. & Havsteen, B., (1973). The normal temperature range. In: Temperature and Life. Precht, H., Christophersen, J., Hensel, H., & Larcher, W., (Eds.). Springer-Verlag, Berlin. Pp: 302-470.

Ratkowsky, D.A., (1983). Nonlinear Regression Modelling. Marcel Dekker, New York. Pp.: 143-149.

Ratkowsky, D.A., Lowry, R.K., McMeekin, T.A., Stokes, A.N. & Chandler, R.E., (1983). Model for bacterial culture growth rate throughout the entire biokinetic temperature range. J. Bacteriol. 154: 1222-1226.

Ratkowsky, D.A., Olley, J., McMeekin, T.A. & Ball, A., (1982). Relationship between temperature and growth rate of bacterial cultures. J. Bacteriol. 149: 1-5.

Reichardt, W. & Morita, R.Y., (1982). Temperature characteristics of psychrotrophic and psychrophilic bacteria. J. Gen. Microbiol. 128: 565-568.

Reichelt, J.L. & Baumann, P., (1974). Effect of sodium chloride on growth of heterotrophic bacteria. Arch. Microbiol. 97: 329-345.

Roberts, T.A. & Jarvis, B., (1983). Predictive modelling of food safety with particular reference to Clostridium botulinum in model cured meat systems. In: Food Microbiology. Advances and prospects. Roberts T.A., & Jarvis B., (Eds.). Academic Press, New York. 85-95.

Robinson, R.A. & Stokes, R.H., (1949). Tables of osmotic and activity coefficients of electrolytes in aqueous solution at 25°C. Trans. Faraday Soc. 45: 612-624.

Ronsivalli, L.J. & Charm, S.E., (1975). Spoilage and shelf life prediction of refrigerated fish. Mar. Fis. Rev. 37(4): 32-34.

Ross, T., (1986). Bělehrádek temperature functions and fish spoilage. Paper given at: CSIRO-DSIRO Joint Workshop on Seafood Processing. Nelson, New Zealand. April, 1986.

Schoolfield, R.M., Sharpe, P.J.H. & Magnusson, C.E., (1981). Non-linear regression of biological temperature-dependent rate models based on absolute reaction-rate theory. J. Theor. Biol. 88: 719-731.

Scott, W.J., (1953). Water relations of Staphylococcus aureus at 30°C. Aust. J. Biol. Sci. 6: 549-564.

Scott, W.J., (1957). Water relations of food spoilage microorganisms. Adv. Food Res. 7: 83-127.

Sharpe, P.J.H. & DeMichele, D.W., (1977). Reaction kinetics of poikilotherm development. J. Theor. Biol. 64: 649-670.

Shaw, M.K., (1967). Effect of abrupt temperature shift on the growth of mesophilic and psychrophilic yeasts. J. Bacteriol. 93: 1332-1336.

Shaw, M.K., Marr, A.G. & Ingraham, J.L., (1971). Determination of the minimal temperature for growth of Escherichia coli. J. Bacteriol. 105: 683-684.

Shehata, T.E., Duran, A. & Collins, E.B., (1971). Influence of temperature on the growth of psychrophilic strains of Bacillus. J. Dairy Sci. 54: 1579-1582.

Sherman, J.M., Cameron, G.M. & White, J.C., (1941). The bacteriological spoilage of milk held near the freezing point. J. Dairy Sci. 24: 526-527.

Shewan, J.M., (1962). The bacteriology of fresh and spoiling fish and some related chemical changes. In: Recent Advances In Food Science. Vol. I. Commodities. Hawthorn, J. & Leitch, J.M., (Eds.). Butterworths, London. Pp: 167-193.

Shewan, J.M., Hobbs, G. & Hodgkiss, W., (1960). A determinative scheme for the identification of certain Gram-negative bacteria, with special reference to the Pseudomonadaceae. J. Appl. Bacteriol. 23: 379-390.

Singleton, R.Jr. & Amelunxen, R.E., (1973). Proteins from thermophilic microorganisms. Bacteriol. Rev. 37: 320-342.

Smith, M.G., (1985). The generation time, lag time and minimum temperature of growth of coliform organisms on meat and the implications for codes of practice in abattoirs. J. Hyg. (Camb.). 94: 289-300.

Smith, M.G., (1987). Calculation of the expected increases of coliform organisms, Escherichia coli and Salmonella typhimurium in raw, blended mutton tissue. Epidem. Inf. In press.

Spencer, R. & Baines, C.R., (1964). The effect of temperature on the spoilage of wet white fish. Food Technol. 18: 769-773.

Sperber, W.H., (1983). Influence of water activity on foodborne bacteria - a review. J. Food Prot. 46: 142-150.

Stannard, C.J., Williams, A.P. & Gibbs, P.A., (1985). Temperature / growth relationships for psychrotrophic food-spoilage bacteria. Food Microbiol. 2: 115-122.

Strong, D.H., Foster, E.F. & Duncan, C.L., (1970). Influence of water activity on the growth of Clostridium perfringens. Appl. Microbiol. 19: 980-987.

Tan, K.H. & Gill, C.O., (1981). A note on the estimation of microbial growth rates from rates of substrate utilization. J. Appl. Bacteriol. 51: 95-99.

Theron, D.P., Prior, B.A. & Lategan, P.M., (1987). Effect of minimum growth temperature on the adenosine triphosphate content of bacteria. Int. J. Food Microbiol. 4: 323-329.

Thomas, C.J., (1984). Temperature function integration and its role in monitoring spoilage. In: Proceedings of the First Short Course on "Rapid Methods in Analysis in Food Microbiology". Brooks, J.D., (Ed.). August 1983. Massey University, New Zealand. Pp: 38-57.

Thomas, C.J. & McMeekin, T.A., (1980). Contamination of broiler carcass skin during commercial processing procedures: an electron microscopic study. Appl. Environ. Microbiol. 40: 133-144.

Thomas, C.J. & McMeekin, T.A., (1981). Spoilage of chicken at 2°C: electron microscopic study. Appl. Environ. Microbiol. 41: 495-503.

Tindall, B.J. & Trüper, H.G., (1986). Ecophysiology of the aerobic halophilic Archaebacteria. System. Appl. Microbiol. 7: 202-212.

Tinuoye, O.L. & Harmon, L.G. (1975). Growth of thermoduric psychrotrophic bacteria in refrigerated milk. Amer. Dairy Rev. 37: 26-28.

Troller, J., (1983). Methods to measure water activity. J. Food Prot. 46: 129-134.

Troller, J.A., (1986). Water relations of foodborne bacterial pathogens - an updated review. J. Food Prot. 49: 656-670.

Troller, J.A. & Christian, J.H.B., (1978). Microbial growth. In: Water Activity and Food. Academic Press, New York. Pp: 86-102.

Waters, J.R., (1972). Sensitivity of the $^{14}CO_2$ radiometric method for bacterial detection. Appl. Microbiol. 23: 198-199.

Wodzinski, R.J. & Frazier, W.C., (1960). Moisture requirements of bacteria. I. Influence of temperature and pH on requirements of Pseudomonas fluorescens. J. Bacteriol. 79: 572-578.

6. APPENDICES.

APPENDIX 6.1: MEDIA AND METHODS.

6.1a: 0.9% (w/v) Saline.

NaCl (AJAX UNIVAR)	9.0g
Deionised Water	1.0L

Sterilised for 15 minutes at 103.4kPa (121°C).

6.1b: Plate Count Agar (PCA).

Plate Count Agar (Oxoid CM325)	17.5g
Deionised Water	1.0L

Sterilised for 15 minutes at 103.4kPa (121°C).

Incubation of PCA plates was for 96 hours at 25°C.

6.1c: Pseudomonas Agar (PsA).

Pseudomonas Agar Base (Oxoid CM559)	24.2g
Glycerol (AJAX UNIVAR)	5.0mL
Deionised Water	500.0mL

Brought to boil, then sterilised for 15 minutes at 103.4kPa (121°C). Allowed to cool to approximately 50°C and one vial of C-F-C Supplement (Oxoid SR103) was aseptically added and mixed well. Incubation of plates was for 48 hours at 25°C (Mead & Adams, 1977).

6.1d: Gram Reaction.

A suspension of bacteria was smeared onto a glass slide, allowed to dry and heat fixed. The smear was then covered with crystal violet for 1 minute, washed with water, flooded with iodine for 1 minute, washed, decolourised with acetone/alcohol (1/1), washed, counterstained with safranin, washed and allowed to dry. Gram positive cells stained blue and Gram negative cells stained red (Marshall, 1964).

6.1e: Hugh and Leifson Medium.

For Gram Negative Bacteria:

Bacteriological Peptone (Oxoid L37)	2.0g
NaCl (AJAX UNIVAR)	5.0g
K_2HPO_4 (BDH AnalaR)	0.3g
Agar (Davis Grade J)	3.0g
Bromothymol Blue (1% alc sol) (BDH Indicators)	3.0mL
Glucose (BDH AnalaR)	10.0g
Deionised Water	1.0L

Brought to boil, pH adjusted to 6.9, then sterilised for 20 minutes at 69kPa (115°C). Tubes of medium were stab inoculated and incubated both aerobically and anaerobically for 48 hours at 25°C.

Tubes initially green in color:

oxidative reaction: yellow at top of aerobic tube only;

alkaline reaction: blue at top of aerobic tube only;

fermentative: both tubes totally yellow (gas production may be observed) (Hugh & Leifson, 1953).

For Gram Positive Bacteria:

Tryptone (Oxoid L42)	10.0g
Yeast Extract (Oxoid L21)	1.0g
Glucose (BDH AnalaR)	10.0g
Bromocresol Purple (AJAX LABCHEM)	0.04g
Agar (Davis Grade J)	2.0g
Deionised Water	1.0L

Brought to boil, pH adjusted to 7.2, then sterilised for 20 minutes at 69kPa (115°C). Tubes of medium were stab inoculated and incubated both aerobically and anaerobically for up to 7 days.

Tubes initially purple in colour:

oxidative reaction: yellow at top of aerobic tube only;

fermentative reaction: both tubes totally yellow (gas may be observed) (Baird-Parker, 1976).

6.1f: Flagella Arrangement.

Flagella arrangement was determined by the use of transmission electron microscopy (TEM) on motile isolates. Collodion coated copper grids were prepared: the 85/15 ether/ethanol solvent was evaporated off the collodion and a 2%(w/v) solution in amyl acetate was prepared. Two drops of this solution were allowed to fall onto the surface of a dish filled with deionised water and the copper grids were floated dull side down on the resultant collodion film. The grids were picked up using parafilm and air dried. The coated grids were placed on drops of cultures for 30 seconds, removed and placed on drops of aqueous uranyl acetate for 15 seconds. Excess stain was washed off using distilled water and the grids allowed to air dry before examination using an Hitachi H-300 TEM, operated at 75kV.

6.1g: Kovacs' Oxidase Reagent.

A freshly prepared 1%(w/v) aqueous solution of N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride (SIGMA T 3134) was prepared and stored in the absence of light. Using a platinum loop, a portion of a colony of each isolate was transferred to a piece of filter paper which had been previously soaked in Kovacs' oxidase reagent. A dark blue colouration within 10 seconds was recorded as a positive test (Kovacs, 1956).

6.1h: Catalase.

A loop of a 24 hour bacterial colony was transferred into a drop of 3% H_2O_2 (AJAX UNIVAR). The formation of gas bubbles was taken as a positive result.

6.1i: Psychrotrophic Growth.

Isolates were recorded as being positive for psychrotrophic growth if they were capable of growing to visible colony size on PCA, after incubation at 4°C for 14 days (Thomas & McMeekin, 1980).

6.1j: King's Medium B for Fluorescein b.

Proteose Peptone (Oxoid L46)	20.0g
Glycerol (AJAX UNIVAR)	10.0mL
K_2HPO_4 (BDH AnalaR)	1.5g
$MgSO_4.7H_2O$ (BDH AnalaR)	1.5g
Agar (Davis Grade J)	15.0g
Deionised Water	1.0L

Sterilised for 15 minutes at 103.4kPa (121°C).

Members of the genus Pseudomonas were grown on King's B medium for 48 hours at 25°C. The production of fluorescent pigments was observed by using ultra violet light of wavelength 254nm (King et al.,1954).

6.1k: Most Probable Number Medium (MPN) for the Detection of Pseudomonads.

Double strength MPN medium was made up:

(a)	Bacteriological Peptone (Oxoid L37)	16.0g
	K_2SO_4 (BDH AnalaR)	10.0g
	$MgCl_2$ (BDH AnalaR)	2.5g
	Peptonised Milk (Oxoid L32)	2.0g
	Glucose (BDH AnalaR)	5.0g
	Glycerol (AJAX UNIVAR)	10.0mL
	Deionised Water	500.0mL

Brought to boil, pH adjusted to 7.0, then sterilised for 20 minutes at 69kPa (115°C).

(b) 0.5g of 2,3,4-triphenyltetrazolium chloride (TTC) (SIGMA T 8877) was dissolved in 5mL of deionised water and filter sterilised through a 0.4 bk Millipore filter.

(c) Two vials of C-F-C Supplement (Oxoid SR103) were rehydrated with sterile deionised water.

(b) and (c) were aseptically added to (a). The resultant double strength (DS) MPN medium was dispensed into sterile tubes:

10.0mL DS	+			10.0mL milk
2.5mL DS	+	2.5mL sterile water	+	1.0mL milk
2.5mL DS	+	2.5mL sterile water	+	0.1mL milk

Five tubes of each dilution were incubated at 25°C and read after 24, 48 and 72 hours. Microbial growth resulted in the MPN medium turning pink. The correlation between positive tubes and bacterial numbers was determined using a table from Anonymous (1982).

6.11: Plate Count Broth (PCB).

Tryptone (Oxoid L42)	5.0g
Yeast Extract (Oxoid L21)	2.5g
Glucose (BDH AnalaR)	1.0g
Peptonised Milk (Oxoid L32)	2.0g
Deionised Water	1.0L

Sterilised for 20 minutes at 69kPa (115°C).

6.1m: Moderate Halophile Broth (MHB).

Basal moderate halophile broth:

Proteose Peptone (Oxoid L46)	5.0g
Tryptone (Oxoid L42)	5.0g
Yeast Extract (Oxoid L21)	3.0g

Na_2HPO_4 (BDH AnalaR)	7.1g
KH_2PO_4 (BDH AnalaR)	2.7g
$MgSO_4.7H_2O$ (BDH AnalaR)	250.0mg
Glucose (BDH AnalaR)	1.8g
Deionised Water	1.0L

The water activity was calculated (Appendix 6.2a) to be:

0.996

The water activity of the basal broth was decreased either by the addition of NaCl (AJAX UNIVAR) or glycerol (AJAX UNIVAR) and the pH adjusted to approximately 7.0, then sterilised for 20 minutes at 69kPa (115°C).

6.1n: Moderate Halophile Agar (MHA).

Yeast Extract (Oxoid L21)	10.0g
Proteose Peptone (Oxoid L46)	5.0g
Tri-Sodium Citrate (BDH AnalaR)	2.0g
KCl (BDH AnalaR)	1.0g
$MgSO_4.7H_2O$ (BDH AnalaR)	8.0g
$MgCl_2.6H_2O$ (BDH AnalaR)	2.0g
Agar No.1 (Oxoid L11)	15.0g
NaCl (AJAX UNIVAR)	80.0g
Deionised Water	1.0L

Brought to boil, pH adjusted to 7.2, then sterilised for 15 minutes at 103.4kPa (121°C).

Incubation of plates was for 2 weeks at 30°C.

6.1o: Extreme Halophile Broth (XHB).

Basal extreme halophile broth:

Tryptone (Oxoid L42)	7.5g
Yeast Extract (Oxoid L21)	3.0g
Tri-Sodium Citrate (BDH AnalaR)	3.0g
$MgSO_4.7H_2O$ (BDH AnalaR)	20.0g
Fe^{2+} (as $FeSO_4.7H_2O$) (BDH AnalaR)	10.0ppm
Mn^{2+} (as $MnSO_4.H_2O$) (BDH AnalaR)	0.1ppm
KCl (BDH AnalaR)	2.0g
Glucose (BDH AnalaR)	1.8g
Deionised Water	1.0L

The water activity was calculated to be (Appendix 6.2a):

0.995

The water activity of the basal broth was decreased by the addition of NaCl (AJAX UNIVAR). The pH was adjusted to approximately 7.0, then sterilised for 30 minutes at 34.5kPa (110°C). The brine was then treated with 10g of activated charcoal, the pH readjusted to 7.0 and filtered under reduced pressure through Whatman No. 42 filter paper.

6.1p: Extreme Halophile Saline (XHS):

NaCl (AJAX UNIVAR)	225.0g
Deionised Water	1.0L

Sterilised for 15 minutes at 103.4kPa (121°C).

6.1q: Extreme Halophile Agar (XHA).

Yeast Extract (Oxoid L21)	10.0g
Proteose Peptone (Oxoid L46)	5.0g
Tri-Sodium Citrate (BDH AnalaR)	2.0g
KCl (BDH AnalaR)	2.0g
$MgSO_4.7H_2O$ (BDH AnalaR)	20.0g

$MgCl_2.6H_2O$ (BDH AnalaR)	5.0g
Agar No.1 (Oxoid L11)	15.0g
NaCl (AJAX UNIVAR)	225.0g
Deionised Water	1.0L

Brought to boil, pH adjusted to 7.2, then sterilised for 15 minutes at 103.4kPa (121°C).

Incubation of plates was for 4 weeks at 30°C.

APPENDIX 6.2: CALCULATIONS.

Appendix 6.2a: Calculation of the Water Activity of a Solution.

The water activity of a solution can be obtained from the relation (Robinson & Stokes, 1949):

$$\log_{10} a_w = -0.007824 \; \nu \infty \Phi$$

where ν = the number of dissociated ions,

∞ = concentration in mole/1000g of water,

Φ = osmotic coefficient.

When there are two or more contibuting solutes, the water activity of the resultant solution is given by the product of the individual calculated water activities.

Sample Calculation:

$\nu = 2$ for NaCl,

$\infty = 2$ molal = 116.8g NaCl

$\Phi = 0.983$,

$a_w = 0.932$

When 116.88g NaCl is added to 1L of MHB, the resultant water activity:

$a_w = 0.932 * 0.996$

$a_w = 0.928$

Appendix 6.2b: Calculation of Relative Incubation Times Using the Square Root Model.

The Square Root Model is of the form:

$$r_T = b(T - T_o)^2$$

For a TTFI set to operate using a T_o value of 263K and display storage time at 4°C, equivalent storage times at other temperatures (T), are calculated:

$$r_T = b(T - 263)^2 \text{ and}$$

$$r_4 = b(277.16 - 263)^2$$

The equivalent time at 12°C is calculated:

$$r_4 = b(200.5) \text{ and}$$

$$r_{12} = b(491.1)$$

Hence the rate at 12°C is,

$$(491.1)/(200.5) = 2.45$$

time faster than at 4°C,

If the time to reach organoleptic spoilage is 9.0 days on the TTFI then the equivalent time at 12°C is 3.7 days.

Appendix 6.2c: Calculation of Lag Time from Generation Time and Optical Density Data.

Lag times can be calculated from GT and OD data in the following manner:

At 298.9K the time required for a change in OD of 0.3 was 164 min and the generation time was 29.4 min.

It was determined in Section 3.2a that a change in OD of 0.3 corresponded to 2.1 generations.

Therefore the lag time = change in OD 0.3 - (2.1 * GT)

= 164 - (2.1 * 29.4)

= 102.3 min

Appendix 6.2d: Calculation of the Cell Volume of Halobacterium sp. Strain HB9, After Growth in XHB Containing 3.5 molal NaCl.

Approximate cell volumes were calculated:

Cell volume = length * $(\text{width})^2$

= 3.46 * $(0.64)^2$

= $1.417\mu^3$

APPENDIX 6.3: DATA FOR CARBON DIOXIDE CONCENTRATIONS VERSUS MICROBIAL NUMBERS (FIGURE 3.1).

Log(cfu)	CO_2	Log(cfu)	CO_2	Log(cfu)	CO_2
8.19	6.33	8.41	10.97	6.30	1.36
8.91	9.56	6.03	0.85	8.00	4.92
9.00	9.95	5.20	0.81	5.20	0.81
8.30	1.86	8.70	4.41	9.30	7.80
6.00	0.84	7.00	1.72	8.00	3.80
9.47	12.88	6.20	0.66	7.00	1.88
8.57	9.89	6.65	0.65	7.88	2.12
9.25	6.15	6.60	0.90	9.18	8.42
9.43	6.38	9.73	7.99	6.80	0.42
8.39	3.83	8.80	5.10	9.80	7.38
8.50	1.52	9.55	5.70	7.43	0.50
8.02	1.40	9.51	5.78	9.49	10.81
7.06	0.39	8.74	4.16	9.14	7.65
7.45	0.27	7.96	6.66	8.85	8.96
8.70	0.13	8.46	0.81	9.34	3.53

Microbial numbers are cfu mL^{-1}.

Carbon dioxide concentrations are $\%CO_2$ in head space.

APPENDIX 6.4: DATA FOR THE SQUARE ROOT PLOTS OF THE EFFECT OF TEMPERATURE ON THE SPOILAGE OF PASTEURISED, HOMOGENISED MILK (FIGURE 3.2, FIGURE 3.3, FIGURE 3.4, FIGURE 3.5, AND FIGURE 3.6).

Temp.: temperature (°C).

ttSp.: time to reach spoilage level of log(7.5) cfu mL^{-1}.

O.RR.: observed square root of spoilage rate.

P.RR.: predicted square root of spoilage rate using least squares analysis.

All times in hours.

Appendix 6.4a: Data for Figure 3.2a, Figure 3.2b and Figure 3.2c.

Figure 3.2a:

Temp.	ttSp.	O.RR.	P.RR.	Residual
21.5	33.5	0.172774	0.171188	0.001586
19.5	38.6	0.160956	0.159763	0.001193
18.1	38.4	0.161374	0.151766	0.009608
16.6	52.9	0.137490	0.143197	-0.005707
14.9	60.2	0.128885	0.133486	-0.004601
13.3	64.8	0.124226	0.124546	-0.000120
12.1	75.2	0.115316	0.117492	-0.002176
11.5	84.8	0.108593	0.114064	-0.005471
10.5	88.9	0.106059	0.106638	-0.000579
9.6	96.5	0.101797	0.103211	-0.001413
8.8	102.3	0.098869	0.098641	0.000228
7.3	117.9	0.092096	0.090072	0.002024
5.7	146.9	0.082507	0.080932	0.001575
4.3	169.6	0.076787	0.072935	0.003852

Figure 3.2b:

21.5	33.5	0.172774	0.174853	-0.002079
19.5	38.6	0.160956	0.161979	-0.000923
18.1	38.4	0.161374	0.152796	-0.008578
16.6	52.9	0.137490	0.143065	0.005575

Figure 3.2c:

14.9	60.2	0.128885	0.129196	-0.000311
13.3	64.8	0.124226	0.121186	0.003040
12.1	75.2	0.115316	0.115178	0.000138
11.5	84.8	0.108593	0.112175	-0.003582
10.5	88.9	0.106059	0.105666	0.000393
9.6	96.5	0.101797	0.102663	-0.000866
8.8	102.3	0.098869	0.098658	0.000211
7.3	117.9	0.092096	0.091148	0.000948
5.7	146.9	0.082507	0.083138	-0.000631
4.3	169.6	0.076787	0.076129	0.000658

Appendix 6.4b: Data for Figure 3.3a, Figure 3.3b and Figure 3.3c.

Figure 3.3a:

Temp.	ttSp.	O.RR.	P.RR.	Residual
23.4	28.5	0.187317	0.183794	0.003523
21.8	30.5	0.181071	0.172957	0.008114
18.9	45.7	0.147925	0.153314	-0.005389
17.1	49.5	0.142134	0.141122	0.001012
15.5	59.5	0.129641	0.130284	-0.000643
13.9	79.4	0.112225	0.119447	-0.007222
12.6	96.3	0.101903	0.110641	-0.008738
10.7	97.7	0.101170	0.097771	0.003399
9.3	116.0	0.092848	0.088289	0.004559
6.8	298.0	0.057928	0.071355	-0.013427
4.2	216.6	0.067947	0.053744	0.014213
3.4	519.0	0.043895	0.048325	-0.004430
2.7	423.0	0.048622	0.043584	0.005038

Figure 3.3b:

23.4	28.5	0.187317	0.188638	-0.001321
21.8	30.5	0.181071	0.176413	0.004658
18.9	45.7	0.147925	0.154256	-0.006331
17.1	49.5	0.142134	0.140503	0.001631
15.5	59.5	0.129641	0.128278	0.001363

Figure 3.3c:

13.9	79.4	0.112225	0.113486	-0.001261
12.6	96.3	0.101903	0.105802	-0.003899
10.7	97.7	0.101170	0.094572	0.006598
9.3	116.0	0.092848	0.086297	0.005551
6.8	298.0	0.057928	0.071520	-0.013592
4.2	216.6	0.067947	0.056152	0.011795
3.4	519.0	0.043895	0.051424	-0.007529
2.7	423.0	0.048622	0.047286	0.001336

Appendix 6.4c: Data for Figure 3.4a, Figure 3.4b and Figure 3.4c.

Figure 3.4a:

Temp.	ttSp.	O.RR.	P.RR.	Residual
23.4	23.2	0.207614	0.194854	0.012760
20.7	32.1	0.176501	0.176712	-0.000211
18.8	37.3	0.163737	0.163946	-0.000209
17.1	44.5	0.149906	0.152523	-0.002617
15.4	49.8	0.141705	0.141100	0.000605
13.8	63.6	0.125392	0.130350	-0.004958
12.5	76.0	0.114708	0.121615	-0.006907
11.1	76.3	0.114482	0.112208	0.002274
10.7	95.1	0.102544	0.109520	-0.006976
10.1	101.2	0.099405	0.105488	-0.006083
8.8	112.6	0.094239	0.096753	-0.002514
7.4	150.4	0.081541	0.087347	-0.005806
6.6	169.5	0.076810	0.081971	-0.005161
5.9	150.3	0.081568	0.077268	0.004300
4.9	192.2	0.072131	0.070549	0.001582
4.2	168.1	0.077129	0.065845	0.011284
3.4	209.4	0.069105	0.060470	0.008635

Figure 3.4b:

23.4	23.2	0.207614	0.203350	0.004264
20.7	32.1	0.176501	0.181189	-0.004688
18.8	37.3	0.163737	0.165594	-0.001857
17.1	44.5	0.149906	0.151641	-0.001735
15.4	49.8	0.141705	0.137688	0.004017

Figure 3.4c:

13.8	63.6	0.125392	0.122124	0.003268
12.5	76.0	0.114708	0.115125	-0.000417
11.1	76.3	0.114482	0.107587	0.006895
10.7	95.1	0.102544	0.105433	-0.002889
10.1	101.2	0.099405	0.102202	-0.002797
8.8	112.6	0.094239	0.095203	-0.000964
7.4	150.4	0.081541	0.087665	-0.006124
6.6	169.5	0.076810	0.083358	-0.006548
5.9	150.3	0.081568	0.079589	0.001979
4.9	192.2	0.072131	0.074205	-0.002074
4.2	168.1	0.077129	0.070436	0.006693
3.4	209.4	0.069105	0.066128	0.002977

Appendix 6.4d: Data for Figure 3.5a, Figure 3.5b and Figure 3.5c.

Figure 3.5a:

Temp.	ttSp.	O.RR.	P.RR.	Residual
25.1	19.5	0.226455	0.221695	0.004760
22.8	24.9	0.200401	0.204070	-0.003669
20.2	29.0	0.185695	0.184146	0.001549
18.5	30.4	0.181369	0.171119	0.010250
17.1	36.7	0.165070	0.160390	0.004680
15.6	47.4	0.145248	0.148896	-0.003648
14.2	55.3	0.134474	0.138167	-0.003693
12.7	69.6	0.119866	0.126673	-0.006807
11.6	76.2	0.114557	0.118243	-0.003686
11.0	85.2	0.108338	0.113645	-0.005307
10.2	101.0	0.099504	0.107515	-0.008011
9.6	109.9	0.095390	0.102917	-0.007527
8.7	117.5	0.092253	0.096020	-0.003767
8.1	119.5	0.091478	0.091422	0.000056
6.6	128.7	0.088148	0.079928	0.008220
5.9	139.1	0.084788	0.074654	0.010224
5.0	182.4	0.074044	0.067667	0.006377

Figure 3.5b:

25.1	19.5	0.226455	0.224264	0.002191
22.8	24.9	0.200401	0.206530	-0.006128
20.2	29.0	0.185695	0.186482	-0.000787
18.5	30.4	0.181369	0.173373	0.007996
17.1	36.7	0.165070	0.162578	0.002492
15.6	47.4	0.145248	0.151012	-0.005764

Figure 3.5c:

14.2	55.3	0.134474	0.128397	0.006077
12.7	69.6	0.119866	0.119570	0.000296
11.6	76.2	0.114557	0.113097	0.001460
11.0	85.2	0.108338	0.109566	-0.001228
10.2	101.0	0.099504	0.104859	-0.005355
9.6	109.9	0.095390	0.101328	-0.005938
8.7	117.5	0.092253	0.096032	-0.003779
8.1	119.5	0.091478	0.092501	-0.001023
6.6	128.7	0.088148	0.083674	0.004474
5.9	139.1	0.084788	0.079555	0.005233
5.0	182.4	0.074044	0.074259	-0.000215

Appendix 6.4e: Data for Figure 3.6a, Figure 3.6b and Figure 3.6c.

Figure 3.6a:

Temp.	ttSp.	O.RR.	P.RR.	Residual
25.0	19.7	0.225303	0.221112	0.004191
23.7	22.1	0.212718	0.211610	0.001108
21.2	25.9	0.196494	0.193337	0.003157
19.7	30.8	0.180187	0.182373	-0.002186
18.0	35.1	0.168790	0.169947	-0.001157
17.2	36.9	0.164622	0.164099	0.000523
15.6	46.4	0.146805	0.152404	-0.005599
14.8	48.1	0.144187	0.146557	-0.002370
13.2	54.5	0.135457	0.134862	0.000595
12.6	56.7	0.132803	0.130476	0.002327
11.5	69.4	0.120038	0.122436	-0.002398
10.9	76.5	0.114332	0.118050	-0.003718
9.9	84.1	0.109044	0.110741	-0.001697
9.4	87.4	0.106966	0.107086	-0.000120
8.2	110.6	0.095087	0.098315	-0.003228
7.4	118.8	0.091747	0.092468	-0.000721
6.3	141.0	0.084215	0.084427	-0.000212
5.8	139.0	0.084819	0.080773	0.004046
5.2	147.7	0.082283	0.076387	0.005896
4.6	184.8	0.073561	0.072001	0.001560

Figure 3.6b:

25.0	19.7	0.225303	0.224924	0.000379
23.7	22.1	0.212718	0.214421	-0.001703
21.2	25.9	0.196494	0.194222	0.002272
19.7	30.8	0.180187	0.182103	-0.001916
18.0	35.1	0.168790	0.168368	0.000422
17.2	36.9	0.164622	0.161904	0.002718
15.6	46.4	0.146805	0.148977	-0.002172

Figure 3.6c:

14.8	48.1	0.144187	0.144155	0.000032
13.2	54.5	0.135457	0.133148	0.002309
12.6	56.7	0.132803	0.129020	0.003783
11.5	69.4	0.120038	0.121452	-0.001414
10.9	76.5	0.114332	0.117324	-0.002992
9.9	84.1	0.109044	0.110444	-0.001400
9.4	87.4	0.106966	0.107004	-0.000038
8.2	110.6	0.095087	0.098748	-0.003661
7.4	118.8	0.091747	0.093244	-0.001497
6.3	141.0	0.084215	0.085676	-0.001461
5.8	139.0	0.084819	0.082236	0.002583
5.2	147.7	0.082283	0.078108	0.004175
4.6	184.8	0.073561	0.073981	-0.000420

Appendix 6.5: IDENTIFICATION OF ISOLATES FROM THE INITIAL AND SPOILAGE MICROBIOTA OF PASTEURISED, HOMOGENISED MILK.

Key:

Isol: Isolate Number.

NDP : Non-Diffusible Pigment.

- PY : pale yellow.
- Cr : cream.
- Y : yellow.
- Wh : white.

ClM : Cell Morphology.

- c : coccus.
- r : rod.
- sr : short rod.
- src: short rods in chains.
- srp: short rods in pairs.

Gr : Gram Reaction.

- \+ : positive character.
- \- : negative character.

OF : Mode of Glucose Utilisation.

- O : oxidative.
- F : fermentative.
- A : alkaline products.
- N : no change.

Flg : Flagella arrangement.

- Pol: polar flagellum.
- Per: peritrichous flagella.

Ox : Kovak's Oxidase Reaction.

Ca : Catalase Reaction.

PG : Psychrotrophic Growth.

KA : Diffusible Pigment Produced on King's B Medium.

Space: Character Not Determined.

Appendix 6.5a: Identification of the Initial Microbiota [2.1b(iii)].

Isol	NDP	ClM	Gr	OF	Mo	Ox	Ca	PG	Identification
1.1	PY	sr	+	F	-	-	+	-	coryneform
1.2	PY	sr	+	F	-	-	+	-	coryneform
1.3	PY	sr	+	F	-	-	+	-	coryneform
1.4	PY	sr	+	F	-	-	+	-	coryneform
1.5	PY	sr	+	F	-	-	+	-	coryneform
1.6	PY	sr	+	F	-	-	+	-	coryneform
1.7	Cr	sr	+	F	-	-	+	-	coryneform
1.8									No Growth
1.9									No Growth
1.10	PY	sr	+	N	-	-	+	-	coryneform
1.11	PY	sr	+	F	-	-	+	-	coryneform
1.12	PY	sr	+	F	-	-	+	-	coryneform
1.13	PY	sr	+	F	-	-	+	-	coryneform
1.14	PY	sr	+	F	-	-	+	-	coryneform
1.15	PY	c	+	O	-	-	+	-	*Micrococcus* sp.
1.16	PY	sr	+	F	-	-	+	-	coryneform
1.17	PY	sr	+	F	-	-	+	-	coryneform
1.18	PY	sr	+	F	-	-	+	-	coryneform
1.19	PY	sr	+	F	-	-	+	-	coryneform
1.20	PY	sr	+	F	-	-	+	-	coryneform
1.21	PY	sr	+	F	-	-	+	-	coryneform
1.22	PY	sr	+	F	-	-	+	-	coryneform
1.23	PY	sr	+	F	-	-	+	-	coryneform
1.24	PY	sr	+	F	-	-	+	-	coryneform
1.25	PY	sr	+	O	-	-	+	-	coryneform
1.26	PY	sr	+	F	-	-	+	-	coryneform
1.27	PY	sr	+	F	-	-	+	-	coryneform
1.28	PY	sr	+	F	-	-	+	-	coryneform
1.29	PY	sr	+	F	-	-	+	-	coryneform
1.30	PY	sr	+	F	-	-	+	-	coryneform
1.31	PY	sr	+	F	-	-	+	-	coryneform
1.32	PY	sr	+	F	-	-	+	-	coryneform
1.33	PY	sr	+	F	-	-	+	-	coryneform
1.34	PY	sr	+	F	-	-	+	-	coryneform
1.35	PY	c	+	O	-	-	+	-	*Micrococcus* sp.
1.36	PY	sr	+	F	-	-	+	-	coryneform
1.37	Y	sr	+	F	-	-	+	-	coryneform
1.38	PY	sr	+	F	-	-	+	-	coryneform
1.39	PY	sr	+	F	-	-	+	-	coryneform
1.40	PY	c	+	O	-	-	+	-	*Micrococcus* sp.
1.41	PY	sr	+	F	-	-	+	-	coryneform

Appendix 6.5b: Identification of the 23.4°C Spoilage Microbiota [2.1b(iii)].

Isol	NDP	ClM	Gr	OF	Mo	Flg	Ox	Ca	PG	Identification
2.1										No Growth
2.2	Y	c	+	O	-		-	+	-	*Micrococcus* sp.
2.3										No Growth
2.4	PY	sr	+	O	-		-	+	-	coryneform
2.5										No Growth
2.6	PY	sr	+	F	-		-	+	-	coryneform
2.7	PY	sr	+	F	-		-	+	-	coryneform
2.8										No Growth
2.9										No Growth
2.10	Cr	src	+	N	-		-	-	-	*Lactobacillus* sp.
2.11	Cr	src	+	N	-		-	-	-	*Lactobacillus* sp.
2.12	Cr	src	+	N	-		-	-	-	*Lactobacillus* sp.
2.13	Cr	src	+	N	-		-	-	-	*Lactobacillus* sp.
2.14	Cr	src	+	N	-		-	-	-	*Lactobacillus* sp.
2.15	Cr	src	+	N	-		-	-	-	*Lactobacillus* sp.
2.17	Cr	lr	+	F	+	Per	+	+	-	*Bacillus* sp.
2.16	Cr	lr	+	F	+	Per	+	+	-	*Bacillus* sp.
2.17	Cr	src	+	N	-		-	-	-	*Lactobacillus* sp.
2.18	Cr	src	+	N	-		-	-	-	*Lactobacillus* sp.
2.19	Cr	src	+	N	-		-	-	-	*Lactobacillus* sp.
2.20	Cr	src	+	N	-		-	-	-	*Lactobacillus* sp.
2.21	Cr	src	+	N	-		-	-	-	*Lactobacillus* sp.
2.22	Cr	src	+	N	-		-	-	-	*Lactobacillus* sp.
2.23										No Growth
2.24	Cr	src	+	N	-		-	-	-	*Lactobacillus* sp.
2.25	Cr	lr	+	F	+	Per	+	+	-	*Bacillus* sp.
2.26										No Growth
2.27	Cr	src	+	N	-		-	-	-	*Lactobacillus* sp.
2.28	Cr	src	+	N	-		-	-	-	*Lactobacillus* sp.
2.29	Cr	src	+	N	-		-	-	-	*Lactobacillus* sp.
2.30										No Growth
2.31										No Growth
2.32	Cr	lr	+	F	+	Per	+	+	-	*Bacillus* sp.

Appendix 6.5c: Identification of the 15.4°C Spoilage Microbiota [2.1b(iii)].

Isol	NDP	ClM	Gr	OF	Mo	Flg	Ox	Ca	PG	KA	Identification
3.1	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
3.2	Cr	lr	+	F	+	Per	+	+	-		Bacillus sp.
3.3	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
3.4	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
3.5	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
3.6	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
3.7	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
3.8	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
3.9	Wh	src	+	F	-		-	-	-		Lactobacillus sp.
3.10	Wh	src	+	O	-		-	-	-		Lactobacillus sp.
3.11	Cr	lr	+	F	+	Per	+	+	-		Bacillus sp.
3.12	Cr	src	+	F	-		-	-	-		Lactobacillus sp.
3.13	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
3.14	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
3.15	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
3.16	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
3.17	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
3.18	Cr	src	+	O	-		-	-	-		Lactobacillus sp.
3.19	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
3.20	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
3.21	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
3.22	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
3.23	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
3.24	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
3.25	Cr	src	+	F	-		-	-	-		Lactobacillus so.
3.26	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
3.27	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
3.28	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
3.29	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
3.30	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
3.31	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
3.32	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.

Appendix 6.5d: Identification of the 10.1°C Spoilage Microbiota 2.1b(iii)].

Isol	NDP	ClM	Gr	OF	Mo	Flg	Ox	Ca	PG	KA	Identification
4.1	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
4.2	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
4.3	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
4.4	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
4.5	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
4.6	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
4.7	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
4.8	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
4.9	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
4.10	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
4.11	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
4.12	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
4.13	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
4.14	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
4.15	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
4.16	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
4.17	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
4.18	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
4.19	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
4.20	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
4.21	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
4.22	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
4.43	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
4.24	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.

Appendix 6.5e: Identification of the 6.6°C Spoilage Microbiota [2.1b(iii)].

Isol	NDP	ClM	Gr	OF	Mo	Flg	Ox	Ca	PG	KA	Identification
5.1	Cr	r	-	O	+	Pol	+	+	+	-	*Pseudomonas* sp.
5.2	Cr	r	-	O	+	Pol	+	+	+	-	*Pseudomonas* sp.
5.3	Cr	r	-	O	+	Pol	+	+	+	-	*Pseudomonas* sp.
5.4	Cr	r	-	O	+	Pol	+	+	+	-	*Pseudomonas* sp.
5.5	Cr	r	-	O	+	Pol	+	+	+	-	*Pseudomonas* sp.
5.6	Cr	r	-	O	+	Pol	+	+	+	-	*Pseudomonas* sp.
5.7	Cr	r	-	O	+	Pol	+	+	+	-	*Pseudomonas* sp.
5.8	Cr	r	-	O	+	Pol	+	+	+	-	*Pseudomonas* sp.
5.9	Cr	r	-	O	+	Pol	+	+	+	-	*Pseudomonas* sp.
5.10	Cr	r	-	O	+	Pol	+	+	+	-	*Pseudomonas* sp.
5.11	Cr	r	-	O	+	Pol	+	+	+	-	*Pseudomonas* sp.
5.12	Cr	r	-	O	+	Pol	+	+	+	-	*Pseudomonas* sp.
5.13	Cr	r	-	O	+	Pol	+	+	+	-	*Pseudomonas* sp.
5.14	Cr	r	-	O	+	Pol	+	+	+	-	*Pseudomonas* sp.
5.15	Cr	r	-	O	+	Pol	+	+	+	-	*Pseudomonas* sp.
5.16	Cr	r	-	O	+	Pol	+	+	+	-	*Pseudomonas* sp.
5.17	Cr	r	-	O	+	Pol	+	+	+	-	*Pseudomonas* sp.
5.18	Cr	r	-	O	+	Pol	+	+	+	-	*Pseudomonas* sp.
5.19	Cr	r	-	O	+	Pol	+	+	+	-	*Pseudomonas* sp.
5.20	Cr	r	-	O	+	Pol	+	+	+	-	*Pseudomonas* sp.
5.21	Cr	r	-	O	+	Pol	+	+	+	-	*Pseudomonas* sp.

Appendix 6.5f: Identification of the 4.2°C Spoilage Microbiota [2.1b(iii)].

Isol	NDP	ClM	Gr	OF	Mo	Flg	Ox	Ca	PG	KA	Identification
6.1	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
6.2	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
6.3	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
6.4	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
6.5	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
6.6	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
6.7	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
6.8	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
6.9	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
6.10	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
6.11	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
6.12	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
6.13	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
6.14	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
6.15	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
6.16	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
6.17	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
6.18	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
6.19	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
6.20	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
6.21	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
6.22	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
6.23	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
6.24	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
6.25	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
6.26	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
6.27	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
6.28	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
6.29	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
6.30	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
6.31	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
6.32	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
6.33	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
6.34	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.

Appendix 6.5g: Identification of the Initial Microbiota [2.1b(v)].

Isol	NDP	ClM	Gr	OF	Mo	Flg	Ox	Ca	PG	Identification
7.1	Cr	sr	+	O	-		-	+	-	coryneform
7.2	Cr	sr	+	N	-		-	+	-	coryneform
7.3	Cr	sr	+	O	-		-	+	-	coryneform
7.4	PY	sr	+	O	-		-	+	-	coryneform
7.5	Cr	sr	+	O	-		-	+	-	coryneform
7.6	Cr	sr	+	O	-		-	+	-	coryneform
7.7	Cr	sr	+	O	-		-	+	-	coryneform
7.8	PY	sr	+	O	-		-	+	-	coryneform
7.9	Cr	sr	+	O	-		-	+	-	coryneform
7.10	PY	sr	+	O	-		-	+	-	coryneform
7.11	BY	sr	+	O	-		-	+	-	coryneform
7.12	BY	c	+	O	-		-	+	-	Micrococcus sp.
7.13	BY	c	+	O	-		-	+	-	Micrococcus sp.
7.14	PY	sr	+	O	-		-	+	-	coryneform
7.15	PY	sr	+	N	-		-	+	-	coryneform
7.16	Cr	sr	+	O	-		-	+	-	coryneform
7.17	PY	sr	+	O	-		-	+	-	coryneform
7.18	BY	c	+	O	-		-	+	-	Micrococcus sp.
7.19	Cr	sr	+	O	-		-	+	-	coryneform
7.20	PY	sr	+	O	-		-	+	-	coryneform
7.21	Cr	sr	+	N	-		-	+	-	coryneform
7.22	Cr	sr	+	N	-		-	+	-	coryneform
7.23	Cr	sr	+	O	-		-	+	-	coryneform
7.24	BY	c	+	O	-		-	+	-	Micrococcus sp.
7.25	PY	sr	+	N	-		-	+	-	coryneform
7.26	Cr	sr	+	O	-		-	+	-	coryneform
7.27	PY	sr	+	N	-		-	+	-	coryneform
7.28	BY	c	+	O	-		-	+	-	Micrococcus sp.
7.29	BY	c	+	O	-		-	+	-	Micrococcus sp.
7.30	PY	sr	+	O	-		-	+	-	coryneform
7.31	Cr	sr	+	O	-		-	+	-	coryneform
7.32	Cr	sr	+	O	-		-	+	-	coryneform
7.33	Cr	sr	+	O	-		-	+	-	coryneform
7.34	BY	c	+	O	-		-	+	-	Micrococcus sp.
7.35	PY	sr	+	O	-		-	+	-	coryneform
7.36	Cr	sr	+	O	-		-	+	-	coryneform
7.37	Cr	sr	+	O	-		-	+	-	coryneform
7.38	PY	sr	+	O	-		-	+	-	coryneform
7.39	Cr	r	+	O	+	Per	+	+	-	Bacillus sp.
7.40	Cr	sr	+	O	-		-	+	-	coryneform
7.41	Cr	sr	+	O	-		-	+	-	coryneform
7.42	PY	sr	+	O	-		-	+	-	coryneform
7.43	PY	sr	+	O	-		-	+	-	coryneform
7.44	PY	sr	+	O	-		-	+	-	coryneform
7.45	Cr	sr	+	N	-		-	+	-	coryneform
7.46	Cr	sr	+	O	-		-	+	-	coryneform
7.47	PY	sr	+	O	-		-	+	-	coryneform
7.48	BY	c	+	O	-		-	+	-	Micrococcus sp.
7.49	Cr	srp	-	O	-		-	+	-	Acinetobacter sp.

Appendix 6.5h: Identification of the 25.0°C Spoilage Microbiota [2.1b(v)].

Isol	NDP	ClM	Gr	OF	Mo	Flg	Ox	Ca	PG	Identification
8.1	Cr	srp	-	O	-		-	+	-	Acinetobacter sp.
8.2	Cr	srp	-	O	-		-	+	-	Acinetobacter sp.
8.3	Cr	lr	+	A	+	Per	+	+	-	Bacillus sp.
8.4	Cr	srp	-	O	-		-	+	-	Acinetobacter sp.
8.5	Cr	srp	-	O	-		-	+	-	Acinetobacter sp.
8.6	Cr	srp	-	O	-		-	+	-	Acinetobacter sp.
8.7	Cr	srp	-	O	-		-	+	-	Acinetobacter sp.
8.8	Cr	srp	-	O	-		-	+	-	Acinetobacter sp.
8.9	Cr	lr	+	O	+	Per	+	+	-	Bacillus sp.
8.10	Cr	srp	-	O	-		-	+	-	Acinetobacter sp.
8.11	Cr	srp	-	O	-		-	+	-	Acinetobacter sp.
8.12	Cr	srp	-	O	-		-	+	-	Acinetobacter sp.
8.13	Cr	srp	-	O	-		-	+	-	Acinetobacter sp.
8.14	Cr	srp	-	O	-		-	+	-	Acinetobacter sp.
8.15	Cr	srp	-	O	-		-	+	-	Acinetobacter sp.
8.16	Cr	srp	-	O	-		-	+	-	Acinetobacter sp.
8.17	Cr	srp	-	O	-		-	+	-	Acinetobacter sp.
8.18	Cr	lr	+	O	+	Per	+	+	-	Bacillus sp.
8.19	Cr	srp	-	O	-		-	+	-	Acinetobacter sp.
8.20	Cr	srp	-	O	-		-	+	-	Acinetobacter sp.
8.21	Cr	srp	-	O	-		-	+	-	Acinetobacter sp.
8.22	Cr	srp	-	O	-		-	+	-	Acinetobacter sp.
8.23	Cr	lr	+	O	+	Per	+	+	-	Bacillus sp.
8.24	Cr	srp	-	O	-		-	+	-	Acinetobacter sp.
8.25	Cr	srp	-	O	-		-	+	-	Acinetobacter sp.
8.26	Cr	srp	-	O	-		-	+	-	Acinetobacter sp.
8.27	Cr	lr	+	O	+	Per	+	+	-	Bacillus sp.
8.28	Cr	srp	-	O	-		-	+	-	Acinetobacter sp.
8.29	Cr	srp	-	O	-		-	+	-	Acinetobacter sp.
8.30	Cr	srp	-	O	-		-	+	-	Acinetobacter sp.
8.31	Cr	srp	-	O	-		-	+	-	Acinetobacter sp.
8.32	Cr	srp	-	O	-		-	+	-	Acinetobacter sp.
8.33	Wh	srp	-	A	-		-	+	-	Acinetobacter sp.
8.34	Cr	srp	-	O	-		-	+	-	Acinetobacter sp.
8.35	Cr	srp	-	O	-		-	+	-	Acinetobacter sp.
8.36	Cr	srp	-	O	-		-	+	-	Acinetobacter sp.
8.37	Cr	srp	-	O	-		-	+	-	Acinetobacter sp.
8.38	Wh	lr	+	O	+	Per	+	+	-	Bacillus sp.
8.39	Wh	srp	-	O	-		-	+	-	Acinetobacter sp.
8.40	Cr	srp	-	O	-		-	+	-	Acinetobacter sp.

Appendix 6.5i: Identification of the 19.7°C Spoilage Microbiota [2.1b(v)].

Isol	NDP	ClM	Gr	OF	Mo	Flg	Ox	Ca	PG	KA	Identification
9.1	Cr	r	-	O	+	Pol	+	+	+	+	Pseudomonas sp.
9.2	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
9.3	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
9.4	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
9.5	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
9.6	Cr	srp	-	O	-		-	+	-		Acinetobacter sp.
9.7	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
9.8	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
9.9	Cr	r	-	F	+	Per	-	+	+		Enterobacteriaceae sp.
9.10	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
9.11	Cr	r	-	O	+	Pol	+	+	+	+	Pseudomonas sp.
9.12	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
9.13	Cr	srp	-	N	-		-	+	-		Acinetobacter sp.
9.14	Cr	r	-	F	+	Per	-	+	+		Enterobacteriaceae sp.
9.15	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
9.16	Cr	r	-	F	+	Per	-	+	+		Enterobacteriaceae sp.
9.17	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
9.18	Cr	r	-	F	+	Per	-	+	+		Enterobacteriaceae sp.
9.19	Cr	srp	-	O	-		-	+	-		Acinetobacter sp.
9.20	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
9.21	Cr	r	-	F	+	Per	-	+	+		Enterobacteriaceae sp.
9.22	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
9.23	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
9.24	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
9.25	Cr	r	-	F	+	Per	-	+	+		Enterobacteriaceae sp.
9.26	Cr	r	-	F	+	Per	-	+	+		Enterobacteriaceae sp.
9.27	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
9.28	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
9.29	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
9.30	Cr	r	-	F	+	Per	-	+	+		Enterobacteriaceae sp.
9.31	Cr	srp	-	N	-		-	+	-		Acinetobacter sp.
9.32	Cr	r	-	F	+	Per	-	+	+		Enterobacteriaceae sp.
9.33	Cr	srp	-	O	-		-	+	-		Acinetobacter sp.
9.34	Cr	r	-	F	+	Per	-	+	+		Enterobacteriaceae sp.
9.35	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
9.36	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
9.37	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
9.38	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
9.39	Cr	r	-	F	+	Per	-	+	+		Enterobacteriaceae sp.
9.40	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
9.41	Cr	r	-	F	+	Per	-	+	+		Enterobacteriaceae sp.
9.42	Cr	r	-	O	+	Pol	+	+	+	+	Pseudomonas sp.
9.43	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
9.44	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
9.45	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
9.46	Cr	r	-	F	+	Per	-	+	+		Enterobacteriaceae sp.
9.47	Cr	r	-	O	+	Pol	+	+	+	+	Pseudomonas sp.
9.48	Cr	r	-	F	+	Per	-	+	+		Enterobacteriaceae sp.

Appendix 6.5j: Identification of the 15.6°C Spoilage Microbiota [2.1b(v)].

Isol	NDP	ClM	Gr	OF	Mo	Flg	Ox	Ca	PG	KA	Identification
10.1	Cr	r	-	F	+	Per	-	+	+		Enterobacteriaceae sp.
10.2	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
10.3	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
10.4	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
10.5	Cr	r	-	F	+	Per	-	+	+		Enterobacteriaceae sp.
10.6	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
10.7	Cr	r	-	F	+	Per	-	+	+		Enterobacteriaceae sp.
10.8	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
10.9	Cr	r	-	F	+	Per	-	+	+		Enterobacteriaceae sp.
10.10	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
10.11	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
10.12	Cr	srp	-	O	-		-	+	-		Acinetobacter sp.
10.13	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
10.14	Wh	sr	+	N	-		-	-	-		Lactobacillus sp.
10.15	Cr	srp	-	O	-		-	+	-		Acinetobacter sp.
10.16	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
10.17	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
10.18	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
10.19	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
10.20	Cr	r	-	F	+	Per	-	+	+		Enterobacteriaceae sp.
10.21	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
10.22	Cr	srp	-	O	-		-	+	-		Acinetobacter sp.
10.23	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
10.24	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
10.25	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
10.26	Cr	r	-	F	+	Per	-	+	+		Enterobacteriaceae sp.
10.27	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
10.28	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
10.29	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
10.30	Cr	srp	-	O	-		-	+	-		Acinetobacter sp.
10.31	Wh	sr	+	O	-		-	-	-		Lactobacillus sp.
10.32	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
10.33	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
10.34	Cr	r	-	F	+	Per	-	+	+		Enterobacteriaceae sp.
10.35	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
10.36	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
10.37	Cr	r	-	F	+	Per	-	+	+		Enterobacteriaceae sp.
10.38	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
10.39	Cr	srp	-	N	-		-	+	-		Acinetobacter sp.
10.40	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
10.41	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
10.42	Cr	r	-	F	+	Per	-	+	+		Enterobacteriaceae sp.
10.43	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
10.44	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
10.45	Cr	r	-	F	+	Per	-	+	+		Enterobacteriaceae sp.
10.46	Cr	r	-	F	+	Per	-	+	+		Enterobacteriaceae sp.
10.47	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
10.48	Cr	sr	-	F	+	Per	-	+	-		Enterobacteriaceae sp.

Appendix 6.5k: Identification of the 11.5°C Spoilage Microbiota [2.1b(v)].

Isol	NDP	ClM	Gr	OF	Mo	Flg	Ox	Ca	PG	KA	Identification
11.1	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
11.2	Cr	r	-	F	+	Per	-	+	+		Enterobacteriaceae sp.
11.3	Cr	r	-	F	+	Per	-	+	+		Enterobacteriaceae sp.
11.4	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
11.5	Cr	r	-	F	+	Per	-	+	+		Enterobacteriaceae sp.
11.6	Cr	r	-	O	+	Pol	+	+	+	+	Pseudomonas sp.
11.7	Cr	r	-	O	+	Pol	+	+	+	+	Pseudomonas sp.
11.8	Cr	srp	-	O	-		-	+	-		Acinetobacter sp.
11.9	Cr	srp	-	O	-		-	+	-		Acinetobacter sp.
11.10	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
11.11	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
11.12	Cr	r	-	F	+	Per	-	+	+		Enterobacteriaceae sp.
11.13	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
11.14	Cr	r	-	F	+	Per	-	+	+		Enterobacteriaceae sp.
11.15	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
11.16	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
11.17	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
11.18	Cr	srp	-	N	-		-	+	-		Acinetobacter sp.
11.19	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
11.20	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
11.21	Cr	r	-	F	+	Per	-	+	+		Enterobacteriaceae sp.
11.22	Cr	srp	-	N	-		-	+	-		Acinetobacter sp.
11.23	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
11.24	Cr	r	-	O	+	Pol	+	+	+	+	Pseudomonas sp.
11.25	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
11.26	Cr	r	-	F	+	Per	-	+	+		Enterobacteriaceae sp.
11.27	Cr	r	-	F	+	Per	-	+	+		Enterobacteriaceae sp.
11.28	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
11.29	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
11.30	Cr	r	-	F	+	Per	-	+	+		Enterobacteriaceae sp.
11.31	Cr	r	-	F	+	Per	-	+	+		Enterobacteriaceae sp.
11.32	Cr	r	-	F	+	Per	-	+	+		Enterobacteriaceae sp.
11.33	Cr	r	-	F	+	Per	-	+	+		Enterobacteriaceae sp.
11.34	Cr	r	-	O	+	Pol	-	+	+	+	Pseudomonas sp.
11.35	Cr	r	-	O	+	Pol	-	+	+	+	Pseudomonas sp.
11.36	Cr	r	-	O	+	Pol	-	+	+	-	Pseudomonas sp.
11.37	Cr	r	-	O	+	Pol	-	+	+	+	Pseudomonas sp.
11.38	Cr	r	-	O	+	Pol	-	+	+	-	Pseudomonas sp.
11.39	Cr	r	-	O	+	Pol	-	+	+	-	Pseudomonas sp.
11.40	Cr	r	-	O	+	Pol	-	+	+	+	Pseudomonas sp.
11.41	Cr	r	-	O	+	Pol	-	+	+	-	Pseudomonas sp.
11.42	Cr	r	-	O	+	Pol	-	+	+	-	Pseudomonas sp.
11.43	Cr	r	-	O	+	Pol	-	+	+	-	Pseudomonas sp.
11.44	Cr	r	-	O	+	Pol	-	+	+	-	Pseudomonas sp.

Appendix 6.51: Identification of the 6.3°C Spoilage Microbiota [2.1b(v)].

Isol	NDP	ClM	Gr	OF	Mo	Flg	Ox	Ca	PG	KA	Identification
12.1	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.2	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.3	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.4	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.5	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.6	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.7	Cr	r	-	O	+	Pol	+	+	+	+	Pseudomonas sp.
12.8	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.9	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.10	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.11	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.12	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.13	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.14	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.15	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.16	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.17	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.18	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.19	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.20	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.21	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.22	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.23	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.24	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.25	Cr	r	-	O	+	Pol	+	+	+	+	Pseudomonas sp.
12.26	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.27	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.28	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.29	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.30	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.31	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.32	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.33	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.34	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.35	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.36	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.37	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.38	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.39	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.40	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.41	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.42	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.43	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.44	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.45	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.46	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.47	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.
12.48	Cr	r	-	O	+	Pol	+	+	+	-	Pseudomonas sp.

APPENDIX 6.6: DATA FOR MICROBIAL NUMBERS VERSUS TIME/TEMPERATURE FUNCTION INTEGRATOR READINGS (FIGURE 3.7).

PCA	PsA	TTFI	PCA	PsA	TTFI
7.66	7.66	9.02	8.17	8.17	8.87
7.28	7.28	9.20	7.00	7.00	9.00
7.78	7.78	9.33	8.07	8.07	10.63
7.48	7.48	8.20	6.93	6.93	9.82
7.99	7.99	10.42	7.26	7.26	8.78
7.92	7.92	11.87	7.69	7.69	10.40
7.23	7.23	9.85	7.49	7.49	9.82
7.00	7.00	9.90	6.90	6.90	9.68
5.85	5.85	9.96	6.30	6.30	9.92
6.64	6.64	7.61	3.21	-	1.23
2.97	1.54	2.47	2.83	2.64	3.72
3.90	3.90	5.07	3.41	1.00	1.20
3.42	2.08	2.40	3.11	3.15	3.67
4.05	4.00	5.03	3.58	-	1.02
3.60	1.03	2.43	3.55	2.61	3.70
3.08	3.98	5.02	5.03	5.17	6.17
3.01	0.70	1.78	2.82	3.12	3.72
5.39	5.70	5.78	7.07	7.32	7.67
7.44	7.61	8.50	7.59	7.71	9.68
3.09	-	1.68	3.03	2.06	2.67
2.85	2.23	3.67	3.16	3.02	4.47
4.22	4.06	5.40	4.65	4.78	6.28
6.41	6.36	7.52	7.18	6.88	8.42
8.09	8.07	9.87	2.95	-	1.72
3.00	-	2.93	3.01	1.48	3.88
3.35	3.13	4.85	7.28	7.13	9.83
6.20	6.10	8.13			

PCA : log of microbial numbers (cfu mL^{-1}) on PCA.

PsA : log of pseudomonad numbers (cfu mL^{-1}) on PsA.

TTFI: time/temperature function integrator reading in days.

APPENDIX 6.7: DATA FOR CALIBRATION OF NEPHELOMETER FOR PSEUDOMONAS SP. STRAIN E5.2 (FIGURE 3.8).

Appendix 6.7a: Data for Figure 3.8a.

OD	PCA
0.267	1.27×10^8
0.131	5.20×10^7
0.041	2.11×10^7
0.026	1.07×10^7

Appendix 6.7b: Data for Figure 3.8b.

Time	OD
260.2	0.020
299.1	0.061
328.5	0.111
348.1	0.210
358.7	0.275
374.7	0.526

OD : optical density.

PCA : microbial numbers (cfu mL^{-1}) on PCA.

Time: time in minutes.

APPENDIX 6.8: DATA FOR THE SQUARE ROOT PLOTS OF TWO BACTERIA ISOLATED FROM PASTEURISED, HOMOGENISED MILK (FIGURE 3.9).

Temp.: temperature (K).

OD0.3: time for change in OD = 0.3 (rate = 1/OD0.3).

O.RR.: observed square root of growth rate.

P.RR.: predicted square root of growth rate using least squares analysis.

GT : generation time (rate = 1/GT).

All times in minutes.

Appendix 6.8a: Data for Figure 3.9a and Figure 3.9b.

Figure 3.9a:

Temp.	ODO.3	O.RR.	P.RR.	Residual
278.8	1745	0.023939	0.023536	0.000403
279.9	1389	0.026832	0.026435	0.000397
281.7	1163	0.029323	0.031179	-0.001856
283.0	877	0.033768	0.034605	-0.000837
284.1	751	0.036491	0.037504	-0.001013
285.2	604	0.040689	0.040403	0.000286
285.9	521	0.043811	0.042248	0.001563
287.3	454	0.046932	0.045938	0.000994
289.0	381	0.051232	0.050418	0.000814
290.2	352	0.053300	0.053581	-0.000281
291.4	308	0.056980	0.056743	0.000237
292.3	287	0.059028	0.059115	-0.000087
293.5	256	0.062500	0.062278	0.000222
295.4	216	0.068041	0.067285	0.000756
297.1	197	0.071247	0.071765	-0.000518
298.1	186	0.073324	0.074401	-0.001077

Figure 3.9b:

Temp.	GT	O.RR.	P.RR.	Residual
281.7	246	0.063758	0.067782	-0.004024
283.0	178	0.074953	0.075817	-0.000864
284.1	153	0.080845	0.082616	-0.001771
285.2	125	0.089443	0.089414	0.000029
285.9	103	0.098533	0.093741	0.004792
287.3	87	0.107211	0.102394	0.004817
289.0	77	0.113961	0.112901	0.001060
290.2	68	0.121268	0.120317	0.000951
291.4	62	0.127000	0.127734	-0.000734
292.3	56	0.133631	0.133297	0.000334
293.5	54	0.136083	0.140713	-0.004630
295.4	43	0.152499	0.152457	0.000042

Appendix 6.8b: Data for Figure 3.9c and Figure 3.9d.

Figure 3.9c:

Temp.	OD0.3	O.RR.	P.RR.	Residual
280.4	853.7	0.034225	0.033350	0.000875
281.7	686.8	0.038158	0.036956	0.001202
283.0	568.0	0.041959	0.040562	0.001397
284.4	496.0	0.044901	0.044445	0.000456
285.8	434.2	0.047990	0.048329	-0.000339
287.2	382.7	0.051118	0.052212	-0.001094
288.5	338.1	0.054385	0.055818	-0.001433
289.7	299.1	0.057822	0.059146	-0.001324
290.7	272.7	0.060556	0.061920	-0.001364
291.9	244.4	0.063966	0.065249	-0.001283
293.0	220.0	0.067420	0.068300	-0.000880
294.0	195.6	0.071502	0.071074	0.000428
295.0	182.9	0.073942	0.073847	0.000095
296.1	167.4	0.077290	0.076899	0.000391
297.6	152.4	0.081004	0.080227	0.000777
298.2	144.4	0.083218	0.082723	0.000495
299.1	135.3	0.085971	0.085220	0.000751
300.0	127.6	0.088527	0.087716	0.000811
301.3	119.8	0.091363	0.091322	0.000041

Figure 3.9d:

Temp.	GT	O.RR.	P.RR.	Residual
280.4	182.0	0.074125	0.075442	-0.001317
281.7	139.0	0.084819	0.083174	0.001645
283.0	117.0	0.092450	0.090905	0.001500
284.4	97.0	0.101535	0.099231	0.002304
285.8	84.0	0.109109	0.107558	0.001551
287.2	76.0	0.114708	0.115884	-0.001176
288.5	67.0	0.122169	0.123616	-0.001447
289.7	60.0	0.129099	0.130753	-0.001654
290.7	56.0	0.133631	0.136700	-0.003069
291.9	51.0	0.140028	0.143837	-0.003809
293.0	46.0	0.147442	0.150379	-0.002937
294.0	41.0	0.156174	0.156326	-0.000152
295.0	36.0	0.166667	0.162274	0.004393
296.1	34.5	0.170351	0.168816	0.001535
297.3	31.0	0.179605	0.175952	0.003653
298.2	30.0	0.182574	0.181305	0.001269
299.1	29.0	0.185695	0.186658	-0.000963
300.0	27.0	0.192450	0.192010	0.000440
301.3	25.5	0.198030	0.199742	-0.001712

APPENDIX 6.9: DATA FOR THE SQUARE ROOT PLOTS OF THE EFFECT OF TEMPERATURE ON THE LAG PHASE AND GROWTH RATE OF PSEUDOMONAS SP. STRAIN E5.2 (FIGURE 3.11 and FIGURE 3.12).

Temp. : temperature (K).

OD0.3 : time for change in OD = 0.3 (rate = 1/OD0.3).

O.O.RR.: observed square root of growth rate (OD).

P.O.RR.: predicted square root of growth rate (OD).

GT : generation time (rate = 1/GT).

O.G.RR.: observed square root of growth rate (GT).

P.G.RR.: predicted square root of growth rate (GT).

Lag : duration of lag phase (rate = 1/Lag).

O.L.RR.: observed square root of rate (Lag).

P.L.RR.: predicted square root of rate (Lag).

All times in minutes.

All predicted values determined by least squares linear regression.

Appendix 6.9a: Data for Figure 3.11a, Figure 3.11b and Figure 3.11c.

Figure 3.11a:

Temp.	OD0.3	O.O.RR.	P.O.RR.	Residual
280.6	861	0.034080	0.034233	-0.000153
282.3	693	0.037987	0.038486	-0.000499
283.8	590	0.041169	0.042238	-0.001069
285.1	479	0.045691	0.045902	-0.000211
286.5	407	0.049568	0.048993	0.000575
288.0	364	0.052414	0.052745	-0.000331
289.4	309	0.056888	0.056248	0.000640
290.9	282	0.059549	0.060000	-0.000451
291.9	257	0.062378	0.062502	-0.000124
293.1	225	0.066667	0.065504	0.001163
294.4	200	0.070711	0.068756	0.001955
295.8	189	0.072739	0.072259	0.000480
297.4	175	0.075593	0.076261	-0.000668
300.8	145	0.083046	0.084767	-0.001721

Figure 3.11b:

Temp.	GT	O.G.RR.	P.G.RR.	Residual
280.6	154.5	0.080452	0.079985	0.000467
282.3	120.0	0.091287	0.089694	0.001593
283.8	106.0	0.097129	0.098261	-0.001132
285.1	93.5	0.103418	0.105686	-0.002268
286.5	82.5	0.110096	0.113682	-0.003586
288.0	68.0	0.121268	0.122249	-0.000981
289.4	58.5	0.130744	0.130245	0.000499
290.9	52.0	0.138675	0.138812	-0.000137
291.9	45.0	0.149071	0.144524	0.004547
293.1	43.0	0.152499	0.151377	0.001122
294.4	37.5	0.163299	0.158802	0.004497
295.8	36.0	0.166667	0.166798	-0.000131
297.4	32.5	0.175412	0.175936	-0.000526
300.8	27.3	0.191390	0.195355	-0.003965

Appendix 6.9a: Data for Figure 3.11a, Figure 3.11b and Figure 3.11c Continued.

Figure 3.11c:

Temp.	Lag	O.L.RR.	P.L.RR.	Residual
280.6	536.6	0.043169	0.043712	-0.000543
282.3	441.0	0.047619	0.049205	-0.001586
283.8	367.4	0.052171	0.054051	-0.001888
285.1	282.7	0.059475	0.058252	0.001223
286.5	233.8	0.065400	0.062775	0.002625
288.0	221.2	0.067237	0.067622	-0.000385
289.4	186.2	0.073284	0.072145	0.001139
290.9	172.8	0.076073	0.076992	-0.000919
291.9	162.5	0.078446	0.080223	-0.001777
293.1	134.7	0.086162	0.084100	0.002062
294.4	121.3	0.090685	0.088300	0.002385
295.8	113.4	0.093906	0.092824	0.001082
297.4	106.8	0.096764	0.097994	-0.001230
300.8	87.7	0.106783	0.108979	-0.002196

Appendix 6.9b: Data for Figure 3.12a, Figure 3.12b and Figure 3.12c.

Figure 3.12a:

Temp.	OD.0.3	O.O.RR.	P.O.RR.	Residual
277.6	1746	0.023932	0.023252	0.000680
282.8	758	0.036322	0.036774	-0.000452
284.4	628	0.039904	0.040934	-0.001030
285.6	561	0.042220	0.044055	-0.001835
286.8	460	0.046625	0.047175	-0.000550
288.2	379	0.051367	0.050815	0.000552
289.8	331	0.054965	0.054976	-0.000011
291.0	291	0.058621	0.058096	0.000525
292.3	249	0.063372	0.061476	0.001896
293.5	234	0.065372	0.064597	0.000775
294.8	203	0.070186	0.067977	0.002209
296.4	192	0.072169	0.072138	0.000031
297.7	176	0.075378	0.075518	-0.000140
298.9	164	0.078087	0.078638	-0.000551
300.0	156	0.080064	0.081499	-0.001435
301.3	141	0.084215	0.084879	-0.000664

Figure 3.12b:

Temp.	GT	O.G.RR.	P.G.RR.	Residual
277.6	260.0	0.062017	0.059918	0.002099
282.8	124.0	0.089803	0.090519	-0.000716
284.4	103.0	0.098533	0.099935	-0.001402
285.6	94.0	0.103142	0.106996	-0.003854
286.8	83.0	0.109764	0.114058	-0.004294
288.2	60.0	0.129099	0.122297	0.006802
289.8	55.0	0.134840	0.131712	0.003128
291.0	58.0	0.131306	0.138774	-0.007468
292.3	49.0	0.142857	0.146424	-0.003567
293.5	40.0	0.158114	0.153486	0.004628
294.8	35.8	0.167132	0.168774	-0.001642
296.4	33.0	0.174078	0.170552	0.003526
297.7	30.8	0.180188	0.178202	0.001985
298.9	29.4	0.184428	0.185264	-0.000836
300.0	28.0	0.188982	0.191737	-0.002755
301.3	26.0	0.196116	0.199387	-0.003271

Appendix 6.9b: Data for Figure 3.12a, Figure 3.12b and Figure 3.12c Continued.

Figure 3.12c:

Temp.	Lag	O.L.RR.	P.L.RR.	Residual
277.6	1200.0	0.028868	0.028098	0.000770
282.8	497.6	0.044829	0.045597	-0.000768
284.4	411.7	0.049284	0.050982	-0.001698
285.6	363.6	0.052443	0.055020	-0.002577
286.8	285.7	0.059162	0.059058	0.000104
288.2	253.0	0.062869	0.063780	-0.000911
289.8	215.5	0.068120	0.069154	-0.001034
291.0	169.2	0.076878	0.073192	0.003686
292.3	146.1	0.082732	0.077567	0.005165
293.5	150.0	0.081650	0.081605	0.000045
294.8	127.8	0.088457	0.085980	0.002477
296.4	122.7	0.090277	0.091365	-0.001088
297.7	111.3	0.094788	0.095740	-0.000952
298.9	102.3	0.098869	0.099778	-0.000909
300.0	97.2	0.101430	0.103480	-0.002050
301.3	86.4	0.107583	0.107854	-0.000271

APPENDIX 6.10: DATA FOR THE SQUARE ROOT PLOTS OF THE EFFECT OF TEMPERATURE ON THE LAG PHASE OF PSEUDOMONAS SP. STRAIN E5.2 (FIGURE 3.13).

Temp. : temperature (K).

OD0.02: time for a change in OD = 0.02 (rate = 1/OD0.02).

O.RR. : observed square root of rate (OD).

P.RR. : predicted square root of rate (OD).

All times in minutes.

All predicted values determined by least squares linear regression.

Appendix 6.10a: Data for Figure 3.13a.

Temp.	OD.02	O.RR.	P.RR.	Residual
282.8	377.0	0.051503	0.051710	-0.000207
284.1	326.5	0.055343	0.056963	-0.001620
285.4	252.0	0.062994	0.062216	0.000778
286.3	218.0	0.067729	0.065853	0.001876
288.1	190.0	0.072548	0.073127	-0.000579
289.1	170.5	0.076584	0.077168	-0.000584
290.8	131.6	0.087179	0.084037	0.003142
291.5	128.3	0.088285	0.086866	0.001419
292.7	113.7	0.093782	0.091715	0.002067
294.1	101.5	0.099258	0.097372	0.001886
295.3	98.5	0.100759	0.102221	-0.001462
296.3	91.5	0.104542	0.106262	-0.001720
297.6	79.5	0.112154	0.111515	0.000639
298.6	75.2	0.115316	0.115556	-0.000240

Appendix 6.10b: Data for Figure 3.13b.

Temp.	OD.02	O.RR.	P.RR.	Residual
283.3	409.0	0.049447	0.047871	0.001576
284.5	383.0	0.051098	0.051822	-0.000726
285.9	296.0	0.058124	0.056432	0.001692
287.2	292.8	0.058441	0.060712	-0.002271
288.5	245.5	0.063823	0.064992	-0.001169
290.0	218.8	0.067605	0.069930	-0.002325
291.0	181.5	0.074227	0.073223	0.001004
292.1	167.0	0.077382	0.076844	0.000538
293.3	146.5	0.082619	0.080795	0.001824
295.9	123.5	0.089984	0.089355	0.000629
297.1	116.3	0.092728	0.093306	-0.000578
298.2	105.2	0.097497	0.096927	0.000570
299.2	101.1	0.099455	0.100220	-0.000765

Appendix 6.10c: Data for 3.13c.

Temp.	OD.02	O.RR.	P.RR.	Residual
288.5	382.2	0.051150	0.051376	-0.000226
289.8	330.9	0.054973	0.054682	0.000291
290.8	316.0	0.056254	0.057225	-0.000971
291.9	278.0	0.059976	0.060023	-0.000047
292.9	247.0	0.063629	0.062566	0.001063
294.1	227.5	0.066299	0.065617	0.000682
295.4	217.5	0.067806	0.068924	-0.001118
296.3	197.0	0.071247	0.071212	0.000035
297.5	188.6	0.072816	0.074264	-0.001448
298.6	162.5	0.078447	0.077062	0.001385

APPENDIX 6.11: DATA FOR THE CALIBRATION OF NEPHELOMETER FOR STAPHYLOCOCCUS XYLOSUS STRAIN CM21/3 (FIGURE 3.14).

Appendix 6.11a: Data for Figure 3.14a.

Optical Density	Reciprocal of Dilution
0.608	1
0.305	2
0.175	4
0.084	8
0.039	16

Appendix 6.11b: Data for Figure 3.14b.

Optical Density	Reciprocal of Dilution
0.703	1
0.335	2
0.186	4
0.102	8
0.057	16

Appendix 6.11c: Data for Figure 3.14c.

Optical Density	Reciprocal of Dilution
0.510	1
0.277	2
0.123	4
0.058	8
0.028	16

APPENDIX 6.12: DATA FROM THE MEASUREMENT OF CELLS OF STAPHYLOCOCCUS XYLOSUS STRAIN CM21/3, GROWN IN MHB, MHB + 3.5 MOLAL SODIUM CHLORIDE AND MHB + 4.5 MOLAL GLYCEROL.

MHB		3.5		4.5	
0.93	0.86	0.66	0.73	1.06	1.06
0.99	0.93	0.93	0.99	0.80	0.93
0.86	0.86	0.86	1.06	0.93	0.86
0.73	0.93	0.86	0.93	0.86	0.99
0.86	0.73	0.99	1.06	0.93	0.93

MHB: moderate halophile broth.

3.5: MHB + 3.5 molal NaCl.

4.5: MHB + 4.5 molal glycerol.

All cell diameters in μ.

Mean for MHB = 0.91 +/- 0.13μ.

Mean for MHB + 3.5 molal NaCl = 0.87 +/- 0.09μ.

Mean for MHB + 4.5 molal glycerol = 0.93 +/- 0.09μ.

APPENDIX 6.13: DATA FOR THE SQUARE ROOT PLOTS OF STAPHYLOCOCCUS XYLOSUS STRAIN CM21/3 AT DIFFERENT SODIUM CHLORIDE CONCENTRATIONS (FIGURE 3.15, FIGURE 3.16, FIGURE 3.17, FIGURE 3.18 AND FIGURE 3.19).

Temp.: temperature (K).

OD0.3: time for change in OD = 0.3 (rate = 1/OD0.3).

O.RR.: observed square root of growth rate.

P.RR.: predicted square root of growth rate using least squares analysis.

GT : generation time (rate = 1/GT).

GTAW : generation time predicted by the modified Square Root Model to incorporate a_w.

%DIF : absolute % difference between GT and GTAW.

All times in minutes.

Appendix 6.13a: Data for Figure 3.15a and Figure 3.15b.

Figure 3.15a:

Temp.	ODO.3	O.RR.	P.RR	Residual
300.8	179.0	0.074744	0.074017	0.000727
299.3	207.1	0.069488	0.069367	0.000121
298.0	222.1	0.067101	0.065618	0.001483
296.8	260.7	0.061934	0.061868	0.000066
295.5	292.1	0.058511	0.058118	0.000393
294.5	335.5	0.054595	0.054968	-0.000373
293.7	368.9	0.052065	0.052569	-0.000504
292.7	407.0	0.049568	0.049569	-0.000001
291.8	451.8	0.047047	0.047169	-0.000122
291.1	516.5	0.044001	0.047769	-0.003768
290.2	570.5	0.041867	0.042069	-0.000202
289.6	641.5	0.039482	0.040269	-0.000787
288.8	731.8	0.036966	0.037869	-0.000903
288.2	799.5	0.035366	0.036070	-0.000704
287.4	916.0	0.033041	0.033820	-0.000779
286.8	1032.0	0.031129	0.032020	-0.000891
286.2	1102.5	0.030117	0.030220	-0.000103
285.5	1297.5	0.027762	0.027970	-0.000208
284.9	1411.5	0.026617	0.026170	0.000447
284.3	1680.0	0.024398	0.024370	0.000028
284.0	1840.0	0.023313	0.023470	-0.000157
283.1	2210.0	0.021272	0.020920	0.000350
282.5	2560.0	0.019764	0.018971	0.000793
281.9	2945.0	0.018427	0.017321	0.001106
281.2	4020.0	0.015772	0.015221	0.000551
280.6	5322.0	0.013708	0.013271	0.000437

Appendix 6.13a: Data for Figure 3.15a and Figure 3.15b Continued.

Figure 3.15b:

Temp.	GT	O.RR.	P.RR.	Residual
300.8	31.1	0.179316	0.179187	0.000129
299.3	34.1	0.171247	0.167758	0.003489
298.0	37.8	0.162650	0.158540	0.004110
296.8	43.8	0.151000	0.149323	0.001777
295.5	50.1	0.141280	0.140106	0.001174
294.5	59.4	0.129750	0.132363	-0.002613
293.7	66.9	0.122261	0.126464	-0.004203
292.7	72.6	0.117363	0.119090	-0.001727
291.9	79.4	0.112225	0.113191	-0.000966
291.1	89.0	0.106000	0.107292	-0.001292
290.2	102.0	0.099015	0.100655	-0.001640
289.6	115.5	0.093048	0.096231	-0.003183
288.8	130.0	0.087706	0.090332	-0.002626
288.2	134.0	0.086387	0.085907	0.000480
287.4	161.0	0.078811	0.080377	-0.001566
286.8	186.0	0.073324	0.075953	-0.002629
286.2	197.0	0.071247	0.071528	-0.000281
285.5	224.0	0.066815	0.065998	0.000817
284.9	191.5	0.072263	0.061574	0.010689
284.3	313.0	0.056523	0.057149	-0.000626
284.0	361.0	0.052632	0.054937	-0.002305
283.1	444.0	0.047458	0.048669	-0.001211
282.5	506.0	0.044455	0.043876	0.000579
281.9	539.0	0.043073	0.039821	0.003252
281.2	858.0	0.034139	0.034659	-0.000520
280.6	1057.0	0.030758	0.029866	0.000892

Appendix 6.13b: Data for Figure 3.15c and Figure 3.15d.

Figure 3.15c:

Temp.	OD0.3	O.RR.	P.RR.	Residual
300.9	175.6	0.075464	0.076986	-0.001522
299.1	192.4	0.072094	0.071452	0.000642
297.8	218.5	0.067651	0.067455	0.000196
296.7	242.9	0.064163	0.064073	0.000090
295.6	270.2	0.060836	0.060692	0.000144
294.5	296.2	0.058104	0.057310	0.000794
293.5	337.6	0.054425	0.054235	0.000190
292.6	373.8	0.051723	0.051469	0.000254
291.8	417.9	0.048918	0.049009	-0.000091
290.9	458.0	0.046727	0.046242	0.000485
289.9	576.4	0.041652	0.043168	-0.001516
288.7	633.0	0.039746	0.039479	0.000267
288.0	714.0	0.037424	0.037327	0.000097
287.2	821.0	0.034900	0.034867	0.000033
286.6	899.0	0.033352	0.033023	0.000329
286.0	1014.0	0.031404	0.031178	0.000226
284.7	1349.0	0.027227	0.027181	0.000046
284.1	1524.0	0.025616	0.025337	0.000279
283.6	1817.0	0.023460	0.023800	-0.000340
283.0	2089.0	0.021879	0.021955	-0.000076
282.4	2526.0	0.019897	0.020110	-0.000213
281.7	3098.0	0.017966	0.017958	0.000008
281.1	4110.0	0.015598	0.016114	-0.000516
280.6	4584.0	0.014770	0.014577	0.000193

Appendix 6.13b: Data for Figure 3.15c and Figure 3.15d Continued.

Figure 3.15d:

Temp.	GT	O.RR.	P.RR.	Residual	GTAW	%DIF
300.9	27.6	0.190347	0.190711	-0.000364	27.6	0.1
299.1	31.4	0.178458	0.176855	0.001603	32.0	2.0
297.8	35.0	0.169031	0.166848	0.002183	36.2	3.7
296.7	38.1	0.162008	0.158381	0.003628	39.9	4.6
295.6	39.3	0.159516	0.149913	0.009603	44.4	13.1
294.5	49.3	0.142422	0.141446	0.000976	49.8	1.1
293.5	53.8	0.136336	0.133748	0.002588	55.7	3.5
292.6	59.5	0.129641	0.126820	0.002821	61.8	3.9
291.8	74.2	0.116091	0.120662	-0.004571	68.2	8.1
290.9	89.0	0.106000	0.113734	-0.007734	76.6	13.9
289.9	99.0	0.100504	0.106036	-0.005532	88.0	11.1
288.7	133.5	0.086549	0.096799	-0.010250	105.6	21.2
288.0	129.5	0.087875	0.091411	-0.003536	117.8	9.1
287.2	163.0	0.078326	0.085253	-0.006927	135.0	17.2
286.6	167.5	0.077267	0.080634	-0.003367	150.6	10.1
286.0	186.0	0.073324	0.076015	-0.002691	169.0	9.1
283.6	260.0	0.062017	0.057541	0.004476	290.8	11.9
283.0	326.0	0.055385	0.052922	0.002463	342.1	4.9
282.4	403.0	0.049814	0.048304	0.001510	408.1	1.3
281.7	456.0	0.046829	0.042915	0.003914	512.6	12.4
281.1	530.0	0.043437	0.038297	0.005140	637.7	20.3
280.6	674.0	0.038519	0.034448	0.004071	780.6	15.8

Appendix 6.13c: Data for Figure 3.16a and Figure 3.16b.

Figure 3.16a:

Temp.	OD0.3	O.RR	P.RR.	Residual
299.6	203.3	0.070134	0.070453	-0.000319
298.5	228.3	0.066183	0.067150	-0.000967
297.3	254.1	0.062733	0.063546	-0.000813
296.0	280.3	0.059729	0.059643	0.000086
294.8	312.3	0.056587	0.056040	0.000547
294.0	342.7	0.054019	0.053638	0.000381
293.1	380.2	0.051285	0.050935	0.000350
292.2	424.4	0.048541	0.048233	0.000308
291.3	475.0	0.045883	0.045531	0.000352
290.5	543.0	0.042914	0.043129	-0.000215
289.6	607.0	0.040589	0.040426	0.000163
289.0	647.0	0.039314	0.038625	0.000689
288.2	743.0	0.036686	0.036223	0.000463
287.7	829.0	0.034731	0.034721	0.000010
286.9	955.0	0.032359	0.032319	0.000040
286.3	1080.0	0.030429	0.030518	-0.000089
285.7	1154.0	0.029437	0.028716	0.000721
285.0	1377.0	0.026948	0.026614	0.000334
284.5	1512.0	0.025717	0.025113	0.000604
284.0	1802.0	0.023557	0.023612	-0.000055
283.4	2164.0	0.021497	0.021810	-0.000313
282.8	2705.0	0.019227	0.020008	-0.000781
282.2	3406.0	0.017135	0.018207	-0.001072
281.7	3774.0	0.016278	0.016706	-0.000428

Appendix 6.13c: Data for Figure 3.16a and Figure 3.16b Continued.

Figure 3.16b:

Temp.	GT	O.RR	P.RR.	Residual	GTAW	%DIF
299.6	40.2	0.157720	0.157283	0.000437	38.2	5.1
298.5	46.5	0.146647	0.150143	-0.003496	42.0	9.7
297.3	49.2	0.142567	0.142355	0.000212	46.8	4.9
296.0	58.5	0.130744	0.133917	-0.003173	53.1	9.3
294.8	60.1	0.128992	0.126128	0.002864	60.0	0.1
294.0	68.5	0.120824	0.120936	-0.000112	65.4	4.5
293.1	68.7	0.120648	0.115094	0.005554	72.5	5.5
292.2	83.4	0.109501	0.109253	0.000248	80.7	3.3
291.3	94.0	0.103142	0.103411	-0.000269	90.4	3.8
290.5	103.0	0.098533	0.098219	0.000314	100.6	2.4
289.6	117.0	0.092450	0.092377	0.000073	114.2	2.4
289.0	130.0	0.087706	0.088483	-0.000777	124.9	3.9
288.2	146.0	0.082761	0.083290	-0.000529	141.7	2.9
287.7	159.0	0.079305	0.080045	-0.000740	154.0	3.2
286.9	186.0	0.073324	0.074853	-0.001529	177.2	4.7
286.3	200.0	0.070711	0.070958	-0.000247	198.2	0.9
285.7	198.0	0.071067	0.067064	0.004003	223.2	12.7
284.5	293.0	0.058421	0.059275	-0.000854	289.9	1.1
284.0	323.0	0.055642	0.056030	-0.000388	326.7	1.2
283.4	359.0	0.052778	0.052136	0.000642	381.1	6.2
282.8	437.0	0.047837	0.048241	-0.000405	450.3	3.0
282.2	535.0	0.043234	0.044347	-0.001113	540.3	1.0
281.7	613.0	0.040390	0.041102	-0.000712	637.3	4.0

Appendix 6.13d: Data for Figure 3.16c and Figure 3.16d.

Figure 3.16c:

Temp.	OD0.3	O.RR.	P.RR.	Residual
300.1	335.3	0.054611	0.054517	0.000094
298.7	371.0	0.051917	0.051782	0.000135
297.3	419.6	0.048818	0.048747	0.000071
296.3	460.3	0.046610	0.046579	0.000031
295.3	510.5	0.044259	0.044412	-0.000153
294.1	575.5	0.041685	0.041810	-0.000125
292.9	624.0	0.040032	0.039209	0.000823
292.0	702.5	0.037729	0.037258	0.000471
291.3	775.0	0.035921	0.035740	0.000181
290.3	890.5	0.033511	0.033573	-0.000062
289.6	971.5	0.032083	0.032055	0.000028
287.3	1436.0	0.026389	0.027069	-0.000680
286.6	1602.0	0.024984	0.025552	-0.000568
285.9	1831.0	0.023370	0.024034	-0.000664
285.2	2025.0	0.022222	0.022517	-0.000295
284.6	2315.0	0.020784	0.021216	-0.000432
283.8	2763.0	0.019024	0.019482	-0.000458
283.1	2990.0	0.018288	0.017964	0.000324
282.4	3386.0	0.017185	0.016447	0.000738
281.8	4183.0	0.015462	0.015146	0.000316
281.1	4993.0	0.014152	0.013629	0.000523

Figure 3.16d:

Temp.	GT	O.RR.	P.RR.	Residual	GTAW	%DIF
300.1	38.5	0.161165	0.157537	0.003628	45.2	17.3
298.7	41.6	0.155043	0.148603	0.006440	50.9	22.3
297.3	49.9	0.141563	0.139668	0.001895	57.7	15.7
296.3	53.8	0.136336	0.133286	0.003050	63.5	18.1
295.3	64.0	0.125000	0.126904	-0.001904	70.2	9.8
294.1	73.0	0.117041	0.112461	0.004580	79.8	9.3
292.9	73.5	0.116642	0.111588	0.005054	91.5	21.5
292.0	97.0	0.101535	0.105844	-0.004309	102.0	5.2
291.3	109.0	0.095783	0.101377	-0.005594	111.5	2.3
290.3	132.5	0.086874	0.094995	-0.008121	127.5	3.8
289.6	159.0	0.079305	0.090528	-0.011223	140.9	11.4
287.3	179.0	0.074744	0.075849	-0.001105	203.4	13.7
286.6	175.0	0.075593	0.071382	0.004211	230.9	32.0
285.9	241.0	0.064416	0.066915	-0.002499	264.4	9.7
285.2	238.0	0.064820	0.062458	0.002372	305.7	28.4
284.6	340.0	0.054233	0.058618	-0.004385	349.3	2.7
283.8	351.0	0.053376	0.053513	-0.000137	423.6	20.7
282.4	357.0	0.052926	0.044578	0.008348	625.8	75.3
281.8	536.0	0.043193	0.040749	0.002444	759.5	41.7
281.1	615.0	0.040324	0.036282	0.004042	977.8	59.0

Appendix 6.13e: Data for Figure 3.17a and Figure 3.17b.

Figure 3.17a:

Temp.	ODO.3	O.RR.	P.RR.	Residual
300.9	332	0.054882	0.055367	-0.000485
299.4	375	0.051640	0.052029	-0.000389
298.1	427	0.048393	0.049135	-0.000742
296.9	461	0.046575	0.046464	0.000111
295.8	513	0.044151	0.044015	0.000136
294.8	541	0.042993	0.041789	0.001204
293.8	632	0.039778	0.039564	0.000214
292.9	714	0.037424	0.037560	-0.000136
292.0	784	0.035714	0.035668	0.000046
291.3	851	0.034280	0.033999	0.000281
290.5	941	0.032599	0.032218	0.000381
289.8	1095	0.030220	0.030771	-0.000551
289.0	1220	0.028630	0.028879	-0.000249
288.2	1389	0.026832	0.027210	-0.000378
287.4	1471	0.026073	0.025429	0.000644
286.8	1711	0.024176	0.023982	0.000194
286.2	1923	0.022804	0.022647	0.000157
285.5	2213	0.021257	0.021200	0.000057
284.8	2426	0.020303	0.019531	0.000772
284.2	3222	0.017617	0.018195	-0.000578
283.5	3150	0.017817	0.016637	0.001180
283.0	3827	0.016165	0.015524	0.000641
282.4	5046	0.014078	0.014188	-0.000110
282.0	6336	0.012563	0.013298	-0.000735
281.4	8240	0.011016	0.011963	-0.000947
280.7	10415	0.009799	0.010516	-0.000717

Appendix 6.13e: Data for Figure 3.17a and Figure 3.17b Continued.

Figure 3.17b:

Temp.	GT	O.RR.	P.RR.	Residual	GTAW	%DIF
300.9	62	0.127000	0.132891	-0.005891	53.6	13.5
299.4	69	0.120386	0.124899	-0.004513	60.7	12.0
298.1	71	0.118678	0.117972	0.000706	68.0	4.2
296.9	76	0.114708	0.111577	0.003131	76.0	0.0
295.8	88	0.106600	0.105716	0.000884	84.6	3.8
294.8	85	0.108465	0.100387	0.008078	93.8	10.4
293.8	113	0.094072	0.095059	-0.000987	104.6	7.4
292.9	136	0.085749	0.090263	-0.004514	116.0	14.7
292.0	138	0.085126	0.085734	-0.000608	129.3	6.3
291.3	161	0.078811	0.081738	-0.002927	141.3	12.2
290.5	120	0.091287	0.077475	0.013812	157.2	31.0
289.8	176	0.075378	0.074011	0.001367	173.2	1.4
289.0	243	0.064150	0.069482	-0.005332	195.3	19.6
288.2	241	0.064416	0.065486	-0.001070	221.5	8.1
286.8	258	0.062257	0.057760	0.004497	282.1	9.3
286.2	335	0.054636	0.054562	0.000074	315.9	5.7
284.8	462	0.046524	0.047103	-0.000579	423.1	8.4
283.5	596	0.040962	0.040176	0.000786	580.2	2.6
283.0	631	0.039809	0.037511	0.002298	664.8	5.4
282.4	915	0.033059	0.034314	-0.001255	793.2	13.3
282.0	1307	0.027661	0.032183	-0.004522	900.7	31.1
281.4	1363	0.027086	0.028986	-0.001900	1307.9	18.7
280.7	1738	0.023987	0.025522	-0.001535	1454.6	16.3

Appendix 6.13f: Data for Figure 3.17c and Figure 3.17d.

Figure 3.17c:

Temp.	ODO.3	O.RR.	P.RR.	Residual
300.7	355.9	0.053007	0.052799	0.000208
299.4	382.2	0.051151	0.050120	0.001031
298.2	438.5	0.047755	0.047647	0.000108
296.9	487.0	0.045314	0.044968	0.000346
295.9	543.5	0.042894	0.042907	-0.000013
294.8	607.0	0.040589	0.040640	-0.000051
294.0	676.2	0.038456	0.038991	-0.000535
293.0	786.0	0.035669	0.036931	-0.001262
292.2	819.0	0.034943	0.035282	-0.000339
291.3	897.0	0.033389	0.033530	-0.000141
290.5	938.0	0.032651	0.031882	0.000769
289.8	1079.0	0.030443	0.030439	0.000004
289.0	1197.0	0.028904	0.028687	0.000217
288.4	1337.0	0.027349	0.027451	-0.000102
287.6	1481.0	0.025985	0.025802	0.000183
287.0	1790.0	0.023636	0.024669	-0.001033
286.5	1885.0	0.023033	0.023535	-0.000502
285.8	2227.0	0.021190	0.022093	-0.000903
285.2	2283.0	0.020929	0.020856	0.000073
284.4	2713.0	0.019199	0.019208	-0.000009
283.8	3100.0	0.017961	0.017971	-0.000010
283.2	3379.0	0.017203	0.016735	0.000468
282.6	4135.0	0.015551	0.015498	0.000053
282.0	4900.0	0.014286	0.014262	0.000024
281.3	5535.0	0.013441	0.012922	0.000519
280.8	5910.0	0.013008	0.011892	0.001116
280.2	9370.0	0.010331	0.010552	-0.000221

Appendix 6.13f: Data for Figure 3.17c and Figure 3.17d Continued.

Figure 3.17d:

Temp.	GT	O.RR.	P.RR.	Residual	GTAW	%DIF
300.7	105.0	0.097590	0.109555	-0.011965	75.9	27.8
299.4	97.5	0.101274	0.103938	-0.002664	84.5	13.3
298.2	101.5	0.099258	0.098753	0.000505	93.8	7.6
296.9	111.5	0.094703	0.093135	0.001568	105.8	5.1
295.9	134.0	0.086387	0.088814	-0.002427	116.6	12.9
294.8	147.0	0.082479	0.084061	-0.001582	130.6	11.1
294.0	158.0	0.079556	0.080604	-0.001048	142.4	9.9
293.0	116.5	0.092648	0.076283	0.016365	159.6	37.0
292.2	160.0	0.079057	0.072826	0.006231	180.0	12.5
291.3	188.0	0.072933	0.069154	0.003779	202.0	7.4
290.5	183.0	0.073922	0.065697	0.008225	218.9	19.6
289.8	218.0	0.067729	0.062672	0.005057	241.5	10.8
289.0	301.0	0.057639	0.058999	-0.001360	271.9	9.7
288.4	353.0	0.053225	0.056406	-0.003181	298.6	15.4
287.6	382.0	0.051165	0.052950	-0.001785	340.8	10.8
287.0	407.0	0.049568	0.050573	-0.001005	378.7	7.0
286.5	653.0	0.039133	0.048196	-0.009063	415.3	36.4
285.8	433.0	0.048057	0.045172	0.002885	476.1	9.9
285.2	716.0	0.037372	0.042579	-0.005207	539.5	24.7
284.4	611.0	0.040456	0.039122	0.001334	645.8	5.7
283.8	1266.0	0.028105	0.036530	-0.008425	747.6	40.9
283.2	531.0	0.043396	0.033937	0.009459	875.5	64.9
282.6	1011.0	0.031450	0.031344	0.000106	1039.4	2.8
282.0	1218.0	0.028653	0.028752	-0.000099	1253.9	3.0
281.3	1600.0	0.025000	0.025943	-0.000943	1600.1	0.0
280.8	2012.0	0.022294	0.023783	-0.001489	1943.3	3.4
280.2	3190.0	0.017705	0.020974	-0.003269	2523.4	20.9

Appendix 6.13g: Data for Figure 3.18a and Figure 3.18b.

Figure 3.18a:

Temp.	ODO.3	O.RR.	P.RR.	Residual
294.9	1436	0.026389	0.025929	0.000460
294.3	1705	0.024218	0.025170	-0.000952
293.8	1624	0.024815	0.024410	0.000405
293.1	1763	0.023816	0.023513	0.000303
292.6	1807	0.023525	0.022754	0.000771
292.0	1975	0.022502	0.021925	0.000577
291.1	2272	0.020980	0.020683	0.000297
290.3	2744	0.019090	0.019578	-0.000488
289.9	3021	0.018194	0.019026	-0.000832
289.0	3360	0.017252	0.017783	-0.000531
288.2	3929	0.015954	0.016679	-0.000725
287.1	4600	0.014744	0.015160	-0.000416
285.9	5500	0.013484	0.013572	-0.000088
284.5	6700	0.012217	0.011640	0.000577
283.3	8855	0.010627	0.009980	0.000647

Figure 3.18b:

Temp.	GT	O.RR.	P.RR.	Residual	GTAW	%DIF
294.9	209	0.069171	0.066033	0.003138	212.6	1.7
294.3	170	0.076697	0.064240	0.012457	226.7	33.4
293.8	358	0.052852	0.062447	-0.009595	239.6	33.1
293.1	325	0.055470	0.060329	-0.004859	259.5	20.2
292.6	251	0.063119	0.058536	0.004583	275.2	9.7
292.0	316	0.056254	0.056580	-0.000326	296.1	6.3
291.1	381	0.051232	0.053647	-0.002415	332.2	12.8
290.3	471	0.046078	0.051039	-0.004961	370.2	21.4
289.9	467	0.046275	0.049736	-0.003461	391.6	16.1
289.0	460	0.046625	0.046802	-0.000177	447.3	2.8
288.2	496	0.044901	0.044195	0.000706	507.4	2.3
287.1	556	0.042409	0.040609	0.001800	611.9	10.1
285.9	785	0.035692	0.036861	-0.001169	767.6	2.2
284.5	760	0.036274	0.032298	0.003976	1037.9	36.6
283.3	1215	0.028689	0.028386	0.000303	1401.8	15.4

Appendix 6.13h: Data for Figure 3.18c and Figure 3.18d.

Figure 3.18c:

Temp.	GT	O.RR.	P.RR.	Residual
297.9	3257	0.017522	0.017718	-0.000196
297.4	3472	0.016971	0.017304	-0.000333
296.9	3490	0.016927	0.016890	0.000037
296.4	3743	0.016345	0.016476	-0.000131
295.9	4055	0.015704	0.016061	-0.000357
295.3	4089	0.015638	0.015564	0.000074
294.8	4369	0.015129	0.015150	-0.000021
294.1	4337	0.015185	0.014571	0.000614
293.5	4758	0.014497	0.014074	0.000423
292.9	5248	0.013804	0.013577	0.000227
292.1	5825	0.013102	0.012914	0.000188
291.3	6055	0.012851	0.012250	0.000601
290.7	7035	0.011923	0.011755	0.000168
290.0	9250	0.010398	0.011175	-0.000777
289.0	10485	0.009766	0.010347	-0.000581
287.9	11085	0.009498	0.009430	0.000068

Figure 3.18d:

Temp.	GT	O.RR.	P.RR.	Residual	GTAW	%DIF
297.9	311	0.056705	0.062003	-0.005298	245.8	21.0
297.4	306	0.057166	0.060433	-0.003267	257.4	15.9
296.9	332	0.054882	0.058864	-0.003982	269.8	18.7
296.4	350	0.053452	0.057294	-0.003842	283.1	19.1
295.9	352	0.053300	0.055724	-0.002424	297.4	15.5
295.3	306	0.057166	0.053841	0.003325	316.1	3.3
294.8	289	0.058824	0.052271	0.006553	333.1	15.2
294.1	288	0.058926	0.050074	0.008852	359.2	24.7
293.5	325	0.055470	0.048190	0.007280	384.1	18.1
292.9	474	0.045932	0.046307	-0.000375	411.7	13.2
292.1	425	0.048507	0.043795	0.004712	453.4	6.7
291.3	510	0.044281	0.041284	0.002997	501.7	1.6
290.7	730	0.037012	0.039400	-0.002388	543.4	25.6
290.0	945	0.032530	0.037203	-0.004673	598.5	36.7
289.0	1160	0.029361	0.034063	-0.004702	693.3	40.2
287.9	1290	0.027842	0.030610	-0.002768	826.2	36.0

Appendix 6.13i: Data for Figure 3.19a and Figure 3.19b.

Figure 3.19a:

Temp.	ODO.3	O.RR.	P.RR.	Residual
299.8	2050	0.022086	0.022273	-0.000187
298.2	2435	0.020265	0.020680	-0.000415
296.7	2832	0.018791	0.019186	-0.000395
295.5	3012	0.018221	0.017991	0.000230
294.3	3312	0.017376	0.016796	0.000580
293.2	3884	0.016046	0.015701	0.000345
292.1	4115	0.015589	0.014605	0.000984
291.0	5890	0.013030	0.013510	-0.000480
289.9	6120	0.012783	0.012414	0.000369
288.8	8505	0.010843	0.011319	-0.000476
287.8	10595	0.009715	0.010323	-0.000608
286.9	11710	0.009241	0.009420	-0.000179
285.8	13610	0.008572	0.008330	0.000242

Figure 3.19b:

Temp.	GT	O.RR.	P.RR.	Residual	AWGT	%DIF
299.8	318	0.056077	0.055426	0.000651	416.6	31.0
298.2	447	0.047298	0.052004	-0.004706	478.5	7.1
296.7	411	0.049326	0.048796	0.000530	550.0	33.8
295.5	481	0.045596	0.046230	-0.000634	619.4	28.8
294.3	472	0.046029	0.043664	0.002365	702.8	48.9
293.2	519	0.043895	0.041312	0.002583	795.1	53.2
292.1	569	0.041922	0.038959	0.002963	906.7	59.3
291.0	885	0.033615	0.036607	-0.002992	1043.6	17.9
289.9	855	0.034199	0.034254	-0.000055	1214.1	42.0
288.8	870	0.033903	0.031902	0.002001	1429.9	64.4
287.8	1060	0.030715	0.029763	0.000952	1680.4	58.5
286.9	1325	0.027472	0.027839	-0.000367	1966.6	48.4
285.8	2030	0.022195	0.025486	-0.003291	2427.9	19.6

APPENDIX 6.14: ACTIVATION ENERGIES PREDICTED BY THE ARRHENIUS EQUATION AT DIFFERENT TEMPERATURES (FIGURE 3.20).

Temperature	Activation Energy
301.2	59.6
299.4	63.4
298.2	66.3
297.6	67.9
293.5	81.4
290.9	93.8
287.3	120.4
284.6	154.8
283.4	178.1

Temperatures in K.

Activation energies in kJ mol^{-1}.

APPENDIX 6.15: DATA FOR THE SQUARE ROOT PLOTS OF STAPHYLOCOCCUS XYLOSUS STRAIN CM21/3 AT DIFFERENT GLYCEROL CONCENTRATIONS (FIGURE 3.22 AND FIGURE 3.23).

Temp.: temperature (K).

OD0.3: time for change in OD = 0.3 (rate = 1/OD0.3).

O.RR.: observed square root of growth rate.

P.RR.: predicted square root of growth rate using least squares analysis.

GT : generation time (rate = 1/GT).

GTAW : generation time predicted by the modified Square Root Model to incorporate a_w.

%DIF : absolute % difference between GT and GTAW.

All times in minutes.

Appendix 6.15a: Data for Figure 3.22a and Figure 3.22b.

Figure 3.22a:

Temp.	OD0.3	O.RR.	P.RR.	Residual
292.7	439.3	0.047711	0.048292	-0.000581
291.8	487.7	0.045282	0.045543	-0.000261
290.8	545.5	0.042816	0.042487	0.000329
289.7	636.5	0.039637	0.039126	0.000511
288.7	752.0	0.036466	0.036070	0.000394
287.6	913.0	0.033095	0.032710	0.000385
286.5	1184.0	0.029062	0.029349	-0.000287
285.3	1567.0	0.025262	0.025683	-0.000421
283.9	2202.0	0.021310	0.021406	-0.000096
282.5	3398.0	0.017155	0.017128	0.000027

Figure 3.22b:

Temp.	GT	O.RR.	P.RR.	Residual	GTAW	%DIF
292.7	47.5	0.145095	0.133635	0.011460	60.6	27.7
291.8	57.5	0.131876	0.125259	0.006617	68.0	18.3
290.8	78.0	0.113228	0.115952	-0.002724	77.9	0.1
289.7	107.0	0.096674	0.105714	-0.009040	91.6	14.4
288.7	124.0	0.089803	0.096408	-0.006605	107.4	13.4
287.6	151.0	0.081379	0.086170	-0.004791	129.9	14.0
286.5	202.0	0.070360	0.075933	-0.005573	160.4	20.6
285.3	260.0	0.062017	0.064764	-0.002747	207.7	20.1
283.9	330.0	0.055048	0.051735	0.003313	295.0	10.6
282.5	420.0	0.048795	0.038705	0.010090	451.5	7.5

Appendix 6.15b: Data for Figure 3.22c and Figure 3.22d.

Figure 3.22c:

Temp.	OD0.3	O.RR.	P.RR.	Residual
299.1	432	0.048113	0.048823	-0.000710
298.2	453	0.046984	0.047067	-0.000083
297.3	472	0.046029	0.045310	0.000719
296.2	540	0.043033	0.043163	-0.000130
295.3	569	0.041922	0.041407	0.000515
294.3	634	0.039715	0.039455	0.000260
293.2	716	0.037372	0.037308	0.000064
292.3	811	0.035115	0.035552	-0.000437
291.4	918	0.033005	0.033795	-0.000790
290.0	1026	0.031220	0.031062	0.000158
288.6	1225	0.028571	0.028330	0.000241
287.3	1456	0.026207	0.025793	0.000414
286.3	1749	0.023911	0.023841	0.000070
285.1	2180	0.021418	0.021499	-0.000081
283.8	2680	0.019317	0.018962	0.000355
282.4	4075	0.015665	0.016229	-0.000564

Figure 3.22d:

Temp.	GT	O.RR.	P.RR.	Residual	GTAW	%DIF
298.2	37.0	0.164399	0.148649	0.015750	45.1	22.0
297.3	48.0	0.144338	0.142382	0.001956	49.2	2.4
296.2	55.0	0.134840	0.134722	0.000118	54.8	0.3
295.3	66.5	0.122628	0.128455	-0.005827	60.2	9.4
294.3	71.0	0.118678	0.121492	-0.002814	67.2	5.3
293.2	81.5	0.110770	0.113832	-0.003062	76.4	6.2
292.3	98.0	0.101015	0.107565	-0.006550	85.4	12.8
291.4	96.0	0.102062	0.101298	0.000764	96.2	0.2
290.0	122.0	0.090536	0.091549	-0.001013	117.3	3.9
288.6	207.0	0.069505	0.081800	-0.012295	146.3	29.3
287.3	182.0	0.074125	0.072747	0.001378	184.0	1.1
286.3	239.0	0.064685	0.065784	-0.001099	223.9	6.3
285.1	302.0	0.057544	0.057428	0.000116	291.5	3.5
283.8	412.0	0.049267	0.048375	0.000892	406.3	1.4
282.4	395.0	0.050316	0.038626	0.011690	626.1	58.5

Appendix 6.15c: Data for Figure 3.23a and Figure 3.23b.

Figure 3.23a:

Temp.	OD0.3	O.RR.	P.RR.	Residual
300.3	416	0.049029	0.050137	-0.001108
299.4	439	0.047727	0.048430	-0.000703
298.5	475	0.045883	0.046724	-0.000841
297.4	505	0.044499	0.044638	-0.000139
296.4	532	0.043356	0.042742	0.000614
295.4	581	0.041487	0.040845	0.000642
294.6	637	0.039621	0.039328	0.000293
293.6	695	0.037932	0.037432	0.000500
292.6	762	0.036226	0.035536	0.000690
291.6	861	0.034080	0.033640	0.000440
290.6	923	0.032915	0.031744	0.001171
288.5	1250	0.028284	0.027761	0.000523
287.4	1520	0.025650	0.025676	-0.000026
286.2	1818	0.023453	0.023400	0.000053
285.0	2388	0.020464	0.021125	-0.000661
283.9	2952	0.018405	0.019039	-0.000634
282.4	4228	0.015379	0.016194	-0.000815

Figure 3.23b:

Temp.	GT	O.RR.	P.RR.	Residual	GTAW	%DIF
300.3	56	0.133631	0.132255	0.001376	53.6	4.3
299.4	62	0.127000	0.127511	-0.000511	57.9	6.6
298.5	70	0.119523	0.122768	-0.003245	62.7	10.4
297.4	77	0.113961	0.116970	-0.003009	70.0	9.7
296.4	80	0.111803	0.111699	0.000106	76.8	4.1
295.4	89	0.106000	0.106428	-0.000428	85.1	4.3
294.6	97	0.101535	0.102211	-0.000676	92.9	4.3
293.6	104	0.098058	0.096940	0.001118	104.1	0.1
292.6	113	0.094072	0.091670	0.002403	117.5	4.0
291.6	126	0.089087	0.086399	0.002688	133.7	6.1
290.6	147	0.082479	0.081128	0.001351	153.5	4.4
288.5	186	0.073324	0.070059	0.003265	212.5	14.2
287.4	218	0.067729	0.064261	0.003468	258.0	18.3
286.2	270	0.060858	0.057936	0.002922	326.5	20.9
285.0	572	0.041812	0.051611	-0.009799	426.4	25.4
283.9	504	0.044544	0.045913	-0.001371	564.6	12.0
282.4	687	0.038152	0.037907	0.000245	894.4	30.2

Appendix 6.15d: Data for Figure 3.23c and Figure 3.23d.

Figure 3.23c:

Temp.	ODO.3	O.RR.	P.RR.	Residual
300.5	1350	0.027217	0.027913	-0.000696
299.5	1429	0.026454	0.026856	-0.000402
298.6	1502	0.025803	0.025904	-0.000101
297.7	1643	0.024671	0.024953	-0.000282
296.7	1754	0.023877	0.023896	-0.000019
295.6	1876	0.023088	0.022732	0.000356
294.7	2022	0.022239	0.021781	0.000458
293.7	2233	0.021162	0.020723	0.000439
292.7	2522	0.019913	0.019666	0.000247
291.7	2780	0.018966	0.018609	0.000357
290.7	3077	0.018028	0.017551	0.000477
289.7	3735	0.016363	0.016494	-0.000131
288.6	4059	0.015696	0.015331	0.000365
287.5	4785	0.014456	0.014168	0.000288
286.5	5970	0.012942	0.013110	-0.000168
285.4	7375	0.011645	0.011947	-0.000302
284.0	9520	0.010249	0.010467	-0.000218
282.7	14075	0.008429	0.009090	-0.000661

Figure 3.23d:

Temp.	GT	O.RR.	P.RR.	Residual	GTAW	%DIF
300.5	178	0.074953	0.077550	-0.002597	167.6	5.8
299.5	180	0.074536	0.074431	0.000105	182.6	1.4
298.6	192	0.072169	0.071624	0.000545	197.8	3.0
297.7	211	0.068843	0.068817	0.000026	215.0	1.9
296.7	228	0.066227	0.065698	0.000529	237.0	3.9
295.6	250	0.063246	0.062267	0.000979	265.2	6.1
294.7	285	0.059235	0.059459	-0.000224	292.2	2.5
293.7	331	0.054965	0.056340	-0.001375	327.4	1.1
292.7	348	0.053606	0.053221	0.000385	369.3	6.1
291.7	350	0.053452	0.050102	0.003350	419.9	20.0
290.7	438	0.047782	0.046983	0.000799	481.6	9.9
289.7	562	0.042183	0.043864	-0.001681	566.5	0.8
288.6	605	0.040656	0.040433	0.000223	664.9	9.9
287.5	715	0.037398	0.037002	0.000396	805.8	12.7
286.5	825	0.034816	0.033883	0.000933	976.8	18.4
285.4	1125	0.029814	0.030452	-0.000638	1236.3	9.9
284.0	1510	0.025734	0.026009	-0.000275	1748.3	15.8
282.7	2350	0.020628	0.022030	-0.001402	2572.9	9.5

APPENDIX 6.16: DATA FOR THE CALIBRATION OF NEPHELOMETER FOR HALOBACTERIUM SP. STRAIN HB9 AND HALOBACTERIUM SALINARIUM STRAIN CM42/12 (FIGURE 3.26).

Appendix 6.16a: Data for Figure 3.26a.

Optical Density	Reciprocal of Dilution
0.413	1
0.227	2
0.109	4
0.040	8

Appendix 6.16b: Data for Figure 3.26b.

Optical Density	Reciprocal of Dilution
0.379	1
0.204	2
0.092	4
0.041	8

Appendix 6.16c: Data for Figure 3.26c.

Optical Density	Reciprocal of Dilution
0.309	1
0.182	2
0.060	4
0.022	8

Appendix 6.16d: Data for Figure 3.26d.

Optical Density	Reciprocal of Dilution
0.331	1
0.161	2
0.080	4
0.034	8

APPENDIX 6.17: DATA FROM THE MEASUREMENT OF CELLS OF HALOBACTERIUM SP. STRAIN HB9 AND HALOBACTERIUM SALINARIUM STRAIN CM42/12, GROWN IN XHB + 3.5 MOLAL SODIUM CHLORIDE AND XHB + 6.0 MOLAL SODIUM CHLORIDE.

3.5				6.0			
HB9		CM42/12		HB9		CM42/12	
L	W	L	W	L	W	L	W
3.2	0.6	2.2	0.6	3.5	0.7	2.7	0.7
2.4	0.8	2.7	0.7	4.2	0.6	4.3	0.6
2.9	0.7	3.5	0.5	2.8	0.7	3.5	0.5
2.5	0.8	2.9	0.7	4.1	0.6	2.5	0.5
2.8	0.7	3.1	0.6	2.4	0.5	2.9	0.6
3.3	0.6	2.1	0.8	3.4	0.6	3.9	0.5
3.0	0.6	2.4	0.9	3.0	0.6	2.8	0.7
2.7	0.7	2.7	0.7	3.5	0.7	3.6	0.5
2.1	0.9	3.7	0.8	4.0	0.8	3.3	0.8
2.4	0.8	2.9	0.6	3.7	0.6	3.4	0.5

3.5 : XHB + 3.5 molal NaCl.

6.0 : XHB + 4.5 molal glycerol.

HB9 : Halobacterium sp. strain HB9.

CM42/12: Halobacterium salinarium strain CM42/12.

L : Length (μ).

W : Width (μ).

Mean for 3.5 HB9 L/W = 2.74 +/- 0.38μ / 0.72 +/- 0.10μ.

Mean for 3.5 CM42/12 L/W = 2.82 +/- 0.52μ / 0.69 +/- 0.12μ.

Mean for 6.0 HB9 L/W = 3.46 +/- 0.59μ / 0.64 +/- 0.08μ.

Mean for 6.0 CM42/12 L/W = 3.29 +/- 0.57μ / 0.59 +/- 0.11μ.

APPENDIX 6.18: DATA FOR THE SQUARE ROOT PLOTS OF HALOBACTERIUM SP. STRAIN HB9 AT DIFFERENT SODIUM CHLORIDE CONCENTRATIONS (FIGURE 3.27, FIGURE 3.28, FIGURE 3.29 FIGURE 3.30 AND FIGURE 3.31).

Temp.: temperature (K).

OD0.3: time for change in OD = 0.3 (rate = 1/OD0.3).

O.RR.: observed square root of growth rate.

P.RR.: predicted square root of growth rate using least squares analysis.

GT : generation time (rate = 1/GT).

All times in minutes.

Appendix 6.18a: Data for Figure 3.27a and Figure 3.27b.

Figure 3.27a:

Temp.	ODO.3	O.RR.	P.RR.	Residual
304.5	4510	0.014891	0.014807	0.000084
303.6	5055	0.014065	0.014100	-0.000035
302.6	5665	0.013286	0.013314	-0.000028
301.7	6450	0.012452	0.012607	-0.000155
300.8	6970	0.011978	0.011900	0.000078
299.9	8025	0.011163	0.011193	-0.000030
298.9	9115	0.010474	0.010407	0.000067
298.0	10640	0.009695	0.009690	0.000005
297.0	12100	0.009091	0.008910	0.000181
296.1	15080	0.008143	0.008200	-0.000057
295.1	18930	0.007268	0.007420	-0.000152
294.1	22280	0.006700	0.006630	0.000070

Figure 3.27b:

Temp.	GT	O.RR.	P.RR.	Residual
304.5	540	0.043033	0.043458	-0.000425
303.6	600	0.040825	0.041247	-0.000422
302.6	620	0.040161	0.038790	0.001371
301.7	750	0.036515	0.036579	-0.000064
300.8	810	0.035136	0.034368	0.000768
299.9	1075	0.030500	0.032157	-0.001657
298.9	1140	0.029617	0.029700	-0.000083
298.0	1300	0.027735	0.027489	0.000246
297.0	1420	0.026537	0.025033	0.001504
296.1	2140	0.021617	0.022822	-0.001205
295.1	2610	0.019574	0.020365	-0.000791
294.1	2870	0.018666	0.017909	0.000757

Appendix 6.18b: Data for Figure 3.27c and Figure 3.27d.

Figure 3.27c:

Temp.	ODO.3	O.RR.	P.RR.	Residual
307.3	1831	0.023370	0.023635	-0.000265
306.1	2008	0.022316	0.022426	-0.000110
305.2	2133	0.021652	0.021519	0.000133
304.2	2345	0.020650	0.020511	0.000139
303.4	2555	0.019784	0.019705	0.000079
302.4	2842	0.018758	0.018697	0.000061
301.5	3101	0.017958	0.017790	0.000168
300.6	3462	0.016996	0.016883	0.000113
299.7	3928	0.015956	0.011598	0.004358
298.8	4425	0.015033	0.015068	-0.000035
297.9	5165	0.013914	0.014161	-0.000247
296.8	5650	0.013304	0.013053	0.000251
295.9	6985	0.011965	0.012146	-0.000181
295.1	7990	0.011187	0.011339	-0.000152
294.1	9670	0.010169	0.011033	-0.000864
292.9	11440	0.009350	0.009120	0.000230

Figure 3.27d:

Temp.	GT	O.RR.	P.RR.	Residual
306.1	309	0.056888	0.058106	-0.001218
305.2	362	0.052559	0.055583	-0.003024
304.2	323	0.055642	0.052780	0.002862
303.4	375	0.051640	0.050538	0.001102
302.4	421	0.048737	0.047735	0.001002
301.5	468	0.046225	0.045212	0.001013
300.6	531	0.043396	0.042690	0.000706
299.7	674	0.038519	0.040167	-0.001648
298.8	704	0.037689	0.037645	0.000044
297.9	930	0.032791	0.035122	-0.002331
296.8	915	0.033059	0.032391	0.000668
295.9	1005	0.031544	0.029517	0.002027
295.1	1375	0.026968	0.027274	-0.000306
294.1	1800	0.023570	0.024471	-0.000901
292.9	2320	0.020761	0.021108	-0.000347

Appendix 6.18c: Data for Figure 3.28a and Figure 3.28b.

Figure 3.28a:

Temp.	ODO. 3	O.RR	P.RR	Residual
310.2	1942	0.022692	0.023115	-0.000423
308.9	2088	0.021884	0.022046	-0.000162
307.8	2223	0.021210	0.021141	0.000069
306.8	2458	0.020170	0.020319	-0.000149
305.7	2585	0.019668	0.019415	0.000253
304.7	2789	0.018936	0.018593	0.000343
303.8	3092	0.017984	0.017853	0.000131
302.9	3399	0.017152	0.017113	0.000039
302.1	3724	0.016387	0.016455	-0.000068
301.3	4041	0.015731	0.015797	-0.000066
300.5	4290	0.015268	0.015140	0.000128
299.7	4584	0.014770	0.014482	0.000288
299.0	5058	0.014061	0.013906	0.000155
298.3	5568	0.013401	0.013331	0.000070
297.5	6375	0.012525	0.012673	-0.000148
296.7	6865	0.012069	0.012015	0.000054
296.0	7855	0.011283	0.011440	-0.000157
295.3	8585	0.010793	0.010864	-0.000071
294.4	9835	0.010084	0.010125	-0.000041
293.6	11525	0.009315	0.009460	-0.000145
292.3	14505	0.008303	0.008390	-0.000087

Figure 3.28b:

Temp.	GT	O.RR.	P.RR.	Residual
306.8	275	0.060302	0.060624	-0.000322
305.7	280	0.059761	0.059555	0.000206
304.7	330	0.055048	0.056945	-0.001897
303.8	315	0.056344	0.054597	0.001747
302.9	359	0.052778	0.052248	0.000530
302.1	402	0.049876	0.050160	-0.000284
301.3	408	0.049507	0.048072	0.001435
300.5	455	0.046881	0.045984	0.000897
299.7	509	0.044324	0.043897	0.000427
299.0	594	0.041031	0.042070	-0.001039
298.3	580	0.041523	0.040243	0.001280
297.5	680	0.038348	0.038155	0.000193
296.7	755	0.036394	0.036067	0.000327
296.0	830	0.034711	0.034241	0.000470
295.3	850	0.034300	0.032414	0.001886
294.4	1215	0.028689	0.030065	-0.001376
293.6	1470	0.026082	0.027977	-0.001895
292.3	1765	0.023803	0.024585	-0.000782

Appendix 6.18d: Data for Figure 3.28c and Figure 3.28d.

Figure 3.28c:

Temp.	ODO.3	O.RR.	P.RR.	Residual
312.5	1783	0.023682	0.023602	0.000080
311.3	1903	0.022924	0.022672	0.000252
309.6	2093	0.021858	0.021355	0.000503
308.2	2304	0.020833	0.020270	0.000563
306.9	2681	0.019313	0.019262	0.000051
305.8	2897	0.018579	0.018410	0.000169
304.8	3198	0.017683	0.017635	0.000048
304.0	3440	0.017050	0.017015	0.000035
302.9	3780	0.016265	0.016163	0.000102
302.1	4227	0.015381	0.015543	-0.000162
301.3	4703	0.014582	0.014923	-0.000341
300.4	5378	0.013636	0.014225	-0.000589
299.8	5787	0.013145	0.013760	-0.000615
298.9	6388	0.012512	0.013063	-0.000551
298.2	6955	0.011991	0.012520	-0.000529
297.6	7335	0.011676	0.012055	-0.000379
296.8	8035	0.011156	0.011435	-0.000279
296.1	8770	0.010678	0.010893	-0.000215
295.3	9750	0.010127	0.010273	-0.000146
294.7	10645	0.009692	0.009800	-0.000108
293.9	11715	0.009239	0.009180	0.000059
293.1	13255	0.008686	0.008560	0.000126
292.2	15525	0.008026	0.007870	0.000156
291.3	16985	0.007673	0.007170	0.000503
290.2	20845	0.006926	0.006320	0.000606
289.4	24530	0.006385	0.005700	0.000685

Appendix 6.18d: Data for Figure 3.28c and Figure 3.28d Continued.

Figure 3.28d:

Temp.	GT	O.RR.	P.RR.	Residual
312.5	195	0.071612	0.075179	-0.003567
311.3	201	0.070535	0.072280	-0.001745
309.6	207	0.069505	0.068183	0.001322
308.2	226	0.066519	0.064805	0.001714
306.9	248	0.063500	0.061668	0.001832
304.8	254	0.062746	0.056602	0.006144
304.0	282	0.059549	0.054671	0.004878
302.9	368	0.052129	0.052017	0.000112
302.1	455	0.046881	0.050087	-0.003206
301.3	550	0.042640	0.048157	-0.005517
300.4	434	0.048002	0.045986	0.002012
299.8	628	0.039904	0.044538	-0.004634
298.9	671	0.038605	0.042366	-0.003761
298.2	630	0.039841	0.040678	-0.000837
297.6	620	0.040161	0.039230	0.000931
296.8	640	0.039529	0.037300	0.002229
296.1	725	0.037139	0.035611	0.001528
295.3	850	0.034300	0.033681	0.000619
294.7	940	0.032616	0.032233	0.000383
293.9	1075	0.030500	0.030303	0.000197
293.1	1250	0.028284	0.028373	-0.000089
292.2	1630	0.024769	0.026201	-0.001432
291.3	1660	0.024544	0.024030	0.000514
290.2	2110	0.021770	0.021376	0.000394
289.4	2650	0.019426	0.019446	-0.000020

Appendix 6.18e: Data for Figure 3.29a and Figure 3.29b.

Figure 3.29a:

Temp.	OD0.3	O.RR.	P.RR.	Residual
311.6	1719	0.024119	0.023433	-0.000686
310.2	1838	0.023325	0.022272	0.001053
308.9	2108	0.021780	0.021194	0.000586
307.7	2453	0.020191	0.020198	-0.000007
306.5	2678	0.019324	0.019203	0.000121
305.6	2968	0.018356	0.018456	-0.000100
304.7	3222	0.017617	0.017709	-0.000092
303.7	3509	0.016881	0.016880	0.000001
303.0	4056	0.015702	0.016299	-0.000597
302.2	4293	0.015262	0.015635	-0.000373
301.2	5093	0.014012	0.014806	-0.000794
300.6	5393	0.013617	0.014308	-0.000691
299.8	6020	0.012889	0.013644	-0.000755
299.0	6442	0.012459	0.012980	-0.000521
298.3	6825	0.012105	0.012400	-0.000295
297.5	7725	0.011378	0.011736	-0.000358
296.7	8720	0.010709	0.011072	-0.000363
296.1	9665	0.010172	0.010575	-0.000403
295.3	10585	0.009720	0.009910	-0.000190
294.4	10990	0.009539	0.009169	0.000379
293.7	12855	0.008820	0.008580	0.000240
293.0	14465	0.008315	0.008000	0.000315
292.0	16845	0.007705	0.007170	0.000535
291.1	19045	0.007246	0.006420	0.000826
290.1	24290	0.006416	0.005900	0.000516

Appendix 6.18e: Data for Figure 3.29a and Figure 3.29b Continued.

Figure 3.29b:

Temp.	GT	O.RR. R	P.RR.	Residual
311.6	174	0.075810	0.075226	0.000584
310.2	200	0.070711	0.071428	-0.000717
308.9	183	0.073922	0.067900	0.006022
307.7	260	0.062017	0.064644	-0.002627
306.5	229	0.066082	0.061388	0.004694
305.6	172	0.076249	0.051891	0.024358
304.7	202	0.070360	0.049721	0.020639
303.7	244	0.064018	0.047007	0.017011
303.0	362	0.052559	0.045379	0.007180
302.2	432	0.048113	0.043209	0.004904
301.2	537	0.043153	0.041038	0.002115
300.6	606	0.040622	0.039138	0.001484
299.8	689	0.038097	0.036968	0.001129
299.0	560	0.042258	0.034797	0.007461
298.3	725	0.037139	0.033169	0.003970
297.5	895	0.033426	0.036968	-0.003542
296.7	1030	0.031159	0.034797	-0.003638
296.1	1080	0.030429	0.033169	-0.002740
295.3	1075	0.030500	0.030998	-0.000498
294.4	1355	0.027166	0.028556	-0.001390
293.7	1795	0.023603	0.026657	-0.003054
293.0	2375	0.020520	0.024787	-0.004267
292.0	1690	0.024325	0.022044	0.002281
291.1	2490	0.020040	0.019602	0.000438
290.1	2650	0.019426	0.016889	0.002537

Appendix 6.18f: Data for Figure 3.29c and Figure 3.29d.

Figure 3.29c:

Temp.	ODO.3	O.RR.	P.RR.	Residual
316.3	984	0.031879	0.031873	0.000006
315.1	1018	0.031342	0.030770	0.000572
313.8	1112	0.029988	0.029574	0.000414
312.6	1162	0.029336	0.028470	0.000866
311.2	1294	0.027799	0.027182	0.000617
310.0	1432	0.026426	0.026078	0.000348
308.8	1630	0.024769	0.024974	-0.000205
307.9	1718	0.024126	0.024146	-0.000020
306.8	1962	0.022576	0.023135	-0.000559
305.9	2052	0.022076	0.022307	-0.000231
304.7	2320	0.020761	0.021203	-0.000442
303.8	2555	0.019784	0.020375	-0.000591
302.8	2775	0.018983	0.019455	-0.000472
301.8	3030	0.018167	0.018535	-0.000368
301.0	3300	0.017408	0.017799	-0.000391
300.0	3775	0.016276	0.016879	-0.000603
299.1	4150	0.015523	0.016051	-0.000528
298.1	4710	0.014571	0.015132	-0.000561
297.3	5195	0.013874	0.014396	-0.000522
296.3	5810	0.013119	0.013476	-0.000357
295.2	5850	0.013074	0.012464	0.000610
294.2	7645	0.011437	0.011544	-0.000107
293.1	8755	0.010687	0.010532	0.000155
291.9	10175	0.009914	0.009420	0.000494
290.8	12025	0.009119	0.008410	0.000709
289.5	14165	0.008402	0.007220	0.001182

Appendix 6.18f: Data for Figure 3.29c and Figure 3.29d Continued.

Figure 3.29d:

Temp.	GT	O.RR.	P.RR.	Residual
308.8	204	0.070014	0.071911	-0.001897
307.9	229	0.066082	0.069509	-0.003427
306.8	234	0.065372	0.066574	-0.001202
305.9	221	0.067267	0.064172	0.003095
304.7	262	0.061780	0.060970	0.000810
303.8	267	0.061199	0.058568	0.002631
302.8	273	0.060523	0.058590	0.001933
301.8	308	0.056980	0.053231	0.003749
301.0	395	0.050316	0.051096	-0.000780
300.0	465	0.046374	0.048428	-0.002054
299.1	473	0.045980	0.046026	-0.000046
298.1	534	0.043270	0.043357	-0.000083
297.3	663	0.038837	0.041222	-0.002385
296.3	740	0.036761	0.038554	-0.001793
295.2	980	0.031944	0.035618	-0.003674
294.2	990	0.031782	0.032950	-0.001168
293.1	1160	0.029361	0.030014	-0.000653
291.9	1330	0.027420	0.026812	0.000608
290.8	1550	0.025400	0.023877	0.001523
289.5	1970	0.022530	0.020408	0.002122

Appendix 6.18g: Data for Figure 3.30a and Figure 3.30b.

Figure 3.30a:

Temp.	ODO.3	O.RR.	P.RR.	Residual
312.5	882	0.033672	0.032387	0.001285
311.0	1076	0.030486	0.030827	-0.000341
309.5	1142	0.029592	0.029267	0.000325
308.0	1278	0.027973	0.027707	0.000266
306.9	1432	0.026426	0.026563	-0.000137
305.8	1642	0.024678	0.025419	-0.000741
304.7	1699	0.024261	0.024275	-0.000014
303.8	1884	0.023039	0.023339	-0.000300
302.9	2028	0.022206	0.022403	-0.000197
302.1	2141	0.021612	0.021570	0.000042
301.3	2222	0.021214	0.020738	0.000476
299.6	2967	0.018359	0.018970	-0.000611
298.9	3154	0.017806	0.018242	-0.000436
298.2	3137	0.017854	0.017514	0.000340
297.6	3776	0.016274	0.016890	-0.000616
296.9	4143	0.015536	0.016162	-0.000626
296.0	4329	0.015199	0.015226	-0.000027
295.4	4853	0.014355	0.014602	-0.000247
294.7	5232	0.013825	0.013874	-0.000049
294.1	5658	0.013294	0.013250	0.000044
293.3	6775	0.012149	0.012418	-0.000269
292.4	7205	0.011781	0.011482	0.000299
291.6	9045	0.010515	0.010649	-0.000134
290.5	8515	0.010837	0.009500	0.001337
289.7	10855	0.009598	0.008670	0.000928

Appendix 6.18g: Data for Figure 3.30a and Figure 3.30b Continued.

Figure 3.30b:

Temp.	GT	O.RR.	P.RR.	Residual
312.5	157	0.079809	0.080281	-0.000471
311.0	176	0.075378	0.076724	-0.001346
309.5	201	0.070535	0.073166	-0.002631
308.0	224	0.066815	0.069609	-0.002794
306.9	245	0.063888	0.067000	-0.003112
305.8	212	0.068680	0.064391	0.004289
304.7	231	0.065795	0.061783	0.004012
303.8	263	0.061663	0.059648	0.002015
302.9	260	0.062017	0.057137	0.004880
302.1	310	0.056796	0.055616	0.001180
301.3	350	0.053452	0.053719	-0.000267
299.6	400	0.050000	0.049687	0.000313
298.9	401	0.049938	0.048027	0.001911
298.2	554	0.042486	0.046367	-0.003881
297.6	479	0.045691	0.044944	0.000747
296.9	566	0.042033	0.043284	-0.001251
296.0	589	0.041204	0.041149	0.000055
295.4	658	0.038984	0.039726	-0.000742
294.7	751	0.036491	0.038066	-0.001575
294.1	734	0.036911	0.036643	0.000268
293.3	890	0.033520	0.034746	-0.001226
292.4	960	0.032275	0.032612	-0.000337
291.6	1100	0.030151	0.030714	-0.000563
289.7	1360	0.027116	0.026208	0.000908

Appendix 6.18h: Data for Figure 3.30c and Figure 3.30d.

Figure 3.30c:

Temp.	OD0.3	O.RR.	P.RR.	Residual
311.5	1278	0.027973	0.028575	-0.000602
310.2	1361	0.027106	0.027379	-0.000273
308.7	1432	0.026426	0.025999	0.000427
307.5	1643	0.024671	0.024894	-0.000223
305.2	1657	0.024566	0.022778	0.001788
304.3	1850	0.023250	0.021950	0.001300
303.4	2167	0.021482	0.021121	0.000361
302.6	2183	0.021403	0.020385	0.001018
301.8	2475	0.020101	0.019649	0.000452
300.1	3412	0.017120	0.018085	-0.000965
299.4	3188	0.017711	0.017440	0.000271
298.5	3420	0.017100	0.016612	0.000488
297.8	4557	0.014814	0.015968	-0.001154
297.0	4073	0.015669	0.015232	0.000437
296.2	5415	0.013589	0.014496	-0.000907
295.7	5667	0.013284	0.014036	-0.000752
295.0	5812	0.013117	0.013391	-0.000274
294.1	5920	0.012997	0.012563	0.000434
293.3	7093	0.011874	0.011827	0.000047
292.7	7960	0.011208	0.011275	-0.000067
291.8	8810	0.010654	0.010447	0.000207
290.8	9020	0.010529	0.009520	0.001009
290.1	12330	0.009006	0.008880	0.000126

Appendix 6.18h: Data for Figure 3.30c and Figure 3.30d Continued.

Figure 3.30d:

Temp.	GT	O.RR.	P.RR.	Residual
311.5	210	0.069007	0.072305	-0.003298
310.2	235	0.065233	0.069144	-0.003911
308.7	225	0.066667	0.065482	0.001185
307.5	246	0.063758	0.062557	0.001201
305.2	255	0.062622	0.056952	0.005670
304.3	378	0.051435	0.054759	-0.003324
303.4	340	0.054233	0.055257	-0.001024
302.6	440	0.047673	0.050616	-0.002943
301.8	368	0.052129	0.048666	0.003463
300.1	504	0.044544	0.044524	0.000020
299.4	469	0.046176	0.042818	0.003358
298.5	699	0.037824	0.040624	-0.002800
297.8	626	0.039968	0.038918	0.001050
297.0	775	0.035921	0.036969	-0.001048
296.2	650	0.039223	0.035019	0.004204
295.7	698	0.037851	0.033801	0.004050
295.0	988	0.031814	0.032095	-0.000281
294.1	1143	0.029579	0.029901	-0.000322
293.3	1325	0.027472	0.027952	-0.000480
292.7	1810	0.023505	0.026490	-0.002985
291.8	1735	0.024008	0.024296	-0.000288
290.8	3120	0.017903	0.021859	-0.003956
290.1	2520	0.019921	0.020153	-0.000232

Appendix 6.18i: Data for Figure 3.31.

Figure 3.31:

Temp.	OD0.3	O.RR.	P.RR.	Residual
302.3	4073	0.015669	0.015831	-0.000162
301.5	4189	0.015451	0.015139	0.000312
300.7	4781	0.014462	0.014524	-0.000062
299.7	5442	0.013756	0.013556	0.000200
298.8	5774	0.013194	0.013064	0.000130
297.9	6530	0.012375	0.012372	0.000003
296.9	7454	0.011583	0.011604	-0.000021

APPENDIX 6.19: DATA FOR THE SQUARE ROOT PLOTS OF HALOBACTERIUM SALINARIUM STRAIN CM42/12 AT DIFFERENT SODIUM CHLORIDE CONCENTRATIONS (FIGURE 3.33, FIGURE 3.34 AND FIGURE 3.35).

Temp.: temperature (K).

OD0.3: time for change in OD = 0.3 (rate = 1/OD0.3).

O.RR.: observed square root of growth rate.

P.RR.: predicted square root of growth rate using least squares analysis.

GT : generation time (rate = 1/GT).

All times in minutes.

Appendix 6.19a: Data for Figure 3.33a and Figure 3.33b.

Figure 3.33a:

Temp.	ODO.3	O.RR.	P.RR.	Residual
307.2	2554	0.019787	0.019169	0.000618
306.0	2792	0.018925	0.018345	0.000580
305.2	3076	0.018031	0.017795	0.000236
304.3	3332	0.017324	0.017177	0.000147
303.5	3381	0.017198	0.016627	0.000571
302.4	3915	0.015982	0.015871	0.000111
301.6	4280	0.015285	0.015322	-0.000037
300.9	4875	0.014322	0.014841	-0.000519
300.0	5275	0.013769	0.014222	-0.000453
299.2	5800	0.013131	0.013673	-0.000542
298.4	6430	0.012471	0.013123	-0.000652
297.6	7315	0.011692	0.012527	-0.000835
296.6	7830	0.011301	0.011886	-0.000559
295.8	8280	0.010990	0.011336	-0.000346
295.0	8725	0.010706	0.010787	-0.000081
294.1	10065	0.009968	0.010168	-0.000200
293.2	11075	0.009502	0.009540	-0.001038
292.3	11710	0.009241	0.008930	0.000311
291.1	13310	0.008668	0.008100	0.000568
289.8	16720	0.007734	0.007210	0.000524
288.7	19570	0.007148	0.006450	0.000698

Figure 3.33b:

Temp.	GT	O.RR.	P.RR.	Residual
309.5	294	0.058321	0.060214	-0.001893
308.3	296	0.058124	0.057625	0.010499
307.2	347	0.053683	0.055253	-0.001570
306.0	350	0.053452	0.052665	0.000787
305.2	385	0.050965	0.050939	0.000026
304.3	423	0.048622	0.048998	-0.000376
303.5	457	0.046778	0.047272	-0.000494
302.4	460	0.046625	0.044900	0.001725
301.6	500	0.044721	0.043174	0.001547
300.9	510	0.044281	0.041665	0.002616
300.0	565	0.042070	0.039723	0.002347
299.2	740	0.036761	0.037998	-0.001237
298.4	825	0.034816	0.036272	-0.001456
293.2	1700	0.024254	0.025057	-0.000803
292.3	2380	0.020498	0.023115	-0.002617
291.1	3035	0.018152	0.020527	-0.002375
289.8	2720	0.019174	0.017723	0.001451
288.7	3390	0.017175	0.015351	0.001824

Appendix 6.19b: Data for Figure 3.33c and Figure 3.33d.

Figure 3.33c:

Temp.	OD0.3	O.RR.	P.RR.	Residual
310.7	1783	0.023682	0.023830	-0.000148
309.5	1914	0.022858	0.022790	0.000068
308.3	2176	0.021437	0.021750	-0.000313
307.1	2313	0.020793	0.020710	0.000083
306.2	2517	0.019932	0.019930	0.000002
305.3	2848	0.018738	0.019150	-0.000412
304.3	2918	0.018512	0.018284	0.000228
303.5	3187	0.017714	0.017590	0.000124
302.7	3409	0.017127	0.016897	0.000230
301.7	3694	0.016453	0.016030	0.000423
301.0	4183	0.015462	0.015424	0.000038
300.1	4781	0.014463	0.014644	-0.000181
299.2	5228	0.013830	0.013864	-0.000034
298.5	5885	0.013036	0.013257	-0.000221
297.5	6533	0.012372	0.012391	-0.000019
296.6	7268	0.011730	0.011611	0.000119
295.8	7395	0.011629	0.010917	0.000712
295.0	9705	0.010151	0.010224	-0.000073
294.2	10230	0.009887	0.009530	0.000357
293.5	14105	0.008420	0.008920	-0.000500
292.6	16005	0.007905	0.008140	-0.000235
291.7	19685	0.007127	0.007360	-0.000233

Appendix 6.19b: Data for Figure 3.33c and Figure 3.33d Continued.

Figure 3.33d:

Temp.	GT	O.RR.	P.RR.	Residual
310.7	235	0.065233	0.062469	0.002764
309.5	248	0.063500	0.059880	0.003620
308.3	290	0.058722	0.057289	0.001433
307.1	335	0.054636	0.054699	-0.000063
306.2	400	0.050000	0.052757	-0.002757
305.3	471	0.046078	0.050814	-0.004736
304.3	514	0.044108	0.048656	-0.004548
303.5	544	0.042875	0.046930	-0.004055
302.7	461	0.046575	0.045203	0.001372
301.7	643	0.039436	0.043045	-0.003609
301.0	479	0.045691	0.041534	0.004157
300.1	598	0.040893	0.039582	0.001311
299.2	743	0.036686	0.037649	-0.000963
298.5	787	0.035646	0.036139	-0.000493
297.5	830	0.034711	0.033980	0.000731
296.6	757	0.036346	0.032038	0.004196
295.7	809	0.035158	0.030311	0.004847
295.0	1065	0.030643	0.028585	0.002058
294.2	1115	0.029948	0.026858	0.003090
293.5	2140	0.021617	0.025347	-0.003730
292.6	2065	0.022006	0.023405	-0.001399
291.7	3040	0.018137	0.021463	-0.003326

Appendix 6.19c: Data for Figure 3.34a and Figure 3.34b.

Figure 3.34a:

Temp.	ODO.3	O.RR.	P.RR.	Residual
310.8	1767	0.023789	0.023872	-0.000083
309.3	1805	0.023538	0.022644	0.002736
308.3	2090	0.021874	0.021825	0.000049
307.3	2234	0.021157	0.021006	0.000151
306.1	2489	0.020044	0.020023	0.000017
305.3	2581	0.019684	0.019368	0.000316
304.4	2864	0.018686	0.018631	0.000055
303.5	3218	0.017628	0.017894	-0.000266
302.6	3400	0.017150	0.017157	-0.000007
301.8	3822	0.016175	0.016502	-0.000327
301.0	4313	0.015227	0.015847	-0.000620
300.1	4406	0.015065	0.015109	-0.000044
299.4	4923	0.014252	0.014537	-0.000285
298.6	5393	0.013617	0.013882	-0.000265
297.8	6258	0.012641	0.013226	-0.000585
297.1	6738	0.012182	0.012653	-0.000471
296.4	7188	0.011795	0.012080	-0.000285
295.5	7678	0.011412	0.011343	0.000069
294.6	8535	0.010824	0.010606	0.000218
293.6	9770	0.010117	0.009780	0.000337
292.7	11055	0.009511	0.009050	0.000461
291.8	13630	0.008566	0.008310	0.000256
290.5	17000	0.007670	0.007240	0.000430

Appendix 6.19c: Data for Figure 3.34a and Figure 3.34b Continued.

Figure 3.34b:

Temp.	GT	O.RR	P.RR.	Residual
309.3	267	0.061199	0.059018	0.002181
308.3	270	0.060858	0.056750	0.004108
307.3	301	0.057639	0.054482	0.003157
306.1	366	0.052271	0.051760	0.000511
305.3	509	0.044324	0.049945	-0.005621
304.4	435	0.047946	0.047904	0.000042
303.5	450	0.047141	0.045862	0.001279
302.6	556	0.042409	0.043821	-0.001412
301.8	650	0.039223	0.042006	-0.002783
301.0	869	0.033923	0.040192	-0.006269
300.1	676	0.038462	0.038150	0.000312
299.4	789	0.035601	0.036562	-0.000961
298.6	940	0.032616	0.034748	-0.002132
297.8	938	0.032651	0.032933	-0.000282
297.1	907	0.033205	0.031345	0.001860
296.4	1110	0.030015	0.029758	0.000257
295.5	1411	0.026622	0.027716	-0.001094
294.6	1452	0.026243	0.025675	0.000568
293.6	1313	0.027597	0.023405	0.004192
292.7	1559	0.025327	0.021365	0.003692
291.8	3235	0.017582	0.019324	-0.001742
290.5	3790	0.016244	0.016375	-0.000131

Appendix 6.19d: Data for Figure 3.34c and Figure 3.34d.

Figure 3.34c:

Temp.	ODO.3	O.RR.	P.RR.	Residual
313.9	1514	0.025700	0.025920	-0.000220
312.6	1598	0.025016	0.024894	0.000122
311.2	1747	0.023925	0.023790	0.000135
309.9	1914	0.022858	0.022764	0.000094
308.8	2071	0.021974	0.021896	0.000078
307.3	2358	0.020593	0.020712	-0.000119
306.3	2533	0.019869	0.019923	-0.000054
305.4	2830	0.018798	0.019213	-0.000415
304.7	2553	0.019791	0.018661	0.001130
304.1	3128	0.017880	0.018818	-0.000938
302.8	3431	0.017072	0.017162	-0.000090
302.0	3703	0.016433	0.016531	-0.000098
301.2	3985	0.015841	0.015899	-0.000058
300.4	4462	0.014971	0.015268	-0.000297
299.7	4712	0.014568	0.014716	-0.000148
298.9	4689	0.014604	0.014085	0.000519
298.1	5960	0.012953	0.013453	-0.000500
297.2	6295	0.012604	0.012743	-0.000139
296.5	6618	0.012292	0.012191	0.000101
295.7	7695	0.011400	0.011560	-0.000160
294.9	8535	0.010824	0.010928	-0.000104
294.0	9435	0.010295	0.010218	0.000077
293.1	10835	0.009607	0.009500	-0.003430
292.2	12045	0.009112	0.008790	0.000322

Appendix 6.19d: Data for Figure 3.34c and Figure 3.34d Continued.

Figure 3.34d:

Temp.	GT	O.RR.	P.RR.	Residual
313.9	205	0.069843	0.066086	0.003757
312.6	238	0.064820	0.063410	0.001410
311.2	270	0.060858	0.060528	0.000330
309.9	344	0.053916	0.057851	-0.003935
308.8	380	0.051299	0.055587	-0.004288
307.3	279	0.059868	0.052499	0.007369
306.3	419	0.048853	0.050440	-0.001587
305.4	452	0.047036	0.048587	-0.001551
304.7	635	0.039684	0.047146	-0.007462
304.1	438	0.047782	0.045911	0.001871
302.8	522	0.043769	0.043235	0.000534
302.0	732	0.036961	0.041588	-0.004627
301.2	577	0.041631	0.039941	0.001690
300.4	803	0.035289	0.038294	-0.003005
299.7	639	0.039559	0.036853	0.002706
298.9	716	0.037372	0.035206	0.002166
298.1	510	0.044281	0.033559	0.010722
297.2	1040	0.031009	0.031706	-0.000697
296.5	1277	0.027984	0.030265	-0.002281
295.7	1380	0.026919	0.028618	-0.001699
294.9	1530	0.025566	0.026971	-0.001405
294.0	1450	0.026261	0.025118	0.001143
293.1	1930	0.022763	0.023265	-0.000502
292.2	2310	0.020806	0.021413	-0.000607

Appendix 6.1e: Data for Figure 3.35a and Figure 3.35b.

Figure 3.35a:

Temp.	ODO.3	O.RR.	P.RR.	Residual
315.5	1436	0.026389	0.026335	0.000054
313.9	1736	0.024001	0.025058	-0.001057
312.2	1818	0.023453	0.023621	-0.000168
311.0	1821	0.023434	0.022743	0.000691
309.6	2026	0.022217	0.021625	0.000592
308.5	2235	0.021153	0.020747	0.000406
307.4	2452	0.020195	0.019869	0.000326
306.4	2826	0.018811	0.019071	-0.000260
305.6	3138	0.017852	0.018432	-0.000580
303.7	3382	0.017195	0.016915	0.000280
302.8	3663	0.016523	0.016197	0.000326
302.1	4197	0.015436	0.015638	-0.000202
301.5	4432	0.015021	0.015159	-0.000138
300.7	4709	0.014573	0.014520	0.000053
299.8	5524	0.013455	0.013802	-0.000347
298.2	5815	0.013114	0.012525	0.000589
297.4	7390	0.011633	0.011886	-0.000253
296.7	8235	0.011020	0.011327	-0.000307
295.7	9335	0.010350	0.010529	-0.000179
294.9	10175	0.009914	0.009890	0.000024
293.4	12775	0.008848	0.008690	0.000158

Figure 3.35b:

Temp.	GT	O.RR	P.RR.	Residual
315.5	220	0.067420	0.067891	-0.000471
313.9	287	0.059028	0.064668	-0.005640
312.2	356	0.053000	0.061043	-0.008043
311.0	315	0.056344	0.058828	-0.002484
309.6	280	0.059761	0.056009	0.003752
308.5	279	0.059868	0.053793	0.006075
307.4	296	0.058124	0.051578	0.006546
306.4	385	0.050965	0.049564	0.001401
305.6	387	0.050833	0.047953	0.003038
303.7	420	0.048795	0.044126	0.004669
302.8	509	0.044324	0.042314	0.002010
302.1	672	0.038576	0.040904	-0.002328
301.5	707	0.037609	0.039696	-0.002087
300.7	554	0.042486	0.038085	0.004401
299.8	926	0.032862	0.036272	-0.003410
298.2	1030	0.031159	0.033050	-0.001891
297.4	1105	0.030083	0.031439	-0.001356
296.7	1120	0.029881	0.030029	-0.000148
295.7	1290	0.027842	0.028015	-0.000173
294.9	1600	0.025000	0.026404	-0.001404
293.4	2250	0.021082	0.023383	-0.002301

Appendix 6.19f: Data for Figure 3.35c.

Figure 3.35c:

Temp.	ODO.3	O.RR.	P.RR.	Residual
302.1	4820	0.014404	0.014546	-0.000142
301.2	5011	0.014127	0.013952	0.000175
300.1	5770	0.013165	0.013226	-0.000061
299.2	6078	0.012827	0.012632	0.000192
298.3	7125	0.011847	0.012038	-0.000191
297.3	7687	0.011406	0.011378	0.000028